Screening for Antibacterial Activity of Twenty Two Iraqi Wild plants

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Abstract

The crude ethanolic extracts of twenty two wild plants growing in the Iraqi south regions was evaluated for antibacterial activities against five bacterial species:Gram positive (*Staphylococcus aureus*ATCC25923) and Gram negative (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922)*(Klebsiella pneumonia* and *Proteus volgars*) bacteriaby agar well diffusion method .The results showed that the *Arnebiadecumbens* exhibit broad spectrum activity against all bacterial species. Of 22plants tested, 10 showed encouragingantibacterial results against one or more species of bacteria .On the other hand 11 plants species have no activity.

Keywords: Antibacterial activity, Agar well diffusion assay,Wild plants

Introduction

Since the beginning of civilization, survival of the human race was dependent on plants, not only as a source of food and oxygen, but also as a source of natural remedies (Muthu*et al*., 2010).

According to world health organization (WHO), more than 80% of the world'spopulation relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in

some chemical substances that produce a definite physiologic

action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds(Chhetri*et al*., 2008) .

The antimicrobial activity of plants had been received attentionmany years ago as one of the most effective mechanism for the control ofmicroorganisms . In Iraq , many studies have been attempted to evaluatethe antibacterial activity of

some plant extracts (AL-Ani*et al* ., 1996 ; AL-Thahab,1998).

Al-Rawi and Chakravarty (1988) study considered the first pioneering study in Iraq classified many wild Iraqi plants.

We chose this plants in our study because its exist in south of Iraq and no any study investigated in biological activity.

MATERIALS AND METHODS

Plant materials

Fresh plant parts were collected randomly from various locations in Basra in March and April 2006, 2007,2008 and classified in Department of Biology, College of Education. University of Basra (Table:1).

Table 1: Plant Species

No.	Species	Family	Part used
1	***Anthemismelampodium***	Asteraceae	Leaves+ Flowers
2	***Cistanchetubulosa***	Orobanchaceae	Whole plant
3	***Rumaxvesicarius***	Polygonaceae	Leaves
4	***SileneArabica***	Chenopodiaceae	Whole plant
5	***Althaealudwigii***	Malvaceae	Whole plant
6	***Astragalusspinosus***	Fabaceae	Leaves+ Flowers
7	***Plantagolanceolata***	Plantaginaceae	Whole plant
8	***Sacrophylariadeserti***	Sacrophylaceae	Whole plant
9	***Bassiaeriophora***	Ameranthaceae	Whole plant
10	***Fagoniaindica***	Zygophyllaceae	Whole plant
11	***Reseda lutea***	Resedaceae	Whole plant
12	***Aervajavanica***	Amaranthaceae	Flowers
13	***Lyciumbarbarum***	Solanaceae	Leaves
14	***Phyllanthusrotundifolius***	Phyllanthaceae	Whole plant
15	***Erodiumpulverulentum***	Geraniaceae	Whole plant
16	***Aizoonhispanicum***	Aizoaceae	Whole plant
17	***Echinosciadium Arabicum***	Umbelliferae	Whole plant
18	***Cynomoriumcoccineum***	Balanphoraceae	Whole plant
19	***Mesembrycrystallinum***	Aizoaceae	Whole plant
20	***Zygophyllum Mandavillei***	Zygophyllaceae	Leaves
21	***Arnebiadecumbens***	Boraginaceae	Whole plant
22	***Diplotaxixharra***	Brassicaceae	Whole plant

Microorganisms

The test organisms used in this study were as followed: Gram positive (*Staphylococcus aureus* ATCC25923) and Gram negative bacteria (*Escherichia coli*ATCC25922) and (*Pseudomon-asaerugenosa* ATCC27853) These reference bacteria were obtained from the Immunological lab, Biology department , Science college, University of Basra, Iraq. And others clinical Gram negative bacteria *Klebsiella pneumonia, Proteus volgars*obtained from the Bacteriological lab, Biology department , Education college, University of Basra, Iraq ,(Table:2) and identified according to (Holt , *et al*., 1994)and(Collee , *et al*.,1996).

Table 2: Bacterial culture

Bacterium Name	Type	ATCC NO.
Staphylococcus aureus	Gram positive	ATCC25923
Escherichia coli	Gram negative	ATCC25922
Pseudomonas aerugenosa	Gram negative	ATCC27853
Klebsiella pneumonia	Gram negative	-
Proteus volgars	Gram negative	-

Preparation of plant extract

The plant parts of each samples (50g)were air-dried and then powdered. The powder was extracted by reflux with ethanol(250ml) for 15 min followed by evaporation of combined extracts using rotary evaporator under vacuum. The residue of each extract was kept in refrigerator until use(Harborne , 1984).

AntibacterialActivity

Antibacterial activity tested against Gram positive bacteria and Gram

negative bacteria by the hole agar diffusion method(Cappuccino and Sherman, 1998). The bacteria were grown on Nutrient agar media. Muller-Hinton agar media were poured into the plates to uniform depth of 5 mm and allowed tosolidify.The bacteria suspensions at 1 x 10^6cfu ml^{-1} (0.1 light density on 540 nm wave length) were streaked over the surface of Mueller-Hinton agar media using a sterile cotton swab to ensure confluent growth of the organism. The holes made by cookporar, 6 mm in diameter. 100 µL aliquots of the sample 33.3% (v/v), which were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates were incubated at 37 ºC for 24 h and then the inhibition zone diameters were measured.

RESULTS AND DESCUSSION

In the present investigation study , extracts of 22 plants belonging to different families were screened as antibacterial against five bacterial species, eleven of which showed activity against at least three of the test bacteria. (Table:3)

The antibacterial assay results showed that the gram-positive bacteria species are more sensitive than the gram-negative which is in agreement with many previous studies (Cosentino*et al*., 1999; Karman *et al*., 2003).

Gram negative microorganisms are less susceptible to active compounds than Gram positive ones because they posses outer membrane surrounding the cell membrane (Ratledge& Wikinson,1988) which restricts diffusion of hydrophobic compounds through its lopoplysaccharide covering (Vaara, 1992). This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity. The negative results obtained against Gram negative bacteria were not unexpected since this class of bacteria is usually more

resistant than Gram positive bacteria(Turnbull *et al*.,1991).

The active compounds in plant extracts have many mechanisms to inhibit bacterial growth. It plays many roles in attacking target sits found in bacteria like cell wall, cell membrane, cellular synthetic processes (DNA,RNA) and protein or enzymes.

The results showed that the *Arnebiadecumbens*exhibit high activity against all bacterial species.

The activity of *Arnebiadeumbens* extract against all bacteria may come from to active compounds like shikoninand this agreement with previous study (Singh *et al*., 2003).

The extracts of *Cistanchetubulosa , Astragalusspinosus , Fagoniacretica , Erodiumpulverulentum , Cynomoriumcoccineum, Sacrophylariadeserti, Bassiaeriophora , Reseda lutea, Diplotaxixharra and Rumexnervosus*showed moderate inhibited the growth of one or more bacteria.

Table 3:Inhibition zone of of 11 ethanol extracts exhibit activity against bacterial species

No.	Species	Inhibition Zone (mm)				
		E.coli	*S.aureus*	*P.aeruginosa*	*K. pneumonia*	*P. volgars*
1	***Arnebiadecumbens***	**40**	**43**	**46**	**28**	**18**
2	***Cistanchetubulosa***	**22**	**26**	**14**	**12**	**11**
3	***Rumaxvesicarius***	**24**	**30**	**-**	**18**	**-**
4	***Erodiumpulverulentum***	**13**	**23**	**11**	**-**	**16**
5	***Cynomoriumcoccineum***	**22**	**32**	**14**	**8**	**3**
6	***Astragalusspinosus***	**8**	**17**	**7**	**-**	**6**
7	***Sacrophylariadeserti***	**7**	**14**	**8**	**-**	**8**
8	***Bassiaeriophora***	**7**	**12**	**8**	**7**	**9**
9	***Fagoniaindica***	**8**	**13**	**6**	**-**	**-**
10	***Reseda lutea***	**6**	**11**	**8**	**10**	**10**
11	***Diplotaxixharra***	**9**	**11**	**13**	**11**	**10**
12	**CONTROL**	**-**	**-**	**-**	**-**	**-**

***Bacteria: S. aureus = Staphylococcus aureus, E. coli = Escherichia coli, P. aeruginosa = Pseudomonas aeruginosa , K. pneumonia= Klebsiella pneumonia, P. volgars= Proteus volgars,* control= Ethanol**

On the other hand, 11 plant extracts showed no effect (Table :4).

Table 4: ethanol extracts of some Iraqi wild plants with no antibacterial activity

No.	Species	Inhibition Zone (mm)				
		E.coli	*S.aureus*	*P.aeruginosa*	*K. pneumonia*	*P. volgars*
1	***Anthemismelampodium***	-	-	-	-	-
2	***Silenearabica***	-	-	-	-	-
3	***Althaealudwigii***	-	-	-	-	-
4	***Plantagolanceolata***	-	-	-	-	-
5	***Aervajavanica***	-	-	-	-	-
6	***Lyciumbarbarum***	-	-	-	-	-
7	***Phyllanthusrotundifolius***	-	-	-	-	-
8	***Aizoonhispanicum***	-	-	-	-	-
9	***Echinosciadium arabicum***	-	-	-	-	-
10	***Mesembrycrystallinum***	-	-	-	-	-
11	***Zygophyllum mandavillei***	-	-	-	-	-
12	**CONTROL**	-	-	-	-	-

***Bacteria: S. aureus = Staphylococcus aureus, E. coli = Escherichia coli, P. aeruginosa = Pseudomonas aeruginosa , K. pneumonia= Klebsiella pneumonia, P. volgars= Proteus volgars,* control= Ethanol**

In conclusion, the present investigationstudy confirmed that many wild Iraqi plants contains the potential antibacterial components that may be of great use to the development of new drugs, as a therapy against various diseases.

It is recommended that further studies should be carried out on Iraqi wild plants to further purify the actual bioactive compounds that have the antibacterial activity and to ascertain their toxicity level before recommending for consumption.

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دراسة الفعالية ضد الجرثومية لاثنين وعشرين نباتا بريا عراقيا

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الخلاصة

تم في هذه الدراسة اختبار تأثير المستخلص الايثانولي لاثنين وعشرين نباتا بريا تنمو في جنوب العراق ضد بعض الانواع البكتيرية الموجبة والسالبة لصبغة كرام وباستخدام طريقة انتشار الاكار .

استخدات خمسة انواع بكتيرية احدها موجب لصبغة كرام(*Staphylococcus aureus* ATCC25923) واربعة سالبة (*Escherichia coli*, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* and *Proteus volgars* ATCC25922)

بينت النتائج ان نبات *Arnebiadecumbens* كان له تأثير واسع الطيف ضد جميع الانواع البكتيرية ، ومن ناحية اخرى اظهرت عشرة مستخلصات نباتية تأثيرا متوسطا ضد واحد او اكثر من الانواع البكتيرية . وقد لوحظ أيضا ان احد عشر نباتا لم تظهر أية فعالية ضد بكتيرية.