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American skullcap (*Scutellaria lateriflora* L): a study of its effects on mood in healthy volunteers

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**American skullcap (*Scutellaria lateriflora* L):
a study of its effects on mood in healthy
volunteers**

Christine A Brock

**A thesis submitted in partial fulfilment of the requirements of the
University of Westminster for the degree of Doctor of Philosophy**

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Abstract

American skullcap (*Scutellaria lateriflora*) has long been an important herb in North American traditional medicine systems and western materia medica for anxiety and related disorders. A previous clinical study assessing acute effects of *S. lateriflora* indicated it has anxiolytic actions with minimal loss of energy or cognition. The aim of the present study was to extend these findings by determining the putative mood enhancing effects and safety of *S. lateriflora* following its chronic administration to healthy volunteers. Based on a randomised, double-blind, placebo-controlled crossover design, it examined the effects of *S. lateriflora* on psychologic symptoms using the Beck Anxiety Inventory (BAI) and the Profile of Mood States (POMS) questionnaires, and its potential physiological effects on stress by measuring free cortisol levels in saliva, at baseline and following administration of *S. lateriflora* or placebo. Liver function was assessed at the same time points by measuring levels of serum alanine aminotransferase (ALT). Pre and post intervention blood pressure and pulse rate were also measured. Freeze-dried *S. lateriflora* for use in the present study was authenticated by the use of high performance liquid chromatography.

Participants (n = 43) were randomised to either freeze-dried *S. lateriflora* (350 mg) or *Urtica dioica folia* (300 mg) placebo three times daily for 14 days. There was a 7 day washout period prior to crossover. Analysis of results was from 31 participants completing the study. Results from the BAI demonstrated no significant difference between *S. lateriflora* and placebo ($p = 0.191$) in anxiety scores. The results from the POMS factors demonstrated there was no significant difference between *S. lateriflora* and placebo in scores for Tension-Anxiety ($p = 0.473$), Anger-Hostility ($p = 0.070$) or for Depression-Dejection ($p = 0.067$). Overall results from subjective measures of global mood as measured by Total Mood Disturbance (TMD) on the POMS also demonstrated no significant difference between *S. lateriflora* and placebo ($p = 0.137$). Additionally, the POMS showed there was no reduction in energy or cognition following chronic administration of *S. lateriflora*, evidenced by there being no difference between *S. lateriflora* and placebo for Vigour-Activity ($p = 0.244$) and Confusion-Bewilderment ($p = 0.838$) scores respectively. From inspection of the means for both BAI and the POMS factors, however, there was an enhanced effect of *S. lateriflora* compared to placebo in those who took the placebo first (n = 15) and an enhanced effect of placebo compared to *S. lateriflora* for those who took *S. lateriflora*

first ($n = 16$) suggesting a residual effect of *S. lateriflora* due to insufficient washout. There was no significant difference between *S. lateriflora* and placebo ($p = 0.524$) in salivary cortisol measurements, suggesting no attenuation of the hypothalamic-pituitary adrenal (HPA) axis by *S. lateriflora*. The ALT measurements revealed no significant difference between *S. lateriflora* and placebo ($p = 0.801$), suggesting there was no acute toxicity from *S. lateriflora*. Furthermore, there was no significant difference between *S. lateriflora* and placebo in effects on systolic ($p = 0.410$) or diastolic ($p = 0.834$) blood pressures or pulse rate ($p = 0.144$), all of which remained within the normal range for healthy adults. According to the participants' diary reports, there were no adverse reactions and only mild and infrequent side-effects, which may not have been attributable to *S. lateriflora*.

The results suggested that *S. lateriflora* may have anxiolytic and mood enhancing effects in some individuals without notable side-effects or reduction in energy or cognition. Contrary to anecdotal evidence, there was no worsening of depression following administration of *S. lateriflora*. Further research is needed in a larger study population, using a sample with self-reported and/or diagnosed anxiety and mood disturbances, in order to further determine the effects of the herb on these paradigms.

Key words: *Scutellaria lateriflora*, skullcap, anxiety, stress, mood, flavonoids, high performance liquid chromatography, practitioner survey, salivary cortisol, randomised, placebo-controlled crossover

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Publications

Brock C., Whitehouse J., Tewfik I. & Towell T. (2010). American Skullcap (*Scutellaria lateriflora*): An ancient remedy for today's anxiety? *British Journal of Wellbeing* 1 (4), 25-30.

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Poster presentations

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Author's Declaration

I declare that the material contained in this material is my own work.

Signed:

Christine Brock

Date: 26th April 2012

List of abbreviations

5-HT ₇	5-hydroxytryptamine 7 or serotonin 7
ACTH	adrenocorticotrophic hormone
ACR	awakening cortisol response
A-H	Anger-Hostility
AHP	American Herbal Pharmacopoeia®
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BAI	Beck Anxiety Inventory
BBB	blood-brain barrier
b.i.d	bis in die (twice daily)
BDZ	benzodiazepine
BMA	British Medical Association
BNF	British National Formulary
BP	blood pressure
CAM	complementary and alternative medicine
CMD	common mental disorder
CNS	central nervous system
C-B	Confusion-bewilderment
COSSH	Control of Substances Hazardous to Health

COX	cyclooxygenase
CRF	corticotrophin-releasing factor
CRH	corticotrophin-releasing hormone
CSM	Committee on Safety of Medicines
CYP	cytochrome P450 (group of enzymes)
DAD/ESI-MS photodiode array and electrospray ionization tandem mass spectrometric	
DCM	dichloromethane
DMY	dry matter yield
DST	dexamethasone suppression test
D-D	Depression-dejection
D ₂	Dopamine ₂
ELISA	enzyme-linked immunosorbent assay
EtOH	ethanol
F-I	Fatigue-inertia
GABA	gamma-aminobutyric acid
GAD	Generalised Anxiety Disorder
GP	General Practitioner
GPT	glutamate pyruvate–transaminase
HADS	Hospital Anxiety and Depression Scale
HAART	high active anti-retroviral therapy
HCAs	heterocyclic amines

HPA	hypothalamic-pituitary-adrenal
HPLC	high performance liquid chromatography
LC/MS	liquid chromatography with mass spectrometry
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder
MeOH	methanol
MIC	minimum inhibitory concentration
ORAC	oxygen radical absorbance capacity
PAs	peak areas
PNC	parvocellular neuroendocrine cells
POMC	pro-opiomelanocortin
POMS	Profile of Mood States
PTSD	post-traumatic stress disorder
PG	prostaglandin
RCTs	randomised controlled trials
RTs	retention times
SD	standard deviation
SEM	standard error of the mean
SNRI	serotonin-norepinephrine reuptake inhibitor
SPSS	Statistical Package for Social Sciences
SSRI	selective serotonin reuptake inhibitor

ST	specific tincture
T-A	Tension-anxiety
TCA	Tricyclic antidepressant
t.d.s	ter die sumendum (thrice daily)
TLC	thin layer chromatography
TMB	3,3',5,5'-tetramethylbenzidine
TMD	Total Mood Disturbance
UV/MS	ultraviolet/mass spectrometry
V-A	Vigour-activity

Chapter 1. Introduction

1.1 Introduction

Traditional herbal medicines have been in use for thousands of years and are still relied upon for healthcare by an estimated 4 billion people in developing countries (around 80% of the world's population) (Lambert *et al.* 1997).

Whole plant extracts were still the most common form of medicine in the western world until the 19th century, when the advent of modern, orthodox medicine led to a decline in its popularity (Stuart 1979). Recent years have seen a reversal of this trend, with use of herbal medicine rapidly increasing for a variety of reasons (Vickers *et al.* 2006): people want to feel they are in control of their own health; some are dissatisfied with the orthodox health services (Ernst & White 2000; Vickers *et al.* 2006); others worry about the side-effects of conventional drugs (Astin 1998) or the failure of orthodox medicine to cure their chronic illnesses (Rawsthorne *et al.* 1999). It is also a common belief that herbal medicines are more 'natural' and therefore safer (Vickers *et al.* 2006).

'Nervine' herbs (those that act on the nervous system) are used traditionally for the alleviation of anxiety and stress and comorbidities. Few of these herbs have been well researched and there is scant scientific evidence for their efficacy (Sarris *et al.* 2011). In particular, American skullcap (*Scutellaria lateriflora*) has long been an important herb in North American traditional medicine culture and western materia medica for anxiety and related disorders (Cole *et al.* 2008). It is also used for barbiturate and tranquilliser withdrawal symptoms (Joshee *et al.* 2002). *In vitro* studies of *S. lateriflora* and its phytochemicals (Liao *et al.* 1998; Hui *et al.* 2000; Gafner *et al.* 2003b) and a small clinical study (Wolfson & Hoffmann 2003) suggest a positive therapeutic benefit of the herb for anxiety and its comorbidities. However, a dearth of large, high quality clinical studies of sufficient duration means there is insufficient scientific evidence for its efficacy and safety, particularly in the long-term (Gao *et al.* 2008; Natural Medicines Comprehensive Database 2011; Sarris *et al.* 2011). While the results of the *in vitro* and chemical studies provide clues to the pharmacological actions of *S. lateriflora*, clinical evidence is necessary to support findings. It is therefore appropriate and timely for more efforts to be made in the conduction of clinical studies in support of the therapeutic value of *S. lateriflora*. In the short-term the results may provide evidence of

the potential value of this herb in reducing anxiety and stress and improving negative mood states and may precipitate further research into the herb. Sarris (2007) suggested *S. lateriflora* is potentially of significant use for generalised anxiety disorder (GAD), proposing that urgent research is needed on the herb in this area.

Using subjective test measures at baseline and during administration of placebo or *S. lateriflora*, the present study, based on a randomised, double-blind, placebo-controlled crossover design, examines the effects of *S. lateriflora* on anxiety and mood over a period of two weeks' administration. It also examines its potential physiological effects on stress by measuring free cortisol levels in saliva; using a non-invasive method of saliva sampling before and during administration of *S. lateriflora* and placebo. Measuring cortisol in participants' saliva may determine whether or not secretory patterns alter following putative therapeutic interventions in those with high anxiety. A crossover study was chosen to determine within subjects as well as between subjects comparisons. There is less variability and more precision within a subject and fewer participants are needed than with a parallel study (Wang *et al.* 2006).

The study is innovative as to date there have been no other clinical studies into the chronic effects of *S. lateriflora* on anxiety, stress or various mood states. Furthermore, for the first time salivary cortisol measurements are used to determine the putative benefits of the herb on stress. The results of this study may help to establish an evidenced-based herbal treatment for attenuation of anxiety and stress and promotion of wellbeing. It also hopes to inform herbal and other health professionals and users about the significance of quality control of the herb. To the author's knowledge it is unusual (but not exceptional) for a herb to be authenticated prior to use in a clinical study.

1.2 General aims

The general aims of this study were to authenticate whole extract of *S. lateriflora* and to conduct a randomised, controlled clinical study to evaluate its effectiveness in reducing mood disturbance and related conditions, with particular reference to anxiety and stress.

1.3 General methods

In order to assess the likelihood of achieving the aims and objectives of the study a literature review, reported in Chapter 2, was conducted to gather information on the background of *S. lateriflora*, particularly in relation to its traditional uses and preclinical

and clinical research on the herb supporting psychopharmacological uses as well as wider therapeutic indications. Knowledge of previous research provides a scientific basis for the uses of *S. lateriflora* and helps to inform reasons for the putative efficacy of the herb on anxiety, stress and positive and negative mood states as well as for potential anxiety and/or stress related comorbidities. Chapter 2 also discusses the chemical constituents of *S. lateriflora*; preparations and doses used; the current safety profile of the herb including side-effects, contra-indications and suggested toxicity; and bioavailability where known. There is also a brief botanical description and an outline of *S. lateriflora*'s historical background.

Results of a survey amongst herbal practitioners in the UK and Ireland (Brock *et al.* 2010) indicate that *S. lateriflora* is considered to be an effective intervention for anxiety and stress and is commonly prescribed for these conditions and related comorbidities. Chapter 3 provides the results of this survey conducted amongst herbal medicine practitioners on their use of *S. lateriflora*.

Botanical descriptions in the literature are often from secondary sources and may not be accurate. *S. lateriflora* is possibly the most substituted species in western materia medica (Wolfson & Hoffmann 2003). Therefore, it is important, for purposes of identification of the true species, to have first-hand knowledge of the macroscopic defining characteristics of the herb. Chapter 4 outlines methods to gain this knowledge - such as visits to herbariums to view authenticated voucher specimens.

Because of frequent substitution *or* adulteration of *S. lateriflora* with other skullcaps or potentially harmful herbs such as *Teucrium* spp. (germander) (Wolfson & Hoffmann 2003; Gorman 2008) the literature was also extensively reviewed in relation to the importance of quality control of the herb. The findings are in a chapter of their own (Chapter 5) in order to provide a background and rationale for the results of an analysis of the *S. lateriflora* material used in the clinical study, also reported in Chapter 5. This chemical analysis was conducted using high performance liquid chromatography (HPLC). Commercial herbal products have been found to contain significant variations in phytochemical profile within a species. Although this may sometimes be the result of misidentification in the case of *S. lateriflora*, the effects of growing, harvesting, storage, extraction and processing methods are likely to also play a role (Ciddi 2006; Gao *et al.* 2008). Therefore, optimum conditions for production of high quality commercial products of the herb are also discussed within this chapter.

Chapter 6 reports a randomised, placebo-controlled crossover study of the effects of *S. lateriflora* on mood, with particular reference to anxiety states. Healthy participants, both males and females aged between 18 and 75 years, were recruited for the study. Study time for each participant was 38 days including suitability screening and a 7 day washout period. Participants were randomly assigned to receive either freeze-dried *S. lateriflora* test or freeze-dried *Urtica dioica folia* (common stinging nettle leaf) placebo three times daily for half of the intervention study duration (14 days) and then, following a washout period of 7 days, crossed over to receive the other intervention for comparison. In other words, participants acted as their own controls. Clinical reporting scales, participant diaries and salivary cortisol measurements were used to compare anxiety and mood symptoms, quality of life and physiological measurements of stress at the beginning, before the end of the first half prior to crossover, and at the end of the clinical study. The safety profile of the herb was assessed using serum analysis of alanine aminotransferase (ALT), an enzyme found primarily in the liver (Kaplan 2002). Blood pressure and pulse were also monitored to assess any changes in either following administration of *S. lateriflora*.

Chapter 7 reports the results of the clinical study, Chapter 8 provides a discussion, summary and conclusion of findings from the clinical study and Chapter 9 reports on the potential for future work.

1.4 Statement of the problem

1.4.1 The problem of anxiety

In any given year, up to one in six adults in the United Kingdom may suffer from a medically unexplained psychological disorder, the most common being anxiety (men 4%; women 5%), depression (men 2%; women 3%) or both experienced at the same time (men 7%; women 11%) (Office for National Statistics 2006). Two recent reports (Halliwell 2009; McManus & Bebbington 2009) indicate anxiety disorders are on the increase (a 12.8% increase between 1993 and 2007). The results of an online survey by the Mental Health Foundation (Halliwell 2009) of 2,246 UK adults, indicated 37% of the population (18 million people) are feeling more fearful (frightened, worried or anxious) than they used to – largely due to social and economic problems. No timescale was attributed to this increase in fear. However, a UK government report (McManus &

Bebbington 2009) suggests 2% more of the adult (> age 16) population in England experienced common mental disorders (CMD) in 2007 (17.6%) than in 1993 (15.5%). Of all CMDs, mixed anxiety and depression was the most prevalent (9%) followed by generalised anxiety disorder (4.4%). The proportions of other categories of CMDs in the general adult population of England in 2007 were depression (1.9%), phobias (1.4%), obsessive compulsive disorder (1.1%) and panic disorder (1.1%). There was no increase between 2000 and 2007 overall but CMDs significantly increased in women. The greatest increase was amongst women between the ages of 45-64, rising by a fifth (McManus & Bebbington 2009). For anxiety-related CMDs the increase amongst all adults in England was estimated to be 12.8%. Extrapolating to the UK population, 800,000 more adults were experiencing an anxiety disorder in 2007 than in 1993 (Halliwell 2009).

Anxiety is ‘an unpleasant emotional state ranging from mild unease to intense fear’ (B.M.A. 2002). It is a normal response to stressful situations but can become chronic as a result of a modern lifestyle that includes repeated daily stressors, whether through work, travel, family or social situations (Pavlovich 1999). If present for no known reason, chronic anxiety may be a symptom of a psychiatric condition such as depression, phobias or generalised anxiety disorder (B.M.A. 2002). Although anxiety is common it is a potentially serious disorder as it can lead to numerous health problems and difficulties in social and occupational functioning (Fricchione 2004).

Research suggests that anxiety positively correlates to increased systemic inflammation. Pitsavos *et al.* (2006), for example, found strong evidence for a positive association between severity of state anxiety in both men and women and increased levels of plasma pro-inflammatory cytokines, coagulation factors, C-reactive protein and white blood cells. In addition, studies have identified anxiety as being linked to unhealthy lifestyle choices such as smoking, drinking alcohol and over or under eating (Halliwell 2009; McManus & Bebbington 2009). For example, in 2007 6% of alcohol-dependent adults were in psychotherapy compared with 2% without alcohol dependence; and 24% of those with eating disorders had been treated for CMDs compared to 10% without an eating disorder (Halliwell 2009; McManus & Bebbington 2009). The association of smoking and anxiety is well documented (Halliwell 2009) and some studies have supported the hypothesis for a causal relationship, with anxious subjects being more likely to start smoking than non-anxious subjects (Patton *et al.* 1996; Senol *et al.* 2006;

Mykletun *et al.* 2008). A six year study of 93 medical students, for example, found those who started regular smoking had a significantly ($p = < 0.05$) higher mean trait anxiety score on the Spielberger State-Trait Anxiety Inventory (Spielberger *et al.* 1970) administered once each year (45.6 ± 6.0 points) than those who did not start during that time (42.2 ± 7.3 points) (Senol *et al.* 2006).

Physical symptoms of anxiety include pallor, sweating, hyperventilation, diarrhoea, irritable bowel, flushing, dysphagia, palpitations, nausea, muscle tension and back pain (B.M.A. 2002). Anxiety sufferers may also be easily fatigued and/or experience sleep disturbances (Fricchione 2004). Alleviation of these adverse effects on health is important. Orthodox anxiolytic treatments, however, have been linked to unwanted side-effects (B.N.F. 2008).

An important factor is the known comorbidity of chronic anxiety with mood disorders. One rarely exists without the other (Brown *et al.* 2001) and McNair *et al.* (1971) identified tension-anxiety as one of six mood factors. The impact of the problems and prevalence of anxiety and stress, with current therapeutic strategies, and an outline of the necessity for their attenuation with safe, effective interventions are reviewed.

1.4.2 Therapeutic strategies for anxiety

Benzodiazepines, which are commonly prescribed anxiolytics, have been linked to muscle weakness, amnesia, headaches, vertigo, urinary retention, slurred speech and gastro-intestinal disturbances. They may lead to tolerance and physical and psychological dependence (both manifested by withdrawal symptoms) and are considered by the Committee on Safety of Medicines (CSM) to be dangerous to use long-term. In addition to anxiety, benzodiazepines are frequently prescribed for mood disorders such as anger, hostility and agitation despite CSM advice to the contrary and the known potential paradoxical effects of increased anger and hostility during use of benzodiazepines (Haw & Stubbs 2007; B.N.F. 2008). Given the known side-effects of benzodiazepines (B.N.F. 2008), safer therapeutic strategies for negative mood disturbances are also important.

The side effects of antipsychotics, sometimes prescribed in the short-term for severe anxiety, include tremor, dystonia (abnormal face and body movements) and restlessness. Beta-blockers may be prescribed for relief of physical symptoms, such as

tremors and palpitations, associated with anxiety. However, side effects are similar to those of benzodiazepines and may additionally include bradycardia, vasoconstriction and heart failure. Chronic, severe anxiety disorders such as generalised anxiety disorder (GAD), panic disorder and obsessive compulsive disorders are sometimes treated with SSRI anti-depressants such as paroxetine and fluoxetine but these may lead to dependency and unpleasant side effects such as gastro-intestinal disturbances, dry mouth, dizziness, headache, sweating and insomnia (B.N.F. 2008). There is therefore an urgent need for safe alternatives to orthodox anxiolytics, without unwanted side-effects.

1.5 Stress

Stress may be defined as “a state in which a strong demand is made on the nervous system” (Longman English Dictionary 1976). According to Cox (1978) humans appear to function optimally at moderate conditions of demand and a departure from this state can impair performance. When there is an imbalance between demand and capability, stress is experienced. “What is important for man is his cognitive appraisal of the potentially stressful situation and his ability to cope” (Cox 1978). Stimuli may be physical or emotional (B.M.A. 2002). Weitz (1970) described eight types of situations that may elicit a stress response: perceived threat, noxious external stimuli, increased processing of information, abnormal physiological function, frustration, pressure from others, confinement and isolation, and hopelessness (Weitz 1970). Personality type may influence stress response (Pavlovich 1999), particularly the more ‘driven’ type A personality who is generally more impatient and competitive than type B personality. The type A personality has been shown by research to experience more cardiovascular problems (Pavlovich 1999).

Selye (1946) proposed three stages of response to stress, which he termed the General Adaptation Syndrome: Alarm, in which the body prepares for action; Resistance, in which there is maximum adaptation followed by a return to equilibrium; and Exhaustion, in which the adaptive mechanisms cease to function (Selye 1946).

During the Alarm stage, activation of sympathetic nerve fibres occurs and adrenalin and noradrenalin are released from the adrenal medulla to prepare the body for a fight/flight response. Sympathetic responses include glycogenolysis, dilation of blood vessels in skeletal muscles, constriction of skin vessels, speeding up of heart rate and force, and

release of insulin and glucagon (Greenstein 1994). Irritable bowel, erectile dysfunction and anorexia may occur through suppression of the parasympathetic nervous system (Marieb 2003).

During the Resistance stage, stress stimuli arriving via afferent inputs activate the parvocellular neuroendocrine cells (PNC) in the paraventricular nucleus of the hypothalamus. Increased activity of PNC cells stimulates release of corticotrophin-releasing factor (CRF) or hormone (CRH) from nerve terminals into the hypothalamo-hypophyseal portal system of the median eminence of the hypothalamus into the anterior lobe of the pituitary gland, thus stimulating pro-opiomelanocortin (POMC) production, a precursor to adrenocorticotrophic hormone (ACTH). Secretion of ACTH in turn stimulates glucocorticoid production (principally cortisol in humans) from the adrenal cortex (Greenstein 1994; Wamsteeker & Bains 2010).

Cortisol from the *zona fasciculata* (middle layer) of the adrenal cortex is released throughout the day in a pulsatile manner and secretion and inhibition is controlled by a negative feedback mechanism, believed to be responsible for its circadian rhythm and secretion amplitude. Cortisol output is at its highest in the morning and lowest in the evening. Only the morning peak has been shown by various studies to be relatively stable, regardless of external stressors (Kirschbaum & Hellhammer 1989), although in individuals suffering ‘burnout’ and exhaustion morning secretion may be reduced (Clow 2004).

1.5.1 The HPA axis

Stress increases the activity of the hypothalamic-pituitary-adrenal (HPA) axis, with stimulation of CRF and ACTH and hence plasma cortisol levels (Figure 1.1). Feedback mechanisms are overridden by stressors, resulting in cortisol hypersecretion, with increased amplitude and at a greater frequency than the usual 15 pulses daily (Kirschbaum & Hellhammer 1989). The gamma-aminobutyric acid (GABA)/benzodiazepine receptor system mediates the inhibition by GABA of CRF and consequently the HPA axis (Fries *et al.* 2006). Conversely, over activity of the HPA axis has been postulated to be associated with anxiety states (Tallman *et al.* 2002). This hypothesis is supported by clinical findings that patients with post traumatic stress disorder (PTSD), a severe anxiety disorder in which there is chronic CRF and cortisol output, have a smaller hippocampal volume (as a result of unrestrained cortisol receptor

binding to hippocampal neurones - see also 1.5.2.1) than healthy subjects (Mann *et al.* 2006). For example, in a study of post-combat patients with PTSD the right hippocampal volume measured a statistically significant mean 8% smaller than healthy comparison subjects (Bremner *et al.* 1996). Furthermore, suppression of the HPA axis following pre-treatment with the benzodiazepine alprazolam was observed in a laboratory study of induction of acute stress in healthy volunteers (Fries *et al.* 2006).

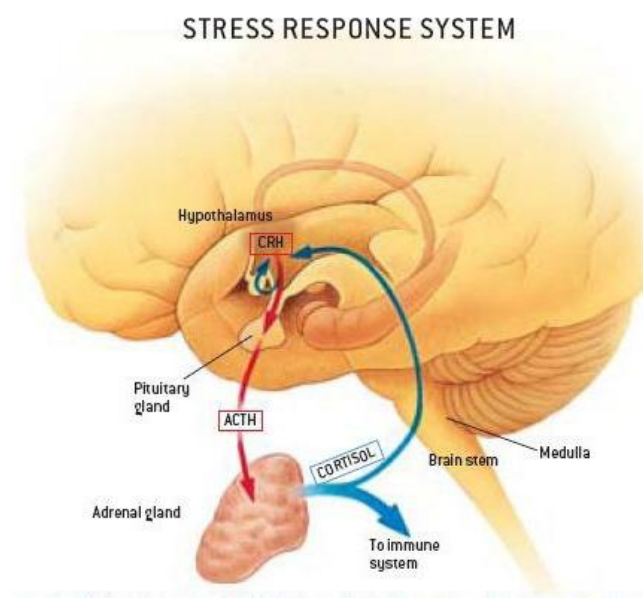


Figure 1.1 The HPA axis

Roberto Osti art in Sternberg and Gold (2002)

Because of the association between cortisol hypersecretion and anxiety there has been much interest in the development of CRF/CRH antagonists as anxiolytics (Mann *et al.* 2006). From results of pre-clinical studies the anxiogenic (and depressive) action of CRH is hypothesised to be by continuous activation of the CRH₁ receptor subtype (Zobel *et al.* 2000), which is expressed in the hippocampus, amygdala and other areas of the neocortex (Refojo & Holsboer 2009) leading pharmaceutical companies to develop CRH₁ receptor antagonists (Zobel *et al.* 2000). They are still in the development stage (Heilig 2012) and although results of clinical studies have been encouraging, with significant reductions in anxiety and depression in one trial, there have nonetheless been some unpleasant worsening of symptoms of anxiety and depression on cessation of the drug in some patients (Zobel *et al.* 2000).

In this research, because of the ability of certain anxiolytics to attenuate stress by decreasing the activity of the HPA axis, the potential of *S. lateriflora* to alter changes in

cortisol output was explored. Changes in cortisol output due to administration of *S. lateriflora* may be indicative of decreased anxiety and stress.

1.5.2 Negative effects of stress

Repeated, prolonged cortisol hypersecretion promotes increased gluconeogenesis, resulting in protein catabolism with consequent muscle and bone atrophy and easy bruising; hyperglycaemia and insulin resistance (steroid diabetes); mobilisation of fat stores and hyperlipidaemia as cortisol is lipolytic; and production of cytokines is reduced, leading to lowered immunity; there may also be hippocampal atrophy, leading to depression (Greenspan 1991).

1.5.2.1 Cortisol effects on the C.N.S.

Hypercortisolism causes an initial euphoria but with prolonged exposure to cortisol (as exemplified by Cushing's disease) further behavioural changes may occur. Depression, insomnia, poor memory, anxiety and irritability are common (Greenspan 1991). Glucocorticoids enter the brain easily and central nervous system toxicity may ensue, particularly in the hippocampus, a part of the brain that is essential for memory formation (Spinella 2001) and which has a high density of corticosteroid receptors (Sapolsky 2000). Cortisol hypersecretion (morning plasma level >50 nmol/L), diagnosed by a dexamethasone suppression test (DST) in which exogenous steroids suppress endogenous secretion in a healthy subject via negative feedback mechanism inhibiting ACTH output (Hurley & Ho 2004), may lead to dysfunction of the hippocampus by causing hippocampal atrophy (Brown *et al.* 2004). Hippocampal atrophy with up to 16% loss of in volume and cognitive impairment has been seen in Cushing's disease and Cushing's syndrome and in those with major depressive disorder (MDD), conditions which are associated with DST non-suppression (Brown *et al.* 2004).

Repeated and/or sustained stress with concurrently increasing/high cortisol output and consequent cumulative exposure has also been associated with decreased hippocampal volume and cognitive impairment. Lupien *et al.* (1998), for example, measured basal plasma cortisol levels in 51 aged volunteers annually over 5 or 6 years. Those with high and increasing levels (initial 12.8 µg/dl ± 3.1) showed significant ($p = < 0.001$) cognitive impairment in memory tests and a decrease in hippocampal volume of 14% when compared to study volunteers with moderate or decreasing levels (initial 9.1 µg/dl

± 2.9). It is clear that stress may lead to physical and mental illness. Its attenuation is therefore important.

1.6 The importance of herbal psychopharmacology

Surveys suggest that herbal medicine is used more frequently by women than by men (Gunther *et al.* 2004). Furthermore statistics indicate that anxiety and depression are more prevalent in women (Office for National Statistics 2006). According to a survey in the United States (del Mundo *et al.* 2002) one of the most common conditions for which complementary and alternative medicine (CAM) is used (30% of users) is anxiety and/or stress. Of 664 respondents (74% of whom were females), 314 were CAM users. Of these, only back pain was a more common reason for CAM use (31% of users). As chiropractors were the most visited CAM therapists, with medical herbalists a close second, it may be deduced from these results that since the chiropractors are more likely to treat back pain (its aetiology perhaps sometimes related to anxiety (B.M.A. 2002)), the majority of visits to an herbal medicine practitioner were by women with anxiety and stress (del Mundo *et al.* 2002).

1.7 Research rationale and justification

The effectiveness of herbal remedies in psychopharmacology can be demonstrated by the fact that St John's wort (*Hypericum perforatum*) has been shown in numerous randomised controlled trials (RCTs) to be effective for even major depression and is widely prescribed both in the herbal clinic and by orthodox physicians (Linde *et al.* 2008).

There have been few randomised, placebo-controlled clinical studies of herbal anxiolytics. A systematic review, published in 2006, located only 7 RCTs (and one other systematic review) of single, oral herbal preparations being tested specifically for anxiety (Ernst 2006), including a study on *S. lateriflora* (Wolfson & Hoffmann 2003). The author (Ernst 2006) concluded that, other than Kava kava (*Piper methysticum*), for which there was a systematic review (Pittler & Ernst 2002), there is no evidence of efficacy for any of the herbs studied, mainly because of poor methodological techniques such as small sample sizes (18 - 40) and poor reporting methods - uncharacterised herbal interventions for example (Ernst 2006). Results of a systematic review of RCTs of herbs for psychiatric disorders (Sarris 2007), of which 9 had been clinically studied for anxiety, were in agreement with those of Ernst (2006) in that only *Piper*

methysticum research provided sound evidence for the herb's anxiolytic properties. The author suggested more research is urgently needed on two of the herbs studied for anxiety disorders, *Passiflora incarnata* (passionflower) and *Scutellaria lateriflora*. Although results were promising for both herbs, methodology and reporting were poor (Sarris 2007). There were only 36 participants in the *Passiflora* study and it was not equivalent, with oxazepam and *Passiflora* in one group and placebo and *Passiflora* in the other (Akhondzadeh *et al.* 2001). The *S. lateriflora* study (Wolfson & Hoffmann 2003) will be discussed in this research in Chapter 2.

A recent review of psychopharmacology found little new clinical evidence for herbs for anxiety (Sarris *et al.* 2011). It was found that, although the evidence for efficacy and safety of whole herb preparations for anxiety disorders is encouraging, in many cases the methodology and/or reporting and good pharmaceutical manufacturing practices were lacking in robustness. Sample sizes were typically small, averaging 50 participants, and studies had not been replicated (Sarris *et al.* 2011). One promising study was a recent 8 week double-blind, placebo-controlled RCT study (n = 57) on *Matricaria recutita* (chamomile) for anxiety, which found a significant reduction in anxiety scores on the Hamilton Anxiety Scale (Hamilton 1959) compared to placebo ($p = 0.047$) (Amsterdam *et al.* 2009).

Poor quality studies of potentially anxiolytic herbs can be exemplified by systematic reviews of RCTs of *Passiflora* (Miyasaka *et al.* 2007) and *Valeriana officinalis* (valerian) (Miyasaka *et al.* 2006), which determined the studies on these herbs were too few in number and sample sizes in monotherapy studies of the single herbs were too small for conclusions to be drawn on their effectiveness for attenuation of anxiety. In the only RCT of *Valeriana* for anxiety (Andreatini *et al.* 2002) that was suitable for review there were only 12 participants in each arm of the study, a sample size considered too small to yield valid results (Miyasaka *et al.* 2006). One *Passiflora* RCT was reviewed by Miyasaka *et al.* (2007). In this study with 36 participants, comparing herb with the benzodiazepine oxazepam (Akhondzadeh *et al.* 2001), the sample size was also considered too small, the randomisation process was not explained and efficacy data could not be re-analysed. There was no placebo for additional comparison (Miyasaka *et al.* 2007).

Piper methysticum on the other hand has been extensively researched for its anxiolytic properties in a clinical setting and a meta-analysis has shown it to be an effective anxiolytic when compared to placebo (Pittler & Ernst 2003). There were, however, a number of reported side-effects such as drowsiness and indigestion. Following reports of possible hepatotoxic reactions to some preparations, *P. methysticum* has been withdrawn from sale in the UK pending further evidence (Whitton *et al.* 2003). It is therefore timely for research to be channelled towards other herbal anxiolytics. Perhaps due to it being favoured as a sedative alternative to *P. methysticum*, demand for *S. lateriflora* in world markets appears to be growing (Greenfield and Davis 2004). Its popularity (Bergner 2002) therefore makes it an ideal candidate for this purpose (see also Chapter 3). Well conducted clinical trials of *S. lateriflora* may prove it to be as effective for anxiety as *P. methysticum* and as *H. perforatum* is for depression.

The current research study is innovative as it aims, for the first time, to conduct a study of sufficient duration to demonstrate long-term effects of *S. lateriflora* on anxiety, stress and other negative mood states as well as determining its effects on cognition and energy. Furthermore, there have been very few crossover studies of herbal preparations used for anxiety. Only four randomised controlled trials (RCTs) involving crossover studies of ‘nervine’ herbs were located by this author:

- A study of *Piper methysticum* in healthy volunteers. The quality of the study was lacking as there was no washout period between interventions, increasing the risk of the carry-over effect seen in the results (Sarris *et al.* 2009a).
- A study of *Trifolium pratense* (red clover) for menopausal depression and anxiety. Either *T. pratense* or placebo was taken for 90 days before a 7 day washout and then crossover. The results were statistically significant (Lipovac *et al.* 2010).
- A study of a *Piper methysticum*/*Hypericum perforatum* combination for anxiety and depression. Only 18 participants completed the study and there was a placebo run-in period phase (Sarris *et al.* 2009b), which may have introduced bias (Berger *et al.* 2003).
- Wolfson and Hoffmann (2003) assessed the acute anxiolytic effects of *S. lateriflora* in healthy volunteers. The study had its limitations, which will be discussed in Chapter 2.

To this author's knowledge this is the first time a crossover study of an anxiolytic herb's effects on salivary cortisol has been conducted. There was a parallel study of the effects of *Magnolia officinalis* (magnolia) and *Phellodendron amurense* (cork-tree) on stress, published in 2008, in which salivary cortisol was measured at baseline and towards the end of a 5 week study. This was quite different from the current research as it was a study of obese women who ate in response to stressful situations (Kalman *et al.* 2008). There were no significant alterations in salivary cortisol with the intervention (*Magnolia-Phellodendron*) when compared to placebo.

1.8 The research question

Can American skullcap (*Scutellaria lateriflora*) contribute to improvement of mood?

1.9 Aims of the research

The study aimed to evaluate the effectiveness of *S. lateriflora* in enhancing mood with particular reference to attenuation of anxiety and stress and other negative mood states.

1.10 Objectives of the research

- To conduct a survey amongst UK and Ireland herbal medicine practitioners on their use of *S. lateriflora*.
- To analyse the phytochemical and botanical profile of *S. lateriflora* to authenticate and verify quality for medicinal use.
- To identify the macroscopic defining features of *S. lateriflora* to verify its identification at source of supply.
- To deliver an evidence-based herbal intervention with extracts of *S. lateriflora* for promotion of wellbeing in a series of tests, using a randomised, double blind, placebo-controlled, crossover design with subjective measures of mood in healthy volunteers.
- To measure changes in levels of cortisol in saliva samples, following administration of an extract of authenticated *S. lateriflora* to the selected group.
- To assess the safety profile of *S. lateriflora* by comparing liver function tests at baseline and following administration of the test herb and placebo.

Chapter 2. Scutellaria lateriflora: background and literature review

2 American skullcap (*Scutellaria lateriflora* L)

2.1 Introduction

Scutellaria lateriflora (Figure 2.1) is a perennial herb belonging to the *Lamiaceae* (mint) family (also known as *Labiatae*), sub-family *Lamioideae*, and is one of 360 known *Scutellaria* species worldwide (Cole *et al.* 1991; Malikov & Yuldashev 2002) many of which are used medicinally (Joshee *et al.* 2002). It grows on wetlands and is indigenous to North America and Canada where it is widely distributed - from Alaska to Florida and British Columbia to Quebec (the only places it is not found are the Northwest Territories, Alberta and Yukon in Canada and Wyoming, Nevada and Utah in the US) (U.S.D.A. 2012). It has also been reported to grow on riverbanks and marshes in northern Iran (Yaghmai 1988) and is grown commercially worldwide, particularly in the USA, Australia and New Zealand (Wills & Stuart 2004).

Demand for *S. lateriflora* in world markets appears to be growing. Harvesting and sales of the herb in 2001 had increased over 1997 by around 250% and amounted to 15, 875.733 kilograms. Between 2000 and 2001 the increase was 23%. According to a market report by Greenfield and Davis (2003) there was a sharp increase (figures not stated) in demand in the last quarter of 2002, which suggested a continued growth in demand of 20-30% annually over a period of 5 years (Greenfield & Davis 2003). This increased demand for *S. lateriflora* is believed to be attributable to it being favoured as a sedative alternative to *Piper methysticum* (kava kava), which, due to toxicity fears, is no longer widely prescribed by herbalists in Europe (Greenfield & Davis 2004).

Although *S. lateriflora* is a popular herb in western herbal medicine and contained in many herbal formulations (Joshee *et al.* 2002), particularly for anxiety and stress, few scientific studies of this herb exist. Therefore its safety, efficacy and pharmacology are not well established (Gao *et al.* 2008).

Considering potentially increasing demand for *S. lateriflora*, new research on this herb is timely and important. *Scutellaria baicalensis* (Georgi) root from baical skullcap is extensively prescribed in traditional Chinese and Japanese (kampo) medicines,

particularly to treat inflammatory diseases, and has been widely researched in relation to its efficacy and pharmacological properties. Some of these findings may be extrapolated to *S. lateriflora*, which is phytochemically similar (Joshee *et al.* 2002).

2.2 Selected common names

Popular names include American skullcap, Virginian skullcap, blue skullcap, dogweed, mad-dog, blue pimpernel, helmet flower, hoodwort, madweed and Quaker bonnet (Felter & Lloyd 1898; Wolfson & Hoffmann 2003; Cole *et al.* 2008; Hull 2010). The term ‘skullcap’ refers to the medieval helmet shaped calyx (Figure 2.2) and the genus name *Scutellaria* means ‘little dish’, due to the appearance of the lid of the calyx (Joshee *et al.* 2002).

2.2.1 Synonyms

Cassida lateriflora (L.) Moench, *Scutellaria lateriflora* f. *albiflora* Fernald, *Scutellaria lateriflora* var. *albiflora* Farw., *Scutellaria lateriflora* var. *axillaris* Jenn., *Scutellaria lateriflora* f. *rhodantha* Fernald (The Plant List 2010)



Robert H. Mohlenbrock @ USDA-NRCS PLANTS Database

Figure 2.1 *Scutellaria lateriflora*



Figure 2.2 *Scutellaria lateriflora* showing helmet-shaped calyces

2.3 Botanical description

S. lateriflora grows up to 30-90 cm in height and has a smooth, many branched, slender stem with four angles. The stem turns brown when exposed to the sun. Its leaves are ovate, smooth and with crenate-serrate margins. They are 2-5 cm long and 1-2 cm wide, opposite, and are rounded at the base and acute at the apex. The leaves are non-sessile, the leaf stalk or petiole being around 2 cm in length. The tubular, two-lipped (upper hooded and lower shallow), blue to purple flowers, which appear from May to September, are about 2-7 mm long and are clustered in one-sided opposite racemes. The short, creeping roots are fibrous and yellow (Felter & Lloyd 1898; Joshee *et al.* 2002; Greenfield & Davis 2004). *Scutellaria lateriflora* is so called because the flowers are turned to one side but it is often erroneously called *Scutellaria laterifolia* (Wohlmuth 2001). It should be noted that the above description notes of *S. lateriflora* were taken from literature and may not be entirely accurate. A more authentic description of *S. lateriflora* taken from primary sources is detailed in Chapter 4.

2.4 Historical background

S. lateriflora was mentioned in the first American *Materia medica* in 1785 but had been in longstanding use as a home remedy before then (Lloyd 1911). The first European to record its use as a medicinal herb was German Physician Johann David Schöppf, who noted its use as a tonic and for fevers and as an abstergent (detergent) in 1787 (Upton *et al.* 2009). Dr Lawrence Van Derveer (1740-1815) studied *S. lateriflora* extensively and used it as a treatment for rabies (hydrophobia); it was then used for this purpose both professionally and as a home remedy. In 1819 Dr Lyman Spalding wrote ‘*A history of the introduction and use of Scutellaria lateriflora* (scullcap), *as a remedy for preventing and curing hydrophobia, occasioned by the bite ...*’ (Lloyd 1911; New Jersey Historical Society 2001).

Medical doctor Joseph Bates wrote in 1855 in the Boston Medical Journal that Van Derveer was believed to have cured more than 300 people of ‘canine madness’... ‘in secret’(Bates 1855). Millspaugh (1892) quoted the number being as high as 1,400. Bates tells how, because of these claims, *S. lateriflora* was eventually thoroughly tested as a cure for rabies. When it was found to be utterly useless for this purpose it fell into disrepute and was consequently removed from pharmacopoeias. Bates nevertheless described using *S. lateriflora* in his practice, claiming a fluid extract had great value in the treatment of nervousness, irritability and restlessness, particularly in children. He also held it in high regard for hysteria and for relieving symptoms of inflammation in patients with arthritis or convalescing from fevers. He prophesied that it would be found to be highly successful in treating many diseases in the future, particularly those for which opium was currently prescribed (Bates 1855). In 1860 *S. lateriflora* was introduced into the official *United States Pharmacopoeia* as *Extractum Scutellariae Fluidum* (Millspaugh 1892; Upton *et al.* 2009) but was dropped in 1910. It was in the *US National Formulary* in 1916 and removed in 1942 when interest in natural remedies declined. It is not included in the *European Pharmacopoeia* (Upton *et al.* 2009).

In 1869 John Shapley and AD Hutchinson applied to patent an ‘Alterative and invigorating cordial’ containing mainly *S. lateriflora* and *Cimicifuga racemosa* (black cohosh), with small amounts of eight other herbs, which were mostly carminatives (Shapley & Hutchinson 1869).

2.5 Therapeutic uses of *Scutellaria lateriflora*

2.5.1 Ethnobotanical uses

The herb has been traditionally used by Native Americans for anxiety and potentially related disorders such as nervous exhaustion, anxiety-related muscular tension, hysteria, hypertension, epilepsy, tremors and convulsions, *delirium tremens*, neuralgia, insomnia, diarrhoea and headaches (Felter & Lloyd 1898; Joshee *et al.* 2002; Hull 2010). Today it is also smoked ceremonially and sometimes used to induce visions (Joshee *et al.* 2002).

King's American Dispensatory (Felter & Lloyd 1898) describes *S. lateriflora* as an antispasmodic nervine tonic that is calming in all cases of '*nervous excitability, restlessness, or wakefulness... ...When its soothing effects have ceased it does not leave an excitable, irritable condition of the system as is the case with some other nervines*'; and for '*functional cardiac disorders due to purely nervous causes*'. Jethro Kloss, who wrote the classic natural medicines text '*Back to Eden*' in the 1920s and 1930s, depicted *S. lateriflora* as '*one of the best nerve tonics we have*' (Kloss 1995).

It has been used for centuries in Cherokee folk medicine for nervous disorders of the digestive tract, such as irritable bowel and related colic, flatulence, heartburn, constipation and diarrhoea. It is believed to normalise peristalsis by the effects of its volatile oils, which may possess spasmolytic and carminative actions (Khosh 2000). However, the effects are also likely to be due to an anxiolytic action of the herb.

It was also used for fever and the Iroquai tribe use the herb '*to keep the throat clear*' (Joshee *et al.* 2002). Additionally, Kloss (1995) advocated its use for rheumatism; and as an antidote to insect and snake bite venom. These uses suggest possible direct anti-inflammatory, antipyretic, immunomodulatory and even antimicrobial effects. According to Wojcikowski *et al.* (2007) it is traditionally used for disorders of the urinary tract. It is unclear whether this application relates to its traditional use as a diuretic (Greenfield & Davis 2004) or whether due to an antimicrobial or anti-inflammatory action.

2.5.2 The potential value of *S. lateriflora* in gynaecology

Known as a woman's herb by Native Americans, the Cherokees used it as an emmenagogue, thus promoting menses and aiding expulsion of the placenta following

childbirth. Native American women also use it to ensure general menstrual health (Joshee *et al.* 2002; Russell *et al.* 2003; Hull 2010) as well as for mastalgia and premenstrual tension (Greenfield & Davis 2004; Hull 2010). Some tribes still use it in ceremonies to induct girls into womanhood and for purification rituals (Joshee *et al.* 2002). According to Kloss (1995) *S. lateriflora* is useful for suppressing sexual desire.

Despite its traditional use for problems relating to the menstrual cycle and for mastalgia, there appears to be little reference in modern herbal medicine texts to current use of *S. lateriflora* in gynaecology. Hoffmann (2003) states that it can be safely used to ease premenstrual tension and Harrar and O'Donnell (1999) suggest it has healing potential for premenstrual syndrome and menopausal mood changes. Due to a lack of research in this area, however, it is unclear whether these effects are due to direct modulation of hormone levels or on neurochemicals that affect mood. Liao *et al.* (1995) found *S. baicalensis* root extract bound to dopamine 2 (D₂) receptors *in vitro*. As mouse striatum preparations were used it is not known whether there would be similar findings in the human brain *in vivo*. Some future research could focus on whether *S. lateriflora* binds to D₂ receptors with (if a D₂ agonist) a consequent lowering of prolactin, itself a stress response hormone (Axelrod & Reisine 1984), with concomitant measurements of salivary prolactin levels (Nadeem *et al.* 2004). However, it does contain vitexin (Lin *et al.* 2009), an important active constituent of *Vitex agnus castus* herb, commonly used for menstrual disturbances (Hoffmann 2003).

Following its use in both folk and professional medicine for hundreds of years, the popularity of *S. lateriflora* has not waned. It is believed to be one of the most commonly used medicinal herbs by western medical herbalists today (Bergner 2002).

2.5.3 Contemporary uses of *S. lateriflora*

Modern herbal medicine's application of *S. lateriflora* appears to be based upon its traditional use as an anti-spasmodic, diuretic, anti-inflammatory, anti-pyretic and sedative herb. Greenfield and Davis (2004), for example, suggest it is used most commonly for insomnia, nervous disorders such as anxiety, and for digestive disturbances. Bergner (2002) proposes its action is primarily as a trophorestorative on the central nervous system (CNS), allowing relaxation following nervous exhaustion, whether from post-traumatic stress, organic diseases, overwork, too many stimulants, lack of sleep, or drug or alcohol abuse (Bergner 2002). It is also prescribed by western

herbalists for epilepsy, fibromyalgia and anorexia nervosa (Hull 2010), post-stroke paralysis, atherosclerosis, hyperlipidaemia, allergies, skin conditions and inflammation (Natural Medicines Comprehensive Database 2011) and as a mild bitter and antispasmodic for digestive disturbances (Bergner 2002). Other than for anxiety (Wolfson & Hoffmann 2003), to date no clinical trials have been conducted to provide evidence of efficacy for these conditions.

2.6 The Chemical constituents of *S. lateriflora*

Since 1998 a large number of compounds have been isolated from *S. lateriflora* (Shang *et al.* 2010), which could have far reaching implications for research into its actions and indications. In common with all *Scutellaria* species studied, *S. lateriflora* is rich in flavonoids, a group of phenolic compounds that are highly active physiologically, with a wide range of pharmacological actions (Malikov & Yuldashev 2002; Li *et al.* 2012). The many biological actions of flavonoids that are beneficial to the plants may also have therapeutic potential in herbal medicine for humans (Cody *et al.* 1986).

Flavonoids are derived from flavone, which consists of two benzene rings connected by a 3 carbon chain, as methoxy or hydroxyl compounds. These compounds are secondary plant metabolites and occur widely in plants (Hoffmann 2003). They are pigments and provide plants with many of their natural colours, attracting pollinators. Their diverse biological effects (e.g. antioxidant, antimicrobial) afford protection to plants from disease and predation by insects but also have numerous physiological actions in humans when consumed as part of the diet or as medicines. Their actions include anti-inflammatory, anti-allergy, antioxidant, spasmolytic, anti-hepatotoxic, antimicrobial and anti-carcinogenic (Gabor 1986; Glusker & Rossi 1986). It is their phenolic properties, for example, which provide them with antioxidant potential (Swain 1986).

An in-depth review (Malikov & Yuldashev 2002) indicated that up until the year 2000, of 208 phenolic compounds isolated from *Scutellaria* species only one (scutellarin: scutellarein 7-*O*-glucuronide) had been isolated from *Scutellaria lateriflora* (it had also been isolated in 26 other skullcap species). However, Nishikawa *et al.* (1999) had also isolated the flavone glycoside baicalin (baicalein 7-*O*-glucuronide) and the aglycone wogonin (5,7-dihydroxy-8-methoxyflavone) (Figure 2.3) from this species. Wogonin was first isolated in 1930 by Hattori (Joshee *et al.* 2002) from the roots of *S. baicalensis*. Considering its history of widespread use for medicinal purposes, this lack

of information about the chemistry of *S. lateriflora* was surprising and reinforces a view that more research on this species was needed.

More recently, a number of other flavonoids have been isolated from *S. lateriflora*. In addition to baicalin and wogonin, baicalein (5,6,7-trihydroxyflavone) - the aglycone of baicalin, lateriflorin (5,6,7-trihydroxy-2'-methoxyflavone-7-*O*-glucuronide), lateriflorein (5,6,7-trihydroxy-2'-methoxyflavone) are quantitatively the major compounds in *S. lateriflora* (Gafner *et al.* 2000). Perhaps due to their greater abundance in the herb, baicalin, baicalein and wogonin (Figure 2.3) are considered the most important compounds in *S. lateriflora*. Of all skullcap flavonoids they have been most widely researched for their actions and related indications *in vitro* (see 2.12.1, p31), research that has been facilitated by their also being major flavonoids in *S. baicalensis* (Joshee *et al.* 2002).

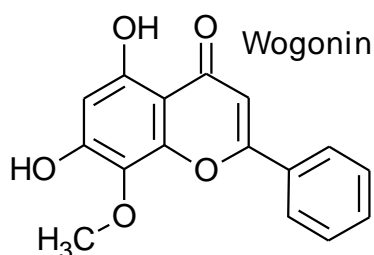
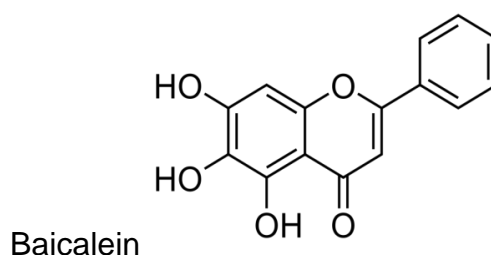
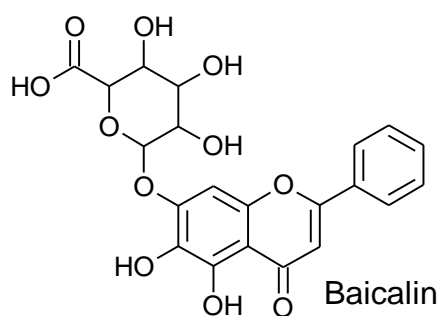


Figure 2.3 Three important flavonoids found in *S. lateriflora*

(See 2.12.1, p31 and 5.5.3.7, p81)

Other flavonoids present are said to include apigenin; hispidulin and luteolin (Barnes *et al.* 2007) but their presence in *S. lateriflora* aerial parts does not appear to have been substantiated by laboratory methods although Parajuli *et al.* (2009) found 5.56 µ/mg apigenin in methanol root extract; both flavonoids have been chemically recovered from the aerial parts of some other skullcap species (Zgorka & Hajnos 2003; Shang *et al.* 2010); vitexin (apigenin C8-glucoside) (Lin *et al.* 2009); viscidulin III-2'-O-β-D-glucopyranoside (Zhang *et al.*, 2009); viscidulin III; *trans*-martynoside; oroxylin A-7-O-β-D glucopyranoside; wogonoside; chryin; chryin-6-C-α-L-arabionopyranosyl-8-C-β-D-glucopyranoside; chrysin (5,7-dihydroxyflavone), dihydrochrysin (5,7-dihydroxyflavanone); oroxylin A; ikonnoside (5,6,7,2'-tetrahydroxyflavone-7-O-glucuronide); ikkonikoside I (7-glucuronyloxy-5,6,2'-trihydroxyflavone) and dihydrobaicalin (Awad *et al.* 2003; Bergeron *et al.* 2005; Li & Khan 2008; Zhang *et al.* 2009); 5,7-dihydroxy-8,2'-dimethoxyflavone, dihydrooroxylin A (Li *et al.* 2009), oroxylin A-7-O-glucuronide, and dihydrowogonin-7-O-glucuronide (Li *et al.* 2012).

Very recently (Li *et al.* 2012), using a sensitive method of high performance liquid ultraviolet photodiode array and electrospray ionization tandem mass spectrometric detection (HPLC-DAD/ESI-MS), seven more flavonoids have been isolated from methanolic extracts of authenticated *S. lateriflora* aerial parts, none of which have been previously identified in this species. The newly detected flavonoids are norwogonin-7-O-glucuronide, wogonin-7-O-glucuronide, 5,7-dihydroxy-6,8-dimethoxy-flavone-7-O-glucuronide, dihydrooroxylin A-7-O-glucuronide, galangin-7-O-glucuronide, and 5,6,7-trihydroxy-flavanone-7-O-glucuronide (Li *et al.* 2012).

According to Zhang *et al.* (2009) *trans*-verbascoside (acteoside) was also found in *S. lateriflora*. However, the skullcap samples (n=10) used for this analysis were purchased from various natural product suppliers and shops (Zhang *et al.* 2009). There is therefore no guarantee that any of the samples were authentic *S. lateriflora* and it is possible that the verbascoside content was due to contamination as this phenylethanoid glycoside is found in Germander (*Teucrium*) species but not in *Scutellaria* species (Lin *et al.* 2009). In the 1970s and 1980s there were reports of liver damage from use of *S. lateriflora* (McCaleb 2004). The cause of hepatotoxicity was subsequently attributed to *Teucrium* being used in some European commercial preparations in place of *S. lateriflora* (de Smet 1999).

The essential oil is at low (0.01-0.02% w/w) concentrations (Wohlmuth *et al.* 2011). According to Yaghmai (1988) the essential oil is dominated by sesquiterpenes (78%), which are principally (in descending order of quantity) δ -cadinene, calamenene, β -elemene, α -cubebene, α -humulene and α -bergamotene. Monoterpenes (2.8%) include camphene and β -pinene. Other aromatic compounds exist in small quantities and include cinnamaldehyde and safrole (Yaghmai 1988). It is not definite that this analysis (Yaghmai 1988) was conducted from authentic *S. lateriflora* material (Wohlmuth *et al.* 2011), however, as the skullcap used in this study was collected in Northern Iran. The species that is known to grow in this region is *S. pinnatifida* (Barceloux 2008). The results differ from those arrived at by Wohlmuth *et al.* (2011), who analysed three authenticated *S. lateriflora* samples and found highest levels of 1-octen-3-ol (29-68%) and acetophenone (6-20%) (Wohlmuth *et al.* 2011).

S. lateriflora also contains small quantities of the indoleamines melatonin and serotonin (Murch *et al.* 1997; Cole *et al.* 2008). *S. baicalensis* contains around eight times more melatonin than *S. lateriflora* (Cole *et al.* 2008)*. The amino acids γ -aminobutyric acid (GABA) (~ 0.55%) and glutamine (~ 0.34%) have also been detected in *S. lateriflora* (Awad *et al.* 2003; Bergeron *et al.* 2005) in both aqueous and ethanolic extracts, with markedly high levels of glutamine in aqueous extract (Awad *et al.* 2003) and a 70% ethanolic extract (Bergeron *et al.* 2005).

*(see Chapter 5, Quality Control section, 5.2.2, p72 & 5.5, p79 for differentiation between skullcap species)

Other known constituents are triterpenoids, including lupenol, ursolic acid, sitosterol and dauchosterol (Li & Khan 2008) and diterpenoid scutelaterins A-C, ajugapitin and scutecyprol A (Bruno *et al.* 1998); *p*-coumaric acid (Awad *et al.* 2003); a mixture of arachic, behenic and lignopalmitic acid (Li & Khan 2008); and wax hydrocarbons (Yaghmai & Benson 1979). Barnes *et al.* (2007) state the iridoid glycoside catalpol as being present but this was not detected (by paper chromatography) in *S. lateriflora*; although found to be present in numerous (13) other species of skullcap (Cole *et al.* 1991).

Some of the most recent compounds to be isolated from authenticated *S. lateriflora* aerial parts (Li *et al.* 2009), are two previously unknown dihydropyrano coumarins,

named scuteflorins A and B respectively, which may be neuroprotective, cytotoxic and protein kinase C activating, and another coumarin, decursin (Li *et al.* 2009).

A metabolomic analysis (the detection, identification and quantitation of all compounds in a sample) of *S. baicalensis* shoots revealed 2,400 compounds. Of these compounds 781 may have medicinal actions, including hyperforin; a compound found in *Hypericum perforatum* (St John's Wort) and believed to be effective against depression (Murch *et al.* 2004). A similar analysis of authentic *S. lateriflora* might be useful. Considering problems of substitution there is no guarantee that all of the analyses of *S. lateriflora* so far are correct.

2.6.1 Flavonoid metabolism

Hydrolysis of flavonoid glycosides to the corresponding aglycone is believed to be by micro-organisms in the intestinal walls (Hackett 1986). This hypothesis is supported by studies demonstrating glycosides remained unhydrolysed in the faeces of germ-free rats (Griffiths & Barrow 1972). Suppression of normal indigenous gut flora such as occurs following antibiotic therapy (George *et al.* 1977) may perhaps result in compromised efficacy of the herb. It could be argued that patients using antibiotics whilst taking *S. lateriflora* (or any herbal medicines in which flavonoids are the active components) should be advised to take probiotic *Lactobacilli* and/or *Bifidobacteria* to modify gut bacteria, and prebiotics (fructo-oligosaccharides) such as inulin to increase probiotic growth (Hart *et al.* 2002) as an adjunct therapy. Inulin is found in *Taraxacum officinale radix* (dandelion root), for example (Hoffmann 2003).

2.7 Preparations and dosages used

Preparations of *S. lateriflora* are made from the aerial parts and are sold in the form of tinctures, teas and tablets; and capsules containing powders, liquids or freeze-dried material (Eclectic Institute Inc. 2003; Wills & Stuart 2004; Natural Medicines Comprehensive Database 2011). Curiously, in Japan, medicines made from the roots of *S. lateriflora* are officially classified as pharmaceuticals, whereas products made from the aerial parts are considered non-pharmaceuticals (Makino *et al.* 2008). Dosages vary according to extraction, marc: menstruum ratio, practitioner preference and preparations used but average at around 1 g equivalent dry weight per dose three times daily (Table 2-1). Preparations from fresh herb are thought to be most effective (Felter & Lloyd 1898; Kuhn & Winston 2001; Yarnell & Abascal 2001). In 1871, Eclectic physician

John Scudder proposed in *Specific Medication* that anything other than fresh *S. lateriflora* was ‘worthless’ (Upton *et al.* 2009).

Table 2-1 Preparations, extractions and recommended doses for *S. lateriflora*

Practitioner/ company/ publication	Fresh herb	Dried herb	Extraction/ processing	Marc: menstruum ratio/ % EtOH:H ₂ O	Dosage (t.d.s. unless otherwise stated)	Weight in crude, dried herb/ dose
Yarnell and Abascal (2001)	Infusion		Hot water		2-3 g	2-3 g
	Tincture		EtOH:H ₂ O	NK	3-5 ml	?
		Capsules 500mg			1 g	1 g
Yarnell (2002)	-	Infusion	Hot water	-	1-2 g	1-2 g
	-	Liquid extract	NK	1: 2	0.6-1.5 ml	300-750 mg
	-	Tincture	EtOH:H ₂ O	1: 5	1-2 ml	200-400 mg
Gaia herbs*	Fresh OG tincture	-	EtOH:H ₂ O	1:1.5	1.5 ml	667 mg
Mediherb*	-	Tincture	EtOH:H ₂ O	1:2 / 45%	5 ml	2.5 g
Solaray (2011)	-	Capsules 425 mg	NK	-	2-3 caps b.i.d.	850 mg - 1.275 g
Eclectic Institute Inc. (2003)	Capsules 350 mg	-	Freeze-dried OG	-	1-3 /day	350 mg
Kuhn and Winston (2001)	Tincture	-	EtOH:H ₂ O	1:2 / 30%	3-6 ml	1.5-3 g
	Capsules (mg?)	-	Freeze-dried OG	-	2 caps	?
British Herbal Medicine Association (1983)	-	Liquid extract	EtOH:H ₂ O	1:1 / 25%	2-4 ml	1-4 g
	-	Tincture	EtOH:H ₂ O	1:5 / 45%	1-2 ml	200-400 mg
	-	Infusion	Hot water	-	1-2 g/150 ml	1-2 g
	-	Infusion	Hot water	-	300 mg /50ml	300 mg
Chevallier (2000)	Infusion	-	Hot water	-	400-600 mg/50ml	400-600 mg
	-	Capsules 200 mg	-	-	1 cap b.i.d	200 mg
Hoffmann (2003)	-	Tincture	EtOH:H ₂ O	1:5 / 40%	2-4 ml	400-800 mg
	-	Infusion	Hot water	-	1-2 g/150 ml H ₂ O	1-2 g
Barnes <i>et al.</i> (2007)	Infusion	NK	Hot water	-	1-2 g/150 ml H ₂ O	1-2 g
	Extract	NK	EtOH:H ₂ O	1:1/ 25%	2-4 ml	2-4 g
	Tincture	NK	EtOH:H ₂ O	1:5/ 45%	1-2 ml	200-400 mg

Abbreviations: EtOH: H₂O = ethanol: water; OG = organic; t.d.s. = 3 times daily; b.i.d = twice daily; NK = not known. *In Natural Medicines Comprehensive Database (2011)

A survey of major botanical and nutritional supplies companies in Europe and the USA found that 22% of these companies sell *S. lateriflora* as a single product and 33% sell it both as singles and as part of a formula (Greenfield & Davis 2004). It is often combined with various other anxiolytic herbs in commercial preparations for anxiety and stress. ‘Serenity’ liquid-filled capsules (Gaia Herbs 2011), for example, contain 60 mg of *S. lateriflora* extract per daily dose of 3 capsules combined with *Passiflora incarnata* (passion flower), *Matricaria recutita* (chamomile), *Piper methysticum* (Kava-kava), *Humulus lupulus* (hops), *Artemisia vulgaris* (mugwort), *Crataegus* spp (hawthorn), *Mentha piperata* (mint) and *Avena sativa* (oats) (Gaia Herbs 2011).

Many products contain standardised extracts. A problem with standardisation is that the process is based on commercially available chemical ‘marker compounds’ so a product may contain irrelevant or insufficient ingredients or they may be in the wrong ratio for a synergistic effect. Concomitant ingredients in an herb may not be majorly bioactive but they may enhance the actions of the main active phytochemicals by, for example, increasing their bioavailability or stability (Andrade *et al.* 2000; Gafner & Bergeron 2005; Cole *et al.* 2008).

2.8 Contraindications and drug interactions

Potential side-effects of *S. lateriflora* were monitored during a clinical trial of its efficacy and none were reported. However, those taking any kind of medication were excluded from the study (Wolfson & Hoffmann 2003) and effects were only monitored for two hours. It is therefore important for the safety of the herb to be verified in a study of longer duration.

It may be advisable for the use of *S. lateriflora* to be avoided with some conditions and medications where it might be contraindicated. Awad *et al.* (2003) found that extracts of *S. lateriflora* equivalent to those used commercially strongly inhibited the CYP3A4 enzyme *in vitro*. As this is a drug metabolising enzyme the herb may be contraindicated with some orthodox medicines; for example calcium channel blockers, barbiturates, cimetidine, ciprofloxacin, cisapride, corticosteroids, paclitaxel and tamoxifen – to name a few drugs that interact with CYP3A4 (Vanin 2008). Also, baicalein and wogonin inhibited diclofenac metabolism by CYP2C9 *in vitro* (Kim *et al.* 2002). Another report (Kron 2004) suggests that *S. lateriflora* may be metabolised by the same CYP450

enzyme as high active anti-retroviral therapy (HAART). It is possible that concurrent administration may increase serum concentration of HAART (Kron 2004). It is unknown whether the evidence for effects of *Scutellaria* species on CYP450 enzymes gathered from *in vitro* studies, however, has clinical significance as *in vitro* drug metabolism studies have been demonstrated sometimes to be poor predictors of the situation in human patients (Piscitelli & Gallicano 2001).

Because of its traditional use as an emmenagogue (Joshee *et al.* 2002) it is not possible to comment on the safety of using *S. lateriflora* during pregnancy.

2.9 The safety profile of *S. lateriflora*

There have been reports of hepatotoxicity associated with *S. lateriflora*. Although believed to be attributed to its adulteration with *Teucrium* species (McCaleb 2004), it is important to confirm the safety profile of *S. lateriflora* by ensuring that all participants in a clinical study of the herb undergo analysis of blood for liver function pre- and post-intervention (see section 6.11.3, p105). In addition, adequate quality control of the herb prior to clinical use in order to ensure it is not adulterated or substituted with potentially toxic botanicals, microbes or heavy metals is essential (de Smet 1999). (See also Chapter 5: 5.2.1, p72.)

Some research has suggested toxicity associated directly with *S. lateriflora*'s phytochemicals. Results of an *in vitro* toxicity study on rat hepatocytes, for example, suggested diterpenoids from *S. lateriflora*, including scutelaterins A-C, were hepatotoxic. Hepatocytes were incubated for two hours with *S. lateriflora* diterpenoids. The diterpenoids reduced cell viability (forming membrane blebs and increasing apoptosis), and decreased ATP levels and protein thiols. The apparent toxic effects were cytochrome P4503A (CYP 3A) dependent. The enzyme activated the diterpenoids, leading to toxic effects (Haouzi *et al.* 2000). The experimental results may not be relevant to the human situation and do not warrant the designation of *S. lateriflora* as a toxic herb. Firstly, *in vitro* systems do not take first pass metabolism into consideration. CYP 3A enzymes are abundant in the human small intestine and play a major role in xenobiotic metabolism here. Secondly, the rat is a poor model for human CYP 3A enzymes, as isoforms of CYP 3A are species specific. In humans the majority of drug biotransformation in the liver is by CYP 3A4 and 3A5 whereas in rats CYP 3A1 and CYP 3A2 predominate. Even minor differences between species in

amino acid sequences in CYP enzymes can result in major differences in CYP activity in relation to substrate specificity and catalytic activity (Martignoni *et al.* 2006). Finally, the use of plant medicines (which the authors refer to as a ‘fad’), are seldom in the form of isolated constituents and there is a potential role for other phytochemicals in *S. lateriflora* acting in synergy with its diterpenoids and having a protective effect.

Contrary to being hepatotoxic, the antioxidant potential of *S. lateriflora* (Cole *et al.* 2008) may help protect hepatocytes from lethal damage.

In Japan, there are also concerns regarding potential toxicity of *S. lateriflora*. This is because the use of *S. baicalensis* root has been linked to a few cases of hepatotoxicity and interstitial pneumonia. Baicalin, also found in *S. lateriflora*, is the compound believed to be responsible for the toxic effects. Initial reports of toxicity were based on the inclusion of the root in ‘shosaikoto’ (Makino *et al.* 2008). This is, ironically, an herbal formula popular in kampo medicine as a hepatoprotective, particularly in cases of hepatitis and hepatocellular carcinoma. As it contains six other herbs: *Bupleurum chinensis* (Chinese thorowax), *Pinellia* spp. (green dragon), *Panax ginseng*, *Zizyphus jujuba* (jujube), *Glycyrrhiza glabra* (liquorice) and *Zingiber officinale* (ginger) (LiverSupport.com 2011), there is no evidence that baicalin is the toxic component linked to hepatopathy and interstitial pneumonia.

2.10 Side-effects

There were no reported side-effects in a clinical study of *S. lateriflora* (Wolfson & Hoffmann 2003). However, in view of purported efficacy and indications from experimental data (Wolfson & Hoffmann 2003) and traditional use, caution may be advisable in some circumstances. For example, due to its potential sedative action (Greenfield & Davis 2004) it may be advisable to refrain from using *S. lateriflora* with other sedatives, including alcohol and benzodiazepines. Although there have been no reported herb-drug interactions with *S. lateriflora* (Kuhn & Winston 2001), warnings could be given regarding effects on driving or operating machinery as all anxiolytics have the capacity to cause drowsiness in some individuals (B.N.F. 2008). According to Joshee *et al.* (2002) overdose may result in ‘giddiness, stupor, confusion and twitching’. However, with normal therapeutic doses (Table 2-1, p26) there have been no reported side-effects (Kuhn & Winston 2001).

2.11 Bioavailability, biotransformation and elimination

Very few pharmacokinetic studies of skullcap flavonoids have been conducted in humans. The following were based on flavonoids in *S. baicalensis*, also found in *S. lateriflora*.

Following oral administration to 10 healthy volunteers of commercial skullcap root (*S. baicalensis*) powder, urinary excretion of conjugated metabolites of baicalein and wogonin was 7.2% and 11.6% of the original dose respectively. The half-life eliminations for these conjugates were approximately 8 hours for baicalein and 10 hours for wogonin (Lai *et al.* 2003). An indication of the length of time some flavonoids remain in the body may be a useful indicator for a washout period in a clinical study.

Studies using rats have shown that very little baicalin enters the blood in its primary form but is first mainly hydrolysed by intestinal flora to baicalein and reconstituted to baicalin in plasma (Akao *et al.* 2000). As baicalin is a prodrug of baicalein and because baicalein was shown in other studies using rats to be the main bioactive metabolite in rat plasma following its oral administration, baicalein was previously believed to be more useful than baicalin as a therapeutic for oral administration (Guo *et al.* 2011). However, a recent study (Guo *et al.* 2011) investigating the metabolites of baicalein in human plasma 3 hours and 5 hours following its oral administration in 3 healthy volunteers showed the two flavonoids may be interchangeable with regard to bioactivity. Five metabolites were identified by HPLC/MS. These were glucuronides of baicalein, including baicalin, which was the main metabolite (Guo *et al.* 2011). Although the results suggest the two flavonoids are interchangeable as major active compounds of *S. lateriflora*, polar glycoside flavonoids such as baicalin and wogonoside are hydrophilic and poorly lipophilic so are unlikely to cross the blood brain barrier; unlike their aglycones baicalein and wogonin respectively (Barceloux 2008). Therefore, the anxiolytic properties may be due mainly to aglycones in the blood. It is noteworthy that benzodiazepines are highly lipophilic and can cross the blood brain barrier (Longo & Johnson 2000).

(See also 2.6.1, p25 & 2.12.1.3, p34)

2.12 Research supporting anxiolytic and thymoleptic effects

2.12.1 In vitro studies

2.12.1.1 Benzodiazepine receptor affinity

Benzodiazepines are allosteric ligands for the GABA_A receptor (Figure 2.4), a chloride channel that is gated by GABA. They bind to the benzodiazepine site of the GABA_A receptor, thus increasing the affinity of the inhibitory neurotransmitter GABA for the GABA site of the GABA_A receptor, decreasing the likelihood of action potentials by excitatory neurotransmitters such as adrenalin and noradrenalin, implicated in anxiety and stress (Rabow *et al.* 1995; Paladini *et al.* 1999).

A study (Liao *et al.* 1998), using four isolated compounds from *S. baicalensis* indicated oroxylin A, skullcapflavone II, baicalein and wogonin bound to the benzodiazepine (BDZ) site of GABA_A receptors in tissue of neuronal origin *in vitro*. Oroxylin A, baicalein and wogonin, which are also found in *S. lateriflora* (Bergeron *et al.* 2005), had weak affinities for the BDZ binding sites (Liao *et al.* 1998).

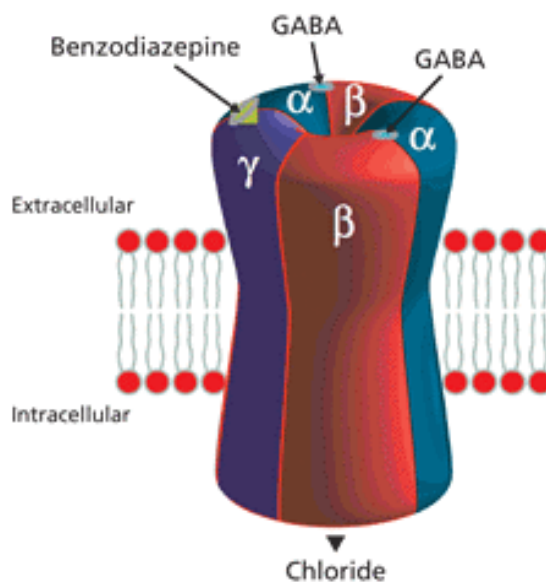


Figure 2.4 The GABA_A receptor complex. BDZs bind to the α and γ subunits

(Dawson *et al.* 2005)

In another study Hui *et al.* (2000) tested the binding capacity of baicalin, baicalein, scutellarein and wogonin, isolated from *S. baicalensis*, to the BDZ site of the GABA_A receptor. Affinity to BDZ receptors for scutellarein was moderate and weak for

baicalin. Contrary to results of the study by Liao *et al.* (1998) they found the binding affinities of wogonin and baicalein were strong and wogonin, which, as with benzodiazepines, bound to the γ subunit of the GABA_A receptor, had the strongest affinity. The discrepancy may be due to differences in species and assay models used. Cerebral cortex membrane of murine origin was used in the study by Liao *et al.* (1998) and homogenised rat brain was used in the study by Hui *et al.* (2000). As there are interspecies variations in regional distribution and binding affinity for receptors, related studies on species other than humans may be misleading (Dodd 1995). In a comparison between rats and humans of neuroanatomical BDZ receptor binding capacity, for example, there were marked species differences (Duncan *et al.* 1998). It would be more appropriate to study the potential of skullcap flavones as neurotransmitter ligands on human CNS tissue. Kopp *et al.* (1990), for example, used homogenised human post-mortem tissue for BDZ binding affinity studies.

2.12.1.2 Structure-activity relationship

BDZs have a seven membered imino ring (B or diazepine ring) with 2 nitrogen atoms and studies have indicated that this ring is essential for its affinity towards the BDZ binding site (Macdonald & Olsen 1994; Patel *et al.* 2009) although lipophilicity is also important (Wang *et al.* 1999).

The binding sites to the BDZ receptor specifically relating to skullcap flavonoids have not been elucidated but studies have indicated the important BDZ binding sites of flavonoids are the phenyl ring (B ring), the methoxy group at C5 and position 4 of the carbonyl group (Wang *et al.* 1999). It has been proposed that activity is via hydrophobic interactions and hydrogen binding causing a change in protein structure (Li *et al.* 2011).

There are some similarities between flavonoid and benzodiazepine structures (Figure 2.5) in that both have a benzene ring (A). In benzodiazepines this is fused to a diazepine ring (B). Ring C is an aryl ring. The B ring on flavonoids is a phenyl ring and ring C is a chroman ring (Ophardt 2003).

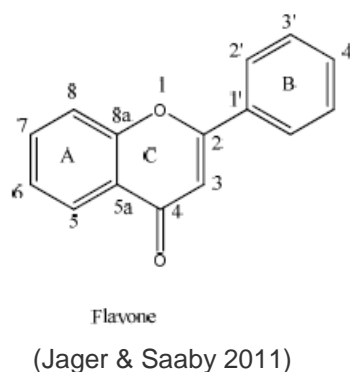
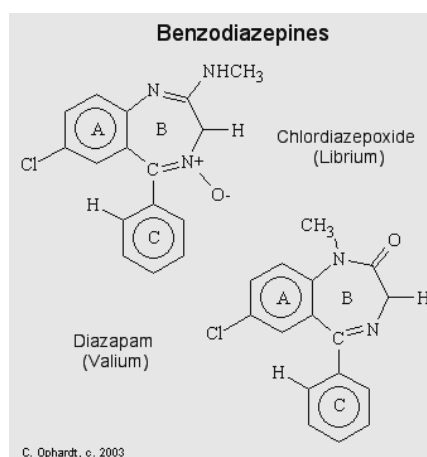


Figure 2.5 Comparison of the structures of common BDZs and flavones

Because flavonoids, including baicalein, baicalin, wogonin and scutellarein from *S. baicalensis* bind to GABA_A-BDZ receptor sites *in vitro* (Liao *et al.* 1998; Hui *et al.* 2000), the presence of these flavonoids also in *S. lateriflora* (Zhang *et al.* 2009) indicates a possible anxiolytic action for both herbs, although relevancy for baicalin is doubtful as it does not cross the blood brain barrier (Barceloux 2008). Benzodiazepines have a similar core structure to flavonoids (Figure 2.5), which may explain the skullcap flavonoid binding capacity to BDZ receptors. The results also present the possibility of baicalein being more strongly implicated in having an anxiolytic effect than its glycoside baicalin, notwithstanding the likely inability of flavone glycosides to cross the blood-brain barrier (Barceloux 2008) and the hydrolysis of baicalin to baicalein at first pass metabolism (Hackett 1986).

2.12.1.3 Serotonin-7 receptor affinity

Gafner *et al.* (2003b) investigated the ability of hot water and 70% ethanol extracts and major isolated flavonoids of dried *S. lateriflora* aerial parts to bind to serotonin-7 (5-HT₇) receptors *in vitro*. Both the hot water and the 70% ethanol extracts as well as the flavonoids baicalin, scutellarin, wogonin, 5,6,7-trihydroxy-2'-methoxyflavone (lateriflorein), ikonnikoside I and dihydrobaicalin, were shown (by radioligand binding assay) to have high affinity for the 5-HT₇ receptor in human 5-HT₇ - transfected Chinese hamster ovary cell lines. The highest affinity was for the hot water extract and for scutellarin (Gafner *et al.* 2003b).

Although the findings by Gafner *et al.* (2003b) suggest that *S. lateriflora* may possess 5HT₇ modulating activity and may therefore assist in regulation of sleep, mood, pain and migraine (Gafner *et al.* 2003b; Agosti 2007), it is not clear which phytochemicals in *S. lateriflora* can cross the blood-brain barrier (BBB) (Gafner *et al.* 2003b). Potential relaxant effects of *S. lateriflora* would be dependent on some of them being able to do so and there is evidence to suggest that some flavonoid aglycones and their metabolites can cross the BBB. For example hispidulin, a GABA_A receptor agonist found in sage, valerian and *S. baicalensis* (Johnston & Beart 2004), and reported to be present in *S. lateriflora* (Barnes *et al.* 2007) was found to cross the BBB in both *in vitro* and *in vivo* studies (Youdim *et al.* 2004). It is possible, however, that the melatonin and serotonin contained in *S. lateriflora* would bind to 5HT₇ receptors *in vitro* (Cole *et al.* 2008).

It was not clear whether the binding effect of *S. lateriflora* flavonoids on the 5-HT₇ receptor was as agonists or antagonists. They are likely to be 5-HT₇ receptor antagonists or inverse agonists as, according to a patent application (Bright & Coffman 2004), 5-HT₇ receptor antagonists or inverse agonists are useful in the treatment of CNS and cardiovascular disorders, premenstrual syndrome, sleep disorders, appetite disorders, anxiety, phobias, panic, depression and for stress-related disorders such as irritable bowel syndrome; these are also conditions for which *S. lateriflora* is traditionally used (Felter & Lloyd 1898; Khosh 2000; Hull 2010).

As flavone glycosides are poorly lipid soluble it is likely that diffusion of baicalin across the BBB is limited (Barceloux 2008). Furthermore, research suggests that scutellarin, also a flavone glycoside, is unable to cross the BBB in its primary form (Chen *et al.* 2006).

Clinical studies were conducted (Chen *et al.* 2006) to determine the bioavailability and pharmacokinetics of scutellarin, following its oral administration to 20 healthy male volunteers. Each participant swallowed with 200 ml of water a ‘dripping pill’ containing 60 mg of scutellarin. Blood samples were taken prior to dosing and hourly for 15 samples and then at 24 hours. Urine was collected prior to dosing and at 8 and 12 hours. Liquid chromatography with mass spectrometry (LC/MS) was used to identify the scutellarin and its metabolite isoscutellarin in the plasma and urine (Chen *et al.* 2006).

Scutellarin was barely detectable in plasma. Only its isomer (isoscutellarin or scutellarein-6-*O*-glucuronide) was present as a major metabolite, at a mean of 7.85 hours. This indicated that bacterial hydrolysis occurred at first pass metabolism and the aglycone of scutellarin was absorbed from the intestines and re-conjugated with glucuronic acid in intestinal and/or liver cells. Four metabolites of scutellarin were detected in human urine and it was determined that urine analysis could be used as a marker for scutellarin ingestion. The results suggest the ability of scutellarin to bind to 5-HT₇ receptors *in vitro* (Gafner *et al.* 2003b) may not be implicated in its anxiolytic effects. Work needs to be conducted to assess binding of isoscutellarin to 5-HT₇ receptors.

The results of the study (Chen *et al.* 2006) conflicted with an earlier study on rats (Zhang *et al.* 2003), in which the metabolites of scutellarin appeared in the urine in different ratios to those detected in human urine (Chen *et al.* 2006). Due to species specificity, more human bioavailability studies of *S. lateriflora* compounds are needed.

Of the serotonin receptors, 5-HT₇ is the most recently discovered and was first characterised in 1993. Since then there has been a considerable amount of research conducted to investigate the effects of 5-HT₇ receptor ligands (Pittala *et al.* 2007) but little is known about the function of human 5-HT₇ receptors. They are known to be widely distributed throughout the human brain (Martin-Cora & Pazos 2004) and vascular system (Cifariello *et al.* 2008) and based in part on anatomic distribution, functional implications have been postulated. It has been proposed, for example, they may play a role in learning and memory and that novel therapeutic interventions selective for 5-HT₇ receptors may potentially treat psychiatric disorders and epilepsy

(Martin-Cora & Pazos 2004). It is worth reiterating that *S. lateriflora* is traditionally used for the very same conditions (Hull 2010).

Results of a series of *in vitro* studies (Cohen *et al.* 1999) of cultured human brain endothelial cells, human brain smooth muscle cells (microvascular) and human brain astrocytes strongly suggested the presence of functional 5-HT₇ receptors on these cell types. The authors hypothesised that the presence of these receptors on non-neuronal cells in the brain may be to help regulate cerebrovascular tone, BBB permeability and brain blood perfusion and, consequently, anti-migraine drugs accessing the brain parenchyma may act by stimulating these receptors (Cohen *et al.* 1999). It could also be hypothesised that scutellarin's affinity for 5-HT₇ receptors may also increase BBB permeability, perhaps facilitating access of certain *S. lateriflora* flavonoid metabolites.

The ability of scutellarin to bind to 5-HT₇ receptors raises the possibility that this flavonoid, found in small amounts in *S. lateriflora* (Cole *et al.* 2008), may be partly implicated in the herb's reported anti-headache and anti-hypertensive (Joshee *et al.* 2002) effects (along with the anxiolytic effects of other flavonoids in the herb).

Another compound found in *S. lateriflora* that does not cross the BBB in mammals, is the indoleamine serotonin (Polo *et al.* 2007; Cole *et al.* 2008). It may, however, contribute synergistically to other physiological activity.

2.12.1.4 Melatonin in *S. lateriflora*

Melatonin, which has important physiological actions in animals, was only discovered in plants in 1995. It is secreted by the mammalian pineal gland at night and plays a role in regulation of sleep, the circadian rhythm, immune function and mood modulation. It is also an antioxidant (Arnao & Hernandez-Ruiz 2006). According to Murch *et al.* (1997), the melatonin content of *S. lateriflora* has implications for treating migraine and insomnia. Although melatonin is, like serotonin, an indoleamine it readily crosses the BBB (Jacob *et al.* 2002). The melatonin content of *S. lateriflora* is only around 90 ng/g (Cole *et al.* 2008) and it was argued by prominent ethnobotanist Duke that the quantities of melatonin present in *S. lateriflora* are insufficient to exert any effects (Levy 2001). Results of a clinical trial (Zhdanova *et al.* 2001) suggested a single oral dose of 0.1 mg melatonin can improve sleep efficiency in insomniacs. Doses lower than this do not appear to have been tested (no publications were located) in clinical trials. Further, the

potential synergistic effect of melatonin with other phytochemicals in *S. lateriflora* is unknown and cannot be discounted. Synergistic actions with herbs in remedies including *S. lateriflora* may be important in the therapeutic action.

2.12.1.5 GABA and glutamine in *S. lateriflora*

Significant amounts of the amino acids GABA, an inhibitory neurotransmitter that modulates anxiety, sleep, convulsions and mood (Rabow *et al.* 1995), and glutamine, a non-essential amino acid that plays an important role in immune function – particularly in response to stress (Griffiths 2001), occur in *S. lateriflora*. They have been detected in both the aqueous and ethanolic extractions, with markedly high levels of glutamine (31mg/g) in aqueous extract (Awad *et al.* 2003) and 0.55% GABA expressed in dry weight (5.5 mg/g) of a 70% ethanolic extract (Bergeron *et al.* 2005). Although GABA does not readily cross the blood-brain barrier (Spinella 2002), glutamine *can* and may be biosynthesised to GABA by GABA-ergic neurons. The presence of glutamine in the herb may therefore add to its anxiolytic activity by increasing the availability of GABA in the central nervous system (Bergeron *et al.* 2005).

2.12.2 Clinical trials

To date only one clinical trial has been conducted on *S. lateriflora*. Wolfson and Hoffmann (2003) assessed the anxiolytic properties of the herb in a double-blind, placebo-controlled crossover study of 19 healthy volunteers. Participants were aged 20 to 70 years and consisted of 15 women and 4 men. They took either 2 placebo capsules, one capsule containing 100 mg of organic freeze-dried *S. lateriflora* extract (Phytos), two capsules of these, or one capsule of 350 mg organic freeze-dried *S. lateriflora* made by a different manufacturer (Eclectic Institute) than the 100 mg capsules. No mention was made of the composition of the placebo but one of the authors (Hoffmann 2008) later revealed in a personal communication it was *Anthriscus cerefolium* (chervil)*.

Participants' energy, cognition and anxiety were self-rated at various time points up to 2 hours following administration of test or placebo substances, when measurements tended to return to baseline. The three herb tests all had notable effects in reducing subjective anxiety scores when compared to placebo, the most effective being the two 100 mg capsules. There was only a very mild decline in cognition and energy with the herbs, with no adverse reactions or side-effects, suggesting that *S. lateriflora* could be a valuable anxiolytic - as many anxiolytics impair cognitive function and physical

performance (Wolfson & Hoffmann 2003). It is unclear whether the results were statistically significant as this was not determined.

Although the study results indicate an anxiolytic effect for *S. lateriflora* the short timescale of the study could not demonstrate any long-term effects, whether negative such as side-effects, tolerance or dependence, or positive such as prolonged well-being. According to the study the anxiolytic effects of the intervention wore off by 2 hours in most subjects but this was self-reported following instructions that the study period was for 2 hours and it is unclear whether the participants may have subconsciously believed the effects were likely to diminish in that time period. The study also does not indicate whether regular doses throughout the day could have an additive effect. More research needs to be conducted in order to assess this paradigm.

Another confounding factor was that the tests used in the study were non-validated. The authors (Wolfson & Hoffmann 2003) acknowledged that the use of validated psychometric tests is needed to determine the herb's clinical anxiolytic effects. Additionally, self-reported changes in anxiety were in healthy subjects so cannot be confidently extrapolated to clinically anxious subjects. With regard to the perceived greater effect on anxiety with the 200 mg *S. lateriflora* regime over the 350 mg single capsule, the authors suggest this may be due to differing potencies resulting from variations in storage, harvesting etc. However, it could be argued that participants believed they were being provided with more powerful treatment with two capsules than with one.

*There is very little written in the literature in relation to therapeutic actions of *Anthriscus cerefolium* but there is a possibility it may be psychoactive as traditional uses are as a memory enhancer and anti-depressant. It is also reported to be diuretic and is traditionally used as an anti-hypertensive. (Plants for a Future 2010a).

2.13 Wider therapeutic implications

2.13.1 Anti-inflammatory activity

Experiments to determine the anti-inflammatory properties of *S. lateriflora* were conducted in two different *in vitro* systems (Gafner *et al.* 2004b). Both hot water (1:20 w: v) and 70% (1:10 w: v) ethanol extracts inhibited cyclooxygenase 1 (COX-1) and

cyclooxygenase 2 (COX-2) in a cell-free medium. Although it contained fewer flavonoids, the hot water extract was more potent than the ethanolic extract, which suggested involvement of other compounds in the anti-inflammatory process. Hot water and 70% ethanol extracts of *S. lateriflora* assayed on human keratinocytes in culture for anti-inflammatory effects significantly reduced activity of PGE2 (prostaglandin E2) (Gafner *et al.* 2004b)

A US patent application for a polypharmacy anti-inflammatory formula (Jia *et al.* 2007) describes the anti-inflammatory activity of extracts of *Scutellaria orthocalyx* (root), *S. baicalensis* (root) and *S. lateriflora*. Whole dried organic *S. lateriflora* plant (presumably including roots) was ground to a powder and extracted with methanol. The extracts were incubated with both ovine and human COX-1 and COX-2 and the IC₅₀ values (the quantity of substance required to inhibit 50% of enzyme activity in comparison to a control) were calculated. The extract of *S. lateriflora* showed greater inhibition of human COX-2 enzymes than of the other two species. It also showed preferential inhibition of human COX-2 over ovine COX-1 and COX-2 with respective IC₅₀ values of 20, 30 and 80 µg/ ml (Jia *et al.* 2007). These results confirm the earlier findings by Gafner *et al.* (2004b) and suggest anti-inflammatory properties for *S. lateriflora*. The herb may therefore have direct as well as indirect positive benefits for attenuation of comorbidities associated with anxiety (Pitsavos *et al.* 2006).

Glutamine is a modulator of tissue inflammation (Coëffier *et al.* 2002) so it could be hypothesised that it may also play a role in the anti-inflammatory properties of *S. lateriflora*. Gafner *et al.* (2004b) discovered that an aqueous extract of *S. lateriflora* had a more potent anti-inflammatory effect than an ethanolic extract containing more flavonoids and suggested that compounds other than the flavonoids contributed to this activity. Furthermore, Awad *et al.* (2003) found high levels of glutamine in an aqueous extract of the herb. A study on cultured duodenal biopsies from human volunteers administered enteral glutamine or amino acids indicated a strong inhibitory effect by glutamine on pro-inflammatory cytokines *in vitro*. Inhibition of COX-2 in this particular study was not significant but the volunteers did not have inflammatory bowel disease (Coëffier *et al.* 2002).

Li *et al.* (2000) investigated the effect of baicalin (isolated from *S. baicalensis*) on human chemokines (which induce leukocyte migration and contribute to inflammatory

reactions) *in vitro*. Human leukocytes were incubated with chemokines in the presence of baicalin isolated from *S. baicalensis*. In a dose-dependent manner, baicalin inhibited the binding of chemokines to human leukocytes as well as reducing the receptor binding capacity of chemokine receptor ligands. There was no observed toxicity to leukocytes (Li *et al.* 2000). The results suggest a molecular mechanism for the anti-inflammatory properties of baicalin in *S. lateriflora* as well as in *S. baicalensis*.

2.13.2 Antimicrobial, larvicidal and molluscicidal activity

Using an agar plate method, the minimum inhibitory concentration (MIC) of a 60:40 (v/v) ethanol/water extract of *S. lateriflora* root against *Bacillus subtilis* and *Escherichia coli* was determined. The results demonstrated 5 mg/ml MIC for *B. subtilis* and a significant MIC at 25 mg/ml for *E. coli* (Duffy & Power 2001). The authors did not clarify why they used the root instead of aerial parts but stated that the experiments were conducted on Chinese herbs. Furthermore, as no quality controls were conducted it was not confirmed that the species used was in fact *S. lateriflora*.

Bergeron *et al.* (1996) screened dichloromethane (DCM), methanol and water extracts of *S. lateriflora* for fungicidal, bactericidal, larvicidal and molluscicidal activities. The methanol and DCM extracts quantitatively inhibited the fungi *Cladosporium cucumerinum* and *Escherichia coli* bacteria on a thin layer chromatography (TLC) plate and the methanol extract also inhibited *Candida albicans*. None of the extracts had any inhibitory effects on *Bacillus subtilis* on TLC plates, or were larvicidal (*Aedes aegypti*) or molluscicidal (*Biomphalaria glabrata*) in solution. A confounding factor in this experiment may have been the use of dried plant material, which is believed to have little activity (Yarnell & Abascal 2001).

2.13.3 Antioxidant effects

The antioxidant potential of tissues from *S. lateriflora* was assessed *in vitro* and compared with *S. baicalensis* and *S. racemosa* (Cole *et al.* 2008). All three herbs, which were propagated in identical conditions, had similar antioxidant capacity although less tissue was required for *S. lateriflora* to produce a 50% reduction in oxygen free radicals than the other two herbs.

In a controlled study of the antioxidant and antimicrobial potential of 12 different herbs, water, water and ethanol (60:40 v/v), and ethanol extracts were prepared from root of *S.*

lateriflora purchased from China and then ground in a blender. The antioxidant properties of skullcap root were assessed by its ability to prevent formation of thiobarbituric acid reactive species from linoleic acid. It had a 1.3 % antioxidant index for the water extract, 1.2% for the ethanol extract and 66.1% for the ethanol/water extract (Duffy & Power 2001).

Wojcikowski *et al.* (2007) studied the antioxidant capacity of 55 medicinal herbs traditionally used to treat urinary tract disorders. The oxygen radical absorbance capacity (ORAC) *in vitro* assay was used. The ORAC of the 55 herbs was compared with that of *Silybum marianum* (milk thistle) and *Camellia sinensis* (tea), herbs known to have significant antioxidant properties. *S. lateriflora* was among the 5 herbs with the most potent free radical scavenging activity, which in all 5 was higher than either of the control herbs (Wojcikowski *et al.* 2007).

Further to the above study (Wojcikowski *et al.* 2007) an investigation of potential antioxidant or toxic effects of extracts of 47 herbs, including *S. lateriflora*, was conducted on kidney fibroblast and renal tubule cell lines. Of all herbs tested, again *S. lateriflora* was amongst the top 5 herbs for antioxidant capacity (with both ethyl acetate and methanol extracts), suggesting it may have a protective action on renal tubular epithelium and may therefore be beneficial in kidney disease (Wojcikowski *et al.* 2009).

The *in vitro* studies of *S. lateriflora* indicate its potential as an herb with strong antioxidant capacity. It may therefore be of use in conditions in which a protective effect on tissues is required as in age-related macular degeneration, various types of dementias, hepatic impairment, kidney disease, and cardiovascular disorders such as atherosclerosis (Meydani 2001; Middleton & Yaffe 2009). It is unclear which compounds were responsible for its antioxidant and antimicrobial effects.

2.13.4 Anti-neoplastic effects

S. lateriflora leaf and root extracts (500 µg/ ml) were independently found to significantly inhibit proliferation of human malignant glioma cells *in vitro* in comparison to controls (100% proliferation), reducing proliferation of cancer cells by 51% and 44% respectively. The stems showed minimal inhibitory activity at 16% (Parajuli *et al.* 2009). The difference in potency may have been due to the maturity of the plants, which were harvested before flowering. Immature *S. lateriflora* has higher

estimated flavonoid content in the leaves (69.3 mg/g) and roots (40.4 mg/g) and negligible in the stems, whereas in fully mature plants the flavonoid content is significant in the stems (21 mg/g) although still higher in the leaves (44.4 mg/g) and roots (37.6 mg/g) than in the stems. At all stages of plant growth the leaves of *S. lateriflora* contain the most flavonoids (Wills & Stuart 2004).

Individual phytochemicals isolated from *S. baicalensis* have been studied for their anti-cancer properties. The studied phytochemicals are also in *S. lateriflora* although in different ratios (Cole *et al.* 2008). Therefore, demonstration of their anti-cancer effects suggests similar actions and indications for *S. lateriflora*. For example, in a controlled experiment wogonin was incubated at different concentrations with human leukaemia cell lines derived from a histiocytic lymphoma. It inhibited cancer cell growth in a concentration- and time-dependent manner, reduced cell viability and nuclear to cytoplasmic ratio, and appeared to have the ability to induce normal cell differentiation (Zhang *et al.* 2008a). Another study demonstrated the inhibition of proliferation and induction of differentiation of human promyelocytic cell lines by 55-60% (Zhang *et al.* 2008b). The results indicate a possible beneficial action for *S. lateriflora* in the treatment of haematopoietic tumours.

Baicalein has also been studied for its anti-cancer properties. Chao *et al.* (2007) found it to be cytotoxic to human bladder cancer cells *in vitro*. They also compared its cytotoxic properties on bladder cancer cell lines with those of baicalin, rutin, catechin and quercetin and found it to be the most potent cytotoxic flavonoid - but without being cytotoxic to normal cultured human fibroblasts. Baicalein induced cancer cell apoptosis, inhibited cancer cell proliferation; therefore arresting the cancer cell cycle. It prevented expression of survivin (an apoptosis-inhibitory protein secreted by cancer cells) in bladder, breast and lung cancer cells (Chao *et al.* 2007).

Conversely, Kumagai *et al.* (2007) compared the anti-cancer effects of baicalin and baicalein on various haemopoietic cell lines and baicalin was more potent than baicalein. This suggests potential flavonoid specificity for cancer cell types.

Baicalin, baicalein and wogonin induced apoptosis and reduced proliferation in human hepatoma cell lines, suggesting a role for these compounds in protection against human liver cancers (Chang *et al.* 2002).

Heterocyclic amines (HCAs) are toxic mutagenic/carcinogenic substances, which have recently been in the news (Bates 2011) relating to warnings about their production by overcooked meats (Layton *et al.* 1995). A study (Kim *et al.* 2002) found baicalein, wogonin and oroxylin A inhibited CYP1A2 activity in human liver microsomes. As this enzyme is capable of catalysing HCAs with the production of toxic or carcinogenic intermediates *S. lateriflora* may have a protective effect against carcinogens produced from HCAs.

In consideration of the results of experiments involving wogonin, baicalin and baicalein and oroxylin A it could be hypothesised that the flavonoids of some skullcap species endow the plants with inhibitory actions against a variety of human cancers. It is noteworthy that in one study (Parajuli *et al.* 2009) extract of *S. ocmulgee* leaf showed the strongest antiproliferative activity of thirteen *Scutellaria* species tested. However, HPLC analysis of *S. ocmulgee* leaf for the presence of apigenin, baicalin, baicalein, chrysin, scutellarein and wogonin found it contained only wogonin, which, when tested alone against human glioma cells, also showed strong anti-cancer activity. Its wogonin content was lower than that of some of the other *Scutellaria* species tested, suggesting a synergistic effect of other flavonoids or other phytochemicals in this species or an antagonistic effect in others (Parajuli *et al.* 2009).

Interestingly, scutellarein was first isolated in 1890 from the seeds of the Tree of Damocles (*Oroxylum indicum*) (Malikov & Yuldashev 2002). They also contain baicalin, baicalein and chrysin (Chen *et al.* 2003). These seeds are used in Bangladeshi folk medicine for their purported anticancer properties (Costa-Lotufo *et al.* 2005). All these compounds are found in significant amounts in *S. lateriflora* (Li & Khan 2008), which suggests it may also have anti-cancer properties.

The results of both antioxidant and anti-tumour studies indicate a role for *S. lateriflora* as a prophylactic agent for carcinogenesis, but clinical trials are needed for confirmation of efficacy and to determine optimal dosage. As the western medical herbalist may not treat cancer, *S. lateriflora* may be a useful adjunct in oncology care.

2.13.5 Endocrine actions

Chrysin has been demonstrated in a number of *in vitro* studies to be a potent inhibitor of human aromatase. It is sold as an extract (of blue passion flower) for body builders as it is believed to increase testosterone levels (Ta & Walle 2007).

2.13.6 Cardiovascular effects

Scutellarin is considered a major bioactive flavonoid and is used in cardiovascular medicine. It is the main constituent of the drug breviscapine, which is used in traditional Chinese medicine for cerebrovascular and cardiovascular disease as a platelet aggregation inhibitor and to reduce platelet count as well as being a vasodilator and benefiting microcirculation (Chen *et al.* 2006; Cole *et al.* 2008). *S. lateriflora* may therefore also have anti-platelet activity. Findings that scutellarin is present in much higher quantities (x 800) in *S. baicalensis* than in *S. lateriflora* (Cole *et al.* 2008), may be more significant for the reported cardiovascular effects of *S. baicalensis* (Liao *et al.* 1998) than of *S. lateriflora*.

There have been many more *in vitro* studies of the actions of individual phytochemicals isolated from the root of *S. baicalensis*, and which are also found in *S. lateriflora*, but they are too numerous to include in this review.

2.14 Evaluation and discussion

Results of *in vitro*, chemical and clinical studies of *S. lateriflora* and its phytochemicals suggest it has antineoplastic, anti-inflammatory, antioxidant, antibacterial, antifungal, aromatase-inhibitory and anxiolytic properties as well as potentially potent effects on modulation of mood, sleep, pain and blood vessel tone. These actions support its traditional uses for insomnia, inflammatory conditions, urinary tract disorders, neurological disorders, headaches, hypertension, stress and anxiety, premenstrual syndrome and possibly other gynaecological complaints (due to aromatase inhibition). It could potentially be used by the modern western medical herbalist for inflammatory conditions such as arthritis, cardiovascular disorders, general debility and exhaustion e.g. myalgic encephalomyelitis, bacterial infections, fungal diseases such as *Candida albicans*, and oestrogen-dependent gynaecological complaints such as uterine leiomyomas and endometriosis as well as an adjunct for certain cancers e.g. leukaemias and hepatocellular carcinoma.

Based on the known phytochemical components it has potentially wider therapeutic value. For example, as some of its major flavonoids are ligands for GABA_A receptors (Liao *et al.* 1998; Hui *et al.* 2000) it could have benefit for tinnitus, for which GABA-ergic compounds are reported to be of known benefit (Smith *et al.* 2005).

Speculation on the anti-inflammatory (Li *et al.* 2000; Gafner *et al.* 2004b), anti-cancer (Chang *et al.* 2002; Chao *et al.* 2007; Kumagai *et al.* 2007; Zhang *et al.* 2008a) and BDZ-binding (Liao *et al.* 1998; Hui *et al.* 2000) properties of *S. lateriflora* is due, in part, to the similarities between *S. lateriflora* and *S. baicalensis* in phytochemical constitution. These studies used isolated phytochemicals from *S. baicalensis*, which are also present in *S. lateriflora*.

It is not known whether effects on physiological function of whole tincture or dried herb administered from the herbal clinic, either alone or with other herbs would be similar to those of isolated phytochemicals in the laboratory.

While orthodox medicine tends to employ isolated phytochemicals, herbal medicine uses extracts of whole herbs in order to provide the synergistic benefits of multiple active constituents in a single herb. *S. lateriflora*'s phytochemicals may act in synergy with each other as well as with concomitantly administered herbs. It has also been suggested (Parajuli *et al.* 2009) that certain phytochemicals may even have an antagonistic action towards each other.

Research shows evidence supporting the theory of synergy; medicinal effects of herbs are due to the actions of constituent chemicals working together (Cole *et al.* 2008). Valerian's phytochemicals, for example, are believed to act together on multiple GABA-ergic systems, and the pharmacodynamic synergy of kavalactones in *Piper methysticum* increases the bioavailability of one another, resulting in a modulation of GABA_A and amine systems in the brain (Spinella 2002). Similarly, the numerous active phytochemicals in *S. lateriflora* may have multiple pharmacodynamic actions.

While the results of the *in vitro* and chemical studies are interesting and provide clues to the pharmacological actions of *S. lateriflora*, clinical evidence is necessary to support findings. The *in vitro* findings alone cannot indicate how the herb influences physiological, environmental, genetic and pathological characteristics inherent in a

human patient. Results of *in vitro* studies of individual phytochemicals as an indication of the action of a whole herb or extract cannot be relied upon to confirm the pharmacology of the combined phytochemicals; nor do they indicate whether or not the compounds under scrutiny cross the blood-brain barrier. More clinical research on *S. lateriflora* is therefore essential in order to assess these parameters.

2.15 Conclusions

The majority of findings of *in vitro* research have been conducted on the pharmacology of the individual phytochemicals of *S. lateriflora*, mostly due to recognition of the therapeutic value of *S. baicalensis*. It is unlikely, however, that the potential efficacy of *S. lateriflora* is due entirely to any of these phytochemicals acting in isolation, but to its multiple constituents acting in synergy. This can perhaps be exemplified by the large number of chemical compounds that have been isolated from *S. baicalensis*, many with putative medicinal value (Murch *et al.* 2004). It should be borne in mind that as similar metabolomics have not yet been conducted on *S. lateriflora* it is not certain whether parallels can be drawn between the two species with regard to medicinal actions. There is still a need for more research into the chemistry and pharmacology of the whole aerial parts of the herb.

The few *in vitro* studies on extracts of whole aerial parts of *S. lateriflora* and a small-scale clinical study described above do suggest a positive therapeutic benefit of the herb for decreasing anxiety but a dearth of larger, high quality, controlled clinical trials of sufficient duration means there is insufficient scientific evidence for its efficacy and safety in humans, particularly in the long-term. It is therefore appropriate and timely for more efforts to be made in the conduction of clinical trials in support of the therapeutic value of *S. lateriflora* for anxiety, stress and comorbidities. In the short-term such studies may provide an awareness of the potential of the value of this herb as an anxiolytic and may precipitate further research. In the long-term it may lead to the production of a useful therapeutic intervention to replace currently used anxiolytic pharmaceuticals available on prescription and perhaps reduce the concurrent financial burden on the NHS as a bonus.

Chapter 3. The use of *S. lateriflora*: A pilot survey amongst herbal medicine practitioners

3.1 Abstract

An email survey was conducted amongst herbal medicine practitioners in the UK and Ireland. The survey aimed to gather information on the extent of, and indications for, current use of *S. lateriflora*, its perceived effectiveness and its safety. Herbal medicine practitioners were selected from the membership list of the National Institute of Medical Herbalists (NIMH). All members with identifiable email addresses were contacted (n = 377) and responses were received from 62 (a 16% response rate).

The results of the survey suggested that *S. lateriflora* is highly regarded among herbal medicine practitioners as an effective intervention for reducing anxiety and stress and is commonly prescribed for these conditions and related comorbidities. The results were not conclusive as the response rate was low and respondents were only those with email access.

3.2 Introduction

American or Virginian skullcap (*Scutellaria lateriflora*) (Figure 3.1) is a perennial herb belonging to the *Lamiaceae* (mint) family (also known as *Labiatae*), sub-family *Lamioideae*, and is one of 360 known *Scutellaria* species worldwide (Cole *et al.* 1991; Malikov & Yuldashev 2002), many of which are used medicinally (Joshee *et al.* 2002). It grows on wetlands and is indigenous to North America and Canada where it is widely distributed - from Alaska to Florida and British Columbia to Quebec (the only places it is not found are the North-west Territories, Alberta and Yukon in Canada and Wyoming, Nevada and Utah in the US) (U.S.D.A. 2012). It is grown commercially worldwide, including in Australia and New Zealand (Wills & Stuart 2004).



Figure 3.1 *Scutellaria lateriflora* (© C. Brock)

S. lateriflora is used extensively and has been highly valued in traditional western herbal and ethnobotanical medicines for anxiety, hysteria, phobias, panic attacks, tension, depression, sleep disorders and stress (Felter & Lloyd 1898; Joshee *et al.* 2002). Known as a woman's herb by Native Americans, the Cherokees used it as an emmenagogue, thus promoting menses and aiding expulsion of the placenta following childbirth. Native American women also used it to ensure general menstrual health (Joshee *et al.* 2002) as well as for mastalgia and premenstrual tension (Greenfield & Davis 2004). Some tribes still use it in ceremonies to induct girls into womanhood and for purification rituals (Joshee *et al.* 2002).

The first European to record its use (in 1787) as a medicinal herb was German Physician Johann David Schöpff, who noted its use as a tonic, for fevers and as an abstergent (detergent) (Upton *et al.* 2009). It was mentioned in the first American *Materia medica* in 1785 but had been in longstanding use as a home remedy before then (Lloyd 1911). Dr Lawrence Van Derveer (1740-1815) studied *S. lateriflora* extensively and used it as a treatment for rabies (hydrophobia); it was then used for this purpose both professionally and as a home remedy. In 1819 Dr Lyman Spalding wrote 'A history of the introduction and use of *Scutellaria lateriflora* (scullcap), as a remedy for

preventing and curing hydrophobia, occasioned by the bite ... ' (Lloyd 1911; New Jersey Historical Society 2001). Medical doctor Joseph Bates wrote in 1855 in the Boston Medical Journal (Bates 1855) that because *S. lateriflora* was eventually scientifically proved to be utterly useless for this purpose it fell into disrepute and was consequently removed from pharmacopoeias. He nevertheless described using *S. lateriflora* in his practice, claiming a fluid extract had great value in the treatment of nervousness, irritability and restlessness, particularly in children. He also held it in high regard for hysteria and for relieving symptoms of inflammation in patients with arthritis or convalescing from fevers. He prophesied that it would be found to be highly successful in treating many diseases in the future, particularly those for which opium was currently prescribed (Bates 1855). In 1860 it was introduced into the official *United States Pharmacopoeia* as *Extractum Scutellariae Fluidum* (Millspaugh 1892; Upton *et al.* 2009) but was dropped in 1910. It was in the US *National Formulary* in 1916 and removed in 1942 when interest in natural remedies declined. It is not included in the *European Pharmacopoeia* (Upton *et al.* 2009).

Modern herbal medicine's application of *S. lateriflora* appears to be based upon its traditional uses for anxiety states. Greenfield and Davis (2004) for example, suggest it is used most commonly for insomnia, nervous disorders and digestive disturbances. Bergner (2002) proposes its action is primarily as a trophorestorative on the central nervous system (CNS), allowing relaxation following nervous exhaustion (Bergner 2002). It is also prescribed by western herbalists for epilepsy (British Herbal Medicine Association 1983), post-stroke paralysis, atherosclerosis, hyperlipidaemia, allergies, skin conditions and inflammation (Natural Medicines Comprehensive Database 2011).

A small clinical study assessing acute effects of *S. lateriflora* indicated it has anxiolytic actions with minimal loss of cognition (Wolfson & Hoffmann 2003). The authors assessed the anxiolytic properties of the herb in a double-blind, placebo-controlled crossover study of 19 healthy volunteers. Participants took either 2 placebo capsules, one capsule containing 100 mg of organic freeze-dried *S. lateriflora* extract, two capsules of these, or one capsule of 350 mg organic freeze-dried *S. lateriflora*. Participants' energy, cognition and anxiety were self-rated at various time points up to 2 hours following administration of test or placebo substances, when measurements tended to return to baseline. The three herb tests all had notable effects in reducing subjective anxiety scores when compared to placebo, the most effective being the two

100 mg capsules. There was only a very mild decline in cognition and energy with the herbs, with no adverse reactions or side-effects, suggesting that *S. lateriflora* could be a valuable anxiolytic - as many anxiolytics impair cognitive function and physical performance (Wolfson & Hoffmann 2003). It is unclear whether the results were statistically significant as this was not determined.

Commercial herbal products have been found to contain significant variations in phytochemical profile within a species. Such variation may be according to geographic region, biodiversity, ecological variations, cultivation, seasonality, harvesting, and storage time affecting stability; processing method, marc to menstruum ratio and alcohol concentration (Ciddi 2006; Gao *et al.* 2008).

Quality control and correct identification may be of particular importance in the case of *S. lateriflora* because of the large number of *Scutellaria* species (Malikov & Yuldashev 2002) and frequent substitution or adulteration with other skullcaps or potentially harmful herbs such as germander (*Teucrium*) (Wolfson & Hoffmann 2003). Respondents to the practitioner survey said they used a variety of suppliers, mainly for price, convenience or habit but it is important to ensure the herb has been authenticated.

Anxiety, stress and related disorders are problems treated most frequently in the herbal medicine clinic (del Mundo *et al.* 2002). As *S. lateriflora* is reported to be one of the most used herbs in western materia medica (Bergner 2002) it is likely that this is the herb of choice by western medical herbalists for these conditions. The purpose of the survey was to provide further evidence for its popularity in relation to other herbs used for the same conditions, to gather anecdotal evidence of its effectiveness and to gain information about prescribing practices such as dosage and duration of treatment, why it is prescribed and any reported issues in using it. The results may help to inform treatment protocols in clinical studies. A brief version of the survey was reported previously (Brock *et al.* 2010) along with a more detailed scientific basis for the use of *S. lateriflora*.

3.3 Materials and methods

UK and Ireland herbal medicine practitioners with an email address identifiable from the register of the National Institute of Medical Herbalists (NIMH) were contacted. NIMH members are qualified herbalists who have undergone several years' rigorous

scientific training, following which they take consultations with patients with a wide range of conditions, using the same diagnostic skills and examination techniques as orthodox doctors. This organisation was chosen as it is the largest organisation representing medical herbalists in the UK. As a number of herbalists belong to more than one representing organisation there was a risk of some receiving a survey questionnaire twice if more than one register was used. Each of the 377 practitioners selected received an electronic letter (Appendix I) and survey questionnaire form (Appendix II).

A mixed methods approach was used with mainly open questions in order to gather as much information as possible about practitioners' experiences with, and beliefs about, *S. lateriflora*. Respondents were asked:

- Whether they regularly prescribe *S. lateriflora* and, if not, reasons for not doing so;
- what they thought were its main actions and indications;
- what they prescribed it for and for how long;
- the length of time in which they expect to see a response in patients and what responses they expected to see;
- what patients reported from its use;
- whether there have been any reported side-effects and what were perceived contra-indications;
- type of preparation used and reasons for choices;
- dosage and strength;
- whether used alone or in combination;
- What was their favourite herb for anxiety?
- They were also asked how long they had been in practice and to add any additional comments should they wish to do so.

3.4 Results and discussion

Of 377 questionnaires sent, 62 practitioners responded (16%). Of all responders, 57 (92%) said they regularly prescribe *S. lateriflora*. Length of time in practice and experience varied widely but generally the responders comprised a group of highly experienced herbal practitioners. The average length of time in practice was 9.03 years

(SD = \pm 7.14) and the average time spent with patients per week was 10 hours per practitioner.

3.4.1 Actions and indications attributed to *S. lateriflora*

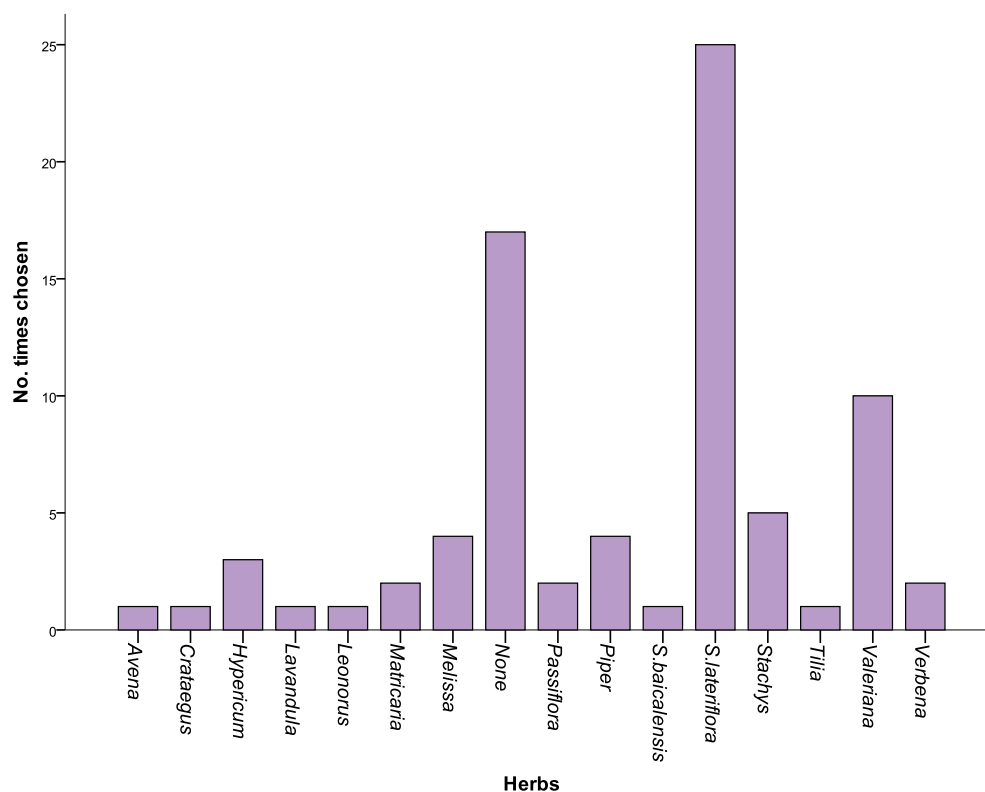
The majority of respondents provided more than one indication and/or action and use of *S. lateriflora* (Table 3-1). There was some confusion over the difference between actions and indications and most common conditions for which it is prescribed. For example, 77% of respondents said they would prescribe it for anxiety but only 35% suggested it has an anxiolytic action and only 18% gave anxiety as an indication for the herb. For simplicity, ‘indications’ and ‘reasons for prescribing’ have been amalgamated in Table 3-1. The confusion between actions and indications and reasons for prescribing is interesting and deserves consideration by herbalists generally; it calls into question how a misunderstanding of the language might potentially impose restrictions to the way in which herbs are used. If so, such an impediment may impact upon herbal practice as a whole. Rigorous scientific research could explore this issue.

3.4.2 The practitioners’ choice for anxiety

Results indicate the principal use of *S. lateriflora* is for relief of anxiety, stress or associated conditions with most (77%) survey respondents stating they would prescribe it specifically for anxiety and all said they would prescribe it for anxiety-related comorbidities. When asked what is their preferred herb for anxiety, twenty five (40%) chose *S. lateriflora* (Figure 3.2). It is interesting to note that one respondent indicated their preferred anxiolytic as being *S. baicalensis* as, although *S. baicalensis* root is most commonly used for inflammation (Joshee *et al.* 2002), it is reported to have been used as a sedative (Liao *et al.* 1998). Conversely, *S. lateriflora* is reported to have been traditionally used for inflammation. The Iroquai tribe, for example, used it ‘to keep the throat clear’ (Joshee *et al.* 2002). Additionally, both *S. lateriflora* and *S. baicalensis* have been found to inhibit cyclooxygenases *in vitro* (Gafner *et al.* 2004a; Jia *et al.* 2007).

Common reasons for prescribing *S. lateriflora* are provided in Table 3-1. All respondents who regularly prescribe *S. lateriflora* (92%) identified use for anxiety as distinct from depression and whilst some reported it useful also in depression, two respondents reported it as unsuitable for significant depression (described as “mentally depressed states” and “severe depression”) as in their opinion it tended to exacerbate it.

Usefulness for insomnia and sleep-related disorders was reported by 33 respondents. Practitioners who prescribe for poor sleep invariably described it to be related to an overactive mind, obsessive or racing thoughts, worry and anxiety.



Key: Avena = *A. sativa* (oats); Crataegus = *Crataegus* spp. (hawthorn); Hypericum = *H. perforatum* (St John's wort); Lavandula = *Lavandula* spp. (lavender); Leonorus = *L. cardiaca* (motherwort); Matricaria = *M. recutita* (German chamomile); Melissa = *M. officinalis* (balm); None = no preference; Passiflora = *P. incarnata* (passion flower); Piper = *Piper methysticum* (kava-kava); *S. baicalensis* (baical skullcap); *S. lateriflora* (American skullcap); Stachys = *S. betonica* (wood betony); Tilia = *Tilia* spp (linden); Valeriana = *Valeriana officinalis* (valerian); Verbena = *Verbena officinalis* (vervain).

NB: Many survey respondents gave more than one choice but overall the herb of choice was *S. lateriflora*.

Figure 3.2 Anxiolytic herbs as preferred by survey respondents

Table 3-1 Actions and indications attributed to *S. lateriflora*

Actions attributed to <i>S. lateriflora</i> (n = 62)	N	Indications/reasons for prescribing <i>S. lateriflora</i> (n= 62)	N
Nervine/ nerve trophorestorative	40	Anxiety	59
Anxiolytic	22	Insomnia and other sleep-related disorders	33
Relaxant	20	Stress	21
Mildly sedative	14	Migraine and other types of headaches	13
Calming	8		
Antispasmodic or spasmolytic	8	Depression	9
Cooling	4	Drug withdrawal and addiction	8
Tranquilising	4	Nervous and muscular tension	7
Anti-inflammatory	3	Panic attacks	7
Anti-stress	2	Fear and phobias	7
Mood lifting	2	Eczema, psoriasis/stress-related skin conditions	6
Anti-allergy	2	Physical and mental exhaustion	5
Parasympathetic	1	Excessive thought processes	5
Anti-panic	1	Hypertension	5
An emotional balancer	1	Palpitations	5
Antidepressant	1	Irritability	3
Supporting	1	Twitches and spasms of nervous origin/convulsions	2
Digestive tonic	1	Premenstrual syndrome (PMS)	2
Sustaining	1	Allergies	2
Anti busy brain	1	To put things into perspective	2
Bringing focus	1	Nervousness	2
		Chronic fatigue syndrome	2
		Irritable bowel syndrome	2

N = number of practitioners stating actions and/or indications/reasons for prescribing.

Other anxiety-related conditions given by practitioners as reasons for prescribing included for: ‘those who sigh a lot’, frustration, menopausal mood swings, despondency, neuralgia, nerve weakness, emotional instability, shock, feelings of not coping, tinnitus, debility, hot flashes triggered by stress, low mood, grand mal, Attention Deficit Hyperactivity Disorder, Obsessive Compulsive Disorder, “liver heat rising” and to “relax constriction where there is heat.”

3.4.3 Duration of treatment

The survey respondents reported the herb as being used over a range of time periods from immediate short-term use to several years, with positive response expected to be experienced by the patient within the first two weeks and persisting throughout the period of use.

According to 8% of respondents who did not state a timescale this is dependent on the health of a patient, the condition presented by a patient and its severity, how long they have been ill and patient compliance. In the words of one practitioner: ‘as long as it takes’. All other respondents gave a lower and/or an upper limit. Rarely, it may be prescribed as a one-off but in general the minimum length of time it is prescribed is for 1 or 2 weeks (11%). At least 30% of user respondents consider prescribing it long-term (6 months or more), sometimes for years (8%), although some stated ‘long term’ without being explicit about the timescale. Those who stated ‘several months’ were not included as long-term prescribers as it is unknown whether this could mean up to 6 months or more than 6 months. Of those who set an upper limit on treatment length 31% stated they would treat for up to 4 months. It is difficult to ascertain average treatment length as many replies were not explicit. What is clear, however, is that it ranges from one-offs to several years.

3.4.4 Responses looked for in patients

Sleep quality was a major factor in assessing mental state improvement with 53% of *S. lateriflora* prescribers stating they would expect patients to sleep better; for example by sleeping longer, with less waking in the night, an ability to get to sleep and feeling refreshed in the morning. Many (37%) felt they would be likely to notice that their patients were better able to cope with the stresses daily life. A reduction in anxiety and feeling calmer would be looked for in patients by 33% and 32% of practitioners respectively. In general, respondents expected their patients to feel happier, less depressed or tearful, calmer, more relaxed, less irritable, less stressed, tense or nervous and more in control of their emotions with elevated mood. They also expected a concomitant resolution or reduction of stress- and anxiety-related physical symptoms such as headaches, digestive disturbances, PMS, inflammatory skin conditions, twitches and spasms, and cardiovascular problems such as hypertension, tachycardia and palpitations.

3.4.5 Benefits reported by patients

The benefits most often reported by patients to their practitioners were feeling calmer, improved sleep patterns and sleep quality, and better able to cope in stressful situations. Other positive effects were mood elevation, increased energy, being more focused and feeling generally more relaxed. Some user respondents (19%) answered that it is

difficult to determine what patients have reported from using *S. lateriflora* as they never prescribed it as a single herb but always mixed it with others.

3.4.6 Effectiveness response time

When asked: “after what length of time would you expect to see a response?” answers were again often implicit rather than explicit. Most provided a range of timescales e.g. “one to two weeks”. Some practitioners did not provide a minimum or maximum timescale, only ‘one month of treatment for every year they have been ill’ or ‘longer term’ and many responded to the question with ‘few days or ‘few weeks’. One said it was difficult to say as they always prescribe *S. lateriflora* in combination with other herbs and therefore values for neither the average nor minimum and maximum expected response time could be ascertained.

Most said it was dependent on the condition being treated and many said that improvement was time and/or dose dependent - practitioners’ own words but suggesting improvement over the minimum response time provided and/or with what they perceive as adequate doses (see Figure 3.3) - but usually noticeable within a few days; and that continuation of treatment for periods beyond the first signs of improvement was beneficial to the patient. Responses by 12 practitioners suggest, however, that *S. lateriflora* may show an effect immediately or within hours. Twenty practitioners questioned expect to see some results in one or two weeks. Of total expected minimum response times provided (n = 56), only 11 practitioners did not expect *S. lateriflora* to show an effect before 3 weeks. The maximum expected response time was 12 weeks (n = 2) but, as practitioners are very keen to say: “it depends on the individual”.

3.4.7 Dosing strategies (posology)

The weekly ethanol: water tincture dose of *S. lateriflora* most commonly prescribed (Figure 3.3) by user respondents is 20 ml (33.3 %) followed by 30 ml (19%). As the average marc: menstruum ratio was 1: 3 in 25% ethanolic extract the most commonly prescribed weekly dose expressed as crude, dried herb was around 7g and 10 g respectively. The range of doses represents approximately 1.7-35 g crude, dried or fresh herb in 1:3 ethanol: water (w/v) per week, and the median daily dose is thus 3 ml, which is equivalent to 1 g at this marc to menstruum ratio. Tinctures are the preferred mode of administration by the majority (96%) of respondents who prescribe the herb. The main reasons given were that tinctures are generally more convenient than dried herb and

therefore better for patient adherence. There is also a belief that tinctures are more effective than dried herb. Tinctures may be made from either fresh or dried organic and non-organic herb and many respondents (63%) said they prefer to use organic *S. lateriflora* and 42% prefer tinctures made from the fresh herb, believing this to be the most effective.

All *S. lateriflora* users responding to the survey prescribe the herb in combination with other herbs. Only 9% regularly prescribed *S. lateriflora* as a single herb so it is difficult to draw conclusions about the perceived actions of *S. lateriflora* used on its own. Nevertheless the practitioners appeared to be confident in attributing specific anxiolytic and related actions and responses to *S. lateriflora* as distinct from other herbs in a mixture. Reasons are unclear but this perhaps reflects a combination of empirical, anecdotal and scientific knowledge relating to each of the herbs prescribed simultaneously. Furthermore, respondents (9% of users) prescribing it as a single herb reported positive feedback from their patients such as reduced anxiety, fewer and less intense panic attacks, feeling of well-being, feeling more positive, more able to cope. Significantly, a relapse of anxiety symptoms in some patients was noticed by one practitioner prescribing the herb in combination with other (non-anxiolytic) herbs whenever it was removed from the mixture.

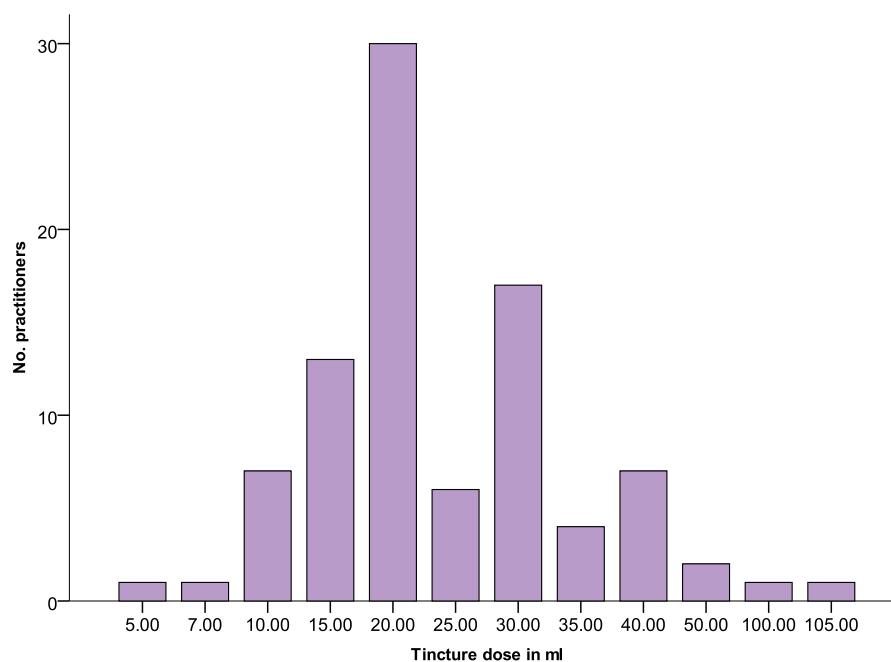


Figure 3.3 Most commonly administered weekly doses of ethanol: water *S. lateriflora* tincture

3.4.8 Contraindications and side-effects

While the majority of respondents (81%) did not consider *S. lateriflora* to be contraindicated for any conditions, the other 19% said they would not prescribe it to severely depressed patients, those with bipolar disorder or other specific mental conditions, to those with epilepsy, thyroid conditions, in pregnancy or to children. It may be possible that herbalists are unlikely to prescribe any herbs for situations where safety is unknown.

The herb was reported as being well tolerated and with only minor and infrequent side effects (reported by 7 prescribers); including mild digestive upsets, worsening of depression, daytime drowsiness, light-headedness and vivid dreaming. It is uncertain whether any of these side effects could definitely be attributed to *S. lateriflora*. There were no reports of toxicity.

3.4.9 Survey response rate

It is not clear why there was such a low response rate to the survey questionnaire (despite two follow-ups, an oral presentation alert at a NIMH conference and an advertisement in a NIMH newsletter). Several reasons for low feedback could be postulated. One is that medical herbalists tend to receive a number of survey response requests during any given year, mainly from undergraduates embarking upon their dissertations, and may therefore be suffering from 'survey fatigue'. It could be that some didn't feel the survey was relevant to their practice if they were non-prescribers of *S. lateriflora*. This is difficult to assess but if this was the case then generalisability across herbal medicine practitioners would have been affected by non-response bias (Cummings *et al.* 2001). Some may have been deterred by the length of the questionnaire and the fact that questions were in the main open-ended, requiring considerable time and effort on the part of already busy practitioners, many of whom are doing more than one job or are studying. Studies of response rates to surveys have demonstrated that the more user-friendly a survey is the more likely it is to have a high response rate (LaGarce & Kuhn 1995). A fair amount of typing was required to fully answer the questions so it could not be considered to be as user-friendly as a quantitative scaling method, for example.

3.5 Conclusions

This study has added to the literature in that it has shown that *S. lateriflora* is prescribed for a range of related conditions and its widespread use for sleep disorders has broadened the indications in the British Herbal Pharmacopoeia. Furthermore, the revelation that herbalists ascribe the action of a compound mixture to the herb has provided empirical evidence of its efficacy.

It is recognised that the response rate (16%) for this pilot survey was low and respondents include only those with access to email. It may therefore not be representative of all UK and Ireland herbal medicine practitioners. An advantage of email surveys is that they are fast and cost-effective and provide geographical diversity but exclusion includes those without access to email. Furthermore, responses may have been mainly from those who have had experience of the herb. Non-responders could possibly be mostly non-prescribers. Importantly, it is possible that those who do not prescribe *S. lateriflora* may not do so because they do not find the herb useful. Although this is speculation it could be a confounding factor. The poor response rate and the propensity of respondents to administer *S. lateriflora* in combination with other herbs make it impossible to rely on evidence regarding the efficacy of the herb from the pilot practitioner survey alone. A future survey could include herbal practitioners from other professional bodies such as the Council of Practitioners of Phytotherapy. In addition, contact with herbalists internationally may provide a more useful indication of the benefits of this herb. Because of the low response rate a future survey could contain fewer open questions and use a world-wide web electronic format so that respondents could have the option to simply click multiple choices with a computer mouse. Furthermore, it would be helpful to urge survey recipients to respond regardless of whether or not they use the herb.

Chapter 4. Botanical identification: macroscopic differences between S. lateriflora and other skullcaps and germander

4.1 Introduction

Botanical descriptions in herbal text books are general and tend to be gathered from secondary sources. As *S. lateriflora* is frequently adulterated with *Teucrium* (germander) species or substituted with other skullcap species (Gorman 2008) an understanding of the macroscopic characteristics of the aerial parts of the plant is necessary to ensure its correct identification. In particular, for the purpose of the present clinical study, it is important to ensure the freeze-dried skullcap sourced from Oregon, USA was correctly identified by the growers/suppliers (Eclectic Institute Inc.). A specification sheet describing macroscopic characteristics relating to each batch of freeze-dried *S. lateriflora* was supplied by the Eclectic Institute on request. In addition to botanical identification, quality control at source was with regard to organoleptics, bacteria, viruses, heavy metals and insects. It was therefore necessary to confirm identification at the University of Westminster using HPLC (Chapter 5).

4.2 Aims

The aim of this study was to gain informed knowledge, using primary sources, regarding the botanical identification of *Scutellaria lateriflora* L.

4.3 Objectives

- To understand the identifying characteristics of *Scutellaria lateriflora*.
- To compare the macroscopic identifying features of *Scutellaria lateriflora* with those of some related (skullcap) species.
- To compare the macroscopic identifying features of *Scutellaria lateriflora* with *Teucrium* species.

4.4 Method

Email contact was made (February 2009) with the curators of both the Natural History Museum (John Clayton) and Linnaean Society herbariums i.e. Dr Mark Spencer and Dr

Charlie Jarvis respectively. Viewing of both private collections was subsequently arranged for the same month. Voucher specimens of skullcap species were viewed, drawings were made and notes taken. Original botanical literature regarding skullcap classification was studied in the herbarium libraries.

Contact was also made with the American Herbal Pharmacopoeia® (AHP), where a *S. lateriflora* voucher specimen is kept (Scott's valley, California). An image of this was received by email from the AHP (Appendix III). An image of a voucher specimen was also supplied by the curators of the John Clayton collection at the Natural History Museum (Figure 4.1.).

In order to recognise the defining characteristics of fresh specimens as opposed to dried or photographic, *S. lateriflora* was grown in ideal conditions of partial shade and frequent irrigation (Similien *et al.* 2008) from seed (Chiltern Seeds) planted in April 2009 and also in April 2011 from propagations derived from division (Arne Herbs Ltd), originally sown in April 2005, repeatedly split and cultivated indoors down to -8 °C in Sinclair Alpine. For purposes of authenticity a trail was made pertaining to the origins of the plants. The Chiltern seeds were originally sourced from Arne Herbs nurseries, whose first *S. lateriflora* plants were originally from Hollington Herb Garden in Berks, UK and expertly identified by botanists and horticulturists Judith and Simon Hopkinson (Lyman-Dixon 2011).

Mature specimens were photographed *in situ* in September 2009 (Figure 4.2) and July 2011 (Figure 4.3) when flowering and the leaves from both years were made into 1: 3 (1 part marc: 3 parts menstruum) ethanol: water (50: 50 v/v) tinctures for possible future HPLC analysis. In addition, a specimen was harvested at the end of June, 2011 when in full flower, and pressed (Figure 4.4). Additionally, a wild-growing *S. galericulata* specimen was located (identified against a wildflowers handbook) on Tresco by the Abbey Pool in the Isles of Scilly in September 2011 and a description was noted.

4.4.2 Voucher specimens viewed:

American Herbal Pharmacopoeia®: Image sent by email PDF attachment (Appendix III) and contained within a full report of botanical identification by the AHP.

Natural History Museum: The following species of *Scutellaria* were published by Linnaeus in *Species Plantarum* (Linnaeus (1753): *S. albida*, *S. alpina*, *S. altissima*, *S.*

cretica, *S. galericulata*, *S. hastifolia*, *S. hyssopifolia*, *S. indica*, *S. integrifolia*, *S. lateriflora*, *S. lupulina*, *S. orientalis*, *S. peregrina* and *S. supina*. The voucher specimen of *S. lateriflora* in the John Clayton herbarium is attributed as being the original, definitive type or lectotype classified by Linnaeus (1753), who stated its habitat as Virginia USA and Canada.

S. lateriflora examples in the John Clayton Herbarium collection:

- *Scutellaria palustris repens*, Virginia major, flora minore – pre 1737 Clayton Herbarium
- *Scutellaria cassida aquatica flora minimo pallide carutro folus, veronica, autumnna floret* – Clayton number 280 ex Virginia (1753): Lectotype of *Scutellaria lateriflora* (Figure 4.1).

Polynomials were given before Linnaeus introduced the binomial system.

Also in the collection are *S. hyssopifolia* and *S. integrifolia*

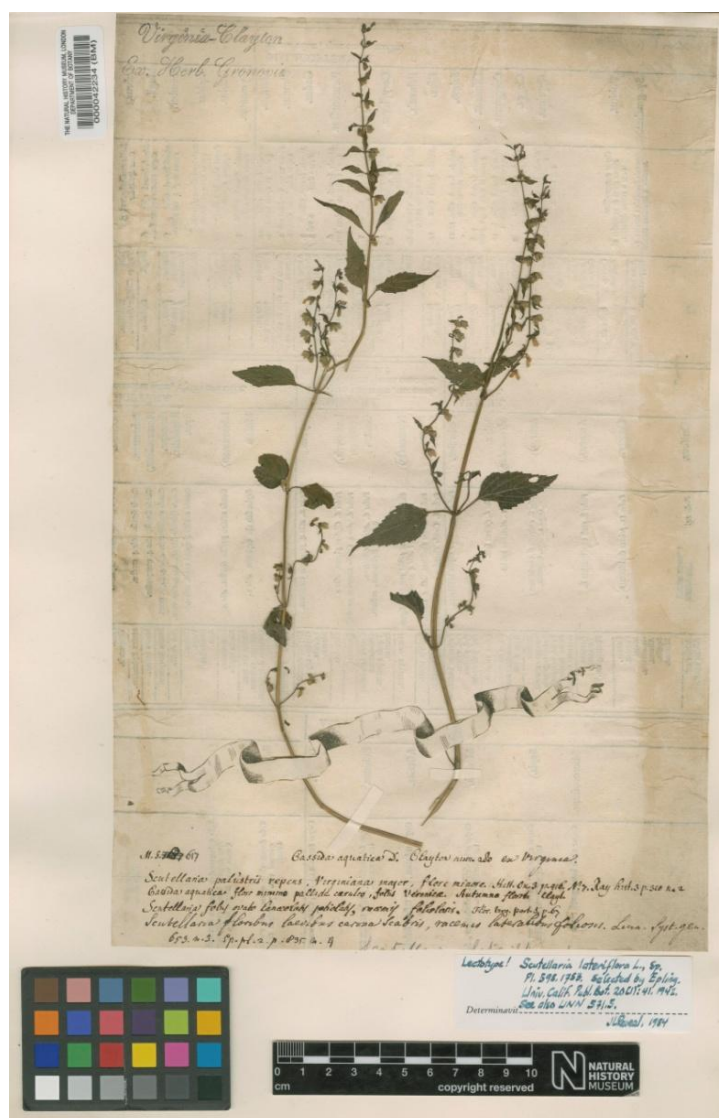


Figure 4.1 John Clayton Herbarium voucher specimen of *S. lateriflora*

© The Natural History Museum, London

Linnaean Society collection: Within this collection is a voucher specimen of *S. lateriflora*, cultivated and collected by Carl Linnaeus (1707-1778) in Uppsala, Sweden and classified by the binomial system, which he invented (genus, species) (Natural History Museum 2011). Also in the collection are *S. albida*, *S. alpina*, *S. cretica*, *S. galericulata*, *S. hastifolia*, *S. hyssopifolia*, *S. integrifolia*, *S. lupulina*, *S. minor*, *S. orientalis* and *S. peregrina*.

A large number of *Teucrium* species are also housed in the Linnaean Society Herbarium. *Scutellaria* specimens in both collections and the *Teucrium* specimens were viewed for comparison with *S. lateriflora*.

4.5 Results

The description and artist's impression of *S. lateriflora* in the Eclectic institute specification sheet correlated to the voucher specimens in the *S. lateriflora* herbariums visited. The fresh herbs grown from seed (Figure 4.2) and plantlet divisions (Figure 4.3 and Figure 4.4) could also be identified as *S. lateriflora*.



Figure 4.2 *S. lateriflora* grown from seed in the UK, September 2009



Figure 4.3 *S. lateriflora* grown from a plantlet division, UK, July 2011



Figure 4.4 *S. lateriflora* harvested in UK, July 2011

A macroscopic comparison of some of the above herbarium species was made from observation (Table 4-1) with additional information from reputable authentication texts. Briefly, the main difference between *S. lateriflora* and other skullcaps is the distinctive way the flower stalks grow from the leaf axillae. The leaves (Figure 4.6 and Figure 4.7) have a distinct venation and crenate dentition with longer petioles (~ 2 cm) than most skullcaps and the flowers are smaller (<10 mm). According to the Eclectic Institute

plant specification sheet used for quality control (Nagel 2008) the size of the corolla at <10 mm is unique amongst skullcaps. The most commonly confused species, *S. galericulata* (Figure 4.8) has almost sessile leaves - the petioles are up to 13 mm in length whereas those of *S. lateriflora* are up to 25 mm long - with sparse dentition. The leaves are smaller (up to 5 cm) and more lanceolate than those of *S. lateriflora*, which has leaves up to 10 cm in length (Upton *et al.* 2009). *S. ovata* (Figure 4.5), another commonly confused species has broad, heart-shaped leaves. The flowers of this species are up to 25 mm in length (Freckmann 2011a) and are therefore much larger than those of *S. lateriflora* (<10 mm). Differences between *Teucrium* (Figure 4.9) and *Scutellaria* species are more pronounced. For example the flowers of *Teucrium* are pink, not purple or blue and the corolla appears to have no upper lip because it is fused with the lower lip (Upton *et al.* 2009).



Figure 4.5 *S. ovata*

©Mark Mittelstadt, Robert Frickmann Herbarium, University of Wisconsin – Stevens Point



Figure 4.6 *S. lateriflora* leaf venation



Figure 4.7 *S. lateriflora* before flowering, July 2011

Robert H. Mohlenbrock @ USDA-NRCS PLANTS Database / USDA NRCS. 1995. *Northeast wetland flora: Field office guide to plant species*. Northeast National Technical Center, Chester.



Figure 4.8 *S. galericulata*

Table 4-1 Main macroscopic characteristics and differences between *S. lateriflora* and 2 common skullcap and 2 *Teucrium* species

Botanical part	<i>S. lateriflora</i>	<i>S. galericulata</i>	<i>S. ovata</i>	<i>Teucrium spp</i>
Stem	Square angled and deeply grooved, branching, erect, glabrous. Turns brown in sun.	Square angled, erect, slightly pubescent.	Square angled, erect, pubescent.	Square angled. Pubescent
Leaf	Opposite. Pinnate-reticulate veins. Glabrous. Crenate, serrated margins with deep dentition. Ovate-lanceolate, round at the base with a pointed apex. Length 5-10 cm, width 3-5 cm. Long petioles ~ 2 cm	Opposite. Pinnate veins. Slightly pubescent, fine, crenate dentition. Oblanceolate. Rounded at the base. Length up to 5 cm, width up to 2 cm. Sub-sessile with petioles ~ 1 cm in length.	Opposite. Pinnate-reticulate veins. Heart-shaped i.e. cordate at the base. Long petioles ~5 cm. Coarsely serrated, crenate margins. Length 3-10 cm and 5-7 cm wide.	<i>T. canadense</i> : Ovate-lanceolate, crenate. Up to 5 cm long and 3 cm wide. Short petioles ~ 1 cm. <i>T. chamaedrys</i> : Oblanceolate. Up to 3 cm long and Leaf margins are scalloped. Waxy cuticle. Very short petioles ~ 0.5 cm (Upton <i>et al.</i> 2009)
Flowers	Bluish-purple. Less than 1 cm in length. 2 lipped with 3 lobes on lower lip. Exterior pubescent	Blue. Up to 2 cm in length. 2 lipped with 3 lobes on lower lip. Exterior slightly pubescent.	Purple with white base. 2 lipped with 3 lobes on lower lip. Length ~ 2.5 cm (Freckmann 2011a)	Pink to pinkish purple. 2 lips. Length ~ 15 mm <i>T. canadense</i> : 2 lobed upper lip, 3 lobed lower lip <i>T. chamaedrys</i> : 5 lobed lower lip. Upper lip not hooded
Fruit	4 nutlets	4 nutlets	1 nutlet (Freckmann 2011a)	1 nutlet (Freckmann 2011b)
Height	Up to 90 cm	Up to 75 cm (Upton <i>et al.</i> 2009)	Up to 70 cm (Freckmann 2011a)	Up to 100 cm (Freckmann 2011b)



Figure 4.9 *Teucrium canadense*

4.6 Discussion

The number of skullcap voucher specimens housed in the two London herbariums visited was miniscule relative to the large number of known species, amounting to only 3.6 % of around 360 worldwide (Malikov & Yuldashev 2002). Although differences between the herbarium species were apparent it is difficult to ascertain whether, of 360 species, there may be some in existence that appear more similar to *S. lateriflora*. It is therefore important for identification to be confirmed by sensitive techniques such as HPLC, random amplified polymorphic DNA (RAPD) markers (Hosokawa *et al.* 2000), estimation of genome size (Cole *et al.*, 2008) and/or or high-powered microscopy (Upton *et al.* 2009).

Misidentification may occur with small scale herb farming and the possibility of those with an untrained eye gathering skullcap herb from the wild. Perhaps there is also a trait amongst herbalists and horticulturalists alike to grow the ‘wrong’ species and pass them on to one another.

Of particular interest is the difference between *S. lateriflora* and *S. galericulata* as *S. galericulata* is a common European species most frequently mistaken for *S. lateriflora*

(Gorman 2008). HPLC analysis can distinguish between the two species by the use of the compound 2'-methoxychrysin, which is found in *S. galericulata* but not *S. lateriflora* (Gafner *et al.* 2003c).

A study, however, which compared flavonoid content of 13 skullcap species, found *S. galericulata* contained a considerably greater quantity (11.31 mg/g) of baicalin than *S. baicalensis* (5.07 mg/g), which is commonly believed to have particularly high baicalin content (Zgorka 2006). The baicalin content of *S. lateriflora* was not determined in this study but its concentration demonstrated in *S. galericulata* compares well with that of the freeze-dried *S. lateriflora* (Eclectic Institute) authenticated by HPLC methods in the present study (see 5.5.4, p82), found to be 11.71 mg/g. Because baicalin is considered to be a physiologically active flavonoid (Joshee *et al.* 2002) it may be useful in the future to conduct clinical efficacy studies of *S. galericulata*.

It is also interesting to note that exacerbation of accidental substitution of *S. lateriflora* in the UK may have begun in the 1930s when, it is believed, a well-known herb farmer misidentified the herb and consequently instigated the introduction of the wrong species, thought to be *S. ovata* (Figure 4.5), into the UK market (Lyman-Dixon 2011).

4.7 Conclusions

S. lateriflora may be correctly identified by the trained botanist. However, for reasons of quality assurance, confirmation of the identity of commercial crops with HPLC is advisable to ensure there is no accidental contamination with *Teucrium* or substitution with other skullcap species.

Chapter 5. Quality control of S. lateriflora

5.1 Introduction

Due to the risk of contamination with potentially toxic botanicals and other hazardous substances, including pesticide residues, bacteria, fungi, toxic metals and radioactive chemicals, the importance of strict quality control of all herbal preparations cannot be underestimated (de Smet 1999).

5.1.1 Identity issues surrounding *S. lateriflora*

Quality control and correct identification may be of particular importance in the case of *S. lateriflora* because of the large number of *Scutellaria* species (around 360 species worldwide) (Malikov & Yuldashev 2002) and frequent substitution or adulteration with other skullcaps or potentially harmful herbs such as germander (*Teucrium*) (Wolfson & Hoffmann 2003; Gorman 2008). Studies have shown wide variations in phytochemical profile of herb claimed to be *S. lateriflora*.

Zhang *et al.* (2009), for example, discovered a wide variation in individual and total phenolic content in 10 different commercial preparations of *S. lateriflora* (analysed by HPLC and compared with *S. baicalensis* and whole, freeze-dried extract). Baicalin content, for example, varied from 0.48% to 10.10% (compared to 18.95% for whole extract). Total phenolic content for these two preparations was 1.11% (extract – type not described) and 20.55% (fine powder in a capsule) respectively (41% for whole extract) (Zhang *et al.* 2009).

Another HPLC analysis (Gao *et al.* 2008), of seven commercial preparations (obtained from five companies), which compared baicalin, baicalein and wogonin content of *S. lateriflora* (four samples of *S. baicalensis* were also analysed), also found wide variations in individual and total content of these flavonoids. One sample contained no baicalin, baicalein or wogonin. Of all three flavonoids, µg/ml ranged from 180, 280 and 97.5 to 12700, 629 and 152 respectively (Gao *et al.* 2008).

Considering reports (Wills & Stuart 2004; Gorman 2008) of a high rate of substitution of *S. lateriflora* with other skullcap species, it is not certain whether the commercial

products tested by Gao *et al.* (2008) and Zhang *et al.* (2009) were definitely *S. lateriflora*.

5.1.2 Authentication problems in the scientific literature

Issues surrounding authenticity of *S. lateriflora* in the market have probably been a problem for hundreds of years. Wohlmuth (2001) pointed out that King's American Dispensatory, published in 1898, describes *S. versicolor* and *S. canescens* as being two substitute species that constituted the majority of the commercial drug. Problems of substitution may have been exacerbated by authentication problems in the scientific literature, some relating to analyses of the chemical composition of *S. lateriflora*. For example, according to Yaghmai (1988) *S. lateriflora* grows on riverbanks and marshes in northern Iran. However, the species known to grow in this region is *S. pinnatifida* (Barceloux 2008). When Wohlmuth (2011) compared the composition of essential oil from morphologically and LC-MS authenticated *S. lateriflora* specimens grown in North America and Australia with that reported from the Iranian samples the results differed regarding which were the dominant oils (Wohlmuth *et al.* 2011). (See 2.6, p21)

Another study (Zhang *et al.* 2009), in which the chemical ingredients of *S. lateriflora* were characterised, used 10 samples of plant material purchased from herbal product stores and companies. There is therefore no guarantee that any of this material, including the whole extract against which other samples were compared, was authentic *S. lateriflora*. The authors even used one of the samples to test for anti-convulsant activity in a rat seizure model (Zhang *et al.* 2009) without first verifying the material against a voucher specimen. In agreement with Rader and Delmonte (2007), the results of a study may be invalid if the botanical material tested has not been authenticated as it may have been misidentified or be adulterated with other species.

Accidental substitution of *S. lateriflora* in the market may occur and perpetuate when the wrong species is cultivated by commercial growers. For example, it is a belief among some growers of authentic *S. lateriflora*, that one UK herb farm had been flooding the market with a considerable amount of misidentified skullcap species, possibly *S. ovata*, since at least the 1930s (Lyman-Dixon 2011). It is not known how widely spread is the use of this particular species amongst herbalists today.

Whilst researching background information for this thesis it became apparent to this author that authors of both popular and academic herbal text books tend to cite from one

another when providing information on the chemical constituents of *S. lateriflora*, as do some authors when publishing research on the herb. This no doubt adds to the identity issues surrounding the herb.

5.2 Adulteration and substitution of *S. lateriflora*

5.2.1 Reports of adulteration of *S. lateriflora* with *Teucrium* species

In the 1970s and 1980s there were reports of liver damage, including hepatitis and fibrosis, from use of *S. lateriflora* (McCaleb 2004). The cause of hepatotoxicity was subsequently attributed to American Germander (*Teucrium canadense*), which is similar in appearance, being used in some European commercial preparations in place of *S. lateriflora* (de Smet 1999). *T. canadense* is a possible substituted species but it is now considered non hepatotoxic. A European *Teucrium* species, *T. chamaedrys*, which contains hepatotoxic furan neoclerodane diterpenoids such as teucrin, may have been substituted for *S. lateriflora* (Bedir *et al.* 2003; McCaleb 2004; Lin *et al.* 2009). *T. chamaedrys* was formerly thought to contain hepatotoxic pyrrolizidine alkaloids (McCaleb 2004). According to researchers at Southern Cross University in Australia, deliberate adulteration of *S. lateriflora* with *Teucrium* species is mainly because it has a heavier dry weight than *S. lateriflora* and non-deliberate adulteration may occur through accidental harvesting when the different genera grow side by side (Gorman 2008).

Gafner *et al.* (2003a) developed a method of detecting adulteration with *Teucrium* species. This involves photomicroscopy in combination with liquid and thin layer chromatographic methods and UV/MS (ultraviolet/mass spectrometry), with the use of phenolic marker compounds e.g. teucrioside and verbascoside, found in germander species but not in skullcap. The method has been further developed by Lin *et al.* (2009), who showed that chromatographic profiling can not only distinguish the two genera separately but also when they are mixed together (Lin *et al.* 2009). A rapid and simple method of determining contamination with *Teucrium* species, flow injection/mass spectrometry (FI/MS) fingerprinting, has been developed recently (Sun & Chen 2011).

5.2.2 Substitution with other skullcap species

S. lateriflora crop damage may occur due to its susceptibility to a number of diseases such as leaf spot, stem rot and powdery mildew (Greenfield & Davis 2004); also, adult

species of the leaf beetle (*Phyllobrotica* genus) are known to attack the aerial parts and their larvae attack the roots (Farrell & Mitter 1990). When such damage leads to crop failure, *S. lateriflora* is sometimes substituted with other *Scutellaria* species, particularly *S. galericulata*, *S. cordifolia* [syn. *S. ovata* (U.S.D.A. 2011)] and *S. canescens*. Substitution may also occur through misidentification (Wolfson & Hoffmann 2003; Gorman 2008). Although *S. galericulata* is rarely used consciously in herbal medicine it is believed to have similar actions and indications as *S. lateriflora* (Plants for a Future 2010b).

More than one species within a genus may possess exactly the same or similar flavonoids. For example, in common with many other *Scutellaria* species, *S. lateriflora* and *S. baicalensis* have similar phytochemical constituents, although in different ratios and quantities, which may explain the differing traditional uses amongst *Scutellaria* species (Cole *et al.* 2008). HPLC methods can quickly and easily distinguish between the species by qualitative analysis of the flavonoids contained in extracts of the plants (de Oliveira *et al.* 2001). This is preferable to TLC as the latter lacks sensitivity and has poor resolution (Sun & Chen 2011). Additionally, HPLC analysis can distinguish between *S. galericulata* and *S. lateriflora*, by the use of the compound 2'-methoxychrysin, which is found in *S. galericulata* but not *S. lateriflora* (Gafner *et al.* 2003c).

Using HPLC, Cole *et al.* (2008) quantitatively compared the content of scutellarin, wogonin, baicalin, baicalein, melatonin, serotonin and indole-acetic acid in stem and leaf tissue of *S. lateriflora*, *S. baicalensis* and *S. racemosa* grown in identical conditions. *S. lateriflora* contained the highest amount of baicalein while *S. baicalensis* contained 800 times more scutellarin than the other two herbs. Of the three herbs *S. racemosa* had the highest wogonin content. Baicalin content was similar for the three herbs (Cole *et al.* 2008), which differs from the findings of Makino *et al.* (2008) who compared baicalin, baicalein, wogonin and wogonin-7-*O*-glucoronide content in roots, stems and leaves of *S. baicalensis* and *S. lateriflora*. In this study, baicalin content in the leaves and stems of *S. baicalensis* was insignificant in comparison to *S. lateriflora*. There was slightly more baicalin in *S. baicalensis* root than in *S. lateriflora* leaves (Makino *et al.* 2008). It is possible that differences between studies in flavonoid concentrations may have been due to differences in growing conditions. Interestingly, of five skullcap species analysed (*S. baicalensis*, *S. lateriflora*, *S. racemosa*, *S.*

tormentosa and *S. wrightii*), Islam *et al.* (2011) found a greater baicalin content in the roots of *S. wrightii* than in root, stem or leaf of the other species and 5-fold higher than in *S. baicalensis* root. They also demonstrated that *S. wrightii* and *S. tormentosa*, previously uncharacterised species, are good sources of the flavonoids scutellarin, baicalin, baicalein and wogonin. As these flavonoids are also found in *S. lateriflora* there are implications that these species also have medicinal properties.

When German plant extraction company Extract Chemie tested 12 samples of material from different sources using HPLC in 2008, none were *S. lateriflora* but were in fact other *Scutellaria* species. This was apparently a surprise to the producers of these plant materials. The company has now produced an HPLC ‘fingerprint’ of *S. lateriflora* from expertly identified raw plant material (Gorman 2008).

In another HPLC study (Wills & Stuart 2004) a liquid commercial product, which was also claimed to be *S. lateriflora*, had the chromatographic profile of *Scutellaria incana*. Of five combination commercial preparations claimed to contain *S. lateriflora*, when tested, none had the typical profile of the herb and may therefore either have contained different *Scutellaria* species or may have lost a considerable amount of flavonoids in processing (Wills & Stuart 2004).

Gafner *et al.* (2003c) have developed a flavonoid fingerprinting technique, using HPLC-UV/MS, to distinguish between five *Scutellaria* species. It is also possible to identify species within a genus using modern genetic techniques. For example, Hosokawa *et al.* (2000) used random amplified polymorphic (RAPD) DNA markers of three species of *Scutellaria*, including *S. lateriflora* to differentiate between DNA isolated from their leaves; and Cole *et al.* (2008) distinguished between three *Scutellaria* species by estimating genome size using flow cytometry.

5.3 Variation of phytochemical constituents dependent on methods of growing, harvesting, storage, extraction and processing

Commercial herbal products have been found to contain significant variations in phytochemical profile within a species. Such variation may be according to geographic region, biodiversity, ecological variations, cultivation, seasonality, harvesting, and storage time affecting stability; processing method, marc to menstruum ratio and alcohol concentration (Ciddi 2006; Gao *et al.* 2008).

5.3.1 Effects of growing and harvesting conditions

According to Greenfield and Davis (2004) *S. lateriflora* grows best in fertile soil and with constant irrigation. They suggest sowing the seeds indoors in shallow flats containing moist soil, placed in a refrigerator for a week at 4-10°C and then germinating them in a greenhouse before planting outside. Seeds can also be planted directly outside in the spring. Other methods of cultivation include root division and transplanting. When harvested, cut plants should be kept out of the sun or they will quickly turn brown (Greenfield & Davis 2004). As cellular disruption may cause enzymatic breakdown of flavonoids, mechanical stress during harvesting should also be avoided to prevent their loss (Wills & Stuart 2004).

In a controlled experiment, Similien *et al.* (2008) demonstrated the importance of growing conditions on dry matter yield (DMY) and flavonoid content of *S. lateriflora*. Seeds planted in April were harvested when in full bloom at two and four months. The highest concentrations of the major flavonoid baicalin (1.8 mg/g) and its aglycone baicalein (0.29 mg/g) (Similien 2009) were at first harvest of fertilised, irrigated plants grown in full sun (32% and 50% higher respectively than at harvest 2). Partial shade (40%) did not affect DMY at first harvest but increased it by 63.4% at second harvest (in comparison to *S. lateriflora* grown in full sun). Irrigation and nutrients increased DMY by 23.7% and 45.7% respectively at first harvest but only nutrients increased DMY at second harvest, by 10.4%. Overall, the concentration of flavonoids was greater in full sun but the flavonoid yield was greater in partial shade because of a higher DMY. There was a very low concentration of wogonin (< 0.6 mg/g) and chrysin (< 0.5 mg/g) at all experimental conditions (Similien *et al.* 2008).

The findings of Similien *et al.* (2008), that maximum flavonoid concentration is in immature plants, agree with those of Wills and Stuart (2004) who assayed flavonoid content at four growth stages. They found, when averaged over the different stages of plant growth, highest flavonoid concentration was in the leaves (50.8 mg/g) but particularly in immature leaves (69.3 mg/g). The stems contained a lower flavonoid concentration (21.3 mg/g) than the roots (35.8 mg/g). Due to an increase in leaf weight of mature plants, the third harvest, which is the usual 'commercial harvest' before fruiting, yielded the highest total *S. lateriflora* flavonoids per weight of whole plants. At full maturity as a percentage of total flavonoids the highest baicalin content is in the

leaves (54.5%), followed by the stems (45.2%) and roots (38.1%). Of total flavonoid content of *S. lateriflora*, baicalin constitutes 40-50% (14-18 mg/g, calculated as 40-50% of a mean concentration of 36mg/g total flavonoids from whole plant at 4 different stages of growth). (Wills & Stuart 2004)

It may be deduced that, although flavonoid concentration is higher in the young plants grown in full sun, as *S. lateriflora*'s DMY is increased over time when grown in partial shade the total flavonoid yield in one harvest may be higher in more mature plants. According to Foster and Johnson (2006) *S. lateriflora* plants are usually harvested when they are three or four years old.

Differences in individual flavonoid concentrations between the leaves, stems and roots result in differences in their chromatographic profiles, which can be used to assist in identification of the plant part used for medicinal purposes. Additionally, adulteration of the plant material will alter the characteristic profiles (Wills & Stuart 2004).

5.3.2 Extraction methods affecting flavonoid concentration

One commercial *S. lateriflora* tincture tested by Gao *et al.* (2008), which had the highest flavonoid content (12.66 mg/ml), was extracted in 45% ethanol, whereas the others were extracted in 25% ethanol. When the investigators later compared extraction in 25% and 45% ethanol from the same batch of *S. lateriflora* plant material they found that 45% ethanol yielded around five times more flavonoids than 25% ethanol (Gao *et al.* 2008). Similarly, Wills and Stuart (2004) found 40 - 60% ethanol extractions from dried powder preparations yielded the maximum flavonoid content of around 70%.

An earlier study (Awad *et al.* 2003) using HPLC analysis also found higher alcohol concentrations extracted *S. lateriflora* flavonoids well. Baicalin content was greater at 50% than at 95% ethanol extraction (40.7 mg/g and 21.3 mg/g respectively) whereas 95% ethanol was better at extracting baicalein than was 50% ethanol (32.7 mg/g and 23.5 mg/g respectively). The analysis also indicated that an aqueous extract contained no baicalein and minimal baicalin (Awad *et al.* 2003).

5.3.3 Variation of Phytochemical constituents on storage

Stability of an herbal product is important with regard to efficacy and safety and may be affected by various factors, such as pH, light, enzymatic degradation (for example due to harvesting stress, heat or insects) and temperature (Gafner & Bergeron 2005).

Flavonoids in glycerite (65% glycerine: 35% water) extracts from dried *S. lateriflora* (1:12 w/v) have demonstrated extreme instability over time with 50.03% total flavonoid loss at 6 weeks total maceration time and up to 66.4% loss after 6 months from initiation of maceration. While heat treatment destabilises the enzymes responsible for degradation of some flavonoids, for example hydrolysis of baicalin to baicalein by endogenous beta- glucuronidase, not all *S. lateriflora* flavonoid degradation is prevented in this way. Some, such as degradation of dihydrobaicalin, can only be prevented by addition of antioxidants, indicating oxidation is also involved in flavonoid instability (Russell *et al.* 2003). Wills and Stuart (2004) found total flavonoid loss from dried herb extracted in 40 - 60% EtOH to be considerable, at 0.17% per day at room temperature (this would amount to over 30% loss of flavonoids in 6 months) and about 50% greater than with dried herb under the same storage conditions (Wills & Stuart 2004).

Although dry heat is believed to negatively affect the activity of herbal medicines, little research has been done to this affect. However, the flavonoid profile of freeze-dried extracts of *S. lateriflora* was not affected by exposure to dry heat for 8 hours at 77°C (Gafner & Bergeron 2005).

There appears to be no data available on flavonoid loss from fresh *S. lateriflora* extracted in EtOH although Russell *et al.* (2003) assert that fresh are more unstable than dried extractions. Future studies on this aspect are important as fresh material is believed to be most efficacious (Felter & Lloyd 1898; Kuhn & Winston 2001; Yarnell & Abascal 2001).

5.4 Discussion and conclusions

Strict quality control must be ensured before the commencement of any *in vitro* or clinical studies. This should include botanical identification to rule out adulteration with different *Scutellaria* species or with *Teucrium* spp. as has occurred in the past. A problem with plants used for medicinal purposes is those that are harvested from their

natural habitats (wildcrafted) are not uniform in the balance of active phytochemicals. Successful modern herb farming needs to pay attention to this possibility and be selective with breeding programmes, particularly with regard to phytochemistry (Lambert *et al.* 1997).

Storage, harvesting, extraction and processing methods, and marc to menstruum strength should be at their optimum for avoidance of flavonoid loss. Many companies extract using 25% ethanol in water. However, it is clear from HPLC studies (Awad *et al.* 2003; Wills & Stuart 2004; Gao *et al.* 2008) that *S. lateriflora* flavonoid extraction and stability is poor at this strength. Gao *et al.* (2008) suggest that more research into the anxiolytic properties of *S. lateriflora* is needed in order to convince manufacturing companies to extract the herb at higher alcohol strength.

To provide valid data it is important that prior to carrying out a clinical trial on *Scutellaria lateriflora*, the plant material should be selected from an identified source with a full botanical authentication and chemical analysis to confirm quality. The plant material should not be subject to deterioration during the trial period. Empirical evidence and research have indicated that fresh freeze-dried or fresh herb extract is likely to be more efficacious than the dried herb (Kuhn & Winston 2001; Yarnell & Abascal 2001; Gafner & Bergeron 2005), due to instability of the latter and hence fewer flavonoids (Wills & Stuart 2004).

Freeze dried material is not widely available as it needs to be processed at harvest by immediately freezing freshly harvested plants, which have been washed in spring water, at low temperature e.g. -18°C. They then undergo sublimation, a process whereby the frozen water is vaporised under vacuum. The vaporised water is passed through condenser plates, a process which converts the water vapour back to a solid and removes it from the vacuum chamber. Only the dried plant material remains and the separation process is complete. Any remaining moisture is removed (around 5%) by gentle heat. Active constituents remain, including flavonoids, enzymes, oils and fatty acids. The colour, smell and taste of the plant material are not affected and it has a long shelf life (Eclectic Institute Inc. 2003; Luthria 2006)

5.5 An optimised HPLC method for confirming the identity, quality and safety of *Scutellaria lateriflora* aerial parts.

5.5.1 Introduction

S. lateriflora is believed to be safe to use but samples must be authenticated (Upton *et al.* 2009). Evidence suggests that *S. lateriflora*'s flavonoids provide it with its therapeutic actions (Gorman 2008). Therefore, the concentration of flavonoids in any commercial preparation of the herb is important in defining its quality and efficacy. Flavonoids in extracts from dried *S. lateriflora* have demonstrated extreme instability over time (Wills & Stuart 2004).

As *S. lateriflora*'s flavonoids are unstable in both dried herb and extracts and because of the risk of its adulteration, some bodies of work have been devoted to developing reliable HPLC methods for verifying its purity and quality. A characteristic profile of the HPLC chromatogram or 'fingerprint', which is altered by adulteration, can be used for accurate identification of the herb. The pattern's relative percentage of flavonoids is the key point to ascertain the quality and identity of *S. lateriflora* (Wills & Stuart 2004).

The purpose of conducting this present HPLC analysis was to ensure the quality, identity and safety of freeze-dried whole aerial parts of *S. lateriflora* to be used in this study to test its efficacy in healthy volunteers. A freeze-dried preparation was selected as providing the optimum preservation of the whole plant phytochemistry (Gafner & Bergeron 2005; Luthria 2006).

5.5.2 Aims and Objectives

The main aims of this HPLC analysis were to confirm the botanical identity of the commercial product and to verify its quality and safety. The specific objectives were to:

- Develop a characteristic chromatogram or 'fingerprint' from authenticated *S. lateriflora*;
- Compare the chromatographic profile of the commercial product with the fingerprint of authenticated herb;
- Qualify and quantify major flavonoids (baicalin, baicalein and wogonin: Figure 2.3, p22) found in the commercial product;
- Verify the absence of germander or other adulterants.

The following protocol is modified from Wills and Stuart (2004), who developed a reliable HPLC method for the purpose of aiding the production of high quality *S. lateriflora* to be grown commercially in Australia.

5.5.3 Materials and Methods

5.5.3.1 Plant materials

Dried, expertly authenticated *S. lateriflora* reference material was donated by the American Herbal Pharmacopoeia® (AHP) and freeze-dried herb was purchased from the Eclectic Institute Inc., Sandy, Oregon. The herb used in the commercial product had been botanically identified at source and was grown on the supplier's own farm in Oregon, USA (Nagel 2008). The reference *S. lateriflora* was grown in Colorado, USA. A voucher specimen for the reference sample is deposited at the herbarium of the AHP.

5.5.3.2 Materials

HPLC grade baicalin, wogonin and verbascoside were purchased from Extrasynthese, Genay, France. Baicalein (98%) and HPLC grade methanol and phosphoric acid were purchased from Sigma-Aldrich, Dorset, UK. HPLC water was obtained from a deioniser.

5.5.3.3 General

Aerial parts of *Scutellaria lateriflora* reference material, ground to a fine powder, and a freeze-dried, powdered commercial sample were extracted with methanol: water and their UV spectra were compared. Qualitative and quantitative analyses of flavonoids were based on retention times (RTs) and peak areas (PAs) respectively of flavonoid biomarkers; baicalin, baicalein and wogonin. RT of verbascoside was also established.

5.5.3.4 Extraction of *S. lateriflora*

Using a coffee grinder (Braun™) the dried herb (AHP) was ground to a fine powder. The powdered plant material was extracted with methanol: water (80: 20 v/v) at a solvent/solute ratio of 100: 1 (1g plant material in 100 ml methanol/water). Maceration was augmented by placing in a sonicator twice for 15 minutes. The extract was filtered through Whatman™ filter paper. The residue on the filter paper was then washed 3 times through the filter paper with 80% methanol until the extract reached 100 ml. The extract was then filtered through a Spartan® membrane filter (Sigma-Aldrich), pore size

0.45µ and then centrifuged for 60 minutes at 6000 r.p.m. The procedure was repeated with the commercial product (Eclectic Institute) with the omission of grinding to a fine powder.

5.5.3.5 HPLC analysis

Samples were analysed using a Dionex A550 BioLC HPLC apparatus. Mobile phase = linear gradient of 30% - 90% methanol/water (v/v). A continuous gradient elution was used. Methanol was acidified with 0.007M phosphoric acid, final pH 3.2; water was acidified with 1% 0.001M phosphoric acid, final pH 4.5. Injection volume = 20 µl. Stationary phase = silica C18 column (Polaris® 5 µ C18-A, 250 x 4.6 mm, Varian Ltd) fitted with a pre-column and a 2 µ pre-column filter (Metasaver, Varian/Agilent Technologies). Flow rate = 1 ml/minute. Optimum flavonoid peak detection was at wavelength = 280 nm. Total run time = 30 minutes. The column temperature was set at 25°C. Reference and test samples were prepared fresh daily and injected in triplicate.

5.5.3.6 HPLC analysis of marker compounds and verbascoside

Retention times (RTs) and calibration curves of the flavonoid reference standards were developed in order to determine their presence and concentration in the known (identified) *S. lateriflora* samples and for comparison with the freeze-dried sample (Eclectic Institute Inc.). Retention time of verbascoside was also determined to ensure there was no adulteration of the commercial product with germander species.

From stock solutions of 100 µg/ ml concentration, serial dilutions of (5, 10, 20, 50, 60, 80, 100) µg/ml were made of the marker flavonoids and (40, 60, 100) µg/ml for verbascoside. Each sample was injected into the column three times and an average peak area (PA) was taken. The average retention time was noted for each reference flavonoid and for verbascoside in order to identify the major relevant peaks of *S. lateriflora* and to determine potential adulteration with germander respectively. The calibration curves also enabled dry weight (mg/g) calculation of the concentration of major flavonoids for each of the identified *S. lateriflora* samples analysed by HPLC.

5.5.3.7 HPLC analysis of plant materials

The supernatant of the MeOH/H₂O extracted *S. lateriflora* reference material (AHP) was used for obtaining a ‘fingerprint’ of the herb for comparison with the commercial

product. Quantification of flavonoids was based on peak areas of the flavonoid glycoside baicalin and the aglycones baicalein and wogonin, all considered to be important flavonoid biomarkers in *S. lateriflora* (Gao *et al.* 2008), which were used as working flavonoid reference standards.

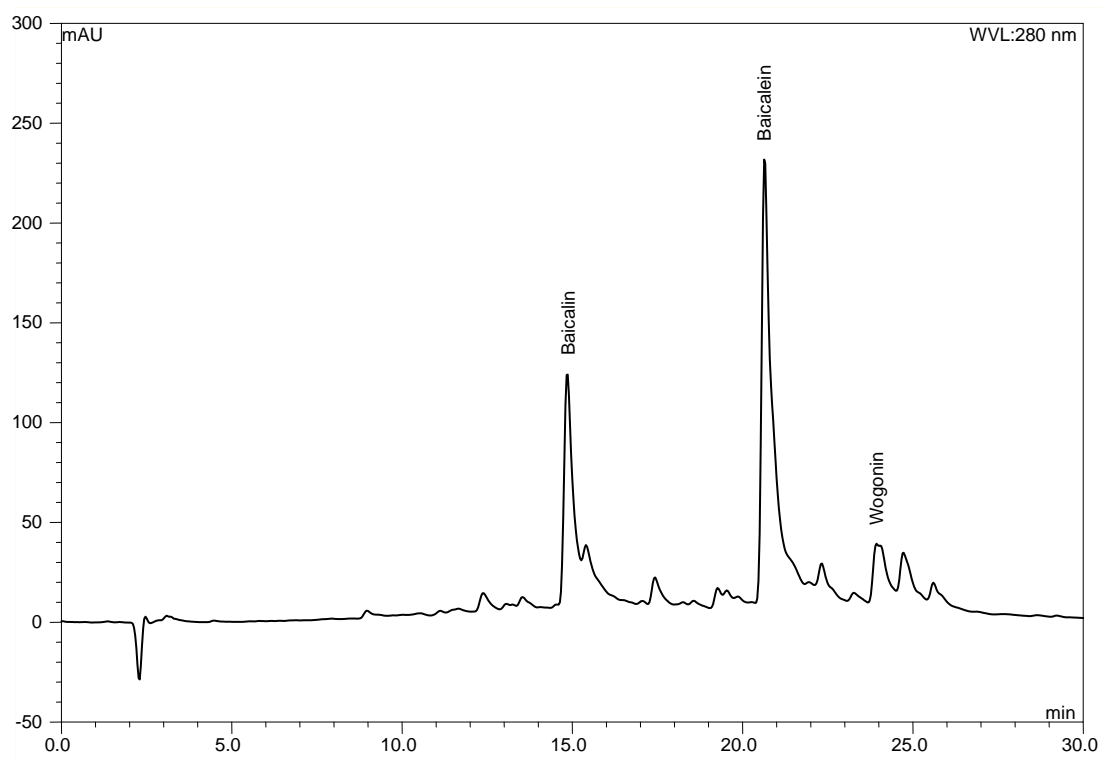
5.5.4 Results

HPLC analysis of the commercial sample showed reproducible RTs of baicalin (RT= 14.8 min; mean \pm SD = 11.71 ± 1.16 mg/g); baicalein (RT= 20.4 min; 7.67 ± 0.89 mg/g); wogonin (RT= 23.7 min; 0.18 ± 0.01 mg/g). The commercial sample appeared to be free from adulteration with germander (verbascoside was not detected; RT= 9.1 minutes) and its phytochemical profile was consistent with that of the *S. lateriflora* reference standard Figure 5.1 (A & B).

5.5.5 Discussion

The concentration of baicalin in ethanol extracts of *S. lateriflora* aerial parts compares favorably with the results of other workers. Awad *et al.* (2003) found 21.3 mg/g and 40.7 mg/g in 95% and 50% ethanol respectively in powdered, dried *S. lateriflora* extract; Wills and Stuart (2004) 14-18 mg/g (calculated as 40-50% of a mean concentration of 36 mg/g total flavonoids from whole *S. lateriflora* at 4 different stages of growth), extracted in 80% MeOH; Wohlmuth *et al.* (2009) found a mean of 1.8 ± 5.5 mg/g (Wohlmuth *et al.* 2009) in a total of 27 authenticated samples extracted in 70% ethanol (Wohlmuth 2011) including genuine commercial *S. lateriflora* and herbarium specimens (Wohlmuth *et al.* 2009); Parajuli *et al.* (2009) found only .0098 mg/g while Gao *et al.* (2008) reported 12.6 mg/ml tincture (drug to ethanol ratio of the original commercial tincture was not stated) as the highest amount of baicalin in one of seven different products analysed. As the samples in this latter study were not linked to a voucher specimen for authentication, however, its identity as *S. lateriflora* was not verified.

A.



B.

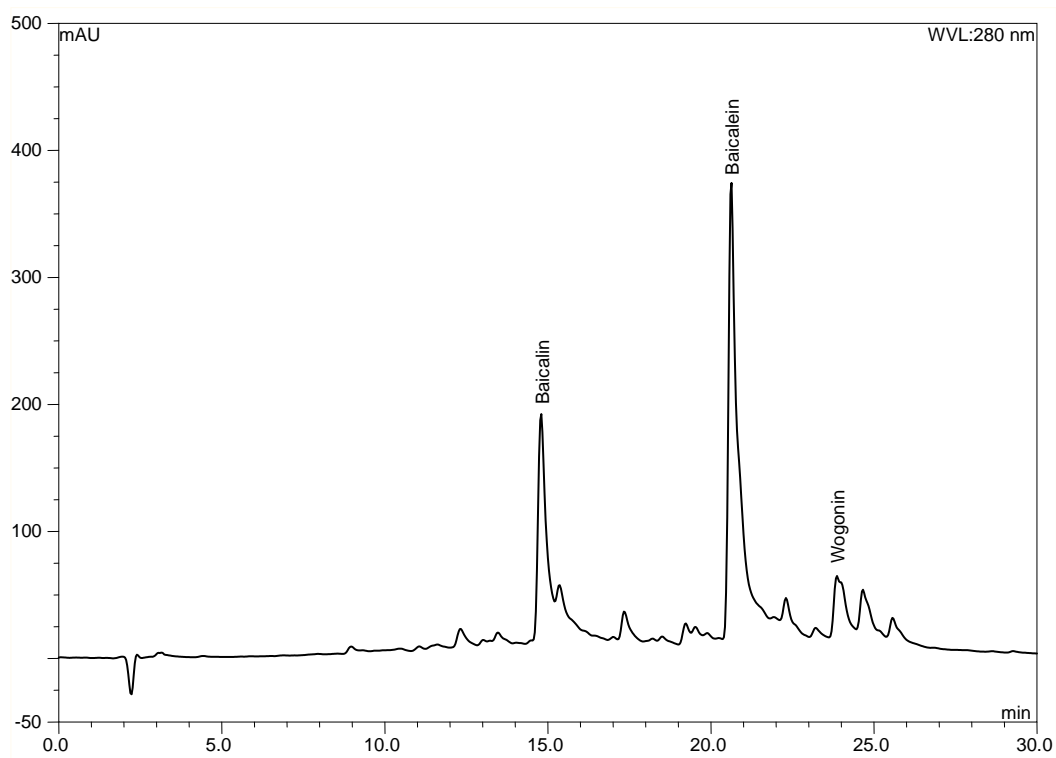


Figure 5.1 HPLC chromatograms demonstrating consistency between the flavonoid profiles of a commercial product (A) and reference material (B)

5.5.6 Conclusions

S. lateriflora has been plagued by problems of substitution and adulteration for many years and must therefore be rigorously authenticated morphologically (macroscopic and microscopic) against a voucher specimen and/or by HPLC or other advanced laboratory methods (such as GC-MS, TLC or DNA analysis) before entry onto the market. Furthermore, it is important when carrying out a phytochemical analysis study that samples are compared with authenticated reference material.

The above study represents a simple and effective method for assessing the authenticity of a sample of the herb and demonstrates the importance, when carrying out a phytochemical analysis study, of comparing samples with authenticated reference material. The identity and quality of a commercial product of *S. lateriflora* to be used in a clinical study have been verified through the matched patterns of investigated flavonoid biomarkers in the reference material and freeze-dried sample. Additionally, HPLC chromatograms enabled the quantification of flavonoids (baicalin; baicalein and wogonin) in the Eclectic Institute product, which proved to be free from adulterants (germander) and/or other skullcap species. The results justify the potential use of the freeze-dried product in future clinical efficacy studies.

Chapter 6. American skullcap (Scutellaria lateriflora): a randomised, placebo-controlled crossover study of its effects on mood in healthy volunteers: aims, objectives, materials and methods

6.1 Introduction

Findings from a review of the available literature on *S. lateriflora*, as outlined in Chapter 2 (see 2.5, p19) indicate this herb has been used extensively in traditional medicine systems for anxiety, stress and related disorders for hundreds of years. Furthermore, results of a survey of herbal medicine practitioners on their use of the herb (Brock *et al.* 2010) suggest it is one of the most widely prescribed herbs in western materia medica for these conditions (see 3.4.2, p52). To date, only one clinical study (see 2.12.2, p37) has been conducted for the purpose of determining the psychopharmacological effects of *S. lateriflora* (Wolfson & Hoffmann 2003). The study indicated a short-term anxiolytic effect of the herb with minimal reduction in energy or cognition. In addition, chemical studies revealing CNS-active amino acid content of *S. lateriflora*, particularly glutamine (Bergeron *et al.* 2005) (see 2.12.1.1, p31) and melatonin (Murch *et al.* 1997) (see 2.12.1.4, p36) and *in vitro* studies demonstrating benzodiazepine binding properties (Liao *et al.* 1998; Hui *et al.* 2000) (see 2.12.1.1, p31) and 5HT-7 binding properties (Gafner *et al.* 2003b) (see 2.12.1.3, p34) have also indicated it has anxiolytic and mood enhancing effects.

The purpose of the following randomised, double-blind, placebo-controlled crossover study was to extend the findings of Wolfson and Hoffmann (2003) (2.12.2) by conducting a study of longer duration and to increase the parameters of their study by determining its effect on mood factors and stress in addition to anxiety.

6.2. Ethics approval and trial registration

The clinical study was approved by the University of Westminster Research Ethics Sub-Committee (ref: 08/09/21) and registered with the International Standard Randomised Controlled Trial Number Register (ISRCTN48078312) and entered onto the UK Current Controlled Trials database. Ethical clearance is essential to protect the rights, confidentiality and welfare of study participants (Eckstein 2003).

6.3. Aims of the research

The aim of the research outlined in this chapter was to determine, using a crossover design, the effects of *S. lateriflora* on anxiety, stress and other negative, as well as positive, mood states. With verified self-administered scales of subjective mood and anxiety, and salivary cortisol measures of stress, the study assessed and compared any changes within and between subjects from baseline in subjective mood, anxiety and salivary cortisol levels following administration of skullcap test and placebo control.

6.4. Objectives of the research

- To deliver an evidence-based herbal intervention with extracts of American skullcap (*S. lateriflora*) for promotion of wellbeing in a series of tests, using a randomised, double blind, placebo-controlled, crossover design with subjective measures of mood and anxiety.
- To measure changes in levels of cortisol in saliva samples as a physiological measure of stress, following administration of *S. lateriflora* extract to a test group, alongside a placebo control group.
- To assess the safety profile of *S. lateriflora* by comparing liver function tests at baseline and following administration of the test herb and placebo.

6.5. Study hypotheses

- *S. lateriflora* will have a superior mood enhancing effect than placebo (when compared with baseline measures).
- *S. lateriflora* will have a superior anxiolytic effect than placebo (when compared with baseline measures).
- *S. lateriflora* will attenuate negative mood states without a marked diminution of cognition or energy.
- *S. lateriflora* will alter cortisol profiles in stressed but otherwise healthy volunteers.
- *S. lateriflora* will have no toxic effects on the liver.

6.6 Study design (Figure 6.1)

The study was based on a simple 2 x 2 crossover design with baseline measurement in which each participant was randomised to a sequence of two treatments over two periods with a washout period in between treatments. This is known as an AB/BA design. Participants act as their own controls and there is less variability within subjects

than between subjects and fewer participants are required for the study than if it were a parallel study (Dallal 2000). (See 8.9, p189 for disadvantages of crossover studies). An equal number of participants (a group) for each time period receives one treatment (either placebo or test) while the other group receives the other treatment. In this research all in Group 1 (n = 22) received placebo in the first period and skullcap in the second period, while all in Group 2 (n = 21) received skullcap first followed by placebo.

Following psychometric testing and health screening, eligible participants (see 6.8.1.2 & 6.8.1.3, pp90-91) were randomly assigned to receive either freeze-dried *Scutellaria lateriflora* (Eclectic Institute) test, or freeze-dried *Urtica dioica folia* (Eclectic Institute) placebo, three times daily for 14 days. In this research it was judged from empirical evidence of the effects of *S. lateriflora*, both onset and duration (see Chapter 3: practitioner survey 3.4.3 & 3.4.6) and evidence from a previous clinical study (Wolfson & Hoffmann 2003) that periods of 2 weeks administration would be sufficient for determination of treatment effects. They then had a washout period of 7 days. As a precautionary measure and without data from any previous study to inform the length of washout, it was judged that a washout period of a week would minimise potential physiological or psychological carry-over effects (see 8.8.1 'minimising carry-over', p187) following repeated dosing whilst being short enough to not inconvenience participants and also to avoid the possibility of spontaneous resolution of negative mood states. Following washout, participants crossed over to receive the other type of capsule for comparison. They took their first saliva samples for two days for baseline cortisol measurements, at the end of the first intervention prior to washout and again at the end of the second intervention.

Each participant made three visits in all (Figure 6.1). Psychometric tests (6.10, p96) and salivary cortisol measurements to compare changes in mood, anxiety symptoms and stress levels were re-administered before the end of the first half just prior to washout and at the end of the clinical study. Blood pressure, pulse and ALT levels (6.9.3 p95) were also retaken. Levels of salivary cortisol were assessed using enzyme-linked immunosorbent assay (ELISA) (6.12.2, p108).

Subjective changes in quality of life determined by participant self-completion of a mood and physical effects diary were reported mostly qualitatively. This is the

preferred method as in keeping a daily diary participants can note any effects that may or may not be as a result of the interventions, with a view to providing new information.

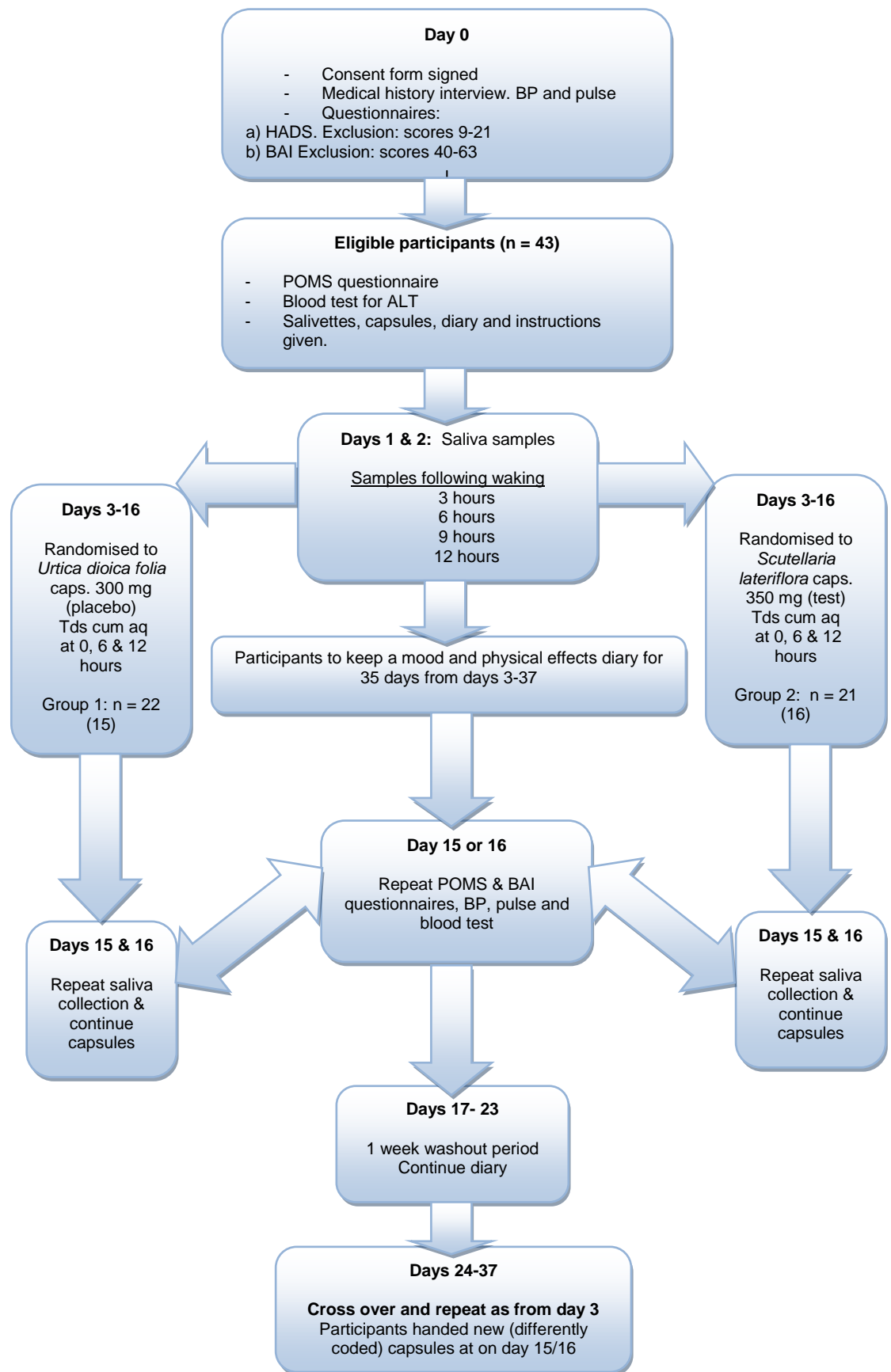
Participants also completed a personality test, the Big 5 Mini-Marker (Saucier 1994b) to determine whether or not there is a typical personality trait correlating to reduction in anxiety scores on BAI, one of the primary outcome measures (the other is the POMS).

6.6.1 Randomisation and blinding

Participants were each randomised to one of two sequences. These were either *S. lateriflora* followed by placebo or placebo followed by *S. lateriflora*. Randomisation was drawn from a list of numbers attached to randomly generated assignment of equal numbers of skullcap test (T) or placebo (P), which signified which intervention each participant was given first. The list was labelled 'A' accordingly. An opposite list was produced and labelled 'B'. The randomisation process was carried out by a person independent of the study, the clinic manager in the University of Westminster Polyclinic, using RANDOM.ORG, a website offering high quality "real" randomization processes for researchers. As capsule codes A and B did not correspond to capsule type it was not possible for either the participants or the researcher to know the sequence for each participant. Blinding was carried out by a Polyclinic herbal medicine dispensary technician by placing the two different capsules into two sealed envelopes for each participant. Envelopes were labelled with the participant code and capsule codes A and B respectively.

6.7 Main outcome measures

- Participants were assessed for changes in anxiety levels and mood and salivary cortisol measures of stress under double-blind placebo controlled conditions.
- The primary outcome measures for assessment of effectiveness of *Scutellaria lateriflora* were changes in mean scores from baseline on Beck Anxiety Inventory (BAI) (Beck & Steer 1993) and the Profile of Mood States (POMS) (Lorr *et al.* 2005).
- Secondary outcomes were mean changes from baseline in salivary cortisol measurements.
- Observational outcome measures were changes in ALT levels, blood pressure and pulse as well as symptom reports from the participants' diaries.



Key: HADS= Hospital Anxiety and Depression Scale; BAI = Beck's Anxiety Inventory; POMS = Profile of Mood States; BP = blood pressure; tds cum aq = 3 times daily with water. Days 3 – 16: Figure in parentheses denotes final No. participants per group

Figure 6.1 Study design

6.8 Participants, Materials and Methods

6.8.1 Participants

The sample group consisted of 43 healthy volunteers; males and females, aged 18-75. A power calculation determined this number of participants would provide sufficient statistical power to yield valid results (see section 6.13.1, p109).

6.8.1.1 Recruitment

A recruitment drive began in May 2010 by placement of 50 advertising posters (Appendix IV) divided between 3 University of Westminster sites. All University of Westminster staff and students were also notified of the need for volunteers by email. In addition, flyers (Appendix IV) were sent by email to 300 herbal and other complementary therapies practitioners in and around London. Advertisements were placed in a national magazine (Appendix V), the University of Westminster Student Union website, social networking sites Twitter and Facebook and free advertising site Gumtree. Although not compulsory, recruitment advertising was on the basis of experiencing persistent stress, anxiety, mood swings, irritability, poor sleep or difficulty in coping. The study inclusion criteria specified that non anxious participants were also included. Those expressing interest were sent an information sheet (Appendix VI) with a cover letter (Appendix VII). It was ensured that a period of at least one week had elapsed prior to each enquirer volunteering to participate in the study. There were 400 enquiries in total; of these, 51 were interviewed and 43 were positively recruited to the study; 31 completed.

Participants were males ($n = 9$) and females ($n = 34$); age range 19 - 66 years; mean \pm SD age 34 ± 13 ; median age 31. Of those completing the study ($n = 31$, males: $n = 6$; females: $n = 25$) the age range was 20 – 65 years; mean \pm SD age was 35 ± 12 ; median age 34. Twenty eight participants were students at the University of Westminster and 15 were members of the general public. Eighteen finishing participants were students at the University of Westminster and 13 were members of the general public.

6.8.1.2 Inclusion criteria and rationale

- Good general health. It was important to minimise any unnecessary risks to health and to ensure there are no endogenous stress chemicals that might affect levels of salivary cortisol, as may occur in those with severe or chronic illnesses. Altered

cortisol responses have been detected in patients with chronic disease (Cooper & Stewart 2003; Kudielka & Kirschbaum 2003). It would be difficult to ascertain whether levels at baseline are as a result of systemic stress or anxiety-related stress. Furthermore, those with severe or chronic illnesses are likely to be taking medications, which might interfere with skullcap and *vice versa* and be a confounding factor (See 2.8: Contraindications and drug interactions).

- Participants were volunteers and had given informed consent.
- Agree to undergo a finger prick blood test for analysis of liver health.
- Understanding of written and spoken English.

6.8.1.3 Exclusion criteria and rationale

- Heavy alcohol (> 4 units daily), tobacco (> 20 cigarettes daily) or recreational drug dependence, which may otherwise interfere with salivary cortisol concentrations (Kirschbaum & Hellhammer 1994) or have an additive effect or other interaction with the test intervention. Heavy alcohol intake increases cortisol concentrations, both during intoxication and withdrawal (Adinoff *et al.* 2003).
- Known hypersensitivity to any herbal medicines (when taken orally).
- Current use or use within the past month of medication affecting the CNS.
- A history of (diagnosed) severe, psychiatric disorders. Those with current mild depression were not excluded. If there is a severe psychosis care must be taken with regard to the potential adverse reaction such individuals may have to skullcap.
- Liver disease, kidney disease, cancer, endocrine disorders, severe (>150/90 mm/Hg) or malignant hypertension or any other serious medical condition that might affect cortisol levels or require medication that might interact with *S. lateriflora*.
- Moderate-high depression i.e. Hospital Anxiety and Depression Scale scores 9-21 (Zigmond & Snaith 1983). Depression may affect the hypothalamic-pituitary-adrenal (HPA) axis and result in an abnormal cortisol response profile (Polk *et al.* 2005). Clinically depressed individuals have a flattened diurnal profile and consistently high levels of plasma cortisol (Weber *et al.* 2000). Furthermore, results of a survey of herbal medicine practitioners (Brock *et al.* 2010) indicated *S. lateriflora* may exacerbate depression. It is known that benzodiazepines worsen symptoms of severe depression (Vanin 2008) so, considering *S. lateriflora* has benzodiazepine receptor binding affinity *in vitro* (Liao *et al.* 1998; Hui *et al.* 2000), caution must be exercised in case it has actions similar to benzodiazepines.

- Initial scores of above 40 in the Beck Anxiety Inventory. As this indicates very severe anxiety (Beck & Steer 1993) there is a responsibility to be aware of referral cases.
- Those currently on, or with a recent history of using synthetic glucocorticoid hormones, including sprays or topical corticosteroid analogues. Exogenous corticosteroids appear to inhibit their endogenous secretion (Masharani *et al.* 2005).
- Those taking herbs or certain supplements as some may have either a direct or indirect effect on the HPA axis (e.g. dopaminergic, serotonergic, GABA-ergic) or interact with *S. lateriflora*.
- Pregnancy or lactation. *S. lateriflora*'s effects on the unborn or babies is unknown.
- Those under 18 or over 75 as this raises further ethical considerations.
- Refusal to undergo blood tests.
- ALT level at 25°C > 22 U/L (men); > 17 U/L women (Roche 2008).
- Those who do not understand the spoken and written English language. Not only are some questions of a sensitive nature but responses to psychometric questionnaires should be spontaneous.
- Participation in another clinical study with oral intervention within the past 30 days.

6.9 Materials

6.9.1 The test and placebo interventions

Organic freeze-dried American skullcap (*Scutellaria lateriflora*) (Eclectic Institute Inc.) in 350 mg capsules is the test herb. A freeze-dried preparation (Eclectic Institute Inc.) was selected as freeze-drying at low temperatures (-18°C) is superior to air drying methods at preserving plant phytochemistry, particularly the phenolic content, because it is faster at removing water content, which could otherwise allow for enzymatic degradation and oxidation (Eclectic Institute Inc. 2003; Maisuthisakul & Pongsawatmanit 2004; Luthria 2006), and has superior storage time (Gafner & Bergeron 2005) (see 5.3.3, p77). The 350 mg capsules (Eclectic Institute Inc.) were found to be effective (Wolfson & Hoffmann 2003) and can deliver controlled doses, recommended at 1-3 capsules daily (Eclectic Institute Inc.).

Freeze-dried stinging nettle leaf (*Urtica dioica folia*) capsules (300 mg) is the placebo of choice as it has no known effects on the CNS, is identical in appearance (a fine,

green powder in a clear capsule) and is similar in taste and smell to the skullcap capsules. Furthermore, capsules are better for participant adherence than tinctures or teas and their contents are less likely to be recognisable by appearance, taste or smell. The *S. lateriflora* used for the clinical study was proved by HPLC analysis (Chapter 5) to be the true herb and of suitable quality for the study.

NB: Lyophilisation is carried out by immediately freezing freshly harvested plants, which have been washed in spring water, at low temperature e.g. -18°C. They then undergo sublimation, a process whereby the frozen water is vaporised under vacuum. The vaporised water is passed through condenser plates, a process which converts the water vapour back to a solid and removes it from the vacuum chamber. Only the dried plant material remains and the separation process is complete. Any remaining moisture is removed (around 5%) by gentle heat. Active constituents remain, including flavonoids, enzymes, oils and fatty acids. The colour, smell and taste of the plant material are not affected and it has a long shelf life (Eclectic Institute Inc. 2003; Luthria 2006).

6.9.2 Saliva sampling materials

Serum cortisol is approximately 90 – 95% bound to cortisol binding globulin (transcortin). Bound cortisol is physiologically inactive whereas salivary cortisol is mainly the free, active form (Adinoff *et al.* 2003). Measurement of free, unbound cortisol levels can provide an insight into the physiological activity of this glucocorticoid hormone. As an alternative to analysis of blood-borne free cortisol, collection and measurement of unbound salivary cortisol is a viable, non-invasive and stress-free method, which accurately reflects serum or plasma levels of unbound cortisol. Urinary cortisol on the other hand does not always correctly reflect the free cortisol concentration in serum as it relies upon accurate 24 hour urine collection (Aardal & Holm 1995). Chewing on a cotton wool or synthetic swab (Figure 6.3) collects a sample volume 0.5-2 ml within 30-60 seconds. Samples may be stored at 20°C for up to 4 weeks, but preferably frozen (Kirschbaum & Hellhammer 1994).

All materials for saliva sampling and analysis were obtained from Salimetrics™ Europe Ltd., including storage tubes (Figure 6.2), oral swabs (Figure 6.3), boxes for storage and freezing (Figure 6.5), and ELISA kits (Figure 6.6). Storage tubes consist of an inert polymer oral swab (10 mm x 30 mm) for saliva collection, a small inner tube for swab

storage and a larger outer centrifuge tube (17 mm x 100 mm) with a snap cap (Figure 6.4). ELISA kits contain 96 well microtitre plates, cortisol standards, cortisol controls (high and low), phosphate wash buffer, assay diluent, cortisol enzyme conjugate, 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution, stop solution (containing sulphuric acid) and non-specific binding wells (see 6.12 *Salivary cortisol assay* for details). Software used for data analysis of salivary cortisol was Gen5™ from Biotek®.



Figure 6.2 Salimetrics™ swab storage tubes
Image used with permission from www.salimetrics.com



Figure 6.3 Salimetrics™ oral swabs
Image used with permission from www.salimetrics.com

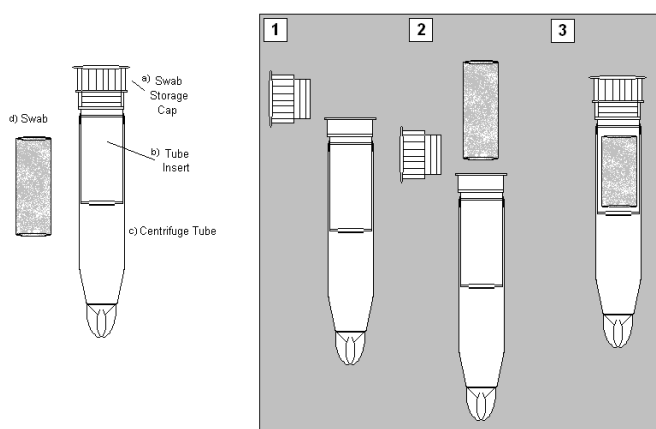


Figure 6.4 Diagrammatic representations of Salimetrics™ storage tube construction
Image used with permission from www.salimetrics.com



Figure 6.5 Salimetrics™ freezer storage box
Image used with permission from www.salimetrics.com



Figure 6.6 Salimetrics™ salivary cortisol kit
Image used with permission from www.salimetrics.com

6.9.3 Reflotron® blood analysis

The Reflotron® Plus (Roche) system (Figure 6.7), originally obtained from Una Health Ltd, Stoke-on-Trent, UK, was available for use in the University of Westminster Polyclinic. Working on the principle of reflectance photometry it allows the quantification of 17 single parameters in whole blood using test strips (Hamer 2010). A study (James & Price 1987) that compared the Reflotron (dry chemistry) analytical method with conventional laboratory methods found results agreed favourably. Reflotron ALT (GPT) test strips, glass capillary tubes (30 μ) and applicator for holding capillary tubes and blood dispensing were sourced from Una Health Ltd. Accu-Chek sterile lancets and an Accu-Chek Softclix-Pro pen for holding them were sourced from Biostat Ltd, Stockport, UK. All instruments were manufactured by Roche Diagnostics (Mannheim, Germany).



Figure 6.7 The Reflotron® Plus blood analyser
Used with permission from <http://www.unahealth.co.uk>

6.10 Questionnaires

Initially, a variety of validated anxiety instruments were evaluated from the literature to determine the most appropriate to use in this study (Table 6-1, p98). Cronbach's alpha coefficient ratings and main findings from validity assessments were compared and the advantages and disadvantages for each in relation to the proposed research were weighted (Table 6-1). Instruments to differentiate anxiety from depression for screening purposes (in order to exclude depressed individuals) and to measure anxiety almost exclusively, including somatic symptoms, were desirable for this research in order to determine anxiolytic effects of *S. lateriflora*.

Following the choice of Beck Anxiety Inventory (BAI) (Beck & Steer 1993) for a primary outcome measure of anxiety as a mood state and the Hospital Anxiety and Depression scale (HADS) (Zigmond & Snaith 1983) for screening, a pilot study on the use of these instruments was conducted on 12 volunteers with self-reported anxiety. The purpose was to determine the ease of use of the HADS and BAI and to assess the correlation between the two questionnaires with regard to anxiety scores - and also to assess their construct validity in those with self-reported anxiety. The scores indicated a high correlation between the questionnaires and confirmed reported levels of anxiety.

6.10.1 The Hospital Anxiety and Depression Scale (HADS)

The HADS (Appendix VIII) is a 14 item scale questionnaire that differentiates between anxiety (A) and depression (D). HADS-A and HADS-D are weighted equally in the questionnaire. Each statement has a choice of 4 responses, scoring 0-3. The maximum score for anxiety or depression is 21 points. A score of 8 for either reflects moderate anxiety or depression respectively, while a score of 11-21 indicates severe anxiety or depression (Zigmond & Snaith 1983). Cronbach's alpha for HADS-A and HADS-D are 0.78 – 0.93 for HADS-A and 0.82 – 0.90 for HADS-D (Mykletun *et al.* 2001). Two

month test-retest reliability for the whole scale was reported by Bjelland *et al.* (2002) to be 0.78. Administration takes approximately 5 minutes.

The instrument does not measure somatic symptoms of anxiety (Mykletun *et al.* 2001) so it was decided to use Beck Anxiety Inventory as an additional anxiety test measuring somatic symptoms (Beck *et al.* 1988) for the intervention outcome.

6.10.1 The Beck Anxiety Inventory (BAI)

The BAI (Beck *et al.* 1988; Beck & Steer 1993) (Appendix IX) measures the severity of self-reported anxiety. The questions are designed to measure general anxiety symptoms, including somatic, not to discriminate between pathological states such as phobias or panic disorders. It excludes symptoms that overlap with depression so is specific for anxiety (Beck & Steer 1993; McDowell 2006) and was constructed for the purpose of minimally sharing depression symptoms as measured by the Beck Depression Inventory (Beck & Steer 1987). The 21-item BAI scale lists statements of anxiety symptoms and each response is rated on a 4-point scale: “Not at all” (0); “Mildly; it did not bother me much” (1); “Moderately; it was very unpleasant, but I could stand it” (2); and “Severely; I could barely stand it” (3) (Beck & Steer 1993). The maximum score is 63 points. Below 8 = “Minimum anxiety”; 8 -15 = “Mild anxiety”; 16 – 25 = “Moderate anxiety”; 26 – 63 = “Severe anxiety”. The BAI has a high internal consistency (correlations between different items on the test), average Cronbach’s alpha .92, and a high external validity (can be held true for different cases, types of people) and high test-retest reliability (.75) (Beck & Steer 1993). Administration takes around 5 minutes.

6.10.2 Profile of Mood States (POMS)

The POMS (McNair *et al.* 1971; Lorr *et al.* 2005) standard questionnaire is regarded to be appropriate for monitoring responses to therapeutic interventions (Lorr *et al.* 2005), not only for psychiatric patients but for normal adults, college students and geriatrics. Controlled outpatient drug trials have demonstrated its construct validity (measures accurately what it is designed to measure) and predictive validity (scores are sensitive to change following intervention). Internal consistency for items within each factor is high (.87 - .95) and 3 week retest reliability is between .66 and .74 (Lorr *et al.* 2005).

Table 6-1 Comparison of popular anxiety rating scales to determine the most appropriate for assessing primary outcome of *S. lateriflora* as a therapeutic intervention

Test	Description and application	Time frame	No. of items	How administered	Cronbach's alpha	Reliability and validity study	Main findings	Rationale for choice: Advantages (A) Disadvantages (D)	Rate for proposed study
Manifest Anxiety Scale: TMAS (Taylor 1953)	Selection of participants for psychology experiments (McDowell 2006)	N/K	50	Self testing	NK	(Kendall 1954)	Only a 'coarse measure' of manifest anxiety	A) Measures trait anxiety D) Considered outdated (McDowell 2006)	*
Hamilton Anxiety Rating Scale: HARS or HAM-A (Hamilton 1959)	Not intended as a diagnostic instrument. Measures severity of anxiety in patients previously diagnosed (Maier <i>et al.</i> 1988)	Past week	14	Clinical rating	* * *	(Maier <i>et al.</i> 1988)	Did not sufficiently distinguish between anxiety and depression	A) Accurate measure of anxiety severity. D) Does not specifically differentiate between anxiety and depression (McDowell 2006). A) Measures somatic and psychological symptoms. Consistency of scores. Ease of use. Therapeutic outcome measure (Shear <i>et al.</i> 2001)	* *
						(Shear <i>et al.</i> 2001)	Good test and retest reliability		
Hospital Anxiety and Depression Scales: HADS-A and HADS-D (Zigmond & Snaith 1983)	Clinical diagnosing and differentiating between anxiety and depression. Rates psychiatric and general medical patients. (McDowell 2006).	Past week	14	Clinician interview or self-reporting.	* * *	(Mykletun <i>et al.</i> , 2001)	Good in terms of homogeneity, consistency and factor structure when tested in a large population	A) Differentiates between anxiety and depression. D) Physical symptoms of anxiety are not addressed. (Mykletun <i>et al.</i> 2001)	*
Self-rating Anxiety Scale and the Anxiety Status Inventory (Zung 1974)	Clinical assessment of anxiety as a psychiatric disorder. Not intended to assess trait anxiety (McDowell 2006)	Not specified	20	Clinical interview or self rating	* *	None found	N/A	A) Assesses anxiety severity specifically. D) Includes mainly physical symptoms and very few 'feelings'. D) Appears to concentrate on severe anxiety (McDowell 2006)	*
Beck Anxiety Inventory (Beck & Steer 1993)	Measures severity of self-reported anxiety. Survey or clinical screening (McDowell 2006). Discriminates between anxious and non-anxious groups (Pearson 2008).	Past week	21	Self-reporting or clinical interview. (Clinician interpretation).	* * *	(Wetherell <i>et al.</i> 1997)	<i>'High internal consistency and no significant differences by sex or race, suggesting that it is appropriate to use with diverse populations'</i>	A) Excludes depression questioning so avoids depression as a confounding factor. Measures general anxiety symptoms, not pathological states such as phobias or panic disorders or social anxiety (McDowell 2006)	* * *
Depression Anxiety Stress Scales: DASS (Lovibond & Lovibond 1995)	Used to distinguish between anxiety and depression. Survey, clinical (McDowell 2006)		42	Interview (non-clinician but clinician interpretation)	* * *	(Nieuwenhuijsen <i>et al.</i> 2003)	High internal validity with high Cronbach-alpha. Useful for distinguishing between anxiety and depression.	A) Useful for assessing changes over time following therapeutic intervention. D) One third loaded towards depression (McDowell 2006).	*
State-trait Inventory: STAI (Spielberger <i>et al.</i> 1970)	Differentiates between state (acute) and trait anxiety (chronic). Research, screening (McDowell 2006)		40	Self- administered	* * *	Numerous studies	Consistently high internal consistency.	A) Can be used for general population. Good for stressful testing conditions. Widely used for measures of anxiety D) Correlated highly with Beck depression scale (McDowell 2006)	* * *

Cronbach-alpha as reported by McDowell (2006) > 60 *: 60-80 **: 80-90 ***. Rating: * low; *** high.

Since its introduction in 1971 the POMS has been widely used in various research settings, for example cancer studies e.g. (Cella *et al.* 1987; Blesch *et al.* 1991; Baker *et al.* 2002; Landsbergen *et al.* 2012), nicotine research e.g. (Hughes *et al.* 1984; Levin *et al.* 1996; Benowitz *et al.* 2012), exercise and fitness and sports studies e.g. (Berlin *et al.* 2006; Chtourou *et al.* 2012; Kumae *et al.* 2012) and memory and cognition research e.g. (Elixhauser *et al.* 1999; Samuels *et al.* 2008; Lopez *et al.* 2012). Between 1995 and 2005 it was cited in at least 1,000 reports in 400 journals by a minimum of 3,800 authors (Lorr *et al.* 2005). Electronic searches on Google Scholar and PubMed revealed that from the time of its introduction up to February 2012 author citations amounted to 6,000 with a tally of 16,400 articles.

For the present study the instrument was selected not only for its popularity and reliability but also because it can measure multiple mood states concurrently. Furthermore, it has been found to be sensitive to changes in mood following administration of mild benzodiazepines (Lorr *et al.* 2005). During early development of the instrument, in a double-blind placebo-controlled study of psychiatric outpatients, following 4 x 10 mg daily chlorthalidone (Librium) over five weeks there were significant reductions in Tension-Anxiety and an increase in Vigour-Activity compared to placebo and no treatment groups ($p = 0.05$) at one week and five weeks (Lorr *et al.* 1963). Considering that phytochemicals found in *S. lateriflora* bind to benzodiazepine receptors *in vitro* (Liao *et al.* 1998; Hui *et al.* 2002) these early findings are relevant to the present study.

On the Standard POMS (Lorr *et al.* 2005) there are six mood factors: Tension-Anxiety (T-A); Depression-Dejection (D-D); Anger-Hostility (A-H); Vigour-Activity (V-A); Fatigue-Inertia (F-I); and Confusion-Bewilderment (C-B). There are 65 items consisting of words or statements and for each, participants circle the score that best describes how they are feeling right now, previously, in the past week including today or a time-scale of the assessors choice. For each item there are two alternative descriptions, which are offered by the assessor should the respondent not understand the meaning of the given item. “Grouchy” for example has the alternatives “Crabby” or “Grumpy” (Lorr *et al.* 2005). “The past week, including today” was chosen for this study because it is long enough to assess participants’ persistent mood and short enough, taking potential idiosyncratic latency into consideration, to determine the effects of the intervention over a period of time (2 weeks) consistent with the study.

6.10.1.1 Scoring the POMS

Scores are as follows: “Not at all” (0); “A little” (1); “Moderately” (2); “Quite a bit” (3); “Extremely” (4). Administration: about 10 minutes (Lorr *et al.* 2005).

Scores for each of the six mood factors, T-A, D-D, A-H, V-A, F-I and C-B are summed and totalled at the end of their respective columns. For total mood disturbance (TMD) the factor scores are summed together with V-A being negatively weighted because it suggests a mood of high energy and friendliness and represents a positive mood factor and is negatively related to the other factors. The TMD scores are considered highly reliable in clinical studies where an overall affective state is required (Lorr *et al.* 2005).

It was expected that participants would score low on depression-dejection as moderately and severely depressed people were excluded from the study by HAM-D (Zigmond & Snaith 1983). Also, it is possible there may be gender differences in scores for some factors. For example, in a healthy, (non-psychiatric) sample women may score higher on fatigue than men (Lorr *et al.* 2005). The main outcomes hoped for were:

- Tension-anxiety – reduction
- Depression-dejection - reduction
- Anger-hostility – reduction
- Vigour activity – increase or stable
- Confusion bewilderment – decrease or stable (effects of skullcap on cognition)
- Fatigue inertia – decrease or stable

6.10.2 Big 5 Mini-Marker

Following decades of research and use of a variety of personality tests without a common framework and which, for example, might have used anything between 2 and 20 main characteristics, the field of personality research appears to have reached a general consensus of the “Big Five” factor structure being the accepted dimensions (John *et al.* 2008). In other words, five personality traits are widely accepted as being a common framework, integrating multiple dimensions. These dimensions were derived from the terminology used by people to describe themselves and others (John *et al.* 2008) and a search for additional factors has demonstrated the Big 5 to be the only replicable traits (Saucier 1997; John *et al.* 2008).

The Big 5 structure is now widely accepted as providing a representation of personality traits that are generalisable across different languages, cultures and population samples using variable testing methods (John *et al.* 2008).

The Big 5 factor structure was first developed as an assessment tool by Tupes and Christal (1961), who termed the factor classifications as I: Surgency, II: Agreeableness, III: Dependability, IV: Emotional Stability and V: Culture it was further developed by Goldberg (1992), who simplified it by replacing sentences with adjectives; 100 unipolar adjectives divided into 20 for each dimension on the scale.

The Big 5 Mini-Marker (Appendix X), a shortened and simplified form of Goldberg's test (Goldberg 1992) was developed by Saucier (1994b) and was used in this research as it is simple to administer, easier to understand than Goldberg's model (adjectives such as 'imperturbable' have been removed) and has been shown (Saucier 1994b) to correlate highly (0.91 – 0.96) to the 100 adjective model and to be reliable. Cronbach's alphas were 0.86, 0.82, 0.84, 0.78 and 0.78 for each factor respectively (Mooradian & Nezlek 1996). This bipolar model uses 40 adjectives divided into equal numbers of 'desirable and undesirable attributes' in sets of 8 for each personality dimension, which are termed Factors I: Extraversion, II: Agreeableness, III: Conscientiousness, IV: Emotional Stability and V: Intellect or Openness. Of each set of 8 adjectives, 4 items each mark the positive and negative poles of each of factors I, II and III. For Factor IV (Emotional Stability) there are 2 positive and 6 negative pole items and for Factor V (Intellect or Openness) there are 6 positive and 2 negative pole items (Saucier 1994b).

6.10.2.1 Mini-Marker procedure and scoring

Participants self-assessed their personalities whilst in the University of Westminster Polyclinic, during their first testing session, following the written instructions on the instrument (Saucier 1994a): *"Describe yourself as you see yourself at the present time, not as you wish to be in the future. Describe yourself as you are generally or typically, as compared with other persons you know of the same sex and of roughly your same age. Before each trait, please write a number indicating how accurately that trait describes you, using the following rating scale"*(Saucier 1994a).

Scores were from 1-9 and participants entered the relevant number next to each adjective. These were then transferred to an Excel grid (Appendix XI), which had been

constructed by this author for ease of interpretation of results, using reverse-scoring to reflect values for negatively loaded items (e.g. a score of 8 becomes 2 and a score of 2 becomes 8). The scores were then totalled for each factor and then each factor total was divided by 8 (the number of items in each factor) to obtain the mean value (Saucier 1994a).

A regression analysis was carried out to determine whether or not there is a predominant personality trait responding to *S. lateriflora*, putatively demonstrable with a correlating reduction in anxiety scores on BAI (Beck & Steer 1993).

6.10.3 Participant diary

Participants were provided with a symptom diary (Appendix XV) in which they were requested to list any physical or mental symptoms they experienced during the study, whether new or old, both negative and positive, including the disappearance of old symptoms. They were also asked to write on a separate piece of paper if necessary and to report anything unusual they felt about their experiences whilst taking part in the study should they wish to do so. They were asked to show the assessor their diaries at follow-up and to hand it in at the end of their participation. In addition to qualitative reporting from participant statements, symptoms were grouped into categories and graphically illustrated.

6.10.3.1 Adverse effects reporting

The purpose of the diary was to provide potentially new information about the effects of *S. lateriflora*, in particular to determine: whether there are any unforeseen side-effects of the herb; any useful effects such as alleviation of chronic symptoms (which may or may not be anxiety-related); and to monitor for serious adverse reactions, which, although unexpected, it cannot be ruled out with any intervention that someone may have an idiosyncratic reaction to it. *S. lateriflora* herb and the placebo *Urtica dioica* herb are both in Schedule 1 of the General Sales List (Medicines Control Agency 1984), which states:

‘There are hereby specified classes of medicinal products which in the opinion of the Ministers can with reasonable safety be sold or supplied otherwise than by or under the supervision of a pharmacist, namely, medicinal products which are not prescription only medicines...’

An electronic literature search did not reveal any reports of human toxicity or serious side-effects directly attributable to *S. lateriflora* and *U. dioica* is widely sold in supermarkets as a popular beverage. Participants were, however, asked to contact their doctor immediately should they suspect a serious reaction related to either of the interventions and to report to the investigator also. If such adverse reactions should occur the participant would be withdrawn from the study immediately.

6.11 Study procedure and rationale

6.11.1 General – the interview

The contents of the information sheet (Appendix VI) were reiterated to volunteers, who were then asked if they had any questions. It was made clear to volunteers that they had a right to refuse to participate, inclusive of a right to not respond to any questions and a right to withdraw at any time. After signing a consent form (Appendix XII) they were screened for eligibility with a health and drug questionnaire (Appendix XIII) and the Hospital Anxiety and Depression Scale (Appendix VIII). Those with moderate-high depression (scores > 8) were excluded. They self-administered Beck Anxiety Inventory (Beck & Steer 1993) (Appendix IX) as the next step in the screening process and those with scores > 40 were excluded. Exclusion was based on the premise that it was important to be aware of clinical cases of anxiety and depression with potential comorbid mood disorders with a propensity to suicide (Beck & Steer 1993) and to refer immediately to their general practitioner (G. P.) in the first instant for treatment if necessary. Scores to specifically reflect clinical cases have not been assessed (Beck & Steer 1993) but it was judged that a score below median of the severe anxiety category (26-63) would be the cut-off point for exclusion. It was, however, important for the interviewer to use judgement from experience of treating anxious patients, which was based on obvious signs of distress and/or very low mood.

Volunteers then underwent blood pressure and ALT level measurements. For health and drug exclusions, see 6.8.1.3, p91: *Exclusion criteria and rationale*. Exclusion criteria were maintained throughout the study as participants' blood pressure and ALT levels were monitored at each subsequent visit. Predominantly somatic symptoms on BAI were also compared and assessed within subjects at each visit in case of previously undetected, underlying medical conditions (Beck & Steer 1993).

Eligible volunteers then became participants and BAI scores used in the screening process were counted as baseline measurements. They then self-administered the Profile of Mood States (standard) questionnaire (Lorr *et al.* 2005). For each psychometric questionnaire, participants were asked how they had been feeling in the past week, including today. They also self-administered the Big 5 Mini-Marker personality test (Saucier 1994b).

The whole procedure lasted about an hour. On occasion it took a while longer if particularly anxious participants volunteered to discuss the nature of their anxiety and/or stress. Medical history questioning and psychometric testing may potentially cause some distress to volunteers as they are asked sensitive questions about their mood, anxiety, feeling state, sleeping habits, medication (including contraception), pregnancy, lactation, alcohol intake, cigarette smoking and recreational drug use. Confidentiality and participant welfare was maintained at all times during each participant visit. This interviewer is an experienced practitioner of herbal medicine who sees anxious patients on a frequent basis and is therefore used to dealing with them in a sensitive and empathetic manner. The interview room was warm, quiet and well illuminated to ensure optimum relaxation and concentration.

Following their first interview and assessments, participants were issued with instruction sheets (Appendix XIV) outlining how to take their capsules, collect and store saliva samples, fill in the saliva sample recording sheets and participant diary and how to contact this researcher; a participant diary (Appendix XV); saliva sample recording sheets (Appendix XVI); saliva collection tubes, which were labelled according to the day of study and hours following waking; saliva collection swabs (in the tubes); and their first coded capsules. For the majority of participants the first day of the study was to be the day following baseline testing (one participant wished to avoid starting at the weekend). To help maximise adherence the interviewer filled in all dates on the diary and saliva sample recording sheets during the participants' first visit to the Polyclinic.

For avoidance of potential herb-drug interactions study participants were advised not to take any medications, including herbal or grapefruit juice, whilst participating in the study. Grapefruit juice is known to inhibit the drug metabolising enzyme CYP3A4 (Vanin 2008). (See 2.8, p27: 'Contraindications and drug interactions'). On the day of

the first testing session and with the consent of each participant, a letter was sent to their G. P. informing them of their patient's participation in the study (Appendix XVII). This was considered necessary to guarantee the physical and mental health of each volunteer. A participant information sheet (Appendix VI) was enclosed with the letter. In addition, participants were requested to fill in a data sheet (Appendix XVIII) to provide personal details, including contact details for their G.P. This was also useful for some demographic information. For reasons of confidentiality any personal information was stored separately from the study data.

6.11.2 Blood pressure and pulse monitoring

Volunteers' blood pressure and radial pulse were taken to exclude those with severe hypertension at screening and to monitor potential changes in either as a result of the interventions during the study. To avoid false high readings it was ensured that participants were suitably rested prior to blood pressure and pulse measurements by waiting until towards the end of the visit. At each visit, for all participants, the same upper arm (right) was used for blood pressure measurements, using the same digital blood pressure monitor, and the right radial artery was used for pulse measurement each time for consistency. Time-matched results (for each of the three time points) mean (SD) systolic and diastolic blood pressures and pulse in beats per minute (BPM) were recorded to see if there were any notable changes from baseline. There is very little recorded evidence of *S. lateriflora* being useful for hypertension although Joshee *et al.* (2002) state it is traditionally used for this purpose in cases of 'excessive heat'. A recent publication, however, of a 22 year longitudinal study appears to dispel previous hypotheses that anxiety and stress raises blood pressure (Hildrum *et al.* 2011).

6.11.3 Liver health monitoring

To exclude those with current liver disease and to confirm the safety profile of *S. lateriflora* participants also underwent a finger-prick blood test for alanine aminotransferase (ALT), a transaminase enzyme found primarily in the liver (Kaplan 2002), before testing began and again at the period end of taking both test and placebo herbs. According to Pratt and Kaplan (2000) *Scutellaria* (species not given) is reported to cause elevation of liver enzyme levels - although no citation was provided. It has not been revealed in the literature that there has been any hepatotoxicity related to authenticated *S. lateriflora* preparations.

ALT, which catalyses the transfer of an amino group from alanine to form glutamate and pyruvate, is also known as glutamate pyruvate–transaminase (GPT). Although AST (aspartate aminotransferase) may indicate liver damage and both enzymes are found in many other organs, high levels of blood ALT (at 25°C > 22 U/L men; > 17 U/L women (Roche 2008) are more likely to relate exclusively to liver damage as the greater quantities are in the liver (Kaplan 2002). These levels relate to a 30 µ volume of blood. Quantities slightly above and less than twice the normal value are unlikely to be significant in an asymptomatic subject, however. The test should be repeated for confirmation and the degree and possible reasons for the elevation should be evaluated. Recent alcohol consumption for example may give a higher reading (Pratt & Kaplan 2000). Elevation of ALT fifteen times the normal upper reference would indicate serious liver disease such as cirrhosis, hepatitis or liver tumour (Hamer 2010).

A drop of blood, extracted by pricking the finger with a sterile lancet, is collected on a Reflotron (magnetic) strip, which is placed in the chamber of a Reflotron® blood analyser (Figure 6.7, p96) for measurement of ALT liver enzymes. Firstly, the depth of puncture was set on the lancet pen. In order to avoid unnecessary pain the shallowest setting was used initially. Participants were asked whether they had a finger preference and the digit pad was wiped with alcohol and dried with a sterile cotton swab. The hand was rubbed to ensure warmth and an adequate blood flow and the lancet puncture was made 1-2 mm from the nail bed. The first drop of blood was wiped away with a cotton wool swab and the finger was squeezed until a bead of blood appeared. This was allowed to flow up a 30 µ capillary tube placed into an applicator until it reached an exact level (marked by a black line) and the blood was expelled onto the gauze of the ALT strip by pushing a button on the applicator. After placing the strip in the chamber of the machine results were ready within 2 minutes.

Results for all measured parameters were recorded on a data sheet individual to each participant (Appendix XIX).

6.11.4 Saliva sampling

Cortisol is released throughout the day in a pulsatile manner and secretion and inhibition is controlled by a negative feedback mechanism, believed to be responsible for its circadian rhythm and secretion amplitude. Stress increases the activity of the hypothalamus-pituitary-adrenal (HPA) axis, with stimulation of corticotrophin-releasing

factor (CRF) and adrenocorticotrophic hormone (ACTH) and hence plasma cortisol levels (Kirschbaum & Hellhammer 1989).

Participants were each provided with 24 tubes for saliva collection. To reflect diurnal sensitivity of the HPA axis to stressors and concomitant changes in cortisol amplitude samples were taken at 3, 6, 9 and 12 hours following waking (Pruessner *et al.* 1997). Due to normal intra-individual variations in HPA activity samples were taken on two consecutive days (Kirschbaum & Hellhammer 1989) and an average nmol/L was taken for each time point for the two days. Of 31 participants, 5 did not produce samples. One forgot to freeze them and they decayed, one forgot to take samples, one did not return them, one was excluded from this part of the study as he was using inhaled steroids and one declined due to travelling with no access to refrigeration.

Participants (n = 26; 21 females, 5 males) were asked to take their first samples the following two days and to commence the capsules on day 3 for 14 days, collecting saliva samples for the last 2 days of capsules prior to a one week washout period. Saliva was collected again after crossover on the last two days of taking the other type of capsules.

To avoid sample contamination, participants were requested to avoid food or drink, other than water, not to smoke, brush their teeth or take vigorous exercise for at least 30 minutes prior to sampling; and to rinse their mouth with water about 10 minutes before sampling (Pruessner *et al.* 1997). Brushing teeth may cause contamination with blood, although evidence that this can falsely elevate levels of cortisol in saliva is inconclusive. Studies (Kivlighan *et al.* 2004; Schwartz & Granger 2004; Granger *et al.* 2007) found the effects of mild to moderate blood leakage from minor oral micro-injury on salivary cortisol levels to be minimal. The presence of relatively large amounts of blood in a saliva sample, however, may significantly raise salivary cortisol levels. For example, 10% of blood present in a sample has been demonstrated to increase levels by 37% (Schwartz & Granger 2004). Exercise may have a positive effect on mood states, believed to be due to increased endorphin levels (Daniel *et al.* 1992). Participants were asked to place the swab under the tongue for saliva collection as the submandibular glands are more likely than the parotids to secrete saliva containing quantities of cortisol reflecting serum levels (Salimetrics 2009).

Due to the number of samples that would be necessary, for reasons of compliance and as the awakening cortisol response (ACR) is relatively stable, this was not assessed in the healthy volunteers. As the ACR peaks at around 30 minutes and then rapidly declines (Kudielka *et al.* 2003), a number of samples would be taken to capture the response before a decline occurred; for example at 0, 15, 30, 45 and 60 min (Schulz *et al.* 1998).

6.12 Salivary cortisol assay

6.12.1 Legal aspects

On completion of saliva sampling participants returned their samples at their convenience. The samples were then placed into labelled freezer storage boxes (Figure 6.5) and stored at -18°C . To comply with the Control of Substances Hazardous to Health (COSHH) Regulations 2002 (Health & Safety Commission 2002), a COSHH assessment form (Appendix XX) was completed prior to commencement of the research. Any tissue, bodily fluid (e.g. saliva, blood or bile) or waste product (e.g. sputum, faeces or urine) derived from a human body and containing human cells is classified as ‘relevant material’ (Human Tissue Authority 2006) and is covered by Human Tissue Act 2004 (C 30) (Human Tissue Authority 2004), which states that consent must be obtained for storage or use of any relevant material for research purposes. Therefore, saliva tracking forms - for inspection by the Human Tissue Authority and to comply with The Human Tissue Act 2004 regulations (Human Tissue Authority 2006) - were completed, to include samples from each participant with regard to dates of appropriate consent, receipt, storage, movement, use for scheduled purposes and disposal of specimens.

6.12.2 ELISA: principle and method

The Salimetrics™ cortisol kit measures salivary cortisol quantitatively *in vitro* and is sensitive to levels of 0.0828 – 82nmol/L in 25 μ volume of saliva per test. It has been validated to accurately reflect circulating free serum cortisol levels (Salimetrics 2008).

All saliva samples are tested in duplicate to minimise error. They are brought to room temperature and centrifuged for 15 minutes (this removes the saliva from the oral swabs) before being applied to the plates with a multi-channel pipette.

The cortisol assay works on the principle of a competitive ELISA method. The 96 plate wells are pre-coated with monoclonal antibodies to cortisol (the antigen). The cortisol (the analyte) in the samples of saliva competes for binding sites with cortisol conjugated to an enzyme, horseradish peroxidase. After incubation the plate is washed with a phosphate wash buffer to remove unbound analyte and conjugate. Conjugated cortisol-antibody binding sites turn blue when TMB substrate for the enzyme is applied.

Blue colouration is inversely proportional to the amount of analyte present in the wells i.e. the less blue colour there is, the more cortisol there is in the saliva samples. This is because the cortisol in the samples has taken up the binding sites on the antibody instead of the enzyme-bound cortisol.

The reaction is stopped with a sulphuric acid solution and the colour changes to yellow. The optical density is read with a plate reader at 450 nm within 10 minutes of stopping the reaction. To determine the amount of analyte in the samples a range of known concentrations are also analysed on each well plate and a calibration curve is established from the results from these standards and controls. Standards contain cortisol concentrations of 82.77, 27.59, 9.19, 3.06, 1.02 and 0.33 nmol/L and the two controls represent high (29 ± 7.23 nmol/L) and low (2.9 ± 0.72 nmol/L) concentrations. Results for the unknowns are derived from the calibration curve. A plate reader attached to Gen5™ data analysis software was used. This software generates a calibration curve and the results for each plate of samples, measured in nmol/L.

6.13 Statistics

6.13.1 Power calculation and sample size estimation

This is based on a cut-off point of 40 at baseline on BAI and a projected mean decrease of 10 points during skullcap test. Projected approximate \pm SD is derived from Prasko (2006). Given the mean and standard deviation for two independent samples of equal size, for a mean (SD) on BAI of 20.0 (5.0) at baseline and a mean of 15.0 (5.0) for the skullcap test, the effect size for Student's t-test (Cohen's d), is 1.0 Using an effect size for Student's t-test (Cohen's d), of 1.0 at 80% power to show a difference \pm SD at alpha 5% and $p = 0.05$ (Cohen 1988):

For a two-tailed (non-directional) hypothesis

Minimum total required sample size: 34

Minimum required sample size per group: 17

For a paired sample t-test (for within subject differences in crossover studies) and an effect size of 1.0 the minimum sample size required is 17.

A minimum sample size of 17 was calculated to be necessary to show a clinically significant minimum 25% decrease in anxiety levels at power 80% and alpha 5%. Considering the test-retest reliability of BAI is estimated to be 0.75 (Beck *et al.* 1988) it was determined that the planned sample size of 30 was likely to show significant results for the study. A total sample size of 40 would allow for a dropout rate of 25%.

6.13.2 Primary outcome measures

Changes in scores as defined by the Profile of Mood States (POMS) (McNair *et al.* 1971), a 65 item questionnaire that measures fluctuations in mood states in normal adults, and Beck Anxiety Inventory (BAI) (Beck & Steer 1993) were used to monitor changes in mood. The POMS has 6 identifiable mood states: tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment with a 5 point self-report scale for each item, a mean score of normal as indicated by the manual, of an average of 13 per mood state, each assessed individually. A statistically significant change is considered to be a mean of around 9 points (Derderian *et al.* 1988). The Total Mood Disturbance score is calculated by summing the scores for each mood factor and subtracting the score for Vigour-Activity (Lorr *et al.* 2005). This is negatively weighted as it is considered to be a positive mood state and the opposite to the other 5 mood factors on the instrument (Daniel *et al.* 1992).

There are 40 possible scores (range = 0-40, mean = 20) in the minimal to a cut-off severe interval on BAI (clinical cases judged to be above this were excluded). An overall response will be reflected by changes in baseline from a mean of 20 points to a mean decrease of 25% over time, projected to be a mean of 15 points on BAI. A drop of 10 points for any participant would be clinically significant as such a decrease to a lower category on the BAI is likely to indicate a level of enhanced calmness.

Based on previous studies, it was judged that following treatment with *S. lateriflora*, a mean drop in subjective anxiety scores of 10 from baseline is a realistic expectation. A drop of 10 points would be clinically significant as such a decrease could indicate a level of mild as opposed to moderate or a change from severe to moderate anxiety. In a previous study of 20 participants (Prasko *et al.* 2006) a mean reduction (SD) of 8-12 points (3-5) in subjective anxiety on BAI (Beck & Steer 1993) following moclobemide (a monoamine oxidase inhibitor) pharmacotherapy was calculated to be statistically significant. The sharpest decline was within the first month of treatment (8 points). Case reports of four patients who were treated for various anxiety disorders with the GABA transporter inhibitor tiagabine demonstrated a decrease of 17, 21, 14 and 12 points respectively on BAI within days or weeks following treatment (Schaller *et al.* 2004).

6.13.3 Secondary outcome measures

Saliva sampling was repeated over 2 days for each period to allow for normal intra-individual variation and the mean (SD) of salivary cortisol in nmol/L for each diurnal time point following waking over each 2 day period was taken prior to use in the final analysis. The means (SD) for each diurnal time point and overall was compared between baseline, test and placebo. Of 26 participants who completed saliva sampling 11 missed the occasional sampling time (totalling 20 of a potential 624 samples from all 26 participants). Missing variables were computed by means analysis for each time point for each period (3 hours, 6 hours, 9 hours and 12 hours for baseline, test and placebo) using the Statistical Package for Social Sciences (SPSS).

The toxicity data would only be analysed in depth if it existed. Similarly blood pressure and pulse rate would be analysed in depth only if there are obvious changes. To determine this, the results of these tests were analysed using paired sample Student's t-tests followed by analysis of Group effects, using a 2 x 3 mixed ANOVA.

6.13.4 Statistical analysis

Differences from baseline between skullcap and placebo across primary and secondary measures were investigated using a mixed method 2 x 3 (sequence x treatment) analysis of variance (ANOVA).

A first analysis establishes that there are no baseline shifts or order effects using the cross-over design. If significant baseline shifts or order effects are present, change scores are calculated against each participant's baseline score. Change score analysis was selected in preference to analysis of co-variance (ANCOVA) because when there is a true baseline imbalance ANCOVA can produce biased treatment effects in observational studies (Metcalf 2010).

Subsequently, the interaction between group sequence (skullcap-placebo and placebo-skullcap) and treatment was evaluated by a two-way ANOVA. To determine whether differences in the mean scores (skullcap versus placebo) are statistically significant pair-wise comparisons between means were carried out using paired sample t-tests.

The relationship between one of the primary outcome variables, measurements from Beck Anxiety Inventory, and personality dimensions, was explored using regression analysis methods.

For all statistical tests, a 5% significance level was used. The Statistical Package for Social Sciences (SPSS) was used for conducting the analyses.

For the purpose of description and discussion in this study the following terms are denoted:

- Group = sequence in which participants received the treatment
- Treatment = baseline, placebo or skullcap
- Period = time period

Chapter 7. RCT Results

7.1 Participants and dropouts

Eligible participants were adults aged over 18 years meeting the criteria of good physical and mental health. Participants were males (n = 9) and females (n = 34); age range 19 - 66 years; mean \pm SD age 34 ± 13 ; median age 31. Of those completing the study (n = 31, males: n = 6; females: n = 25) the age range was 20 – 65 years; mean \pm SD age was 35 ± 12 ; median age 34. Twenty eight participants were students at the University of Westminster and 15 were members of the general public. Eighteen finishing participants were students at the University of Westminster and 13 were members of the general public.

Of 51 screened (see Table 7-1 and Figure 7.1) seven potential volunteers were excluded because they did not meet the eligibility criteria: 5 were using prescribed anti-depressant medication; 1 was on thyroid medication (thyroxin); one was pregnant and one was excluded following initial testing due to severe hypertension ($> 180/100$ mm/Hg). The latter was referred to his G. P. for medical investigations. This participant was followed up and he informed this author that his hypertension was being treated.

Table 7-1 Participant selection and dropouts

Participants	No.
Screened and evaluated	51
Did not meet inclusion criteria	7
Refused study at the beginning	1
Commenced the study	43
Dropped out during the study	12
Adverse effects of medication	0
Completed the study	31
Participants who completed saliva sampling	26

Only participants who completed the trial (n = 31) were included in the analysis. Because the trial was comparatively small participants were approached to ask if they wished to volunteer the reason/s for withdrawal and reasons were recorded.

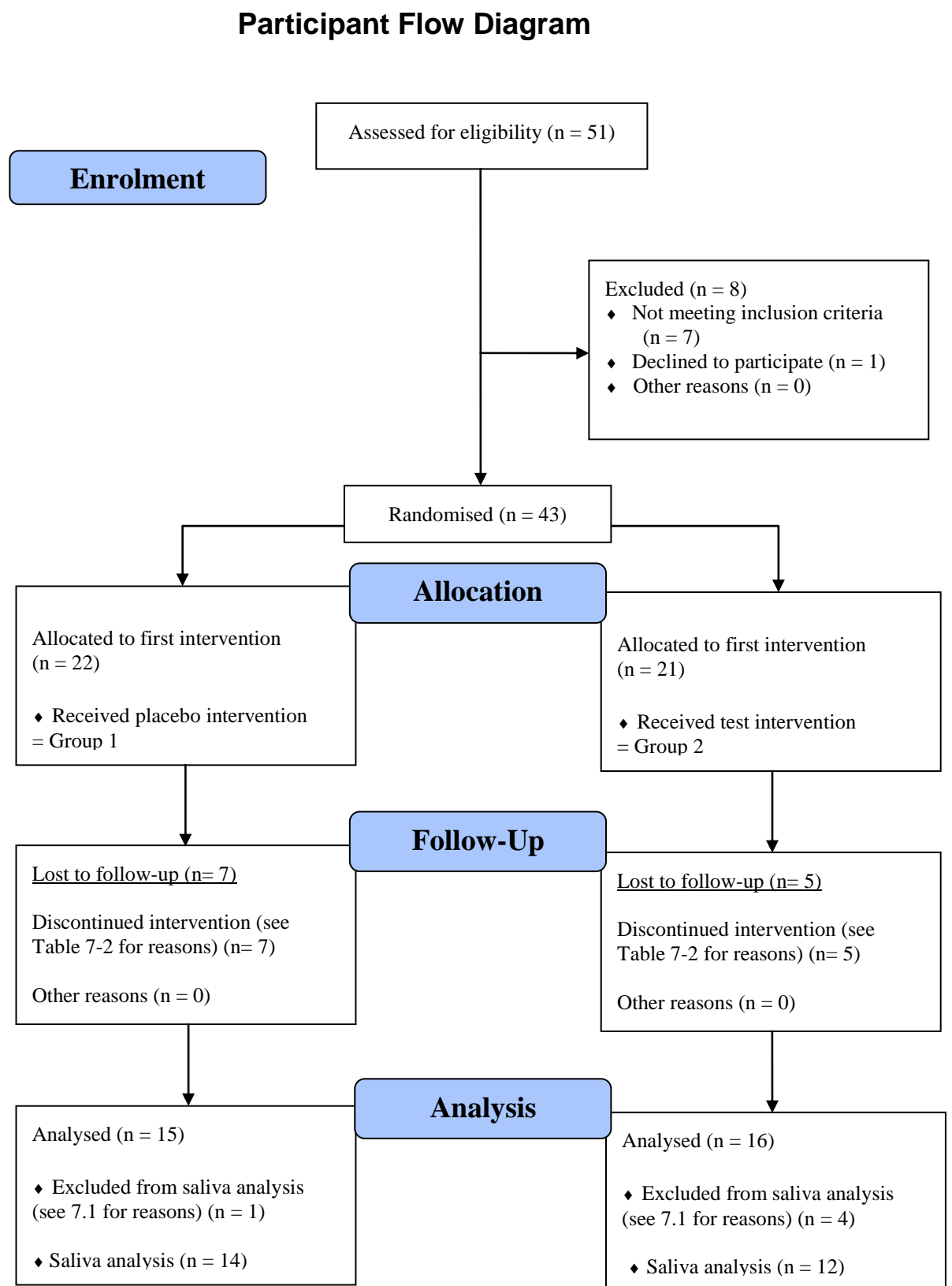


Figure 7.1 Consort trial profile for recruitment, randomisation and dropouts

There was a 28% dropout rate (n = 12). Withdrawals were for a variety of reasons (Table 7-2). There was no particular order in relation to intervention with the dropout rate; 7 left during placebo and 5 left during the test treatment period. Of the 12 dropouts, 10 of whom left during the first treatment period, there were two non-adherent participants. One of these stopped after a couple of days because she forgot to take the capsules and the other said ‘they were not doing him any good’. One participant with high anxiety levels and one with mild depression decided they wanted to commence orthodox treatment for their conditions so were no longer able to take part in the study.

Of 31 participants completing the study for primary outcome measures (the effects of *S. lateriflora* on mood as measured by BAI and POMS), 5 did not produce saliva samples. One forgot to freeze them and they decayed, one forgot to take samples, one did not return them, one was excluded from this part of the study as he was occasionally using inhaled steroids and one declined due to travelling with no access to refrigeration.

Table 7-2 Reasons for dropout

Participant ID	Order of intervention	Reason for dropout (Treatment period)
P.03	P-T	Bereavement (2)
P.06	P-T	Non-adherence (1)
P.07	P-T	DNA for second test session no reason offered (1)
P.09	T-P	Prescribed antidepressants; relationship breakup (1)
P.12	P-T	Time issues (1)
P.22	T-P	DNA for second test session; no reason offered (1)
P.26	T-P	Commenced orthodox anxiolytic medication (1)
P.27	T-P	Illness not related to intervention – influenza (1)
P.31	P-T	Non-adherence (1)
P.33	P-T	Personal circumstances (2)
P.34	T-P	Time issues (1)
P.42	P-T	Felt 'weird', difficulty in concentrating (1)

Key: DNA = did not arrive; P = placebo; T = test

7.2 Blinding

No participants reported noticing any difference between test and placebo capsules with regard to taste, smell or appearance.

7.3 Beck Anxiety Inventory

According to the BAI (Beck & Steer 1993), of the 31 participants who completed the study, 11 were initially experiencing minimal anxiety (BAI scores 0 -7), 14 were mildly

anxious (BAI scores 8-15), 3 were moderately anxious (BAI scores 16-25) and 3 had severe anxiety (≥ 26).

The mean (SD) score for all participants before test intervention was 12.16 (8.59). This decreased to 6.29 (4.79), a mean decrease of 48% in anxiety scores following 2 weeks of *S. lateriflora* (350 mg) three times daily. This would appear to imply a significant decrease in anxiety due to *S. lateriflora*; paired t (df 30) = 5.537 ($p < 0.001$). However, for placebo the mean (SD) scores decreased from baseline to 7.19 (5.99), a 42.6 % decrease in anxiety scores over a period of 2 weeks on placebo; paired t (df 30) = 4.343 ($p < 0.001$). There was no significant difference between test and placebo; mean diff = 0.90; paired t (df 30) = -1.145 ($p = 0.261$).

However, this analysis is misleading in that it does not take into account the possibility of order effects and baseline shifts in anxiety scores. It can be seen in Figure 7.2 that 1 and 2 relate to the sequence (group) in which the intervention was taken. Group1 ($n = 15$) = placebo-test; group 2 ($n = 16$) = test-placebo. The two variables showed differences between baseline measurements, with those in group 1 showing higher initial mean (SD) anxiety scores of 15.73 (10.71) than those in Group 2 at 8.81 (3.99).

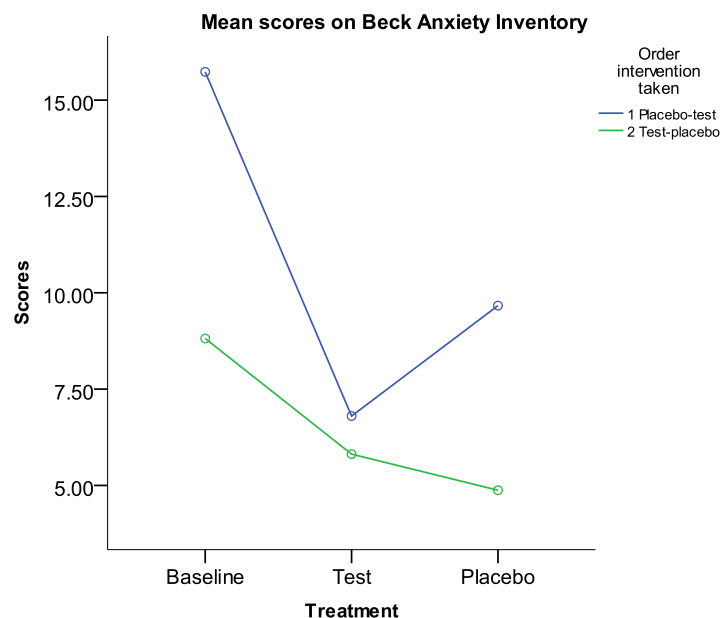


Figure 7.2 Mean scores on Beck Anxiety inventory showing differences between groups according to the order in which the interventions (test herb and placebo) were taken.

A 2 x 3 mixed ANOVA revealed that there was a significant order effect ($F_{1, 29} = 4.614$; $p = 0.040$) as well as a significant effect of treatment ($F_{2, 58} = 22.8$; $p < 0.001$) and a

significant group x treatment interaction ($F_{2, 58} = 5.03$; $p = 0.010$) and indicated an enhanced effect of placebo over skullcap in Group 2 (Figure 7.2).

Because of the shifts in baseline between the 2 groups as described above, change scores were calculated (by subtracting pre-scores from post-scores) and analysed using a 2 x 2 mixed ANOVA. This showed that, within subjects, there was no significant effect of treatment ($F_{1, 29} = 1.791$; $p = 0.191$). There was however a significant between subjects group effect ($p = 0.049$) and a significant group x treatment interaction ($F_{1, 29} = 6.96$; $p = 0.013$). Mean changes from baseline (Table 7-3) demonstrated an enhanced anxiolytic effect for skullcap compared to placebo for Group 1 (placebo-test) and no significant change for Group 2 (test-placebo). Independent sample t-tests showed differences between groups in change scores from baseline for skullcap to be significant ($t(df 29) = -3.108$; $p = 0.003$) but not for placebo ($t(df 29) = -.928$; $p = 0.361$). Paired sample t-tests showed a significant difference between skullcap and placebo for Group 1 ($t(df 14) = -2.323$, $p = 0.036$ (SE 1.23) but not for Group 2 ($t(df 15) = 1.198$, $p = 0.249$ (SE 0.782) confirming an enhanced effect overall for skullcap for Group 1 compared with placebo.

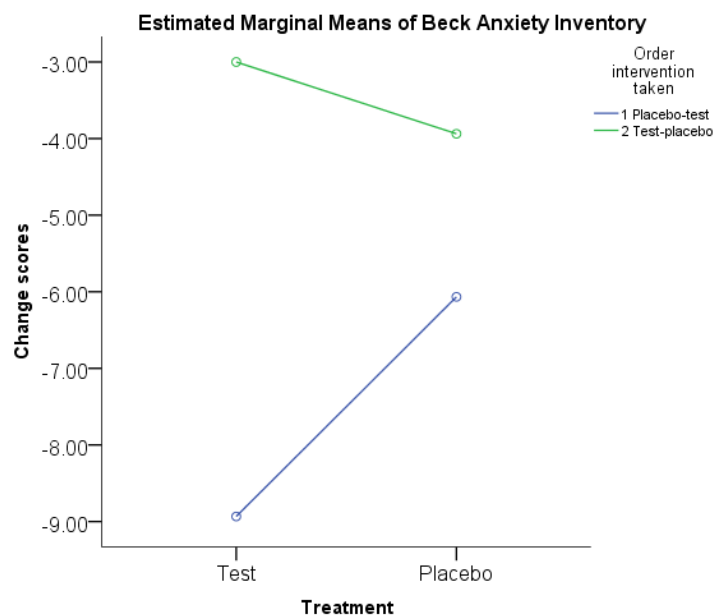


Figure 7.3 Mean differences in change scores between test and placebo according to the order in which the interventions were taken.

Table 7-3 Mean baseline scores and change scores on Beck Anxiety Inventory following skullcap and placebo, showing an enhanced effect of skullcap compared to placebo for Group 1

	Mean initial scores (SD)	Mean change scores (SD)				
		Skullcap	Placebo		Skullcap-placebo (mean diff)	
			SE		SE	P value
Group 1 (n = 15)	15.73 (10.71)	-8.93 (6.13)	1.58	-6.07 (7.87)	2.03	0.036
Group 2 (n = 16)	8.81 (3.99)	-3.0 (4.05)	1.01	-3.94 (4.58)	1.15	0.249

SD = standard deviation; SE = standard error of the mean

A 2 x 2 ANOVA demonstrated that although there was a significant group x treatment interaction ($F_{1,29} = 6.96$; $p = 0.013$), there was no significant overall difference ($F_{1,29} = 1.791$, $p = 0.191$) between skullcap, mean (SD) = 6.29 (4.79) and placebo, mean (SD) = 7.19 (5.99)

7.4 Profile of Mood States

In addition to mean scores for the 5 mood factors Tension-anxiety (T-A), Depression-dejection (D-D), Anger-hostility (A-H), Vigour-activity (V-A), Fatigue-inertia (F-I) and Confusion-bewilderment (C-B) the means \pm SD of the Total Mood Disturbance (TMD) score for the same periods were calculated for assessment of an overall effect of *S. lateriflora* on negative mood. This was obtained for each individual participant by summing the scores of each factor and subtracting the score for Vigour-Activity (Table 7-4), which was negatively scored because it is a positive rather than a negative mood state (Lorr *et al.* 2005).

Table 7-4 Differences in mean (M) scores (SD) with the POMS according to skullcap (test) or placebo treatment (n = 31).

Mood factor	Baseline M (SD)	Test M (SD)	%Change	Placebo M (SD)	% Change
T-A	12.61 (6.76)	8.74 (5.85)	- 31%	9.74 (6.98)	- 23%
D-D	11.74 (11.33)	5.83 (6.84)	- 50%	8.03 (8.85)	- 32%
A-H	13.13 (10.16)	7.13 (5.83)	- 46%	9.97 (8.63)	- 24%
V-A	16.26 (5.8)	17.42 (5.51)	7%	15.90 (5.80)	- 2%
F-I	8.03 (4.84)	6.16 (4.52)	- 23%	7.45 (6.39)	- 7%
C-B	8.67 (4.56)	6.41 (3.63)	- 26%	6.54 (5.14)	- 24%
TMD	37.94 (31.54)	16.54 (23.21)	- 56%	25.80 (36.21)	- 32%

From inspection of the percent change scores (Table 7-4) it appears that, overall, skullcap had a greater effect on wellbeing than placebo within subjects (although the increased energy with skullcap as suggested by Vigour-activity scores appeared minor).

It was important to determine whether there was a baseline shift (as discussed previously in relation to BAI scores) in accordance with the order in which the intervention was taken as otherwise these POMS results could be misleading. To determine whether these effects were present a mixed method 2 x 3 ANOVA for each mood state and also for TMD was conducted. As a further step towards ascertaining the relevance of the results for test and placebo, paired samples t-tests and independent samples t-tests were also carried out for each mood state and for Total Mood Disturbance.

7.4.1 Tension-Anxiety

To see whether there were significant differences between groups a mixed method 2 x 3 ANOVA (Figure 7.4) was carried out. This revealed that there was no significant order effect according to group ($F_{1, 29} = 2.070$; $p = 0.161$). It also confirmed that there was a significant treatment effect ($F_{2, 58} = 4.925$; $p = 0.011$) but there was no significant group x treatment interaction ($F_{2, 58} = 2.548$; $p = 0.087$).

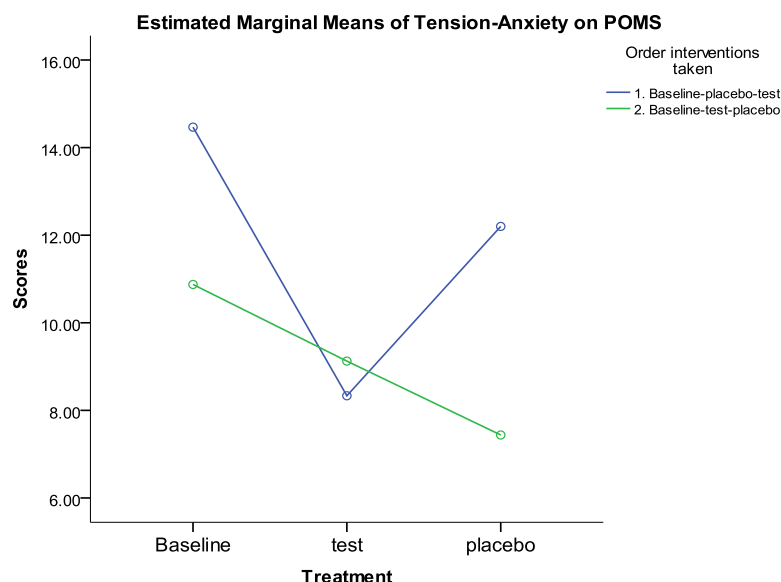


Figure 7.4 Mean scores per group for the Tension-Anxiety factor on POMS in accordance with the order in which skullcap test or placebo were taken

Paired sample t-tests indicated there was a significant effect of treatment from baseline for both skullcap, (t (df 30) = 3.345; p = 0.002) and placebo (t (df 30) = 2.314; p = 0.028) and there was no significant difference in treatment effects between skullcap and placebo (t (df 30) = - 0.642; p = 0.526).

Changes were from a mean (SD) baseline score of 14.47 (7.4) to skullcap treatment score of 8.33 (7.34) = 42% for Group 1 and from 10.88 (5.8) at baseline to skullcap treatment score of 9.13 (4.24) = 16% for Group 2. The changes from baseline scores to placebo were to 12.20 (7.8) = 16% for Group 1 and 7.48 (5.38) = 31% for Group 2.

Independent sample t- tests also confirmed there were no significant differences in baseline scores between groups (t (df 29) = 1.509; p = 0.142) and revealed a significant difference between groups in the effect of placebo (t (df 29) = 1.991; p = 0.056) but not for skullcap (t (df 29) = -0.371; p = 0.713). Overall the results suggest no significant difference between skullcap and placebo in changes from baseline. There was an enhanced effect of placebo over skullcap for Group 2.

A 2 x 2 ANOVA conducted to determine overall treatment effects demonstrated there was no significant difference ($F_{1, 29} = 0.529$; p = 0.473) between skullcap, mean (SD) = 8.74 (5.85) and placebo, mean (SD) = 9.74 (6.97).

7.4.2 Depression-dejection

Similarly for Depression-dejection, a mixed method 2 x 3 ANOVA (Figure 7.5) showed there was no significant difference between groups ($F_{1, 29} = 1.305$; p = 0.263). It also confirmed there was a significant effect of treatment ($F_{2, 58} = 7.435$; p = 0.001) and no significant group x treatment interaction ($F_{2, 58} = 2.069$; p = 0.136).

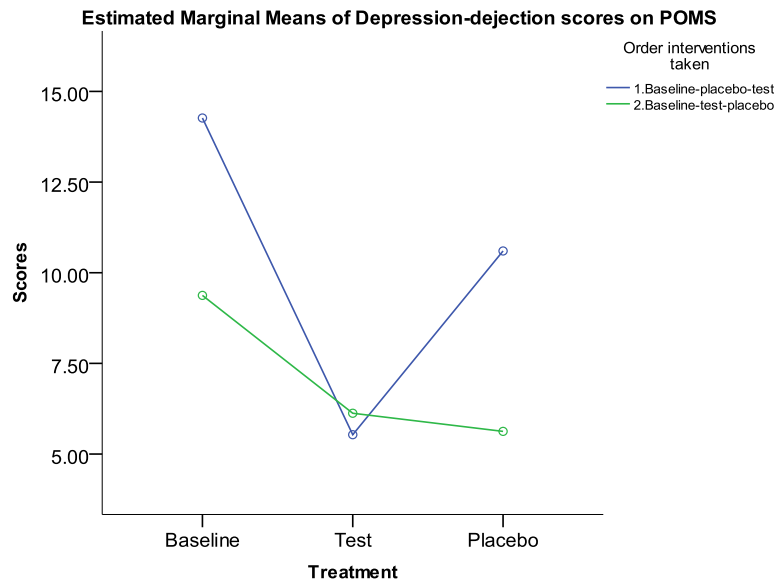


Figure 7.5 Mean scores per group for the Depression-Dejection factor on POMS in accordance with the order in which skullcap test or placebo interventions were taken

Paired sample t- tests revealed that although there was a significant effect of treatment for both skullcap (t (df 30) = 3.65; p = 0.001) and placebo (t = 2.02; p = 0.052) there was no significant difference in treatment effects between skullcap and placebo (t (df 30) = - 1.706; p = 0.098).

Independent sample t-tests revealed differences in mean (SD) baseline scores between Group 1 = 14.28 (13.15) and Group 2 = 9.38 (9.11) but these were not significant (t (df 29) = 1.210; p = 0.236). There was no significant difference between Group 1 = 5.53 (8.95) and Group 2 = 6.13 (6.55) in the effect of skullcap (t (df 29) = - 0.244; p = 0.809) or between Group 1 = 10.6 (9.35) and Group 2 = 5.56 (7.89) in the effect of placebo (t (df 29) = 1.605; p = 0.119) but there was an enhanced effect of skullcap compared to placebo for Group 1 and an enhanced effect of placebo compared to skullcap for Group 2. The percent change scores from baseline following administration of skullcap were 61% for Group 1 and 35% for Group 2 and for placebo they were 26% for Group 1 and 40% for Group 2. Overall the results confirmed there was no significant difference between the effects of skullcap and placebo on Depression-dejection.

A 2 x 2 ANOVA conducted to determine overall treatment effects demonstrated, although there was a significant group x treatment interaction ($F_{1, 29} = 5.364$; p = 0.028), there was no significant difference ($F_{1,29} = p = 0.067$) between skullcap, mean (SD) = 5.84 (6.64) and placebo, mean (SD) = 8.03 (8.85).

7.4.3 Anger-Hostility

A 2 x 3 mixed method ANOVA (Figure 7.6) showed no significant between subjects effects according to group ($F_{1, 29} = 3.462$; $p = 0.073$). In addition there was no significant group x treatment interaction ($F_{2, 58} = 1.412$; $p = 0.252$) and the tests confirmed a significant effect of treatment ($F_{2, 58} = 8.235$; $p = 0.001$). Mean (SD) scores at baseline for Group 1 reduced from 15.93 (11.03) to 7.93 (6.90) with skullcap (50%) and 13.2 (8.34) for placebo (17%). For Group 2 mean (SD) scores reduced from baseline 10.5 (8.82) to 6.38 (4.72) for skullcap (39%) and to 6.94 (7.99) for placebo (33%). The scores indicated an enhanced effect of skullcap for Group 1 compared to Group 2.

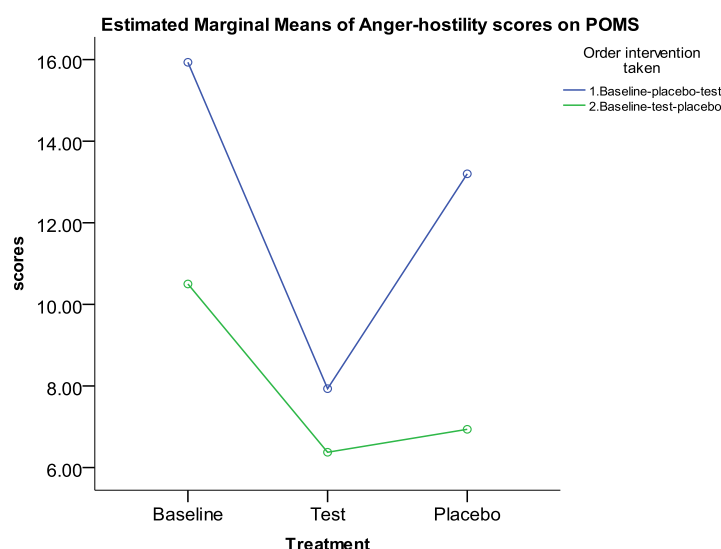


Figure 7.6 Mean scores for Anger-Hostility according to the order in which the interventions skullcap test or placebo were taken

Anger-hostility scores as assessed by paired sample t - tests revealed no significant difference between skullcap and placebo in treatment effects (t (df 30) = -1.793; $p = 0.083$) but there was a significant effect of treatment from baseline for both skullcap (t (df 30) = 4.055; $p < 0.001$) and placebo (t (df 30) = 2.188; $p = 0.037$).

Independent sample t-tests confirmed the differences between groups at baseline (t (df 29) = 1.519; $p = 0.139$) and with skullcap treatment (t (df 29) = 0.738; $p = 0.466$) were not significant. They also showed a significant difference between groups in the effect of placebo, confirming an enhanced effect of placebo for Group 2 compared to Group 1 (t (df 29) = 2.135; $p = 0.041$).

Overall, these results suggest the reduction in anger and hostility scores from baseline following skullcap compared to placebo treatment was not significant.

A 2 x 2 ANOVA was conducted to determine the main treatment effects. This showed there was no significant difference ($F_{1, 29} = 3.534, p = 0.070$) between skullcap, mean (SD) = 7.13 (5.83) and placebo, mean (SD) = 9.97 (8.63).

7.4.4 Vigour-Activity

To determine whether these treatment effects were significant according to the order in which skullcap and placebo were taken a 2 x 3 mixed method ANOVA was conducted (Figure 7.7). This showed significant differences according to the order in which the interventions were taken as there was a significant between subjects effect according to group ($F_{1, 29} = 5.286; p = 0.029$). However, there was no significant treatment effect ($F_{2, 58} = 0.785; p = 0.461$) or group x treatment interaction ($F_{2, 58} = 1.615; p = 0.208$).

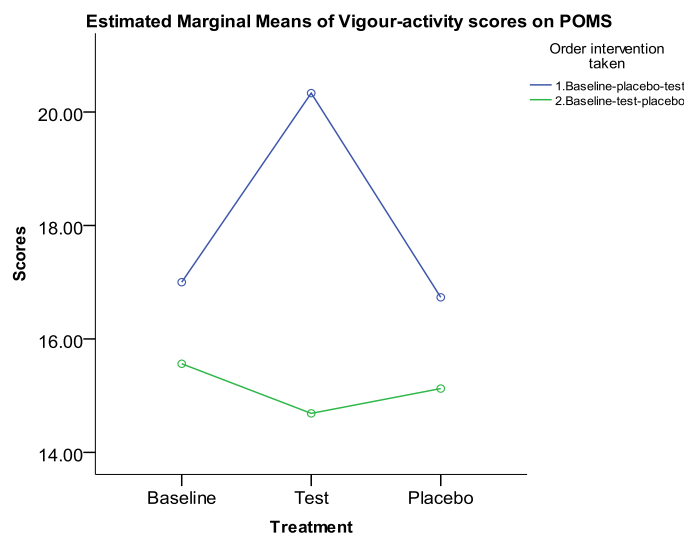


Figure 7.7 Group differences in the effects of skullcap test or placebo interventions on Vigour-Activity scores on POMS

Paired sample t-tests confirmed there were no significant changes in Vigour-activity scores from baseline for either skullcap ($t(df 30) = -1.219; p = 0.232$) or placebo ($t = 0.219; p = 0.828$) or differences between skullcap and placebo ($t(df 30) = 0.118; p = 0.213$). Mean (SD) changes in scores from baseline for Group 1 were from 17 (6.65) to 20.33 (3.83) for skullcap (20%) and to 16.73 (6.29) for placebo (- 2%). For Group 2 they were from 15.56 (4.97) to 14.69 (5.55) for skullcap (- 6%) and to 15.13 (5.4) for placebo (- 2.5 %).

Independent sample t-tests were conducted to analyse the group differences and these demonstrated a significant difference between groups in the effect of skullcap (t (df 29) = 3.276; p = 0.003) but not for baseline scores (t (df 29) = 0.685; p = 0.499) or for placebo (t (df29) = 0.788; p = 0.450). There was an enhanced effect of skullcap in increasing Vigour-activity for Group 1 compared to Group 2, in which there was a slight decrease in Vigour-activity scores with skullcap.

The results overall indicate that there was no significant difference between test and placebo in alteration of Vigour-activity scores but an enhanced effect of skullcap for Group 1 (in increasing energy levels).

A 2 x 2 ANOVA was conducted to determine overall treatment effects. This confirmed overall there was no significant difference ($F_{1, 29} = 1.416$, p = 0.244) between skullcap, mean (SD) = 17.42 (5.51) and placebo, mean (SD) = 15.90 (5.80).

7.4.5 *Fatigue-Inertia*

A 2 x 3 mixed methods ANOVA (Figure 7.8) showed there was no significant order effect according to group ($F_{1, 29} = 0.002$; p = 0.969), no significant treatment effect ($F_{2, 58} = 1.688$; p = 0.194) and no significant group x treatment interaction ($F_{2, 58} = 0.956$; p = 0.391).

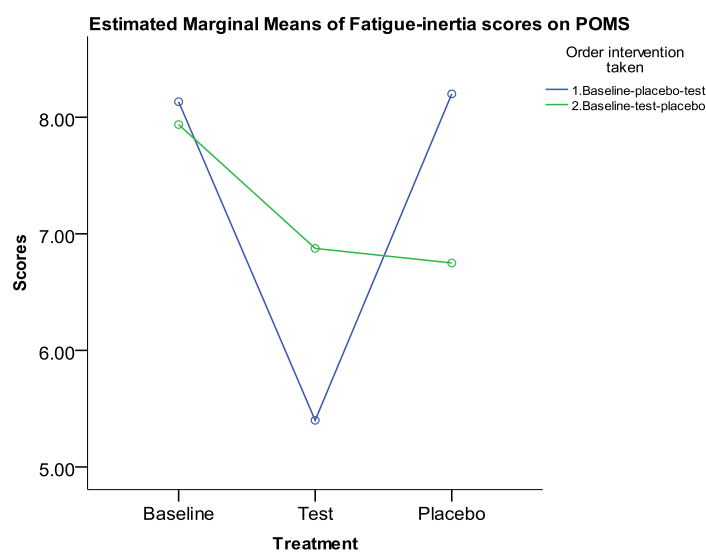


Figure 7.8 A 2 x 3 ANOVA demonstrating group differences in the effects of skullcap test or placebo interventions on Fatigue-inertia scores on POMS

Scores for Fatigue-inertia were demonstrated by the paired sample t-tests to show a significant change (decrease) from baseline scores for skullcap ($t(df\ 30) = 2.246; p = 0.032$) but not for placebo ($t(df\ 30) = 0.465; p = 0.645$). However, overall there was no significant difference in treatment effect between skullcap and placebo ($t(df\ 30) = -1.220; p = 0.232$).

A further analysis using independent sample t-tests showed there was no significant difference between groups in baseline ($t(df\ 30) = 0.116; p = 0.909$), skullcap test ($t(df\ 30) = -0.904; p = 0.373$) or placebo ($t(df\ 30) = 0.625; p = 0.537$) scores. Mean (SD) change scores for Group 1 were from 8.13 (4.5) to 5.4 (4.45) for skullcap (33%) and to 8.2 (6.36) for placebo (-1%); and for Group 2 from 7.94 (4.91) to 6.88 (4.62) for skullcap (13%) and to 6.75 (6.54) for placebo (14%). Overall, although there was an enhanced effect of skullcap compared to placebo in decreasing fatigue and inertia, the results showed the difference in treatment effects between skullcap and placebo for Fatigue-inertia were not significant, indicating overall that skullcap does not cause fatigue when taken orally.

To determine overall treatment effects a 2 x 2 ANOVA was conducted. This confirmed there was no significant difference ($F_{1, 29} = 1.609, p = 0.209$) between skullcap, mean (SD) = 6.16 (4.52) and placebo, mean (SD) = 7.45 (6.39).

7.4.6 Confusion-Bewilderment

For Confusion-bewilderment a 2 x 3 mixed method ANOVA (Figure 7.9) to see if there was a group sequence effect on results. This confirmed there was a significant overall treatment effect from baseline within subjects ($F_{2, 58} = 6.290; p = 0.003$), no significant order effect according to group ($F_{1, 29} = 1.078; p = 0.308$) and no significant group x treatment interaction ($F_{2, 58} = 1.055; p = 0.355$), indicating no significant difference overall between skullcap and placebo in their effects on Confusion-bewilderment.

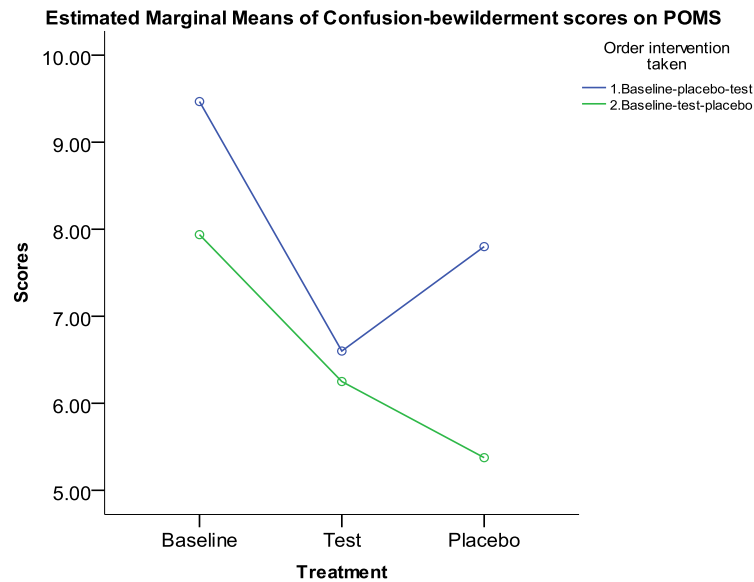


Figure 7.9 Group differences in the effects of skullcap test or placebo interventions on Confusion-bewilderment scores on POMS

The results were further analysed by paired sample t-tests, which showed there were significant differences in scores from baseline for both skullcap test (t (df 30) = 4.116; p < 0.001) and placebo (t (df 30) = 2.745; p = 0.010) but there was no significant difference in treatment effects between skullcap and placebo (t (df 30) = - 0.162; p = 0.873).

Independent sample t-tests carried out to analyse the mean (SD) differences in scores between Group 1 and Group 2 confirmed the differences between groups were not significant. A mean (SD) baseline score of 9.47 (5.24) for Group 1 and 7.94 (3.84) for Group 2 (t (df 29) = 0.930, p = 0.360) reduced to 6.6 (3.85) for Group 1 and 6.25 (3.53) for Group 2 (t (df 29) = 0.264; p = 0.794) with skullcap test (30% and 21% respectively). Changes with placebo from baseline were to 7.8 (5.49) for Group 1 (17.5%) and to 5.38 (4.65) for Group 2 (32%) (t (df 29) = 1.330, p = 0.194).

Overall skullcap had no significant effect on Confusion-bewilderment because there was no significant difference between skullcap and placebo. There was, however, an enhanced effect of placebo compared to skullcap for Group 2.

A 2 x 2 ANOVA to analyse main treatment effects revealed no significant difference (p = 0.838) between skullcap, mean (SD) = 6.42 (3.63) and placebo, mean (SD) = 6.54 (5.14).

7.4.7 Total Mood Disturbance

Mean Total Mood Disturbance (TMD) was calculated as the mean sum of all mood factors minus mean Vigour-Activity scores. On inspection of the percent decrease in scores from baseline TMD it appeared that there might be a significantly enhanced effect of skullcap over placebo in positively altering negative mood states overall as the percent change for skullcap was a mean decrease of 56% from baseline following administration of skullcap and 32% for placebo.

A 2 x 3 mixed method ANOVA (Figure 7.10) revealed no overall order effect for group ($F_{1,29} = 0.963$; $p = 0.335$). There was a significant treatment effect ($F_{2,58} = 7.117$; $p = 0.002$) but no significant group x treatment interaction ($F_{2,58} = 2.508$; $p = 0.090$) indicating the enhanced effect of placebo for those in Group 2 (Figure 7.10) was not significant. Changes in mean (SD) scores for Group 1 from baseline were from 45.27 (37.41) to 13.33 (27.99) with skullcap (70%) and to 34.93 (37.51) with placebo (23%). For Group 2 the scores changed from 31.06 (24.08) at baseline to 19.56 (18.06) with skullcap (37%) and to 17.25 (33.87) with placebo (44.5%) suggesting an enhanced effect for skullcap for Group 1 compared with placebo and compared with Group 2; and an enhanced effect of placebo for Group 2 compared with Group 1.

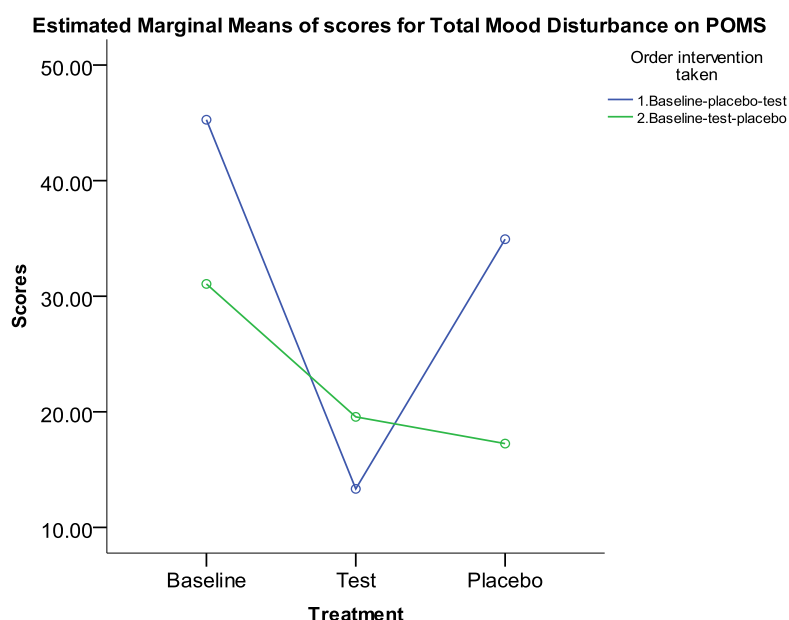


Figure 7.10 Mean scores of Total Mood Disturbance on POMS for within and between subjects in accordance with the order in which skullcap test or placebo interventions were taken

A paired sample t-test indicated significantly decreased scores on POMS TMD with skullcap from baseline (t (df 30) = 4.884; p = <0.001) and the decrease in scores from baseline for placebo to be not significant (t (df 30) = 1.864; p = 0.072). However, although there was an enhanced effect for the skullcap versus placebo, for overall treatment effects the difference between skullcap and placebo appeared to be not significant (t (df 30) = -1.408; p = 0.169).

Subsequently a 2-way ANOVA (Figure 7.11) was conducted on change scores from baseline to further analyse the differences between groups and skullcap and placebo treatment periods. This showed there was no significant difference in groups ($F_{1, 29}$ = 0.897; p = 0.351), no significant effect of treatment ($F_{1, 29}$ = 2.335; p = 0.137) and no significant group x treatment interaction ($F_{1, 29}$ = 3.589; p = 0.068).

Change scores (SD) with skullcap for Group 1 were – 32 (22) and -11.5 (23) for Group 2; with placebo change scores for Group 1 were -10.3 (37) and -13.8 (36.6) for Group 2. Independent sample t-tests to analyse change scores from baseline showed overall the results for Total Mood Disturbance suggest a significant difference between groups in the response to skullcap (t (df 29) = -2.534; p = 0.017) but not placebo (t (df 29) = 0.263; p = 0.794). From inspection of Figures 7.10 & 7.11 it can be seen there was an enhanced effect of skullcap for Group 1 compared to placebo and an enhanced effect of placebo for Group 2 compared to skullcap.

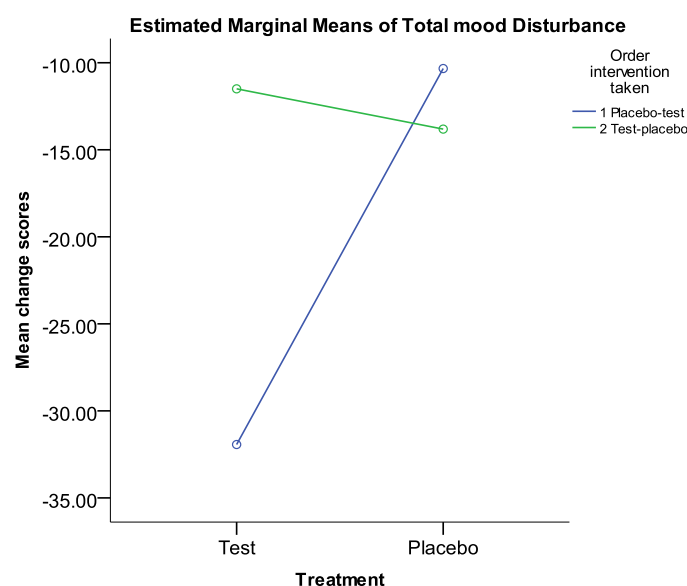


Figure 7.11 Mean differences in change scores between skullcap test and placebo for Total Mood Disturbance according to the order in which the interventions were taken

A 2 x 2 ANOVA was conducted to analyse the main treatment effects. This demonstrated that there was no significant difference ($F_{1,29} = 2.335, p = 0.137$) between skullcap, mean (SD) = 16.54 (23.21) and placebo, mean (SD) = 25.80 (36.21).

7.5 Salivary cortisol measures

In order to analyse mean changes in salivary cortisol concentration (nmol/L) in saliva at baseline, skullcap test and placebo, it was necessary to first replace missing variables. These were computed by means analysis for each time point for each period (3 hours, 6 hours, 9 hours and 12 hours for baseline, test and placebo) using the ‘replace missing variables’ in the transform function in SPSS.

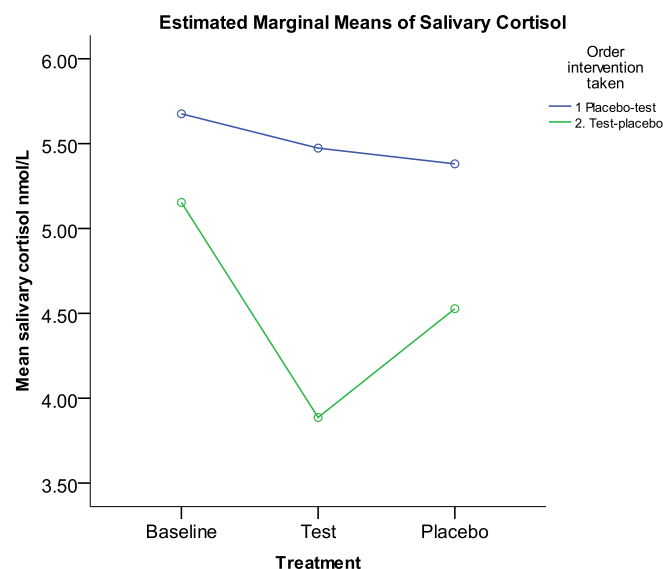


Figure 7.12 Mean salivary cortisol concentration (nmol/L) within and between subjects in accordance with the order in which skullcap test and placebo were taken

The 2 x 3 x 4 ANOVA (group x treatment x time) (Figure 7.12) showed no significant differences between Group 1 (placebo-test, $n = 14$) and Group 2 (test-placebo, $n = 12$) ($F_{1,24} = 0.981; p = 0.332$), no significant treatment effect ($F_{2,48} = 1.870; p = 0.165$) for either group and no significant within subjects group x treatment interaction ($F_{2,48} = 1.008; p = 0.373$).

It also showed that although both groups changed significantly for the 4 diurnal time points ($F_{3,72} = 29.702; p = < 0.0001$) as expected (Figure 7.13), with salivary cortisol levels being at their highest 3 hours following waking and declining throughout the day (Kudielka & Kirschbaum 2003), there were no significant group x time ($F_{3,72} = 0.310; p = 0.818$), treatment x time ($F_{6,144} = 0.164; p = 0.986$) or group x time x treatment ($F_{6,144} = 0.164; p = 0.986$) or group x time x treatment ($F_{6,144} = 0.164; p = 0.986$).

$_{144} = 1.942$; $p = 0.078$) interactions, indicating no significant diurnal effects of skullcap and no significant diurnal differences between skullcap and placebo.

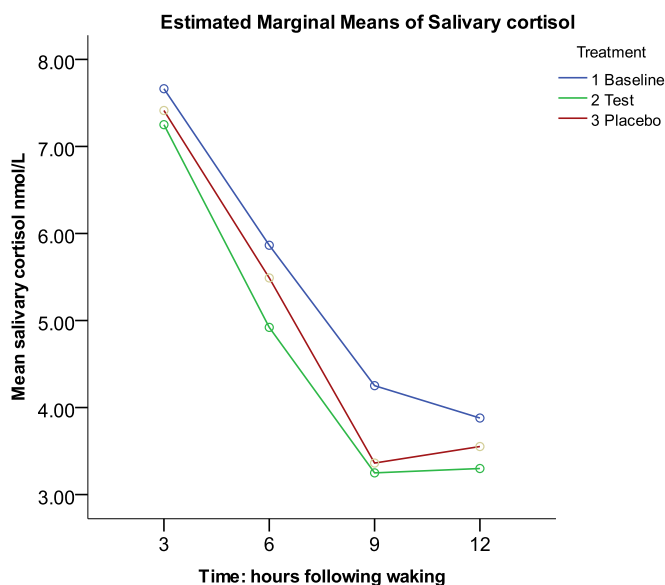


Figure 7.13 Comparison of changes in salivary cortisol concentration from baseline over time according to treatment (skullcap test or placebo)

Paired sample t tests ($n = 12$) for each time point (3, 6, 9 and 12 hours following waking) between baseline and skullcap, baseline and placebo, and skullcap and placebo, showed no significant differences between any pairs: ($p > 0.091$):

Baseline – skullcap (3h, $p = 0.769$; 6h, $p = 0.156$; 9h, $p = 0.091$; 12h, $p = 0.298$);
 Baseline – placebo (3h, $p = 0.870$; 6h, $p = 0.587$; 9h, $p = 0.099$; 12h, $p = 0.515$);
 Skullcap – placebo (3h, $p = 0.098$; 6h, $p = 0.545$; 9h, $p = 0.694$; 12h, $p = 0.649$).

To analyse the main treatment effects a 2×2 ($\times 4$) (group \times treatment \times diurnal time points) ANOVA was conducted. The results demonstrated that skullcap had no significant effect on salivary cortisol measurements. There was no significant difference ($F_{1, 24} = 0.418$; $p = 0.524$) between skullcap, mean (SD) = 4.74 (3.68) and placebo, mean (SD) = 4.98 (3.89).

7.6 Safety and tolerability

7.6.1 ALT levels

A 2×3 mixed ANOVA (Figure 7.14), showed there was no significant effect of group ($F_{1, 29} = 0.166$; $p = 0.687$). There were no overall significant changes within subjects in

ALT levels according to treatment ($F_{2, 58} = 2.192$; $p = 0.121$) and no significant group x treatment interaction ($F_{2, 58} = 0.426$; $p = 0.655$).

Mean values (SD) in U/L at baseline = 9.72 (4.61), following skullcap = 11.74 (5.32) and following placebo = 12.14 (9.11). Paired sample t-tests revealed the ALT changes from baseline with skullcap were significant (t (df 30) = - 2.513; $p = 0.018$) but not the ALT changes from baseline with placebo (t (df 30) = - 1.825; $p = 0.078$). However, the tests also demonstrated that there was no significant difference in ALT levels between skullcap and placebo in treatment effects (t (df 30) = - 275, $p = 0.785$).

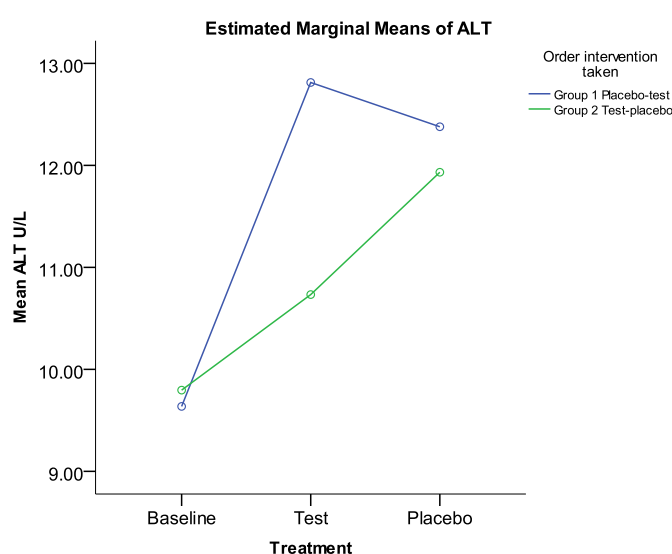


Figure 7.14 Mean plasma ALT levels (U/L) for treatment x group with *S. lateriflora* test and *Urtica dioica folia* placebo

Although there was a significant increase in ALT levels following administration of skullcap according to the results of the t-tests, levels were still well within the normal range of ≤ 22 U/L (men) and ≤ 17 U/L (women) at 25°C (Roche 2008).

A 2 x 2 ANOVA to analyse main treatment effects demonstrated there was no significant difference between skullcap and placebo ($F_{1, 29} = 0.065$, $p = 0.801$).

One male participant (13) had raised ALT levels to 51 U/L, a level slightly above the normal range, on his second visit (the final day of placebo treatment), (baseline = 12.5; test = 11.3; placebo = 51.0 U/L) but admitted to drinking alcohol a few hours previously. Recent alcohol consumption may give a higher reading (Pratt & Kaplan 2000). The test was repeated using a different Reflotron machine to ensure there was no error due to

faulty equipment or to operator error and the reading was the same. He was advised to see his G. P. for follow-up.

7.6.2 Blood pressure

Changes in blood pressure were not particularly expected and blood pressure tests were for purposes of observation and health monitoring. A plot demonstrating means of systolic and diastolic blood pressures at each treatment period (Figure 7.15) provides a visual representation of minimal change from baseline or difference between skullcap and placebo in their effects on blood pressure or pulse pressure (the difference between systolic and diastolic). The recommended average healthy blood pressure in adult humans is 120/80 mm/Hg (Beers *et al.* 2006) so the results indicate there were no alterations due to either skullcap or placebo. Paired sample t-tests were carried out for confirmation. However, to determine whether there might be significant changes in blood pressure within and between groups according to the order in which the interventions were taken a 2 x 3 mixed method ANOVA was also carried out for both mean systolic (Figure 7.16) and diastolic (Figure 7.17) blood pressures.

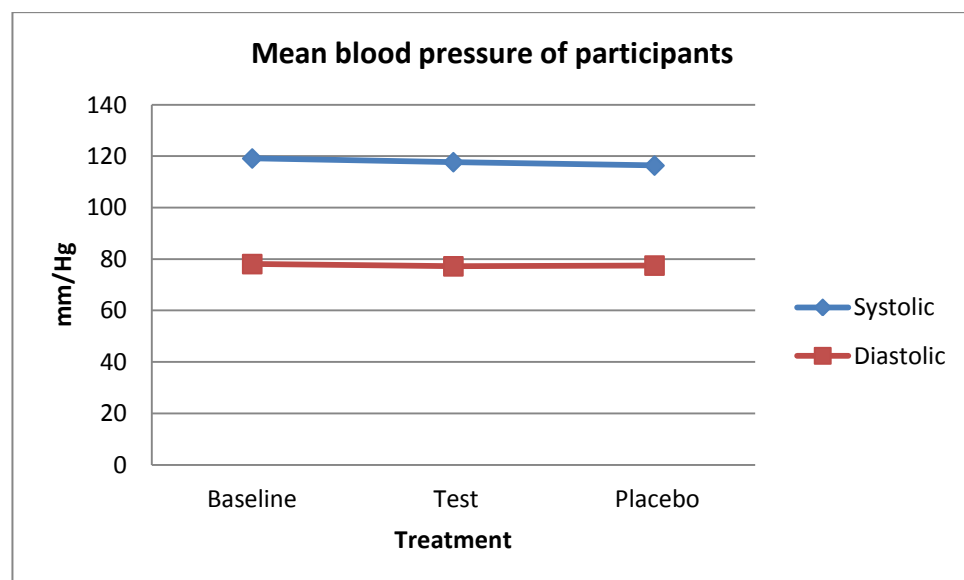


Figure 7.15 Plots demonstrating the mean systolic and diastolic blood pressures (mm/Hg). Note that mean scores remained within the recommended healthy range for adults (Beers *et al.* 2006) following skullcap and placebo treatments, with no significant changes according to treatment x time

7.6.2.1 Systolic

A 2 x 3 mixed method ANOVA (Figure 7.16) demonstrated, although Group 2 (test-placebo) showed a greater decrease in mean (SD) systolic blood pressure from baseline; difference = 117.31 (16.15) – 113.56 (13.86) with skullcap (3%) and 117.31 (16.15) – 111.5 (13.08) with placebo (5%) than Group 1 (placebo-test), mean (SD) difference from baseline = 121.13 (18.43) – 122.13 (20.54) with skullcap (an increase of < 1%) and 121.13 (18.43) – 121.6 (17.37) with placebo (an increase of < 5%) this was not significant. There was no significant order effect of group ($F_{1, 29} = 1.76$; $p = 0.195$), no significant effect of treatment ($F_{2, 58} = 1.16$; $p = 0.320$) and no significant group x treatment interaction ($F_{2, 58} = 3.06$; $p = 0.091$).

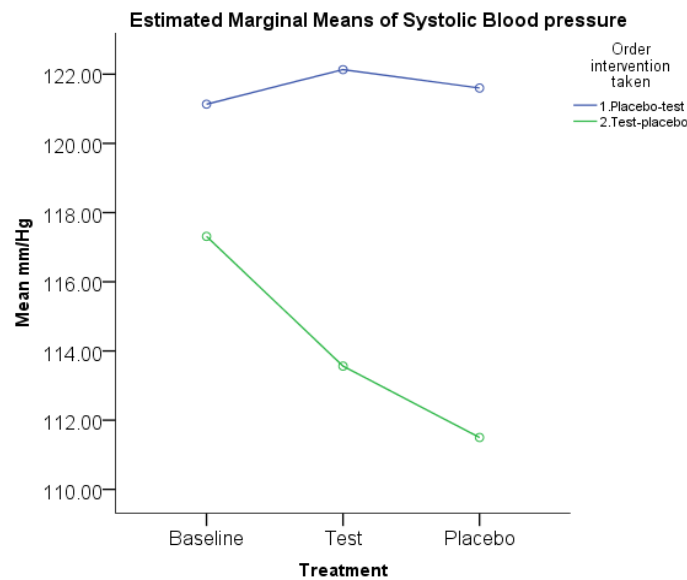


Figure 7.16 Mean systolic blood pressure (mm/Hg) showing within and between subjects effects to be non-significant in accordance with the order in which the interventions were taken

A paired sample t-test confirmed there was no significant change from baseline in systolic blood pressure following either *S. lateriflora* or placebo. Mean (SD) systolic pressure at baseline was 119.61 (17.1), with skullcap 117.71 (17.66) and with placebo 116.39 (15.9). The differences between baseline and skullcap ($t(df 30) = 0.76$; $p = 0.454$), baseline and placebo ($t(df 30) = 1.497$; $p = 0.145$) and skullcap and placebo ($t(df 30) = 0.86$; $p = 0.395$) were not significant.

A 2 x 2 ANOVA to determine main treatment effects confirmed the difference between skullcap and placebo was not significant ($F_{1, 29} = 0.700$, $p = 0.410$).

7.6.2.2 Diastolic

Similarly for diastolic blood pressure, a 2 x 3 mixed method ANOVA (Figure 7.17) confirmed there was no order effect between groups: Group 1 mean (SD) difference from baseline = 79.2 (11.01) – 79.2 (10.98) with skullcap (0%) and 79.46 (10.37) with placebo (< 0.5% increase); group 2 mean (SD) difference from baseline = 77.06 (10.20) – 75.375 (10.41) with skullcap (2%) and 77.06 (10.20) – 75.69 (10.64) with placebo (< 2%).

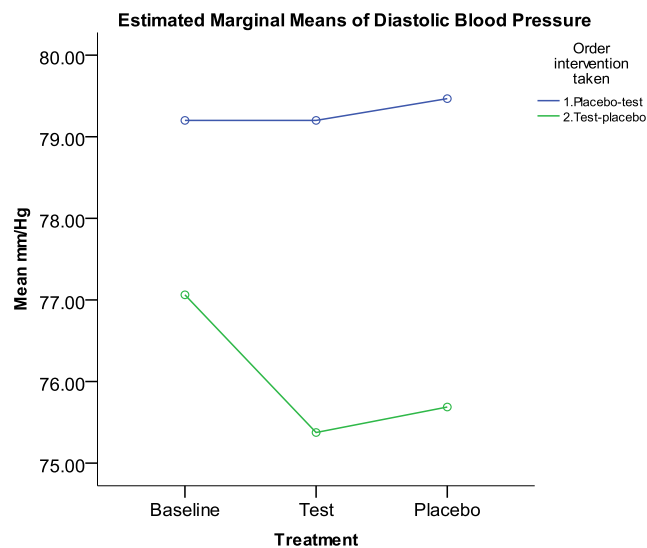


Figure 7.17 Mean diastolic blood pressure (mm/Hg) showing within and between subjects effects in accordance with the order in which the intervention was taken

There was no significant group effect ($F_{1, 29} = 0.874$; $p = 0.358$), no significant effect of treatment ($F_{2, 58} = 0.201$; $p = 0.819$) and no significant group x treatment interaction ($F_{2, 58} = 0.253$; $p = 0.778$), confirming there were no significant effects on diastolic blood pressure for either skullcap or placebo or significant differences between skullcap and placebo in their effects on diastolic blood pressure.

Paired sample t-tests showed there were no significant changes. Mean (SD) diastolic pressure was 78.1 (10.47) at baseline, 77.22 (SD 10.69) following skullcap and 77.51 (10.51) after placebo. Differences between baseline and skullcap ($t(df 30) = 0.663$; $p = 0.513$), baseline and placebo ($t(df 30) = 0.43$; $p = 0.669$) and skullcap and placebo ($t(df 30) = -0.216$; $p = 0.830$) were not significant.

A 2 x 2 ANOVA, which was conducted to determine main treatment effects, confirmed there was no significant difference overall between skullcap and placebo ($F_{1,29} = 0.045$, $p = 0.834$).

7.6.3 Pulse rate

No irregularities or abnormalities such as bounding or rates that were too high (> 100 BPM) or too low (< 60 BPM), except in trained athletes (normal BPM = 40 – 60) (Vorvick 2012) were palpated in the radial pulse of any participants during initial screening. Changes from a mean (SD) of 69.65 (9.56) baseline beats per minute (BPM) were to 68.58 (8.09) with skullcap (1.5% decrease) and to 71.16 (9.76) with placebo (1.5% increase).

To see if the results might be significant according to group a 2 x 3 mixed method ANOVA was conducted (Figure 7.18). This revealed there was no significant effect of group ($F_{1,29} = 0.090$; $p = 0.766$), no significant group x treatment interaction ($F_{2,58} = 0.172$; $p = 0.842$) and no overall effect of treatment ($F_{2,58} = 1.054$; $p = 0.355$), confirming neither skullcap nor placebo had any significant effects on pulse rate overall.

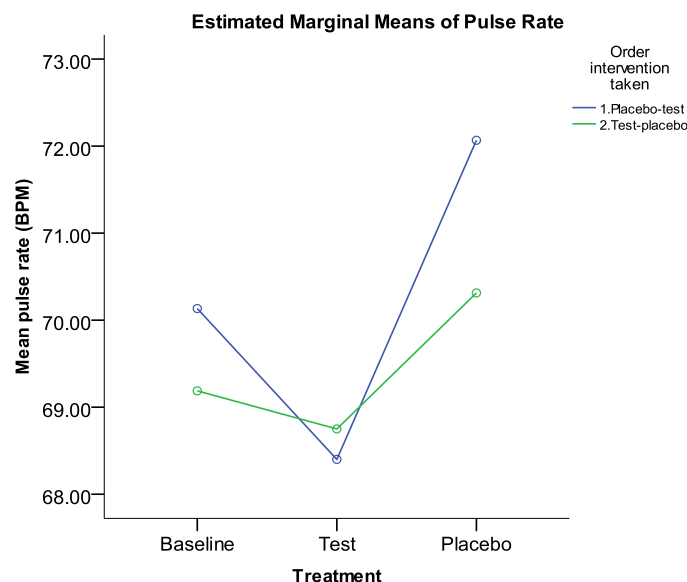


Figure 7.18 Mean pulse rate (BPM) showing within and between subjects effects in accordance with the order in which the interventions were taken

Paired sample t-tests indicated there was an insignificant mean change in pulse rate from baseline for either skullcap ($t(df 30) = 0.594$; $p = 0.557$) or placebo ($t(df 30) = -0.827$; $p = 0.415$) and suggested the difference in pulse rate between skullcap and placebo ($t(df 30) = -1.498$; $p = 0.145$) was also not significant.

A 2 x 2 ANOVA also confirmed there was no significant difference between skullcap and placebo in treatment effects ($F_{1,29} = 2.251, p = 0.144$).

Results demonstrated that skullcap has no effect on pulse rate, which remained within the normal range (Vorvick 2012), in healthy adults.

7.6.4 Summary of main effects

To assess the main differences between skullcap and placebo a series of 2 x 2 ANOVAs was conducted on all variables. The results (Table 7-5) demonstrated no significant differences overall between skullcap treatment and placebo for any variables analysed.

Table 7-5 Main differences in treatment effects between skullcap and placebo

Variables tested	Skullcap mean (SD)	Placebo mean (SD)	Significance of treatment effect: $p =$
BAI	6.29 (4.79)	7.19 (5.99)	0.191
POMS T-A	8.74 (5.85)	9.74 (6.97)	0.473
POMS D-D	5.84 (6.64)	8.03 (8.85)	0.067
POMS A-H	7.13 (5.83)	9.97 (8.63)	0.070
POMS V-A	17.42 (5.51)	15.90 (5.80)	0.244
POMS F-I	6.16 (4.52)	7.45 (6.39)	0.209
POMS C-B	6.41 (3.63)	6.54 (5.14)	0.838
POMS TMD	16.54 (23.21)	25.80 (36.21)	0.137
Salivary cortisol	4.74 (3.68)	4.98 (3.89)	0.524
Systolic BP	117.71 (17.66)	116.38 (15.90)	0.410
Diastolic BP	77.22 (10.69)	77.51 (10.51)	0.834
Pulse	68.58 (8.09)	71.16 (9.76)	0.144
ALT	11.74 (5.32)	12.14 (9.11)	0.801

Key: BAI = Beck Anxiety Inventory; POMS = Profile of Mood Disturbances: T-A = tension Anxiety; D-D = Depression-Dejection; A-H = Anger-Hostility; V-A = Vigour Activity; F-I = Fatigue-Inertia; C-B = Confusion-Bewilderment; TMD = Total Mood Disturbance; BP = Blood Pressure; ALT = Alanine aminotransferase

7.6.5 Toxicity and side – effects

No participants experienced any serious adverse reactions during the study and only minor and infrequent side-effects were reported by seven participants whilst taking *S. lateriflora*. Of these, one had vivid dreams, one felt ‘spaced out’, four reported mild digestive disturbances and one reported a constant taste of salt in her mouth. For details see sections 7.7.5 & 7.7.6 of participant diary symptom reports. Although it is uncertain whether these side-effects can be attributed to skullcap, mild digestive upset and vivid dreaming were symptoms also reported by two practitioners responding to a survey on their use of *S. lateriflora*, in one each of their patients (see 3.4.9).

7.7 Participant symptom diary report: results and discussion notes

Participants were given a symptom diary (Appendix XVI) in which they were requested to list any physical or mental symptoms they experienced during the study, whether new or old, both negative (Figure 7.19 & Figure 7.20) and positive (Figure 7.21 & Figure 7.22), including the disappearance of old symptoms. They were also asked to write on a separate piece of paper if necessary and to report anything unusual they felt about their experiences whilst taking part in the study should they wish to do so. Participant statements are reported separately below (see 7.8). All completed a diary except participants 14, 16, 29, 30 and 32 (n = 26).

7.7.1 Negative mood symptoms reported by participants

Diary reports of negative mood symptoms and low energy will only be briefly alluded to; stress, anxiety, anger and depression have been assessed in this clinical study by the use of validated instruments, Beck Anxiety Inventory (Beck & Steer 1993) and the Profile of Mood States (Lorr *et al.* 2005), and physiological measurements of stress, and will be discussed in the main discussion section (Chapter 8).

7.7.1.1 Stress

Stress was reported by 7 participants, 2 during skullcap treatment for one day each - the first and last day of treatment respectively and 1 of these participants reported stress during the skullcap period only. Of the others 1 reported stress at baseline, 4 during placebo treatment for 2 days each and 2 of these were also stressed during washout. There was generally no correlation between the days stress was reported and the days on which saliva samples were taken in these participants.

7.7.1.2 Depression

Two responders in a survey of herbal medicine practitioners (Chapter 3) said they found *S. lateriflora* worsens severe depression. In this research seven participants reported feeling low in mood; described as tearful, upset, emotional, sad, despondent or depressed and one of these said they felt depressed (for a day) only during skullcap treatment and one (for a day) only during placebo. Of 5 who experienced low mood during skullcap, 4 experienced symptoms during placebo treatment with one of these also feeling low at washout and at baseline. At a total of 11 participant days amongst 5 participants periods of depression whilst on skullcap were short-lived however, compared to a total of 16 participant days for placebo. There is no indication from the above results that it was the skullcap that caused the depression in the individuals experiencing it during the study.

7.7.1.3 Anxiety

Anxiety symptoms, described by participants as feeling anxious, worried, nervous or apprehensive, were experienced by 5 people. Two reported symptoms during skullcap treatment for one day each as opposed to 3 during placebo for a total of 10 days. One person reported feeling anxious only whilst taking skullcap. One person had a panic attack - on the second day of the washout period following cessation of skullcap treatment. As this coincided with what she described as bad premenstrual syndrome it cannot be linked to cessation of skullcap treatment.

7.7.1.4 Anger

Anger, which can manifest during intense anxiety (Pary *et al.* 2003), was described as feeling irritable, bad tempered, grumpy, annoyed or angry by 6 participants. This was mostly during the placebo treatment - by 5 participants for a total of 18 participant days and during a 7 day washout – by 2 participants for a total of 7 participant days. The two who felt anger during washout had taken the interventions in reverse order to one another so there was no relevance regarding whether there was a residual effect of either skullcap or placebo. Only 2 felt angry whilst on skullcap (for 1 day and 2 days respectively) but felt angry for longer periods during placebo (4 days each) and washout (4 days and 3 days respectively). Overall there was less anger experienced by participants whilst on skullcap.

7.7.1.5 Other negative mood symptoms reported

Other negative mood symptoms reported by participants were poor concentration and feeling unmotivated. Poor concentration was reported by 2 participants, one for one day of taking skullcap. However, this same person reported improved concentration whilst on skullcap for another 4 days during the remainder of the skullcap period. The other participant reported poor concentration during the washout period for 2 days.

Feeling ‘foggy-headed’ (unable to think clearly) was reported by 2 participants, one during skullcap for one day. The other reported foggy-headedness for 3 days during skullcap and 4 days during placebo treatment, she also felt ‘spaced out’ on 2 separate days during skullcap treatment. Foggy-headedness cannot be attributed to skullcap as it was experienced also with placebo for an equal number of days. Although “feeling spaced out” is a possible idiosyncratic reaction to skullcap by one participant, it cannot be definitively attributed to the herb.

Feeling unmotivated was reported only during placebo treatment (2 participants for 9 days) and during washout (1 participant for 1 day). There is therefore no evidence from the diary alone that skullcap affects concentration or motivation.

7.7.2 Low energy

Of 8 participants who reported states of low energy such as feeling tiredness, fatigue, lethargy or excessive yawning, 6 of these experienced the symptoms whilst on skullcap, 2 reported at baseline, 5 whilst on placebo and 5 during washout. Only 1 participant reported extreme tiredness only whilst on skullcap but this participant also reported disturbed sleep whilst taking skullcap, which could account for the tiredness. Those reporting these symptoms experienced them for an equal number of days in total (44) for both skullcap and placebo and for 4 days at baseline and 11 days during the washout period. High energy was reported by 3 whilst on skullcap, 5 on placebo and 1 during washout. It appears there was no difference between skullcap and placebo in relation to energy levels but the effects of skullcap on energy were measured by the Vigour-Activity and Fatigue-Inertia factors on the POMS and will be discussed in Chapter 8.

7.7.3 Headaches

Headaches or migraines were reported by 12 participants (11 headache, 1 migraine), with 5 experiencing the symptoms whilst taking skullcap for a total of 14 participant

days. Nine had headaches during placebo for a total of 18 participant days; 1 at baseline (both days) and 1 during washout (4 days). Of those reporting headaches whilst on skullcap, 2 had them only during this period and one of these had a migraine on two separate days. All who had headaches during the skullcap period reported them during the first 5 days, with none thereafter for the remaining 9 days. Those with headaches during placebo reported them scattered throughout the placebo treatment period.

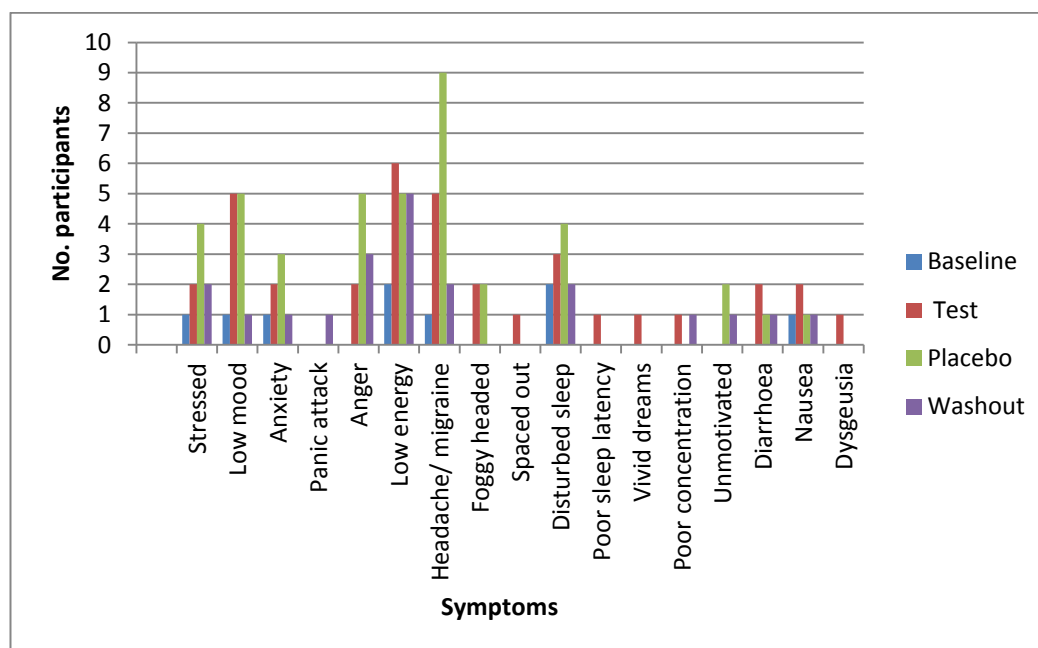


Figure 7.19 No. participants reporting negative symptoms in their diaries during the randomised controlled trial

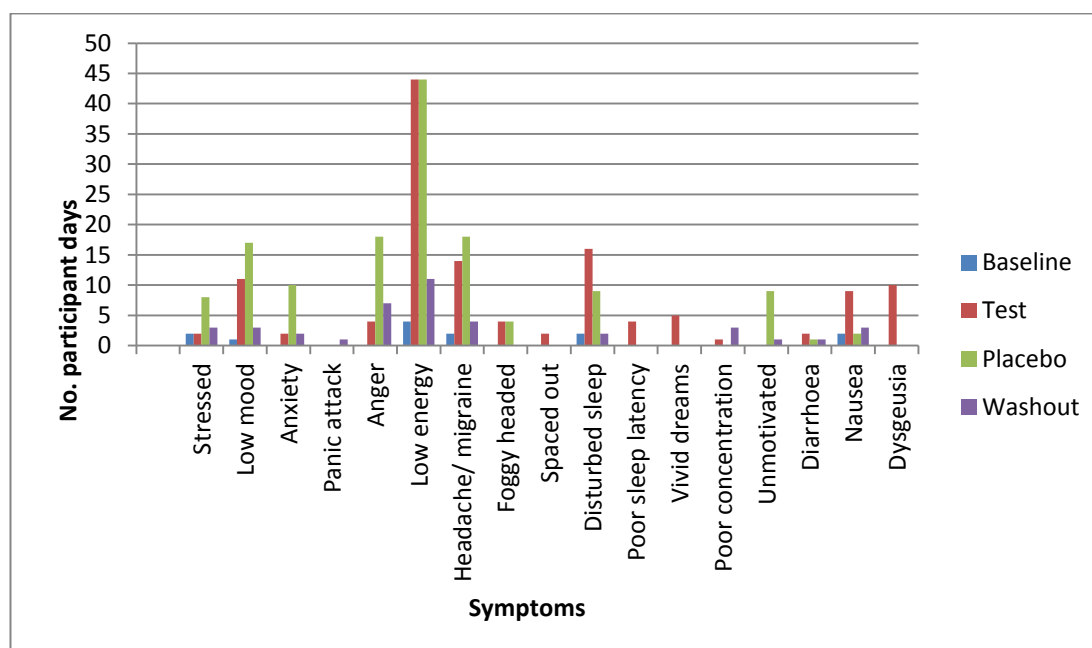


Figure 7.20 No. participant days on which negative symptoms were reported in the diaries during the randomised controlled trial

7.7.4 Positive mood symptoms reported in participant diaries

Symptoms reported by participants in their diaries were grouped together by closest match, using their own words. None reported any positive mood symptoms during the first 2 days (no treatment). Feeling “calm” or “relaxed”, “chilled” or “no anxiety” were grouped as calm/relaxed and reported by a total of 11 participants; 7 whilst taking skullcap for a total of 45 participant days, 6 on placebo for a total of 36 days and 1 during washout for 1 day. Although there was little difference between skullcap and placebo in the number of participants with these states of low anxiety as reported in their diaries, it lasted for longer periods during skullcap treatment. It is notable that during skullcap treatment calm feelings were reported for 45 participant days in contrast to feelings of anxiety reported for 2 days (net 43 participant days calm). During placebo treatment feelings of calm were reported for 36 days in the diaries in contrast to feelings of anxiety reported for 10 days (net 26 participant days calm).

The main positive symptom reported during skullcap treatment was an enhanced mood, reported as feeling happy, in a good mood or contented, reported by 8 participants for a total of 54 participant days (compared with a total of 49 participant days by 11 people for placebo and washout periods together). Furthermore, positive or hopeful feelings whilst taking skullcap were reported by 4 participants - for a total of 14 participant days. This was not reported during placebo, washout or baseline periods.

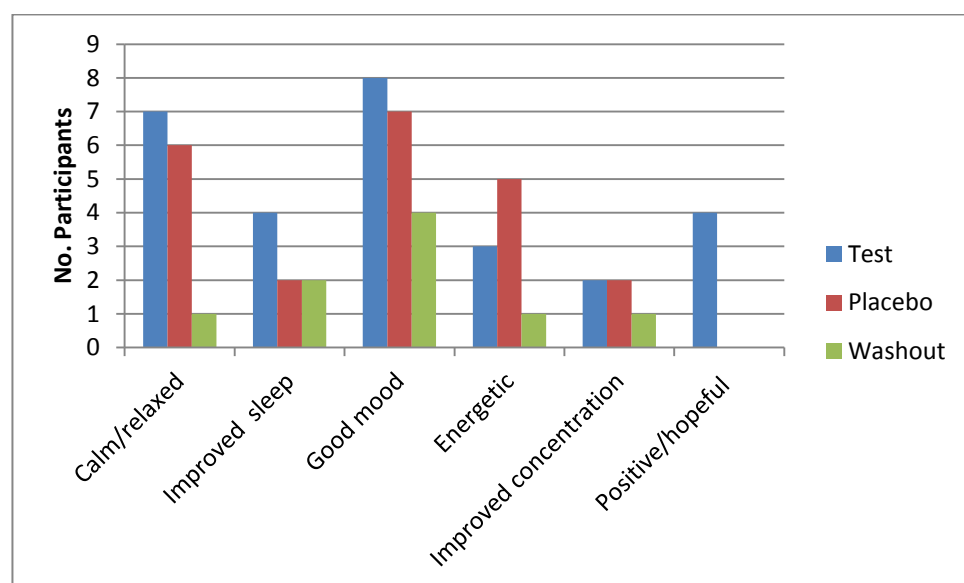


Figure 7.21 No. participants reporting positive mood symptoms in their diaries during the randomised controlled trial

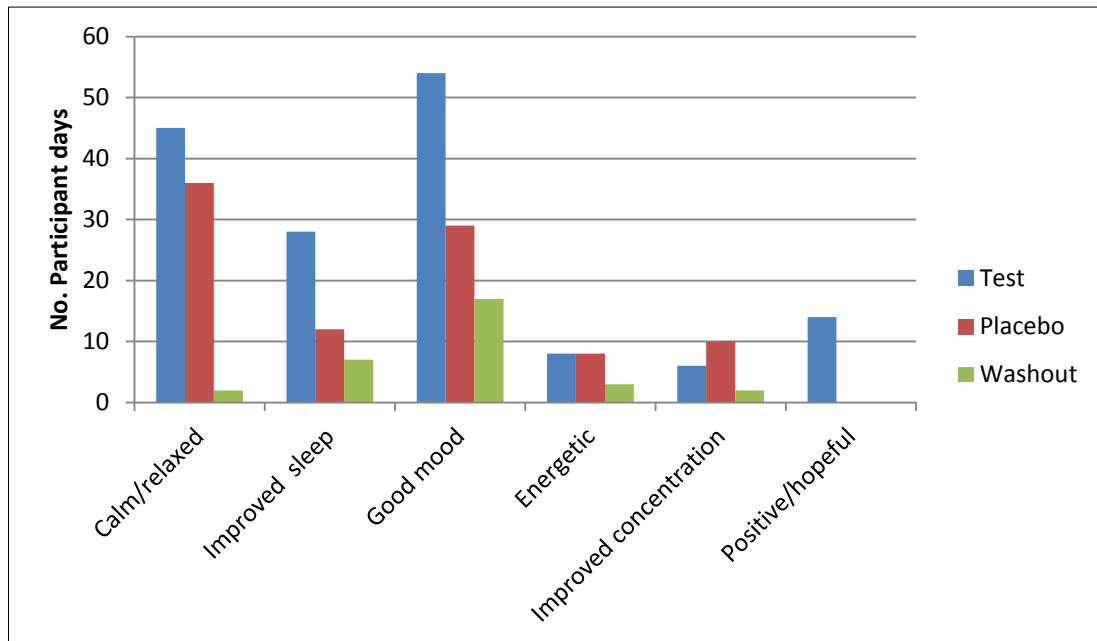


Figure 7.22 No. participant days on which positive mood symptoms were reported by participants in their diaries during the randomised controlled trial

7.7.5 Sleep experiences

Disturbed sleep was experienced by 6 participants; for a total of 16 participant days by 3 participants during skullcap treatment, 9 days by 4 participants during placebo treatment and for 2 days by 2 participants during both baseline and washout. Two of the participants who experienced sleep disturbances during skullcap experienced it during placebo too and had taken the skullcap first and placebo second so it is unclear whether the restlessness was due to a carryover effect of the skullcap. One of these also reported 2 days good night's sleep whilst on skullcap and these same two participants also reported sleep disturbances prior to commencement of any treatment (baseline). Only one participant reported sleep disturbance whilst on skullcap alone – waking after only 5 hours and not being able to get back to sleep on the 8th to 11th night inclusively before sleep patterns normalised. Interestingly, poor sleep latency during skullcap treatment reported by one participant was also on the 8th to 11th night inclusively - before sleep patterns normalised.

Another participant had vivid dreams for five days whilst taking skullcap, from the 10th to 14th night inclusively on the herb and stopping immediately the washout period

commenced. This person also reported having deep sleep from 5th to 14th day of using skullcap.

Insomnia is a common symptom of anxiety (Pary *et al.* 2003) so it was hypothesised that overall there would be an improvement in sleep patterns amongst the study participants whilst on skullcap. However, better sleep whilst taking skullcap was reported by only 4 participants for a total of 28 days. They also reported feeling refreshed in the morning. Two reported improved sleep whilst on placebo for a total of 12 days and also during washout for a total of 7 days. Of the 5 participants who reported better sleep, only one reported this only whilst on placebo and also during washout. Of the 4 reporting improved sleep whilst on skullcap, the first day of improvement was on days 3, 3, 5 and 7 respectively. One participant said she felt she needed no more than 5 hours sleep as she has so much energy (whilst on skullcap).

Although the sleep data is subjective and reported in a number too small to have any statistical significance, overall it might be that it may take up to a week for skullcap to begin to improve sleep quality or, in certain individuals, to begin to cause restlessness during the second week. It is not known however, how many participants were previously poor sleepers so it is possible that those reporting improved sleep whilst taking skullcap were unique amongst the sample in suffering from chronic poor sleep. A future study could assess the effectiveness of *S. lateriflora* on insomnia.

Only one participant reported extreme tiredness only whilst on skullcap but this participant also reported disturbed sleep whilst taking skullcap, which could account for the tiredness. Of interest regarding the wakefulness during skullcap treatment is that it is known that *Valeriana officinalis*, another herbal anxiolytic, extracts of which interact with GABA_A receptors *in vitro* (Cavadas *et al.* 1995), can also cause a similar problem in certain individuals, who find it stimulating (Mills & Bone 1999).

7.7.6 Miscellaneous symptoms reported

Various other symptoms reported in the diaries include 4 participants with attacks of diarrhoea for one day each; 2 during skullcap, one during placebo and one during washout. Nausea was reported by another 2 participants. One had the symptoms only whilst on skullcap (one day) while the other experienced episodes of nausea throughout the study but particularly during skullcap treatment (8 days) and both baseline days. It

is not certain whether the symptoms were as a result of either of the interventions as nausea is a known potential comorbidity of anxiety (Pary *et al.* 2003).

One participant had a constant taste of salt in her mouth for 10 days during skullcap treatment. The reason for this is unclear. A large number of medications (> 75 types including CNS drugs such as anti-epileptics, antidepressants and antipsychotics) can cause taste disorders or dysgeusia in some individuals, amounting to around 32% of cases. Some diseases, for example gastro-oesophageal reflux disease, diabetes, endocrine disease and Bell's palsy are known to be a cause of dysgeusia, which may manifest as a persistent salty taste in the mouth (Collet *et al.* 2007). However, the participant was otherwise well and not taking any other medications.

7.7.7 Disappearance, or reduced severity of, old symptoms

Five participants reported a resolution or improvement in non serious but chronic conditions. One reported her irritable bowel syndrome had cleared up from the 10th day of skullcap treatment (first intervention) and it did not return for the remainder of the study. Another reported that she normally experiences intense premenstrual mastalgia and dysmenorrhoea, yet this did not occur for the usual few days prior to menstruation; only for 10 minutes at onset of menstruation, which was at 2 days following skullcap treatment (during the washout period). This same participant reported her hay fever symptoms, described as runny nose and sneezing, as being milder than usual during skullcap treatment.

Another participant reported itching eczema throughout placebo treatment (first intervention) followed by an improvement for 5 days from the fifth day of skullcap treatment. The itching did, however, return for the remaining 5 days of the study.

Two participants reported a disappearance of muscular aches and pains. One experienced muscle aches throughout the 2 days of no treatment at baseline with a disappearance of the pain from day 3 of skullcap treatment. The pains did not return during the 7 day washout period but reappeared intermittently during the placebo period. The other participant reported a return of muscle pains on day 11 of placebo treatment (second intervention).

The above conditions are some of those for which *S. lateriflora* is commonly prescribed. It is used for allergies, skin conditions and inflammation (Natural Medicines Comprehensive Database 2011), which may account for reported improvements in eczema and hay fever symptoms. It is also believed to relieve muscle spasm in irritable bowel syndrome directly by the action of volatile oils on the smooth muscles of the colon (Khosh 2000) as well as due to an anxiolytic action of the herb. Physical symptoms of anxiety include irritable bowel (B.M.A. 2002) and *S. lateriflora* is traditionally prescribed for anxiety-related digestive disturbances (Bergner 2002). Muscle tension and back pain are also manifest symptoms of anxiety and the herb is also commonly prescribed for these conditions (Joshee *et al.* 2002; Hull 2010). It is also used for mastalgia and premenstrual tension (Greenfield & Davis 2004; Hull 2010) which may explain the alleviation of these symptoms reported by one participant. *S. lateriflora*'s antispasmodic actions (Felter & Lloyd 1898) are as likely as its anti-inflammatory and anxiolytic effects (Greenfield & Davis 2004) to have contributed towards the attenuation of her dysmenorrhoea.

Relief of hay fever and eczema symptoms reported by two participants during skullcap treatment is potentially explained by the well known phenomenon that onset or exacerbation of symptoms of allergies are often preceded by emotional or physical stress (Jefferies 1994). *S. lateriflora* is traditionally used to alleviate emotional stress (Bergner 2002; Gao *et al.* 2008).

7.8 Participant comments: with discussion notes on unexpected responses to interventions

According to the BAI (Beck & Steer 1993), of the 31 participants who completed the study, 11 were initially experiencing minimal anxiety (BAI scores 0 -7), 14 were mildly anxious (BAI scores 8-15), 3 were moderately anxious (BAI scores 16-25) and 3 had severe anxiety (≥ 26).

The following comments are reported exactly the way in which they were written by participants. No comments have been omitted from the sample of 13 participants who wrote down their experiences in the form of a commentary. In addition to comments from participants, any unusual responses, in particular when there was a likely placebo response, are noted in this section. A full discussion on placebo effects is in the main discussion section in Chapter 8.

7.8.1 Possible placebo responders

All participants completed a personality test, the Big 5 Minimarker (Saucier 1994b) and for interest the predominant personality traits will be noted for each participant mentioned in this section in order to determine whether there is a particular personality type that responds to placebo.

The following 2 participants, for whom there were notable alterations in scores whilst taking placebo as the first intervention, intimated satisfaction with the corresponding change in their mood.

One participant (P.05) said that after 5 days of taking capsules (first intervention):

“I felt more sociable, not anxious - not worried about anything. I had no panic attacks. I felt good even though I was ill with a cold, I had problems at work and my boyfriend was away. My mind was not racing and I was thinking clearer”. (P.05)

She also reported that she was sleeping better and did not take a long time to fall asleep as she would normally.

In her diary she marked the second day of taking the placebo with a large, black ‘X’ and *“last strong feeling of anxiety”*.

On later revelation of the code it was evident that this participant was taking placebo first. Her score on BAI had reduced by 19 points with the placebo from 32 (severe anxiety) to 13 (mild anxiety), which is considered to be a statistically significant change. In a previous study of 20 participants (Prasko *et al.* 2006) a mean reduction (SD) of 8-12 points (3-5) in subjective anxiety on BAI (Beck & Steer 1993) following moclobemide (a monoamine oxidase inhibitor) pharmacotherapy was calculated to be statistically significant.

Her BAI score increased slightly to 14 on skullcap. Furthermore, whilst taking skullcap she reported difficulty in falling asleep. Her Tension-Anxiety (T-A) scores on POMS also decreased significantly, from 27 points at baseline to 11 with placebo, increasing to 24 whilst on skullcap. Depression-Dejection (D-D) scores decreased with placebo from a score of 25 at baseline to 9 and increased to 18 whilst on skullcap. Anger-Hostility

(A-H) scores were 25 at baseline, decreasing to 12 with placebo and increasing to 22 with skullcap. This participant's placebo response requires special attention. Her Total Mood Disturbance (TMD) score on POMS was 84 at baseline, 26 with placebo and 74 with skullcap. A statistically significant change is considered to be 9 points (Derderian *et al.* 1988). At 84, her baseline TMD score was outside the normal range, the cut-off TMD commonly being 68, which is based on normative data of a mean of 18 with 67% falling between -16 and 52 and only 7% of scores being above 68 (Lorr *et al.* 2005). Her response with the placebo (26 points) amounted to her score being within the normal range, returning with skullcap to being outside the normal range at 74.

Personality (P.05): Her personality score range on the Big 5 Minimarker (Saucier 1994a) was highest for Intellect/Openness (8.0; normative = 6.55) and lowest for Emotional Stability (3.0; normative = 5.79) (Saucier 1994c).

There appeared to be a placebo response in a number of other participants who took the placebo capsules first:

P.01 also thought (as reported to assessor) she was taking skullcap first when she was in fact taking the placebo and her scores reduced from 31 (severe anxiety) to 14 (mild anxiety). They reduced by 1 more point to 13 on skullcap. She said she was sleeping better and “not bothering too much about anything” whilst taking the placebo.

P.01 showed little change in Tension-Anxiety on POMS during skullcap or placebo (15 at baseline, 16 on placebo and 15 on skullcap) as her changes on BAI were due to a reduction in somatic symptoms. Her Total Mood Disturbance score on POMS remained constant with placebo at 36 (36 at baseline) but decreased to 23 with skullcap, mainly because of a reduction in Anger scores from 10 at baseline and placebo to 4 with skullcap and an increase in Vigour from 14 at baseline and placebo to 18 with skullcap.

Personality (P.01): Her personality score range on the Big 5 Minimarker (Saucier 1994a) were highest on Conscientiousness (7.88; normative = 6.74) and lowest on Extraversion (4.5; normative = 5.7) (Saucier 1994c).

Both of these participants (P.05 and P.01) disclosed at initial interview they were usually anxious so although remission may have been spontaneous it is unlikely that this was the reason for reduced anxiety scores on BAI during placebo treatment.

P.08: Scores on BAI also dropped considerably for this participant who took the placebo first – from 17 (moderate anxiety) at baseline to 8 (mild anxiety) at follow-up (reducing slightly to 7 points with skullcap). Her initial TMD score on POMS was 80, which was outside the normal range, reducing to 22 with placebo and increasing a little to 28 with skullcap. The most notable changes with placebo were a decrease in D-D scores from 36 at baseline to 4 with placebo, further decreasing to 2 with skullcap; a reduction in T-A scores from 20 at baseline to 10, increasing to 12 with skullcap and a reduction in A-H scores from 18 at baseline to 10, increasing to 14 with skullcap.

She wrote whilst taking placebo:

“Paid council tax and was told that my rebate hadn’t been paid. Instead of getting angry and upset I felt clam and laughed about the situation.” (P.08)

Whilst on skullcap she wrote:

“I had a surprise visit from the Department of X (name provided by participant but anonymised here). Very calm and not as stressed as I thought I would be. However I was very tearful afterwards.” (P.08)

Personality (P.08): Her range of scores on the Big 5 Minimarker (Saucier 1994a) were highest for Agreeableness (7.87; normative = 7.32) and lowest for Emotional stability (3.6; normative = 5.79) (Saucier 1994c).

Other possible placebo responders were:

P.20: BAI score reduced from 19 (moderately anxious) to 9 (mildly anxious) whilst on placebo as the first intervention and increased to 12 during skullcap treatment. However, the POMS scores of this participant show a different story, albeit not for T-A, which showed little change (baseline = 17, placebo = 20, skullcap = 18). Her TMD decreased from 50 at baseline to 40 with placebo and to 28 with skullcap. The reduction in POMS scores was mostly related to a fall in D-D (baseline = 14, placebo = 13, skullcap = 4), A-H (baseline = 15, placebo = 13, skullcap = 9) and F-I (baseline = 10, placebo = 8, skullcap = 5) scores. No comments were made by this participant, other than being wakeful until 3 a.m. whilst on placebo.

Personality (P.20): Score range on the Big 5 Minimarker (Saucier 1994a) was highest for Intellect/Openness (8.38; normative = 6.55) and lowest for Emotional Stability (4.38; normative = 6.55) (Saucier 1994c).

P.21: BAI score reduced by 8 points from 14 (mildly anxious) to 6 (minimally anxious) on placebo as the first intervention - but further reducing to 4 on skullcap. His POMS scores, however reduced considerably whilst on both placebo and skullcap when compared to baseline for TMD (baseline = 41, placebo = 10, skullcap = 12), the major decrease being for A-H (baseline = 26, placebo = 10, skullcap = 6). There was little change for T-A (9, 5 and 9 respectively).

Personality (P.21): His score range on the Big 5 Minimarker (Saucier 1994a) was highest for Extraversion (7.63; normative = 5.7) and lowest for Conscientiousness (4.38; normative = 6.74) (Saucier 1994c).

P.25: BAI scores reduced from 15 to 4 on placebo (first intervention) and remained at 4 whilst on skullcap. Her baseline POMS TMD scores of 3 were low, reducing to -1 with placebo and -13 with skullcap.

Personality: P.25's score range on the Big 5 Minimarker (Saucier 1994a) personality test was highest for Agreeableness (8.25; normative = 7.32) and lowest for Extraversion (5.5; normative = 5.7).

As the baseline scores for both P.21 and P.25 were in the mild anxiety category, it is quite possible that anxiety in these participants would have resolved regardless of intervention (Vickers & Altman 2001).

P.35, who also took placebo first, was initially in the severe anxiety category at baseline with a BAI score of 37. His score reduced to 27 whilst on placebo but he was still in the severe category. It was not until he took skullcap that his scores reduced to 20 and were in the moderately anxious category. He said he felt 'nothing new' whilst on placebo but reported whilst on skullcap:

"From the beginning of June [3rd day of skullcap] I was acting careless. I didn't care too much about my exams and even about my family - and I am sleeping more than usual." (P.35)

P.35 had a very high TMD score on POMS at 130, the highest possible score on the scale, reducing to 90 with placebo and to 63 whilst taking the skullcap. The reduction was particularly prevalent with D-D, which was 44 at baseline, 32 with placebo (both outside the normal range) and 22 with skullcap (within the normal range). Contrary to beliefs that skullcap may worsen depression (see practitioner survey, Chapter 3), it appears to reduce it in some individuals. However, this is uncertain as although this participant was not excluded for high depression by the HADS (Zigmond & Snaith 1983), the POMS scale includes feelings of dejection concomitant with depression (Lorr *et al.* 2005), unlike the HADS (Zigmond & Snaith 1983) so may not be comparable for true depression. His T-A scores of 24 at baseline (above the normal range) reduced to 17 with placebo and 14 with skullcap (both within the normal range). His A-H score was also outside the normal range at baseline at 39, reducing to 24 with placebo (outside normal) and 19 with skullcap (within normal).

Personality (P.35): His range of scores on the Big 5 Minimarker (Saucier 1994a) was highest for Intellect/Openness (7.63; normative = 6.55) and lowest for Extraversion (3.89; normative = 5.7).

P.40: This participant, who took placebo first, was initially moderately anxious, scoring 21 points on BAI at baseline. He said he did not like to sit opposite people on the train as it made him nervous. He also said he spits a lot when he talks if he feels anxious at the time.

Although he appeared to experience a strong placebo effect with the first capsules (BAI score reduced to 7), his TMD scores on POMS demonstrated an enhanced mood whilst taking skullcap, reducing from 44 points at both baseline and placebo to 21 whilst on skullcap, mainly attributable to T-A scores reducing from 17 at baseline to 10 with skullcap (16 on placebo) and an increase in V-A scores (energy) from 8 at baseline to 21 with skullcap (19 with placebo). It appears his placebo response related to BAI only and not the POMS. The difference could be because those with anxiety may have a heightened awareness of somatic symptoms (Geers *et al.* 2006), which are measured by BAI (Beck & Steer 1993) in contrast to the POMS, which does not specifically do so (Lorr *et al.* 2005).

P.40 kept a diary only during test (skullcap) herb (BAI score reduced to 5).
See below for P.40's self-reported experiences of skullcap:

Day 3 on skullcap: *“In the train sitting in front of people, no anxiety and very happy about it”.*

Day 4 on skullcap: *“Starting to believe I am on the real herbal! Feeling good about myself.”*

Day 8 on skullcap: *“I am doing street fundraising for a charity, speaking to people and no anxiety. Later in the evening I am dining with friends. No anxiety. A little spitting but under control!!!”*

Day 10 on skullcap: *“I am thinking that there are things which are not great in my life such as my finance and my love life but I am not depressed? [sic]. It must be the herbal.”*

(P.40)

Personality: P.40’s score range on the Big 5 Minimarker (Saucier 1994a) were highest for Agreeableness (8.25; normative = 7.32) and lowest for Extraversion (4.0; normative = 5.7) (Saucier 1994c).

It is possible that P.35 and P.40 may have had an initial (BAI) placebo response to the placebo intervention but noticed the difference when they took skullcap.

It can be seen in Table 7-6 and Figure 7.23 (p152) that of possible placebo responders the dominant personality traits are Agreeableness and Intellect/Openness with a low emotional stability. However, this compares closely with normative data derived from 1125 residents of Eugene-Springfield, Oregon tested in 1993 (Saucier 1994c).

Table 7-6 Mean scores on the Big 5 Minimarker personality test of placebo responders in comparison normative data

Factor	Trait	Mean score	Standard deviation	Normative \pm SD
1	Extraversion	5.41	± 1.27	5.7 ± 1.31
2	Agreeableness	7.19	± 1.11	7.32 ± 0.86
3	Conscientiousness	6.47	± 1.28	6.74 ± 1.12
4	Emotional stability	5.09	± 1.45	5.79 ± 1.18
5	Intellect/openness	7.12	± 0.91	6.55 ± 1.09

Table 7-7 and Figure 7.24 (p157), which show means for the factors of the Big 5 Minimarker scores for all study participants, were close to normative values. The results of this research did not yield a demonstrable personality type for placebo response (see also 7.9). See main discussion section (Chapter 8:8.6, p181) for full discussion on placebo responders and personality.

In general, when participants were convinced they were taking skullcap first it is likely they would have been equally convinced that they were taking placebo after crossover. Of course this is notwithstanding the possibility that some may also have had a placebo response to skullcap.

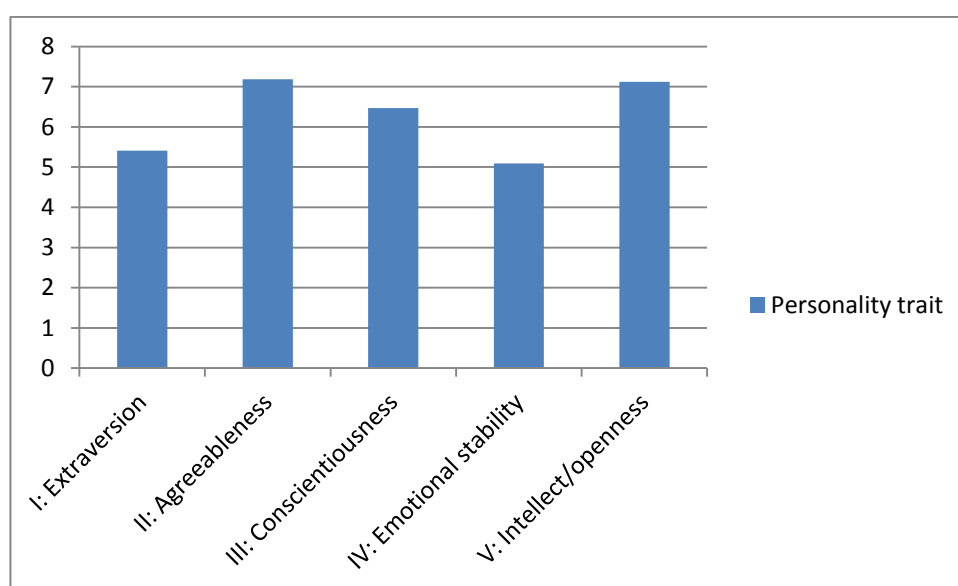


Figure 7.23 Mean scores of Big 5 Minimarker personality factors of placebo capsule responders

7.8.2 Other comments from participants during the study

P.04 (Sequence = test-placebo):

Day 10 (skullcap): *“Felt snappy and irritable. Flew off the handle 3 times”.*

Day 12 (skullcap): *“Felt relaxed and at ease. An easygoing, pleasant day.”*

Day 24 (placebo): *“Tetchy, tetchy, tetchy. Woke up at 5 a.m. and unable to get back to sleep.”* (P.04)

It is notable that this participant showed no significant changes on BAI, reducing from a score of 14 at baseline to 11 with skullcap and increasing by 1 point to 12 with placebo,

therefore remaining within the mild anxiety category. There was a significant difference in her TMD score on POMS with skullcap, however, from a score of 70 at baseline to 39 with skullcap, mainly due to a marked decrease in Anger-Hostility scores from 32 to 14 with skullcap. Her TMD scores on placebo were 64 points, which can be accounted for by decreased energy (Vigour score of 9 from 19 at baseline and 18 on skullcap) and return of depression to 23 points in the D-D factor on POMS (24 at baseline, 16 with skullcap). Her A-H score remained stable at 19.

P.19 (Sequence = placebo-test):

Day 25 (skullcap): *“More hopeful, not as bad feelings as before”.*

Day 32 (skullcap): *“Definitely feeling more positive about most things – dealing with problems more efficiently, so coping better.”*

“With the first capsules [placebo] I felt no different. I was just as angry as ever – but this time [test] I am coping much better”.

(P.19)

Her initial scores on BAI were 15, increasing to 19 with placebo and reducing to 11 with skullcap. Her POMS scores changed considerably with skullcap, reducing from a TMD score of 94 at baseline to 19 (90 with placebo). T-A reduced from 22 at baseline to 7 with skullcap (18 with placebo); D-D reduced from 27 at baseline to 10 with skullcap (22 with placebo); A-H reduced from 34 at baseline to 7 with skullcap (27 with placebo).

P.23 (Sequence = placebo-test):

Day 10 (placebo): *“Woke up feeling hot and bothered, anxious and fearful about the commitments and issues in my life”*

Day 16 (placebo): *“All week lacking motivation and generally feeling low emotionally, negative and angry.”*

Day 27 (skullcap): *“Slept amazingly well, couldn’t remember when last slept so well. Woke up relaxed and not angry. Warm, fuzzy feeling.”*

Day 36 (skullcap): *“Continue to be happy and relaxed and calm and not getting stressed in spite of so much to do”.*

Day 37(skullcap): *“I have really enjoyed doing this study and got so much benefit from it, especially in terms of the disappearance of my stress and anxiety, which previously dominated my life.”* (P.23)

P.23's initial BAI score was 10, rising to 17 on placebo and reducing to 1 with skullcap. This participant had an unusual response to placebo (in comparison with the other study participants) in that her scores considerably increased on the POMS from a TMD of 18 at baseline to 115, reducing to -24 with skullcap. Her POMS T-A score on skullcap, for example, was 0 (16 at baseline and 30 with placebo). Her A-H score with skullcap was also 0 (17 baseline; 32 placebo) as was her F-I score (3 baseline; 21 placebo).

The following two participants appeared to have a more favourable response to the second intervention even though it was placebo:

P.37 (Sequence = test-placebo)

"Days 27-37 should have been stressed due to exams however felt very chilled and focused with very good sleep most nights"
(Placebo) (P.37)

P.39 (Sequence = test-placebo):

"Felt a distinct difference between the capsules. Felt more alert, slightly more energetic [and] able to focus and remain calm during a 'tasking' week (Placebo)." (P.39)

P.37 was in the mild anxiety (< 15) category as measured by BAI, scoring 9 at baseline, reducing to 7 whilst taking skullcap and further to 2 with the placebo. Similarly, her scores for all factors on POMS were low at baseline, with a TMD of 43 (within the normal range; 67% of scores for adult norms fall between -16 and 52), reducing to 4 with skullcap and -9 with placebo. P.39 was also within the mild anxiety category at baseline according to BAI; initial score was 14, reducing to 4 with skullcap and to 3 with placebo. Her POMS TMD scores were 17 at baseline, reducing to 12 with skullcap and -8 with the placebo. Those with mild anxiety are more likely to undergo a spontaneous remission than those with severe anxiety (Vickers & Altman 2001).

P.41 (Sequence = test-placebo):

Day 3: (skullcap): *"I felt so calm about 1 hr after taking my 2nd tablet and went to supermarket and just completed my tasks very smoothly. I felt very friendly towards other people too."*

Day 14: (skullcap): *"Generally feel calmer and go about my duties without issues. When issues arise I deal with them swiftly and without hesitation."* (P.41)

This participant also wrote (not relating to any particular days):

"I feel my head buzz one hour after taking tablet [skullcap]. It disappears after about half an hour. I could stand it."

"My vocabulary usage has improved 10 fold!" (skullcap)

"Felt fine throughout taking second batch of capsules [placebo]. Did not notice any changes in my mood." (P.41)

P.41 scored 2 points on BAI at baseline, minimal anxiety (< 8), reducing to 0 with skullcap and remaining at 0 with placebo. Her TMD scores on POMS were also very low at -9 at baseline, reducing to -7 and -3 with skullcap and placebo respectively. It appears that, although P.41 was not particularly anxious or experiencing any negative mood at the beginning of the study, she nevertheless experienced some benefit from *S. lateriflora*.

7.8.3 Participant symptom diary summary and conclusions

According to the results of the participants' diaries there was no toxicity and only mild side-effects in a few individuals, such as mild digestive disturbances and vivid dreams. There appeared to be a placebo response in 8 participants, which is likely to have affected the overall results of the study. There was no particular personality trait linked to placebo responders.

Fewer participants reported negative mood symptoms such as stress, depression and anger whilst on skullcap than whilst on placebo or at baseline or during the washout period. Negative mood during skullcap treatment was relatively less frequent than during placebo treatment or washout. A predominant feature of taking skullcap was

prolonged feelings of happiness, contentment and feeling hopeful. There was no difference between skullcap and placebo in effects on energy and no negative effects of skullcap on either concentration or motivation. On the other hand, there were more reports of poor motivation during the placebo period.

Headaches were also more prevalent during placebo treatment than with skullcap and it is notable that following a latency period of 5 days skullcap appeared to decrease the likelihood of headaches occurring.

There was also apparent latency in effects on sleep patterns in some individuals whilst taking skullcap; those reporting sleep disturbances experienced the symptoms when they had been taking skullcap for over a week and those who reported improved sleep did so from a minimum of 3 days. As the diary is entirely subjective reporting however, that is not to say those who did not report sleep experiences ($n = 25$) did not sleep well. There was little difference in sleep patterns between skullcap and placebo reported by participants but it should be emphasised that as insomnia is a feature of anxiety (Pary *et al.* 2003) only results from an anxious population would likely yield relevant results.

Perhaps the most important finding from the participant diary results is the revelation of attenuation or resolution of chronic symptoms of longstanding conditions such as allergies, IBS, muscle pain and premenstrual symptoms. These findings correspond with those in the literature review (see 2.5, p19) and practitioner survey (see Table 3-1, p54). Future clinical studies on *S. lateriflora* could assess these paradigms.

7.9 Personality tests

All participants completed a personality test, the Big 5 Mini-Marker (Saucier 1994b) to determine whether or not there is a typical personality trait correlating to reduction in anxiety scores on BAI (Beck & Steer 1993), one of the primary outcome measures (the other is the POMS (Lorr *et al.* 2005)). It can be seen from Table 7-7 and Figure 7.24 that participants' scores on the five Big 5 Mini-Marker dimensions were within the normal range (Saucier 1994c).

Table 7-7 Comparison of normative values of the Big 5 Mini-Marker (Saucier 1994b) and mean values from participants who completed the skullcap study.

Big 5 Mini-Marker Personality Factors	Normative scores (Saucier 1994c)	Mean participant score (SD) (N = 31)	Standard Error Mean
I: Extraversion	5.7 (1.31)	5.62 (1.09)	0.197
II: Agreeableness	7.32 (0.86)	6.87 (1.08)	0.193
III: Conscientiousness	6.74 (1.12)	6.43 (1.20)	0.22
IV: Emotional stability	5.79 (1.18)	5.44 (1.21)	0.22
V: Intellect/openness	6.55 (1.09)	6.88 (1.03)	0.18

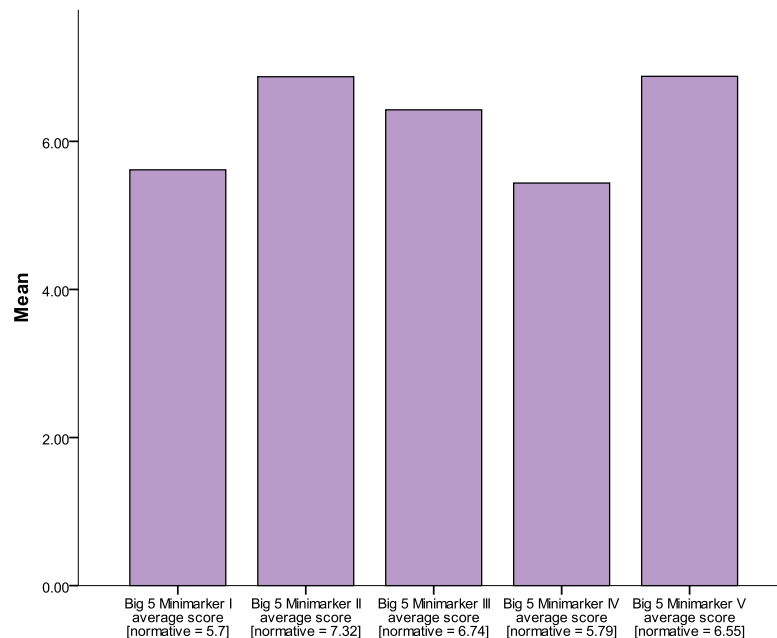


Figure 7.24 Mean values of scores for participants from the Big 5 Minimarker personality tests compared with normative values

Key: I = Extraversion; II = Agreeableness; III = Conscientiousness; IV = Emotional stability; V = Intellect/openness

In order to determine which personality Factor correlated most highly with anxiolytic response to *S. lateriflora*, as determined by change scores, Pearson correlations were carried out against change scores from baseline following skullcap and placebo on Beck's Anxiety Inventory (Beck & Steer 1993).

An initial correlation analysis was conducted by entering all relevant variables (Factors I-V and sequence group: group 1 placebo-test, group 2 test-placebo) with BAI change

scores. This demonstrated a significant correlation with Factor I only ($r = .318$, $n = 31$, $p = 0.041$) and the order in which the intervention was taken ($r = .511$, $n = 31$, $p = 0.002$). Because the order in which the intervention was taken was highly significant it was decided to analyse the 2 order (sequence) groups independently.

It can be seen from the slope in Figure 7.25 there is a high correlation between low scores on Factor I and change scores on BAI following skullcap for the placebo-test Group 1 ($R^2=0.399$, $n = 16$, $p = 0.006$).

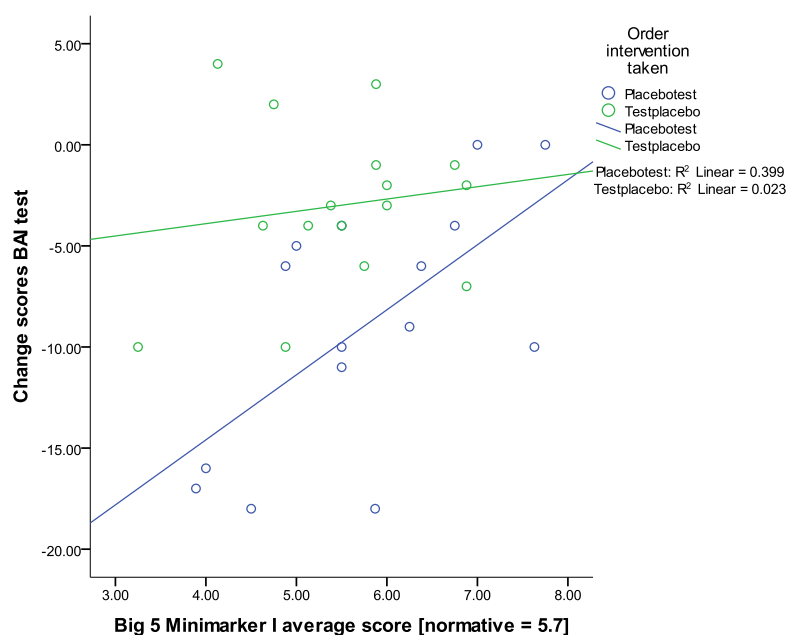


Figure 7.25 Linear relationships between Factor 1 (Extraversion) and BAI change scores with skullcap according to group

A linear regression confirmed lower scores on Factor 1 as a significant predictor of high change scores on BAI ($F_{1, 13} = 8.645$, $p = 0.011$) with 40% of change scores being explained by lower scores on Factor I for Group I ($t = 2.94$, $p = 0.011$).

For Group 2 (test-placebo) there was no significant correlation between Factor 1 and BAI change scores ($r = .150$, $n = 16$, $p = 0.289$).

As with skullcap, a correlation matrix was conducted to see which Factor could predict high change scores on BAI following placebo by entering all relevant variables (Factors I-V and sequence group: group 1 placebo-test, group 2 test-placebo). This demonstrated a significant correlation with Factor I ($r = .323$, $n = 31$, $p = 0.038$) in comparison with Factors II-V ($p = > 0.15$). There was no significance for the order (group 1 or group 2)

in which the placebo was taken for change scores with placebo ($p = 0.181$). However, a regression analysis demonstrated no significant predictor of change scores on BAI for Factor I for placebo ($F_{1,30} = 3.378, p = 0.076, t = 1.838, p = 0.076$).

Overall, the results of the regression analyses demonstrate there is more likelihood of those with low scores on personality Factor I (Extraversion) on the Big 5 Mini-Marker responding to skullcap but not to placebo for anxiety, shown by a reduction in scores on BAI following oral administration of skullcap. The response was only apparent in those who took the placebo first (Group 1).

Chapter 8. Discussion of the RCT results

This research represents the first randomised, double-blind placebo-controlled repeated measures crossover study of the putative efficacy of *Scutellaria lateriflora* on multiple mood states and physiological measures of stress. Although the results did not universally support the study hypotheses, nevertheless the findings make substantial contributions to the scientific and general herbal literature. The positive results achieved in the study were from doses as recommended by the manufacturers (Eclectic Institute Inc.) of the freeze-dried *S. lateriflora* as well as those found to be effective in a previous clinical trial assessing the anxiolytic properties of the herb (Wolfson & Hoffmann 2003). This chapter discusses major findings from the results of this clinical study of *S. lateriflora*.

Participants were assessed for changes in anxiety levels and mood and salivary cortisol measures of stress following administration of *S. lateriflora* or placebo. Of 43 participants commencing the study, results were obtained from those (n = 31) who completed it. The primary outcome measures for assessment of effectiveness of *S. lateriflora* were changes in mean scores from baseline on Beck Anxiety Inventory (Beck & Steer 1993) (Appendix IX) and the Profile of Mood States (POMS) (Lorr *et al.* 2005). Secondary outcomes were mean changes from baseline in salivary cortisol measurements and observational outcome measures were changes in ALT levels, blood pressure and pulse as well as symptom reports from the participants' diaries.

8.1 Beck Anxiety Inventory: does S. lateriflora reduce anxiety?

In any given year, up to one in six adults in the United Kingdom may suffer from a medically unexplained psychological disorder, the most common being anxiety (men 4%; women 5%), depression (men 2%; women 3%) or both experienced at the same time (men 7%; women 11%) (Office for National Statistics 2006). Anxiety is 'an unpleasant emotional state ranging from mild unease to intense fear' (B.M.A. 2002). Physical symptoms of anxiety can include pallor, sweating, hyperventilation, diarrhoea, irritable bowel, flushing, dysphagia, palpitations, nausea, muscle tension and back pain (B.M.A. 2002). Alleviation of these adverse effects on health is important. Orthodox anxiolytic treatments, however, have been linked to unwanted side-effects and may lead

to tolerance and physical and psychological dependence (B.N.F. 2008). There is a need therefore for safe, effective alternatives.

American skullcap (*Scutellaria lateriflora*) has long been an important herb in North American traditional medicine systems and western materia medica for anxiety and related disorders (Cole *et al.* 2008). *In vitro* studies of *S. lateriflora* and its phytochemicals (Liao *et al.* 1998; Hui *et al.* 2000; Gafner *et al.* 2003b) and a small clinical study (Wolfson & Hoffmann 2003) suggest a positive therapeutic benefit of the herb for anxiety and its comorbidities. To support and extend these findings, primary outcome measures for assessment of the effectiveness of *S. lateriflora* for anxiety were changes in scores from baseline on Beck Anxiety Inventory (Beck & Steer 1993). The instrument was used to measure and compare subjective anxiety at baseline and during administration of placebo or *S. lateriflora*. The hypothesis was that *S. lateriflora* would have a superior anxiolytic effect than placebo (when compared with baseline measures).

Mean change scores from baseline (Table 7-3, p118) demonstrated an enhanced anxiolytic effect for *S. lateriflora* compared to placebo for Group 1 (placebo-test) and no significant mean change for Group 2 (test-placebo). There was an enhanced effect of placebo compared to *S. lateriflora* for Group 2 (Figure 7.2, p116) suggesting an insufficient washout period for *S. lateriflora*, causing a sustained effect of treatment for those who took *S. lateriflora* first. Overall, there was no significant difference between *S. lateriflora* and placebo ($p = 0.191$) but the results suggest that *S. lateriflora* may attenuate anxiety in some individuals.

An important point to note, however, is the fact that very few participants could be considered to be in the anxious category. On the other hand, by random chance, all of those in the severe anxious category (BAI scores >26) were in Group 1, which could account in part for the efficacy of *S. lateriflora* in this group, with those in Group 1 showing higher initial mean (SD) anxiety scores of 15.73 (10.71) than those in Group 2 at 8.81 (3.99). Of the 31 participants who completed the study, 11 were initially experiencing minimal anxiety (BAI scores 0-7), 14 were mildly anxious (BAI scores 8-15), 3 were moderately anxious (BAI scores 16-25) and 3 had severe anxiety (≥ 26). Altered scores following intervention in those with an initially low score could be explained by normal daily intra-individual fluctuations, spontaneous remission or regression to the mean (where the scores are likely to increase at follow-up).

Additionally, when there is a floor effect at baseline, as seen in Group 2, which included those with baseline BAI scores of below 8 and considered to have minimal anxiety, there is little room for the scores to go any lower (Ernst & Resch 1995; Vickers & Altman 2001; Twisk & Proper 2004).

It was deemed important for the study to be of a duration that would not only prevent a high dropout rate but also produce less likelihood of remission of anxiety. Although none of the participants had been diagnosed with any anxiety disorder, those on the moderate and high anxiety scales on BAI had self-reported persistent anxiety at their screening interview. Generalised Anxiety Disorder (GAD) for example is a chronic condition characterised by excessive worrying and hypervigilance and is often undiagnosed. GAD is known to have low spontaneous remission rates, even following an intervention, with only one third experiencing full life-time remission (Wittchen & Hoyer 2001). In contrast, those with mild or minimal anxiety during initial testing would be more likely to experience spontaneous remission of anxiety symptoms (Vickers & Altman 2001). Furthermore, those suffering from GAD often present to a clinician with somatic symptoms of anxiety (Wittchen & Hoyer 2001), which will be evident during BAI anxiety tests allowing for higher initial BAI scores than those in a non-anxious group (Beck & Steer 1993).

By chance the randomisation for this current study was not very successful, as evidenced by differences between groups at baseline for BAI and POMS. This chance pre-test difference can be attributed to the small sample size (Metcalf 2010). Comparability between groups tends to be limited when a sample size is small because of pre-test differences occurring between groups even when there is randomisation (Mitte *et al.* 2005). The efforts asked of participants in the present study were quite exacting and the mean dropout rate of 28% was higher than average for studies of anxiolytics. In a meta-analysis of 48 double-blind placebo-controlled trials of anxiolytic drugs (including benzodiazepines, azapirones and antidepressants) in patients with GAD the mean dropout rate was 24.4%. This decreased to 20% for benzodiazepines (BDZs) (Mitte *et al.* 2005). Because the range on the BAI scale between subjects was wide at baseline it is unclear whether or not the results in the present study may have been adequately comparable between groups if the dropout rate had been similar to that in studies of BDZs. It is possible an inclusively anxious

population may not have yielded chance differences at baseline following randomisation.

The BAI was constructed with psychiatric outpatients in mind; so the construct validity assessing the severity of anxiety or detecting it in normal adults was not known at the time of its development (Beck and Steer 1993). McDowell (2006) states that it is not intended for panic or phobias but research has indicated that it is more capable of assessing the severity of panic disorders than of GAD. Its superiority in assessing panic disorders is believed to be related to the somatic symptoms on the scale (Leyfer *et al.* 2006). Cox *et al.* (1996) proposed that it measures mainly panic attacks instead of anxiety in general, whereas evidence suggests that in the field of anxiety disorders as a whole, panic attacks are qualitatively distinct from anxiety symptoms (Cox *et al.* 1996) and similarly panic disorder is physiologically distinct from GAD (Graeff 2007). Steer and Beck responded to Cox *et al.* (1996) by defending their instrument's capability of measuring general anxiety symptoms, asserting that somatic symptoms such as pounding heart, difficulty in breathing or sweating represent anxiety generally, are typically found in those with GAD and are not unique to panic attacks (Steer & Beck 1996). Nevertheless, subjects with any anxiety disorder score significantly higher on BAI ($p = < 0.001$) than those with no anxiety (Muntingh *et al.* 2011).

Our study sample was deliberately a non-psychiatric population and not intended for diagnosis of anxiety with BAI. It was notable that somatic symptoms such as "wobbliness in legs" were rare in our study sample. This may be a confounding factor, resulting in low scores if the scale has a tendency to be loaded towards somatic symptoms of GAD and panic disorder (Cox *et al.* 1996; Steer & Beck 1996; Leyfer *et al.* 2006; Muntingh *et al.* 2011). Therefore, BAI may not be appropriate in a normal, non-psychiatric sample for measuring response to an intervention. Further study is needed to assess its validity in non-clinical samples and it would be appropriate for future studies of *S. lateriflora*'s putative efficacy to be concentrated on clinical samples.

8.2 Profile of Mood States: does *S. lateriflora* enhance mood?

This 65 item validated questionnaire (Lorr *et al.* 2005) measuring subjective mood states (see 6.10.2, p97 for details) was fast and simple to administer. It evaluated six mood states: tension-anxiety, depression-dejection, anger-hostility, vigour-activity,

fatigue-inertia and confusion-bewilderment at baseline and at the end of each intervention period (placebo or *S. lateriflora*). In addition, it assessed participants' Total Mood Disturbance as a global measure of mood. This was obtained for each individual participant by summing the scores of each factor and subtracting the score for Vigour-Activity (Table 7-4, p118), which was negatively scored because it is a positive rather than a negative mood state. Participants were asked how they had been feeling during the past week, including today. The POMS has been found to be sensitive to changes in mood following administration of benzodiazepines (Frey *et al.* 1998; Lorr *et al.* 2005; Uhlenhuth *et al.* 2008), findings considered relevant to the present study as flavonoids found in *S. lateriflora* were found to bind to benzodiazepine receptors *in vitro* (Liao *et al.* 1998; Hui *et al.* 2002) (see 2.12.1.1, p31).

8.2.1 Tension-anxiety

For POMS Tension-Anxiety, although there was a highly significant reduction in scores with *S. lateriflora* ($p = 0.002$) there was also a significant reduction in scores with placebo ($p = 0.028$); the results indicated no significant difference between *S. lateriflora* and placebo ($p = 0.473$) overall and no significant differences in the effects of *S. lateriflora* in T-A attenuation between groups. There was, however, an enhanced effect of placebo over *S. lateriflora* for Group 2, suggesting an insufficient washout period following *S. lateriflora* treatment. Because of the possibility of a continuation of the effects of *S. lateriflora* following washout for Group 2, who took *S. lateriflora* first, the overall efficacy of *S. lateriflora* on T-A may be underestimated by this being a confounding factor. Results suggest, however, *S. lateriflora* attenuates tension/anxiety in some individuals.

8.2.2 Depression-dejection

The results confirmed there was no significant difference between the effects of *S. lateriflora* and placebo on Depression-dejection ($p = 0.067$) but there was an enhanced effect of *S. lateriflora* in reducing D-J scores compared to placebo for Group 1 (order = placebo-test) and an enhanced effect of placebo compared to *S. lateriflora* for Group 2 (order = test-placebo), indicating an insufficient washout period and a sustained effect of *S. lateriflora* for Group 2. As with T-A the carryover effect of *S. lateriflora* may be causing an underestimation of the ability of the herb to reduce depressive symptoms but results suggest that *S. lateriflora* reduces symptoms of depression in some individuals.

An important point to note is that baseline scores for depression in this study were intentionally relatively low. An exclusion criterion was significant depression i.e. > 8 on the HADS (Zigmond & Snaith 1983) as anecdotal evidence from the practitioner survey (3.4.8, p58) suggested *S. lateriflora* might worsen severe depression. However, the results demonstrated a significant reduction in D-J scores for this factor in some individuals. Participant 35, for example, had a high D-J score of 44 at baseline, 32 with placebo (both outside the range for adult norms; mean = 8 ± 9.3 SD; cut-off > 23 requiring special attention) and 22 with *S. lateriflora* (within the normal range) (Lorr *et al.* 2005). This participant did not have an overly high HADS depression score (= 8) but the POMS D-J also measures feelings of inadequacy associated with symptoms of depression (Lorr *et al.* 2005) so these feelings may have been a major factor in this participant. Only 5 participants (3 of whom took *S. lateriflora* first) showed a mild increase in D-J scores following *S. lateriflora* (an increase of 1, 2, 3, 6 and 9 points respectively) and four (2 of whom took placebo first) showed an increase in D-J scores with placebo (an increase of 3, 8, 8 and 18 points respectively).

The results from the D-J scores coupled with findings that *S. lateriflora* does not appear to worsen existing depression suggest that future work assessing its efficacy in those with anxiety disorders need not exclude those with comorbid depression. This is important because there is a high comorbidity rate with anxiety and depression (Mitte *et al.* 2005; Greaves-Lord *et al.* 2007). For example, for a lifetime diagnosis of GAD there is an estimated 50% rate of comorbidity with depression. It is therefore essential that an effective anxiolytic does not exacerbate symptoms of depression. A preferred anxiolytic would attenuate both anxiety and depression (Mitte *et al.* 2005). A reduction in depressive symptoms comorbid with anxiety would be favourable regardless of whether the thymoleptic effects were as a result of *S. lateriflora* or to a psychological effect resulting from reduction in anxiety. It would be worthwhile for future studies of *S. lateriflora* to assess the efficacy of *S. lateriflora* on anxiety and comorbid depression with the use of a validated instrument such as the Beck Depression Inventory (Beck *et al.* 1996) in addition to the Beck Anxiety Inventory (Beck & Steer 1993).

8.2.3 Anger-hostility

Although there was a slightly enhanced effect of *S. lateriflora* in reducing A-H scores ($p < 0.001$) compared with placebo ($p = 0.037$), overall the reduction in A-H scores from baseline following *S. lateriflora* compared to placebo treatment was not significant ($p =$

0.070). A confounding factor, however, may be an insufficient washout period following *S. lateriflora*, demonstrated by the finding that, although there was an enhanced effect of *S. lateriflora* for Group 1, albeit insignificant, the differences, shown by independent sample t-tests, between Group 1 and Group 2 in the effects of *S. lateriflora* treatment were not significant ($p = 0.466$) but there was enhanced placebo effect compared to Group 1 ($p = 0.041$). Overall, *S. lateriflora* reduced feelings of anger and hostility but this was not significant. The results suggest that a sufficient washout period may have yielded more positive results from the effect of *S. lateriflora* on T-A.

8.2.4 Vigour-activity

Importantly, the results showed that chronic administration of *S. lateriflora* does not cause a reduction in energy. The results overall indicated that there was no significant difference between test and placebo in alteration of V-A scores ($p = 0.244$) but an enhanced effect of *S. lateriflora* for Group 1 (who took placebo first), for which energy levels were increased by a mean of 20%. Group 2 (who took *S. lateriflora* first) showed a slight decrease in energy levels by a mean 6%. The difference between the groups in the effect of *S. lateriflora* was significant ($p = 0.003$). The reason for the difference between groups in the effects of *S. lateriflora* is unclear but could perhaps be accounted for by differences in anxiety levels between the groups at baseline. Group 1 was generally more anxious than Group 2 with a mean anxiety score on BAI of 15.73 (10.71), which is at the beginning of the moderate anxiety scale whereas mean scores for those in Group 2 at 8.81 (3.99) were just above the minimally anxious scale of < 8) (Beck & Steer 1993). It is well known that anxiety sufferers, particularly those with GAD, may become easily fatigued and/or experience sleep disturbances such as frequent waking in the night or difficulty in getting to sleep (Fricchione 2004). If attenuation of anxiety in the more anxious participants also led to less fatigue and better sleep the consequence would likely be increased daytime energy levels. The slight decrease in energy following *S. lateriflora* for Group 2 is consistent with the earlier findings of Wolfson and Hoffmann (2003), who observed a mild decrease in energy in 19 healthy volunteers following acute administration of *S. lateriflora*. There was no mean baseline anxiety measurement provided in the Wolfson and Hoffmann (2003) study and no validated anxiety instrument was used so it is unknown whether or not it was an anxious population. It is possible therefore that the study population was similar to Group 2 in the present study in that they were a generally non-anxious population.

According to the British Herbal Pharmacopoeia (British Herbal Medicine Association 1983) *S. lateriflora* is sedating so it could be hypothesised that a sedative effect of the herb may be beneficial in anxious subjects who sleep badly yet it may have a mildly sedating effect in the daytime in those who do not. The difference cannot be entirely explained by greater anxiety and thus less energy for Group 1 at baseline, however, because energy levels as defined by V-A were normal at baseline for both groups. Normal adult populations have been estimated (from a sample of 400) to have a mean V-A of 19.3 ± 6.7 at baseline (Lorr *et al.* 2005) and in the present study, V-A was 17 ± 6.65 for Group 1 and 15.56 ± 4.97 for Group 2. A post-hoc correlation analysis confirmed there was no correlation between anxiety levels at baseline on BAI and change scores on V-A following *S. lateriflora* for either Group 1 ($r = -.125$, $n = 15$, $p = 0.328$) or Group 2 ($r = -.068$, $n = 16$, $p = 0.401$). There is, however, a possibility that the differences in alteration of V-A scores might be explained by differences between groups in predominant personality traits (see 8.5, p179 Personality tests).

8.2.5 Fatigue-inertia

Overall, although there was an enhanced effect of *S. lateriflora* compared to placebo in decreasing fatigue and inertia, the results showed the difference in treatment effects between *S. lateriflora* and placebo were not significant ($p = 0.209$), indicating overall that *S. lateriflora* does not cause fatigue when taken orally. The enhanced effect of *S. lateriflora* in reduction of fatigue and inertia was only apparent in Group 1. As previously discussed in relation to V-A this cannot be convincingly explained by greater anxiety in this group.

8.2.6 Confusion-bewilderment

Overall *S. lateriflora* had no significant effect on C-B; there was no significant difference between *S. lateriflora* and placebo ($p = 0.838$). There was, however, an enhanced effect of placebo compared to *S. lateriflora* for Group 2, suggesting insufficient washout. Importantly the results suggest that chronic administration of *S. lateriflora* does not impair cognitive function. One participant (P.41), for example, said *S. lateriflora* gave her increased mental clarity. She believed her vocabulary had increased while she was taking the herb. Results of the current study conflict with results from the study by Wolfson and Hoffmann (2003) in which there was a mild reduction in cognition. The studies are not directly comparable, however as the latter

assessed the effects following acute administration of the herb and no validated instruments were used.

8.2.7 Total Mood Disturbance

The results for TMD suggest no significant difference between *S. lateriflora* and placebo in improvement of global mood ($p = 0.137$). There was, however, a significant difference between groups in the effect of *S. lateriflora* ($p = 0.017$) but not of placebo ($p = 0.794$). It can be seen from Figure 7.10 (p127) and Figure 7.11 (p128) that there was an enhanced effect of *S. lateriflora* for Group 1 (placebo-test) compared with placebo and there was an enhanced effect of placebo compared to *S. lateriflora* for Group 2 (test-placebo). Group 2 scores continued to decrease, suggesting an insufficient washout period. Although this was not very significant it may nonetheless have caused an underestimation of the global effects of *S. lateriflora* on mood. Despite the confounding factor of a potential carryover effect, the results suggest a significant effect of *S. lateriflora* on global mood in some individuals. Mean TMD (SD) scores at baseline, at 45.27 (37.41) for Group 1 and 31.06 (24.08) for Group 2 were above the mean of 17.7 (33) for adult norms as estimated by Lorr *et al.* (2005) but decreased to 13.33 (27.99) for Group 1 and 19.56 (18.06) for Group 2 (but further decreasing to 17.25 (33.87) with placebo for Group 2).

8.2.8 Main findings from the POMS

The results for the POMS factors (Lorr *et al.* 2005) imply that chronic administration *S. lateriflora* enhances mood in healthy volunteers without a decrease in energy or cognition. Main findings were:

- A significant decrease in T-A following *S. lateriflora* but only in those who took placebo first, suggesting *S. lateriflora* attenuates anxiety in some individuals.
- A significant decrease in D-D following *S. lateriflora* but only in those who took placebo first, suggesting *S. lateriflora* has a thymoleptic or antidepressant effect in some individuals.
- A slight decrease in A-H following *S. lateriflora* but only in those who took placebo first, suggesting *S. lateriflora* calms feelings of anger and hostility in some individuals.
- No decrease in energy or cognition following chronic administration of *S. lateriflora* as evidenced by the results from V-A, F-I and C-B.

- For T-A, D-D, A-H and TMD an insufficient washout period following *S. lateriflora* was evident, which may account for significant results in those who took placebo first but may underestimate the response to *S. lateriflora* overall.

It is difficult to extrapolate findings from the present study of the effects of *S. lateriflora* on mood as reflected by POMS (Lorr *et al.* 2005) results to those of orthodox anxiolytics, particularly considering that a comparison of dosage strength is currently impossible. These findings do, however, suggest some of the effects of *S. lateriflora* on mood are comparable with certain benzodiazepines. For example, consistent with the results for Group 1 (placebo-test) in the present study, during early development of the POMS, in a double-blind placebo-controlled study of 150 male psychiatric outpatients administered 4 x 10 mg daily chlordiazepoxide (Librium) over five weeks there were significant reductions in T-A and an increase in V-A compared to placebo and no treatment groups ($p = 0.05$) (Lorr *et al.* 1963). As the study by Lorr *et al.* (1963) was a parallel study results were not complicated by a potential problem of carryover as in the present study (as seen in Group 2 who took *S. lateriflora* first).

On the other hand, when Frey *et al.* (1998) used the POMS (Lorr *et al.* 2005) in a double-blind, placebo-controlled anxiolytic drug discrimination study in 10 healthy volunteers, they found buspirone and diazepam produced effects on mood differing from *S. lateriflora* and Librium (Lorr *et al.* 1963) and between the two anxiolytics. In this study each participant randomly received ten exposures to each of 3.75, 7.5 and 15 mg buspirone, 2.5, 5.0 and 10.0 mg diazepam, and lactose placebo and completed the POMS immediately before each drug administration and 2 hours following each drug administration. Baseline measurements on the POMS related to how they were feeling ‘right now’ and post-drug measurements were based on feelings during the last 2 hours. In comparison to placebo, the diazepam and buspirone produced dose related mean decreases in V-A scores; and the highest dose of buspirone but not diazepam resulted in significant increase in T-A scores. The high dose diazepam, but not buspirone, significantly increased F-I and C-B scores (Frey *et al.* 1998). The results suggest that *S. lateriflora* is superior to diazepam and buspirone in its ability to produce mood enhancing effects without side-effects such as a reduction in energy or cognition. Furthermore, it has not been known for high doses of *S. lateriflora* to produce anxiogenic effects similar to those seen from a high (15 mg) dose of buspirone. The results of a practitioner survey on the use of *S. lateriflora* did not reveal any such effects

(see 3.4.7, p56), even though administration was up to 105 ml or 35 g dried or fresh herb equivalent per week (approximately 1.7 g/dose). Other side effects produced by buspirone but not diazepam included dizziness and nausea.

Buspirone belongs to the azaspirodecaedione (azapirone) class of anxiolytics and is primarily used to treat GAD. It is pharmacologically distinct from the benzodiazepines such as diazepam in that it modulates serotonin synthesis whereas BDZs act on the GABA_A receptor, increasing availability of GABA. Buspirone acts as a full agonist on the presynaptic serotonin receptor subtype 5-HT_{1A} (decreasing neuronal firing and hence inhibiting serotonin synthesis) and as a partial agonist at postsynaptic serotonin receptors (Frey *et al.* 1998; Vanin 2008).

In another study using the POMS as a subjective measure of mood, 26 panic disorder patients received escalating doses of the benzodiazepine alprazolam for 4 weeks and then a fixed dose for of 1 mg four times daily. Momentary mood was measured at baseline and at 1, 2, 3, 4, 6 and 8 weeks (Uhlenhuth *et al.* 2008). Consistent with the findings of Frey *et al.* (1998) increasing plasma levels of alprazolam was associated with decreasing V-A and increasing C-B scores, said to be reflecting sedation and dysphoria (Uhlenhuth *et al.* 2008). In the current study *S. lateriflora* was not found to worsen symptoms of depression or increase confusion. On the contrary, the results suggested that it may attenuate depression.

8.3 Salivary cortisol: does *S. lateriflora* attenuate stress?

Cortisol is essential to life. It has a role in the regulation of lipid, protein and carbohydrate metabolism; it maintains blood glucose levels by promoting gluconeogenesis in times of food shortage and stress (see also 1.5, p7); maintains the integrity of blood vessels and modulates immune function; its immunomodulatory action is principally as a potent anti-inflammatory agent and prevention of over-activity of the immune system (Sternberg & Gold 2002). Repeated and/or sustained stress with concurrently increasing/high cortisol output and consequent cumulative exposure, however, may result in compromised immune function, exhaustion, depression and behavioural changes (Greenspan 1991). As with other hormones, it is cortisol's interaction with neural pathways that causes it to have an effect on behaviour (McEwen 2006). Cortisol hypersecretion has also been associated with decreased hippocampal volume and cognitive impairment (Lupien *et al.* 1998).

This is the first time measurement of salivary cortisol concentrations has been used to determine the putative benefits of *S. lateriflora* on stress. Negative feedback mechanisms are overridden by stressors and during persistent stress there is increased activity with increased levels of circulating cortisol. In PTSD and panic disorders, for example, there is chronic CRF and cortisol output (Mann *et al.* 2006). Salivary cortisol concentrations reflect the activity of the HPA axis and over-activity of the HPA axis has been postulated to be associated with anxiety states (Tallman *et al.* 2002). Conversely, the (GABA)/benzodiazepine receptor system mediates the inhibition by GABA of CRF and consequently the HPA axis and BDZs are thought to attenuate CRF via the effects of GABA-ergic neurons (Rohrer *et al.* 1994; Fries *et al.* 2006). As *S. lateriflora* flavonoids, including baicalin, baicalein and wogonin, extracted from *S. baicalensis* but also found in *S. lateriflora*, had affinities for the BDZ binding site of GABA_A receptors it was hypothesised that alterations in cortisol profile following administration of the herb would reflect its effectiveness in attenuation of anxiety.

To this author's knowledge, this is the first time depressed individuals have been excluded from a study of the relationship between anxiety, stress and diurnal salivary cortisol levels. Previous studies have not adjusted for overlap of anxiety with depression. Depression has consistently demonstrated higher basal cortisol levels than in healthy adults and furthermore is strongly associated with anxiety. If present, because it has this tendency to affect the HPA axis it may interfere with results desirable from anxiety alone (Greaves-Lord *et al.* 2007).

The results of the salivary cortisol analysis demonstrated that there was no significant difference between *S. lateriflora* and placebo ($p = 0.524$) and the mean diurnal cortisol was within the normal range before and after treatment (see below). The mean results were also consistent with findings stated in the literature that levels are at their highest in the morning and gradually decrease throughout the day (Greaves-Lord *et al.* 2007).

The range in salivary cortisol concentration in normal subjects is broad but in one study of 20 normal adults the mean (\pm SEM) concentration was 15.5 ± 0.8 nmol/L (range 10.2–27.3 nmol/L) at 0800 h and 3.9 ± 0.2 nmol/L (range 2.2–4.1 nmol/L) at 20.00 h (Laudat *et al.* 1988) and Aardal and Holm (1995) established reference ranges in 195 healthy volunteers (121 males, 74 females) as a mean 11.9 nmol/L, range 2.6–42.8 for

morning values (not the awakening response values) and a mean 1.8 nmol/L, range 0.5-9.9 nmol/L for evening values. Participants supplied at least one morning sample and one evening sample over a 24 hour period (Aardal & Holm 1995).

Consistent with the results of (Laudat *et al.* 1988) and (Aardal & Holm 1995), mean salivary cortisol concentrations were within the normal range at baseline and following *S. lateriflora* and placebo. At 3 hours following waking, for example, salivary cortisol concentration in nmol/L for participants in the *S. lateriflora* study was in the range (mean \pm SEM) 1.94-20.51(7.62 \pm 5.24) at baseline, 1.95-22.77 (7.36 \pm 5.21) with *S. lateriflora* and 1.24-18.44 (7.49 \pm 4.67) with placebo. At 12 hours following waking ranges were 0.41-10.75 (3.93 \pm 2.38) at baseline, 0.95-12.51(3.37 \pm 2.79) with *S. lateriflora* and 0.73-11.72 (3.58 \pm 2.37) with placebo. As secretory activity of cortisol declines throughout the day in relation to the awakening response (Edwards *et al.* 2001) the mean salivary cortisol concentration at 3 hours for participants in the *S. lateriflora* study therefore appears within the normal range across all treatment periods when compared to the above values (Laudat *et al.* 1988) for healthy adults. It also compares well with mean salivary cortisol results of Edwards *et al.* (2001) at 3 and 12 hours following waking (in figure ~ 7.0 and 3.5 respectively - exact figures not provided in text). Furthermore, higher concentrations (> 15 nmol/L) were negatively correlated to those reporting stress. For example, participant No. 24 had a mean high of 20.51 nmol/L salivary cortisol at 3 hours of the baseline period but did not report stress in the diary. As subjective reports of stress did not reflect physiological measurements of stress, the diary was therefore not valid in assessing this paradigm. It is also apparent from the diary reports, that stress was intermittent during the study, so assessment at only 3 time points was insufficient. Transitory cortisol elevations could not be measured. A superior method might be to use the Perceived Stress Scale (Cohen 1994), taking measurements over a number of consecutive days with concomitant saliva sampling, for each intervention as well as at baseline and during a washout period.

Because there appeared to be no correlation between subjective reporting of reduced anxiety levels and mean physiological cortisol measurements as demonstrated by no mean change in salivary cortisol measurements, a Pearson's correlation between anxiety scores and salivary cortisol values was not assessed. The association between anxiety, stress and basal cortisol levels in healthy adults is, however, inconclusive. A study of 1,768 adolescents, for example, revealed no correlation between daytime salivary

cortisol levels and anxiety but there was a significantly higher cortisol awakening response in persistently anxious individuals than in those with current or no anxiety (Greaves-Lord *et al.* 2007). Additionally, using self-report participant diaries of stressors and anxiety in a naturalistic setting with concomitant saliva samples, Kurina *et al.* (2004) found no strong association between self reported anxiety and stress and diurnal salivary cortisol patterns.

An electronic search revealed there has been much research relating to the effects of anxiolytic pharmacotherapy, mainly BDZs, on the HPA axis. As it is beyond the scope of this chapter to outline them all a small sample of these studies is discussed here. A number of studies have demonstrated the ability of BDZs to attenuate cortisol output following acute administration; for example, diazepam (Tormey *et al.* 1979; Roy-Byrne *et al.* 1991; Schuckit *et al.* 1992; Pomara *et al.* 2005), temazepam (Beary *et al.* 1983; Korbonits *et al.* 1995), alprazolam (Charney *et al.* 1986; Risby *et al.* 1989; Zemishlany *et al.* 1990; McIntyre *et al.* 1993; Osman *et al.* 1993; Curtis *et al.* 1997; Pomara *et al.* 2003), oxazepam (Gram *et al.* 1984). Results, however, have been conflicting. An early study (Garland & Zis 1989) found no alteration in afternoon serum cortisol levels in comparison to placebo in nine healthy volunteers administered oxazepam. All participants received oxazepam, codeine and placebo orally (30 mg of each). Each intervention was administered on 3 different afternoons separated by 48-72 hours. Neither oxazepam nor placebo attenuated afternoon serum cortisol levels whereas codeine caused serum cortisol levels to decline. The effects of codeine on cortisol levels were hypothesised to be attributable to the inhibitory effects of opioids on the HPA axis (Garland & Zis 1989). In another placebo-controlled study, 6 healthy male volunteers were administered 10 mg diazepam intravenously and blood samples for measurement of serum cortisol were taken at 0, 30, 60 and 120 minutes following administration at around 8 a.m. Although there was a decline in cortisol levels over time for diazepam there was no difference between diazepam and placebo (Laakmann *et al.* 1984). The study samples may have been too small, however, for the results of these two studies to be significant. Furthermore, studies of attenuation of cortisol by anxiolytics in non-stressed individuals may have little value.

Few studies have assessed the acute effects of anxiolytics on the HPA axis following anxiogenic psychological challenge (Fries *et al.* 2006) but results so far have been promising, suggesting that certain BDZs inhibit activation of the HPA axis in stressful

situations. Hellhammer *et al.* (1988) studied the effects of lorazepam (1 mg), alprazolam (0.5 mg) and placebo on the salivary cortisol stress response to harrowing film scenes in 60 healthy adults divided into 3 groups respectively. Only lorazepam was effective in preventing a stress response. These results conflict with those of Rohrer *et al.* (1994), who found 0.5 mg alprazolam significantly attenuated serum ACTH and cortisol levels 15 minutes after a stressful speech by each of 10 healthy volunteers in comparison to placebo, which had no effect on the HPA axis (Rohrer *et al.* 1994). In another study, Benschop *et al.* (1996) observed 1 mg alprazolam significantly reduced serum cortisol and adrenalin levels in comparison to placebo in 25 males following a first-time parachute jump. The study indicated alprazolam may attenuate the sympathetic-adrenal-medullary axis (SAM) in addition to the HPA axis. Fries *et al.* (2006) determined the levels of blood ACTH and cortisol as well as adrenalin and noradrenalin and salivary cortisol in placebo-controlled study of 46 male volunteers administered 1 mg alprazolam prior to the Trier Social Stress Test (Kirschbaum *et al.* 1993), which produces a rise in ACTH and anticipatory anxiety. The test included subjective measures of mood and a free speech situation. Participants administered alprazolam, but not those administered placebo, displayed a strongly blunted ACTH and cortisol secretion. Inconsistent with the study by Benschop *et al.* (1996) the SAM axis was not affected by alprazolam (Fries *et al.* 2006).

Midazolam has been shown to decrease salivary cortisol levels and thus to be of value in lowering anxiety and stress in patients about to undergo oral surgery. In comparison to a control group (n = 18) 7.5 mg midazolam administered sublingually significantly ($p < 0.05$) decreased salivary cortisol levels concurrently with a reduction in anxiety scores on the HADS (Zigmond & Snaith 1983) in otherwise healthy male patients (n = 20) pre-medicated with the drug prior to undergoing molar extraction (Jerjes *et al.* 2005).

There appears to be a paucity of research relating to the chronic effects of anxiolytic pharmacotherapy on the HPA axis. Consistent with findings in the present research, however, a study (Kalman *et al.* 2008) assessing chronic effects of a herbal anxiolytic on stress also yielded no statistically significant changes in salivary cortisol measurements. A placebo-controlled parallel study of 26 obese women suffering from anxiety and who ate in response to stressful situations was conducted to determine the effects of *Magnolia officinalis* (magnolia) and *Phellodendron amurense* (cork-tree) on

anxiety and stress. Saliva samples for salivary cortisol measurements were collected on waking, 30 minutes later and in the evening over 3 consecutive days at baseline and at the end of a 6 week study. There were no significant differences in salivary cortisol measurements between intervention (*Magnolia - Phellodendron*) and placebo after 6 weeks treatment. The *Magnolia* treatment, however, significantly reduced anxiety in comparison with placebo when measured by the Spielberger STATE questionnaire but not the Spielberger TRAIT questionnaire (Spielberger *et al.* 1970), which may account for the inability of chronic administration of the active drug to significantly alter cortisol levels. The authors suggested a larger study over a longer period of time may show efficacy with this variable (Kalman *et al.* 2008). A study of the herb *Rhodiola rosea* (rosenroot), although not strictly considered an anxiolytic but is administered to stressed patients in the herbal clinic as an ‘adaptogen’ (a herb that modulates stress), however, did yield significant results when assessed for its efficacy in lowering salivary cortisol concentrations. In a placebo-controlled parallel study of 60 stressed volunteers, *Rhodiola* administration significantly decreased the cortisol response to awakening in comparison to baseline measurements and placebo ($p = < 0.05$) (Olsson *et al.* 2009). In this study, participants gave saliva samples daily, which might account for the positive results, unlike the present study on *S. lateriflora* and the study by Kalman *et al.* (2008), both of which assessed cortisol levels only at baseline and following interventions.

The results of the above studies suggest that attenuation of the HPA axis is likely to be dependent on anxiolytic type, even within drug group as well as the test situation as certain BDZs appear to affect (block) cortisol secretion in some stressful situations but not in others. It is unclear whether attenuation of the HPA axis is also likely to depend on dosage. In a crossover study of young ($n = 52$, mean age 27) and elderly ($n = 31$, mean age 67) volunteers Pomara *et al.* (2005) found acute and chronic challenge of both high and low dose diazepam (2.5 mg or 10 mg) significantly decreased plasma cortisol levels, but only in the elderly, when compared to placebo. Cortisol response to diazepam was not associated with a GAD status evident in 19 young and 16 elderly volunteers (Pomara *et al.* 2005). Overall, the results of the above studies also suggest, because physiological responses to psychological (and physical) stress are reflected by a rise in cortisol levels (Kirschbaum & Hellhammer 1989) that the effects of *S. lateriflora* on the HPA axis could perhaps be better demonstrated during an acute stressful situation; or when samples are taken on a daily basis in order to capture a stress response.

8.4 Safety and tolerability: is *S. lateriflora* safe to use?

8.4.1 ALT levels

Reports of hepatotoxicity associated with *S. lateriflora* have been attributed to its occasional adulteration with *Teucrium* species (McCaleb 2004). Although the freeze-dried *S. lateriflora* used in the study was found by HPLC analysis (see 5.5, p79) to be authentic and unadulterated it was still important to assess liver function in participants following administration of the herb to ensure there were no toxic effects from *S. lateriflora*. There have been no reports of toxicity attributed to use of authentic, unadulterated *S. lateriflora* but this present clinical analysis heralded the first time this was demonstrated scientifically in human volunteers.

According to Pratt and Kaplan (2000) *Scutellaria* (species not given) is reported to cause elevation of liver enzyme levels. This assertion was not substantiated as no citation was provided by the authors. The results of the present study showed that although there was a statistically significant increase in ALT levels following administration of *S. lateriflora* there was no significant difference ($p = 0.801$) between *S. lateriflora* and placebo in ALT elevation. Although there was a mean elevation of 2.01 U/L ALT levels (from a mean 9.72 U/L) following *S. lateriflora*, in real terms this was not clinically significant and the increase was very mild because ALT levels were still well within the normal range of ≤ 22 U/L (men) and ≤ 17 U/L (women) at 25°C (Roche 2008), indicating that authenticated *S. lateriflora* is safe to use. Serious liver disease is not indicated until elevation of ALT is fifteen times the normal upper reference (Hamer 2010) and elevation up to twice the normal range is considered unlikely to be clinically significant (Pratt & Kaplan 2000).

8.4.2 Blood pressure

There were no significant changes from baseline in mean systolic or diastolic blood pressures following treatment with either *S. lateriflora* ($p = 0.454$ systolic; $p = 0.513$ diastolic) or placebo ($p = 0.395$ systolic; $p = 0.669$ diastolic) and no significant differences in the effects of *S. lateriflora* and placebo on systolic ($p = 0.410$) or diastolic ($p = 0.834$) blood pressure parameters. *S. lateriflora* is therefore unlikely to have the stated traditional action as a diuretic (Greenfield & Davis 2004) as diuresis is a mechanism for lowering BP by elimination of salt and water and reducing blood volume. Diuretics are usually the first line of treatment for hypertension; in orthodox

medicine thiazides are most commonly prescribed (Stone & Darlington 2004) and in herbal medicine *Taraxacum officinale folia* (dandelion leaf) is the treatment of choice when powerful diuresis is required (Hoffmann 2003). It is not clear, however, whether or not *S. lateriflora* would have reduced mean blood pressure readings in a study population with hypertension. For safety reasons this was a normotensive population so it is unclear whether a reduction in blood pressure might be observed in a hypertensive population. Furthermore, *Urtica dioica folia* is mildly diuretic so potentially reduces BP (Hoffmann 2003). One of the exclusion criteria (see 6.8.1.3, p91) was severe hypertension (>150/90) so mean blood pressure parameters for the study were within the normal range at baseline. The recommended average healthy blood pressure in adult humans is 120/80 mm/Hg (Beers *et al.* 2006) was synonymous with baseline readings for the study and the results demonstrated there were no alterations from this 'text book' reading in either mean systolic or diastolic BP following either *S. lateriflora* or placebo.

According to the results of a survey of herbal medicine practitioners (Brock *et al.* 2010) a common response looked for in patients was a lowering of blood pressure concomitant with a reduction in anxiety (see 3.4.4, p55). A recent publication however, of a 22 year longitudinal study, the Nord-Trøndelag Health Study (HUNT) in Norway (Hildrum *et al.* 2011), appears to dispel previous hypotheses dating back to the early 20thth century (Rutledge & Hogan 2002; Hildrum *et al.* 2011) that anxiety and stress raise blood pressure. On the contrary, data from the study by Hildrum *et al.* (2011) of 17,410 men and women aged 20 to 67 demonstrated a higher mean decrease in both systolic (-1.59 mm/ Hg, $p = 0.004$) and diastolic (-0.78 mm/ Hg, $p = 0.019$) blood pressures in those with high symptom scores of anxiety or depression measured on the HADS (Snaith 2003) at baseline, at 11 years and at 22 years, in comparison with those with a low anxiety or depression symptom score. The study results, which were adjusted for health status, smoking and alcohol consumption, indicated overall that those with anxiety and/or depression are 20% less likely to suffer from hypertension (>140 mm/Hg) at year 22 than those without anxiety and/or depression (Hildrum *et al.* 2011).

Furthermore, a quantitative review of 15 prospective studies linking psychological factors such as anger, hostility, anxiety, depression and stress found only moderate support for a link between these psychological factors and development of hypertension. This was in the main due to poor methodological quality, such as use of non-validated psychological questionnaires; for example non-validated anger

questionnaires in 12 studies (Rutledge & Hogan 2002). There was, however a strong association between anger and hypertension in one study (Everson *et al.* 1998) using the Spielberger Anger questionnaire (Spielberger *et al.* 1985). In this study of 537 initially normotensive men, at 4 year follow-up there was a 12% increase in hypertension risk ($\text{mm/Hg} \geq 165/95$, $p = 0.002$) in those with high anger scores (Everson *et al.* 1998). Despite the results of the HUNT study by Hildrum *et al.* (2011) and the current study suggesting that *S. lateriflora* is not a useful herb for attenuation of hypertension, this does not obviate the possibility that it could be a useful hypotensive by indirect means. Habitual, heavy smoking and alcohol drinking are linked to anxiety (Halliwell 2009; McManus & Bebbington 2009) (see 1.4.1, p4) and there is evidence that both cigarette smoking and alcohol abuse raise blood pressure (Arkwright *et al.* 1982; Bonita 1986; MacMahon 1987; Etminan *et al.* 2005) so it could be hypothesised that anxiolysis by *S. lateriflora* might reduce hypertension proneness from these causative variables in anxious individuals.

8.4.3 Pulse rate

There was no significant mean change in pulse rate from baseline following administration of either *S. lateriflora* ($p = 0.557$) or placebo ($p = 0.415$). The difference in pulse rate between *S. lateriflora* and placebo ($p = 0.144$) was also not significant. The results demonstrated that *S. lateriflora* has no effect on pulse rate, which remained within the normal range of 60 - 100 BPM (40-60 BPM in athletes) in healthy adults, indicating a favourable health status of participants. A high pulse rate (> 100 BPM), for example, can indicate tachycardia and conversely, a low pulse (< 60 BPM) may indicate bradycardia (Vorvick 2012). The potential causes of cardiac arrhythmias such as bradycardia and tachycardia are too numerous to discuss here but in relation to the present study, tachycardia may indicate severe anxiety or panic attacks or may result from excessive use of stimulants such as caffeine, alcohol or certain drugs e.g. cocaine, amphetamines and tricyclic antidepressants. Bradycardia can result from use of certain drugs, for example antihypertensive agents, beta blockers and calcium channel blockers (Seller 2007). The absence of high pulse rate in any of the study participants suggests that there were no episodes of severe anxiety during visits and that no participants were using recreational drugs during the study. The lack of decreased pulse rate suggests that *S. lateriflora* does not cause any physiological changes such as hypotension that may result in bradycardia. The evidence from the results that it does not lower blood pressure in healthy subjects is further reinforced by pulse rates remaining stable.

8.5 **Personality tests**

Although much has been written about the placebo/nocebo response e.g. (Beecher 1955; Harrington 1997; Shapiro & Shapiro 1997; Geers *et al.* 2005; Link *et al.* 2006; Weeks & Newman 2011), to this author's knowledge there is little, if anything, in the available literature about the association between personality type and prediction of active response to an active herbal treatment. This appears to be the first time a putative link between responders to a herbal anxiolytic and personality trait has been assessed. The Big 5 Minimarker personality test was employed in conjunction with this current clinical research in an attempt to address this paradigm.

There was a correlation between low Extraversion (Factor I) on the Big 5 Minimarker and high change scores on BAI following *S. lateriflora* for those who took the placebo first ($p = 0.011$). This group had a higher mean anxiety score at baseline than those in the group who took *S. lateriflora* first. However, as only 40% of high change scores could be predicted by personality (low extraversion) and 60% being attributable to something else unknown, the relationship between personality response to the test herb and change scores on BAI appears weak. It would be interesting to repeat the analysis with a larger study population as if a stronger or weaker relationship is found in relation to personality trait and prediction of response to *S. lateriflora* it could help to inform differential psychopharmacological herbal treatment.

There also appears to be a dearth of current information relating to association between personality and active response to orthodox anxiolytics. Eysenck (1963) hypothesized that response to drugs is in accordance with personality types; he conducted a series of experiments to prove his theory and demonstrated that introversion/extraversion is responsible for these individual differences. He found that the sedation threshold is lower in extraverts and that the performance of more highly aroused introverts could be improved by administration of tranquillisers (Eysenck 1963; Netter 2000). These findings are consistent with those in the current research in which participants with higher anxiety and hence higher change scores on BAI, and thus reflecting a greater response to *S. lateriflora*, also correlated with low extraversion. This may also be the reason why those with higher anxiety scores at baseline showed a greater increase in V-A scores and greater decrease in F-I scores in comparison to those with lower anxiety scores at baseline.

The findings that *S. lateriflora* responders generally score low on extraversion corresponds to earlier findings by Golding *et al.* (1983), Golding and Cornish (1987) and Ashton and Golding (1989), who found a significant correlation between use of tranquillisers and higher scores for neuroticism and also for low extraversion on the Eysenck Personality Inventory. The Eysenck Personality Inventory (Eysenck 1975) consists of 4 factors: Extraversion, Neuroticism, Psychoticism and Lie. Neuroticism encompasses anxiety (as well as depression, guilt, tension, emotional, shyness, moodiness and irrationality) (Eysenck 1975; Zuckerman *et al.* 1988). Although those with high neuroticism and low extraversion have an increased likelihood of using tranquillisers or hypnotics, cause and effect cannot be distinguished between these factors (Ashton & Golding 1989). It could be argued, however, as indicated by results of the current research demonstrating a significant correlation between low extraversion and response to *S. lateriflora*, that those who score high on neuroticism and low on extraversion may be more anxious and more likely to respond to anxiolytics (including herbal) than those who score low in neuroticism and high on extraversion and are possibly more likely to become dependent on tranquillisers. Although chronic benzodiazepine users may develop comorbid psychosomatic symptoms (Ashton & Golding 1989) no such adverse effects inherent in tranquilliser use have been reported in *S. lateriflora* to date.

Although success in the search for a personality type as predictor of response to herbal anxiolytics may be of use in informing practice, other factors may need to be taken into consideration. For example, a systematic review (Bagby *et al.* 2002) revealed certain personality traits, have been found to be predictors of response to antidepressants (passive-aggressive, high extraversion and low hostility) but variables studied independently as predictors of response to antidepressants - such as comorbid psychological conditions, personality, age, sex, religion, cultural factors, use of substances such as alcohol and tobacco or concomitant drug use – have not been found to be strong predictors of treatment response. However, the authors suggested that these variables and personality variables may nonetheless interact and complicate the picture (Bagby *et al.* 2002). It could be argued that similar interactions may confuse putative personality predictors of treatment response to anxiolytics.

8.6 Placebo responders

Eight participants (25% of all participants) who took placebo first appeared to have a strong placebo response from the placebo treatment (see section 7.8.1, p146). The majority of potential placebo responders were in the moderate-severe anxiety category (BAI scores > 16; n = 6) (Beck & Steer 1993). Only six of all study participants were in the moderate to severe anxiety category on BAI so it is noteworthy that all six appeared to be placebo responders. The other two likely placebo responders were borderline mild-moderate anxiety with scores of 14 and 15 respectively on BAI. It is not clear why anxious participants had a high placebo response to the placebo capsule. One possible reason is a psychological effect from the first face-to-face interview acting as subtle psychotherapy (Kasper *et al.* 2009) but in general, anxious populations are more likely than non-anxious populations to have high placebo response rates (Sarris *et al.* 2009a).

Over the past 60 years or so the literature has indicated that the positive treatment effects of many drugs and other treatments are as a result of placebo responses (Beecher 1955; Harrington 1997; Shapiro & Shapiro 1997; Geers *et al.* 2005; Weeks & Newman 2011). According to Shapiro and Shapiro (1997) the placebo effect is an important component in drug treatment of anxiety. Beecher (1955) found evidence, citing studies of anxious and stressed patients as examples, that the more severe is a disease state, the greater is a placebo response to a placebo - or to a purportedly active drug that has no more power than a placebo. Geers *et al.* (2006) suggest this phenomenon is linked to expectation when attention is focused on somatic symptoms of anxiety. In their study they found that those who were told they were taking a drug that may produce unpleasant somatic anxiety symptoms (such as nervousness, perspiring and trembling) when it was in fact a placebo, experienced more side-effects than those who were told it was, or might be, a placebo. The authors speculated the response was as a result of unconditional expectation coupled with a heightened awareness of somatic symptoms of anxiety (Geers *et al.* 2006).

In the present research, consistent with previous findings (Beecher 1955; Shapiro & Shapiro 1997; Geers *et al.* 2006) it is possible that those who took the placebo first, if convinced they were on active drug, were more likely to feel a response, particularly considering that by chance this group was more anxious at baseline than the group who took *S. lateriflora* first. Because they were keeping a symptom diary, they would likely

have been paying attention to somatic symptoms on a daily basis. Placebo response is discussed here only in relation to the subjective responses reported from the participant symptom diary because inspection of the means of the BAI and the POMS may not, considering the size of the study population and the baseline imbalances, provide a true picture. Furthermore it was unknown whether the reduction in scores was as a result of regression to the mean in some individuals. A larger study population would perhaps have obviated the baseline imbalance in the present research. According to (Hrobjartsson & Gotzsche 2004):

“The vast majority of reports on placebos, including Beecher’s article, have estimated the effect of placebo as the difference from baseline in the condition of patients in the placebo group of a randomized trial after treatment. With this approach, the effect of placebo cannot be distinguished from the natural course of the disease, regression to the mean, and the effects of other factors. The reported large effects of placebo could therefore, at least in part, be artefacts of inadequate research methods”.

The placebo responders were not significantly linked to any particular personality dimension on the Big 5 Minimarker (see sections 7.8.1, p146 and 7.9, p156). Efforts to determine links between personality type and placebo response appear to have produced insignificant or weak results despite great interest in the subject and by 2005 research had failed to find a specific personality type for placebo responders (Geers *et al.* 2005). Acquiescence and absorption, however, are personality traits that are most reported to be a predictor for placebo responding (Whalley *et al.* 2008). Agreeableness, which is synonymous with these traits, is a personality dimension (Factor 2) on the Big 5 Minimarker (Saucier 1994b) but there was no association between this factor and placebo responders in the present study.

In their study of 54 healthy volunteers, Geers *et al.* (2005) found pessimists were more likely than optimists to respond to placebos. Following completion of the Revised Life Orientation Test, which measures optimism/pessimism (Scheier *et al.* 1994), optimists and pessimists were randomly divided into 3 intervention groups and each participant was provided with a placebo pill. One group were told they were taking a pill that would make them feel unpleasant, a second group (conditional-expectation) were told the pill may or may not make them feel unpleasant and the third group was a control group who were told the pill they were taking was inactive. Fifteen minutes following ingestion of the pills participants filled in an affect questionnaire and of those who believed their pill was an active drug pessimists experienced significantly more side-

effects than optimists. There was no difference between pessimists and optimists in either the conditional-expectation or the control groups. The results suggested that pessimism is a significant predictor of placebo responding (Geers *et al.* 2005). The study was limited by a small sample size and the use of a subjective, non-validated brief affect questionnaire. Consistent with the findings of Geers *et al.* (2005), Whalley *et al.* (2008) found in a study of 81 healthy volunteers that pessimists are more likely than optimists to believe they are taking an active drug (even reporting related negative side-effects) if told it was so when it was in fact a placebo; although there was no difference between optimists and pessimists in expectation of effects when they were told they *might* be taking a placebo (Whalley *et al.* 2008).

Only negative effect placebo responding, known as a nocebo effect (Weeks & Newman 2011), was assessed by Geers *et al.* (2005) and Whalley *et al.* (2008) so direct comparisons with placebo responders in this *S. lateriflora* study cannot be made. Placebo responders in this research had a tendency to high BAI scores at baseline and appeared to have a positive expectation effect from the placebo (placebo effect). It is known that people with chronic worry (such as those with GAD) are pessimists and have negative expectations (Miranda & Mennin 2007) as do those who are pessimists (Geers *et al.* 2005), which might explain the results of Geers *et al.* (2005) and (Whalley *et al.* 2008) but does not explain the positive placebo-proneness in certain individuals in the current research.

It cannot be judged definitively whether or not there was a placebo response to either the placebo or the *S. lateriflora*. As the apparent placebo responders were all participants who took the placebo first it is difficult to ascertain the extent to which positive anxiolytic effects are as a result of *S. lateriflora* treatment in those who took *S. lateriflora* first or in fact due to placebo response or spontaneous remission (Geers *et al.* 2006; Kasper *et al.* 2009). Fewer than 4% of placebo-controlled clinical studies of the effects of medical treatments have included a no-treatment condition in addition to placebo but, notwithstanding financial pressures the addition of this factor to a future controlled trial of *S. lateriflora* might provide valuable information on placebo response (Ernst & Resch 1995; Geers *et al.* 2006). A possible way to reduce the potential placebo response in this study may have been to include a placebo run-in phase. However, it was decided not to have a run-in for the study for exclusion of placebo responders because this potentially introduces bias (Berger *et al.* 2003).

Because anxious participants in a study are likely to have a high placebo response rate (Geers *et al.* 2006; Sarris *et al.* 2009a); for example, approximately 50% of studies of GAD patients have been unable to show efficacy for putative anxiolytic interventions because of a high placebo response (Kasper *et al.* 2009), around 70% of studies of the effects of BDZs eliminate placebo responders during a run-in phase (Sarris *et al.* 2009a). Furthermore, it has been found that up to 50% of participants in clinical trials for antidepressants respond to placebos and this has, according to Eli Lilly, plagued drug development. It is for this reason that the company has decided to exclude placebo responders. Others however, believe that this is a subtle manipulation of results that could lead to the marketing of ineffective drugs. Professor Kay Dickersin, currently Director of the Center for Clinical Trials at John Hopkins Bloomberg Center for Public Health and Director of the US Cochrane Center, said “*such winnowing allows bias to enter in and constitutes "a subtle manipulation" of trial results* (Abboud 2004).

According to a report by Berger *et al.* (2003) “if the intention is to determine how best to treat a patient initially, then response run-in selection should not be used”. Exclusion of placebo responders reduces internal validity of a study i.e. it introduces bias. Participants are no longer representative of an unselected study population and therefore positive results of response to the test intervention may be misleading, as the statistical power of the results could be falsely increased when compared to placebo. Similarly, excluding active non-responders may also give “over inflated results to treatment” (Berger *et al.* 2003). On the other hand, in a meta-analysis of randomised, controlled trials of anxiolytics tested on anxious patients, integrating the results of 48 studies, Mitte *et al.* (2005) found there was no increase in the difference between active drug treatment and placebo following placebo run-ins (Mitte *et al.* 2005).

Although the results of the current study were complicated by a high placebo response, to exclude placebo responders in future studies would be detrimental to the heart of the clinical effectiveness of any herbal medicine intervention. The placebo effect in complementary practice is an integral part of the practitioner-patient relationship and therefore the treatment (Black 2009), where talking and listening may share and partially relieve the ‘burden of illness’ (Paterson & Dieppe 2005). It is true to say that during the face-to-face interviews in the current study some participants shared their

emotions and worries with this interviewer, which may therefore account in part for placebo responses in some individuals.

8.6.1 The *Urtica dioica folia* placebo used in this study

There was some concern when choosing the placebo for this study that it may have some physiological effects that might interfere with the results. Beecher (1955) described a placebo as ‘...*pharmacologically inert substances; i.e. lactose, saline solution, starch*’.

The *Urtica dioica folia* used in this study cannot be said to be pharmacologically inert. It is commonly used as a mild diuretic and is sometimes prescribed for anaemia or for the nutritional properties because of its iron and mineral content (Hoffmann 2003); but there are no reports in the scientific or traditional literature of it having any psychopharmacological effects in humans. It was important to use a convincing placebo. In other words, if it did not smell, look and taste like a herb, participants were likely to have been aware they were taking a placebo. Perhaps for the same reason, Wolfson and Hoffmann (2003), in their RCT of *S. lateriflora* for anxiety, also used another herb (*Anthriscus cerefolium*) as the placebo. One of the difficulties in conducting randomised, controlled trials of herbal medicines is the awareness by participants of particular tastes and smells of herbs, which cannot easily be disguised (Zick *et al.* 2005). Placebos need to be not only unidentifiable but must also be identical to the test substance in colour, taste and smell – or both test and comparator need to be ‘hidden’ by being encapsulated. Our test herb, however, had a definite ‘herbal’ aroma and colour, which was visible through the capsule shell, so the placebo was chosen to look and smell similar whilst having no known effects on mood.

8.7 Onset of efficacy

Onset of efficacy was not determined in this study. It appeared from the participants’ diary reports, however, that this was possibly anything from day 3 in some participants up to day 10 in others, depending on the condition reported to be alleviated; for example three (P.35, P.40 and P.41) noticed an attenuation of anxiety on day 3 and two others (P.19 and P.23) reported the same on day 4 of using *S. lateriflora*. Symptoms of IBS disappeared in one participant after 10 days, muscle aches disappeared in another after 3 days while eczema showed signs of improvement in another after 5 days on *S. lateriflora*. Five participants experiencing headaches whilst on *S. lateriflora* noticed

their disappearance after 5 days. It would be interesting if future work could assess onset of efficacy of different variables in order to inform herbal practice.

8.8 Carryover effects of the test herb

The practitioner survey respondents (Chapter 3) reported the herb as being used over a range of time periods from immediate short-term use to ‘several years’, with positive response expected to be experienced by the patient within the first two weeks and persisting throughout the period of use. This perhaps indicates that people believe it to be effective whilst being used but does not indicate for longer.

In contrast, the present study found there was a residual effect of *S. lateriflora* in those who took it first, particularly in relation to BAI and the factors T-A, D-D, A-H, F-I and C-B and for TMD on the POMS. This finding is important as it signifies a previously unknown beneficial effect of this herb. BDZs are commonly known to cause a rebound anxiolytic reaction on sudden cessation of use (B.N.F. 2008), an obviously undesirable effect. BDZs may also cause a rebound stress reaction, as indicated by findings of Pomara *et al.* (2003) who assessed both acute and chronic (3 week) effects of high and low dose lorazepam (0.25 mg, 1.0 mg b.i.d) and alprazolam (0.25 mg, 0.5 mg b.i.d) in 68 healthy geriatric volunteers. Only the 0.5 mg alprazolam dose attenuated the HPA axis in acute treatment when compared to placebo, as demonstrated by decreased salivary cortisol output but there was a significant increase in pre-dose cortisol output during chronic alprazolam treatment. This effect was not observed with chronic lorazepam treatment (Pomara *et al.* 2003).

Prolonged anxiolysis due to a residual effect of *S. lateriflora* following cessation may be a desirable factor in its administration, which is possibly in contrast to the residual effects of BDZs, which manifest as unpleasant withdrawal symptoms. The noticeable pharmacological effects of some BDZs with long half-lives are considerably shorter than their half-lives and they may leave cumulative residual effects on the body (Ashton 2008). King’s American Dispensatory (Felter & Lloyd 1898) describes *S. lateriflora* as an antispasmodic nervine tonic that is calming in all cases of ‘*nervous excitability, restlessness, or wakefulness*’ without any rebound excitability on cessation of use. This is worth remembering as it might imply that the benefit persists after cessation – something there is generally little comment about.

There is always a risk of carryover in a crossover study. One problem is that sometimes the condition is actually cured by the test treatment to such an extent that placebo may not result in a reversal of symptoms, regardless of the length of washout period. Another is that it may also continue to work if the washout period is not long enough, producing a carryover effect (Kotler & Laster 1998).

It is difficult to accurately assess carryover effects unless the condition is chronic and stable (EMEA 1998). A study on patients with GAD, which is a waxing and waning condition with a lifetime spontaneous remission of only one third, may give more significant results than those from an apparently healthy population who are more likely to have a remission of anxiety (Wittchen & Hoyer 2001). In this present study there were few chronically anxious subjects and even those who scored high on the anxiety scale could not be said categorically to have trait anxiety. There is no guarantee that spontaneous remission did not occur in some participants. One way to test this would be to have another arm of the study in which there was no treatment in order to estimate the extent of the placebo effects (Geers *et al.* 2006) and to assess the possibility of spontaneous remission; or to repeat the anxiety instruments following a prolonged washout period and prior to recommencing either *S. lateriflora* or placebo.

8.8.1 Minimising carry-over

Estimation of washout times is frequently problematic with consequent design faults leading to inadequate results (Wang *et al.* 2006). In this research it is evident that there may have been an under-estimation of the half-life of *S. lateriflora* and perhaps a washout period of at least 5 x 200 hours analogous with diazepam (Ashton 2008) approximately 6 weeks - would be appropriate. This would necessitate a lengthy study period and perhaps more dropouts so would necessitate a larger study population. A way around the problem of participant adherence could be to conduct a parallel study (double-blind RCT) of similar duration to the current study.

In a cross-over design a washout or drug-free period is important for the avoidance of not only carry-over effects of an intervention from one arm of the study to another but also to avoid a combination effect of two drugs (Smith 1992). In the case of the *Urtica* placebo in the present study, although not known to be active for anxiety, it is unknown whether its phytochemicals have the potential to interact with those in the *S. lateriflora*. A washout period must also be of a duration that is sufficient to ensure there is no

psychological carry-over effect e.g. a ‘memory’ of treatment effects, and that all participants have returned to baseline (Wang *et al.* 2006).

The washout period necessary to avoid carry-over effects in a crossover study is usually determined as at least five times the half-life of a study drug and should be for a minimum of 7 days (World Health Organisation 2006). This assumes the half-life is known. The half-life of the active metabolites of some BDZs marketed as anxiolytics, for example, can be as much as 200 hours even though the effects of one dose may appear to wear off within a few hours. When there is repeated dosing an accumulation of a BDZ drug or its metabolites occurs in fatty tissues; there is a cumulative effect and subtle physiological effects may persist (Ashton 2008).

Although subjective reporting by human volunteers in a clinical trial suggests that the effects of a single dose of *S. lateriflora* wear off after around 2 hours (Wolfson & Hoffmann 2003), the half-life of *S. lateriflora* and its possible cumulative effects in humans are unknown - although some of its flavonoids have been studied to this effect. Following oral administration to 10 healthy volunteers of commercial skullcap root (*S. baicalensis*) powder, urinary excretion of conjugated metabolites of baicalein and wogonin was 7.2% and 11.6% of the original dose respectively. The half-life eliminations for these conjugates were approximately 8 hours for baicalein and 10 hours for wogonin (Lai *et al.* 2003). An indication of the length of time some flavonoids remain in the body may be a useful indicator for a washout period in a clinical study. Therefore, as a precautionary measure, it was judged that a washout period of a week would minimise potential physiological or psychological carry-over effects following repeated dosing.

Another reason why a week’s washout was judged to be conducive to a return to baseline mood and desirable prior to taking new capsules is because there is evidence to suggest that mood may be affected by social zeitgebers (environmental factors that synchronize the endogenous cycle or circadian rhythm to 24 hours) of the calendar week (Reid *et al.* 2000). Carry-over effect of an intervention or the change itself could potentially disrupt this entrainment (biological clock) to the calendar week. A week as a unit was judged to be preferable as a washout period than periods between 8 and 13 days because of this influence of social zeitgebers on mood and two weeks would have been too long for reasons of patient compliance.

Washout period would likely depend on whether major active substances are hydrophilic or lipophilic. Baicalin is hydrophilic, whereas the aglycones, such as baicalein and wogonin are lipophilic (Barceloux 2008) and this lipophilicity may account for carry-over effects if the compounds remain in fatty tissues. A random selection of 22 double-blind randomised controlled crossover trials involving flavonoids, whole herbs and/or anxiolytics revealed the mean (SD) washout period for these studies was 11 (9) days. Where washout was included the longest period was 35 days and the shortest for 3 days. No reason for time choice was provided in any of the studies. All studies on whole herbs (8) had a washout period of 10 days or less, usually 7 days (5 studies); one had no washout at all. It appears that washout period is difficult to judge therefore when the half life is unknown and in some cases may be arbitrary. Measurements of subjective anxiety and mood at an additional time point i.e. following washout, is paramount for assessment of carry-over and also providing information on placebo response in future studies.

Overall, the evidence in this study of a carry-over effect of *S. lateriflora* seems to indicate a cumulative effect of the herb, which could be said to be beneficial considering the known rebound excitability and dependability of other anxiolytics, particularly BDZs (B.N.F. 2008). Future studies could determine the duration of effects by repeated subjective measures of mood at regular follow-up time points following cessation of treatment.

8.9 Strengths and limitations of the study

A major strength of the study was the use of freeze-dried herb in controlled doses and its authentication prior to commencement of the RCT. Another was the ability to assess within subjects effects as well as between subjects effects by the use of a crossover design. An advantage of a crossover design is that participants act as their own controls and there is less variability within subjects than between subjects (Dallal 2000). Fewer participants are required for the study than if it were a parallel study. An equal number of participants (group) for each time period receives one treatment (either placebo or test) while the other group receives the other treatment.

Potential disadvantages of crossover studies are that they are of longer duration than a bioequivalent parallel study and there is therefore more chance of dropouts because of

inconvenience to participants; the study might not be long enough to show effects of a drug as each period needs to be kept reasonably short to avoid too much inconvenience and/or instability in a condition. Spontaneous resolution, particularly of acute conditions, may be more likely during a longer period. This situational change is known as a period effect (Wang *et al.* 2006). In this current research, psychometric testing following washout would have been a useful measure of comparison between nothing and test and placebo, to see how much scores return to baseline or if there is a placebo effect.

Potential carry over effect – physiological or psychological – in the group taking the test substance first (Shen & Lu 2006; Wang *et al.* 2006) is another disadvantage as evident in this research. The carry-over effects in the present research indicate that a longer washout period was necessary. Although it was ensured that almost equal numbers received the test intervention during the first and second periods of the study to help balance this effect and avoid bias – adequate washout is important (EMEA 2006). The dropout rate of 28% in this study may have added to the chance imbalance between groups as demonstrated by numerous order effects. Order effects are problems inherent in crossover designs, creating difficulties in the interpretation of results (Mackereth *et al.* 2009). If there is a baseline imbalance, one approach is to abandon the results of the second period and to treat the study as if it were a parallel study. This, however, wastes valuable time and resources as well as reducing the statistical power and the ability to compare within subjects effects (Sibbald & Roberts 1998). Other approaches are to use either change score analysis or analysis of covariance (ANCOVA). A problem with change score analysis is regression to the mean when there are low baseline scores (Vickers & Altman 2001). However, when there is a true baseline imbalance as in the present study ANCOVA can distort treatment effects and result in bias (Metcalf 2010).

One of the major improvements for any future crossover study using healthy volunteers would be to recruit more participants as it was evident due to baseline shifts between subjects that the above study did not have enough power to eliminate this in a healthy sample population. A new power calculation and sample size estimation for a future crossover study in a healthy population would be based on the standard deviations of the results of the present study instead of, as previously, an anxious population (Prasko *et al.* 2006). Out of necessity, because of problems obtaining ethical approval for a study sample with self-reported anxiety, other than 3 who were moderately anxious

(BAI scores 16-25) and 3 who had severe anxiety the results of this study are based on volunteers from a mainly non-anxious population. Baseline scores on BAI ranged from 1-37 and 25 participants completing the study had minimal or mild anxiety at baseline. In this research it happened by chance that there were more non-anxious subjects in the Group who took the *S. lateriflora* first. The two variables showed differences between baseline measurements, with those in group 1 showing higher initial mean (SD) anxiety scores of 15.73 (10.71) than those in Group 2 at 8.81 (3.99). Spontaneous remission after a few weeks is more likely if they had a transient mild or minimal anxiety during baseline testing, for example due to upcoming examinations. This is particularly true for participants with low baseline scores as they are more likely to undergo remission than those with high scores (Vickers & Altman 2001). Recruiting more volunteers may provide inclusion of more anxious volunteers and a more equally distributed baseline anxiety score. Similarly for other parameters (see POMS) an increased number of participants might be an advantage. Recruitment of notably anxious volunteers may also help to eliminate baseline shift.

Another limitation of the study is the anxiety and mood data obtained from the BAI and POMS respectively was retrospective and could therefore not determine the effects of daily stressors and psychological effects on these paradigms. Similarly the physiological data from salivary cortisol samples was obtained at only three time points and did not reflect stressful life situations occurring during the study.

This was a pilot study to determine the effects of *S. lateriflora* on mood states, anxiety and stress. In addition to the above, another limitation was the small sample size. Significant delays at the start of the study due to authorisation and ethical clearance to conduct the study severely limited the time available and hence the number of participants. Ethics and MHRA approval for recruitment of diagnosed anxiety would have required a major change in study design and necessitated external ethics approval, which were explored and were problematic for the research team to achieve. Restricted resources limited the number of visits per participant. Nevertheless the results are still encouraging and merit a stimulus for further research in the area, using improved methodology.

8.10 Summary and conclusions

Despite the problems in analysis revealed by the study design, new information has been obtained from this small, observational study, which can make substantial contributions to the literature and herbal practice as a whole. There is an indication that *S. lateriflora* reduces symptoms of anxiety and enhances mood in some individuals as demonstrated respectively by a reduction in anxiety scores on BAI and T-A scores on the POMS in healthy volunteers. Although the anxiolytic effects were evident only in the group that took placebo first, these were in the main more anxious at baseline than those who took *S. lateriflora* first and an insufficient washout period may have resulted in an underestimation of the effects of *S. lateriflora* in Group 2. Considering the placebo response in the group who took the placebo first it is even more remarkable that there was a significantly ($p = 0.036$) greater response (as shown on BAI) to the *S. lateriflora* compared with placebo in this group. Furthermore, the group that took *S. lateriflora* first experienced a carry-over effect, which precludes the apparently greater response to placebo compared with *S. lateriflora* in this group. It is however, important to note that the possibility of placebo responses following *S. lateriflora* indicates the study results do not confirm a definite anxiolytic effect and emphasises the need for further study. As the results were limited by a small sample size ($n = 43$ and 28% dropout rate; 31 completed) future studies may benefit from a larger sample size as well as recruitment of an anxious population – such as those with primary diagnosis of GAD.

For the first time, personality trait as a predictor of response to *S. lateriflora* was assessed and it was found that low extraversion was a significant predictor of higher change scores on BAI. Although only evident in the group that took placebo first the reason may be because this group was generally more anxious at baseline and therefore had a higher mean BAI change score.

Other major findings were an overall enhancement of mood as demonstrated by TMD scores on the POMS and an indication that, contrary to previous beliefs and in contrast to some orthodox anxiolytics e.g. alprazolam, *S. lateriflora* does not worsen depression shown by D-J scores on POMS. There was no discernible effect of *S. lateriflora* on attenuation of the HPA axis as reflected by no significant mean changes in salivary cortisol measurements ($p = 0.524$) but it was not evident that participants were experiencing stress during saliva sampling. There was also no effect on blood pressure

but participants were normotensive and healthy at baseline so this was to be expected. Additionally, *S. lateriflora* had no effect on pulse rate. Importantly there were no toxic effects from *S. lateriflora* as demonstrated by no clinically significant mean elevation of ALT levels from baseline (2.01 U/L) and no significant difference between *S. lateriflora* and placebo in mean ALT elevation ($p = 0.801$). Of great importance is this is the first time it has been demonstrated that there were no adverse effects on energy or cognition (as shown by V-A, F-I and C-B on the POMS) during chronic administration of *S. lateriflora*. Additionally, the finding, for the first time, that the effects of *S. lateriflora* persisted following cessation of use, may prove to be beneficial.

In conclusion the data suggest, despite the study limitations, that *S. lateriflora* has significant anxiolytic and mood enhancing effects in some people. Also significant is the lack of toxicity, adverse reactions or definite side-effects that could be attributed to the herb and the fact there were no rebound reactions such as tolerance, dependability or excitability. This lack of harmful or unpleasant effects is central to the clinical efficacy of an anxiolytic. Further studies of *S. lateriflora* could prove it to be a safe effective alternative to currently used anxiolytics. As this was an exploratory study with a number of design faults (e.g. carry-over effects, unmatched groups and small sample size) the results should be interpreted with caution. A longer study with a larger sample size, either a parallel study or a crossover with a longer washout period, and using participants with a diagnosis of GAD may yield more significant results. It is not clear, however, whether participants with GAD would yield significant results relating to attenuation of the HPA axis. A sufficient number of well-matched subjects using a pre-defined narrow range of baseline scores on a validated instrument such as BAI would be a parallel study of choice in order to eliminate problems of baseline shift and inadequate washout. A disadvantage of a parallel study is no intra-individual comparison and more variation between subjects, with also a likelihood of a large number of dropouts in the placebo group. Again such problems could be reduced by ensuring a large enough study population.

Chapter 9. Future work

The promising results from this study, for example an indication that *S. lateriflora* attenuates anxiety and enhances mood without a reduction in energy or cognition, could lead to valuable future work for which time and financial constraints of the PhD did not permit. For example:

9.1 Future clinical trials of *S. lateriflora*

9.1.1 Anxiolytic and mood enhancing effects

The regimen of the current study did not allow for longer-term effects to be assessed, required to determine tolerance, dependency or optimum dosage. Tolerance, believed to result from sensitisation of GABA_A receptors, to the anxiolytic effects of benzodiazepines, for example, may take several months (Ashton 2005). A similar randomised, double-blind, placebo controlled crossover study could be conducted with varied dose regimens such as 1-4 x 350 mg capsules of freeze-dried *S. lateriflora* daily. Because it was evident from the results that there was a carryover effect of *S. lateriflora*, a longer washout period would be appropriate as would additional repetition of measures following washout to determine the extent of potential residual effects of the test herb as well as a ‘no treatment’ effect. Furthermore, although the *Urtica dioica* placebo has no known psychopharmacological effects, it was nonetheless not an inert substance and its potential interactions with *S. lateriflora* are unknown. It may be more pertinent to conduct parallel studies, given the carry-over effects seen in the current study and other problems inherent in the crossover design, such as order effects – on the proviso of course that resources allow for a large study population. If a parallel study is conducted it will have three arms in order to include a no-treatment group for comparison with placebo and test group. In addition, participants would have follow-up assessments for determination of long-term benefits or rebound effects in comparison to the no-treatment group.

Assessments on those with a primary diagnosis of generalised anxiety disorder (GAD) are desirable as the study has revealed the difficulties inherent in the assessment of the efficacy of a putative anxiolytic in non-anxious subjects. In addition, when there is a true baseline shift, which in the current study could be attributed to inconsistency between subjects in anxiety severity rather than sample size, between-subjects comparisons are not straightforward (Vickers & Altman 2001; Metcalfe 2010). A

parallel study is likely to be ‘intention to treat’ and would benefit from having 4 arms of the study; skullcap treatment, a mild benzodiazepine control, placebo and no treatment. A mild benzodiazepine for positive control would preferably have a short half-life if a crossover design was used. Oxazepam has a half-life of 5-15 hours, and Alprazolam has a half-life of 10-15 hours (compared to Lorazepam and Clonazepam which have a half-life of 10-20 and 24-56 hours respectively) (Vanin & Helsley 2008) and diazepam has a half-life of up to 200 hours due to the residual effect of its active metabolite (Ashton 2008).

GAD is a severe, chronic anxiety disorder with a lifetime prevalence of 6% of the population (Kasper *et al.* 2009). It is defined by the Diagnostic and Statistical Manual of Mental Disorders IV (American Psychiatric Association 2000) as persistent, excessive worry for at least 6 months and at least 3 of the symptoms of muscle tension, fatigue, insomnia, disturbed sleep, inability to concentrate and irritability. The presence of anxiety is not due to a mood disorder or psychotic disorders and it is not as a result of drug or substance use or environmental factors. The patient cannot control their anxiety (American Psychiatric Association 2000). GAD is usually treated with benzodiazepines, buspirone, SSRIs, serotonin-norepinephrine reuptake inhibitors, monoamine-oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), beta-blockers and cognitive behavioural therapy (CBT) as monotherapy or in various combinations (Helsley 2008). The chronicity of the anxiety in those with GAD deems it to be less likely that spontaneous remission would occur during the course of the study in comparison to mildly anxious patients (Wittchen & Hoyer 2001) and therefore the efficacy of *S. lateriflora* could be assessed with little risk of confusion between efficacy, regression to the mean and spontaneous remission.

Findings that *S. lateriflora* does not appear to worsen existing depression suggest that future work assessing its efficacy in those with anxiety disorders need not exclude those with comorbid depression. This is important because there is a high comorbidity rate with anxiety and depression, with 7% men and 11% women experiencing both at the same time in any given year (Office for National Statistics 2006) and therefore a preferred anxiolytic would attenuate both anxiety and depression (Mitte *et al.* 2005; Greaves-Lord *et al.* 2007). A validated measure of subjective depression such as the Beck Depression Inventory (Beck *et al.* 1996) would be used to assess this paradigm.

Finally, it would be of interest to note the onset of efficacy of *S. lateriflora*. In an 8 week double-blind, placebo-controlled efficacy study of pregabalin (an anti-convulsant) and venlafaxine-XR (an SNRI) in GAD, Kasper *et al.* (2009) used investigator-rated HAM-A scales by telephone interview on day 4 (in advance of the first weekly visit) to determine efficacy onset of the drugs. There was > 20% improvement in anxiety scores from baseline for pregabalin compared with venlafaxine or placebo on day 4. Measurements were not taken on days 1, 2, or 3 but it would be useful to do so with *S. lateriflora*.

As enhancement of global mood following *S. lateriflora* as demonstrated by the results of the current study in some individuals, the Profile of Mood States (Lorr *et al.* 2005) would be a useful instrument to use in conjunction with Beck's Anxiety Inventory (Beck & Steer 1993) when conducting future studies on an anxious population. It would provide new information for general practice if it could be determined that the *S. lateriflora* also enhances mood in those suffering from chronic anxiety, particularly considering studies of administration of benzodiazepines have indicated that some of these tranquillisers have some negative effects on subjective measures of mood e.g. increased confusion and decreased energy, using the POMS (Frey *et al.* 1998).

9.1.2 Clinical studies of *S. lateriflora* for premenstrual syndrome

One participant in the current study reported attenuation of her premenstrual symptoms (see 7.7.7). Furthermore, results of a survey of herbal medicine practitioners found it is commonly prescribed for this condition (see 3.4.1). Despite its traditional use for problems relating to the menstrual cycle and for mastalgia, there appears to be little reference in modern herbal medicine texts to current use of *S. lateriflora* in gynaecology. Hoffmann (2003) states that it can be safely used to ease premenstrual tension and Harrar and O'Donnell (1999) suggest it has healing potential for premenstrual syndrome. Liao *et al.* (1995) found *S. baicalensis* root extract bound to D₂ receptors *in vitro*. Any resultant lowering of prolactin may modulate dysfunctional menstrual cycles and alleviate premenstrual mood disorders, which are implicated in cases of latent hyperprolactinaemia (Trickey 2003). The latter may dysregulate dopamine secretion, leading to a lowering of mood (Greenstein 1994). A randomised controlled trial to assess the efficacy of *S. lateriflora* for premenstrual syndrome would

make a valuable contribution to herbal practice. Research into the binding capacity of *S. lateriflora* on D₂ receptors could also be conducted.

9.1.3 Antioxidant effects

The *in vitro* studies of *S. lateriflora* indicate its potential as an herb with strong antioxidant capacity (Wojcikowski *et al.* 2007; Wojcikowski *et al.* 2009). Future clinical studies could be conducted to determine its efficacy for conditions in which a protective effect on tissues is required; for example Alzheimer's disease and non-Alzheimer's senile dementia (Middleton & Yaffe 2009), or liver, kidney and cardiovascular disorders (Meydani 2001).

9.2 A metabolomic analysis of *S. lateriflora*:

A metabolomic analysis of *S. baicalensis* shoots revealed 2,400 compounds. Of these compounds 781 may have medicinal actions, including hyperforin; a compound found in *Hypericum perforatum* and believed to be effective against depression (Murch *et al.* 2004). A similar study of *S. lateriflora* would provide a useful contribution to the literature and give more insight into potential therapeutic actions and indications of the herb.

9.3 Conclusions

While there are likely to be many other studies that could be conducted on this vital herb, whether chemical, *in vitro* or clinical, it is beyond the scope of this work to ponder on them all. However, this PhD has provided a valuable contribution and insight into future possibilities.

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7 December 2008

Dear Herbal Medicine Practitioner,

I am currently a PhD student at the University of Westminster within the new Westminster Institute for Health and Wellbeing. I am researching the use of skullcap (*Scutellaria lateriflora*) in the treatment of stress and anxiety.

My Director of Studies is Dr Julie Whitehouse, principal lecturer in the School of Integrated Health. (whitehj@wmin.ac.uk)

The Institute aims to provide research-based evidence that informs policy and changes practice so that society reaps benefits in terms of improved understanding and promotion of health and well-being.

I believe that high-quality research in herbal medicine is essential to provide evidence to support its traditional use. Such evidence will hopefully raise the profile of the herbal medicine profession, engender greater acceptance by orthodox medical professionals and the general public, and help to provide patients with informed treatment choice.

Although there is much anecdotal evidence of efficacy, there has been little research conducted on *Scutellaria lateriflora*. I believe it has great potential as a candidate herb to provide scientific evidence in support of the traditional use of herbal medicine.

No animals will be used for the purposes of this research.

I am writing to you in the hope that you will be able to give a little of your time to help with this research by offering your valuable practitioner experience and answering a few questions (see attached) about *Scutellaria lateriflora*.

Please return the completed questionnaire by email attachment. I would be most grateful if it could possibly be returned by 10 January 2009.

All responders will remain anonymous in the use of the data collected

Thank you very much in anticipation.

Yours sincerely,

Christine Brock, BSc (Hons) Herb. Med., MNIMH, MIBMS

c.brock@westminster.ac.uk



Survey amongst UK herbal practitioners on the use of *Scutellaria lateriflora*.

Please answer the following questions as far as possible:


1. Do you regularly prescribe <i>Scutellaria lateriflora</i> ?	Y	N
2. If not, please give reasons:		
3. What do you consider the main actions and indications of <i>S. lateriflora</i> ?		
4. What are the most common uses or conditions for which you prescribe it?		
5. What is the usual length of treatment?		
6. What response do you expect or what do look for in the reports from patients		
7. After what length of time would you expect to see a response?		
8. What have your patients reported from its use?		
9. If there are any conditions for which you consider it contraindicated, please specify:		
10. Please state any side-effects reported.		

<p>11. Please indicate the most likely <u>dose range</u> (min - max) and <u>strength</u> you would use, specifying either single dose, weekly dose, or both, and your preferred mode of administration. Eg:</p> <ul style="list-style-type: none"> - Tincture extracted from dried herb (marc: menstruum and % alcohol) - Fresh (specific tincture) (marc: menstruum and % alcohol) - Fluid extract - Dried, cut - Powder - Freeze-dried capsules <p>(Indicate whether organic/ non organic).</p>
<p>12. What is the reason for your choice (eg cost, most effective)?</p>
<p>13. Who is your supplier for the herbal preparation?</p>
<p>14. Do you use it alone or in combination with other herbs?</p>
<p>15. Do you have a preferred herb for anxiety (<i>Scutellaria lateriflora</i> or any other herb)? Y N</p>
<p>16. If so, which one?</p>
<p>17. Would you be willing to be contacted if this research plans to collect further information form practitioners? Y N</p>
<p>Please add any additional comments you would like to make about <i>Scutellaria lateriflora</i>:</p>
<p>Practitioner data:</p> <p>Name:</p> <p>Length of time in practise:</p> <p>Average number of patients per week:</p> <p>Email:</p> <p>Confidentiality: Your identity will not be revealed in any way, either verbally or in any publication</p>

Appendix III *S. lateriflora* voucher specimen (AHP)



American Herbal Pharmacopoeia© voucher of *S. lateriflora* dated 7 August 2003

UNIVERSITY OF WESTMINSTER RESEARCH	
Research Volunteers Wanted	
	<ul style="list-style-type: none">• Stressed?• Irritable?• Anxious?• Mood swings?• Unable to cope?• Poor sleep?• Feel as though life is getting you down? <p>You can take part in a study of traditional herbal remedy American skullcap, which has long been used for relieving anxiety, tension, insomnia, irritability and stress.</p> <p>To take part you must be:</p> <ul style="list-style-type: none">• In good general health• 18 - 75 years old• Not be taking any medication. <p>Further eligibility criteria will apply.</p>
<p>Contact: Christine Brock C.Brock@westminster.ac.uk Or ring the University's Polyclinic: 020 7911 5041 quoting 'Skullcap Project'</p>	

Research volunteers wanted

- Stressed?
- Irritable?
- Anxious?
- Unable to cope?

Contact: Christine Brock
C.Brock@westminster.ac.uk

Or ring the university's
Polyclinic: **020 7911 5041**
quoting 'Skullcap Project'

You can take part in a University of Westminster study of traditional herbal remedy American skullcap, which has long been used for relieving anxiety, tension, insomnia, irritability and stress.

To take part you must be:

- In good general health
- 18 - 75 years old
- Not taking medication.

Further eligibility criteria apply





Researcher: Christine Brock (PhD student)

The researcher is a qualified medical herbalist, Member of the National Institute of Medical Herbalists. She is knowledgeable in the use of herbal medicines and her training and experience enables her to recognise and assess a wide range of health problems.

Principal supervisor: Dr Julie Whitehouse

Second supervisors: Dr Ihab Tewfik and Professor Anthony Towell

Address: School of Life Sciences, Cavendish Campus, New Cavendish St, London W1W 6UW

Email: C.Brock@westminster.ac.uk **Tel:** 020 7911 5041 (University of Westminster Polyclinic)

Study: American skullcap (*Scutellaria lateriflora*): a study of its effects on mood in healthy volunteers.

Study identifier: 09/10/31/ISRCTN48078312

Ethics: This study has been approved by the University of Westminster Research Ethics Subcommittee

Estimated dates of study: April 2010 – July 2011

Expected duration of study: 38 days

Thank you for volunteering to take part in this study. This form outlines the purposes and methods of the study and provides a description of your involvement and rights as a participant.

PLEASE READ ALL 3 SECTIONS CAREFULLY

Section 1: The purposes of this project:

- A. To gain insight into the potential for American skullcap herb to enhance mood and produce feelings of wellbeing.
- B. To determine the effectiveness of American skullcap in reducing stress.
- C. The information collected from the study will add to scientific knowledge in general and will be used to inform practitioners of herbal medicine and other professionals of the effectiveness and safety of skullcap herb for reducing negative mood states. Results will be published accordingly.
- D. The researcher is undertaking this study as part of the requirement for the qualification of PhD at the University of Westminster Institute of Health and Wellbeing.

Section 2: Outline of the study and methods to be used

This project aims to find out how safe and effective the herb skullcap is in reducing symptoms of stress and improving mood. Skullcap is a medicinal plant widely prescribed over many centuries by medical herbalists and included in over the counter preparations to help people who feel stressed and anxious to relax. It is not known to be harmful.

- Those taking part in the study (participants) will be randomly split into two groups. One group will take a skullcap capsule 3 times a day. For comparison, the other group will take a placebo (dummy/harmless) capsule 3 times a day. This is dried common nettle, often used as a refreshing herbal tea, which has no effects on mood. Neither you nor the investigator will know which type of capsule you are taking until the end of the study. After 2 weeks you will stop taking capsules for a week. You will then be given the other type of capsule for 2 weeks.
- To see if your feelings of wellbeing change over time you will be asked to complete 2 questionnaires about your mood and anxiety levels (about 10 minutes each) before, in the middle, and at the end of the study. We will also measure your blood pressure and pulse at these time points to see if there are any noticeable changes in either.
- To determine whether personality might affect the way you respond to American skullcap you will be asked to complete a short personality test at the beginning of the study.
- You will be asked to provide samples of your saliva. This is because saliva contains a substance (hormone) called cortisol and levels of this may change when you are stressed.

Measurements of the amount of cortisol in your saliva may show whether or not the skullcap herb is reducing your stress. Using a convenient and comfortable method of chewing on a cotton wad contained in a small plastic tube you will need to provide saliva at **3, 6, 9 and 12 hours** after your natural waking time for **2 days running**. I will ask you to do this at the beginning, in the middle, and near the end of the study. You will not be able to eat or drink anything except water, smoke, clean your teeth, or do any rigorous physical exercise for at least 30 minutes before providing each saliva sample.

- I will also take a small drop of blood from one of your fingers before, during and after the study so that chemicals in your blood can be tested to assess the safety of skullcap herb.
- Throughout the study you will be asked to keep a daily diary of your mood and of any changes in your body you might notice.

Section 3: Your rights and your involvement in the study:

1. Your eligibility to take part in the study is not automatic and you will be invited to undergo a selection procedure. This involves being asked questions about your health, having your blood pressure and pulse taken and filling in a brief questionnaire about your mood.
2. If at the initial selection interview or at any later time during the study, there is any reason that you may not start or continue to participate in the study, you will be informed of the reason and offered advice by a qualified herbal practitioner in the Polyclinic.
3. You have been invited to participate in this research because you are in good general health and are interested in helping to promote wellbeing with herbal medicine.
4. Your participation in this study is voluntary. You have the right to refuse to participate and to withdraw at any time and for any reason without prejudice.
5. You have the right to refuse to answer any questions if you do not wish to do so.
6. You are encouraged to ask questions about the study. I will endeavour to respond as soon as possible to any queries or concerns you may have.
7. All questioning, blood pressure, pulse and blood checks will be performed in private at the University of Westminster Polyclinic, which you will be invited to attend at a mutually convenient time and date. Attendance is 3 times in all, for approximately an hour followed by two 30 minute visits.
8. Saliva sampling, diary keeping and capsule taking will be carried out away from the clinic.
9. Saliva samples will be coded, frozen and then destroyed following study completion.
10. Your participation in this study will be completely confidential and your name will never be revealed to anyone, either verbally or in print. Your records will be kept securely locked away at all times with access only by the investigator. Your name will not be included in computerised data.
11. You will have access to your own data at any time during the study, upon request.
12. You may experience some positive effects on your mood during the study. This may be immediate or may occur closer to the end of the study; or you may feel no difference at all.
13. Although not expected, if you experience any adverse effects while taking capsules it is important that you report these as soon as possible to the investigator or to the Polyclinic and if concerned to your GP immediately.
14. In the unlikely event that you experience any undesirable side effects as a result of the study you will be asked to withdraw and further advice will be offered to you.
15. The study may be terminated early by the investigator if deemed appropriate to do so as a result of the investigations.
16. You will be informed of the findings of the research on completion of the study.
17. If there are any changes to the study design you will be informed of these and your consent to continue will be renewable.
18. We request that you inform your GP of your participation in the study.



Christine Brock
School of Life sciences
University of Westminster
115 New Cavendish St
London W1W 6UW

cabrock@westminster.ac.uk

Tel: 020 7911 5041

Dear

Re: American skullcap (*Scutellaria lateriflora*): a study of its effects on mood in healthy volunteers

Thank you for your interest in taking part in this study. The enclosed form outlines the purposes and methods of the study and provides a description of your involvement and rights as a participant.

If after reading the information, and giving yourself a reasonable amount of time to consider it, you would like to take part in this study please contact me at the email address above to arrange an appointment for eligibility screening. You may instead telephone the university Polyclinic to leave your number and I will call you back.

At your appointment, you will be asked to sign a consent form before screening commences. If screening is successful your participation in the study will begin with immediate effect. The first visit will take about an hour and the two subsequent visits are likely to be shorter.

If you have any questions at all about the study please do not hesitate to contact me and I will respond to your queries as soon as possible.

Yours sincerely,

Christine Brock, BSc (Hons) Herbal Medicine, MNIMH, MIBMS

Appendix VIII Hospital Anxiety and Depression Scale and scores (Zigmond & Snaith 1983)

Participant code _____

Date _____

This questionnaire is about how you feel. For each of the following items **tick** the response that comes closest to how you have been **feeling** in the **last week**. Do not take too long to think about your answers - your immediate response will probably be the most accurate.

1. I feel tense or 'wound up':

- ☐ MOST OF THE TIME
- ☐ A LOT OF THE TIME
- ☐ OCCASIONALLY
- ☐ NEVER

2. I still enjoy the things I used to enjoy:

- ☐ AS MUCH AS EVER
- ☐ NOT QUITE AS MUCH
- ☐ ONLY A LITTLE
- ☐ HARDLY AT ALL

3. I get a sort of frightened feeling as if something awful is about to happen:

- ☐ VERY DEFINITELY AND QUITE BADLY
- ☐ YES, BUT NOT TOO BADLY
- ☐ A LITTLE, BUT IT DOESN'T WORRY ME
- ☐ NEVER

4. I can laugh and see the funny side of things:

- ☐ AS MUCH AS I ALWAYS COULD
- ☐ NOT QUITE SO MUCH AS I USED TO
- ☐ A LOT LESS THAN I USED TO
- ☐ NEVER

5. Worrying thoughts go through my mind:

- ☐ A GREAT DEAL OF THE TIME
- ☐ A LOT OF THE TIME
- ☐ FROM TIME TO TIME BUT NOT TOO OFTEN
- ☐ ONLY OCCASIONALLY

6. I feel cheerful:

- ☐ NEVER
- ☐ NOT OFTEN
- ☐ SOMETIMES
- ☐ MOST OF THE TIME

7. I can sit at ease and feel relaxed:

- ☐ DEFINITELY
- ☐ USUALLY
- ☐ NOT OFTEN
- ☐ NOT AT ALL

8. I feel as if I am slowed down:
- ☐ NEARLY ALL THE TIME
 - ☐ VERY OFTEN
 - ☐ SOMETIMES
 - ☐ NOT AT ALL
9. I get a sort of frightened feeling like 'butterflies' in the stomach:
- ☐ NEVER
 - ☐ OCCASIONALLY
 - ☐ QUITE OFTEN
 - ☐ VERY OFTEN
10. I have lost interest in my appearance:
- ☐ COMPLETELY
 - ☐ I DON'T TAKE AS MUCH CARE AS I SHOULD
 - ☐ I MAY NOT TAKE QUITE AS MUCH CARE
 - ☐ I TAKE JUST AS MUCH CARE AS EVER
11. I feel restless as if I have to be on the move:
- ☐ VERY MUCH INDEED
 - ☐ QUITE A LOT
 - ☐ NOT VERY MUCH
 - ☐ NOT AT ALL
12. I look forward with enjoyment to things:
- ☐ AS MUCH AS EVER I DID
 - ☐ LESS THAN I USED TO
 - ☐ A LOT LESS THAN I USED TO
 - ☐ NOT AT ALL
13. I get sudden feelings of panic:
- ☐ VERY OFTEN
 - ☐ QUITE OFTEN
 - ☐ NOT VERY OFTEN
 - ☐ NEVER
14. I can enjoy a good book or radio or TV programme:
- ☐ OFTEN
 - ☐ SOMETIMES
 - ☐ NOT OFTEN
 - ☐ VERY SELDOM

For office use only:

D -----

A -----

HOSPITAL ANXIETY AND DEPRESSION SCALE: SCORES

1. I feel tense or 'wound up': **A**
- 3 MOST OF THE TIME
 - 2 A LOT OF THE TIME
 - 1 OCCASIONALLY
 - 0 NEVER
2. I still enjoy the things I used to enjoy: **D**
- 0 AS MUCH AS EVER
 - 1 NOT QUITE AS MUCH
 - 2 ONLY A LITTLE
 - 3 HARDLY AT ALL
3. I get a sort of frightened feeling as if something awful is about to happen:
- A**
- 3 VERY DEFINITELY AND QUITE BADLY
 - 2 YES, BUT NOT TOO BADLY
 - 1 A LITTLE, BUT IT DOESN'T WORRY ME
 - 0 NEVER
4. I can laugh and see the funny side of things:
- D**
- 0 AS MUCH AS I ALWAYS COULD
 - 1 NOT QUITE SO MUCH AS I USED TO
 - 2 A LOT LESS THAN I USED TO
 - 3 NEVER
5. Worrying thoughts go through my mind:
- A**
- 3 A GREAT DEAL OF THE TIME
 - 2 A LOT OF THE TIME
 - 1 FROM TIME TO TIME BUT NOT TOO OFTEN
 - 0 ONLY OCCASIONALLY
6. I feel cheerful:
- D**
- 3 NEVER
 - 2 NOT OFTEN
 - 1 SOMETIMES
 - 0 MOST OF THE TIME
7. I can sit at ease and feel relaxed:
- A**
- 0 DEFINITELY
 - 1 USUALLY
 - 2 NOT OFTEN
 - 3 NOT AT ALL

8. I feel as if I am slowed down: **D**
- 3 NEARLY ALL THE TIME
 - 2 VERY OFTEN
 - 1 SOMETIMES
 - 0 NOT AT ALL
9. I get a sort of frightened feeling like 'butterflies' in the stomach: **A**
- 0 NEVER
 - 1 OCCASIONALLY
 - 2 QUITE OFTEN
 - 3 VERY OFTEN
10. I have lost interest in my appearance: **D**
- 3 COMPLETELY
 - 2 I DON'T TAKE AS MUCH CARE AS I SHOULD
 - 1 I MAY NOT TAKE QUITE AS MUCH CARE
 - 0 I TAKE JUST AS MUCH CARE AS EVER
11. I feel restless as if I have to be on the move: **A**
- 3 VERY MUCH INDEED
 - 2 QUITE A LOT
 - 1 NOT VERY MUCH
 - 0 NOT AT ALL
12. I look forward with enjoyment to things: **D**
- 0 AS MUCH AS EVER I DID
 - 1 LESS THAN I USED TO
 - 2 A LOT LESS THAN I USED TO
 - 3 NOT AT ALL
13. I get sudden feelings of panic: **A**
- 3 VERY OFTEN
 - 2 QUITE OFTEN
 - 1 NOT VERY OFTEN
 - 0 NEVER
14. I can enjoy a good book or radio or TV programme: **D**
- 0 OFTEN
 - 1 SOMETIMES
 - 2 NOT OFTEN
 - 3 VERY SELDOM

Key: A = Anxiety D = Depression

Interpretation: For either anxiety or depression, scores of 8-10 = possible clinical case;
11-21 = probable clinical case (Zigmond & Snaith 1983)

Beck Anxiety Inventory® (BAI®)
(Sample Items)

	NOT AT ALL	MILDLY It did not bother me much	MODERATELY It was very unpleasant, but I could stand it.	SEVERELY I could barely stand it
1. Frightened.				
2. Heart feels like it is skipping a beat.				
3. Legs like jelly.				

Simulated Items similar to those in the Beck Anxiety Inventory. Copyright © 1990, 1993 by Aaron T. Beck. Reproduced with permission of the Publisher, NCS Pearson, Inc. All rights reserved.

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Information concerning the *BAI* is available from our UK office:

Customer Services

Tel: 0845 630 88 88 (Monday to Friday, 8am to 5pm)

Fax: 0845 630 55 55

Email: info@psychcorp.co.uk

Post:

Pearson Assessment

80 Strand

London, WC2R 0RL

United Kingdom

Participant code: _____

Date _____

MINI-MARKERS

How Accurately Can You Describe Yourself?

Please use this list of common human traits to describe yourself as accurately as possible. Describe yourself as you see yourself at the present time, not as you wish to be in the future. Describe yourself as you are generally or typically, as compared with other persons you know of the same sex and of roughly your same age. Before each trait, please write a number indicating how accurately that trait describes you, using the following rating scale:

1	2	3	4	5	6	7	8	9
Extremely Inaccurate	Very Inaccurate	Moderately Inaccurate	Slightly Inaccurate	Neither Inaccurate nor Accurate	Slightly Accurate	Moderately Accurate	Very Accurate	Extremely Accurate

___ Bashful	___ Energetic	___ Moody	___ Systematic
___ Bold	___ Envious	___ Organized	___ Talkative
___ Careless	___ Extraverted	___ Philosophical	___ Temperamental
___ Cold	___ Fretful	___ Practical	___ Touchy
___ Complex	___ Harsh	___ Quiet	___ Uncreative
___ Cooperative	___ Imaginative	___ Relaxed	___ Unenvious
___ Creative	___ Inefficient	___ Rude	___ Unintellectual
___ Deep	___ Intellectual	___ Shy	___ Unsympathetic
___ Disorganized	___ Jealous	___ Sloppy	___ Warm
___ Efficient	___ Kind	___ Sympathetic	___ Withdrawn

Office use:

Scores are entered on the grid provided. Designated negative traits are reverse scored (e.g. a score of 8 becomes 2 and a score of 2 becomes 8). Enter the total score in each factor column. Then divide the total score for each factor by 8.

How to score the Mini-Markers?

Check signs and factors in the table below. Asterisk indicates which factor each item is scored on. Each scale has 8 items, as groupings below indicate. The group of items that have negative loadings are scored negatively (subtract their total from sum of positive-loading items, OR reflect their values [9-->1, 1-->9, etc.] before summing with positive-loading items. My preferred approach is to reflect values as appropriate, sum, then divide (for each scale) by 8 to arrive at the mean response for items on the given scale.

Varimax-Rotated Factor Loadings of 40 Mini-Marker Scale Items

Item	Factor				
	I	II	III	IV	V
Talkative	.73*	.14	-.12	-.05	-.05
Extroverted	.70*	.07	-.07	.11	-.01
Bold	.51*	-.17	.00	.24	.03
Energetic	.44*	.18	.18	.18	.02
Shy	-.79*	.15	.04	-.08	-.03
Quiet	-.76*	.02	.13	.05	.07
Bashful	-.73*	.19	.04	-.06	-.06
Withdrawn	-.71*	-.15	-.07	-.10	.02
Sympathetic	-.05	.72*	-.06	-.03	.00
Warm	.20	.67*	.08	.00	-.01
Kind	.02	.66*	.14	-.01	-.01
Cooperative	-.11	.52*	.21	.20	-.06
Cold	-.21	-.65*	.03	-.05	-.02
Unsympathetic	-.02	-.64*	.03	.07	-.10
Rude	.14	-.55*	-.18	-.03	-.04
Harsh	.10	-.54*	.00	-.14	-.06

Organized	-.06	-.01	.83*	-.01	-.02
Efficient	.01	.04	.65*	.07	.05
Systematic	-.11	-.02	.63*	.13	.02
Practical	-.08	.13	.51*	.15	-.10
Disorganized	.01	.02	-.82*	.05	-.02
Sloppy	-.01	-.10	-.62*	.13	.02
Inefficient	-.16	-.05	-.62*	-.01	-.05
Careless	.09	-.10	-.61*	-.05	-.05
Unenvious	-.03	.00	.00	.68*	.08
Relaxed	.11	.16	.07	.49*	-.10
Moody	-.06	-.12	-.04	-.64*	.12
Jealous	-.04	-.01	-.03	-.63*	-.15
Temperamental	.03	-.17	-.03	-.62*	.03
Envious	-.10	.07	-.03	-.61*	-.15
Touchy	-.09	.01	.04	-.59*	-.01
Fretful	-.17	.09	-.07	-.54*	-.08
Creative	.05	.01	.01	.15	.69*
Imaginative	.11	.03	.01	.07	.65*
Philosophical	-.08	.04	-.03	.07	.56*
Intellectual	-.03	-.01	.12	.15	.54*
Complex	-.09	.01	-.10	-.13	.51*
Deep	-.13	.22	-.09	.03	.44*
Uncreative	-.13	.06	-.01	.00	-.66*
Unintellectual	-.02	.01	-.09	.09	-.52*

Note. (N = 636). Loadings of .30 and above are listed in boldface type. * Indicates highest factor loading of each item. I - Extraversion; II - Agreeableness; III - Conscientiousness; IV - Emotional Stability; V - Intellect or Openness. Table 3

(Saucier 1994a)

Appendix XI Big 5 Mini-Marker scoring grid

Participant code: P.

Date:

NB: Negative scores for each factor are reverse-scored

I: Extraversion	Score	II: Agreeableness	Score	III: Conscientiousness	Score	IV: Emotional stability	Score	V: Intellect/openness	Score
Positive scores		Positive scores		Positive scores		Positive scores		Positive scores	
Talkative		Sympathetic		Organised		Unenvious		Creative	
Extraverted		Warm		Efficient		Relaxed		Imaginative	
Bold		Kind		Systematic				Philosophical	
Energetic		Cooperative		Practical				Intellectual	
								Complex	
								Deep	
Negative scores		Negative scores		Negative scores		Negative scores		Negative scores	
Shy		Cold		Disorganised		Moody		Uncreative	
Quiet		Unsympathetic		Sloppy		Jealous		Unintellectual	
Bashful		Rude		Inefficient		Temperamental			
Withdrawn		Harsh		Careless		Envious			
						Touchy			
						Fretful			
Totals									
Mean									

CONSENT FORM

Title of Study: **American skullcap (*Scutellaria lateriflora*): a study of its effects on mood in healthy volunteers.**

Lead researcher: Christine Brock

I have read the information in the Participation Information Sheet, and I am willing to act as a participant in the above research study.

Name: _____

Signature: _____ Date: _____

This consent form will be stored separately from any data you provide so that your responses remain anonymous.

I have provided an appropriate explanation of the study to the participant

Researcher Signature _____

Appendix XIII Participant health questionnaire and screening for Skullcap study Part 1

Participant code _____



Date: _____			
Assessor _____	Signed _____		Participant _____
Accepted Part 1: Y/N	Part 2: HADS Y/N	Part 3: BAI Y/ N	Part 4: ALT/BP Y/N

1. Main exclusion criteria If YES to any, do not complete Q

Ever been diagnosed with:

Depression
Bipolar disorder
Generalised anxiety disorder (GAD)
Schizophrenia
Panic disorder
Phobias (state which)
Any other mental condition (please state)

Do you currently suffer from:

Epilepsy?
Diabetes?
Kidney disease?
Heart disease?
Hepatitis?
Cancer?
Any other serious illness?

Females: Pregnancy/lactating

2. Allergies

State how it affects participant

Eg Foods, Drugs, Chemicals, Herbal medicines, Supplements

Any tests and diagnosis with dates:

Exclude participant: allergy to skullcap, nettle, *Lamiaceae* or *Urticaceae* allergy/ allergies to known constituents eg serotonin, histamine.

3. Diagnosis of any other condition; date diagnosed and whether resolved

GIT eg Hepatitis, pancreatitis, Crohn's, UC, Coeliac, ulcer

CVS eg Hypertension, Hypotension, Angina, Stroke, Cardiac problem, Hypercholesterolaemia, Anaemia

Respiratory eg Asthma, Hay fever, Sinusitis, Bronchitis, Emphysema, TB

Urinary Kidney, bladder disease

CNS/ PNS Sensory/ Motor Dysfunction/ disorders

Musculoskeletal eg Rh. arthritis, osteoarthritis, gout

Skin eg eczema, psoriasis

Endocrine Eg Thyroid, Cushing's disease/ syndrome, Prostate

Gyn / Repro / Obs. DLMP, PCO/PCOS; Cycle, Dysfunction, Breasts

Autoimmune (other)

Eg Iritis/uveitis, SLE, HIV/AIDS

Cancer

Hospitalisations/ operations: reason (s), and dates

Taken part in another study in past 30 days? Y/N

Drug check

<i>In the last month (M) week (w) 6 months (6) year (y) ever (e)</i>	<i>Type/ brand/name</i>	<i>Frequency</i>	<i>Quantity</i>
Alcoholic drinks w	N/A		
Cigarettes	N/A		
Recreational (eg cannabis, LSD, ecstasy, heroin, morphine, cocaine, methadone, amphetamines) M			
Pain killers (<i>type</i> , mg/day) w			
CNS drugs: antipsychotics (e) , antidepressants (y) sleeping tablets (m), tranquillisers/ sedatives (m), (<i>type</i> , mg/day)			
Corticosteroids eg prednisolone, nasal sprays, eye drops, skin creams, inhalers. (6)			
Contraception			
Herbal medicines <i>Type, frequency and duration are important. Use judgement but none for past week</i>			
Supplements e.g. vitamins			
Other drug			
Other drug			
Other drug			

American skullcap (*Scutellaria lateriflora*): a study of its effects on mood in healthy volunteers.

Thank you for agreeing to take part in this study, which will involve

- answering questionnaires about how you feel,
- taking herbal capsules,
- undergoing finger-prick blood tests,
- having your blood pressure and pulse taken and
- collecting samples of your saliva.

Please follow these instructions carefully. If you have any questions, please do not hesitate to contact me: Christine Brock: **cabrock@westminster.ac.uk** or: **020 7911 5041** to leave a message and I will ring you back.

I would like to remind you that you may withdraw from the study at any time.

PARTICIPANT DIARY

- Tick and date the checklist for capsule taking and saliva collection
- Record any symptoms you might have.

CAPSULES

How to take your capsules:

- Take **one** capsule **three** times daily with water from **DAY 3**
- Take one on waking, one 6 hours later and one 12 hours later.
- On days 15 and 16 and days 36 and 37 you will be also collecting saliva samples. On these days please take the capsules straight **after** sample collection (6 and 12 hours).
- If you miss one you may take it later but no more than 3 hours before the next one.
- If you miss a capsule altogether please note this on your 'participant diary'.
- Please refrain from using any other medication, including herbs, during the study.
- It is preferable that you also avoid using alcohol during the study - or at least keep your alcohol consumption low.

SALIVA COLLECTION and STORAGE

Please record the times and dates of saliva collection on the sheets provided

What not to do during saliva collection:

Do not:

- eat
- drink (except water)
- smoke
- brush your teeth
- take strenuous exercise

for at least **half an hour** before collecting of the samples

How to collect your saliva sample:

- Collect samples 3, 6, 9 and 12 hours after waking. Enter times on record sheets.
- Rinse mouth with water about 10 minutes before sampling.
- Take the ridged cap off the correctly labelled tube and remove the cotton swab.
- Place the swab under your tongue for 1-2 minutes until it is soaking wet.
- Return the swab to the tube (with your mouth) and seal tightly with the cap.

How to store your saliva samples:

- Place the tube back in its original polythene bag.
- When all 4 tubes have been replaced in the correctly labelled polythene bag (day 1, day 2, day 15, day 16, day 36 or day 37) place the bag in your freezer until all sampling has been completed (24 tubes in all).

At the end of the study:

- On completion of the study please bring the tubes to the Polyclinic on arrangement or, if necessary, place the tubes (still in their polythene bags) in the container and addressed envelope provided and post.

When to take your saliva samples

Please take your first 4 samples on **day 1:** _____
Date

Please take your next 4 samples on **day 2:** _____
Date

Please take your next 4 samples on **day 15:** _____
Date

Please take your next 4 samples on **day 16:** _____
Date

Please take your next 4 samples on **day 36:** _____
Date

Please take your next 4 sample on **day 37:** _____
Date

Appendix XV Participant Diary



Participant code _____

Participant Diary: (Dates) From_____ To_____

Starting on Day 1 and finishing on Day 37 please tick the boxes below the day on which symptoms occurred.

Page 1 (continue on page 2)

[illegible]

[illegible]

American skullcap (*Scutellaria lateriflora*): a study of its effects on mood in healthy volunteers.

SALIVA SAMPLE COLLECTION RECORDING SHEET

Participant code: _____

DAY 1

Date: _____

What time did you wake up this morning? _____

Use your wake up time to calculate the correct sampling times and write these in the first column. Record the actual time of sampling in the second column.

	Time to take samples	Time sample is taken
Tube 1 (3 hrs after awakening)	_____	_____
Tube 2 (6 hrs after awakening)	_____	_____
Tube 3 (9 hrs after awakening)	_____	_____
Tube 4 (12 hrs after awakening)	_____	_____

Do not eat, drink (except water), smoke, brush your teeth or take strenuous exercise for at least **half an hour** before collecting of the samples

DAY 2

Date: _____

What time did you wake up this morning? _____

Use your wake up time to calculate the correct sampling times and write these in the first column. Record the actual time of sampling in the second column.

	Time to take samples	Time sample is taken
Tube 1 (3 hrs after awakening)	_____	_____
Tube 2 (6 hrs after awakening)	_____	_____
Tube 3 (9 hrs after awakening)	_____	_____
Tube 4 (12 hrs after awakening)	_____	_____

Do not eat, drink (except water), smoke, brush your teeth or take strenuous exercise for at least **half an hour** before collecting of the samples



recipient
organisation
address

date

Dear Dr XXX

Re. NAME DOB ADDRESS

The above patient has volunteered to participate in a clinical study of an herbal medicine:

American skullcap (Scutellaria lateriflora): a study of its effects on mood in healthy volunteers.

The study is part of a research project in the School of Life Sciences at the University of Westminster.

The herb has been in long time use and is not reported to have any side-effects.

To participate, your patient must be in good general health and not be taking steroids (e.g. hydrocortisone), or mind-altering drugs (including herbal) such as tranquillisers, sleeping tablets or anti-depressants and be between 18-75 years old.

It is also important that they are not currently participating in any clinical trials and have not done so within the past month.

If you feel that for any reason the above patient should not participate in this study I would appreciate it if you could inform me as soon as possible.

I enclose a participant information sheet. If you would like more information about the study please do not hesitate to contact me.

Yours sincerely,

Christine Brock

Research Scholar

E: C.Brock@westminster.ac.uk

M: 07947 347 396

School of Life Sciences
University of Westminster
115 New Cavendish Street
London W1W 6UW
T: +44 (0)020 7911 5000



A charity and a company limited by guarantee
Registration number 977818 England
Registered office 309 Regent Street London W1B 2UW

**Participant Data Sheet: Strictly confidential**

Title..... Surname.....

First name(s)..... Sex.....M/F

Date of birth.....

Address:

.....

.....

.....

.....

.....

.....

Email.....

Telephone:

Home..... Work..... Mob.....

Contact name and telephone in case of emergency.....

Name and address of GP:

.....

.....

.....

.....

.....

Telephone number of GP.....

Occupation.....

Participant signature:..... Date.....

Participant code.....

Appendix XIX Participant assessment data sheet

***Scutellaria lateriflora* RCT assessments**

Assessor _____

Participant code _____

Screening Part 2 and baseline assessment

Blood pressure 1 _____ Date _____

Pulse 1 _____ Date _____

ALT 1 _____ Date _____

BAI 1 _____ Date _____

POMS1_T_____/D_____/A_____/V_____/F_____/C_____/TMD_____/Date_____

Intermediate assessment

Blood pressure 2 _____ Date _____

Pulse 2 _____ Date _____

ALT 2 _____ Date _____

BAI 2 _____ Date _____

POMS2_T_____/D_____/A_____/V_____/F_____/C_____/TMD_____/Date_____

Final assessment

Blood pressure 3 _____ Date _____

Pulse 3 _____ Date _____

ALT 3 _____ Date _____

BAI 3 _____ Date _____

POMS3_T_____/D_____/A_____/V_____/F_____/C_____/TMD_____/Date_____

Intervention/ placebo code

A	Date	B	Date
---	------	---	------

Salivary cortisol measurements

	Day 1	Day 2	Day 15	Day 16	Day 36	Day 37
3 hours						
6 hours						
9 hours						
12 hours						

Completed Y/ N Date _____

Dropout Y/ N Date _____ Reason:

Appendix XX COSHH form

UNIVERSITY OF WESTMINSTER
School of Biosciences
Control of Substances Hazardous to Health (COSHH)

Sheet of

APPENDIX 10 - MICROBIAL HAZARDS (ACDP GROUPS 1 & 2 ONLY)

Title of Experiment: American skullcap (Scutellaria lateriflora): a study of its effects on stress in healthy volunteers

Room/Laboratory: C.605

1. Analysis of salivary cortisol levels by EIA in healthy volunteers following administration of American skullcap herb or placebo.

2. LIST OF CULTURES USED (continue on a separate sheet if necessary)

NAME OF CULTURE (GENERIC AND SPECIFIC NAME)	CULTURE TYPE	MAX. QUANTITY EXPOSED TO	INSERT ACDP GROUP NUMBER BELOW (1 or 2 ONLY ARE ALLOWED)	KNOWN HAZARDS
Microorganism (ACDP Category 1 or 2 only). Give full name, strain code(s) and UOVW number (if known).	e.g. broth culture, Petri dish, fermenter.	e.g. 20 ml broth for UB, 15 ml agar for plate.		20 (Biological hazard). ACDP = Advisory Committee on Dangerous Pathogens.
A				20 (Biological hazard/infectious material)
B				"
C				"
D				"
E				"
F				"
G				"
H				"
I				"

NOTE: 1) EXPLOSIVE 2) OXIDIZING 3) FLAMMABLE 4) TOXIC 5) HARMFUL 6) CORROSIVE
7) IRRITANT 8) CARCINOGEN 9) MUTAGEN 10) TERATOGEN 11) DUST 12) INHALATION
13) INGESTION 14) SKIN ABSORPTION 15) SKIN OR EYE 16) INJECTION 17) MAXIMUM EXPOSURE
LIMIT 18) OPERATIONAL EXPOSURE STANDARD 19) RADIATION 20) OTHER (specify)

3. PERSONNEL INVOLVED WITH CULTURES (continue on a separate sheet if necessary)

SURNAME/CLASS	INITIALS	STATUS	INVOLVEMENT - DATE OF USE or DAILY/WEEKLY/MONTHLY/OCCASIONALLY
Brock	C A	Research Scholar	Occasionally
Tevfik	I	PhD Supervisor	Occasionally

4. OTHER GROUPS/PERSONS WHO MAY HAVE ACCESS TO THE CULTURES
(e.g. students [name class], cleaners, maintenance staff, contractors, visitors, storekeepers, etc.)

5. EMERGENCY PROCEDURES
if any of the cultures or procedures identified above is likely to pose a special hazard in an emergency, then identify below action to be taken

<u>BRIEFAGE/UNCONTROLLED RELEASE</u> Follow instructions in <i>Appendix 2</i> of UOW Regulations for the Use of Biological Materials (Procedure for dropped or spilled cultures).	<u>RRE:</u> N/A
If personnel are affected (time, contamination, etc.) treatment to be adopted: (N.B. Antidotes and special treatment may be obtained through.....N/A.....) Wash affected area of skin with germicidal soap. Change contaminated clothes and wash with detergent and water. If eyes affected, wash with plenty of water.	

6. CONTROL MEASURES TO BE ADOPTED
(NB. Consider following points, appropriate Biosciences Safety Codes plus any other. ∇ = ESSENTIAL)
1. Limiting exposure time of personnel - state maximum time
2. Partial enclosure with local exhaust ventilation
3. Local exhaust ventilation
 ∇ . Sufficient general ventilation T T
etc.) \diamond . Personal protective equipment (goggles/gloves/clothing/mask/breathing apparatus/safety screen,
NB. gloves essential for human body fluids but consider them for other samples also.
Goggles should also be considered for some samples.
 \diamond . Safe disposal/storage of hazardous substances/cultures
** Regular cleaning of the workplace
II. Adequate washing facilities

<u>STORAGE - SAFETY CONSIDERATIONS</u> Use secure, <u>fully labelled</u> containers in fridge, freezer or incubator.	<u>HANDLING PRECAUTIONS</u> Follow instructions in <i>Appendix 1</i> of UOW Regulations for the Use of Biological Materials (Code of Practice for the Use of Microorganisms). Plus all students must receive adequate practical instruction from the lecturer in charge.
<u>DISPOSAL PROCEDURES DURING AND AT END OF EXPERIMENT</u> Follow instructions in <i>Appendix 1</i> . Technicians to follow instructions in <i>Appendix 8</i> (Instructions for technical staff).	

7. REVIEW AND MONITORING OF CONTROL MEASURES
(Required checks and their frequency, on the adequacy and maintenance of the control measures during the course of the experiment)
(N.B. Also state if any health surveillance is necessary; are exposure time logs/maintenance/inspection test records available for inspection? Medical monitoring not normally required for ACDP Group 1 and 2 organisms but note para 4 of Chemical Safety Regulations (p. 26) and the footnote (p. 28).)
Lecturer in charge of class must ensure that students follow all instructions and safety procedures as detailed in the UOW Regulations for the Use of Biological Materials, the Codes of Practice and the relevant Appendices during the course of the experiment.

medical monitoring required? - **Y / N** occupational health nurse informed? - **Y / N**

8. OTHER RELEVANT INFORMATION (continue on a separate sheet if necessary).
All experiments must comply with the UOW Regulations for the Use of Biological Materials, the Codes of Practice and the relevant Appendices. This form is for ACDP Group 1 and 2 microorganisms only. Microorganisms in ACDP Groups 3 and 4 are not allowed on University property. The use of all microbial cultures and biologically hazardous materials must be cleared with the Biological Safety Officer (Keith Redway) before they are brought onto University property or ordered. COSHH Risk Assessment for chemicals requires a different form (*Appendix 4* of Chemical Safety Regulations.) Assessment for class activity may not be sufficient for technician preparation - consider double risk assessment. For guidance notes on the completion of COSHH forms see page 32.

9. Name of Assessor (BLOCK CAPITALS): Signed:
Status of Assessor (Lecturer/Post-doc/Technician): Date

Glossary

Adaptogen: A herb with a normalising action on the mind and body, particularly with regard to mental and physical stress.

Allosteric ligand: A ligand that binds to a receptor site on a molecule and alters the shape of the molecule to increase its affinity for a particular substrate.

Bleb: An irregular protrusion on a cell membrane

Carminative: A herb that soothes the gut wall and promotes expulsion of gas.

Calyx (pl. Calyces): An outer whorl of floral leaves (sepals) forming the protective covering of the flower petals.

Crenate: Round toothed.

Dripping pill: A herbal extract and a matrix are blended under warm conditions. The mixture is dripped into a cooling liquid in which the droplets are insoluble.

Dysgusia: A disorder of the sense of taste

Dysphagia: Difficulty in swallowing

Dysphoria: Negative emotions such as depression, sadness, melancholy, discontent and indifference.

Enteral delivery: Introduction directly into the stomach, duodenum or jejunum.

Glabrous: Smooth and hairless

Gluconeogenesis: Production of glucose from non-carbohydrate molecules.

Glycogenolysis: Conversion to glucose monomers from glycogen polymers.

Isoform: A protein with a similar function as another protein but which is encoded by a different gene and therefore has a slightly different amino acid sequence.

Lipolysis: Hydrolysis of triglycerides into free fatty acids

Lyophilisation: Freeze-drying

Metabolomics: Detection, identification and quantitation of all compounds in a sample

Microsomes: Small particles of endoplasmic reticulum containing enzymes

Organoleptic: The practice of recognition of herbal quality by the use of the senses, particularly sight e.g. colour, smell and taste.

Pinnate: Structural arrangement on either side of a common stalk e.g. leaf veins or leaves.

Pubescent: Covered in fine hairs

Raceme: Clusters of flowers are arranged on an unbranched stalk, with the youngest growing at the apex

Radioligand binding: A ligand e.g. drug is radioactively labelled for observation of its binding affinity to e.g. receptors, enzymes

Thiols: Sulphur-containing compounds

Trophorestorative: A herb with an affinity for a particular organ or system on which it exerts a nourishing and restorative action.

Thymoleptic: Mood enhancing effect

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