Avicenna J Clin Microb Infec. 2017 August; 4(3):e43457. doi: 10.5812/ajcmi.43457.
Published online 2017 May 7. **Research Article**

Preliminary Evaluation of the Antimicrobial Activity of Total Extract and Fractions of Chloroform, Methanol, and Aqueous from the Aerial Parts of *Salvia aegyptiaca*

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Received 2016 October 29; **Revised** 2017 February 22; **Accepted** 2017 March 29.

Abstract

Background: Increased microbial resistance to conventional medicines and their side effects have led to studying the effect of herbal extracts on microorganisms.
Objectives: The current study aimed at evaluating the antimicrobial activity of total extract and fractions of chloroform, methanol, and aqueous of aerial parts of *Salvia aegyptiaca*.
Methods: The plants were collected from Bandar Abbas, Iran, in April 2015. The methanol extract of aerial parts, in addition to chloroform, methanol, and aqueous fractions, were prepared by the maceration method from *S. aegyptiaca*. The antimicrobial activity of fractions were determined by cup plate and further micro-dilution methods based on the Clinical and Laboratory Standards Institute (CLSI) 2013 against some Gram-positive and -negative bacteria as well as *Candida albicans*. Statistical analysis was conducted by t test.
Results: Significant results were obtained regarding the tested microorganisms only by micro-dilution method.
Conclusions: There were no significant results in plant extract and its fractions against the tested microorganisms in the cup plate method, which may be due to the inability of agar diffusion. In contrast, by excluding the agar's hindrance property, verified results of micro-dilution method were deleted.

Keywords: Gram-Positive Bacteria, Gram-Negative Bacteria, Microbial Drug Resistance, *Candida albicans*

1. Background

Due to extending microbial resistance to chemical medicines and also increase of the side effects of the existing antibiotics, studies on herbs to recognize their antimicrobial effects are increasing (1). Iran is one of the few areas with special geographical situation and climate suitable for the growth of medicinal plants used in traditional medicine (2, 3). Many different documents show the antimicrobial activity of medicinal plants or herbs such as garlic as a natural source of healing (4). Due to the adverse side effects of synthetic drugs and emergence of resistant microbes armed with different intrinsic or acquired antibiotic resistance mechanisms, there is a need to find new natural compounds with antimicrobial activity (5). Salvia is one of the medicinal plants with about 900 species in various parts of the world such as Europe, Asia, and America except the cold areas; 56 species of them are reported in Iran (6, 7). The most known *Salvia* species are *S. officinalis*, *S. macro sipho Boiss*, *S. mirzayuni* and *S. aegyptiaca*. Most of the differences between the species are in their growth conditions, morphology, and physical appearance (8). *Salvia aegyptiaca*, is a floral, angiosperm, and dicotyledon plant that pertains to Tubiflorales class, Verbenales (Lamiaceae) phylum, *Labiatae* family and Salvia genus (9). *Salvia aegyptiaca*, is native to Iran and has dispersed in the South of Iran, especially Hormozgan province (9). Based on the documents, this plant is used in traditional medicine to improve nervous disorders, dizziness, and diarrhea (6, 7).

Since other species of *Salvia* previously showed sig-

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nificant antibacterial effects, the current study aimed at evaluating the antimicrobial activity of total extract and the chloroform, methanol, and aqueous fractions of aerial parts of *Salvia aegyptiaca* against some Gram-positive and -negative bacteria and *Candida albicans* by cup plate, and determination of the minimum inhibitory concentration (MIC) with micro-dilution methods for the first time.

2. Methods

2.1. Plant Collection

Salvia aegyptiaca collected from Bandar Abbas (Hormozgan province) in April 2015 and identified by Dr. J. Asgarpanah. Aerial parts of the plant were washed and dried for 14 days at room temperature under the shade and kept away from direct sunlight and moisture. Then, dried plants were powdered by electric mill and stored in a dark, caped, and waterproof glass jar (2).

2.2. Extraction by Maceration

Extraction was done by maceration method and using a percolator apparatus. Following that, 500 g of plant's powder weighed by a digital scale to the nearest 0.01 g and extraction was done by methanol solvent. After 4 or 5 days, the extract was collected in a glass container. This procedure was repeated 3 times. Finally, the extracts were pooled and concentrated using a rotary evaporator. The concentrated extract was poured into a container and kept away from light and heat to dry completely. Finally 60 g extract was obtained from 500 g of powdered plant and more than half of it was kept for the preparation of fractions. Part of the extract was poured into a large beaker and 25 to 30 mL of chloroform solvent was added. Then, all the fractions were concentrated using a rotary evaporator (10).

2.3. Preparation of Extracts

Two grams of the total methanol extract and each of its different fractions were weighed by an analytical scale to the nearest 0.0001 g, and then, solved in 2% dimethyl sulfoxide (DMSO) as solvent and a concentration of 1000 mg/mL of the total extract and fractions were prepared. All of the extracts were filtered by a 0.45 micron syringe filter and further concentrations of 125, 250, and 500 mg/mL were prepared after double dilution.

2.4. Microbial Strains

The microbial strains were tested in the study were *Staphylococcus aureus* (PTCC 1431), *Staphylococcus epidermidis* (PTCC 1435), *Bacillus cereus* (PTCC 1015), *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1430), and *Candida albicans* (PTCC 5027). All were purchased from Iranian research organization for science and technology (IROST).

2.5. Cup Plate Method

Cup plate method was used to study the antibacterial activity of *S. aegyptiaca* extract and the fractions. For this purpose, based on the clinical and laboratory standards institute (CLSI) 2013 (11) protocol, the MHA (Mueller-Hinton agar) plates containing microorganisms were used. The wells with 6 mm diameter were created by a sterile Pasteur pipet. A microbial suspension was prepared from each microorganism with a turbidity of 0.5 McFarland (1.5×10^8 CFU/mL). Each well was filled by 80 μL of prepared total extract and fractions of chloroform, methanol, and aqueous of concentrations of 125 to 1000 mg/mL. Erythromycin and gentamycin as positive controls and 2% DMSO as the negative control were tested, simultaneously. This antimicrobial procedure was repeated 3 times (2, 11).

2.6. Minimum Inhibitory Concentration by Microdilution Method

To determine the MIC of each extract and fractions, micro-dilution method was used based on CLSI 2013 guidelines12. The 96-well micro-plates (Extra Gene-Company-product No. EL-1190-FCS) were used and microbial suspensions with the turbidity of 0.5 McFarland (1.5×10^8 CFU/mL) were prepared. Then, the bacterial suspensions were diluted 1:20 to yield 5×10^6 CFU/mL. When 0.01 mL of this suspension was inoculated into the broth, the final concentration of the microorganism was approximately 5×10^5 CFU/mL. All the microplates were incubated at 37°C for 24 hours. The eerythromycin and gentamycin (Chemie Darou and Alborz Daru, Iran) and 10% DMSO as controls were tested, simultaneously. This antimicrobial procedure was repeated 3 times.

2.7. Statistical Analysis

Statistical analysis was performed by SPSS software version 16.1 and the paired t test was conducted for analogy extracts and antibiotic analysis.

3. Results

In the current study, the antimicrobial effects of different concentrations (125 to 1000 mg/mL) of total extract and chloroform, methanol, and aqueous fractions of *S. aegyptiaca* in 2% DMSO solvent were determined against some Gram-positive and -negative bacteria and *Candida albicans* by micro-dilution (MIC) and cup plate methods (Tables 1 - 3). To validate the evaluation of inhibition zone, cup plate method was conducted 3 times.

Table 1. Minimum Inhibitory Concentration of Total Extract and Different Fractions of *Salvia aegyptiaca*

Bacteria	MIC, mg/mL							
	Total extract	Chloroform fraction	Methanol fraction	Aqueous fraction	Gentamicin	Erythromycin	Nystatin	2% DMSO
Escherichia coli	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 1000.0	500.0 ± 0.0	0.0 ± 1000.0	-	-	0.0 ± 0.0
Pseudomonas aeruginosa	0.0 ± 1000.0	0.0 ± 1000.0	500 ± 0.0	500 ± 0.0	0.0 ± 1000.0	-	-	0.0 ± 0.0
Staphylococcus aureus	250.0 ± 0.0	0.0 ± 1000.0	500 ± 0.0	0.0 ± 1000.0	-	0.0 ± 1000.0	_	0.0 ± 0.0
Staphylococcus epidermidis	0.0 ± 0.0	0.0 ± 0.0	500 ± 0.0	0.0 ± 1000.0	-	0.0 ± 1000.0	-	0.0 ± 0.0
Bacillus cereus	0.0 ± 0.0	0.0 ± 1000.0	125.0 ± 0.0	0.0 ± 1000.0	-	0.0 ± 1000.0	-	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	0.0 ± 1000.0	0.0 ± 0.0	0.0 ± 0.0	-	0.0 ± 250.0	0.0 ± 1000.0	0.0 ± 0.0

Table 2. Mean ± Standard Deviation of Effect of *Salvia aegyptiaca* Total Extract by the Cup Plate

Bacteria	Total Extract (Concentrations), mg/mL				Gentamicin	Erythromycin	Nystatin	2% DMSO
	1000	500	250	125				
Escherichia coli	5.3 ± 5.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.66 ± 38.33	-	-	0.0 ± 0.0
Pseudomonas aeruginosa	0.88 ± 11.33	1.45 ± 11.33	4.67 ± 4.67	3.33 ± 3.33	0.0 ± 40.0	-	-	0.0 ± 0.0
Staphylococcus aureus	11.67 ± 0.88	6.67 ± 3.33	0.0 ± 0.0	0.0 ± 0.0	-	35.0 ± 0.0	-	0.0 ± 0.0
Staphylococcus epidermidis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	35.0 ± 0.0	-	0.0 ± 0.0
Bacillus cereus	10.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	43.0 ± 0.0	-	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	-	35.0 ± 0.0	0.0 ± 0.0

Table 3. Mean ± Standard Deviation of Effect of *Salvia aegyptiaca* Different Fractions in the Cup Plate Method

Bacteria	Tested Extracts and Antibiotics															
	Chloroform fraction, mg/mL				Methanol extract, mg/mL				Aqueous extract, mg/mL				Gentamycin	Erythromycin	Nystatin	2% DMSO
	1000	500	250	125	1000	500	250	125	1000	500	250	125				
Escherichia coli	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	38.33 ± 1.66			0.0 ± 0.0
Pseudomonas aeruginosa	17.33 ± 1.45	17.33 ± 1.86	16.33 ± 2.96	15.0 ± 3.61	10.67 ± 0.67	11.3 ± 0.67	10.33 ± 0.33	10.67 ± 0.67	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	40.0 ± 0.0	-	-	0.0 ± 0.0
Staphylococcus aureus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 0.0	3.3 ± 3.3	0.0 ± 0.0	0.0 ± 0.0	-	35.0 ± 0.0	-	0.0 ± 0.0
Staphylococcus epidermidis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0					-	30.0 ± 2.0	-	0.0 ± 0.0
Bacillus cereus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.67 ± 2.6	6.67 ± 0.33	8.0 ± 0.0	10.33 ± 1.2	11.33 ± 0.67	11.00 ± 0.0	12.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	40.0 ± 1.50	-	0.0 ± 0.0
Candida albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	-	35.00 ± 0.0	0.0 ± 0.0

4. Discussion

Salvia aegyptiaca is one of the native plants in Iran that its antibacterial effects are not studied yet (12). The current study determined the antimicrobial effect of different concentrations (125 to 1000 mg/mL) of total extract and chloroform, methanol, and aqueous fractions of *S. aegyptiaca* fractions in DMSO10% solvent against some Gram-positive and -negative bacteria and *Candida albicans* by cup plate and micro-dilution methods.

In the study by Farjam et al., the antimicrobial effect

of *S. aegyptiaca* oil was evaluated by determining MIC using the agar dilution method (9). They showed that the MIC ranged from 64 to 256 μg/mL and the best results were against *Klebsiella oxytoca* (64 μg/mL), followed by *S. aureus* and *Fusarium solani* (128 μg/mL) and *Aspergillus niger* and *A. alternaria* (256 μg/mL). However, there were differences among the studies by Farjam et al. and the current study regarding the method of MIC determination (agar vs. broth dilution) and also evaluation of oil in the Farjam vs. methanol extract and the fractions of *S. aegyptiaca* in the current study, both had similar antimicrobial effects against some Gram-negative and -positive bacteria and also fungi.

As mentioned in the results, in the cup plate method, there was no inhibition zone for the total extract against *P. aeruginosa*, *S. aureus*, and *B. cereus*, for chloroform fraction against *P. aeruginosa*, for methanol fraction against *B. cereus* and *P. aeruginosa*, and for aqueous fractions against *S. aureus*, and *B. cereus*. In contrast, in microdilution method, significant results were obtained against all tested bacteria and also *Candida albicans*. With regard to no inhibition zone in the cup plate method, it can be concluded that the total extract and its fractions had no good diffusion on Mueller-Hinton agar. Similarly, according to the study by Chang in 2011, to investigate the antimicrobial activity of chitosan, failure diffusion of chitosan extracts on solid media seemed logical. They concluded that in such conditions, a direct collision method should be used (12). Also, other studies evaluated (13-15) the antioxidant property and the components of *Salvia* spp. and *S. aegyptiaca*. Fifteen phenolic compounds were identified by high-performance liquid chromatography (HPLC). Existence of different phenolic compounds in the methanol extract of this plant may be related to its antimicrobial effect detected in the current study.

Finally, it is hoped that by proven non-toxicity of different extracts of *S. aegyptiaca*, the product could be useful as a medicinal plant in one of the pharmaceutical forms in future. Therefore, the following topics are recommend for further studies: a) Evaluation of the antibacterial effect of *S. aegyptiaca* fraction against other microbial strains, b) Identification and purification of the active substances in each extract of the mentioned plant, c) Survey of *S. aegyptiaca* mutagenicity, d) Isolation of other plant extracts, e) Performing in vivo tests in animal models and also cell cultures.

Footnotes

Conflict of Interests: The authors declared no conflict of interest.

Funding/Support: The current study was supported by faculty of pharmacy, pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, IR Iran.

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