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Antimicrobial Activities of Aqueous and Methanolic Extracts from *Salvia officinalis* and *Salix acmophylla* Used in the treatment of wound infection isolates

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Abstract

The aqueous and methanol extracts of *Salvia officinals* and *Salix acmoplylla* traditionally used for the treatment of infections disease were tested for their active against gram positive and gram negative bacteria isolated from wound infection culture using the broth dilution and disc diffusion melhod. Results of this study revealed the prescence of phytochemical which were active against gram positive and negative bacteria. Methanol extracts of both plants showed the highest activity other the aqueous extract. The minimum inhibitory concentration (MIC) of the aqueous extracts on the test organism was 25- 100 mg\ml,while that of the methanol extract was ranged between 25 -50 mg\ml on the test organisms, the minimum bacterial concentration (MBC) ranging between 25-100 mg\ml for methanol extract , and 25-200 mg\ml for aqueous extracts. The highest activity at 100 and 121 ^{o}C was demonstrated by the methanol extracts of *Salix acmophylla* against *Staphylococcus aureus* and *Klebsiella spp*. While in methanol extracts of *Salvia officinalis* the 45 ^{o}C was the effective temperature. In this study plants extracts against gram negative bacteria showed activity in acidic pH only in contrast of gram positive bacteria which were constant in all plants extract . *Salvia officinalis* contained essential elements at higher levels than *Salix acmoplylla* .Ca and Zn were present of high levels in *Salix acmoplylla* than other. The results of this study suggest the possibility of using the methanolic extracts of these plants in treating diseases caused by the test organisms, especially when prepared at acidic pH.

Introduction

Plants play a vital role in maintaining human health and contribute towards the improvement of human life. They are important components of medicines, cosmetics, dyes,and beverages etc. [1] Although hundreds of plant species wer tested for antimicrobial properties[2].

There are many cases of infection by drug resistant bacteria whereas few drugs are available effective for the treatment of such patients. Thus, it is urgently necessary to discover or develop new drugs that are effective on such drug resistant bacteria. We have been trying to discover novel compounds, such as antimicrobial compounds and inhibitors of drug resistance systems in bacteria, [3] that are effective against multidrug-resistant bacteria. Though *Salvia officinalis* (sage) is known as one of the herbs that has antimicrobial activity, there are few papers that have shown ther antibacterial activity, and have shown anti-fungal, anti-viral properties that make it a useful weapon in combating many illnesses [4]. *Salvia officinalis* is cultivated in several countries mainly to obtain dried leaves to be used as raw material in medicine, perfumery

and food industry [5]. Salvia comprises one of the largest genera of flowering plants in the world with 900 to 950 species occurring worldwide except in Australia[6 and 7].

The European salvias, best known from *Salvia officinalis L*. (common sage), the sage of culinary and herbal uses, also offer several striking ornamental species[8 and 9]. The dried root of Salvia (Dan-Shen in Chinese) is one of the most popular traditional herbal medicines in some Asian countries, and has been used extensively for the treatment of coronary artery diseases angina pectoris, myocardial infarction , cerebrovascular diseases , various types of hepatitis , chronic
renal failure dysmenorrhea, and also to improve microcirculation in human body [10].

An extract from *Salvia officinalis* (Sage) leaves showed antimicrobial activity against vancomycin-resistant enterococci (VRE). We isolated the effective compound and identified it as oleanolic acid, a triterpenoid.. These two compounds also showed antimicrobial activity against *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA)[11]. Willow and "bains," respectively, are common English and vernacular names for a number of sister trees of the genera Salix. These are fast growing and yet medium-sized deciduous trees belonging to the plant family Salicaceae. They are of enormous ecological and economic importance [12]. As per the Unani system of medicine the leaves of willows give "cold dry" effect while the flowers display "cold wet" effect. Sleeping on a bed of willow leaves is beneficial in treating heart problems and body pain. A squash of fresh leaves is believed to control dysentery, earache, worms, etc. Inhaling the aroma of fresh flowers of willow relieves headache and mental tension. A distillate extract of willow flowers is much more effective in relieving the above ailments.[13]. The goal of this investigation was to discover plant products that inhibit micro-organisms, especially that causes wound infection.

Materials and Methods

Collection of plant samples

The medicinal plants used for the experiment were identified according to various literatures, and including other pertinent taxonomic literature .Collected plants were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form. Clean and dry separating funnel was taken.

Test microorganisms

Bacterial species *Shigella dysenteriae ; Aeromonas hydrophila* ; *Escherichia coli* ; *Enterobacter spp* ; *Klebsiella spp* ; *Pseudomonas aeruginosa* and *Staphylococcus aureus* were all obtained from the student laboratory in Mustansiryah University.

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at +4°C. Cell suspensions were prepared by inoculation of each bacteria into 10 ml of Nutrient broth. Incubation was performed at 37°C for 24 h. On the next day Mueller-Hinton Agar (MHA) was prepared and cooled to 45°C. Bacterial suspension was added into MHA to give a final concentration of 10^7 bacteria/ml and plated out.

Phytochemical screening

The two plant extracts were screened for phytochemical constituents by using standard procedures of analysis [14 and 15].

Antibacterial activity

The plate-hole diffusion assay as described by [16] was used to determine the growth inhibition of bacteria by the plant extract. The isolated bacteria from wound infection were obtained. The tests were carried out by using a stock concentration of 500mg/ml prepared by dissolving 1g of the methanol extract (MTE) and aquatic extract into 2ml of distilled water. Nutrient agar was prepared and 25ml each was poured into sterile petri dish. This was allowed to solidify and dry. Using a sterile cock-borer of 9mm diameter three equi-distant holes per plate were made in the set agar and were inoculated with 0.5ml over night suspension of the bacteria. Thereafter, the wells (holes) were filled with the extract solution at varying concentrations of 500mg/ml, 400mg/ml and 300mg/ml respectively. This was done in triplicate and the plates were incubated at 37ºC for 18hours. The antibacterial activities were observed and measured by using a transparent meter rule and recorded if the zone of inhibition was ‹10mm [17].

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The Reuben *et al.*[18] was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and serially diluted (two-fold) to a working concentration ranging from 0.780 mg/ml to 200mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37ºC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Minimum Bacterial Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub culturing to antimicrobial free agar as described by Usman *et al.*, [19]In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

The effect of heat and pH on medicinal plant extract

The samples of plant extract (one vial of 100 ml) were provided to determine the effect of heat on it, test samples were heated 45 ºC, 70 ºC, 100 ºC and 121 ºC for 15 min. [20]. To determine the effect of pH, extracts were treated at pH ranges of 3 to 8 using 1 N HCl and 1 N NaOH solutions respectively in series of test tubes for 1 h and then tested for antibacterial activity [21].

Determination of essential elements

Three grams of dried plants were taken and mixed with 8ml of concentrated H_2So_4 (98%) and 2ml of $HClo_3$ (60%) in conical flask for 24 hours which covered by watch class. Then this mixture was left for 6 hours at the sand bath at 80C° , until the digestion material was converted to a white powder. Then add 8ml of deionized water to this powder and the trace elements were determined by flame atomic absorption spectrophotometer[22].

Result and Discussion

The result of the Phytochemical screening for *Salvia officinalis* and *Salix acmophylla* showed the same results waich are presented in Table 1. This reveals a moderate concentration of alkaloids,coumarines,cardiacglycosides,ratenges, phenols, flavonoids, saponins, tannins,essensial oil and terpenes some of which chemical compounds have been associated to antibacterial activities and thus have curative properties against pathogens [23] except steroids, no one of both plant extracts contains it. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms [24]. This may therefore explain the demonstration of antimicrobial activity of *Salvia officinalis* and *Salix acmophylla.*

Regression analysis of the relationship between size of inhibition zone (mm) and plant crude extract concentration (Log value) showed that there was a significant correlation between concentrations of tested plant extracts and the mean inhibition zone of pathogenic isolates. The invitro antibacterial activities are shown in Table 2. As is shown, a wide spectrum activity against some of bacterial strains was studied. Amongst the Gram-positive and Gram-negative bacteria,Gram positive bacteria *S. aureus* were inhibited by both plant extracts. Indifference methanol extract of *Salvia officinalis* was more effective in compare is on with the extracts for the same plant ,while in *Salix acmophylla* the cold aqueous extract was possessed antibacterial highly than methanol and hot aqueous extract.All Gram negative bacteria i.e. *E. spp ,S. dysenteriae, A . hydrophila* were found to be resistant to all of the extracts of *Salvia officinalis* , Exceptionally *K .spp, E . coli* and *P. aeruginosa* showed zone of inhibition . Where as all gram negative bacteria i.e. *S. Dysenteriae, A . hydrophila, K . spp , E . coli* and *P . aeruginosa* gave antibacterial activity as zone of inhibition around the extract of *Salvia officinalis*, but only *E . spp* was resistant to all of the extracts preparation. The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative for the presence of broad spectrum antibiotic compounds [25].Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, e.t.c. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria [26].

Out of the two solvents used for extraction, the methanol extracts showed the highest activity against the test organisms, followed by the aqueous extracts(hot & cold). Different solvents waer been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent [24]. Methanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine men use water as their solvent in which the decoctions are prepared.

The minimum inhibitory concentration MIC and minimum bactericidal concentration MBC results are shown in Tables 3,4,5 and 6 respectively. These tables reveal that the ranges of activity for both MIC and MBC are 0.780 to 200mg/ml. The highest MIC and MBC values is an indication that either the plant extracts are less effective on some bacteria or that the organism has the

potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficacy of the plant extracts.

Result of the effect of temperature on the plant extracts showed that various temperature ranges of 45, 70, 100 and 121^{o}C had various effects on the antimicrobial activity of the extracts (Figs 1, 2, 3, 4,5and 6), The highest activity (diameter of zone of inhibition 30 mm) at 100 and 121 ^{o}C was demonstrated by the methanol extracts of *Salix acmophylla* against *S. aureus* and *K.spp* , whether as *A . hydrophila* lose activity at the same temperature. While in methanol extracts of *Salvia officinalis* the 45 ^{o}C was the effective temperature (diameter of zone of inhibition 25 mm).,while *A.. hydrophila* had the constant activity in different used temperatures .As can clearly be seen by these figures, the rest bacteria did not hav response to these temperatures in each methanol,and both of aqueous extracts (no zone of inhibition) .

The activity is slightly increased at acidic pH (3 to 5). While at alkaline pH the activity of the plant extracts is reduced except for *A.. hydrophila* in each of plant exracts (Figs. 7,8,9,10,11and 12). The antibacterial activity of the extracts is slightly increased at acidic pH. Increase in activity of phyotoconstituents in the presence of acidic medium has earlier been reported [27]. The local application of these plants involves the addition of high doses of potash which is a strong basic salt, and for the fact that the activity of the extracts reduced at alkaline pH in this study, it may explain why the plant concoction is taken for a longer period of time before any curative effect is noticed. In this study, it was noticed that gram positive bacteria *S. aureus* gave constant result in all plant extracts. While plant extracts against gram negative bacteria especially *E.coli and E.spp* showed activity in acidic pH only, and this activity was stable in all plant extracts application. As well as lactose fermented bacteria *K.spp* is inhibited in different pH ,but with low inhibition zone, similar to *PS.aeruginosa* .

Ten elements, Ca, Co, Cu, Mn, Fe, K, Na, P, Zn and Pb, were determined in *Salvia officinalis* and *Salix acmophylla* Table 7 . *Salvia officinalis* contained essential elements (Mn, Fe, K,Na,P and Pb,) at higher levels than *Salix acmophylla*. Ca and Zn were present at high levels in *Salix acmophylla* than other. Therefore, it may not produce any health risks for human consumption, if other sources of toxic metal contaminated food are not taken at the same time.

References

1-Khare, C.P. (2004). Encyclopedia of Indian Medicinal Plants, Rational Western Therapy and other Traditional Usage, Botany (Springer Verlag Berlin Meidelberg) . 406-407.

2-Nair, M.G. and Burke, B.A. (1990).Antimicrobial metabolite and related compounds. Journal of Agri- cultural and Food Chemistry 38, 1093-1096.

3-Horiuchi, K.; Shiota, S.; Hatano, T. ; Yoshida, T. ; Kuroda, T. and Tsuchiya, T.(2007). Antimicrobial Activity of Oleanolic Acid from *Salvia officinalis* and Related Compounds on Vancomycin-Resistant Enterococci (VRE). Biol. Pharm. Bull. 30(6) 1147—1149 .

4-Eidi,A. ; A. and, Baharc, M.(2006). Effects of *Salvia officinalis L*. (sage) leaves on memory retention and its interaction with the cholinergic system in rats. Applied and Basic Nutritional Science.22(3): 321-326.

5- Santos,P.C.; Seabra, R.M.; Andrade, P.B.and Fernandes,F.M. (2002). Phenolic antioxidant compounds produced by *in vitro* shoots of sage (*Salvia officinalis L*.). Plant Sci. 162: 981 – 987.

6- Clebsch , B . (1997). A book of salvias: Sages for every garden. Timber. Press,. Portland , OR .

7-Lasseigne ,F.T; Stuart, L.W; Frank A .B. and Thomas, G.R.(2007). Day/Night Temperature Affects Growth and Photosynthesis of CultivatedSalvia Taxa. J. Amer. Soc. Hort. Sci. 132(4):492–500.

8-Reales, A. (2004). Numerical taxonomy study of Salvia sect. Salvia (Labiatae). Bot. J. Linn. Soc. 145:353–371.

9-Kintzios, S. E. (2000). Sage: The Genus Salvia.. CRC Press. pp. 27.

10-Jiang ,R.W.;Lau, K.M.; Hon ,P.M.; Thomas ,C.W.M ; Woo ,K.S.;and Fung ,K.P.(2005).Chemistry and Biological Activities of Caffeic Acid Derivatives from *Salvia Miltiorrhiza*. Crr.Med.Chem.12:237-246.

11-

Kumiko,H.;Sumiko,S.H.;Tsutomu,H.;Takashi,Y.;Teruo,K.;andTomofusa,T.A..(2005).Antimicroi al activity of oleanolic acid from *Salvia officinalis* andrelated compounds on vancomycin resistance entero coocci (VRE).Bio. and pharma.ball.30(6):1147-1149.

12- Argus, G. W.(1997). Infrageneric classification of *Salix* (*Salicaceae*) in the New World', Syst. Bot. Monogr. 52: 121.

13- Noatay,K.L.(2003).The bat wood from cold climes.Agriculture Tribune,Chandigarh ,India.

14- Trease,G.E. and Evans W.C. (2002).Pharmacology.15thg Edn.Saunders Publishers ,London.pp :42-393.

15- Harbome , J. B. (1993) . Phytochemistry .Academic Press, London , pp:89-131.

16-Ogundipe, O.O.; Moody, J.O.; Fakeye, T.O. and Ladip, O.B. (2000). Antimicrobial activity of *Mallotus oppositifolium* extractives. Afr. J. Med. Med. Sci. Vol. 29: 3/4, pp 281-283.

17-Kudi, A.C.; Umoh, J.U.; Eduvic, L.O.and Getu, J. (1999). Screening of some Nigerian Medicinal plants for Antibacterial Activity. J. Ethanopharm. 67:225-228

18-Reuben,K.D; Abdulrahman,F.I.; Akan,J.C.; Usman, H.; Sodipo, O.A. and Egwu, G.O. (2008). Phytochemical Screening and *In Vitro* Antimicrobial Investigation of the Methanolic Extract of *Croton Zambesicus* Muell ARG. Stem Bark. European Journal of Scientific Research,23(1):134-140.

19-Usman, H.; Abdulrahman, F.I. and Ladan ,A.H. (2007). Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris L.* (Zygophylaceae). Growing in Nigeria. Res. J. Bio. Sci. Medwell Journals, 2007 2(3): 244-247.

20-Franz,C.M.;Toit,D.M.;Holy,H.A.;Schillinger,U. and Holzapfel, W.H.(1997). Production of nisinlike bacteriocins by *lactococcus lactis* strains isolated from vegetables. J. Basic. Microbiol. 37:187-196.

21-Doughari, J. H., Pukuma, M. S. and De, N.(2007). Antibacterial effects of *Balanites aegyptiaca* L. Drel and *Moringa oleifera* Lam. on *Salmonella typhi* .African Journal of Biotechnology Vol. 6 (19), pp. 221

22-Beyenbach, K.W.(1990). Transport of magnesium across biological membranes.Magnes Trace Elem.9 :233 –254

23-Nweze, E.T.; Okafor, J.I.and Njoku, O. (2004). Antimicrobial Activities of Methanolic extract of Trumeguineesis (Schumm and Thorn) and Morinda lucinda Benth used in Nigerian Herb. Medicinal Practice. J. Bio. Res. Biotechnol. 2 (1): 34-46.

24-Doughari,J.H. (2006). Antimicrobial Activity of *Tamarindus indica* Linn .Tropical Journal of Pharmaceutical Research; 5 (2): 597-603.

25-Srinivasan, D.; Perumalsamy, L.P.and Nathan, S. S. T(2001).Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm; 94: 217-222

26-Bushra, N.R. and. Ganga,T. D.(2003). Antibacterial activity of selected Seaweeds from Kovalam south West coast of India. Asian. J. of Microbiol. Biotech. Env. Sc. 5(3): 319-322

27-Molan, P.C.(1992). The antibacterial activity of honey . The nature of antibacterial activity. Bee World. 73: 59 – 76

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Table (1) Phytochemical screening of Methanol, Hot water and Cold water extract of *Salvia officinalis and* extract of *Salix acmophylla*

Number	Constituents	Methanol extract		Hot water extract		Cold water extract	
		So	Sa	So	Sa	So	Sa
1	**Alkaloids**						
	i.Dragendorff's test ii.Meyer's test	+	+	+	+	+	+
2	**Phenols**						
		+	+	+	+	+	+
3	**Cardiac glycosides**						
	Killer-killanis test	+	+	+	+	+	+
4	**Flavonoids**						
	i.Shinoda's test ii.$FeCl_3$ test	+	+	+	+	+	+
5	**Saponins**						
	Frothing test	+	+	+	+	+	+
6	**Terpenes**						
	Salkowski test	+	+	+	+	+	+
7	**Steroids**						
	Libarman-Burchard's test	-	-	-	-	-	-
8	**Tanins**						
	i.$FeCl_3$ test ii.Lead acetate test	+	+	+	+	+	+
9	**Ratenges**						
		+	+				
10	**Coumarines**						
		+	+				
11	**Essensial oil**						
		+	+				

So : *Salvia officinalis.*

Sa : Salix acmophylla.

Table 2: Antibacterial Activity of Plant Extracts against Test Organisms

Extract/concentration Mg/ml	**Zone of inhibition (mm)**							
	Co.	P.a.	E.spp	K.spp	A.h.	S.d.	E.c.	S.a.
Methanol Extract of *Salvia officinalis*	500 400 300	25 22 20	- - -	10 9 9	- - -	- - -	30 25 22	30 24 20
Hot aqueous Extract of *Salvia officinalis*	500 400 300	20 20 18	- - -	9 - -	- - -	- - -	28 25 21	12 11 9
Cold aqueous Extract of *Salvia officinalis*	500 400 300	20 20 17	- - -	10 9 -	- - -	- - -	20 15 13	20 15 11
Methanol Extract *Salix cmophylla*	500 400 300	25 20 18	- - -	15 10 10	22 22 20	15 15 10	14 13 10	27 18 16
Hot aqueous *Salix acmophylla*	500 400 300	25 25 21	- - -	11 10 10	21 19 18	12 10 9	14 14 10	20 18 16
Cold aqueous *Salix acmophylla*	500 400 300	25 25 20	- - -	18 15 12	20 20 18	15 15 13	13 13 12	30 29 26
Control(water)	-	-	-	-	-	-	-	-
Control(Methanol)	-	-	-	-	-	-	-	-

Table 3:**Minimum Inhibitory Concentration (MIC) values for Bacterial Isolates Against *Salvia officinalis* extracts**

Bacteria	Extract concentration (mg/ml)																											
	0.780			1.560			3.125			6.25			12.5			25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C
P.a.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	+	+	+	+	+	+	+	+	+
E.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.h.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	+	+	I	+	+	+
S.a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	+	-	-	+	I	-	+	+	-

S.d = *S. dysentriae;* S.a = *S. aureus*; A.h.= *A . hydrophila*; *E.c.= E. coli ;* E.spp.= *Enterobacter spp*: K.spp= *Klebsiella spp:* P.a.=*P . æruginosa*

- = Resistance (growth of bacteria)

+ = Concentrations show no turbidity (inhibition of bacterial growth)

I= least concentration showing no turbidity (MIC)

M=Methanol extract

H= Hot aqueous extract

C= Cold aqueous extract

Table 4: Minimum Bacterial Concentration (MBC) Values for Bacterial Isolates Against *Salvia officinalis* extracts

Bacteria	Extract concentration (mg/ml)																											
	0.780			1.560			3.125			6.25			12.5			25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C
P.a.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	B	+	+	+	+	+	+	+	+	+
E.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.h.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	-	+	+	B
S.a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	-	-	+	-	-	+	B	-	+	+	-

S.d = *S. dysentriae;* S.a = *S. aureus*; A.h.= *A . hydrophila*; *E.c.= E. coli ;* E.spp. = *Enterobacter spp*: K.spp = *Klebsiella spp:* P.a.=*P . aeruginosa*

- = Resistance (growth of bacteria)

+ = Concentrations show no turbidity (inhibition of bacterial growth)

B= = Minimum Bactericidal (MBC)

M=Methanol extract, H= Hot aqueous extract C= Cold aqueous extract

Table 5: Minimum Inhibitory Concentration (MIC) values for Bacterial Isolates Against *Salix acmophylla* extracts

Bacteria	Extract concentration (mg/ml)																											
	0.780			1.560			3.125			6.25			12.5			25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C
P.a.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	+	+	+	+	+	+	+	+	+
E.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.h.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	+	I	I	+	+	+
S.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	+	+	I	+	+	+
S.a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	+	I	I	+	+	+	+

S.d = *S. dysentriae;* S.a = *S. aureus*; A.h= *A . hydrophila*; *E.c.= E. coli ;* E.spp.= *Enterobacter spp*: K.spp= *Klebsiella spp:* P.a.=*P . æruginosa*

- = Resistance (growth of bacteria)

+ = Concentrations show no turbidity (inhibition of bacterial growth)

I= least concentration showing no turbidity (MIC)

M=Methanol extract

H= Hot aqueous extract

C= Cold aqueous extract

Table 6: Minimum Bacterial Concentration (MBC) Values for Bacterial Isolates Against *Salix acmophylla* extracts

Bacteria	Extract concentration (mg/ml)																											
	0.780			1.560			3.125			6.25			12.5			25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C
P.a.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	-	-	+	B	B	+	+	+	+	+	+
E.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.h.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	-	-	+	B	B	+	+	+
S.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	+	+	-	+	+	B
S.a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	B	+	+	+	+	+	+	+

S.d = *S. dysentriae;* S.a = *S. aureus*; A.h= *A . hydrophila*; *E.c.= E. coli ;* E.spp.= *Enterobacter spp*: K.spp= *Klebsiella spp:* P.a.=*P . æruginosa*

+ = Concentrations show no turbidity (inhibition of bacterial growth)

= = Minimum Bactericidal (MBC)

M=Methanol extract

H= Hot aqueous extract

C= Cold aqueous extract

Table 7: Essential elements concentration of *Salvia officinalis* and *Salix acmophylla*

Elements	Concentration	*Salvia officinalis*	*Salix acmophylla*
Pb	ppm	0.6	0.4
Na	ppm	594	594
K	%	1.2	0.44
Ca	%	0.92	1.2
Fe	ppm	700	200
Zn	ppm	63.6	90.6
P	%	0.35	0.13
Mn	ppm	5.7	3.1
Co	ppm	2.5	0.5
Cu	ppm	6.5	3.9

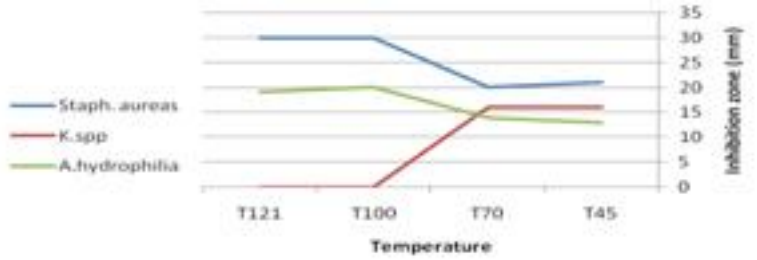

Fig 1. Effects of temperature on antimicrobial activity of Methanol extract *Salix acmophylla*

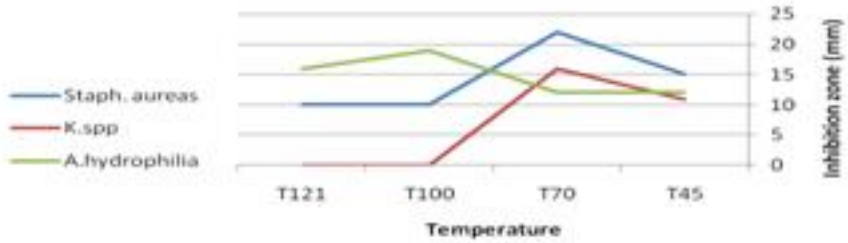

Fig 2. Effect of temperature on antimicrobial activity of Hot aqueous extract *Salix acmophylla*

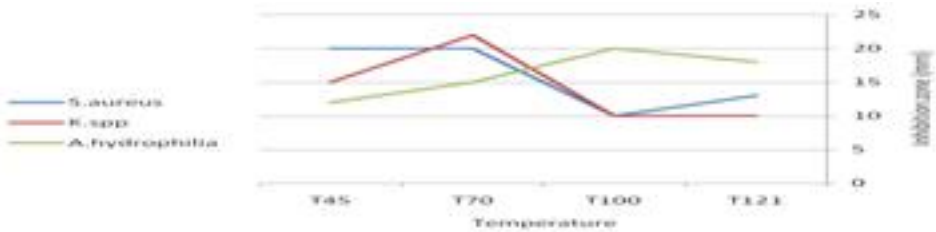

Fig 3. Effect of temperature on antimicrobial activity of Cold aqueous extract *Salix acmophylla*

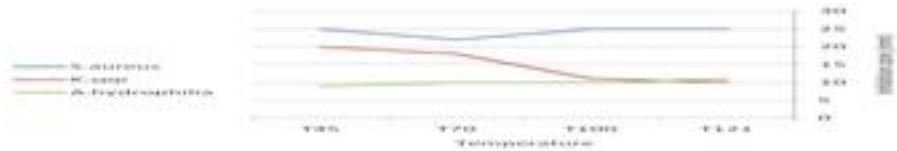

Fig 4. Effects of temperature on antimicrobial activity of Methanol extract *Salvia officinalis*

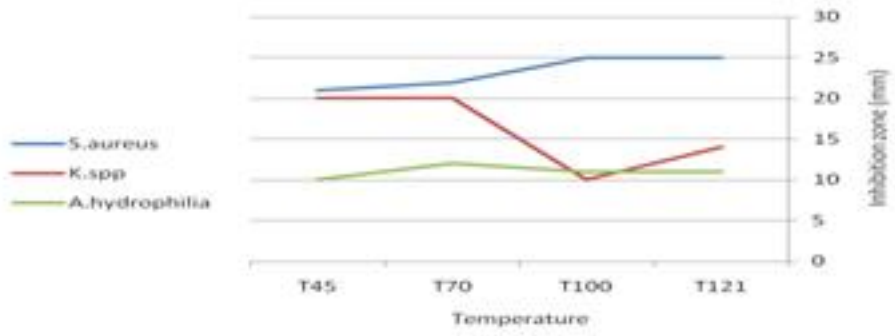

Fig 5. Effect of temperature on antimicrobial activity of Hot aqueous *extract Salvia officinalis*

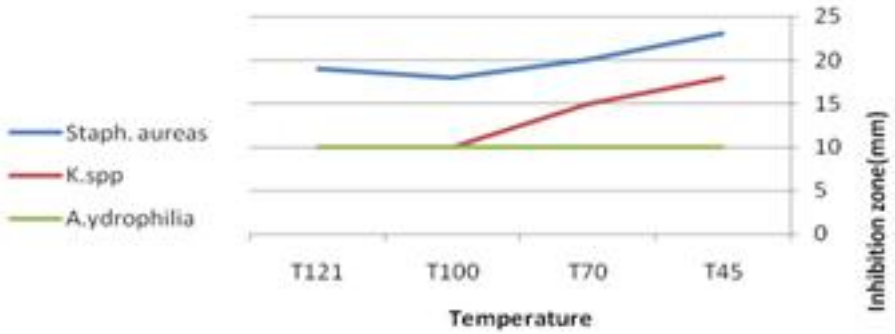

Fig 6. Effect of temperature on antimicrobial activity of Cold aqueous extract *Salvia officinalis*

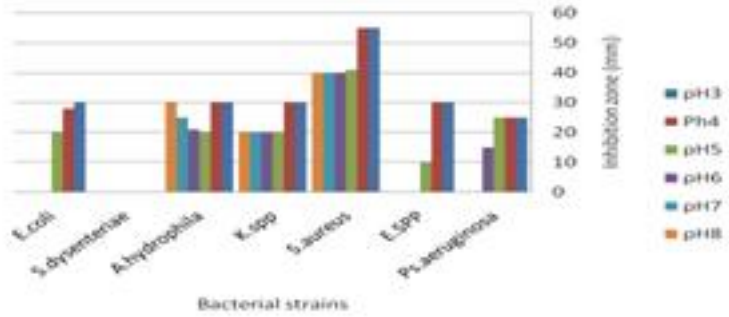

Fig 7. Effects of pH on antimicrobial activity of Methanol extract *Sa Salix acmophylla*

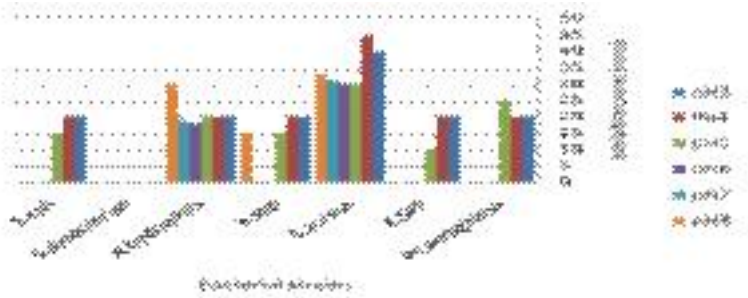

Fig 8. Effect of pH on antimicrobial activity of Hot aqueous extract *Salix acmophylla*

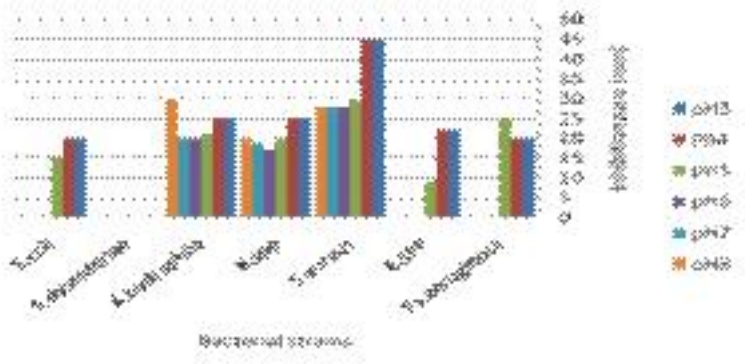

Fig 9. Effect of pH on antimicrobial activity of Cold aqueous extract *Salix acmophylla*

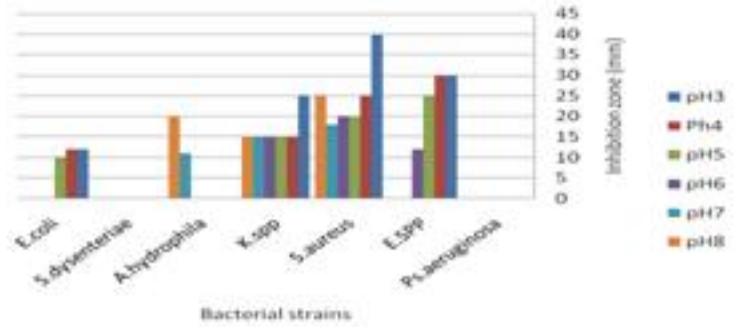

Fig 10. Effects of pH on antimicrobial activity of Methanol extract *Salvia officinalis*

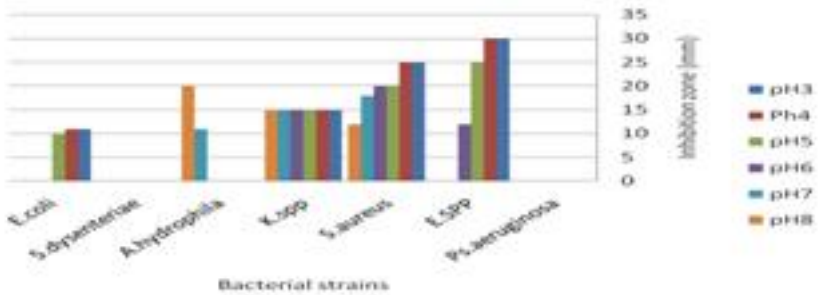

Fig 11. Effect of pH on antimicrobial activity of Hot aqueous extrac*t Salvia officinalis*

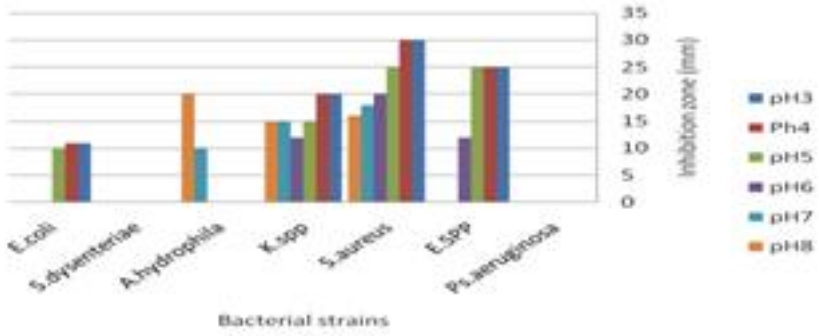

Fig 12. Effect of pH on antimicrobial activity of Cold aqueous extract *Salvia officinal*

الفعالية المضادة للبكتريا للمستخلص المائي والكحولي لنبات الميرمية والصفصاف المستعمله في علاج اخماج الجروح .

منعم رضوان علي ، انمار سعدي عبود

قسم علوم الحياة ، كلية العلوم ، الجامعة المستنصرية

الخلاصة :-

تم في هذه الدراسة استعمال المستخلص المائي والكحولي لنبات الميرمية والصفصاف في علاج الامراض المعدية وذلك باختبار فعاليتها ضد البكتريا الموجبة والسالبة لصبغة كرام المعزولة من اخماج الجروح وذلك باستعمال طريقة الانتشار بالاقراص . اظهرت نتائج الدراسة وجود مركبات كيميائية التي تكون فعالة ضد البكتريا الموجبة والسالبة لصبغة كرام. كما لوحظت فعالية عالية للمستخلص الكحولي لكلا النباتين مقارنة بالمستخلص المائي وقد بلغ التركيز المثبط الادنى للمستخلص المائي تجاه الاحياء المجهرية المختبرة 25 – 100 ملغم / مل، بينما تراوح المستخلص الكحولي من 25 – 50 ملغم / مل وقد بلغ التركز البكتيري الادنى 25 -100 ملغم / مل للمستخلص الكحولي و 200 – 25 ملغم / مل للمستخلص المائي. اظهر المستخلص الكحولي للصفصاف اعلى فعالية عند درجات الحرارة 100، 121 م تجاه بكتريا *S. aureus* و *K .spp* ،بينما كان المستخلص الكحولي للميرمية فعال عند درجة حرارة 45 م. كما لوحظ في هذه الدراسة ان مستخلص النباتين اظهر فعالية ضد البكتريا السالبة لصبغة كرام في الدالة الحامضية وعلى النقيض فان البكتريا الموجبة لصبغة كرام كانت ثابتة في جميع المستخلصات النباتية . كما لوحظ احتواء نبات الميرمية على العناصر الاساسية وبتراكيز عالية مقارنة بنبات الصفصاف في حين لوحظ وجود عنصري الزنك والكالسيوم بمستويات عالية في نبات الصفصاف مقارنة بنبات الميرمية . وقد بينت نتائج هذه الدراسة انه بالامكان استعمال المستخلص الكحولي لكلا النباتين في معالجة الامراض المتسببة بوساطة الجراثيم لاسيما تلك المحضرة في اس هيدروجين حامضي.

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