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Antibacterial activity of different extracts from the aerial parts and roots of *Stachys lavandulifolia* Vahl harvested at two stages of growth

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ABSTRACT

Background & Aim: Nowadays, the application of traditional medicine and herbal medicinal plants instead of chemical and synthetic drugs has captured the attention of the researchers. The purpose of this study was to investigate the antimicrobial effects of the aquatic, ethanolic and methanolic (80%) extracts of the aerial and root parts of *Stachys lavandulifolia* Vahl which were taken from the countryside of Marand (Eastern Azerbaijan, Iran) in two generative and vegetative phases against standard strains of microorganisms.

Experimental: Antimicrobial activity of the alcoholic and aquatic extracts from different parts of *S. lavandulifolia* was investigated against standard strains of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) through well-plate and micro-dilution methods. Ampicillin as antibacterial agent was used as positive control.

Results & Discussion: Results of antimicrobial properties of *S. lavandulifolia* extracts revealed that the aquatic extract of the plant had no antibacterial effects. While the ethanolic and methanolic (80%) extracts showed the growth inhibition effect against standard strains of examined microorganisms. Their effect against Gram-positive bacteria was higher than Gram-negative bacteria. The antimicrobial activity of the ethanolic extract from the inflorescence of *S. lavandulifolia* was higher than the alcoholic extract of the roots.

Recommended applications/industries: The overall results indicated the ethanolic extract of the inflorescence of *Stachys lavandulifolia* has the best antimicrobial effects and it can be employed in manufacturing desirable bactericidal agents.

1. Introduction

Among *Iranica flora*, nearly all plant species have important role in traditional medicine. Almost 9000 plant species exist in *Iranica flora* out of which more than 500 species are extensively used in traditional medicine due to antimicrobial and anticancerous properties (Parsa, 2001; Sengul *et al*., 2005). In fact, it is common for Iranian people to use some plant species for treatment of infectious diseases and other health problems (Adiguzel *et al*., 2005). For centuries, herbal medicinal plants have been known due to curative properties or disease treatments (Skaltsa *et al*., 1999). In addition, there is a vital need for discovering the new antimicrobial compounds (with different chemical structures) in order to deal with re-emerging infectious diseases. Thus, researchers have increasingly paid attention to traditional medicine and have looked for better herbal drugs against the microbial infectious diseases (Parekh and Chanda, 2007). Today, great interest towards traditional medicines has been arisen and even there have been increasingly immense demand for some drugs with herbal origin. This rehabilitation of interest towards herbal drugs could be mainly because of the common belief that herbal medicine is healthy and is more reliable than expensive synthetic drugs that have usually side effects (Nair and Chanda, 2007). This view was a source of motivation for research on new antimicrobial substances out of various herbal medicinal plants.

From this perspective, the effect of different plant extracts on pathogen microorganisms has captured the attention of researchers (Cordell, 2000). The species of *Stachys* L. genus in traditional and local medicine have been explored for the treatment of genital tumors, spleen sclerosis, inflammatory tumors, and cancerous ulcers (Skaltsa *et al*., 1999). *Stachys lavandulifolia* Vahl. is a medicinal plant belonging to the Lamiaceae family and is used as an herbal tea. It is worth noting that the flowering shoots of the herb is extremely important in treatment of diseases such as digestive problems, headache, and stomachache, gastritis, and nerve disorders. Moreover, *S. lavandulifolia* can relieve the primary disminorey problems and reduce the intensity and the time of pain. It also decreases tiredness and nausea caused by primary disminorey, but it has no effect on vomiting (Seyyed-Javadi *et al*.,1998). The quick review of studies showed that there has been no research on antimicrobial activity of *S. lavandulifolia*. Therefore, the purpose of this study was to investigate the antimicrobial effects of the aquatic, ethanolic, and methanolic (80%) extracts of the aerial parts and roots of *S. lavandulifolia* collected from Marand (Eastern Azerbaijan, Iran) at two generative and vegetative phases against standard bacterial strains.

2. Materials and methods

2.1. Plant material

Samples of *S. lavandulifolia* at two generative and vegetative phases were collected during April to June 2013 from Mishudagh region in East Azerbaijan. The plant was identified according to the sample at Herbarium, Department of Plant Biology of Islamic Azad University of Marand Branch (No.19405).

2.2. Preparation of extracts

The collected the plants were separated into two parts, including the aerial organs (stem, leaf, petiole, and flower) and root organs. The samples were kept in shade and were dried far from the direct light of sun. Then, the electronic powdering apparatus was used to powder this plant and the extraction was performed (Kursat and Erecevit, 2009; Dugler and Aki, 2009). Various extracts included aquatic, ethanolic and methanolic (80%) extracts.

2.3. Examined microorganisms

In this research, the antimicrobial studies were done against three standard bacterial strains as follows: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), and *Pseudomonas aeruginosa* ATCC 9027.

2.4. Antimicrobial activity by Well-plate assay

In this method, the inoculation of 100 µl microbial suspension was fulfilled into surface of the plate with a sterile pipette. All culture plates were allowed for dry for about 5 min. The same diameter wells produced on Mueller-Hinton agar. Then, 100 µl the extracts were poured in well separately [one well was allocated to distilled water, ethanol and methanol (80%)]. Later, plates were put in incubator for 18-24 h at 37 °C and then the diameter of zone of growth inhibition were measured that results from diffusion of the extracts into the medium surrounding the wells with millimeter ruler. Additionally, ampicillin disc was used as standard control antibiotic (Shafiee, 1992; Kursat and Erecevit, 2009).

2.5. Antimicrobial activity by micro-dilution assay

The identification of the minimal inhibitory concentration (MIC) was performed with some modifications (Zyoda and Porter, 2001). Then the minimal bactericidal concentration (MBC) was determined. Then, ampicillin as a standard antimicrobial agent was considered in this method. In addition, their dilutions (0.25 to 128 μg/ml) were similar to plates of microliter (Dugler and Aki, 2009).

2.6. Statistical analysis

This study was performed within the framework of factorial experiment 2 ×3 ×2, including two organs of the plant, three extracts (aquatic, ethanolic, and methanolic), and two stages of growth with a completely randomized design with three replications. After analysis of variance in procedure of GLM of SAS software, means comparison was performed by Tukey method ($p \leq 0.05$). Means of the antibacterial activity of the extracts with standard antibiotic were compared using Dunet test at $p \leq 0.05$.

3. Results and Discussion

In this study, the antimicrobial activity of the aquatic, ethanolic, and methanolic (80%) extracts of *S. lavandulifolia* were investigated against the standard strains of bacteria and the antimicrobial capacity of these extracts was evaluated through the existence or non-existence of diameter zone of inhibition. In well-plate assay, the aerial and root organs of *S. lavandulifolia* were determined in two generative and vegetative phases against standard strains of microorganisms through aquatic, ethanolic, and methanolic extracts. Results of this study indicated that the different extracts had a significant growth inhibition effect against examined microorganisms in comparison with standard control antibiotic. The extracts from the aerial parts of *S. lavandulifolia* collected at generative stage had the largest zone of growth inhibition against studied bacteria (Table 1).

In comparison, the extracts of the herb with standard antibiotic showed that the ethanol extract from the aerial parts of *S. lavandulifolia* at vegetative stage acts like antibiotic, while the ethanol extract from the aerial parts at generative stage acts higher than ampicillin (Table 2).

Comparison of minimal inhibitory concentration (MIC) in two groups showed that there was no significant difference between standard antibiotic and ethanol extract of *S. lavandulifolia* (Table 3). The aquatic extract from the aerial parts of *S. lavandulifolia* at the vegetative phase had no antimicrobial effect. Regarding to the antimicrobial effects of different extracts of the plant, the findings revealed that the ethanolic, methanolic, and aquatic extracts from the aerial parts had the higher antimicrobial effect than the extracts from the roots, especially at the generative phase. Results of plate-well assay indicated that *Staphylococcus aureus* was the most sensitive in comparison with other studied bacteria. Probably, some components in the extracts, including phenols, flavonoids, monoterpenes (β-phellandrene, α-pinene, β-pinen, myrcene, and β-ocimene) and sesquiterpenes (germacrene-D and Spathulenol) are caused to antimicrobial activity in *S. lavandulifolia* (Javidnia *et al*., 2004). The phytochemical analysis of *S lavandulifolia* has proved the presence of some secondary metabolites such as diterpenes, phenyl ethanoidglycosides, flavonoids, and saponis (Khanavi *et al*., 2005) in a way that flavonoids might be responsible for antimicrobial activity (Saeedi *et al*., 2008).

In present study, both ethanolic and methanolic extracts could be regarded as organic solvent with favorite polarity because they were able to solve antimicrobial compounds in the plant. In fact, those extracts that were provided during the generative phase of this plant showed more antimicrobial properties. In addition, the generative phase is important for survival of the species so that the above-mentioned substances may increase in this phase. From physicochemical perspective, the cell wall structure of Gram-negative bacteria was more complex than that of Gram-positive bacteria. For this reason, Gram-negative bacteria indicated more resistance against antimicrobial substances. Gram-negative bacteria have external hydrophilic membrane due to presence of lipopolysaccharide (LPS) molecules. The small solution substances of molecules are able to pass this external membrane of bacteria through numerous porin proteins. Thus, this membrane might be regarded as a barrier against macromolecules and hydrophobic compounds, and toxic drugs and it is important for evaluation of the antimicrobial compounds in plants. In total, Gram-positive bacterium (*S. aureus* ATCC 6538) showed more sensitivity towards the extracts as compared to Gram-negative bacteria. These findings are in agreement with results of a study on *Stachys inflata* by Maleki et al. (2001).

The ethanolic and methanolic extracts have a great amount of bactericide properties even in high dilutions, in exception of *E.coli*, which showed a static reaction and was resistant to the extracts. These findings are in agreement with results of a research by Rasooli and Mirmostafa (2002). It has been shown that extracts depend on species, type of chemical substance of the plant and the climate condition. As a result, the antimicrobial activities of extracts in various plant species were different from each other (Lawrence *et al*., 2005; Chi-kuen and Chi-Chiu, 2006).

Table 1. Simple and interaction effects of organs, extracts and stages of growth of *Stachys lavandulifolia* on the zone diameter of growth inhibition of studied bacteria

Factor		***S. aureus* ATCC 6538**	***E. coli* ATCC 8739**	***P. aeruginosa* ATCC 9027**
Organ	Aerial	11.67a¶	6.33a	9.17a
	Root	4.83b	2.67b	4.05b
	SEM	0.204	0.192	0.219
Extract	Ethanol	13.00a	7.00a	10.25a
	Methanol	10.75b	6.00b	8.83b
	Water	1.00c	0.50c	0.75c
	SEM	0.250	0.236	0.268
Stage	Vegetative	6.50b	3.67b	5.55b
	Generative	10.00a	5.33a	7.67a
	SEM	0.204	0.192	0.219
Organ×Extract	Aerial × Ethanol	18.50a	9.50a	14.00a
	Aerial × Methanol	14.50b	8.50a	12.00b
	Aerial × Water	2.00d	1.00c	1.50d
	Root × Ethanol	7.50c	4.50b	6.50c
	Root × Methanol	7.00c	3.50b	5.67c
	Root × Water	0.00e	0.00c	0.00d
	SEM	0.353	0.333	0.379
Organ × Stage	Aerial × Vegetative	9.00b	5.00b	7.33b
	Aerial × Generative	14.33a	7.67a	11.00a
	Root × Vegetative	4.00d	2.33c	3.78c
	Root × Generative	5.67c	3.00c	4.33c
	SEM	0.289	0.272	0.309
Extract × Stage	Ethanol × Vegetative	11.00c	6.00bc	9.00b
	Ethanol × Generative	15.00a	8.00a	11.50a
	Methanol × Vegetative	8.50d	5.00c	7.67c
	Methanol × Generative	13.00b	7.00b	10.00ab
	Water × Vegetative	0.00f	0.00d	0.00d
	Water × Generative	2.00e	1.00d	1.50d
	SEM	0.353	0.333	0.379
Organ × Extract × Stage	Aerial × Ethanol × Vegetative	16.00c	8.00b	12.00c
	Aerial × Ethanol × Generative	21.00a	11.00a	16.00a
	Aerial × Methanol × Vegetative	11.00d	7.00b	10.00d
	Aerial × Methanol × Vegetative	18.00b	10.00d	14.00b
	Aerial × Water × Vegetative	0.00i	0.00h	0.00h
	Aerial × Water × Generative	4.00g	3.00f	3.00f
	Root × Ethanol × Vegetative	6.00h	6.00e	6.00e
	Root × Ethanol × Generative	9.00e	7.00e	7.00e
	Root × Methanol × Vegetative	6.00h	5.33e	5.33e
	Root × Methanol × Generative	8.00f	6.00e	6.00e
	Root × Water × Vegetative	0.00i	0.00h	0.00h
	Root × Water × Generative	0.00i	0.00h	0.00h
	SEM	0.500	0.536	0.536

¶ Different letters in above of means in any column represent significant statistical difference ($p \leq 0.05$)

Table 2. Comparison of antibacterial activity the extracts of *Stachys lavandulifolia* with standard antibiotic against the studied bacteria

Treatments	***S. aureus* ATCC 6538**	***E. coli* ATCC 8739**	***P. aeruginosa* ATCC 9027**
Aerial × Ethanol × Vegetative	2.00ns	0.00ns	0.00ns
Aerial × Ethanol × Generative	7.00*	3.00ns	4.00*
Aerial × Methanol × Vegetative	-3.00*	-1.00ns	-2.00ns
Aerial × Methanol × Vegetative	4.00*	2.00ns	2.00ns
Aerial × Water × Vegetative	-14.00*	-8.00*	-12.00*
Aerial × Water × Generative	-10.00*	-6.00*	-9.00*
Root × Ethanol × Vegetative	-8.00*	-4.00*	-6.00*
Root × Ethanol × Generative	-5.00*	-3.00*	-5.00*
Root × Methanol × Vegetative	-8.00*	-5.00*	-6.67*
Root × Methanol × Generative	-6.00*	-4.00*	-6.00*
Root × Water × Vegetative	-14.00*	-8.00*	-12.00*
Root × Water × Generative	-14.00*	-8.00*	-12.00*

Table 3. Comparison of the ethanolic extract of *Stachys lavandulifolia* Vahl and standard antibiotic against standard strains of bacteria by *t*-student test.

Treatment	***S.aureus* ATCC 6538**	***E.coli* ATCC 8739**	***P. aeruginosa* ATCC 9027**
Ampicilin	9.33	115.33	4.67
Ethanolic extract	18.67	271.33	37.33
t-value	1.18	1.09	2.30
p-value	0.302	0.338	0.145

Results of this study indicated that the ethanol was the best solvent to extract of the plant. Indeed, our findings were in consistent with findings of other studies concerning the antimicrobial effects of ethanolic extracts (Jonathan and Fasidi, 2003; Saeedi *et al*., 2008). It is worth noting that the slight variations between our findings and other researchers might be due to several reasons such as intraspecific variation for producing secondary metabolites and differences in extracted protocols that were used for discovering the active metabolites and even differences in evaluation methods (Dugler and Aki, 2009).

4. Conclusion

The findings of this research indicated that the extracts of *Stachys lavandulifolia* Vahl contain some compounds with antibacterial properties that can be used as an herbal drug for treatment of infectious diseases. Obviously, most species of *Stachys* genus were particularly native and were traditionally used by local people in different regions of Iran. However, little evidence is available about the application of such plants. As a result, ethno-botanical studies in various parts of Iran are essential for recording all traditional medicinal knowledge that was experienced among local people.

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