Phytomedicine 10: 640–649, 2003

http://www.urbanfischer.de/journals/phytomed

Phytomedicine

Phytochemical and biological analysis of Skullcap (*Scutellaria lateriflora* L.): A medicinal plant with anxiolytic properties

R. Awad[1], J. T. Arnason[1], V. Trudeau[2], C. Bergeron[1], J. W. Budzinski[1], B. C. Foster[3] and Z. Merali[4]

[1] Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, Canada
[2] Centre for Advanced Research in Environmental Genomics (CAREG), University of Ottawa, Ottawa, Canada
[3] Office of Science, Therapeutic Products Directoriate, Health Canada, Ottawa, Canada
[4] Institute of Mental Health Research and Department of Psychology, University of Ottawa, Ottawa, Canada

Summary

The phytochemistry and biological activity of *Scutellaria lateriflora* L. (American skullcap) which has been traditionally used as a sedative and to treat various nervous disorders such as anxiety was studied. *In vivo* animal behaviour trials were performed to test anxiolytic effects in rats orally administered *S. laterifolia* extracts. Significant increases in the number of entries into the center of an "open-field arena"; number of unprotected head dips, number of entries and the length of time spent on the open arms of the Elevated Plus-Maze were found. The identification and quantification of the flavonoid, baicalin in a 50% EtOH extract (40 mg/g) and its aglycone baicalein in a 95% EtOH extract (33 mg/g), as well as the amino acids GABA in H_2O and EtOH extracts (~1.6 mg/g) and glutamine in a H_2O extract (31 mg/g), was performed using HPLC. These compounds may play a role in anxiolytic activity since baicalin and baicalein are known to bind to the benzodiazepine site of the $GABA_A$ receptor and since GABA is the main inhibitory neurotransmitter.

Key words: skullcap, anxiety, cytochrome P450 3A4, baicalin, baicalein, γ-aminobutyric acid

Introduction

American skullcap (*Scutellaria lateriflora* L. Lamiaceae) is a perennial member of the mint family and comprises one of the 300 *Scutellaria* species worldwide. It grows in meadows and swampy woods in North America, and the dried aerial parts are used as a sedative/nerve tonic as well as an antispasmodic to treat epilepsy, St. Vitus's dance, insomnia, anxiety, neuralgia and withdrawal from barbiturates and tranquilizers (Foster, 1996). In Canada, skullcap herb is generally sold as a tea in health food stores, but can also be found as a tonic or in combination with other herbs such as valerian and passion flower in sleep-inducing tablets.

The inconsistent use of *S. lateriflora* over the past three centuries has been due to many uncertainties about the herb, and the US FDA presently designates it as an herb of undefined safety. To date, most research has been done on the root of the Baikal skullcap (*Scutellaria baicalensis*) which is used to treat inflammation and atherosclerosis, has antibacterial, antiviral (Hirotani et al. 1998; Nishikawa et al. 1999), antioxidant (Yoshino et al. 1997) and anti-thrombic (Kubo et al. 1985) properties. Very few studies have been conducted on *S. lateriflora* and Foster (1996) has called for further scientific evaluation of this species.

Anxiolytic drugs such as benzodiazepines and buspirone are used to treat anxiety-related disorders (e.g. panic, agoraphobia, obsessions and compulsions, generalized anxiety etc.) which affect over 20 million North Americans today (Kessler et al. 1994). In addi-

0944-7113/03/10/08-640 $ 15.00/0

tion to these conventional drugs, botanicals such as Kava, St. John's Wort and Valerian have been used to treat anxiety. Demand for botanicals with anxiolytic properties has increased more than any other category of medicinal plants in recent years (Brevoort, 1998). Since *S. lateriflora* has been traditionally used to treat nervous conditions, it was of interest to further investigate the phytochemistry and biological activity of this herb and its potential as a medicinal plant with anxiolytic properties in standardized animal models.

Recent research with the anxiolytic herb *Valeriana officinalis* has shown that GABA present in the aqueous extract was responsible for inducing the *in vitro* release of [^{3}H] GABA in rat brain synaptosomes (Santos et al. 1994b). The extract was also found to inhibit GABA reuptake in these cells (Santos et al. 1994a). This may explain the *in vivo* mechanism of inhibitory neurotransmission in valerian root and similar "anxiolytic" plants such as *S. lateriflora*. In addition, studies with *S. baicalensis* have revealed several putative active flavonoids, including baicalin and its aglycone baicalein, that bind to the benzodiazepine site of the $GABA_A$ receptor (Liao et al. 1998; Hui et al. 2000). Baicalein in particular has been reported to bind with very high affinity (Ki = 4 µM) (Paladini et al. 1999). If these compounds are constitutively expressed in *S. lateriflora*, their presence could potentially contribute to the anxiolytic properties of the plant. The efficacy and safety of *S. lateriflora* can be assessed by using some of the validated animal models or paradigms. A positive outcome in such models could then warrant additional tests culminating in clinical trials.

The main objectives of the present study were to *I*) assess the anxiolytic efficacy of *S. lateriflora* in two validated animal test models, in order to test the traditional claims of anxiolytic activity, *II*) determine whether commercially prepared tinctures or crude extracts of *S. lateriflora* inhibit the human cytochrome P450 3A4 enzyme, which has relevance in potential drug-herbal interactions and *III*) identify and quantify the major skullcap flavonoid, baicalin and its aglycone baicalein as well as the amino acids present in various *S. lateriflora* extracts and to compare these levels to known "anxiolytic" plants.

Materials and Methods

Plant material and extractions

Dried *Scutellaria lateriflora* herb (Lot # SKU-S2082-93C) was obtained from Trout Lake Farm Co., Trout Lake, WA., USA. Powdered herb was extracted with distilled water (dH_2O), 50% ethanol and 95% ethanol: ratio of dry plant material-solvent (mg/ml) 1:20, 1:50 and 1:10 respectively. The extracts first shook for 24–48 hours and were filtered in vacuo with Whatman no. 1 filter paper. Ethanolic extracts were evaporated and all samples were freeze dried to a powder. Valerian root (1:4) and passion flower (1:5) alcohol extracts as well as the three commercially prepared alcohol extracts of *S. lateriflora* (1:2): 50% ethanol, 65% glycerin and 25% ethanol-40% glycerin were used to test cytochrome P450 3A4 inhibition. Dried *Oxytenanthera abyssinica* herb from Togo, West Africa was obtained from Dr. F. Gleassor (Université du Benin) and extracted by the same method as *S. lateriflora* with 95% ethanol (1:11). Both *Echinacea pallida* and *Echinacea purpurea* 95% ethanolic extracts were prepared from fresh herb (5 g/ml).

Behavioral tests

Behavioral tests were conducted to determine if *S. lateriflora* imparted anxiolytic responses in rats. The plant extract was dissolved in a vehicle containing sweetened condensed milk (President's Choice Brand) diluted 1:1 milk:dH_2O. Treated rats were orally administered 1 ml of aqueous *S. lateriflora* extract (100 mg extract/ml milk solution). Control rats received 1 ml of the milk solution without herbal extract.

Subjects: Groups of adult male Sprague-Dawley rats (n = 21) (Charles River Canada, St. Constant, Quebec) weighing ~300–400 g (for the Elevated Plus Maze – EPM test) and ~400–500 g (for the Social Interaction – SI test), were individually housed in standard clear Plexiglas cages (24 × 30 × 18 cm) and maintained on 12L:12D cycle in a temperature and humidity controlled room (Animal Care Facility, University of Ottawa). Animals had free access to water and an unlimited supply of food (Purina Lab Chow). All experimental procedures complied with the guidelines of the Canadian Council on Animal Care and were approved by the Research Ethics Committee at the University of Ottawa.

• *Elevated Plus-Maze and Open Field Paradigms:* The Elevated Plus-Maze paradigm (Pellow et al. 1985) was used to determine the relative anxiety level in rats exposed to an aqueous extract of *S. lateriflora*, compared to vehicle treated control animals. The EPM is raised at a height of 50 cm and is composed of two open arms (50 × 10 cm) and two walled (or closed) arms (50 × 10 × 40 cm) arranged so that the arms of the same type were opposite to each other, connected by an open central area (10 × 10 cm). All parts of the apparatus were made from wood, with closed arms lined with clear Plexiglas and the floor covered with black rubber. To prevent distractions to the animal, the EPM was surrounded by a black curtain and a closed circuit camera is mounted above the setup to observe the rat's behaviour and record the trials for later scoring.

Rats were randomly assigned to either control or treatment groups. Extract was administered orally 60

minutes prior to testing. Prior to EPM testing the rats were placed in an open-field box (6 × 6 cm grid) for a five minute habituation. Behaviour in the open-field box was observed and the following parameters were measured: (a) the total number of squares traversed, (b) amount of time in the perimeter (32 outer squares closest to the outer walls), (c) amount of time in the center (4 innermost squares), (d) the number entries into the center of the arena, over the five minute period (Fig. 1).

After habituation, the rats were placed in the center of the EPM, facing towards one of the enclosed arms and were observed again for five minutes from the video-link relay. Behaviours scored included: (a) number of entries into the open arms, (b) time spent in the open arms of the maze, (c) number of entries in the closed arms and (d) time spent in the closed arms. In addition, "risk assessment" behaviours were also scored: (e) number of protected head dips (protruding the head over the edge of the maze with the hind legs still in the closed arms) and (f) number of unprotected head dips (protruding the head over the edge of an open arm) (Fig. 2). The data were statistically analyzed by one-way analysis of variance (ANOVA).

• *Social Interaction Paradigm:* The social interaction paradigm (File, 1980) was used to test the relative anxiety level of rats in a social setting, comparing animals treated with *S. lateriflora* extract and those of the control group. The SI test arena is a 60 × 60 cm white Plexiglas box with 35 cm high walls. The arena is surrounded by a black curtain to prevent distraction and a closed circuit camera is mounted above the set up.

Extract was administered orally 60 minutes prior to testing. Animals were paried randomly, but based on weight (10–15 g apart). Each pair was placed in the SI arena for 7 minutes and their behaviour was monitored. The parameters scored were: amount of time spent interacting with each other (sniffing, grooming, chasing or playing), the amount of time not spent interacting, the number of times the animals initiated contact and the latency to initiate contact (time elapsed prior to the initial contact following their introduction into the test arena). The data were analyzed by one-way analysis of variance (ANOVA).

Cytochrome P450 3A4 enzyme inhibition

Three commercially prepared tinctures of *S. lateriflora* (50% ethanol, 65% glycerin and 25% ethanol–40% glycerin) and two crude extracts (95% ethanol and dH_2O) were tested for their ability to inhibit the CYP 3A4 enzyme *in vitro* using the fluorometric microtitre plate assay according to the method in Budzinski et al. (2000). Results are expressed as the percent of enzyme inhibition caused by the test samples.

HPLC method for quantification of flavonoids

Compounds which were previously isolated from *S. lateriflora were*: *p*-coumaric acid, ikonnoside (5,6,7,2′-tetrahydroxyflavone-7-*O*-glucuronide) and 5,6,7-trihy-

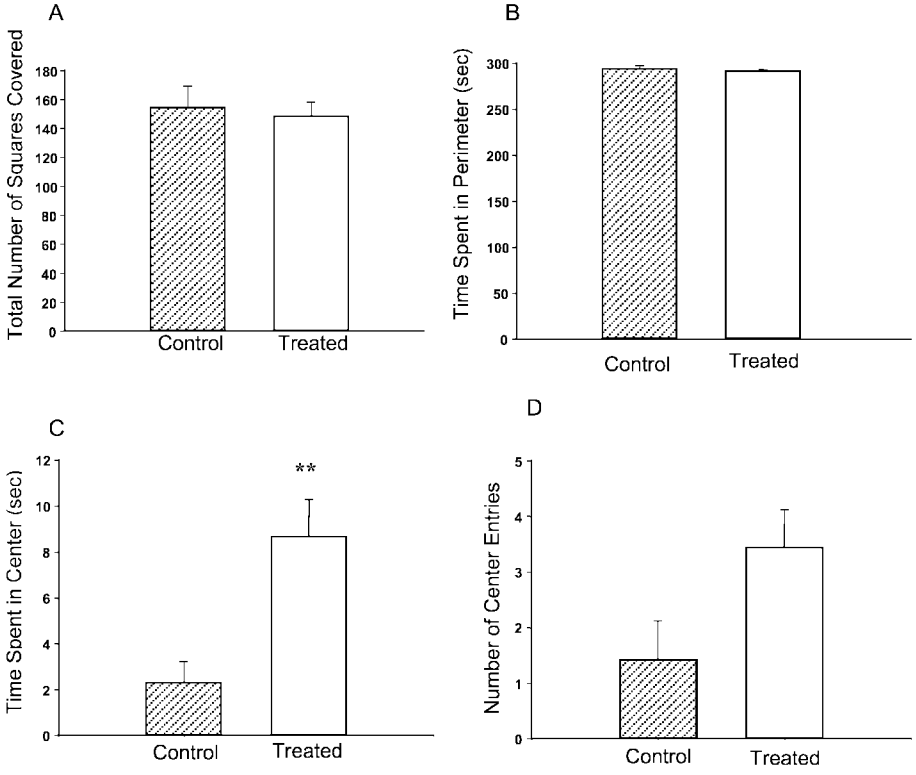

Fig. 1. Rat behaviour observed in open-field trials (5 min). Treated rats (n = 11) were orally administered 1 ml of aqueous *S. lateriflora* extract (100 mg extract/ml of 1:1 milk:dH_2O solution). Control rats (n = 10) received 1 ml of milk solution without herbal extract, 60 minutes prior to testing. **A**) Total number of squares covered. **B**) Time spent in the perimeter. **C**) Time spent in the center. **D**) number of center entries. As shown by the double asterisk, there are significant differences between control and treated rats determined by the ANOVA, on time spent in the center ($F = 0.125$; $p = 0.007$). ** $p < 0.01$

droxy-2′-methyflavone-7-*O*-glucuronide, as well as the standard flavonoids baicalin and baicalein. The three *S. lateriflora* samples analyzed were the aqueous, the 50% ethanol and the 95% ethanol extracts.

HPLC analysis and conditions: A Varian ProStar Model 330 HPLC was used for the flavonoid analysis with the following accessories: an autosampler module 410 with 250 µl loop, a solvent delivery model 230, an absorbance detector module 330 and the Star Chromatographic workstation software (version 5.3). A YMC ODS-AM S 3 µm 120A column with a 2 mm × 50 mm cartridge (Waters, cat# AM12S030502WTA) was used. Solvent A was acetonitrile and solvent B was the buffer (3.45 g/l NaH_2PO_4 and 250 µl/l H_3PO_4, pH 3.0). The column temperature was 55 °C, the flow rate was 0.5 ml/min and the absorbance wavelength was 248 nm for all extracts except aqueous *S. lateriflora* which was at 280 nm. The following table describes the run times and solvent conditions:

Time (min)	Solvent A (%)	Solvent B (%)
0	10	90
8	50	50
10	10	90

HPLC method for quantification of amino acids

Aqueous and 95% ethanolic *S. lateriflora* extracts, valerian root and passion flower tinctures, as well as 95% ethanolic extracts of *E. pallida*, *E. purpurea* and *O. abyssinica* were tested for the following amino acids: gamma-aminobutyric acid (GABA), homoserine, hypotaurine, taurine, β-alanine, glutamine and glutamate (Sigma Chemical Co., St. Louis, MO).

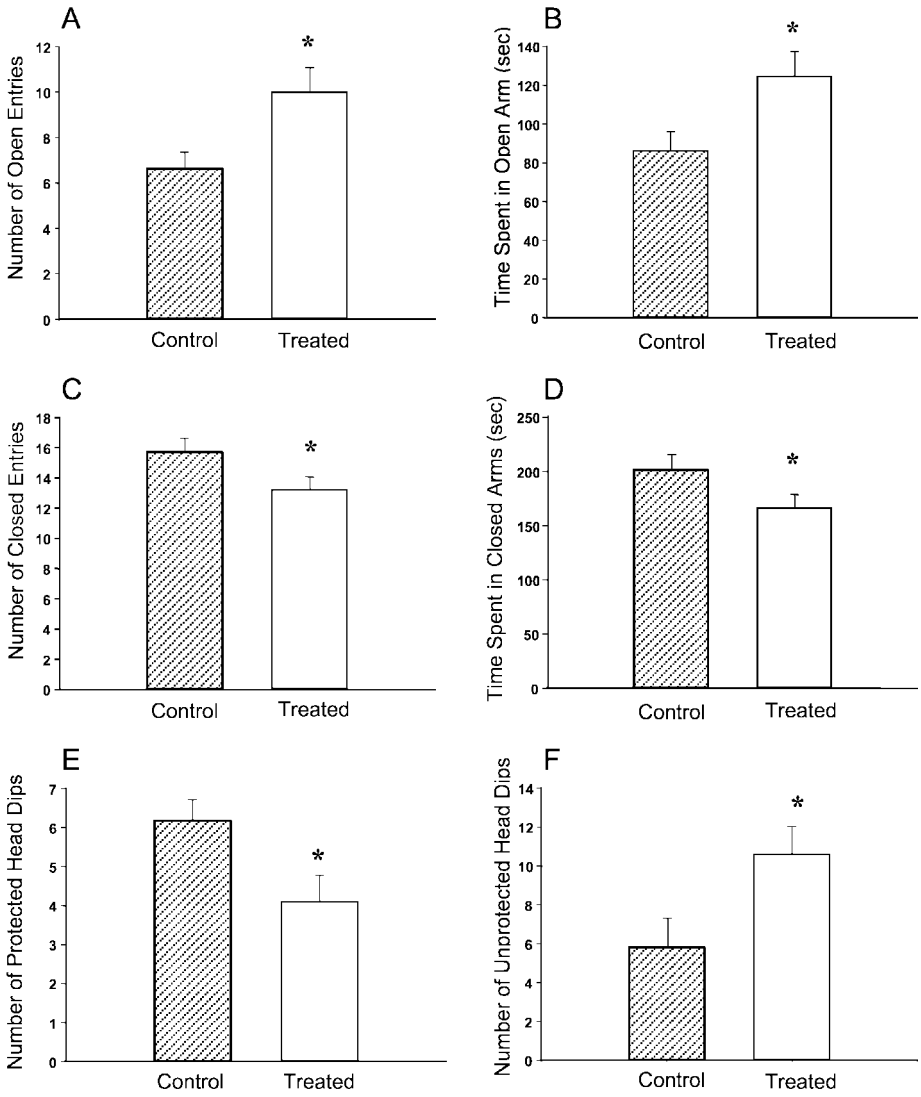

Fig. 2. Rat behaviour observed in Elevated Plus Maze (5 min). Treated rats (n = 11) were orally administered 1 ml of aqueous *S. lateriflora* extract (100 mg extract/ml of 1:1 milk:dH_2O solution). Control rats (n = 10) received 1 ml of milk solution without herbal extract, 60 minutes prior to testing. As shown by the asterisks, treated rats had significant increases in the number of open entries ($F = 6.843$; $p = 0.017$); time spent in the open arm ($F = 5.624$; $p = 0.028$); and the number of unprotected head dips ($F = 5.689$; $p = 0.027$) as well as a significant decrease in the number of protected head dips ($F = 6.293$; $p = 0.021$) as determined by the ANOVA. *$p < 0.05$

OPA reaction: 100 µl of filtered sample was added to 100 µl of *o*-phthalaldehyde (OPA) reagent (Lot # 98070669, Pierce, Rockford, IL), vortexed (10 sec) and allowed to react for 2.5 minutes. 20 µl of the reacted mixture were added to 980 µl 0.1 M sodium acetate buffer, and vortexed. 5 µl of the mixture were injected for HPLC analysis.

HPLC analysis and conditions: The method for amino acid analysis was the same as the flavonoids, with the following modifications: an autosampler module 410 with 100 µm loop and a fluorescence detector module 360. A 4 µm Supersher 100RP-118 column was used with a 4 × 7 mm analytical cartridge and a 5 µm Lichropher 100 C18 column with a 4 × 4 mm guard cartridge. Solvent A was methanol and solvent B was a buffer solution (25 mM NaH_2PO_4 pH 6.0 with 11% MeCN). The flow rate was 1 ml/min and excitation and emittance wavelengths were 360 nm and 460 nm respectively. The following table describes the run time and solvent conditions:

Time (min)	Solvent A (%)	Solvent B (%)
0	10	90
10	20	80
14	10	90

Results

Animal behavioral tests

Initial behavioral trials with rats were performed to assess the potential of *S. lateriflora* as a medicinal plant with anxiolytic properties. In the initial experiment, anxiety-like behaviors were measured after administration of the aqueous extract using the open field exploration and elevated plus maze tests (statistical analysis by ANOVA with one degree of freedom for all tests).

Open field exploration

As seen in Fig. 1, both control and treated rats spent relatively the same amount of time in the perimeter of the open field box. There was no significant difference between control and treated rats for the total number of squares traversed (A) during the five minute period ($F = 0.125$; $p = 0.728$), nor for the amount of time spent in the perimeter (B) ($F = 0.465$; $p = 0.506$). There was a significant difference, however, in the amount of time spent in the center (C) ($F = 9.957$; $p = 0.007$). The control rats spent on the average only 2.3 seconds, whereas the treated rats spent about 8.7 seconds. There was also a minor difference between the groups on the number of entries into the center squares (D). The control animals entered the center on average 1.4 times, whereas the treated animals entered the center about 3.4 times however, their difference did not reach statistical significance ($F = 4.153$; $p = 0.061$).

Elevated plus maze

The drug treatment did not significantly alter the number of entries into the closed arms of the maze (Fig. 2C) ($F = 4.029$; $p = 0.059$) or the amount of time spent in the closed arms (D) ($F = 4.078$; $p = 0.058$). In contrast however, rats treated with aqueous *S. lateriflora* extract entered the open arms (A) of the EPM more often than the control rats; 10 times versus 6.6 times ($F = 6.843$; $p = 0.017$) within a five minute period and also spent more time out in the open (B); 124.6 seconds versus

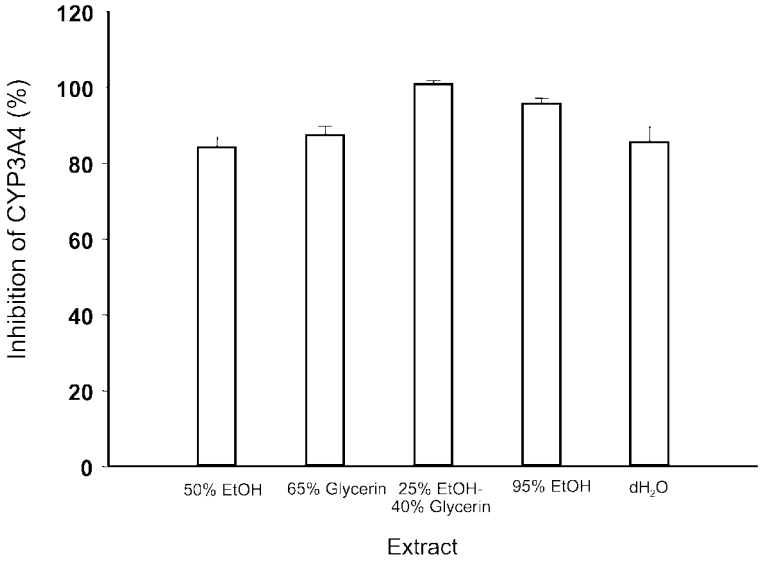

Fig. 3. *Scutellaria lateriflora* extract activity against human cytochrome P450 3A4, determined by the fluorometric microtitre plate assay. All commercial tinctures (50% ethanol, 65% glycerin and 25% ethanol–40% glycerin) and crude extracts (95% ethanol and aqueous) are active *in vitro* (>84% inhibition; n = 3). For conditions see materials and methods.

86.1 seconds (F = 5.624; p = 0.028). The "risk assessment" behaviour was also significantly different between the two groups. Control rats elicited a greater number of protected head dips (E); 6.1 times compared to 4.1 times in the treated group (F = 6.293; p = 0.021). They also had a lower number of unprotected head dips (F) in relation to the treated rats; 5.8 times versus 10.6 times (F = 5.689; p = 0.027).

The results from the EPM paradigm indicate that the rats treated with aqueous *S. lateriflora* extract enter the open arm more frequently, spend more time there and have a greater risk assessment behaviour as outlined by a greater number of unprotected head dips, compared to control rats who were only exposed to the milk placebo. The social interaction paradigm did not yield significant results in the parameters tested (data not shown).

Cytochrome P450 3A4 enzyme inhibition

The fluorometric microtitre plate assay was performed to determine the inhibitory activity of five *S. lateriflora* samples against the human cytochrome P450 3A4 drug metabolizing enzyme. The average percent inhibition of the three commercially prepared tinctures and the two crude extracts are presented in Fig. 3. All *S. lateriflora* samples had extremely high inhibitory activity against the enzyme at their original concentrations. The 25% EtOH/40% glycerin tincture had the highest inhibition at 100%, followed by the 95% EtOH crude ex-

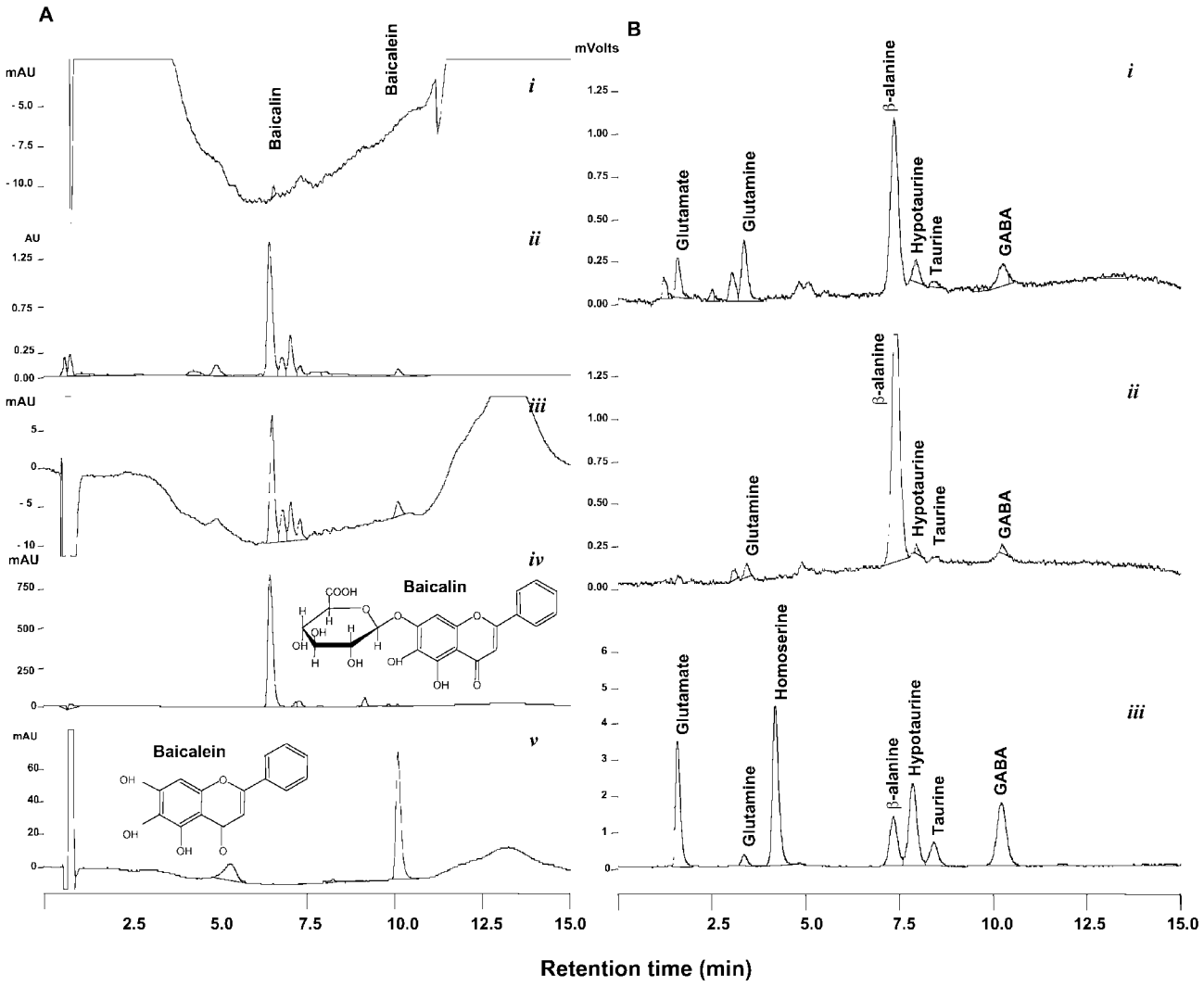

Fig. 4. HPLC chromatographs of active compounds identified in *S. lateriflora*. **A**) Flavonoids: ***i***) aqueous (λ = 280 nm), ***ii***) 50% ethanol and ***iii***) 95% ethanol (λ = 248 nm) extracts compared to the ***iv***) baicalin and the ***v***) baicalein standards. Also shown are the chemical structures of both putative flavonoids. **B**) Amino acids: ***i***) aqueous and ***ii***) 95% ethanol extracts compared to ***iii***) the standard amino acids tested using a fluorescence detector. For conditions see materials and methods.

tract (95.6%), the 65% glycerin tincture (87.3%), the dH_2O crude extract (85.5%) and lastly the 50% EtOH tincture (84.1%). These results indicate *S. lateriflora* inhibits cytochrome P450 3A4 at the commercially prepared tincture doses and the crude extract concentrations.

Phytochemical analyses

Flavonoid quantification

HPLC was performed on three extracts of *S. lateriflora* to quantify the major flavonoid, baicalin and its aglycone baicalein present in the plant. Three compounds,

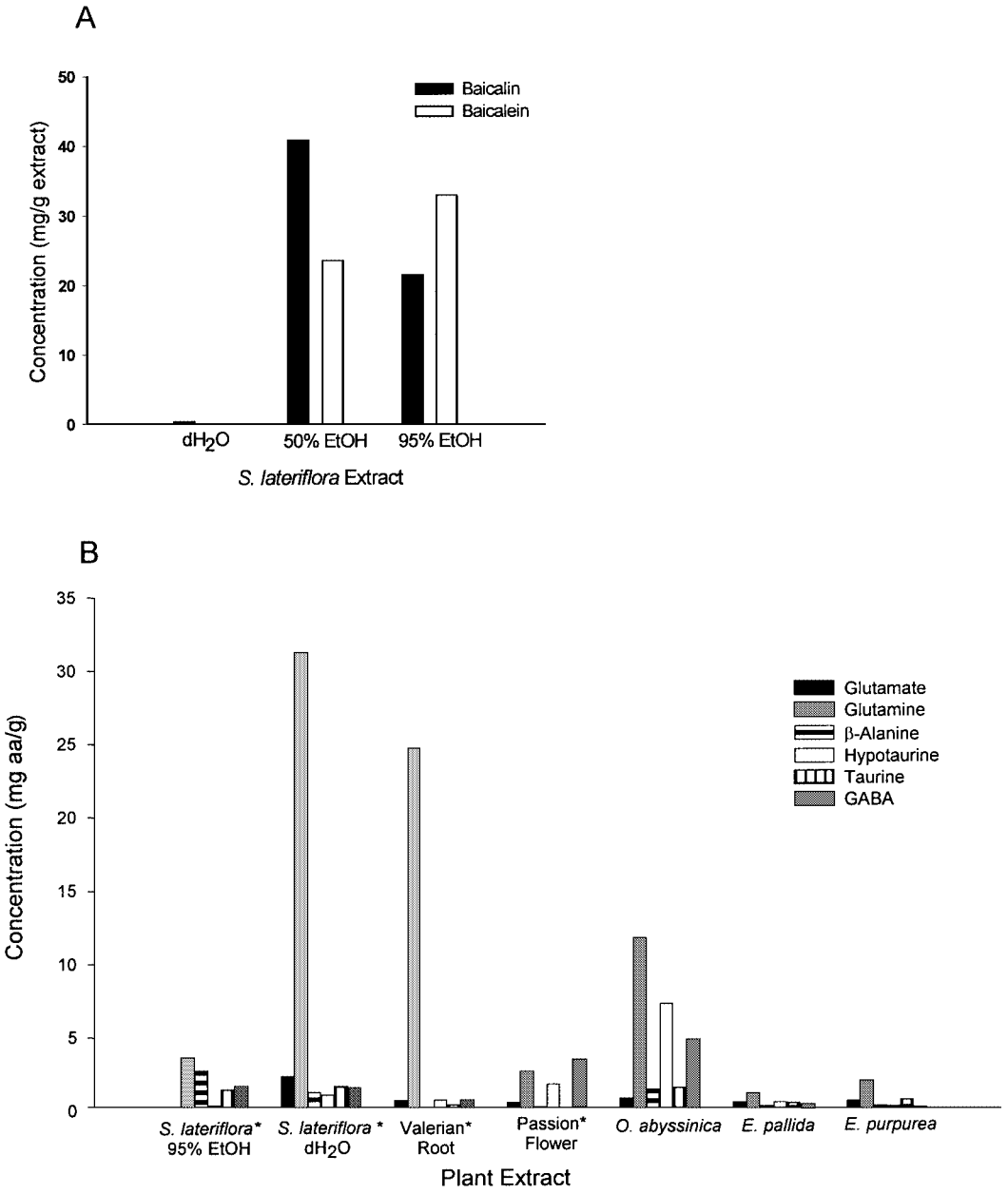

Fig. 5. A) Flavonoid concentrations (mg/g extract) of aqueous and ethanolic extracts as determined by HPLC. Standard compounds were used to calculate baicalin and baicalein concentrations by single point calibration. Both ethanolic extractions yeilded higher flavonoid concentrations than the aqueous extract. **B**) Amino acid concentrations (mg amino acid/g extract) comparing 95% ethanol extracts of *S. lateriflora* with two known "anxiolytic" plants, valerian root and passion flower and three "non-anxiolytic" plant extracts, *O. abyssinica, E. pallida* and *E. purpuea* as determined by HPLC. Standard dilutions of amino acids were used to set up a calibration curve for quantification using β-alanine as the internal standard. Asterisks indicate currently used anxiolytic plants.

p-coumaric acid, ikonnoside I and *5*,6,7-trihydroxy-2′-methylflavone-7-*O*-glucuronide, which have been previously reported from this Scutellaria species (Gafner et al. 2000), were not detected in any of the extracts. The chromatographs obtained for the three *S. lateriflora* extracts (dH_2O, 50% ethanol and 95% ethanol) are compared to those of the baicalin and baicalein standards (Fig. 4A). Baicalin is present in all extracts, however baicalein was only detected in the ethanolic extracts. The aqueous extract contained baicalin at 0.1 mg/g extract and no baicalein (Fig. 5A). The 50% ethanol extraction showed a higher level of baicalin than that obtained in the 95% ethanol extraction: 40.7 mg/g versus 21.3 mg/g respectively. On the other hand, baicalein was higher in the 95% ethanol extract (32.7 mg/g) compared to 23.5 mg/g in the 50% ethanol extract. The two major flavonoids baicalin and baicalein present in *S. lateriflora* are isolated best under ethanolic extraction.

Amino acid quantification

HPLC was performed on various extracts of *S. lateriflora* to identify and quantify the major amino acids present and to compare them to extracts from "anxiolytic" and "non-anxiolytic" plants. Figure 4B outlines the chromatographs obtained for the aqueous and ethanolic extracts of *S. lateriflora* compared to that of the amino acid standards. Of the seven amino acids tested for: glutamate, glutamine, homoserine, β-alanine, hypotaurine, taurine, and GABA, six were detected in the aqueous extract (all except homoserine). Standard amino acid concentrations were measured and five dilutions were made to prepare calibration curves for their quantification in all plant extracts. In general, all amino acids are present in relatively low amounts (less than 5 mg/g extract), with the exception of glutamine which was 31.2 mg/g of aqueous extract. The other main amino acid of interest is GABA, which has similar amounts in both aqueous (1.53 mg/g) and ethanolic (1.65 mg/g) extracts.

In comparison with other plant extracts tested, outlined in Fig. 5B, glutamine was the highest in the aqueous *S. lateriflora* extract. The two "anxiolytic" plants analyzed were commercially prepared tinctures of valerian root, and passion flower. The valerian extract also had high glutamine concentration (24.6 mg/g extract), but had less of the other amino acids compared to both aqueous and ethanolic extracts of *S. lateriflora*. In passion flower, five of the seven amino acids were detected in which GABA was highest at a value of 3.4 mg/g, almost twice as much as both *S. lateriflora* extracts. The next highest concentration was glutamine followed by hypotaurine, 2.6 mg/g and 1.8 mg/g respectively.

Ethanolic extracts of *O. abyssinica*, *E. pallida* and *E. purpurea* were chosen as "non-anxiolytic" test plants for amino acid comparison with *S. lateriflora*. Surprisingly, the *O. abyssinica* extract had high amino acid concentrations overall compared to the other "anxiolytic" and "non-anxiolytic" plant extracts. Again, glutamine was highest (11.7 mg/g) followed by hypotaurine (7.2 mg/g) and GABA (4.7 mg/g). Both *Echinacea* species had low amino acid concentrations compared to *S. lateriflora*, with values all less than 0.6 mg/g except for glutamine which was 1.1 mg/g and 1.9 mg/g for *E. pallida* and *E. purpurea* respectively.

Discussion

The biological activity of *S. lateriflora* extracts were assessed *in vivo* using animal behavioral tests to measure the efficacy of an aqueous extract on inducing anxiolytic actions in rats, and *in vitro* using the fluorometric microtitre plate assay to test for human cytochrome P450 3A4 inhibition. In this study, water extracts were tested in the animal trials because tea is the traditional mode of administration. If it is confirmed that baicalin is a major active principle it is clear that alcohol extracts should be more active and therefore, the animal tests should be repeated with this dosage form.

In vivo, the aqueous extract proved to be active as indicated by significance in two of the three behaviour trials tested. The "open-field" test showed that treated rats entered the center (or anxiogenic) part of the box more often than control rats and they spent more time in the center where they generally feel more vulnerable or afraid. In the EPM paradigm, rats treated with aqueous *S. lateriflora* extract entered the open arms more frequently and spent more time in the open compared to control rats. Since the rat's natural instinct is to protect itself in stressful environments (i.e. stay in enclosed spaces), we would expect to see less foreys into the open arms from rats in the control group. This in fact was observed, indicating that treated rats were less apprehensive or anxious in the unfamiliar environment, a promising behavioural characteristic seemingly caused by *S. lateriflora*. Risk assessment was also measured and there were significant differences between control and treated rats. Rats treated with *S. lateriflora* extract displayed a greater number of unprotected head dips which indicates the lack of fear in exploring an unfamiliar territory. The lack of demonstrable effect in the social interaction paradigm may be attributable to the fact that the drug in question may only be effective in specific forms of anxiety. However, it also remains possible that the extraction procedure, dose or time used in this test may have been less than ideal. Additional tests using dose- and time-course

analyses would be useful. In addition, it may be useful to test the efficacy of this drug in various other tests of anxiety.

In vitro results indicate high biological activity causing inhibition of the CYP 3A4 drug metabolizing enzyme. All *S. lateriflora* commercial tinctures and crude extracts tested had greater than 84% inhibition at their original concentrations. These results suggest that possible drug-herbal interactions may exist between common medications and *S. lateriflora* extracts, but many medicinal plants have similar activity and further studies are warranted *in vivo* to determine whether interactions are a significant issue. Since the use of herbal products is on the rise, it is of great importance to identify potential interactions or side effects of consuming herbal preparations along with traditional medicines regularly prescribed by physicians, so as to avoid any harmful or detrimental effects. HPLC analysis revealed high amounts of flavonoids in both ethanolic extracts of *S. lateriflora*. Initially these compounds were detected in *S. baicalensis*, and have been found to be active ligands of the benzodiazepine (BDZ) site of $GABA_A$ receptors in neurons (Liao et al. 1998; Hui et al. 2000; Paladini et al. 1999). Since BDZ's are widely prescribed for their anxiolytic, muscle relaxant and sedative actions it is of interest to find compounds with active binding ability. Many neuroactive flavonoids have been discovered in plants over the past few years, providing us with a chemical class of compounds that will be useful when designing new treatements for anxiety and related disorders (Medina et al. 1998).

Baicalin concentration (40 mg/g) was approximately twice that of baicalein (21 mg/g) in the 50% ethanol extract. This is consistent with a recent study that obtained approximately two and a half times the amount of baicalin (1418 µg/ml) as baicalein (514 µg/ml) (Gafner et al. 2000). Baicalein however was higher in the 95% ethanol extract, indicating differences in extraction methods will yield varying concentrations of active compounds. The aqueous extracts detected only a minute amount of baicalin and absolutely no baicalein.

In comparison to *S. baicalensis* studies, most of which have been done on root extracts, baicalin is usually present in much higher amounts. For example, 50% ethanol extracts yielded four times (Liu and Shen, 1994) and six times (Hirotani et al. 1998) the amount of baicalin compared to baicalein, in roots and callus tissue derived from cotyledon of *S. baicalensis* respectively. After analyzing tissue cultures of nine *Scutellaria* species, Nishikawa et al. (1999) determined that flavonoids were higher in roots rather than the leaves or stems, which may explain the differences between baicalin and baicalein concentrations in *S. lateriflora*. Determining the most appropriate plant extraction required to obtain the active flavonoids is an important process in formulating safe and effective herbal medicines.

Amino acid composition of *S. lateriflora* ethanolic and aqueous extracts were compared with valerian root and passion flower commercial tinctures. Both of these plants are widely prescribed sedative/anxiolytic plants (Blumenthal et al. 2000) and it was of interest to see if GABA or glutamine were present in high concentrations. The chromatography analysis revealed the presence of these compounds in varying amounts. Santos et al. (1994a, b), found that the high GABA concentration in an aqueous extract of *Valeriana officinalis* root (5 mM) induced the *in vitro* release of [^{3}H] GABA in rat brain synaptosomes, and inhibited its reuptake. These results suggest that an *in vivo* mechanism of GABA release in neurons may act in the same manner. Since GABA is an inhibitory neurotransmitter, increased levels may contribute to the sedative/anxiolytic properties of valerian. It was of interest to find detectable amounts of GABA in *S. lateriflora* as well, although it is not clear how much these levels contribute to the anxiolytic effects. The aqueous extracts of this herb may also be active *in vivo* and cause similar release of GABA in mammalian neurons, thus having the potential anxiolytic properties as depicted in Valerian.

Glutamine however, was the amino acid detected in the highest amount in all plant extracts tested except for passion flower, which had the highest level of GABA. This amino acid is also important because of its ability to pass the blood-brain barrier where it is subsequently metabolized to GABA in GABAergic neurons (Santos et al. 1994b). High levels of glutamine may also explain the *in vivo* mechanism of GABA release, resulting in an inhibitory response in neurons. Passion flower on the other hand, had the highest amount of GABA and relatively high amounts of glutamine however it was less than that found in *S. lateriflora* extracts and the valerian tincture.

It was also of importance to measure amino acid concentrations in control plants which have not been claimed to possess anxiolytic effects. These included ethanolic extracts of *Oxytenanthera abyssinica* a bamboo from Africa, and *E. pallida* and *E. purpurea*, which are used mainly for their immunostimulant activities. Surprisingly, *O. abyssinica* had higher amounts of all amino acids compared to the other plant samples. Glutamine concentration was roughly half of that found in *S. lateriflora* and GABA was more than doubled. Both *Echinacea* extracts were low in all amino acids, however these results are not conclusive. In order to properly identify possible amino acid trends in "anxiolytic" and "non-anxiolytic" plants, further studies are required and should incorporate a larger sample size of plant species as possible.

Traditional use of *Scutellaria lateriflora* as an anxiolytic and sedative plant has been informally recorded over the past few centuries. Due to lack of scientific research on this species, wide scale or commercial applications have yet to be initiated. Initial behaviour trials indicated that the traditionally used aqueous extract has significant effects on anxiety levels in rats and therefore has the potential of becoming a new and popular anxiolytic phytomedicine. It is clear from the results obtained in this study that *S. lateriflora* is a plant with multiple active compounds (flavonoids and amino acids) which may explain the activity of various extracts *in vivo* and *in vitro*. The phytochemical analysis suggests that the alcohol extract has higher concentrations of putative actives and deserves further analysis.

Acknowledgements
This research was supported by the Natural Sciences and Engineering Research Council of Canada.

References

Blumenthal M, Goldberg A, Brinckmann J, Foster S (2000) Herbal medicine: expanded commission E monographs. American Botanical council

Brevoort P (1998) The booming U.S. botanical market: A new overview. Herbal Gram No 44, pp 33–48

Budzinski JW, Foster BC, Vandenhoek S, Arnason JT (2000) An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. Phytomedicine 7: 273–282

File SE (1980) The use of social interaction as a method for detecting anxiolytic activity of chlor-diazepoxide-like drugs. In Current Protocols in Neuroscience. John Wiley & Sons. pp 8.3.3–8.3.4

Foster S (1996) Scullcap: an Herbal Enigma. The Business of Herbs May/June: 14–16

Gafner S, Batcha LL, Bergeron C, Arnason JT, Angerhofer CK (2000) In: Abstracts of plenary lectures, workshops and postersm, International congress and 48th annual meeting of the society for medicinal plant research (GA), Zurich, abstract P1E/08

Hirotani M, Nagashima S, Yoshikawa T (1998) Baicalin and baicalein productions of cultured *Scutellaria baicalensis* cells. Natural Medicines 52: 440–443

Hui KM, Wang XH, Xue H (2000) Interaction of flavones from the roots of *Scutellaria baicalensis* with the benzodiazepine site. Planta Med 66: 91–93

Kessler RC, McGonagle KA, Zhao S, Nelson CB (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: Results from the National Comorbidity Study. Arch Genl Psychiat 51: 8–19

Kubo M, Matsuda H, Tani T, Arichi S, Kimura Y, Okuda H (1985) Studies on Scutellariae radix. XII. Anti-thrombic actions of various flavonoids from Scutellariae radix. Chem Pharm Bull 33: 2411–2415

Liao J-F, Wang H-H, Chen M-C, Chen C-C, Chen C-F (1998) Benzodiazepine binding site-interactive flavones from *Scutellaria baicalensis* root. Planta Med 64: 571–572

Liu Y-M, Sheu S-J (1994) Determination of the six major flavonoids in *Scutellariae radix* by micellar electrokinetic capillary electrophoresis. Anal Chim Acta 288: 221–226

Medina JH, Viola C, Wolfman C, Marder M, Wasowski C, Calvo D, Paladini AC (1998) Neuroactive flavonoids: new ligands for the benzodiazepine receptors. Phytomedicine 5: 235–243

Nishikawa K, Furukawa H, Fujioka T, Fujii H, Mihashi K, Shimomura K, Ishimaru K (1999) Phenolics in tissue cultures of *Scutellaria*. Natural Medicines 53: 209–213

Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH (1998) Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol 51: 519–526

Pellow S, Chopin P, File SE, Briley J (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety of the rat. Neuroscience Methods 14: 144–146

Santos MS, Ferreira F, Cunha AP, Carvalho AP, Ribeiro CF, Macedo T (1994a) Synaptosomal GABA release as influenced by valerian root extract: involvement of the GABA carrier. Arch Int Pharmacodyn Ther 327: 220–231

Santos MS, Ferreira F, Faro C, Pires E, Carvalho AP, Cunha AP, Macedo T (1994b) The amount of GABA present in aqueous extracts of valerian is sufficient to account for [3H]GABA release in synaptosomes. Planta Med 60: 475–476

Yoshino M, Ito M, Okajima H, Haneda M, Murakami K (1997) Role of baicalein compounds as antioxidant in the traditional herbal medicine. Biomed Res 18: 349–352

Address

J. T. Arnason, Ottawa-Carleton Institute of Biology, Department of Biology, University of Ottawa, 30 Marie Curie St., PO Box 450, Stn. A, Ottawa, ON, K1N 6N5, Canada
Tel.: ++613-562-5262; Fax: ++613-562-5765;
e-mail: jarnason@science.uottawa.ca