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An Ethnomedicinal Plant: Antibacterial Activities of Stachys Lavandulifolia Vahl Aqueous Extract against Common Pathogens

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Abstract

Enhancing microbial resistance to antibiotics and their probable side effects leads to the popularity of medicinal plants, so the need for novel antibacterial compounds with plant origin is felt more than ever. The object of the present study was to assess the antibacterial property of Stachys lavandulifolia Vahl aqueous extract (SLVAE) on Escherichia coli O157:H7 (EC), Pseudomonas aeruginosa (PA), Staphylococcus aureus (SA) and Bacillus subtilis (BS). The aqueous extract was obtained using a rotary evaporator. Agar disk and well diffusion methods were used to investigate the antibacterial property of the SLVAE. In the agar disk diffusion test, distilled water was used as a negative control whereas streptomycin, oxytetracycline, gentamicin, difloxacin, chloramphenicol, ampicillin, and amikacin were used as positive controls. Macro broth tube test was accomplished to specified Minimum Inhibitory Concentration (MIC). Statistical comparison among groups means were done through one-way ANOVA followed by Duncan's post-hoc test. $P \leq 0.01$ was considered as significant. Indeed compared with many standard antibiotics, the extract showed the higher antibacterial property. Also SLVAE with 125, 15/62 and 7/81 mg/mL concentrations has prevented the growth of EC, SA/BS and PA,

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respectively, and with 125, 62/5 and 15/62 mg/mL concentrations has destroyed EC, SA/BS and PA, respectively (p≤0.01). SLVAE had the most antibacterial activity on PA. In conclusion *the obtained results indicated the antibacterial effect of SLVAE on EC, PA, SA, and BS. It seems that this plant can be used for the treatment of some bacterial infections as an antibiotic.*

How to cite this paper

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Introduction

Medicinal plants as a source of useful chemical compounds received much attention for prevention, control and treatment of many diseases and promoting human health [1,2]. Many plants are used for their antibacterial property [3,4]. Due to recent developments in the plants extraction methodology, medicinal herbs are prepared and examined in different sorts [5]. Types of extraction methods had high effect on the medicinal property of obtained extracts [6]. One of the medicinal plant compound extraction methods is using of distilled water, which the produced extract is called aqueous extract [7,8]. In recent years, interest in aqueous extract has been incremented for pharmacological studies and it appears that the aqueous extract has been useful for prevention, control and inhibition of animal and human bacterial infections [9,10]. The aqueous extracts are effective on a wide range of Gram-negative and positive bacteria such as Escherichia coli O157:H7 (EC), Pseudomonas aeruginosa (PA), Staphylococcus aureus (SA) and Bacillus subtilis (BS) [11-13].

Animal studies of various plant species have generated promising results. For example, Leea macrophylla has healing effects. It increases the synthesis of collagen, stimulates the production of antioxidants, reduces the levels of proinflammatory factors, and improves cell proliferation [14]. Wrightia tinctoria presented healing activity, with an increase in the contraction rate of induced lesions [14]. Pereskia aculeata accelerated the cicatricial process by increasing blood flow and collagen deposition [14c]. An ointment from Struthanthus vulgaris stimulated the closure of lesions, stimulated the formation of granulated tissue, and stimulated the proliferation and organization of collagen fibers [14]. Cynodon dactylon presented antioxidative activity and stimulated collagen formation and healing [14]. Caesalpinia mimosoides stimulated reepithelialization of the epidermal layer and the contraction of lesions [14f].

Iran has a rich flora that are widely distributed throughout the country, particularly in the west. In Iranian traditional medicine, herbal medicines have been the basis of treatment and cure for the bacterial disease [15-17]. A list of medicinal plants in Iran that are consumed for their antibacterial property including Prunus mahaleb, Torilis leptophylla, Quercus brantii, Ziziphus spina-christi, Callistemon citrinus, Plantago ovate, Oliveria decumbens, Teucrium polium, Euphorbia granulata Forssk, Peganum harmala Esfand, Galium tricornutum Jeghjeghak, Galium tricornutum Jeghjeghak, and Vitex pseudo negundo Hendeh bid [18].

One of the most important herbal medicines, which is widely consumed in Iranian traditional medicine for the treatment of bacterial disease is Stachys lavandulifolia Vahl (SLV) [19]. This plant is grown in many parts of Iran, Iraq, Turkey, Syria, Armenia, and Georgia. SLV has been used in folk medicine as an anti-inflammatory and antipyretic supplementary in the treatment of diarrhea and throat infection [20]. It contains many substances (alkanoids, nicotinic acid, organosulfids, polyphenols, saponines, tannins, and steroids) which act together to prevent several maladies such as fungal, viral, parasitic, and bacterial diseases [21-23]. Also fresh and dried areal parts such as flowers, leaves and roots have been used as traditional drugs to treat wounds, bruises, gum inflammations and mouth ulcers [20-22].

The aim of the present study was the evaluation of the effect of the SLV aqueous extract (SLVAE) against common pathogens (EC, PA, SA, and BS) with broth macro-dilution, agar disk and agar well diffusion methods.

Materials and Method

Source of microorganisms

Four bacterial species namely Escherichia coliO157:H7 (ATCC No. 25922), Pseudomonas aeruginosa (PTCC No. 1707), Staphylococcus aureus (ATCC No. 25923) and Bacillus subtilis (ATCC No. 21332) were procured from Iranian Research Organization for Science and Technology as lyophilized. Each bacterial strain was activated on Tryptic Soy broth (Sigma chemical company, German), constant at 37°C for 18 h. Then 60 µL of the broth was transferred to Nutrient agar (Gibco, New York, USA) and incubated at 37°C for 24 h; cell concentration was 108 cfu/mL.

Culture media

Mueller-Hinton agar and broth (Gibco, New York, USA) were prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 22 ml per plate in 12 x 12cm Petri dishes and tubes. Set plates and tubes were incubated overnight to ensure sterility before use.

Plant sample collection

SL was collected in February 2017 from Kermanshah province (in the west of Iran). The plant was identified by the herbarium of the Research Center of Agriculture and Natural Resources of Kermanshah Province, Iran.

Preparation aqueous extract

Plant sample collection and Preparation of aqueous extract

To obtain the aqueous extract of the plant, 250 gr of the dried branches of the SL leaves were poured in a container containing 2000 mL boiled water, and the container lid was tightly closed for 4 h. Then, the container's content was filtered, and the remaining liquid was placed on a bain-marie to evaporate. Finally, a tar-like material was obtained, which was powdered by a freeze dryer [10,11].

Evaluation of the antibacterial activity

Agar disk and well diffusion were used as screen tests to assess antibacterial property of SLVAE based on the standard protocol [24]. In the macro broth dilution test, a solution of the SLVAE was yielded in 1000 mg/ml from which ten-fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After 24 hours of incubation, the diameters of growth inhibition zones around the disks and wells were measured. Distilled water was used as a negative control whereas streptomycin (10 mg), oxytetracycline (30), gentamicin (10), difloxacin (30), chloramphenicol (30), ampicillin (10) and amikacin (25) were used as positive controls. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) were specified by macro broth dilution assay based on the Clinical Laboratory Standard Institute (CLSI) guidelines [24].

Statistical Analysis

Data are expressed as mean and standard deviation. Statistical comparison between group means were made through one-way ANOVA followed by Duncan's post-hoc test. $P \leq 0.01$ was considered significant.

Results and Discussion

Antibiotics make the fundamental basis for the prevention, control and treatment of bacterial diseases. However excessive use of antibiotics has led to the production of multi-drug resistant strains [1]. One way to limit the resistance of pathogenic bacteria species to antibiotic is using of plants [2,3]. Plants as a rich source of medicinal compounds have continued to play a distinguished role in the maintenance of human health against bacterial diseases [4, 5]. SLV as a traditional Iranian plant has been indicated to have some optimal treatment effect, due to its antioxidant and anti-inflammatory activities in both in vitro and in vivo [20]. But, as far as we know, there is little data about the antibacterial property of SLVAE collected from Kermanshah province, west of Iran.

In this study, the inhibitory zone of the SLVAE in agar disk diffusion as compared to standard antibiotics (streptomycin (10), oxytetracycline (30), gentamicin (10), difloxacin (30), chloramphenicol (30), ampicillin (10), and amikacin (25)) are shown in Figures 1-4.

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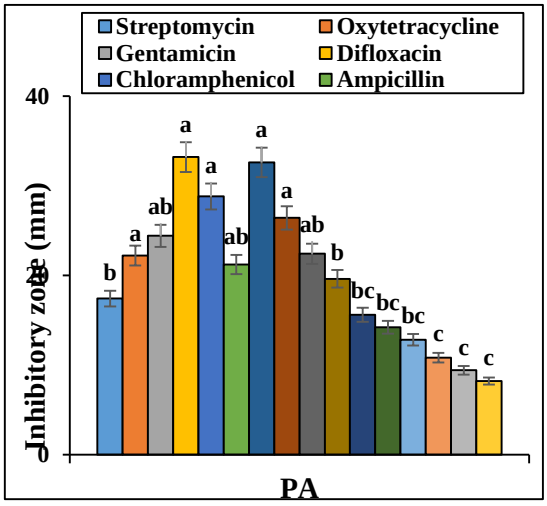

Figure 1. The diameters of growth inhibition zones of EC in agar disk diffusion test in different dilutions of SLVAE (p≤0.01). No inhibitory zone was observed in SLVAE (1.95) and distilled water.

Non-identical letters indicate a significant difference between the groups (p≤0.01).

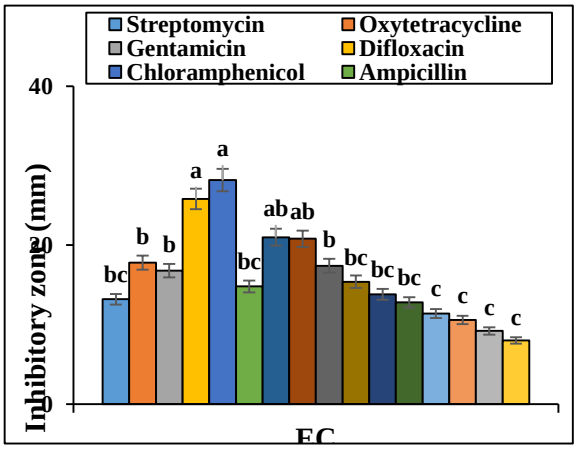

Figure 2. The diameters of growth inhibition zones of PA in agar disk diffusion test in different dilutions of SLVAE (p≤0.01). No inhibitory zone was observed in SLVAE (1.95) and distilled water.

Non-identical letters indicate a significant difference between the groups (p≤0.01).

Non-identical letters indicate a significant difference between the groups (p≤0.01).

The results indicated that the SLVAE exhibited most and least antibacterial activity on tested bacteria with inhibitory zone value ranging from 8 mm (for EC) to 26/4±0.44 mm (against PA), respectively. Also in many levels of confidence there was no significant difference (p≤0.01) between the antibacterial effect of extract of the mentioned plant and tested standard antibiotics on all bacteria. Indeed compared with standard antibiotics (streptomycin (10), oxytetracycline

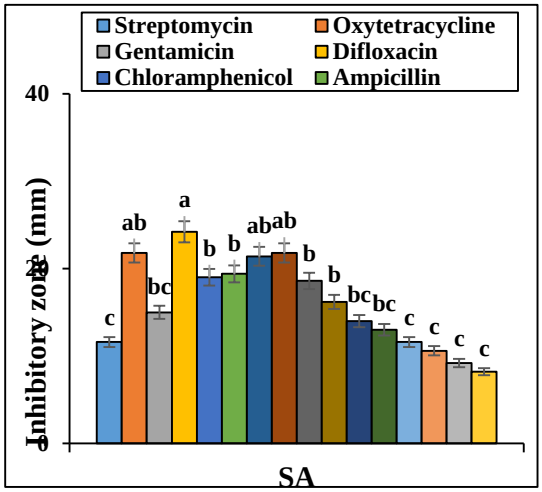

Figure 3. The diameters of growth inhibition zones of SA in agar disk diffusion test in different dilutions of SLVAE (p≤0.01). No inhibitory zone was observed in SLVAE (1.95) and distilled water.

Non-identical letters indicate a significant difference between the groups (p≤0.01).

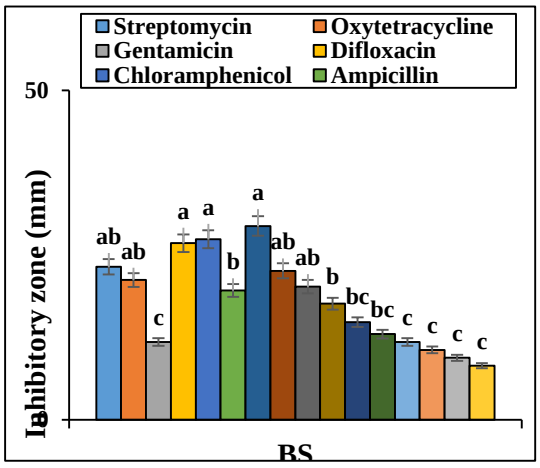

Figure 4. The diameters of growth inhibition zones of BS in agar disk diffusion test in different dilutions of SLVAE (p≤0.01). No inhibitory zone was observed in SLVAE (1.95) and distilled water.

(30), gentamicin (10) and ampicillin (10) (in case of EC), streptomycin (10), oxytetracycline (30), gentamicin (10) and ampicillin (10) (in case of PA), streptomycin (10), gentamicin (10), chloramphenicol (30), ampicillin (10) and amikacin (25) (in case of SA) and oxytetracycline (30), gentamicin (10) and ampicillin (10) (in case of BS)), the extract (1000mg/ml) showed higher antibacterial activity (p≤0.01). Also the widest zone of SLVAE was observed at 1000 mg/ml concentration about PA (p≤0.01). There was no inhibition zone in all of the bacteria due to 1.95 mg/ml

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concentration. No inhibition zone was observed due to distilled water.

About Agar well diffusion test, the widest inhibition zone

Table 1: The diameters of growth inhibition zones of EC, PA, SA and BS in the agar well diffusion test in different dilutions of SLVAE ($p \leq 0.01$).

Microorganism	EC	PA	SA	BS
SLVAE (1000)	18.6 ± 0.54^{ab}	22.8 ± 1^{a}	19.4 ± 0.54^{ab}	20.4 ± 1.3^{a}
SLVAE (500)	16.2 ± 0.89^{b}	20.2 ± 0.89^{a}	17.8 ± 0.54^{ab}	19.2 ± 0.83^{ab}
SLVAE (250)	14.4 ± 0.44^{b}	17.8 ± 0.83^{ab}	15.4 ± 1^{b}	16.6 ± 0.54^{b}
SLVAE (125)	12.8 ± 0.83^{bc}	14.2 ± 0.44^{b}	13.4 ± 0.44^{bc}	14.4 ± 0.89^{b}
SLVAE (62.5)	11 ± 0.44^{bc}	13.6 ± 0.54^{bc}	11.8 ± 0.89^{bc}	$13 \pm 0.44b^{bc}$
SLVAE (31.25)	10.8 ± 0.44^{c}	12 ± 0.54^{bc}	11 ± 0.54^{bc}	11 ± 1^{bc}
SLVAE (15.62)	10 ± 0.54^{c}	10.4 ± 0.83^{c}	10 ± 0.83^{c}	10.4 ± 0.44^{c}
SLVAE (7.81)	8.8 ± 0.83^{c}	8.4 ± 0.44^{c}	8.8 ± 0.44^{c}	9 ± 0.54^{c}
SLVAE (3.9)	8 ± 0.44^{c}	8 ± 0.44^{c}	8.2 ± 0.54^{c}	8.2 ± 0.89^{c}
SLVAE (1.95)	0	0	0	0
Distilled water	0	0	0	0

was observed at 1000 mg/ml concentration about PA ($p \leq 0.01$). Also the inhabitation zone in many of the samples has been increased when the aqueous extract has increased ($p \leq 0.01$). The results of the agar well diffusion test defined that in tested bacteria, there was a considerable discrepancy in terms of sensitivity to SLVAE ($p \leq 0.01$)

No inhibition zone was observed due to distilled water. The details of growth inhibition zones due to different dilutions are listed in Table 1.

As seen above, the most antibacterial effect of the plant extract was shown in the agar disk diffusion method. The agar disk diffusion was described as an assay with relatively high sensitivity. This procedure is one of the most employed to determine the antibacterial activity of SLVAE, because it allows the study of a vast number of samples. In agreement with this study, in various studies have shown that inhibitory zones of EC, PA, SA and BS in agar disk diffusion test is greater than the agar well diffusion method [7-11, 25].

Also, the results of this study show that SLVAE with 125, 15/62 and 7/81 mg/ml concentrations have inhibited EC, SA/BS and PA growth, respectively ($p \leq 0.01$), with 125, 62/5 and 15/62 mg/ml concentrations have eradicated EC, SA/BS and PA, respectively ($p \leq 0.01$) (Table 2).

Table 2: MIC and MBC of SLVAE ($p \leq 0.01$).

Microorganism	EC	PA	SA	BS
MIC (mg/ml)	125^{a}	7.81^{c}	15.62^{b}	15.62^{b}
MBC (mg/ml)	125^{a}	15.62^{c}	62.5^{b}	62.5^{b}

Thus, the results represent the strong antibacterial property of the medical herb on EC, PA, SA and BS. Moreover SLVAE had the most antibacterial effect on PA. Probably, some components in the extracts, including phenols, flavonoids, monoterpenes (β- pinen, β-phellandrene, α-pinene, myrcene, and β-ocimene) and sesquiterpenes (spathulenol and germacrene-D) are caused to antimicrobial effect in SL [26]. The phytochemical analysis of SL has proved

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the presence of some secondary metabolites such as diterpenes, flavonoids, phenyl ethanoidglycosides, and saponis [27] in a way that flavonoids might be responsible for antimicrobial property [28]. There are several studies about antibacterial effect of SLV. A study demonstrated SLV has a variable antibacterial effect against Gram-negative and positive bacteria for the following reasons: Genetic and ecotypic characteristics, environmental conditions and geographic origin [29]. In the study of Zarali *et al.* indicated the SLV had an inhibitory effect on the SA in 2.3 mg/ml concentration [30]. In other study, Taheri *et al.* revealed that SLV alcoholic extract proved to be effective against Gram-positive bacteria such as SA [31]. Mahzooni-kachapi *et al.* reported the antibacterial effect of SLV oil against Gram-negative and positive bacteria. Also in the previous study indicated SLV oil has a greater effect on Gram-negative bacteria and this is the results of our study [32]. 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 [33].

Conclusion

SLV is an ethnomedicinal plant with antibacterial effect toward EC (ATCC No. 25922), PA (PTCC No. 1707), SA (ATCC No. 25923) and BS (ATCC No. 21332). The growth of EC, PA, SA, and BS was prevented by the examined SLVAE. These results indicate that SLVAE has its chemical composition, which is attributed to its antibacterial activity. So SLV can be used as an antibacterial supplement or drug. It is suggested that in this connection supplementary study should be done on experimental animals' models.

Conflict of interest

None of the authors have any conflict of interest to declare.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

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