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Full Length Research Paper

Antibacterial Potential of Twenty-Two Wild Plant Species from Iraq: A Screening Study

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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 vulgaris*) bacteria by agar well diffusion method . The results showed that the *Arnebia decumbens* exhibit broad spectrum activity against all bacterial species. Of 22 plants tested, 10 showed encouraging antibacterial 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's population 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 attention many years ago as one of the most effective mechanism for the control of microorganisms . In Iraq , many studies have been attempted to evaluate the 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 by dr. Ali Aboud Shareef and Louy Hussain Ali, Department of Biology, College of Education. University of Basra (Table1).

Table 1. Plant Species

No.	Species	Family	Part used
1	***Anthemis melampodium***	Asteraceae	Leaves+ Flowers
2	***Cistanche tubulosa***	Orobanchaceae	Whole plant
3	***Rumax vesicarius***	Polygonaceae	Leaves
4	***Silene arabica***	Chenopodiaceae	Whole plant
5	***Althaea ludwigii***	Malvaceae	Whole plant
6	***Astragalus spinosus***	Fabaceae	Leaves+ Flowers
7	***Plantago lanceolata***	Plantaginaceae	Whole plant
8	***Sacrophylaria deserti***	Sacrophylaceae	Whole plant
9	***Bassia eriophora***	Ameranthaceae	Whole plant
10	***Fagonia indica***	Zygophyllaceae	Whole plant
11	***Reseda lutea***	Resedaceae	Whole plant
12	***Aerva javanica***	Amaranthaceae	Flowers
13	***Lycium barbarum***	Solanaceae	Leaves
14	***Phyllanthus rotundifolius***	Phyllanthaceae	Whole plant
15	***Erodium pulverulentum***	Geraniaceae	Whole plant
16	***Aizoon hispanicum***	Aizoaceae	Whole plant
17	***Echinosciadium arabicum***	Umbelliferae	Whole plant
18	***Cynomoriumcoccineum***	Balanphoraceae	Whole plant
19	***Mesembry crystallinum***	Aizoaceae	Whole plant
20	***Zygophyllum mandavillei***	zygophyllaceae	leaves
21	***Arnebia decumbens***	Boraginaceae	Whole plant
22	***Diplotaxix harra***	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 (*Pseudomonas aerugenosa* 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 vulgaris* 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).

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).

Antibacterial Activity

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 to solidify. 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

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 vulgaris	Gram negative	-

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 *nervosus* showed moderate inhibited the growth of one or more bacteria.

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

In conclusion, the present investigation study 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 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 *Arnebia decumbens* exhibit high activity against all bacterial species.

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

The extracts of *Cistanche tubulosa , Astragalus spinosus , Fagonia cretica , Erodium pulverulentum, Cynomorium coccineum, Sacrophylaria deserti, Bassia eriophora , Reseda lutea, Diplotaxix harra and Rumex* compounds that have the antibacterial activity and to ascertain their toxicity level before recommending for consumption.

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	***Arnebia decumbens***	**40**	**43**	**46**	**28**	**18**
2	***Cistanche tubulosa***	**22**	**26**	**14**	**12**	**11**
3	***Rumax vesicarius***	**24**	**30**	**-**	**18**	**-**
4	***Erodium pulverulentum***	**13**	**23**	**11**	**-**	**16**
5	***Cynomoriumcocci neum***	**22**	**32**	**14**	**8**	**3**
6	***Astragalus spinosus***	**8**	**17**	**7**	**-**	**6**
7	***Sacrophylaria deserti***	**7**	**14**	**8**	**-**	**8**
8	***Bassia eriophora***	**7**	**12**	**8**	**7**	**9**
9	***Fagonia indica***	**8**	**13**	**6**	**-**	**-**
10	***Reseda lutea***	**6**	**11**	**8**	**10**	**10**
11	***Diplotaxix harra***	**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. vulgaris= Proteus vulgaris, **control= Ethanol**

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	***Anthemis melampodium***	**-**	**-**	**-**	**-**	**-**
2	***Silene arabica***	**-**	**-**	**-**	**-**	**-**
3	***Althaea ludwigii***	**-**	**-**	**-**	**-**	**-**
4	***Plantago lanceolata***	**-**	**-**	**-**	**-**	**-**
5	***Aerva javanica***	**-**	**-**	**-**	**-**	**-**
6	***Lycium barbarum***	**-**	**-**	**-**	**-**	**-**
7	***Phyllanthus rotundifolius***	**-**	**-**	**-**	**-**	**-**
8	***Aizoon hispanicum***	**-**	**-**	**-**	**-**	**-**
9	***Echinosciadium arabicum***	**-**	**-**	**-**	**-**	**-**
10	***Mesembry crystallinum***	**-**	**-**	**-**	**-**	**-**
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**

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