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Antibacterial activity of *Borago officinalis* extracts on bacteria isolated from burns infected

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Abstract

This study provides a scientific information about the aqueous and methanol extracts of *Borago officinalis* based on its antimicrobial potential againts gram positive and gram-negative bacteria isolated From burns infection using the broth dilution and disc diffusion mehtod. Results of this study indicate the prescence of many phytochemicals which have antimicrobial activity against broad spectrum of bacteria. The methanol extract of *B. officinalis* showed highest activity than aqueous ones. The minimum inhibitory concentration (MIC)of the aqueous extract on the tested organisms was25_100mg/ml while in the methanol extract ranged between 25_50mg/ml on the tested organisms and the minimum bacterial concentration (MBC) of the aqueous extract was 25-200 mg/ml while the methanol extract ranged between 25-100 mg/ml. The highest activity of methanol extract demonstrated at 100 Co, 121 Co against *S.aureus, K.spp, A.hydrophila*, and *S. marcescens*, while there was low activity against *S.dysenteria* and *E. coli*. The activity of plant extract increased at acidic pH5-3 whearus, there are slightly increased of plant extract at alkaline pH 8. *B. officinalis* contained. essential element (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co and Cu) at different concertation. The high preformance liquid chromatograph (HPLC) analysis of *B. officinalis* showed some chemical compounds that have antimicrobial activity against test isolates. The result of this study demonstrate that HPLC analysis of. *B. officinalis* constituent revealed that htis plant have antimicrobial activity against test orgainsm and this may be suggest the use at this extract intreatment of infections disease.

Keywords: Plant Extract; *Borago Officinalis;* HPLC; Antimicrobial Activity

1. Introduction

It is well known that infectious diseases are responsible for a high proportion of health problems, especially in developing countries. The situation has created immense clinical problems for infectious disease treatment. More scientists are in search for new antimicrobial substances derived from plants. Historically, plants provide us with a good source of anti-infective agents [1]. However, an emerging problem associated with misuse of antibiotic therapy is the worldwide emergence of higher level tolerance of target organisms against available broad-spectrum antibiotics. As a result, and in the light of the rapid spread of multidrug resistance, the development of new antimicrobial or antipathogenic agents that act upon new microbial targets has become a very pressing priority [2]. In the traditional systems of medicine, plants are used in the form of crude extracts, infusions and powders to treat common infections without scientific evidence of efficacy [3]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [4]. It is necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases, especially now that there are proposals on the integration of traditional medicine in health care programme in world. In addition, investigations into antimicrobial activities of local medicinal plants will expose the plants as potential sources of therapeutic agents [5]. Madder (*Borago officinalis*) is a plant that historically originated from Ghafghaz and Near East.. Cultivation of madder is prevailed in the world province for dye industry and extracting the drug

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components. Nowadays, industrial color is used instead of madder extracted color [6].The main parts of madder used for mentioned goals are roots and rhizomes, which contain Alizarin, rubestric acid and pourpourines [7]. The red color of madder is due to the Alizarin component Drugs synthesized from products of madder are used as diuretics, laxatives and also to parry the kidney stones.In India, it has been used to redden lips and cheeks. It has a 2000 year history as a medicinal herb in China, India and ancient Greece for breaking kidney stones (it's a diuretic), to promote the flow of menses, cure jaundice and because of its high tannin content, for various intestinal problems. In Europe, it was used to dye urine and bones for medicinal purposes. It is antibiotic and anti-inflammatory [8]. Bao *et al*., (1990) showed certain antibacterial activities compounds were isolated from the roots of *Borago* that is alizarin (I) ,1-hydroxy-2 -methyl-9,10-anthraquinone (II) ,1,3,6-trihydroxy-2-methyl-9,10-anthra-quinone -3-O-(6'-O-acetyl) -alpha- L-rhamnosyl (1----2)-beta-D-glucoside (III),1,3,6- trihydroxy-2-methl -9,10-anthraqueinone-3-O-a lpha- L-rhamnosyl(1----2)-beta-D-glucoside (IV),1,3,6- trihydrozy-2- methyl-9,10- anthraquinone -3-O-(6'-O-acetyl)-beta- D-glucoside (V),2-carbomethyoxy +++-3-prenyl-1,4-naphthohydroquinonedi -beta-D-glucoside (VI) and rubimallin(VII) [9]. A sensitive and reproducible RP-HPLC method was developed for the characterization of madder root and its cell cultures extracts and for the determination of anthraquinone derivatives as glycosides (, lucidin primveroside) and aglycones (alizarin, lucidin, purpurin) in them. The chief components were identified and quantitatively determined. The natural plant and its cell suspension cultures were compared to each other. The preparative fractionation of the extracts was achieved by gel chromatography, HPLC and selective extractions with solvent series, solid-phase extraction techniques [10].

The aim of this study was to determine secondary metabolit of plant products that inhibit micro-organism that was associated with burn infection and analysis of its coustituents by HPLC technique.

Figure 1 *Borago officinalis* plant

2. Materials and Methods

2.1. Collection of plant samples

The medicinal plant in for the experiment was identified according to various literatures, Collected plants from herbous house/ College of Science/ Baghdad university were washed thoroughly and chopped into small pieces shade dried and grinded into powdered form.

2.2. Test microorganisms

Bacterial species *Shigella dysenteriae; Aeromonas hydrophila; Escherichia coli; Klebsiella spp;Serratia marcescens* and *Staphylococcus aureus* were all obtained from Al-Kindi Hospital.

2.3. Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at +4°C. Cell suspansions 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.

2.4. Phytochemical screening

The plant extracts were screened for phytochemical constituents using standard procedures of analysis.These were done at College of Science/ Dept.Biology/ Al-Mustansiryah University [11].

2.5. Antibacterial activity

The plate-hole diffusion assay as described by [12] was used to determine the growth inhibition of bacteria by the plant extract.The isolated bacteria from burn 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 using a transparent meter rule and recorded if the zone of inhibition was ‹10mm [13].

2.6. Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). Reuben et al.[14] 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.

2.7. 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., (2007) 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[15].

2.8. 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. [16]. 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 [17].

2.9. Determination of Essential elements

The work was carried out in the centeral laboratory, College of Science/ Dept.Biology/Baghdad university.Three gram of dried plant 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 left this mixture for 6 hours on the sand bath at 80C° , until the digestion material converted to white powder. Then add 8ml of deionized water to this powder and the trace elements were determined by flame atomic absorption spectrophotometer[18].

2.10. HPLC analysis

The analysis of the sample was performed according to the method of Shalini and Rachana. (2009).The work carried out in Ibn-sina company/Ministery of Industral and Minerals. The HPLC system (Shimadzu LC-10 A, Japan) Reverse-phase chromatographic analysis was carried out in isociratic conditions using a C-18 reverse phase column (250 x 4.6 mm) particle size 5 µm, Luna 5µ C-18 at 30 C°. Running conditions included: injection volume, 5µl; mobile phase, acetic acid 25% ,water 75% , flow rate, 0.4ml/min; and detection at 350 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm) prior to injection in the sample loop.Kampherol and CH33 (2^{-},3^{-},4^{-}–trihydroxy -4 –methoxychalcone) ,Red anthocyanin (3^{-},4^{-},dihydroxy-3,5,7-trihydroxy flavylium chloride) were used as Flavonoids standers.The sampl of plant and standerds determined according to retention times obtained from authentic standerd run at identical condition[19].

3. Results and Discussion

The results of phytochemical screening for *B. officinalis* are shown in Table1 which reveals the presence of Alkaloids, Phenol ,Cardiac glycosides ,Flavonoids , Terpenes, Tanins, Ratenges ,Coumarines,and Essensial oil which were secondary metabolites have been used in traditional Chinese medicine for its antibacterial ,antioxidant and anti-inflammatary activities.[20,21].In other research phytocemical constituents in the roots of Borago was identified with the aid of high-preformance liquid chromatography and liquid chromatography mass spectrometry and by comparsion with authentic standards.[22]. Controlled studies indicate the great potential of phytochemicals to be the richest reservoir of new and novel therapeutics [23]. Although the antimicrobial activities of plant extracts are beyond doubt, in many instances their exact mechanism of antimicrobial functionality is not well understood [24,25]

Table 1 Phytochemical screening of Methanol, Hot and Cold water extract of *B. officinalis*

Number	Constituents	Methanol extract	Hot water extract	Cold water extract
1	Alkaloids			
	i.Dragendorff's test ii.Meyer's test	+	+	+
2	Pheno l			
		+	+	+
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			
		+		

The result of antibacterial activity of plant extract against test organism list in Table2. In this study was correlation between concentration of test plant extract and the inhibition zone of pathogenic isolates. As is shown, the methanol extract of *B. officinalis* was more effective than two aqueous extract (hot and cold) for the same plant ,and the hot aqueous extract of plant was more effective than cold extract. *S.aureus* shwoed zone of inhibition for aqueous and methanol extract. while all gram negative bacteria, *K.spp, A..hydrophila ,S.marcesence, S.dysenteriae* and *E.coli* exhibit difference in zone of inhibition respectively.The result of this study in agreement with other research which showed

that plant extracts with well documented antimicrobial activities could possess antipathogenic as well as antivirulent activities, which may not be linked to the growth and inhibition of the microorganism [26].Al- Fatimi, *et al*., (2007) tudied *In vitro* antimicrobial activity of crude dichloromethane, methanol and aqueous extracts from medicinal plants in Yemeni ethnomedicine and showed good activity against gram positive and negative.[27]. Aysegul, *et al*., (2002) reported that the hydro alcoholic extract of *B. officinalis* has an antioxidant activity and an antimicrobial effect on bacilli, escirichiae and staphylococci [28]. The magnitude of activity varied in terms of the type and number of bacteria and fungi tested and the part of the plant extracted. In addition it is well established that the polarity of these extracts where in our case ethanol is highly polar which probably means getting different profiles in the activity if other extracts of differe polarity were used.[29].

Table 2 Antibacterial Activity of *B. officinalis* Extracts against Test Organisms

Extract/concentration Mg/ml	**Zone of inhibition (mm)**						
	Cone.	*K.spp*	*S. marcescens*	*A.hydrophila.*	*S.dysenteriae*	*E.coli*	*S.aureus*
Methanol Extract	500 400 300	25 22 18	17 15 14	22 20 17	10 9 7	13 11 10	40 35 32
Hot aqueous Extract	500 400 300	19 16 15	15 14 12	20 19 17	10 8 7	11 10 8	38 35 30
Cold aqueous Extrac	500 400 300	16 15 13	14 13 13	14 12 10	8 7 7	9 7 6	35 28 20
Control (water)	-	-	-	-	-	-	-
Control (Methanol)	-	-	-	-	-	-	-

The minimum inhibition concentration MIC and minimum bacterial concentration MBC results are shown in (Table 3,4) respectively. 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 extract. The result of this study was agreement with other research which showed antimicrobial activity of ethanol, methanol, ethyl acetate and water extract of *B. officinalis* by disc diffusion method, from this study it was found that *B. officinalis* revealed antimicrobial activity against some gram positive and gram negative bacteria, yeast, filamentous fungi and actinomycetes [30]. These MIC values for the different bacteria though relatively high, are definitely demonstrative of the potential clinical use [31]. The microorganisms were least sensitive to the aqueous crude extracts due to negligible secondary metabolites in it[32]. Biswas, *et al*., (2004) talk about the use of this extract in the treatment of wounds and Injuries in the traditional medicine of India [33]

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

Bacterial Isolates	**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
K.spp	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	I	+	I	I	+
S. marcescens	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	I	+
A.hydrophila	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	I	+
S.dysenteriae	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	I	_
E.coli	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	_	_

S.aureus	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	I	_	_	+	I	I	I	+	+	+	+	+

- = 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 BacteriaConcentration (MBC) values for Bacterial Isolates Against *B. officinalis* extracts

Bacteria Isolates	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
K.spp	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	B	B	B	+	+	+	+	+	+
S. marcescens	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	B	B	B	+	+	+
A.hydrophila	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	B	+	+	B	B	+	+	+	+
S.dysenteriae	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
E.coli	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
S.aureus	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	B	B	B	+	+	+	+	+	+	+	+

- = 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

Results of the effect of temperature on the plant extracts showed that various temperatures ranging from 45 C°, 70 C° , 100 C°and 121 C° had various effect on the antimicrobial activity of the extracts (fig 1,2,3) The highest activity of methanol extract at 100 C° ,121 C° *against S.aureus, K.spp. A..hydrophila*, and *S.marcesencs* respectively while there was low activity against , *S.dyesnteria* and *E.coli* (no zone of inhibition) While aqueous extract of *B. officinalis* revealed low activity than methanol extract and hot aqueous extract use more effective than cold aqueous.

Result of the effect of pH on the plant extract showed that plant extract was action in pH between (6-7), but the activity of plant extracts increased at acidic pH(5-3) , i.e increased in zone of inhibition of isolates at acidic pH. While at pH 8 there was slightly increase in activity.The methanol extract was more effective than hot and cold aqueous extract as shown in (fig 4 ,5,6,). Suleyman and Huseyin.(1999) showed that *B. officinalis* generally grow on loam and clayey-loam, neutral to slightly alkaline soils and the activity of phytoconstiuents of this plant increased in the presence of acidic medium has earlier been reported[34]. The treatment of plant with high temperature could commence to release simple sugars that could be readily utilized in protein synthesis. Release of hormones such as auxins and ethylene, which could increase nucleic acid metabolism and protein synthesis[35].

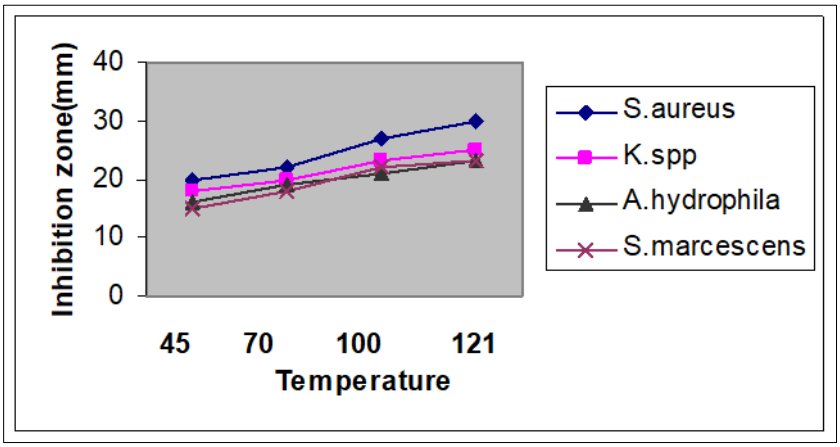

Figure 2 Effects of temperature on antimicrobial activity of Methanol extract *B. officinalis*

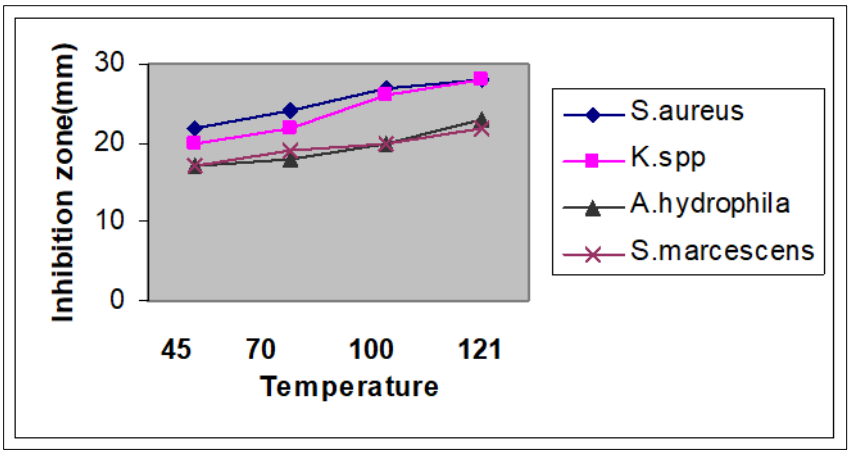

Figure 3 Effect of temperature on antimicrobial activity of Hot aqueous extract *B. officinalis*

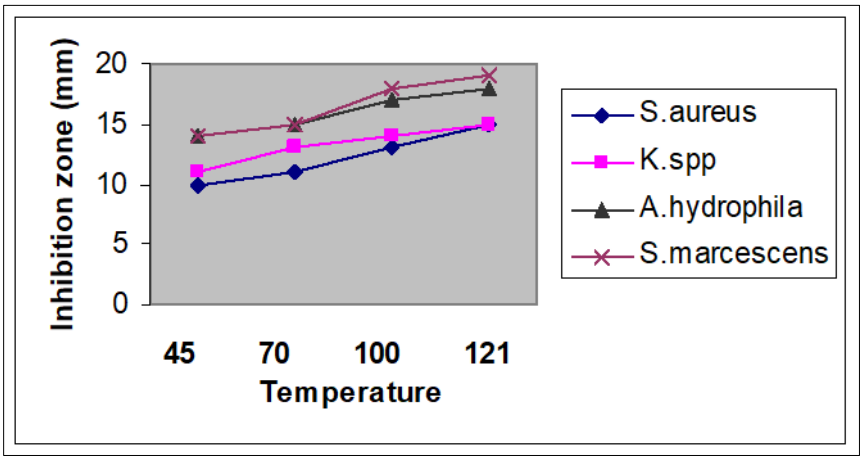

Figure 4 Effect of temperature on antimicrobial activity of Cold aqueous extract *B. officinalis*

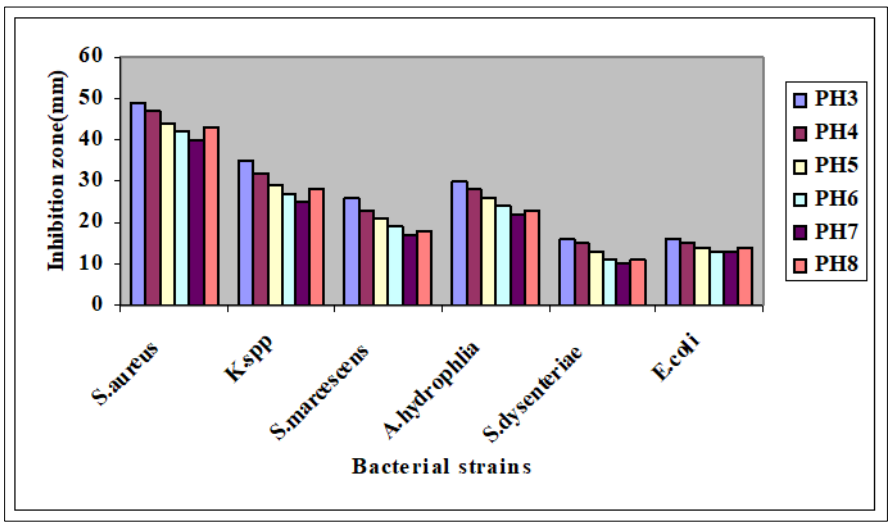

Figure 5 Effects of pH on antimicrobial activity of Methanol extract *B. officinalis*

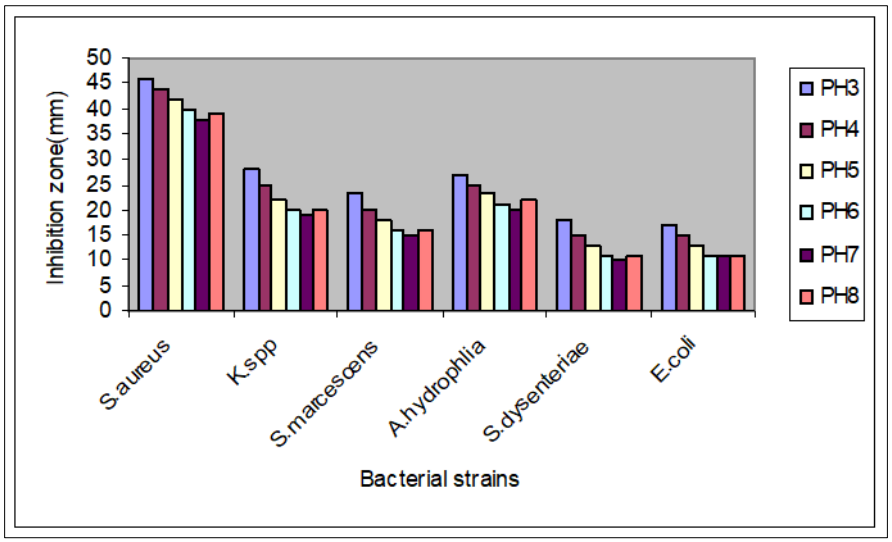

Figure 6 Effect of pH on antimicrobial activity of Hot aqueous extract *B. officinalis*

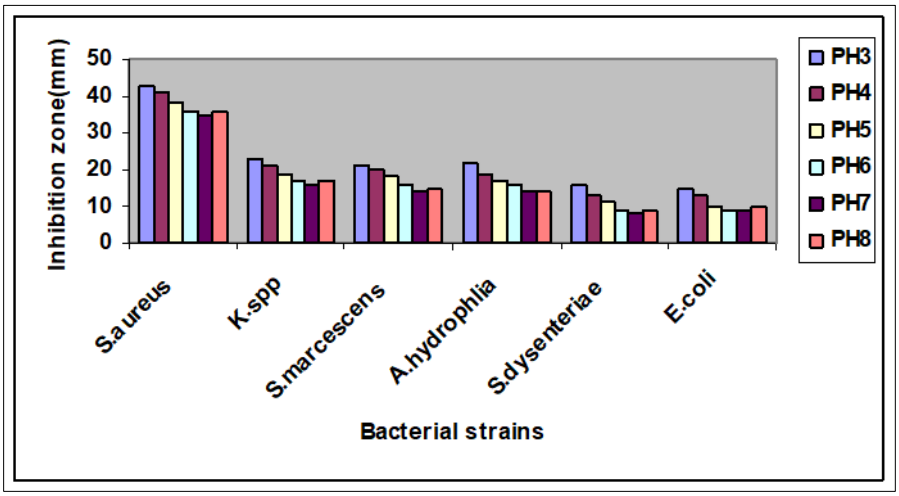

Figure 7 Effect of pH on antimicrobial activity of Cold aqueous extract *B. officinalis*

The results of determined essential elements (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co,and Cu) in *B.officinalis* (Table5) releavel the prescence of this elements at different concentration ,this results agrrement The results of Suleyman and Huseyin.(1999) showed higer concentration of Na, Mn, Zn, and K in *B. officinalis* whereus showed N, P, Ca, at lower concentration. [34].

Table 5 Essential elements concentration of *B. officinalis*

Elements	**Concentration**	***Borago officinalis***
Pb	ppm	0.3
Na	ppm	613
K	%	1.8
Ca	%	0.85
Fe	ppm	620
Zn	ppm	94.2

P	%	0.21
Mn	ppm	6.4
Co	ppm	1.9
Cu	ppm	5.2

The HPLC analysis (fig. 7) of *B. officinalis* shows major peaks at the retention time(min).at a wavelength of 350 nm one of these retention time of this peaks (6.657min) compatibile with two retention time of standared flavonoids with (CH33 AND Campherol).but one of these standared (Anthocyanine) which have peak at the retention time (2.451min) was incompatibile with any one of peaks at the retention time.

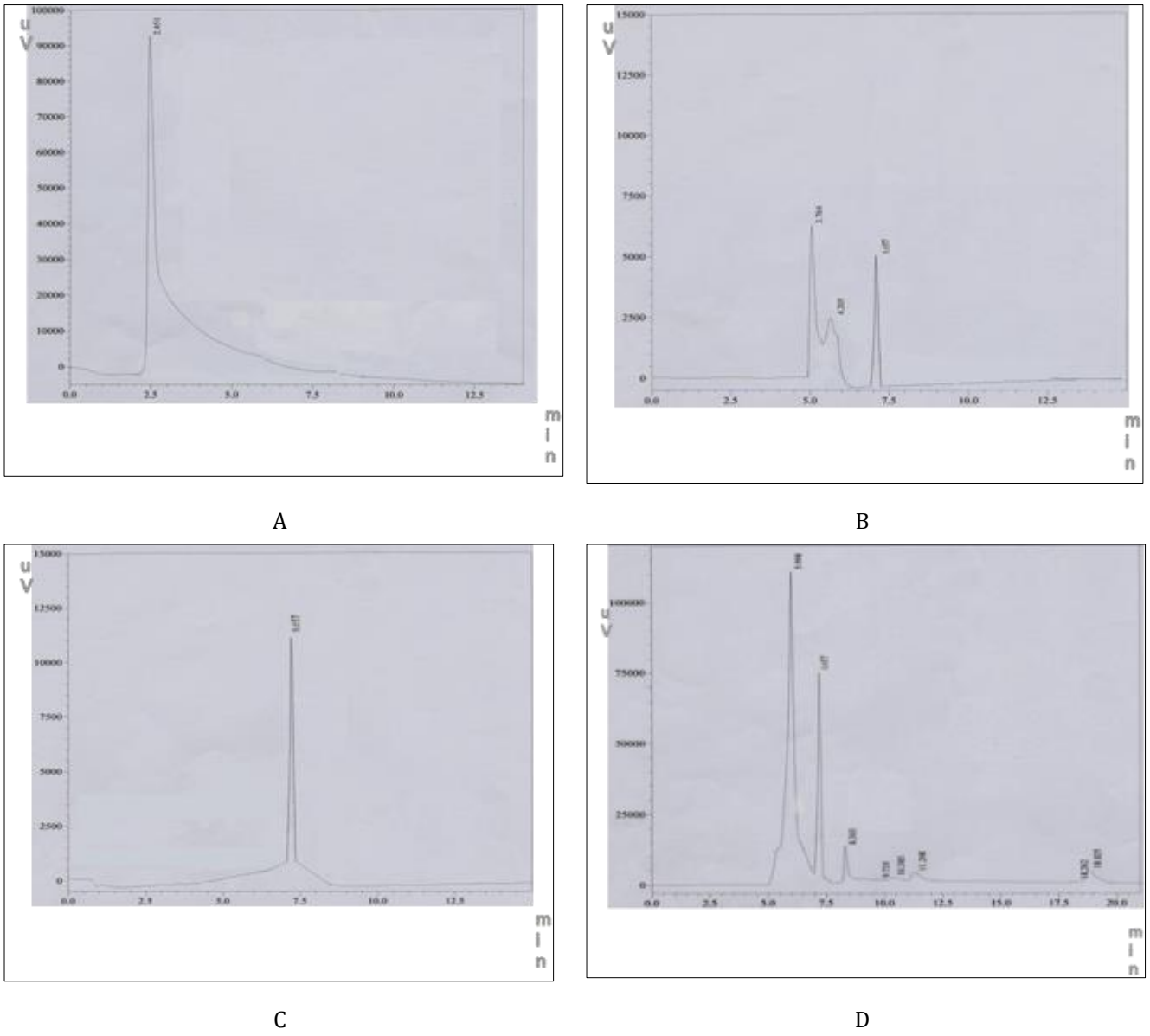

Figure 7 HPLC analysis of *B. officinalis* constituents and the standard a. Anthocyanin b. Kampherol c. CH33 d. Sample plant

This compatibility indicate that the prescence of phytochemical (flavonoids) in this plant which have antimicrobial activity against broad spectrum of microorganism and this agreement with the uses of *B. officinalis* in traditional medicine. HPLC analysis of the plant sample revealed wide variability in their flavonoids content. The constituents of *B. officinalis* called also madder root(pseudopurpurin, purpurin, alizarin, lucidin, munjistin and nordamnacanthal) Was identified and using gas chromatography (GC) ,high-performanace liqud chromatography (HPLC)[36].Yizhong cai *et*

al.,(2004) were identified the phenolic constituents in the root of Borago by (HPLC) and by comparison with authentic standard.A total of 17 hydroxyanthraquinones, gallic acid tannins were separated , and 14 of them were identified. Hydroxyanthraquinones and tannins and gallic acid were the predominant antioxidant phenolic constituents in the roots of Borago.[22].

4. Conclusion

Results of this study demonstrate that HPLC analysis of *B. officinalis* constituent revealed that this plant has antimicrobial activity against test orgainsms and this may be suggest the use at this extract in treatment of infections disease.

Compliance with ethical standards

Disclosure of conflict of interest

Authors confirm no conflict of interest to be disclosed.

References

[1] Priti, M. and Anil, K. "Antimicrobial activity of *Abelmoschus moschatus* leaf extracts" Curr. Tren. in Biotech. and Pharm. 3 (3), 2009, 260 – 266.

[2] Coates, A.; Hu,Y.; Bax, R.and Page, C. "The future challenges facing the development of a new antimicrobial drugs". Nat. Rev. Drug Discov. 1, 2002, 895-910.

[3] Recio, M.C. "A review of some antimicrobial compounds isolated from medicinal plants reported in the literature". Phytother. Res., 3, 1989,117 -125

[4] Cowan, M. M." Plant products as antimicrobial agents.Clin.Microbiol".Rev.12,1999,564– 582.

[5] Ebena, R. V.; Madunaju, R. E.; Ekpe, E. D. and Itugu ,I. " Microbiological exploitation of Cardiac-glycosides and alkaloid from *Garcina kola, Bonveria ocymoides, Kola nitida and Citrus aurantifolia*". J. Appl. Bacteriol.. 71,1991, 398 – 401.

[6] Tripathi, Y.B. and Sharma, M"Comparison of the antioxidant action of the alcoholic extract of *Borago officinalis* ". Indian J. Biochem. Biophys., 35 (5), 1998,313-316.

[7] Sedigheh, S. ; Zoheir , Y. A.; Fakhr ,T.M. and Hassan ,M. A." Study methods of dormancy breaking and germination of common madder (*Borago officinalis*) seed in laboratory conditions". Bot. Res. Inter. 2 (1), 2009, 07-10.

[8] Boldizsar, I. Z. ; Szucs, Z. F. and Molnar-Perl, I." Identification and quantification of the constituents of madder root by gas chromatography and high-performance liquid chromatography". J. Chromatogr A,10; 1133 (1-2), 2006, 259-274.

[9] Bao ,Y.X. ; Wang , S.X. ; Wu, L.J. and Li ,X. Z." Studies on antibacterial constituents from the roots of Borago officinalis ". Chinese.Qiao. 25(11) ,1990,834-839.

[10] Krizs ,K. ; Gy , S. ; Toth , Z.A. ; Holl , F. and Khlafulla , A. "HPLC analysis of anthraquinone derivatives in madder root (*Borago officinalis*) and its cell cultures". Journ.of Liqu. Chromato. & Rel.Techno. C:\Users\suvi\rubia2\title~db=all~content=t713597273~tab=issueslist~branches=19 - v1919 (14) ,1996,2295 - 2314.

[11] Trease,G.E. and Evans W.C.Pharmacology.15thg Edn.Saunders Publishers ,London,2002,pp :42-393.

[12] Ogundipe, O.O., J.O. Moody, T.O. Fakeye and O.B. Ladip, " Antimicrobial activity of Mallotus oppositifolium extractives". Afr. J. Med. Med. Sci. Vol. 29: 3/4, 2000, pp 281-283.

[13] Kudi, A.C., Umoh, J.U., Eduvic, L.O.and Getu, J. "Screening of some Nigerian Medicinal plants for Antibacterial Activity". J. Ethanopharm. 67,1999,225-228.

[14] Reuben,K.D; Abdulrahman,F.I.; Akan,J.C.; Usman, H.; Sodipo, O.A. and Egwu, G.O. "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), 2008,134-140.

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

[16] Franz,C.M., Du Toit ,M., von Holy, A., Schillinger, U.and Holzapfel ,W.H."Production of nisinlike bacteriocins by lactococcus lactis strains isolated from vegetables". J Basic Microbiol;37,1997,187-196.

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

[18] Beyenbach, K.W."Transport of magnesium across biological membranes.Magnes". Trace Elem.9,1990, 233 –254

[19] Shalini and Rachana , S. " Antifungal activity screening and HPLC analysis of crude extract from Tectona grandis, Shilajit, Valeriana wallachi.".EJEAFChe, 8 (4) ,2009, 218-229.

[20] Singh , R.G. and Chauhan , S.M. "Anthraquinones and other biologically active compounds from the genus Rubia". Chem.Biodivers. 1(9), 2004,1241-1264

[21] Lu ,Y.; Liu , R. ; Sun , C. and Pan , Y. "An effective high-speed countercurrent chromatographic method for preparative isolation and purification of mollugin directly from the ethanol extract of the Chinese medicinal plant Borago officinalis ". J. Sep. Sci. 30(9) ,2007,1313-1317.

[22] Yizhoug , C. ; Mei , S. ; Sie , X. and Harold , C. " Antioxidant phenolic constituents in roots of *Rheum officinale* and *Borago officinalis*: structure-radical scavenging activity relationships". J. Agric. Food.Chem. 52(26) ,2004,7884-7890.

[23] Kumar, V.P.; Chauhan, N.S.and Rajani, H.P.M. "Search for antibacterial and antifungal agents from selected indian medicinal plants". J. Ethnopharm. 107,2006,182-188.

[24] Mahasneh, A.M. "Screening of some indigenous Qatari medicinal plants for antimicrobial activity". Phytother. Res.16,2002,751-753.

[25] Rios, J.L.and Recio, M.C. "Medicinal plants and antimicrobial activity". J. Ethnopharm. 100,2005,80-84.

[26] Vattem, D.A.; Mihalik, K.; Crixell, S.H.; McClean, R.J.C. "Dietary phytochemicals as quorum sensing inhibitors". Fitoterapia, 78,2007, 302-310.

[27] Al-Fatimi, M.; Wurster, M.; Shroder, G.; Lindequist, U. "Antioxidant, antimicrobial, and cytotoxic activities of selected medicinal plants from Yemen". J. Ethnopharmacol., 111,2007, 657-666

[28] Aysegul, G., Mustafa, D. Metin, D. and Selahattin S. " The Biological Activity of Dyer,s Madder (Borago officinalis), Proceeding of ICDG, 2002, pp: 255-258

[29] Reema ,H.and Adel, M. M. "Microbial Growth and Quorum Sensing Antagonist Activities of Herbal Plants Extracts". Molecules, 14,2009,3425-3435.

[30] Kalyoncu, F. ; Cetin, B. and Saglam ,H. "Antimicrobial activity of common madder (Borago officinalis). Phytother. Res. 20(6) ,2006,490-942.

[31] Tadeg, H.; Mohammad, E.; Asres, K.; Gebre-Mariam, T. "Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders". J.Ethnopharmacol. 100,2005,168-175.

[32] Priti, M. and Anil, K "Antimicrobial activity of Abelmoschus moschatus leaf extracts Current".Trends in Biotechno. and Pharma. 3 (3) ,2009, 260 – 266.

[33] Biswas, T.K., Maity, L.N. and Mukherjee, B. "Wound healing potential of Pterocarpus santalinus linn: A pharmacological evaluation". Intl. J. low, 3 (3) ,2004,143-150.

[34] Suleyman, B. and Huseyin ,H. "Studies on the Ecology of Chrozophora tinctoria L. and Borago officinalis in Western Anatolia ".Tr. J. of Botany ,23,1999,33-40.

[35] Sedigheh , S. ; Zoheir, Y.A. ; Yaghobi Ashrafi,. Fakhr ,T. and Hassan, M. A. "Study Methods of Dormancy Breaking and Germination of Common Madder (Borago officinalis) Seed in Laboratory Conditions ".Bota. Res. Inter., 2(1) ,2009,07-10.

[36] Boldizs, I. ; Szucs, Z. ; Moln,, Z. and Perl, I. "Identification and quantification of the constituents of madder root by gas chromatography and high-performance liquid chromatography".J. Chromatogr. A. 1133(1-2) ,2006,259-274.