ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Antioxidant activity, total phenolic and flavonoid content, and antibacterial effects of *Stachys lavandulifolia* Vahl. flowering shoots gathered from Isfahan

Elahe Aleebrahim-Dehkordy [1,2], Mahmoud Rafieian-kopaei [3*], Mahmoud Bahmani[4], Safiyeh Abbasi [5]
1 Young Researchers Clubs, Islamic Azad University, Shahrekord Branch, Shahrekord, IRAN.
2Shahrekord Branch, Islamic Azad University, Department of Medicinal Plants, Shahrekord, IRAN.
[3] Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, IRAN.
[3]Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
[5] MSc of Microbiology, Shahrekord University of Medical Sciences, Shahrekord, IRAN.
***Corresponding author: E-Mail: rafieian@yahoo.com**

ABSTRACT

Plants are a rich source of phenolic compounds and one of the most important nature-based antioxidants. The compounds derived from plant-based extracts are an important pharmaceutical resource. This study was conducted to investigate the antioxidant activity and total phenolic and flavonoid content, and to investigate the antibacterial effects of *Stachys lavandulifolia* flowering shoots extract. In this study, *S. lavandulifolia* was gathered from Isfahan and extracted by maceration using ethanol 70%. Total phenol content was measured by Folin-Ciocalcteu reagent and gallic acid standard, and antioxidant activity was investigated by DPPH with reference to butylated hydroxytoluene (BHT). Antibacterial effects were investigated by Broth Microdilution and minimum inhibitory concentration and minimum bactericidal concentration were determined.The effects of different concentrations of *S. lavandulifolia* were investigated on *Staphylococcus aureus* and *Enterococcus faecalis* by disk diffusion with reference to vancomycin and nitrofurantoin. The findings demonstrated that the inhibition of DPPH free radicals was greater by hydroalcoholic *S. aureus* extract than BHT, and therefore the IC50 of this extract was lower than BHT. Total phenolic content was obtained 18.61 (mg gallic acid) and the flavonol and flavonoid content was obtained 2.42 and 8.93 mg/g, respectively. In this study, investigating the effects of different concentrations of hydroalcoholic *S. lavandulifolia* extract on pathogenic bacteria by disk diffusion and Broth Microdilution demonstrated that this extract exerted great inhibitory effects on both bacteria. *S. aureus* was more susceptible to *S. lavandulifolia* extract than *E. faecalis*.

KEY WORDS: Antioxidant activity, total phenolic and flavonoid content, antibacterial effects, *Stachys lavandulifolia* Vahl.

1. INTRODUCTION

The plants from family Laminaceae have long been used conventionally to treat gastrointestinal infections and bloat. Extract is one of the most important products derived from these plants, which is frequently used in traditional medicine, food and pharmaceutical industries, and perfumery (Tabatabaie yazdi, 2016). *Stachys lavandulifolia* Vahl. is a plant from family Lamiaceae that occurs in many regions of Iran, Turkey, and Iraq. This plant spontaneously occurs in several regions of Iran including Isfahan, Chaharmahal va Bakhtiari, Fars, and Lorestan provinces (Mohammadpour-Kanzaq, 2015). *S. lavandulifolia* is a perennial, short, hairy plant and has multiple stems more or less green or grayish, flowers are in a inflorescence and are pink, purple, and rarely white or yellowish (Mozaffarian, 2011). *lavandulifolia* is called variously in different provinces of Iran. Myrcene, alpha-pinene, gama-muurolene, and ogneol are some of the compounds found in *S. lavandulifolia* essential oil (Aghaei Noroozloo, 2015). *S. lavandulifolia* is a tonic agent for stomach and is used to treat infections, asthma, and rheumatic diseases. Moreover, this plant can exert anti-anxiety effects and is effective in treating genital tumors, cancer-induced wounds, and inflammation (Taghikhania, 2012). Boiled *S. lavandulifolia* is used to treat certain diseases such as headache, diarrhea, gastrointestinal diseases, wound, cough, common cold, neuralgia, urinary and bile ducts stones, dyspepsia, bloat, skin infections, and fever (Mohammadpour-Kanzaq, 2015). Recently, medicinal plants have been investigated for antioxidant and antibacterial properties. Some studies have indicated that plants are rich resources of antioxidant and antibacterial compounds and contain large amounts of secondary metabolites including phenolic compounds, flavonols, flavonoids, glycosides, and alkaloids (Zarali, 2016). Flavonoids are the most well-known phenolic compounds with strong antioxidant properties. The protective effects of flavonoids in biological systems have been attributed to their antioxidant capacity, disposing free radicals, activating antioxidant enzymes, and reducing alpha-tocopherol radicals (Rafiee¸2012). Flavonoids can prevent platelets from accumulating and have anti-inflammatory, antibacterial, and antitumor properties (Dehghan, 2013). Phenolic compounds are an important group of plant-based products that are developed in response to environmental stresses. These compounds are able to neutralize free radicals and can act as donors of electron or hydrogen because of having hydroxyl groups (Nazari, 2013). Antioxidant compounds can prevent free radical reaction and decrease cell damage or death, cardiovascular diseases, and cancers (Aleebrahim-Dehkordy, 2016). Today, phythotherapy, as the use of plant-based products or herbal extracts, is a common approach worldwide. Given the side effects due to the use of chemical drugs and large

costs spent for mass production of synthetic drugs, the secondary compounds of medicinal plants can be suitable alternatives to synthetic drugs (Omidbaigi, 2006). In this study, two gram-positive strains, *Staphylococcus aureus* and *Enterococcus faecalis*, were used to investigate the antibacterial effects of *S. lavandulifolia*. *S. aureus* is a gram-positive, catalase-producing bacterium which contributes significantly to the incidence of food poisoning, purulent, systemic, and nosocomial infections (Sepehri, 2015). abscess, third-degree burn, trauma wounds, surgical incisions, bed sore, or atrophy wounds (Hamon-Navard, 2013). *S. aureus* anthrotoxin is heat resistant; therefore, *S. aureus* in foods is not removed by heat (Alizadeh Behbahani, 2014). Enterococci are the fourth leading cause of nosocomial infections and a common cause of urinary tract infections. The Enterococci with antibiotic resistance can colonize in gastrointestinal tract, and the Enterococci that are a constituent of natural flora of the body of patients with malignancy and those treated with broad-spectrum antibiotics are able to proliferate and cause disease (Borjian-Borujeni, 2016). Plant extracts contain different organic compounds that can be used in food and pharmaceutical industries. Since the antimicrobial activities of plants are partly attributed to the presence of secondary metabolites such as flavonoids, phenols, and antioxidants, and given the increasing use and efficacy of these compounds in treating diseases, it is highly important to investigate the extracts derived from plants, especially the plants traditionally used in medicine. In addition, the studies conducted on the medicinal plants gathered from different regions have reported widely inconsistent findings. This may indicate that the plants of different regions may exert different therapeutic effects (Dehghan, 2013). This study was conducted to investigate the antioxidant activity, total phenolic and flavonoid content, and antibacterial effects of *S. lavandulifolia* gathered from Isfahan, central Iran.

2. MATERLAL AND METHODS

Flowering shoots of *S. lavandulifolia* were gathered from Isfahan and dried under natural conditions and aeration, after being identified with reference to botanical keys abd Iranian flora, and Herbarium samples of the Medical Plants Research Center of the Shahrekord University of Medical Sciences. Extraction was done using maceration with ethanol 70% and extract concentration using rotary evaporator at 40°C. Antioxidant property was determined by DPPH using a spectrophotometer at 517 nm wavelength with reference to a synthetic antioxidant, butylated hydroxytoluene (BHT). The results were expressed as the percentage of inhibiting free radicals by the formula below, the graph plotted against the extracts concentration, and IC50 calculated.

DPPH percentage of inhibiting: control absorbance - sample absorbance/control absorbanceTo measure total phenolic content, Folin-Ciocalcteu reagent was used. For this purpose, aluminium chloride 2% and sodium acetate 5% were used. To measure flavonoid compounds, aluminium chloride 2% and potassium acetate 5% were used. sodium carbonate was introduced and absorbance of the samples was read after 30 min at 765 nm wavelength. In this method, gallic acid was used as standard, the gallic acid standard curve plotted at 760 nm wavelength, and total concentration of the phenolic content of the extracts measured. To do microbial tests, two standard (ATCC) gram-positive bacteria, *S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, which were provided from Iranian Research Organization for Science and Technology as lyophilized were used. After culturing bacteria and isolating pure colony, we used 24 h isolates at growth phase to prepare a microbial suspension equal to 0.5 McFarland standard opacity to investigate strains susceptibility. To prepare Stok solution, different concentrations of hydroalcoholic *S. lavandulifolia* were prepared and DMSO 5% was used for dilution. To investigate antimicrobial effects, Broth Microdilution and Mueller-Hinton Broth base culture medium, which was prepared according to the manufacturer (Merck Co., Germany) instructions, were used. According to this method, the first well is considered as negative control and the second well as positive control. After introducing the culture medium, Stok solution, and bacteria into microplate wells and diluting them, we incubated the samples at 37°C for 24 h and the first well in which no opacity was developed was considered MIC. To determine MBC, all of the wells with no opacity were cultured on Blood agar medium separately, and the lowest concentration of the extract in which the bacteria could not grow was considered MBC. Moreover, to investigate antibacterial effects of *S. lavandulifolia* and compare these effects with those of vancomycin and nitrofurantoin, positive controls, disk diffusion was adopted. For this, paper sterile blank discs were kept in different prepared concentrations of *S. lavandulifolia* extract for 24 h to let the extract be completely absorbed by the disks.Then, the disks containing different concentrations of the extract were incubated at 37°C for one h to dry. The dried disks were placed on the plates containing cultured bacteria alongside the standard disks containing the antibiotics: vancomycin (30 g) and nitrofurantoin (300g) and then incubated at 37°C for 24 h to investigate the antibacterial property of each extract. The inhibition rate was measured by measuring the inhibition zone diameter with a ruler (in mm) and the results were compared with CLSI (2012).

3. RESULTS AND DISCUSSION

The inhibition rate of DPPH free radicals was compared between hydroalcoholic *S. lavandulifolia* and BHT (standard) (Table 1). The concentration of the extract that inhibited 50% of DPPH free radicals was obtained 98.8 mg/mL. Moreover, IC50 and inhibition rate of DPPH radicals of BHT was obtained 45.18 mg/mL (Table 1).

Table.1.The total amounts of flavonolic, flavonoid, and phenolic compounds, antioxidant property, and BHT of *Stachys lavandulifolia* Vahl. hydroalcoholic extract

Flavonol (mg/g)	Flavonoid (mg/g)	Total phenol (mg Gallic acid)	Antioxidant (IC50, %)	BHT (%)
2/42	8/93	18/61	8/98	18/45

According to the findings, the inhibition of DPPH free radicals was obtained higher for hydroalcoholic *S. lavandulifolia* extract than BHT, and therefore the IC50 of this extract was lower than BHT. Notably, *S. lavandulifolia* extract is inversely associated with IC50; in other words, the more colorless the color of DPPH solution at presence of the plant, the lower the IC50 and hence the greater the antioxidant property. Table 1 shows the total flavonolic, flavonoid, and phenolic content isolated from *S. lavandulifolia* using ethanol 70%. Total phenolic content was measured 18.61 (mg gallic acid) by Folin-Ciocalcteu reagent according to gallic acid standard. The flavonolic and flavonoid content was obtained 42.2 and 93.8 mg/g, respectively. Some plants are rich in phenolic and flavonoid compounds with strong antioxidant activity and some others contain less effective compounds. Different studies have demonstrated that there are certain differences in the phenolic and flavonoid content and antioxidant properties among different medicinal plants (Baradaran, 2014). Shareen et al studied antioxidant activity of *Carissa opaca* fruit using different solvents, and found the extracts with higher phenolic and flavonoid content to have greater antioxidant activities (Sahreen, 2010). In some plants, antioxidant properties were not proportionate to these compounds, indicating other factors potentially influencing the antioxidant properties of these plants through certain reactions. Mortezaei et al investigated eight medicinal plants for phenolic content and antioxidant activity (Mortazaei, 2013). They found the most phenolic compounds to exist in one g of dry extract and the least in *S. lavandulifolia*. Besides that, *S. lavandulifolia* was found to have antioxidant property, lower than other extrcts. A study investigated antioxidant capacity of ethanolic extract of different tissues (skin, seed, and bulb) of jujube, and indicated that in all tissues, skin had the highest antioxidant capacity and therefore the most phenolic and flavonoid content (Zhang, 2010). Therefore, inconsistency of the findings between our work and other studies can be attributed to the differences among the plants occurring in different regions, because the plants of different regions may have different antioxidant properties and different phenolic and flavonoid content. Notably, many factors, including the time of harvesting, the used organs of plants, climatic conditions of the regions where the plants are gathered, and the type of solvent used for extraction, may contribute to the phenolic, flavonolic, and flavonoid content, antioxidant capacity, and therefore IC50. Investigating the effects of different concentrations of hydroalcoholic *S. lavandulifolia* extract on two pathogenic bacteria, this study demonstrated that hydroalcoholic *S. lavandulifolia* extract exerted considerable inhibitory effects on both studied bacteria, and the inhibitory effect was represented as increase in the inhibition zone diameter. Regarding the longer the inhibition zone diameter, the greater the antibacterial effect, we obtained the greatest antibacterial effects for 1000 and 900 µg/mL. Tables 2 and 3 indicate the effects of different concentrations of hydroalcoholic *S. lavandulifolia* extract according to the diffusion of the respective well on the studied bacteria. As observed in Table 3, the effects of 1000 and 900 of hydroalcoholic *S. lavandulifolia* extract on *S. aureus* were 20 and 18 µg/mL, respectively, which caused a 4-mm increase in the inhibition zone diameter compared to the positive control (30 g vancomycin). The corresponding effects on *E. faecalis* were 30 and 25 µg/mL, which caused a 12-mm increase in the inhibition zone diameter compared to the positive control (300 g nitrofurantoin) (Table 4).

Table.2. Inhibition zone diameter of *Staphylococcus aureus* for different concentrations of *Stachys lavandulifolia* compared to vancomycin

Different concentrations of *Stachys lavandulifolia* (µg/mL)	100	200	300	400	500	600	700	800	900	1000
Inhibition zone diameter (mL)	0	0	0	0	8	10	15	17	18	20

Vancomycin = 16 mm (according to CLSI)

Table.3. Inhibition zone diameter of *Enterococcus faecalis* for different concentrations of *Stachys lavandulifolia* compared to nitrofurantoin

Different concentrations of *Stachys lavandulifolia* (µg/mL)	100	200	300	400	500	600	700	800	900	1000
Inhibition zone diameter (mL)	0	0	0	10	15	19	20	20	25	30

Nitrofurantoine = 18 mm (according to CLSI)

Many studies have recently been conducted on the effects of different plant species on bacteria (Rafieian-Kopaei, 2013; Ghasemi-pirbalouti, 2015). These studies confirmed the inhibitory effects of the studied plants on bacteria. In this study, the effects of hydroalcoholic *S. lavandulifolia* extract were investigated on *S. aureus* and *E. faecalis*. Antimicrobial properties of *S. lavandulifolia* essential oil and extract have already been investigated by

various methods (Shahnama, 2015) Taheri et al studied the antibacterial effects of aqueous, ethanolic, and methanolic (80%) *S. lavandulifolia* extract on *S. aureus*, *Escherichia coli*, and *P. aeruginosa* using disk diffusion and determination of MIC and MBC, and observed no antibacterial effects for aqueous extract, but ethanolic and methanolic (80%) extracts exerted anti-inhibitory effects on growth (Taheri, 2013). Some investigations have indicated that another species of Stachys genus, inflate, exerts antimicrobial effects on *E. coli*, *S. aureus*, *P. aeruginosa*, and Salmonella (Meshkibaf, 2010). An important explanation of medicinal plants inhibiting bacteria is the presence of phenolic compounds in their essential oils and extracts, which enable them play an effective role, thanks to their hydrophobicity, in decomposing cell and mitochondrial membrane lipids and changing membrane permeability and hence bacterial cell death through bonding with amino groups and hydroxylamine proteins (Tavassoli, 2011). Many plants contain phenolic compounds. As well, the phenolic content was investigated in *S. lavandulifolia* in this study. Phenolic compounds have antibacterial effects. Moreover, the number of hydroxyl groups is directly associated with their toxicity on microorganisms. The structures of flavonoids and flavonols are phenolic with antibacterial effects. In addition to exerting antimicrobial activities, these compounds are highly useful because of having antioxidant properties (Sharafati-Chaleshtori, 2010). It can be inferred that most of the antibacterial compounds identified in medicinal plants are aromatic or saturated organic compounds with greater solvency in methanolic solvents such as methanol and ethanol. However, difference in antibacterial effects may be related to different compounds identified in different medicinal plants (Hamon-Navard, 2013). Regarding MBC and MIC, hydroalcoholic *S. lavandulifolia* extract exerted acceptable inhibitory and bactericidal effects on *S. aureus* and *E. faecalis* of which *S. aureus* was more susceptible to *S. lavandulifolia* extract (Table 4).

Table.4. MIC and MBC (µg/mL) of hydroalcoholic *Stachys lavandulifolia* extract gathered from Isfahan

Pathogen	MIC µg/mL	MBC µg/mL
Staphylococcus aureus (ATCC 25923)	300	600
Enterococcus faecalis (ATCC 29212)	400	800

The difference in the effects due to *S. lavandulifolia* extract between *S. aureus* and *E. faecalis* can be related to difference in structures of these two bacteria, the type of culture medium used, and extraction method, and the compounds identified in *S. lavandulifolia* (Tajkarim, 2010). Moreover, this work confirmed that the antibacterial activity of *S. lavandulifolia* was associated with phenolic, flavonolic, and flavonoid compounds.

4. CONCLUSION

Overall, hydroalcoholic *S. lavandulifolia* extract has a high antioxidant property and large amounts of phenolic, flavonolic, and flavonoid compounds, and can inhibit DPPH free radicals. Because antioxidant compounds of plants can exert protective effects on the body's cells, *S. lavandulifolia* extract can be considered an effective factor for human beings' health and used as a nature-based antioxidant to prevent and treat diseases. Besides that, *S. lavandulifolia* extract can be replaced partly with synthetic antioxidants to reduce the risk of liver damage and development of cancer using nature-based antioxidants. Besides that, this study demonstrated that hydroalcoholic *S. lavandulifolia* extract had antibacterial effects on *S. aureus* and *E. faecalis* strains. This finding requires further investigations so that other effective concentration of *S. lavandulifolia* extract can be studied on these two bacteria and clinical strains. In the light of climatic diversity and occurrence of plants in different regions in Iran, a great deal of attention should be paid to identification of the best species in terms of the amounts of effective substances, because climatic conditions, the type of soil and gathered plant in each province can influence the amounts of effective compounds and antibacterial properties of the plants. Regarding the findings of this work, we can argue that hydroalcoholic extract of *S. lavandulifolia* gathered from Isfahan is a rich source of phenolic, flavonolic, flavonoid, and antioxidant compounds which can be used in food, pharmaceutical, and cosmetic industries, as an inhibitory agent of bacterial growth, and a suitable alternative to antibiotics and synthetic drugs.

5. ACKNOWLEDGEMENT

Hereby, we gratefully thank the Medical Plants Research Center of the Shahrekord University of Medical Sciences for cooperation with this work.

REFERENCES

Aghaei Noroozloo Y, Mirjalili M.H, Nazeri V, Moshrefi araghi A.R, Evaluation of some ecological factors, morphological traits and essential oil productivity of *Stachys lavandulifolia* Vahl, in four provinces of Iran . Iranian Journal of Medicinal and Aromatic Plants, 30(6), 2015.

Aleebrahim-Dehkordy E, Khodadadi S, Mousavipanah Z, Nasri H. Herbal antioxidants and kidney, Ann Res Antioxid. 1(1), 2016, e10.

Alizadeh Behbahani B, Tabatabaei Yazdi F, Shahidi F, Mohebbi M, Hossein Zanganeh, Investigation of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Aqueous and Ethanolic Avicennia Marina Extracts on Gram Positive and Gram Negative Bacteria, *in vitro*, Sadra Med Sci J, 2(2), 2014, 123-134.

Baradaran A, Nasri H, Nematbakhsh M, Rafieian-Kopaei M, Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. Clin Ter, 165(1), 2014, 7-11.

Borjian-Borujeni S, Kaveh Baba Heidari E, Mortezaei S, Borjian- Borujeni M, Validi M, Study antibacterial effects of hydroalcoholic extract of acorn fruit´s (Quercus branti) against Listeria monocytogenes and Enterococcus faecalis *in vitro*, J Shahrekord Univ Med Sci, 17, 2016, 98-106.

CLSI (clinical and Laboratory standards Institute), Performance standards for antimicrobial disks susceptibility tests: approved Standards-Eleventh edition. Mo2-A11, CLSI, USA, 2012.

Dehghan E, Dashti H, Baghizadeh A, Antibacterial Effect of Ethanol Extract (Althaea Officinalis) on Streptococcus Pyogenes Compared with Prevalent Antibiotics *In-Vitro*. J Rafsanjan Univ Med Scie, 12(6), 2013, 461-74.

Ghasemi-pirbalouti A, Ahmadzadeh Y, Malekpoor F, Vayiation in antioxidant, and antibacterial activities and total Phenolic content of the bulbs of mooseer (Allium hirtifolium Boiss) Str, 2015,15-22.

Hamon-Navard S, Bahrami AM, Razmjou M, Asadi-Samani M, Hatami-Lak M, Evaluation of Nerium oleander aqueous extract effect on Staphylococcus aureus and Staphylococcus epidermis, J Shahrekord Univ Med Sci, 15(1), 2013, 46-54.

Meshkibaf MH, Abdollahi A, Fasihi Ramandi M, Adnani SA, Moravvej A, Hatami S, Antibacterial effects of hydro-alcoholic extracts of Ziziphora tenuior, Teucrium polium, Barberis corcorde and Stachys inflate.2010, Journal of semnan University of medical Sciences, 4(36), 2010.

Mohammadpour-Kanzaq H, Noroozi M, Mahmoudi R, Mohammadpoorasl A, Zavoshy R, AsadiNadari M, Antimicrobial Effect of *Stachys lavandulifolia* Vachl Essential Oil on Listeria Monocytogenes, Mljgoums, 9 (2), 2015,47-53

Mortazaei S, Rafieian M, Ansary Samani R, Shahinfard N, Comparison of Phenolic Compounds Concentrations and Antioxidant Activity of Eight Medicinal Plants. J Rafsanjan Univ Med Sci, 12(7), 2013, 519-30.

Mozaffarian V, Medicinal and Aromatic Plants recognize Iran. Publications of farhang moaser.Tehran, 2011.

Nazari S, Nazarnezhad N.J, Ebrahimizadeh M.A, Evaluation of antioxidant properties and total phenolic and flavonoids content of Eucalyptus camaldulensis and Pinus sylvestris bark. Iranian Journal of Wood and Paper Science Research, 28(3), 2013.

Omidbaigi R, processing and production of medicinal plants. 3rd ed. Astan Ghods Razavi press.Iran, 2006, 347.

Rafiee M, Naseri L, Bakhshi D, Alizadeh A, Phenolic compounds and antioxidant activity of some Iranian and commercial apple varieties in West Azarbaijan province. Journal of Crops Improvement,14(2), 2012.

Rafieian-Kopaei M, Baradaran A, Rafieian M, Oxidative stress and the paradoxical effects of antioxidants. J Res Med Sci, 18(7), 2013, 628 .

Sahreen S, Rashid Khan M and Ali Khan R, Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food Chemistry, 122, 2010, 1205-1211.

Sepehri Z, Nasiri AK, Hesaraki M. Javadian F, Kiani Z, Fooladvand Z, Evaluation of the effect of antimicrobial activity of ethanol extract of Myrtus communis , Zataria multiflora Boiss and Allium sativum on biofilm formation by Staphylococcus aureus. Quarterly Journal of Sabzevar University of Medical Sciences, 21(6), 2015.

Shahnama M, Azami S, Mohammadhosseini M, Characterization of the Essential Oil and Evaluation of Antibacterial Activity of Methanolic Extract of *Stachys lavandulifolia* Vahl. Int.J.Curr.Microbiol.App.Sci, 4(3), 2015, 275-283.

Sharafati-Chaleshtori R, Sharafati-Chaleshtori F, Sharafat-Chaleshtori A, Ashrafi K. Antimicrobial effects and evaluation of total phenols, flavonoids and flavonols contents of ethanolic extracts of Scrophularia striata , Journal of Shahrekord University of Medical Sciences, 11(4), 2010.

Tabatabaie yazdi F, Alizadeh Behbahani B, Mortazavi S.A. Investigation Effects of Lamiaceae plants (Thymus vulgaris L, Mentha spp. and Ziziphora tenuir L.) Inhibitory Staphylococcus aureus and Geotrichum candidium in Razavi Khorasan Province Industrial Doogh Samples with Response Surface Method (RSM). JFST, 13(51), 2016.

Taghikhania A, Ansarisamani R, Afrogh H, Shahinfard N, Ganji F, Asgari A, Taji F, Rahimi M, Asgharzadeh S, Rafieian-Kopaei M, The Hepatotoxic Effects of *Stachys lavandulifolia* Vahl on Wistar Rat. J Mazandaran Univ Med Sci, 22 (88), 2012, 81-87.

Taheri M, Majd A, Nejadsattari T, Hekmatshoar H & Mehrabian S, Ethanolic extract of aerial organs of *Stachys lavandulifolia* Vahl in generative phase has more efficient antimicrobial effects. Advances in Environmental Biology. 7(13), 2013, 4016-4021.

Tajkarim MM, Ibrahim SA, Cliver DO Antimicrobial herb and spice compounds in food, Food Control 21, 2010, 1199-18.

Tavassoli S, Mousavi SM, Emam-Djomeh Z, Comparative Study of the Antimicrobial Activity of Rosmarinus officinalis L. Essential Oil and Methanolic Extract. Middle-East Journal of Scientific Research, 9 (4), 2011, 467-71.

Zarali M, Hojjati M, Tahmouzi Didehban S, Jooinadeh H, Evaluation of chemical composition and antibacterial activities of Echinophora cinerea Boiss and Stachys lavandulifolia Vahl. essential oils *in vitro*. JFST, 52(13), 2016.

Zhang H, Jiang L, Ye S, Ye Y and Ren F, Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (Ziziphus jujube Mill) from China. Food and Chemical Toxicology, 48, 2010, 1461-1465.