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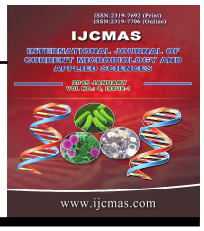

Original Research Article

Characterization of the Essential Oil and Evaluation of Antibacterial Activity of Methanolic Extract of *Stachys lavandulifolia* Vahl.

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Keywords

Stachys lavandulifolia Vahl., Essential oil composition, Methanolic extract, Antibacterial activity

A B S T R A C T

The present study was designed to identify the chemical composition of essential oil and evaluate *in-vitro* antibacterial activity of methanolic extract of *Stachys lavandulifolia* Vahl. against some clinical isolates. The essential oil of *S. lavandulifolia* Vahl. was obtained by headspace solid phase microextraction (HS-SPME) method. The HS-SPME coupled with gas chromatography/mass Spectrometry (GC/MS) was developed for the analysis of essential oil. Components were identified constituting more than 99.63% of the oil. Main components in the essential oil were as follows: myrcene (41.55%), L-α-pinene (33.3%), β-terpinene (12.16%), L-β-pinene (5.06%), sabinene (1.34%) and α-phellandrene (1.27%). Four Gram positive and six Gram negative clinical isolates were identified by standard microbiology techniques. In-vitro antibacterial activity of extract was evaluated against isolates by the broth microdilution method. The flowers of *S. lavandulifolia* methanolic extract exhibited more inhibitory effect against Gram positive isolates. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values ranged between 6.25 and 12.5 mg/ml, 12.5 and 25 mg/ml, respectively. Our findings indicate the promising antibacterial potential of *S. lavandulifolia* Vahl. extract which make it to be considered for pharmaceutical and medicinal purposes.

Introduction

Medicinal plants have renewed interest in their use as alternative source of antimicrobial compounds (Dorman and Deans, 2000) because problems of the uncontrolled use of synthetic antibiotics **(**Mckeegan *et al.,* 2002). The screening of medicinal plants for their antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic uses (Khajehdehi *et al.,* 2012). The use of antimicrobial properties of herbal extracts has been current in the field of pharmacology, pharmaceutical botany and medical microbiology (Conner *et al.,* 1993;

Kung *et al.,* 2012; Rahbar *et al.,* 2005). Some herbs have strong anti-oxidant activities and are rich in anti-microbial components that can be used to treat bacterial infections (Shirzad *et al.,* 2011; Fujisawa *et al.,* 2009).

Stachys genus from Lamiaceae family is represented by about 300 species in the world, mostly in Europe and Asia (Evans, 1996). It has been represented in Iran by 34 species including 13 endemics (Mozaffarian, 2007). This genus contains different natural product classes, including monoterpenes, sesquiterpenes, diterpenes (Chalchat *et al.,* 2001; Kobzar *et al.,* 1986; Paternostro *et al.,* 2000), saponins (Yamamoto *et al.,* 1994), flavonoids, bioflavonoids, glycosides and phenolic acids (Kotsos *et al.,* 2001; Miyase *et al.,* 1996). *Stachys lavandulifolia* Vahl. or Chaye Koohi is widely used in Iranian traditional medicine as an anxiolytic and to treat diarrhea (Meshkatalsadat *et al.,* 2007). Furthermore, experimental studies have demonstrated the anxiolytic effect of it in mice (Rabbani *et al.,* 2003, 2005). The main constituents of *S. lavandulifolia* are α-pinene, myrcene, β- phellandrene and β-caryophyllene (Meshkatalsadat *et al.,* 2007) which some of them have spasmolytic effect (Mata *et al.,* 1997; Camara *et al.,* 2003). The existence of apigenin and luteolin in *S. lavandulifolia* (Safaei, 2004) and the antispasmodic effects of these two flavonoids have been demonstrated (Weimann *et al.,* 2002; Lemmens-Guber *et al.,* 2006; Gharzouli and Holzer, 2004). *S. lavandulifolia* also possesses antimicrobial (Moghim *et al.,* 2014; Saeedi *et al.,* 2008) and antioxidative activities (Taghikhani *et al.,* 2012). Antimicrobial activity of methanol extracts of 4 species of Stachys such as *S. lavandulifolia*have been evaluated against some of bacterial strains and fungal species. The extracts had more activity against Gram positive strains (Saeedi *et al.,* 2008). Composition and antioxidant and antimicrobial activities of essential oil and methanol extract of *S. inflata* have been determined. The plant showed a week antimicrobial activity against three strains of tested microorganisms (Ebrahimabadi *et al.,* 2010). The aims of this study were to identify the essential oil components and evaluate antibacterial activity of methanolic extract of *S. lavandulifolia* Vahl. against some of clinical isolates.

Materials and Methods

Plant material

S. lavandulifolia Vahl. in the flowering stage was collected from the Abr jungle (Shahrood, Semnan province, Iran) in June 2013. The species was identified at the Department of Botany, Research Center of Agriculture and Natural Resources of Shahrood and the voucher specimen was deposited (No. 567) at herbarium.

Isolation of the essential oil by HS-SPME method

The essential oil was obtained by headspace solid phase microextraction (HS-SPME) methodfrom the dried flowers (0.5 g) of *S. lavandulifolia* Vahl. SPME is a fast sample preparation technique based on adsorption, depending on the fiber coating, which is useful for extraction and concentration analyses either by submersion in a liquid phase or by exposure to a gaseous phase (Rocha *et al.,* 2001). The SPME apparatus (Supelco, Inc.) with polydimethylsiloxane (PDMS- thickness 65µm) coating fiber was used for the extraction of volatile oils in grinded flowers of the plant. The sample was transferred into a headspace vial. The vial was placed in a circulating bath and heated to 85°C for 30 min. Then, the needle of the SPME device pierced the vial septum

and the fiber was exposed in the headspace of the sample. Finally, the needle was removed from the vial and injected into the injection port of the GC/MS for 4 min at 280°C. Before analysis, the fiber was conditioned at 280°C for 90 min, in the GC/MS injection port, according to the manufacturer's instructions.

Gas Chromatography/Mass Spectrometry (GC/MS)

The essential oil was analyzed on an Agilent Technologies 6890 (USA) gas chromatograph fitted with a HP-5 MS fused silica capillary column (30m × 0.25mm × 0.25µm film thickness), interfaced with an Agilent mass selective detector 5975C used for mass spectral identification of the components of the oil. Helium was used as carrier gas at a flow rate of 1.2 ml/min and split ratio of 50:1. The oven temperature was initially programmed at 50°C for 3 min, and then was raised at 8°C/min to 200°C, followed by 12°C/min to 290°C for 3 min. Other temperature programs of GC were as follows: injector temperature: 280°C; interface temperature: 280°C; MS quadrupole temperature: 230°C. Mass spectrometry was run in the electron impact (EI) ionization mode at 70 eV with detector voltage of 1.66 Kv, ranged from 30-450 u at 150°C. Mass scan rate was 2.86 scans/s and interval of 0.01 min (20 Hz). A sample (1.0 µl) of diluted oil in hexane was injected into the GC/MS.

Identification of the essential oil components

A mixture of aliphatic hydrocarbons in hexane (Sigma, USA) was injected as under the above-mentioned temperature programmed to calculate the retention indices. Identification of components was based on retention time and indices determined by reference to n-alkenes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored in MS database (Wiely 7 NIST/ ChemStationdata system) with data previously reported in literature (Jennings and Shibamoto, 1980; McLafferty and Stauffer, 1989; Joulain and Koenig, 1998). The chemical compositions of essential oil of *S. lavandulifolia* Vahl. are characterized in Table 1.

Preparation of the extract

Forty gram each of the dried flowers of *S. lavandulifolia* Vahl. was packed in a rotary evaporator (Heidolph, Germany) containing 250 ml methanol. Methanolic extract was concentrated to dryness under vaccum at 50°C for 20 min, yielding 4.33 g. The extract was reconstituted in dimethyl sulphoxide (DMSO) (Merck, Germany) to obtain stock solution of 500 mg/ml concentration. Concentrations (mg/ml) of 100, 50, 25, 12.5, 6.25 and 3.125 were prepared by serial dilution from the stock solution.

Antibacterial assay

The following typed culture and isolated pure microorganisms (from the Microbiology Department, Iran University of Medical Sciences, Tehran, Iran) were used for the current study. In-vitro antibacterial activity of the flower extract was determined against clinical isolates of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *E. coli* ATCC 25922, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella* enteritidis, *Salmonella* typhi and *Pseudomonas aeruginosa* by the broth microdilution method. Overnight cultures (37°C) of the

test isolates were diluted tenfold in tryptic soy broth (Merck, Germany) and incubated at 37°C until they reached exponential growth phase. The 96- well plates were dispensed with 100 µl of Mueller Hinton broth (Merck, Germany). The first well was charged with 100 µl of the DMSO extract solutions. Subsequent concentrations of the extract were prepared with serial dilution as follows: 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml. Suspension was adjusted to 0.5 McFarland turbidity standards. Finally, 100 µl of the diluted inocula containing 1-2 × 10^6 CFU/ml of each isolates were added to each well. *E. coli* ATCC 25922 and gentamicin (Sigma) with concentrations of 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/ml served as positive controls according to the Clinical Laboratory Standard Institute recommendations (CLSI, 2013).

The experiment was carried out in triplicates. MIC was defined as the first well with no visible growth during a 18-20 h incubation period at 37 C. After incubation time 5 µl of the inoculations without growth were dispensed on Mueller Hinton agar (Merck, Germany) plates and after 24 h incubation at 37 C, the lowest concentration without visible growth was regarded as MBC (CLSI, 2013).

Result and Discussion

Essential oil components

Essential oil was successfully isolated from the dried flowers of *S. lavandulifolia* Vahl. Using HS-SPME method and analyzed to determine chemical compositions. GC/MS analysis revealed the occurrence of 13 components constituting 99.63% of the oil. Monoterpene hydrocarbons (96.36%) were major terpenes, whereas oxygenated sesquiterpenes and oxygenated monoterpenes were 2.6% and 0.67%, respectively. Myrcene (41.55%), L-α-pinene (33.3%), β-terpinene (12.16%), L-β-pinene (5.06%), sabinene (1.34%) and α-phellandrene (1.27%) were main constituents. The results are shown in Table 1.

Evaluation of antibacterial activity

The MIC and MBC values were obtained by the broth microdilution method. According to the results given in Table 2, methanolic extract of the *S. lavandulifolia* Vahl. showed moderate antibacterial activity against all tested bacteria. The extract inhibited the growth of the test isolates at 6.25 mg/ml (for *S. aureus*, *S. agalactiae*, *B. cereus* and *E. coli*) and 12.5 mg/ml (for *L. monocytogenes*, *E. coli* ATCC 25922, *K. pneumoniae*, *P. mirabilis*, *Salmonella enteritidis*, *Salmonella* typhi and *P. aeruginosa*). Furthermore, the extract has been found to be bactericidal at 12.5 mg/ml (for *S. aureus*, *S. agalactiae*, *B. cereus*, *E. coli* and *E. coli* ATCC 25922) and 25 mg/ml (for *L. monocytogenes*, *K. pneumoniae*, *P. mirabilis*, *Salmonella* enteritidis, *Salmonella typhi* and *P. aeruginosa*). The MIC and MBC values of gentamicin for *E. coli* ATCC 25922 were 0.78 and 1.56, respectively.

In this study, the recovery of volatile oils using HS-SPME method was satisfactory. Myrcene (41.55%), L-α-pinene (33.3%), β-terpinene (12.16%), L-β-pinene (5.06%), sabinene (1.34%) and α-phellandrene (1.27%) were main constituents in *S. lavandulifolia* Vahl. oil, collected from the Abr jungle in Semnan province, Iran. Monoterpene hydrocarbons (96.36%) were major terpenes, whereas oxygenated sesquiterpenes and oxygenated monoterpenes were 2.6% and 0.67%, respectively. According to the results, the HS-SPME technique is an appropriate and fast method for the recovery of volatile oils in plants. The flowers of *S. lavandulifolia*

methanolic extract exhibited antibacterial activity against both Gram positive and Gram negative bacteria. The results of this study are partially in agreement with previous ones which have proved the inhibitory effects of *S. lavandulifolia* Vahl. against microorganisms (Saeedi *et al.,* 2008; Mahzooni-kachapi *et al.,* 2012).

Composition and antimicrobial activity of essential oil of *S. lavandulifolia* Vahl. collected from Mazandaran, Iran has been determined (Mahzooni-kachapi *et al.,* 2012). Some of the major components identified were hexadecanoic acid (13.9%), α-pinene (18.7%), D-germacrene (8.9%) and β-pinene (7.0%). MIC and MBC values for *S. aureus* and *E. coli* as reference strains were 4.3, 2.15, 8.0 and 4.3 mg/ml, respectively. The essential oils of the flowering aerial parts of four Stachys species collected from Iran were obtained.

The major components of *S. lavandulifolia* oil were 4-hydroxy-4-methyl-2-pentanone, α-pinene and hexadecanoic acid (Morteza-Semnani *et al.,* 2006).The essential oil of the aerial parts of different stages of growth as pre-flowering, flowering and post flowering of *S. lavandulifolia* Vahl. from Lorestan province in Iran has been isolated by hydrodistillation. The chemical composition of volatile oil was analyzed by GC and GC/MS. The major components were found to be α-pinene (27.25, 25.66 and 8.52%), myrcene (17.33, 9.33 and 23.85%), β-phellandrene (21.96, 37.49 and 16.86%) (Sajjadi and Amiri, 2007). In the other study, the essential oil of *S. lavandulifolia* Vahl. collected from the Fasham area near Tehran, Iran, with a yield of 0.25%, was analyzed by the same method. The major components were D-germacrene (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%) and Z-β-ocimene (5.8%) (Javidnia *et al.,* 2004). Similarly, *S. lavandulifolia* oil in our study demonstrated myrcene, α-pinene, L- β-pinene as major components.

Antimicrobicrobial activity of the methanol extracts of the *S. byzanthia*, *S. inflata*, *S. lavandulifolia* and *S. laxa* were evaluated against some of bacterial strains and fungal species (Saeedi *et al.,* 2008). The extracts were found to be more active against Gram positive strains. However, they had no anti-fungal activity. Anti-fungal effects of ethanol extracts of three medicinal plants such as *S. lavandulifolia* were evaluated by the broth microdilution method (Moghim *et al.,* 2014). *S. lavandulifolia* extract exhibited moderate activity on *Candida albicans*. The MIC_{90} and MFC values of extract were 6.55 and 65 mg/ml, respectively.

Antimicrobial activity of *S. lavandulifolia* can be attributed to presence of monoterpenes that are in high amounts in the plant. Uptake monoterpenes will be determined by both their aqueous solubility and the permeability of the membrane of microorganisms (Mann *et al.,* 2000). Instance, α-pinene destroys the cellular integrity of Gram positive bacteria, but Gram negative bacteria are found to be more resistant (Andrew *et al.,* 1980). High concentration of α-pinene in the essential oil of different plants isin relation to antimicrobial activity (Mahboubi and Kazempour, 2009; Mahzooni-kachapi *et al.,* 2012).

The results of various studies have revealed that methanolic extracts have higher activity than the other extracts (Ganga *et al.,* 2012; Razavi *et al.,* 2011; Ejikeugwu *et al.,* 2012). Moreover, high concentration of α-pinene in the flowers of methanolic extract exhibited more bactericidal effect against Gram positive test isolates in our study.

Table.1 Chemical composition of the flower essential oil of *S. lavandulifolia*

Peak No.	Compound	%	[a] KI[C]	[b] t_R
1	α-Thujene	0.86	923	6.393
2	L-α-Pinene	33.03	929	6.533
3	Sabinene	1.34	970	7.423
4	L-β-Pinene	5.06	973	7.484
5	β-Myrcene	41.55	988	7.811
6	α-Phellandrene	1.27	1001	8.096
7	δ-3-Carene	0.75	1007	8.218
8	β-Terpinene	12.16	1027	8.632
9	Cyclofenchene	0.34	1073	8.830
10	(E)-Pinocamphone	0.67	1176	11.609
11	α-Yalangene	1.11	1379	15.202
12	(E)-Caryophyllene	0.78	1424	15.943
13	δ-Cadinene	0.71	1526	17.529
	Total	99.63		
	Monoterpene Hydrocarbon (MH)	99.36		
	Oxygenated Monoterpene (OM)	0.67		
	Sesquiterpene Hydrocarbon (SH)	2.6		

[a]KI: Kovats indice, [b] t_{ret}: retention time in reference to C_8-C_{24} n-alkanes

Table.2 Antibacterial activity of the flower methanolic extract of *S. lavandulifolia*

Microorganism	MIC values (mg/ml)	MBC values (mg/ml)
Staphylococcus aureus	6.25	12.5
Streptococcus agalactiae	6.25	12.5
Bacillus cereus	6.25	12.5
Escherichia coli	6.25	12.5
Listeria monocytogenes	12.5	25
E. coli ATCC 25922	12.5	12.5
Klebsiella pneumoniae	12.5	25
Proteus mirabilis	12.5	25
Salmonella enteritidis	12.5	25
Salmonella typhi	12.5	25
Pseudomonas aeruginosa	12.5	25

The findings of our study in-vitro have justified the antibacterial use of *S. lavandulifolia* for the treatment of bacterial related infections which are caused by some of test isolates (Table 2). In the present study, the flowers of *S. lavandulifolia* Vahl. methanolic extract exhibited moderate antibacterial activity against all of the test isolates. The high concentration of α-pinene in the flower extract is in relation to antibacterial activity in this study and the previous investigations (Grujic-Jovanovic *et al.*, 2004). It was concluded that the characterization of the bioactive components of *S. lavandulifolia* Vahl. can be used in the development of drugs for the treatment of bacterial related infections.

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References

Adams, R.P. 2007. Identification of essential oil components by gas chromatography / mass spectrometry, 4th edn. Allured Publishing Corporation, Carol Stream, Illinois. Pp. 803.

Andrew, R.E., Parks, L.W., Spence, K.D. 1980. Some effects of Douglas fir on certain microorganisms. *Appl. Environ. Microbiol.*, 40(2): 301–304.

Camara, C.C., Nascimento, N.R., Macedo-Filho, C.L., Almedia, F.B.S., Fonteles, M.C. 2003.Antispasmodic effect of the essential oil of *Plectranthus barbatus* and some major constituents on the guinea-pig ileum. *Planta Med.*, 69(12): 1080–1085.

Chalchat, J.C., Petrovic, S.D., Maksimovic, Z.A., Gorunovic, M.S. 2001.Essential oil of *Stachys officinalis* L. Trevis. Lamiaceae from Montenegro. *J. Ess. Oil Res.*, 13(4): 286–287.

Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing. 23th Information supplement; M100–S23. Wayne, PA.

Conner, D.E. 1993.Naturally occurring compounds. In: *Antimicrobials in foods* (Eds.) P.M. Davidson and A.L. Branen. second ed. Marcel Dekker Inc., New York. Pp. 441– 467.

Dorman, H.J.D., Deans, S.G. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. App. Microbiol.*, 88(2): 308–316.

Ebrahimabadi, A.H., Ebrahimabadi, E.H., Djafari-Bidgoli, Z., JookarKashi, J., Mazoochi, A., Batooli, H. 2010. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of *Stachys inflata* Benth. From Iran. *Food Chem.*, 119(2): 452–458.

Ejikeugwu, C., Ikegbunam, M., Ugwu, C., Araka, O., Iroha, I., Adikwu, M., Esimone, C. 2012. Evaluation of antibacterial activity of the leave extracts of *Buchholiziacoriaceae*. *Asian J. Pharm. Biol. Res.*, 2(4): 204–208.

Evans, W.C. 1996. Trease and Evans pharmacognosy, 14th edn. W.B. Sounders Company, London. Pp. 612

Fujisawa, H., Wantanabe, K., Suma, K., Origuchi, K., Matsufuji, H., Seki, T., Ariga, T. 2009. Antibacterial potential of garlic-derived allicin and its cancellation by sulphydryl compounds. *Biosci. Biotechnol. Biochem.*, 73(9): 1948–1955.

Ganga, B.R., Umamaheswara, P.R., Sambasiva, E.R., Mallikarjuna, T.R., Praneeth, V.S.D. 2012. Evaluation of in-vitro antibacterial activity and anti-inflammatory activity for different extracts of *Rauvolfiat etraphylla* L. root

bark. *Asian Pac. J. Trop. Biomed.*, 2(10): 818–821.

Gharzouli, K., Holzer, P. 2004. Inhibition of guinea pig intestinal peristalsis by the flavonoids quercetin, naringenin, apigenin and genistein. *Pharmacology*, 70(1): 5–14.

Grujic-Jovanovic, S., Skaltsa, H.D., Marin, P., Sokovic, M. 2004.Composition and antimicrobial activity of the essential oil of six Stachys species from Serbia.*Flavour Frag. J.*, 19(2): 2004.

Javidnia, K., Mojabb, F., Mojahedi, S.A. 2004.Chemical constituents of the essential oil of *Stachys lavandulifolia* Vahl.from Iran. *Iran. J. Pharm. Res.*, 3: 61–63.

Jennings, W., Shibamoto, T. 1980.Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography, Academic press, New York.

Joulain, D., Koenig, W.A. 1998. The atlas of spectral data of sesquiterpenes hydrocarbons. E.B-Verlag: Hamburg, Germany.

Khajehdehi, P. 2012. Turmeric: Reemerging of a neglected Asian traditional remedy. *J. Nephropathol.*, 1(1): 17–22.

Kobzar, A.Y. 1986. Phytochemical study of *Stachys officinallis*, Isolation of biologically active substances from the aerial parts of the plant. *J. Khim. Prir. Soedin*, 2: 239–240.

Kotsos, M., Aligiannis, N., Mitaku, S., Skaltsounis, A.L., Charvala, C. 2001. Chemistry of plants from Crete: *Stachys pinoside*, a new flavonoid glycoside and iridoids from *Stachys spinosa*. *Nat. Prod. Lett.*, 15(6): 377–386.

Kung, K.H. 2012.Antimicrobial properties of allium species. *Cur. Opin. Biotechnol.*, 23(2): 142–147.

Lemmens-Gruber, R., Marchart, E., Rawnduzi, P., Engel, N., Benedek, B., Kopp, B. 2006.Investigation of the spasmolytic activity of the flavonoids fraction of *Achillamillefo lium* s.1.on isolated guinea-pig ilea. *Arzneimittel forchung*, 56(8): 582–588.

Mahboubi, M., Kazempour, N. 2009.The antimicrobial activity of essential oil from *Perovskia abrotanoides* Karel.and its main components. *Indian J. Pharm. Sci.*, 71(3): 343–347.

Mahzooni-kachapi, S., Mahdavi, M., Roozbeh-Nasira'ei, L., Akbarzadeh, M., Rezazadeh, F., Motavalizadehkakhky, A. 2012. Antimicrobial activity and chemical composition of essential oils of *Stachys lavandulifolia* Vahl. from Mazandaran, Iran. *J. Med. Plants Res.*, 6(24): 4149–4158.

Mann, C.M., Cox, S.D., Markham, J.L. 2000. The outer membrane of *P. aeruginosa* NCTC 6749 contributed to its tolerance to the essential oil of *Melaleuca alternifolia* (Tea Tree Oil) *Lett. Appl. Microbiol.*, 30(4): 294–297.

Mata, R., Rojas, A., Acevedo, L., Estrada, S., Calzada, F., Rojas, I., Bye, R., Linares, E. 1997.Smooth muscle relaxing flavonoids and terpenoids from *Conyza filaginoides. Planta Med.*, 63(1): 31–35.

Mckeegan, K.S., Borges-Walmsley, M.I., Walmsley, A.R. 2002.Microbial and viral drug resistant mechanisms. *Trends Microbiol.*, Suppl. 10: 84–145.

McLafferty, F.W., Stauffer, D.B. 1989. The wiley/NBS registry of mass spectral data. Wiley and Sons, New York.

Meshkatalsadat, M.H., Sajjadi, S.E., Amiri, H. 2007. Chemical constituents of the essential oils of different stages of the growth of *Stachys lavandulifolia* Vahl.from Iran. *Pak. J. Biol. Sci.*, 10(16): 2784–2786.

Miyase, T., Yamamoto, R., Ueno, A. 1996.Phenylethanoid glycosides from *Stachys officinalis*. *Phytochemistry*, 43(2): 475–479.

Moghim, H., Taghipoor, S., Shahinfard, N., Kheiri, S., Heydari, Z., Rafieian, S. 2014. Antifungal effects of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* extracts on *Candida albicans*. *J. Herb. Med. Pharmacol.*, 3(1): 9–14.

Morteza-Semnani, K., Akbarzadeh, M., Changizi, S. 2006.Essential oils composition of *Stachysbyzantia, S. inflata*, *Stachys lavandulifolia* and *S. laxa* from Iran. *Flavour Frag. J.*, 21(2): 300–303.

Mozaffarian, V. 2007. A dictionary of Iranian plant names, Farahang Moaser Press, Tehran. Pp. 522.

Paternostro, M.P., Maggio, A.M., Piozzi, F., Servettaz, O. 2000. Labdanedi terpenes from *Stachys plumosa*. *J. Nat. Prod.*, 63(8): 1166–1167.

Rabbani, M., Sajjadi, S.E., Jalali, A. 2005. Hydroalcoholic extract and fractions of *Stachys lavandulifolia* Vahl.: effects on spontaneous motor activity and elevated plus-maze behavior. *Phytother. Res.*, 19(10): 854–858.

Rabbani, M., Sajjadi, S.E., Zarei, H.R. 2003. Anxiolytic effects of *Stachys lavandulifolia* Vahl. on the elevated plus-maze model of anxiety in mice. *J. Ethnopharmacol.*, 89(2–3): 271–276.

Rahbar, M., Hosseini,Taghavi, S.A., Diba, K., Heidari, A. 2005.In vitro antibacterial activity of shallot (*Allium ascalonicum*) crude juice. *J. Med. Plants*, 4(13): 26–29.

Razavi, S.M., Zarrini, G., Molavi, G., Ghasemi, G. 2011. Bioactivity of *Malvasylvestris* L., a medicinal plant from Iran. *Iranian J. Basic Med. Sci.*, 14(6): 574–579.

Rocha, S., Ramalheira, V., Barros, A., Delgadillo, I., Coimbra, M.A. 2001. Headspace solid phase microextraction (SPME) analysis of flavor compounds in wines. Effect of the matrix volatile composition in the relative response factors in a wine model. *J. Agric. Food Chem.*, 49(11): 5142–5151.

Saeedi, M., Morteza-Semnani, K., Mahdavi, M.R., Rahimi, F. 2008. Antimicrobial studies on extracts of four species of *Stachys*. *Indian J. Pharm. Sci.*, 70(3): 403–406.

Safaei, A. 2004.Identification and quantitative determination of luteolin and apigenin in the aerial parts and an extract of *Stachys lavandulifolia* by HPLC. *Iranian J. Pharm. Res.*, 90–6(Suppl. 2): 274.

Sajjadi, M.H., Amiri, H. 2007. Chemical constituents of the essential oils of different stages of the growth of *Stachys lavandulifolia* Vahl. from Iran. *Pak. J. Biol. Sci.*, 10(16): 2784–2786.

Shirzad, H., Taji, F., Rafieian-Kopaei, M. 2011. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. *J. Med. Food*, 14(9): 969–974.

Taghikhani, M., Nasri, H., Asgari, A., Afrough, H., Namjoo, A.R., Ansari-Samani, R., Shahinfard, N., Rafieian-Kopaei, M. 2012. The renal toxicity of hydroalcoholic extract of *Stachys lavandulifolia* Vahl. inWistar rats. *Life Sci. J.*, 9(4): 3025–3031.

Weimann, C., Goransson, U., Pongprayoon-Claeson, Y., Claeson, P., Bohlin, L., Rimpler, H., Heinrich, M. 2002. Spasmolytic effects of *Baccharis conferta* and some of its constituents.*J. Pharm. Pharmacol.*, 54(1): 99–104.

Yamamoto, R., Miyase, T., Ueno, T. 1994. Stachyssaponins I-VIII new oleanane-type triterpene saponins from *Stachy sriederi* Chamisso. *Chem. Pharm. Bull.*, 42: 1291–1296.