RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2011.37.327

ScienceAsia 37 (2011): **327–334**

Antioxidant and antimicrobial activity of methanol, dichloromethane, and ethyl acetate extracts of *Scutellaria litwinowii*

Bibi Sedigheh Fazly Bazzaz[a,*], Mohamad Hassanzadeh Khayat[b], Seyed Ahmad Emami[c], Javad Asili[c], Amirhossein Sahebkar[a,c], Elahe Javadi Neishabory[c]

[a] Biotechnology Research Centre and School of Pharmacy, Mashhad University of Medical Sciences, PO Box: 91775–1365, Mashhad, Iran
[b] Pharmaceutical Research Centre and School of Pharmacy, Mashhad University of Medical Sciences, PO Box: 91775–1365, Mashhad, Iran
[c] School of Pharmacy, Mashhad University of Medical Sciences, PO Box: 91775–1365, Mashhad, Iran

*Corresponding author, e-mail: fazlis@mums.ac.ir

Received 14 Mar 2011
Accepted 15 Nov 2011

ABSTRACT: Genus *Scutellaria* belongs to the family Lamiaceae and is known to possess different pharmacological properties. *S. litwinowii* is a species from this genus that is endemic to Iran. In this work, antioxidant activities of methanol, dichloromethane, and ethyl acetate extracts of *S. litwinowii* were evaluated using thiobarbituric acid reactive species (TBARS) and 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. In addition, antimicrobial activities of the mentioned extracts were screened against standard strains of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. The minimum inhibitory concentrations and minimum bactericidal concentrations were determined by broth microdilution method and tetrazolium chloride salt. The highest antioxidant index (AI) was observed from the methanolic extract in both TBARS (41.6% at 32 mg/ml) and DPPH (92.29% at 16 mg/ml) assays. The AI of the methanolic extract in DPPH assay was significantly higher than those of other extracts ($p < 0.001$). However, the AI of all extracts were significantly lower than those of the reference compounds, namely vitamin E and butylated hydroxytoluene ($p < 0.001$). The results of this investigation indicated that extracts obtained from the aerial parts of *S. litwinowii* possessed antioxidant and antimicrobial properties. Further research is required to identify the active phytochemicals responsible for these biological activities.

KEYWORDS: flavonoids, broth macrodilution method, *Candida albicans*, DPPH, TBARS

INTRODUCTION

In recent decades, considerable interest has been focused on finding naturally-occurring antioxidants to be used in food industry as safe alternatives for synthetic compounds, for which serious side effects have been reported[1]. Natural crude extracts and biologically active phytochemicals have been long used to treat a number of common infections in developing countries[2]. However, scientific investigation to determine the therapeutic potential of these plants is limited[3].

Many medicinal plants contain large amounts of antioxidant and antimicrobial constituents such as phenols and flavonoids. Phenolic compounds exhibit a wide range of physiological properties such as anti-inflammatory[4], anti-mutagenic[5,6], antimicrobial[7,8], antioxidant[7,9,10], anti-thrombotic, cardioprotective, and vasodilatory effects[9,11,12]. Polyphenols have been shown to exert important antioxidant effects against free radicals and lipid peroxidation via several mechanisms[1]. Flavonoids are another group of secondary metabolites which are widely distributed in plants. These phytochemicals possess diverse biological activities including well-documented antioxidant properties. Interestingly, the antioxidant activity of many flavonoids such as quercetin, luteolin, and catechins is higher than that of some well-known compounds, e.g., vitamins C and E, and β-carotene[13]. Besides, flavonoids such as apigenin and luteolin are active against methicillin-resistant *Staphylococcus aureus*[14].

Genus *Scutellaria* (Lamiaceae) contains around 300 species of plants with 20 species and 2 hybrids in Iran, of which 10 species and 2 hybrids are endemic[15,16]. The genus has been reported to possess a variety of biological activities[17] such as antibac-

terial[14,18], antiviral, cytotoxic, anti-inflammatory[19], antioxidant[13,20], anti-cancer[21,22], anticonvulsant[8,23], and hepatoprotective effects[24].

Although the antioxidant activity of extracts from different *Scutellaria* species is known[13,14,18,21,25], there has been no previous report concerning *S. litwinowii* which is endemic to Iran and Afghanistan[26]. In the present study, total phenolic and flavonoid contents as well as antioxidant and antimicrobial activities of different *S. litwinowii* extracts were investigated.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were purchased from Merck (Germany) unless otherwise stated.

Plant materials

Aerial parts of *S. litwinowii* were collected from Pivehgan (2100 m), Khorasan Razavi province, northeast of Iran, in July 2006. Mr. M. R. Joharchi from the Herbarium of Ferdowsi University of Mashhad identified the species. A voucher specimen of the plant (no. 14 175) was deposited at the Herbarium of Ferdowsi University of Mashhad (Mashhad, Iran).

Plant extraction

The aerial parts of the plants were dried under shade at room temperature and then cut into small pieces. About 200 g of sample was macerated in methanol, dichloromethane, and ethyl acetate[27] at room temperature for 48 h separately. Each solvent was allowed to remain in contact with plant material for 24 h, and replaced with fresh solvent four times. Removal of the solvents under vacuum at 40 °C gave the crude extracts[3].

Qualitative tests for the presence of flavonoids, tannins, and saponins

Methanol extract was first subjected to some rapid tests for qualitative evaluation of the presence of flavonoids, tannins, and saponins. In order to test the presence of flavonoids, concentrated methanol extract (1 g) was dissolved in 10 ml distilled water. Following filtration, magnesium powder (100 mg) was added to 2–3 ml of the filtrate. Then, concentrated HCl (0.5 ml) was added to the mixture. The presence of flavonoids is characterized by the formation of a pale pink to red colour within 2 min. To confirm the presence of flavonoids, amyl alcohol (3-methyl-1-butanol; 2 ml) was added to the aforementioned solution. If flavonoids are present in the solution, the colour will be displaced into the upper (amyl alcohol) layer and thus could be differentiated from anthocyanins[28].

To test the presence of saponins, concentrated methanol extract (1 g) was diluted with 10 ml distilled water in a test tube. After vigorous shaking for 2 min, the presence of saponins was confirmed by the formation of froth which is stable for at least 30 min. The height of the stable froth is proportional to the saponin content of extract. It should be noted that froth obtained from herbal saponins does not disappears by addition of 1 or 2 drops of HCl[29].

The presence of tannins was evaluated by dissolving concentrated methanol extract (1 g) in 10 ml distilled water. Following filtration, the solution was divided into 2 portions. NaCl 10% and aqueous gelatin 1% solutions were added to the first and second parts, respectively. The presence of tannins was detected by formation of precipitate in the gelatin containing tube or both tubes. The tannin content of the extract is proportional to the amount of precipitate. As a confirmation test, one drop of extract was placed on a paper and sprayed with 5% ferric chloride solution. Formation of blue or dark green spots is indicative of the presence of tannins[30].

Determination of total phenolics

Total phenolic contents of methanol, dichloromethane, and ethyl acetate extracts were determined using the Folin-Ciocalteu method[31]. A standard curve was plotted using tannic acid as standard. One-hundred microlitres of each sample and standard was diluted with distilled water to the final volume of 0.5 ml. Then, 0.25 ml of the Folin-Ciocalteu reagent and 1.25 ml of the Na_2CO_3 solution (7.5%) were added to each tube, respectively. The tubes were vortexed and the absorbance of all samples and standards were measured at 725 nm using a UV-vis spectrophotometer (Cecil, UK) after 40 min. Total phenolic content was expressed as tannic acid equivalent (TAE) calculated from the calibration curve[10].

Determination of total flavonoids

The flavonoid content of each extract was determined according to Lamaison and Carnat, using a method based on the formation of a flavonoid-aluminium complex. Different concentrations of quercetin (Sigma, USA) were used to make the calibration curve. One ml of each diluted sample was mixed with 1 ml of 2% $AlCl_3$ methanol solution. Following incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a UV-Vis spectrophotometer. The flavonoid contents

of extracts were expressed as quercetin equivalents (QE)[2]. Quercetin was purchased from Sigma, USA.

ANTIOXIDANT ACTIVITY

Thiobarbituric acid reactive species (TBARS) assay

A modified TBARS assay[32,33], using egg yolk homogenates as lipid rich media, was applied to measure the potential antioxidant capacity of extracts. Briefly, 0.5 ml (10% w/v) of tissue homogenate and 0.1 ml of sample solution (extract concentrations: 0.5, 1, 2, 4, 8, 16, and 32 g/l) were added to a test tube and made up to 1.0 ml with distilled water. Then, 0.05 ml of 2, 2′-azobis (2-amidinopropane) dihydrochloride (Aldrich, Germany) aqueous solution (0.07 M) (for induction of lipid peroxidation), 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulphate solution were added. The resulting mixture was vortexed and heated at 95 °C for 60 min. After cooling, 5.0 ml of *n*-butanol was added to each tube, then extensively vortexed and centrifuged at 2500g for 10 min. The absorbance of the upper organic layer was measured using a spectrophotometer at 532 nm. Butan-1-ol was used as blank and vitamin E (α-tocopherol) as positive control. All tests were carried out in quadruplicate. Values were expressed as antioxidant index (AI) defined as $(1 - A_{\mathrm{T}}/A_{\mathrm{C}})$, where A_{C} is the absorbance of the fully oxidized control and A_{T} is the absorbance of the test sample.

DPPH (2, 2′-diphenyl-1-picrylhydrazyl) assay

The antioxidant activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability, using DPPH as a stable radical[33]. Scavenging of DPPH represents the free radical reducing activity of extracts based on one-electron reduction[10].

Methanol stock solution of each sample (0.1 ml) was added to 3.9 ml of freshly prepared DPPH methanol solution (0.1 mM). Afterwards, all samples were incubated for 30 min at room temperature in the dark and then absorbance was measured at 517 nm using a UV-vis spectrophotometer. Methanol was used as blank and ascorbic acid and butylated hydroxy toluene (BHT) (Sigma, USA) as positive controls. All tests were carried out in quadruplicate.

The absorbance of control was measured daily and AI was calculated as $(A_{\mathrm{C}}(0) - A_{\mathrm{A}}(t))/A_{\mathrm{C}}(0)$, where $A_{\mathrm{C}}(0)$ is the absorbance of the control at $t =$ 0 min and $A_{\mathrm{A}}(t)$ is the absorbance of the antioxidant at $t = 30$ min. IC_{50} values were calculated using CalcuSyn program.

ANTIMICROBIAL ACTIVITY

Determination of the minimum inhibitory concentrations (MIC)

Antimicrobial activities of methanol, dichloromethane, and ethyl acetate extracts of the aerial part of the plant were determined against two Gram-positive bacteria: *S. aureus* (ATCC 6538p) and *Bacillus cereus* (ATCC 10876), two Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 10536), and the fungus *Candida albicans* (ATCC 10231).

Minimum inhibitory concentrations (MICs) were determined by broth macrodilution method in 24-well plates, using a modification of methods reported by Rios et al[34] and Duffy and Power[35]. Initial concentration of each extract was prepared with the aid of bath sonicator (0.8 g extract plus 4 ml solvent, 30% dimethylsulphoxide in sterile distilled water and drop of Tween 80). One ml of diluted extract was infused into macroplate with 1 ml of sterile Mueller-Hinton broth (2× MHB; HiMedia, India) and then serially diluted (50% with MHB) and finally homogenized. Microbial suspension equivalent to 0.5 McFarland standard turbidity was prepared by suspensions of the growth from brain-heart infusion medium (HiMedia, India). Suspensions were further diluted to obtain a concentration of 10^7 colony-forming units (CFU) per ml for the bacteria and 10^6 CFU for fungi[3]. Then, 10 µl of diluted inoculums was added to each well of macro-plate. Gentamicin and ketoconazole media were used as the positive control for bacterial and fungus strains, respectively, and their effects were determined the same as plant extracts. The sterility of the medium was also tested in two wells. Plates were incubated for 24 h at 37 °C for bacteria, and 48 h at 25 °C for *C. albicans*.

The growth of microorganisms was assessed by MTT (2,3,5-triphenyl tetrazolium chloride, Sigma, USA) assay. Briefly, 0.5 ml of MTT (5 mg/ml; dissolved in sterile water) was added to each well and the plates were incubated at 37 °C for bacteria and 25 °C for yeast for 3 h[36]. The results were expressed as the lowest concentration of plant extract that could inhibit any red dye production. The experiment was repeated in triplicate.

Determination of minimum bactericidal concentrations (MCC)

The bactericidal effects of extracts were determined according to the method described by Rios et al[34]. One hundred µl of clear dilutions in wells of macroplate were subcultured on the Mueller-Hinton agar

plates (HiMedia, India) and subsequently incubated at 37 °C for 24 h for bacteria, and at 25 °C for 48 h for yeast. The lowest concentration of each extract with no observable growth represented MCC.

Statistical analysis

The results are presented as means ± SD. Statistical analysis was carried out using INSTAT 3 software. Group comparisons were made by one-way ANOVA. Tukey-Kramer test was used for post-hoc multiple comparisons.

RESULTS AND DISCUSSION

The presence of non-volatile compounds such as saponins, flavonoids, and tannins in the methanol extract of *S. litwinowii* was investigated. The extract was quite rich in flavonoids but free from saponins and tannins.

Total phenolic and flavonoid content

Total phenolic content of extracts was determined by Folin-Ciocalteu method. Total phenols of *S. litwinowii* extracts were calculated according to the equation $y = 0.1074\,x - 0.0027$ ($r^2 = 0.9999$) as tannic acid equivalent (TAE, mg/g dry material), while total flavonoid contents were determined in accordance with the equation $y = 0.0316\,x + 0.0118$ ($r^2 = 0.9987$) obtained by calibration curves as quercetin equivalent (QE, mg/g dry material).

Total phenolics varied in different extracts: 16.9, 17.0, and 35.3 mg TAE/g of the dry material in dichloromethane, ethyl acetate, and methanol extract, respectively. In regard to the amount of flavonoids in the aerial parts of *S. litwinowii*, dichloromethane extract contained a higher content of flavonoids (13.2 mg QE/g of the dry material), compared to ethyl acetate (9.9 mg QE/g) and methanol extracts (7.2 mg QE/g).

Antioxidant activity

There is a great demand for natural antioxidants to be used for dietary, pharmaceutical, and cosmetical purposes. Regarding the diversity of active antioxidant ingredients in plant extracts, it would be difficult to evaluate the activity of different ingredients in separate assays. Besides, the total antioxidant activity of a given herbal extract may be due to the synergism of several active components. Therefore, to ensure the inclusion of the majority herbal antioxidants, several intermediate extractions are usually performed[37]. Phenolic compounds (including flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, and tocotrienols) represent an important class of plant-derived antioxidants. The redox properties of phenolic compounds make them behave as reducing agents, hydrogen donors and singlet oxygen quenchers. They also may have a metal chelating potential[38].

In the present study, using two different methods, it was found that *S. litwinowii* extracts possess antioxidant properties (Table 1 and Table 2).

TBARS assay

TBARS assay is based on the reaction between thiobarbituric acid and malondialdehyde as well as other secondary lipid peroxidation products. Results from this assay indicated that the highest antioxidant activity of methanol, dichloromethane, and ethyl acetate extracts of *S. litwinowii* were at 32, 8, and 8 mg/ml, respectively (Table 1). The highest AI was observed from methanolic extract being 41.63%. However, the AIs of the three extracts were different to that of the vitamin E at either of tested doses ($p < 0.001$).

DPPH free radical scavenging activity

Relatively stable organic DPPH (2,2′-diphenyl-1-picrylhydrazyl) has been widely used for the determination of antioxidant activity of single compounds as well as different plant extracts. This easy, rapid, and inexpensive method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant. Upon pairing with hydrogen from a radical scavenger, the absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant[1,10,33]. This method is faster than TBARS and could provide useful data on the radical scavenging activity and thereby antioxidant activity of compounds. However, in the TBARS method, the test environment is more similar to the in vivo conditions[33].

In the current investigation, vitamin C and BHT had the highest radical scavenging activities while the activity of *S. litwinowii* extracts were significantly lower at all tested concentrations ($p < 0.001$, Table 2). Among the three tested extracts, methanol extract exhibited the strongest activity (AI = 92.3% at 16 mg/ml) compared to other extracts at all tested concentrations ($p < 0.001$). Likewise, the ethyl acetate and dichloromethane extracts showed moderate activity (highest AI = 60.2% and 55.2%, respectively, at 32 mg/ml). Consistent with present results, a previous comprehensive study that evaluated the DPPH radical scavenging activity of the extracts from the aerial parts of 33 Turkish *Scutellaria* species, reported the activity of ethyl acetate and methanol extracts to be low and

Table 1 Antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts from *S. litwinowii* in TBARS assay ($n = 4$).

Sample	AI[a] in different concentrations						
	32[b]	16	8	4	2	1	0.5
Vit E	90.45 ± 0.02	85.14 ± 0.05	80.42 ± 0.28	77.36 ± 0.71	63.56 ± 0.36	58.93 ± 0.53	45.39 ± 0.44
Methanol extract	41.63 ± 0.48 ***	27.45 ± 0.38 ***	21.21 ± 0.19 ***	19.33 ± 0.53 ***	16.51 ± 0.64 ***	13.30 ± 0.93 ***	11.03 ± 0.50 ***
Dichloromethane extract	6.17 ± 0.22 ***§§§	11.57 ± 0.12 ***§§§	31.22 ± 0.81 ***§§§	22.56 ± 0.44 ***§§§	20.56 ± 0.51 ***§§§	16.67 ± 0.36 ***§§§	12.35 ± 0.15 ***§§
Ethyl acetate extract	13.62 ± 0.23 ***§§§†††	17.96 ± 0.13 ***§§§†††	30.41 ± 0.80 ***§§§	25.34 ± 0.89 ***§§§	20.46 ± 0.38 ***§§§	14.81 ± 0.41 ***§††	12.28 ± 0.20 ***§§

[a] Antioxidant effectiveness expressed as antioxidant index (%).
[b] Concentrations (mg/ml).
Comparison with vitamin E: $^{***}p < 0.001$; Comparison with methanol extract: $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$; Comparison with dichloromethane extract: $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$.

Table 2 Antioxidant activity of the methanol, dichloromethane, and ethyl acetate extracts of *S. litwinowii* in DPPH assay.

Sample	IC_{50}[a]	AI[b] in different concentrations						
		32[c]	16	8	4	2	1	0.5
Vit C	0.2	96.80 ± 0.04	96.73 ± 0.05	96.71 ± 0.04	96.64 ± 0.11	96.58 ± 0.04	96.54 ± 0.08	96.45 ± 0.08
BHT	0.3	95.70 ± 0.08	95.55 ± 0.26	94.71 ± 0.14	94.60 ± 0.12	92.94 ± 0.23	84.50 ± 0.36	61.74 ± 0.12
Methanol extract	3.0	89.27 ± 0.03 ***‡‡‡	92.29 ± 0.10 ***‡‡‡	81.56 ± 0.11 ***‡‡‡	50.24 ± 0.19 ***‡‡‡	25.06 ± 0.02 ***‡‡‡	13.34 ± 0.02 ***‡‡‡	6.76 ± 0.07 ***‡‡‡
Dichlorom. extract	16.7	55.24 ± 0.10 ***‡‡‡§§§	31.09 ± 0.19 ***‡‡‡§§§	16.40 ± 0.23 ***‡‡‡§§§	11.56 ± 0.20 ***‡‡‡§§§	7.41 ± 0.21 ***‡‡‡§§§	4.51 ± 0.36 ***‡‡‡§§§	2.54 ± 0.14 ***‡‡‡§§§
Ethyl acetate extract	4.7	60.24 ± 0.12 ***‡‡‡§§§†††	43.44 ± 0.17 ***‡‡‡§§§†††	25.28 ± 0.11 ***‡‡‡§§§†††	12.04 ± 0.12 ***‡‡‡§§§††	5.36 ± 0.12 ***‡‡‡§§§†††	2.90 ± 0.08 ***‡‡‡§§§†††	1.32 ± 0.13 ***‡‡‡§§§†††

[a] Concentration (g/l) of *S. litwinowii* extract for a 50% inhibition.
[b] Antioxidant effectiveness expressed as antioxidant index (%).
[c] Concentrations (mg/ml).
Comparison with vitamin C: $^{***}p < 0.001$; Comparison with BHT: $^{\ddagger\ddagger\ddagger}p < 0.001$; Comparison with methanol extract: $^{\S\S\S}p < 0.001$; Comparison with dichloromethane extract: $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$.

very high, respectively[39]. Table 2 shows the 50% inhibitory concentration (IC_{50} value) of the samples and standards in the radical scavenging assay.

Although phytochemical reports of *S. litwinowii* extracts are scant, much has been performed on other *Scutellaria* species, in particular *S. baicalensis*. The most famous active ingredients of this genus are three flavonoids: baicalin, baicalein, and wogonin. These flavonoids appear to play an important role in the biological activities observed from *Scutellaria* species. For instance, there have been several reports on the free radical (including hydroxyl and DPPH) scavenging activity and inhibition of lipid peroxidation by these compounds[40–43]. In addition, these flavonoids possess anti-bacterial[44], antifungal[45,46], and anti-viral properties[47–49].

The difference in the antioxidant activity of extracts may be attributed to the difference in the total phenolic and flavonoid content as well as the composition of these bioactive phytochemicals[10]. Since the specificity and sensitivity of tests are different, using the same amounts of compounds for each test is very difficult. However, performing the test on multiple concentrations could be helpful to obtain a more complete picture of the antioxidant activity of *S. litwinowii* extracts[33]. It is hypothesized that the antioxidant power depends on the applied method, sample concentration, and the nature and physiochemical properties of studied antioxidant[50].

Table 3 Minimum inhibitory concentrations (MIC, mg/ml) of the extracts of *S. litwinowii*.

Extract	Microorganism				
	C. albicans	*P. aeruginosa*	*E. coli*	*B. cereus*	*S. aureus*
Methanol	100	50	25	25	6.25
Dichloromethane	100	50	50	25	12.5
Ethyl acetate	100	12.5	50	12.5	6.25

Table 4 Minimum bactericidal concentrations (MCC, mg/ml) of the extracts of *S. litwinowii*.

Extract	Microorganism				
	C. albicans	*P. aeruginosa*	*E. coli*	*B. cereus*	*S. aureus*
Methanol	> 100	100	50	50	6.25
Dichloromethane	> 100	100	50	50	12.5
Ethyl acetate	> 100	25	50	25	12.5

Antimicrobial activity

To our knowledge, this is the first report on the antimicrobial activity of *S. litwinowii*. Experimental studies carried out in species of *Scutellaria* have identified phenols and flavonoids as phytochemicals with antimicrobial properties[14].

Tetrazolium salts are frequently used to indicate biological activity. These colourless salts act as electron acceptors and are reduced to colourful compounds (formazan dyes) by biologically active organisms. For instance, bacterial suspension turns into red or blue within 10–60 min where bacterial growth occurs[36].

In the present study, MICs and MCCs of *S. litwinowii* extracts were evaluated (Table 3 and Table 4). The growth of microbial strains was inhibited by extracts. The MICs varied from 6.25 to 100 mg/ml, depending on the susceptibility of the test microorganism (Table 3). As for gentamicin, observed MICs were 0.03, 0.3, 5, and 1.25 µg/ml against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*, respectively. Inhibition of *C. albicans* growth was also confirmed by the single tested concentration of ketoconazole (5 µg/ml). The results indicated that among the three extracts, methanolic and ethyl acetate extracts have greater antimicrobial activity against the tested microorganisms compared to dichloromethane extract. However, it should be noticed that testing multiple microorganisms is required for an accurate comparison among antimicrobial properties of different *S. litwinowii* extracts. Table 3 indicates obtained MIC values of the tested extracts.

According to the present results, Gram-positive bacteria were more sensitive to the extracts than Gram-negative bacteria and yeast. This is almost in line with a previous report on the antimicrobial activity of *S. barbata* essential oil[18]. In regard to *S. baicalensis*, 10% aqueous extract of the plant was reported to possess antimycotic properties against pathological phyla of *Aspergillus fumigatus*, *C. albicans*, *Geotrichum candidum*, and *Rhodotorula rubra*. Besides, the mentioned extract had the highest activity against *C. albicans* among 56 widely used dried Chinese medical plants[51]. In another investigation, ethanol extract of *S. baicalensis* was found to be capable of improving the in vitro antimicrobial activity of four antibiotics (penicillin G, gentamicin, ciprofloxacin, ceftriaxone) against methicillin-resistant *Staphylococcus aureus*[52].

To conclude, the findings of the present study provided evidence with respect to the antioxidant and antimicrobial properties of *S. litwinowii* extracts. Although antioxidant activities of the mentioned extracts were lower than standard reference compounds, this needs to be fully clarified by further assay methods and using additional concentrations of extracts. Further phytochemical studies are also warranted to isolate and characterize active ingredients that are responsible for the antimicrobial and antioxidant activities, and to explore the existence of synergism, if any, among the compounds.

Acknowledgements: This study was conducted with financial support that was provided by the Mashhad University of Medical Sciences (MUMS) and was part of the PhD thesis of Elahe Javadi Neishabory in the School of Pharmacy at MUMS. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

REFERENCES

1. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006) Antioxidant activity of some algerian medicinal plants extract containing phenolic compounds. *Food Chem* **97**, 654–60.
2. Al-Fatimi M, Wurster M, Schröder G, Lindequist U (2007) Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol* **111**, 657–66.
3. Rojas G, Levaro J, Tortoriello J, Navarro V (2001) Antimicrobial evaluation of certain plants used in Mexican traditional medicine for the treatment of respiratory diseases. *J Ethnopharmacol* **74**, 97–101.
4. Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: im-

plications for inflammation, heart disease and cancer. *Pharmacol Rev* **52**, 673–751.
5. González de Mejía E, Castaño-Tostado E, Loarca-Piña G (1999) Antimutagenic effects of natural phenolic compounds in beans. *Mutat Res Genet Toxicol Environ Mutagen* **441**, 1–9.
6. Geetha T, Malhotra V, Chopra K, Kaur IP (2005) Antimutagenic and antioxidant/prooxidant activity of quercetin. *Indian J Exp Biol* **43**, 61–7.
7. Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey K-M (2001) Antimicrobial properties of phenolic compounds from berries. *J Appl Microbiol* **90**, 494–507.
8. Wang HH, Liao JF, Chen CF (2000) Anticonvulsant effect of water extract of *Scutellariae radix* in mice. *J Ethnopharmacol* **73**, 185–90.
9. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA (1997) Uses and properties of citrus flavonoids. *J Agr Food Chem* **45**, 4505–15.
10. Singh R, Singh S, Kumar S, Arora S (2007) Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A Cunn. *Food Chem Toxicol* **45**, 1216–23.
11. Samman S, Lyons Wall PM, Cook NC (1998) Flavonoids and coronary heart disease: Dietary perspectives. In: Rice-Evans C, Packer L (eds) *Flavonoids in Health and Disease*, Marcel Dekker, New York 1998, pp 469–82.
12. Manach C, Mazur A, Scalbert A (2005) Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* **16**, 77–84.
13. Gao Z, Huang K, Yang X, Xu H (1999) Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochim Biophys Acta* **1472**, 643–50.
14. Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H, Higuti T (2000) Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol* **72**, 483–8.
15. Ghahreman A, Attar F (1999) *Biodiversity of Plant Species in Iran*. Tehran Uiversity Publication, Iran, p 384.
16. Mabberley DJ (1993) *The Plant Book*, Cambridge Univ Press, p 532.
17. Shang X, He X, He X, Li M, Zhang R, Fan P, Zhang Q, Jia Z (2010) The genus *Scutellaria* an ethnopharmacological and phytochemical review. *J Ethnopharmacol* **128**, 279–313.
18. Yu J, Lei J, Yu H, Cai X, Zou G (2004) Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. *Phytochemistry* **65**, 881–4.
19. Kim HM, Moona EJ, Lib E, Kimb KM, Namc SY, Chungd CK (1999) The nitric oxide-producing activities of *Scutellaria baicalensis*. *Toxicology* **135**, 109–15.
20. Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Ríos JL (2002) Antioxidant activity of anti-inflammatory plant extracts. *Life Sci* **70**, 1023–33.
21. Himeji M, Ohtsuki T, Fukazawa H, Tanaka M, Yazaki S, Ui S, Nishio K, Yamamoto H, et al(2006) Difference of growth-inhibitory effect of *Scutellaria baicalensis*-producing flavonoid wogonin among human cancer cells and normal diploid cell. *Canc Lett* **245**, 269–74.
22. Li-Weber M (2009) New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents Wogonin, Baicalein and Baicalin. *Canc Treat Rev* **35**, 57–68.
23. Zhang ZZ, Lian XY, Li SY, Stringer JL (2009) Characterization of chemical ingredients and anticonvulsant activity of American skullcap (*Scutellaria lateriflora*). *Phytomedicine* **16**, 485–93.
24. Lin CC, Shieh DE, Yen MH (1997) Hepatoprotective effect of the fraction of Ban-zhi-lian on experimental liver injuries in rats. *J Ethnopharmacol* **56**, 193–200.
25. Gao Z, Huang K, Xu H (2001) Protective effects of flavonoids in the roots of *Scutellaria baicalensis* georgi against hydrogen peroxide-induced oxidative stress in hs-sy5y cells. *Pharmacol Res* **43**, 173–8.
26. Yuzepchuk SV (1954) Scutellaria. In: Shishkin BK (ed) *Flora SSSR*. 20. pp 73–5. Russian: Izdatl'stvo Akademii Nauk SSSR Moskva-Leningrad.
27. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V (2008) Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. *Molecules* **13**, 904–21.
28. Markham RK (1982) *Techniques of Flavonoid Identification*, Academic Press, London pp 15–45.
29. Hostettmann K, Martson A (1995) *Saponin*, Cambridge, Univ Press, p 233.
30. Evans E (2009) *Trease and Evans Pharmacognosy*, 16th edn, Saunders, Edinburgh.
31. Lister E, Wilson P (2001) Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication), Crop Research Institute, Lincoln, New Zealand.
32. Ruberto G, Baratta MT (2000) Antioxidant activity of selected essential oil componenets in two lipid model systems. *Food Chem* **69**, 167–74.
33. Kulisic T, Radonic A, Katalinic V, Milosa M (2004) Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem* **85**, 633–40.
34. Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antimicrobial activity. *J Ethnopharmacol* **23**, 127–49.
35. Duffy CF, Power RF (2001) Antioxidant and antimicrobial properties of some Chines plant extracts. *Int J Antimicrob Agents* **17**, 527–9.
36. Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extract for bacteria. *Planta Med* **64**, 711–3.
37. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J Agr Food*

Chem **47**, 3954–62.
38. Rice-Evans CA, Miller NJ, G, Paganga (1996) Structure antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–56.
39. Şenol FS, Orhan I, Yilmaz G, Çiçek M, Şener B (2010) Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 *Scutellaria* L. taxa from turkey. *Food Chem Toxicol* **48**, 781–8.
40. Hamada H, Hiramatsu M, Edamatsu R, Mori A (1993) Free radical scavenging action of baicalein. *Arch Biochem Biophys* **306**, 261–6.
41. Gao D, Sakurai K, Katoh M, Chen J, Ogiso T (1996) Inhibition of microsomal lipid peroxidation by baicalein: a possible formation of an iron-baicalein complex. *Biochem Mol Biol Int* **39**, 215–25.
42. Yoshino M, Murakami K (1998) Interaction of iron with polyphenolic compounds: application to antioxidant characterization. *Anal Biochem* **257**, 40–4.
43. Gao Z, Huang K, Yang X, Xu H (1999) Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochim Biophys Acta* **1472**, 643–50.
44. Yang D, Hu H, Huang S, Chaumont JP, Millet J (2000) Study on the inhibitory activity, in vitro, of baicalein and baicalin against skin fungi and bacteria. *Zhong Yao Cai* **23**, 272–4.
45. Yang D, Michel D, Bevalot F, Chaumont JP, Millet-Clerc J (1995) Antifungal activity in vitro of *Scutellaria baicalensis* Georgi upon cutaneous and ungual pathogenic fungi. *Ann Pharm Fr* **53**, 138–41.
46. Jianhua W, Hai W (2009) Antifungal susceptibility analysis of berberine, baicalin, eugenol and curcumin on *Candida albicans*. *J Med Coll PLA* **24**, 142–7.
47. Chen F, Chan KH, Jiang Y, Kao RYT, Lu HT, Fan KW, Cheng VCC, Tsui WHW, et al (2004) In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *J Clin Virol* **31**, 69–75.
48. Ma SC, Du J, But PPH, Deng XL, Zhang YW, Ooi VEC, Xu HX, Lee SHS, Lee SF (2002) Antiviral Chinese medicinal herbs against respiratory syncytial virus. *J Ethnopharmacol* **79**, 205–11.
49. Huang RL, Chen CC, Huang HL, Chang CG, Chen CF, Chang CM, Hsieh MT (2000) Anti-hepatitis B virus effects of wogonin isolated from *Scutellaria baicalensis* Georgi. *Planta Med* **66**, 694–8.
50. Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002) Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal* **13**, 8–17.
51. Blaszczyk T, Krzyzanowska J, Lamer-Zarawska E (2000) Screening for antimycotic properties of 56 traditional Chinese drugs. *Phytother Res* **14**, 210–2.
52. Yang ZC, Wang BC, Yang XS, Wang Q, Ran L (2005) The synergistic activity of antibiotics combined with eight traditional Chinese medicines against two different strains of *Staphylococcus aureus*. *Colloids Surf B Biointerfaces* **41**, 79–81.