Cytotoxicity and antimicrobial activity of *Salvia officinalis L.* flowers

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Abstract:
In this study a comparison of the Cytotoxicity and antimicrobial action of the aqueous and 70% methanol extracts from the flower of the herbal species *Salvia officinalis* L. (Lamiaceae), originating from Sudan was carried out.

Material and Methods:
Aqueous, and aquatic methanolic extracts of *S. officinalis* was investigated for its antimicrobial activity and cytotoxicity using brine shrimps lethality assay.

Results:
The methanol extract was found to contain cardiac glycosides, flavonoids, saponins and alkaloids. It exhibited antibacterial activities against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas fluorescence*. It was also found to be potent against brine shrimps with LC50 value of 55.1- 55.6 ppm. The 70% methanol extract has a stronger antimicrobial activity than the aqueous one.

Conclusion:
This work has revealed further potentials of *S. officinalis* L flowers as an antimicrobial agent, especially against *P. aeruginosa* which is resistant to some antibiotics.

Keywords: *Salvia officinalis* L, Lamiaceae, extracts composition, brine shrimps, antimicrobial..

Salvia is a large and polymorphous genus of the family Lamiaceae, comprising about 900 species with almost cosmopolitan dissemination[1]. The Flora of Serbia comprises 14 species of this genus was investigated earlier[2]. They concluded that the chemical contents are varies depending on the locality, extraction procedures and extracting agents, and especial position among them has the herbal species *S. officinalis*

Some components of *S. officinalis* have antimicrobial activity especially essential oils. Shiota *et al*[3] also reported that Linalyl acetate and terpineol extracted from *S. officinalis* L. have the greatest power of bacterial inhibition. Antifungal action of alpha-bisabolol, farnesol, anethole, carvacrol has been proved before[4]. Salvin from acetone extract of the dried flowers is effective against *Staphylococcus aureus, and C. albicans*[4].

In this work a comparison of antimicrobial activity and cytotoxicity using brine shrimps lethality assay was carried out.

MATERIALS AND METHODS

Plant material

Salvia officinalis L flowers were collected from local market in Khartoum Sudan. The plant was identified by Department of Botany,

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Faculty of Science, University of Khartoum; it was authenticated at Medicinal and Aromatic Plants Research Institute of the National Center for Research, Sudan.

Extraction of plant materials

Plant sample was air-dried and ground. 400 grams of the plant material were macerated with 1000ml in the solvent(s) at room temperature and after 24 h filtered through Whatman number 1 filter paper. The procedure was repeated three times to ensure exhaustive extraction of the plant material. The extracts were concentrated, and the solvent removed by evaporation under reduced pressure in a rotary evaporator, at 40°C. The extracts were further dried by freeze-drying and kept in a freezer, at 4°C, until the time of use[5].

Phytochemical screening

The powder of the plants was screened for the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, lavonoids and phlobatannins according to the methods described elsewhere[5].

Antimicrobial activity test:

Preparation of standard bacterial suspensions:

one-ml aliquots of 24 hours broth cultures of the standard organisms were aseptically distributed on to nutrient agar slopes and incubated at 37°C for 24 hours, the bacterial growth was harvested and washed off with sterile normal saline, to produce a suspension containing about 10^8-10^9 colony-forming units per ml. The suspension was stored at 4°C until the time of use[6].

Preparation of standard fungal suspensions:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for three days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored at 4°C until the time of use[6].

Table1: Phytochemical constituents of the plants extracts.

Botanical name part used (family)	Metabolite								
	1	2	3	4	5	6	7	8	9
Salvia officinalis L. Flower (Lamiaceae)	+	+	+	+	±	+	+	+	+

1= triterpene; 2= alkaloid; 3=flavonoid; 4= tannin; 5=saponin; 6= anthraquinone; 7= cyanogenic glycoside; 8= cardiac glycoside; 9 = Steroidal ring;
(+) = detected; (-) = not detected, and (±) = detected in a very low or fain result

Table2: Antimicrobial activities of the aqueous and 70% methanol extracts of *Salvia officinalis* L flowers against standard microorganisms

Plant extract Species Part used Family name	MIC mg/ml	(%) of yield	EC mgl/l	Microorganism (standard strains) Zone of Inhibition (mm)[1]								
				Bacterial strains							*Fugal strains*	
				S a[2]	*Bs*[3]	*Pa*[4]	*C s*[5]	*E c*[6]	*K p*[7]	*B c*[8]	*C a*[9]	*An*[10]
Salvia officinalis L. (Lamiaceae) Aqueous extract	0.32 ± 0.15	7.5	10	20	-	25	10	24	9	-	-	-
			50	25	-	29	19	29	15	-	-	-
			100	30	18	35	24	35	24	8	-	±
			150	40	29	39	24	42	34	15	18	+
Salvia officinalis L. (Lamiaceae) 70% methanol extract	1.30 ± 0.20	3.3	10	28	-	29	-	29	-	-	-	-
			50	33	7	33	10	35	11	-	-	-
			100	38	10	36	17	39	17	-	-	-
			150	48	10	40	21	48	21	7	17	±

EC= Extract Consentration;**= Minimum inhibitory concentration; 1=values are the mean of four replicates; (+) complete inhibition, (±) medium inhibition and (-)= no inhibition. [2]= *Staphylococcus aureus ;*[3]*= Bacillus stearothermophilus ;*[4]*= Pseudomonas aeruginosa* ; [5]*= Clostridium sporogenes;* [6]*= Escherichia coli;;*[7]*= Klebseilla pneumoniae,* [8]*= Bacillus cereus,* [9]*= Candida alicans*; [10]*=aspergillus niger;*

Antibacterial activities

Antibacterial activity was studied by a disc-diffusion method. Each of the innocula (test organisms) (1 ml) was poured into sterile Petri-dish. A medium (about 40°C) was poured into each of the Petri dishes (20 ml). The medium was left to stand for 5 min to allow it to set. Holes were bored on the media with the aid of a sterile cork borer of 10 mm diameter. The holes were marked, and then different concentrations of the plant extract were pipette into the hole using sterile syringes. Plates were then incubated at 37°C for 24 h. The sensitivities of the test organisms to the plant extracts were indicated by clear zone of inhibition around the holes containing the plant extracts and the diameter of the clear zone was taken as an index of the degree of sensitivity[6].

Minimum inhibitory concentration

The minimum inhibitory concentrations of the plant extracts against the sensitive organisms were determined using the agar disc method. Serial dilutions of the plant extracts were prepared to obtain 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mg/ml. Each of the innocula (1 ml) was poured into each Petri-dish and the agar was later poured and allowed to set. Wells were bored using the sterile 3 mm cork borer. Serial dilutions of the extracts were added into the marked wells. The plates were incubated at 37°C for 24 h. The growth was observed to determine the sensitivity of each organism using clear zones of no microbial growth. The least concentration of the plant extract that had inhibitory effect was taken as the minimum inhibitory concentration (MIC) of that plant extract against such organisms[6].

Brine shrimp lethality test of crude extracts

Seawater was put in a soap case (partitioned into dark and light area). Brine shrimp eggs were added to the dark side and covered. The set up was left in a well lit place for 48 h. The hatched eggs, which swarm to the lit side, were used for the bioassay. 20 mg of each of the extracts was dissolved in 2 ml of sea water. From this solution, 500, 50 and 5 ml were transferred into vials and made up to 5 ml. The corresponding concentrations were 1000, 100 and 10 g/ml, respectively. Ten (10) brine shrimps (nauplii) were transferred into each of these vials using Pasteur pipette. Replicates of each of the dose levels were prepared, using seawater as control Number of survivors, deaths, and nauplii with sluggish movement were recorded, 24 h later. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC_{50}) was determined from the graph[7, 8].

Results:

The results of the preliminary phytochemical screening were shown in table 1, while the antimicrobial activities of the extracts obtained from *S. officinalis* flower were shown on table2. The minimal inhibitory concentrations of the 70% methanol extract of *Salvia officinalis* L. flower were demonstrated on table3. Aqueous and aquatic methanol extracts of *S. officinalis* L flowers were found to be potent against brine shrimps with LC50 value of 55.1 and 55.6 ppm respectively. The brine shrimps lethality was found to be concentration-dependent as in table 4.

Discussion:

Preliminary phytochemical screening as shown in table 1 is in agreement with earlier reports[9, 10]. The methanol extract of *S. officinalis* flower showed significantly higher antibacterial activity compared to the aqueous one and compares well with that of gentamycin and streptomycin. However, it is superior to streptomycin which was not able

to inhibit the growth of *E. coli*. This is in keeping with the literature where some components of *S. officinalis* extracted as Linalyl acetate and terpineol compounds were found to have the greatest power of bacterial inhibition[11], while Salvin from acetone extract of the dried flowers is effective against *S. aureus*[3].

Table 3: Values of minimal inhibitory concentration [MIC] of the 70% methanol extract of *Salvia officinalis* L. flower

Micro-organisms	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*
Flower MIC (µL/mL)	70	60	13	14	35

Table 4: Effects of *Salvia officinalis* L flowers extracts on brine shrimps

Plant name extract	Conc. (µg/ml)	No. of subject	No. of Living*	No. of death*	LD50 (ppm) ±SD
Salvia officinalis L. (Lamiaceae) Aqueous extract	1000	10	4	6	55.1±3.12
	100	10	5	5	
Salvia officinalis L. (Lamiaceae) 70% methanol extract	1000	10	3	7	55.6 ± 3.01
	100	10	4	6	

Values are mean ± SD of 3 replicates.

On the other hand methanol extract at concentration of 150 ml\ l exhibited antifungal activity against *C. albicans*. This result goes with other reports which proved the antifungal activity of *S. officinalis*[4, 12]. Those reports concluded that the antifungal activity of *S. officinalis* is due to the presence of alpha-bisabolol, farnesol, anethole, and carvacrol.

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions[13, 14]. A positive correlation between the antimicrobial activity and the brine shrimps lethality was observed in this study.

Conclusion:

This work has revealed further potentials of *S. officinalis* L flowers as an antimicrobial agent, especially against *P. aeruginosa* which is resistant to some antibiotics. Studies regarding the mode of action for these compounds in the bacterial cell should be done. The high LD50 value in brine shrimps lethality and high antimicrobial activity points to the relative safety of the extract as an antimicrobial agent.

REFERENCES:

1. Hedge I C. Advances in Labiate Science,(R. Harley, T. Reynolsd Eds.), Roy. Bot. Gard., Kew., UK, 1992 ;p. 85- 89.
2. Powis J, McGeer A, Green K et al. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Antimicrob Agents Chemother 2004; 48, 3305-3311.
3. Shiota S, Shimizu M, Mizusima T et al. Phytochemical screening of medicinal plants. *FEMS* Microbial Lett 2000; 185, 135-138.
4. Jean F I, Collin G J, Lord D. Phytochemistry and biological activities of Salvia officinalis. Perfurm Flavor 1992; 17: 35- 36
5. Sofowora A (1993). Phytochemical screening of medicinal plants and traditional medicine in Africa, 2nd Edition. Spectrum Books Ltd Nigeria. pp. 150 – 156.
6. Kavangh, F. (1972). Analytical Microbiology. Kavangh (Ed.) New York and London: Academic press, 1:11-14.
7. Meyer BN, Ferrigini RN, Jacobsen LB et al. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982; 45:31-35.
8. Gupta MP, Monge A, Karitas G et al. Screening of Panamanian medicinal Plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA interaction. Int J Pharmacol 1996; 34: 123-127.
9. Dragant TV, Novicav RM, Risti AV et al. Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L. Serb Chem Soc 2003; *68*(1)17–24
10. Kumiko Horiuchi, Sumiko Shiota, Tsutomu Hatano et al.Antimicrobial Activity of Oleanolic Acid from Salvia officinalis and Related Compounds on Vancomycin-Resistant Enterococci (VRE) Biol. Pharm.Bull 2007; 30(6), 1147- 1149
11. Shimizu M, Shiota S, Mizushima T et al. Antimicrob. Agents Chemother, Antibacterial activity of plant extracts 2001; 45, 3198—3201.
12. Takeuchi K, Tomita H, Fujimoto S et al., Antibacterial activity of plant extracts and phytochemicals, *FEMS* Microbiol Lett 2005; 243, 347—354
13. MacLaughin JL, Chnag CJ, Smith DL. "Bench-Top" Bioassays for the discovery of Bioactive Natural Product: An update (Atta Ur- Rahman Ed), Studies in natural product Chemistry. Elsevier Science Publisher BV Amsterdam. 1991; 9: 101-103.
14. Klare I, Konstabel C, Badstubner D et al. Composition and Antimicrobial Activity of the Essential Oil of *Salvia lanigera*. Int J Food Microbiol 2003; 88, 269—290.