

Phytoremediation of wastewater by *Bacopa monnieri* plant growth *in vitro*

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ABSTRACT

This study carried out to evaluate the antibacterial effect of ethanolic and methanolic extract of *Bacopa monnieri* on *Pseudomonas aeruginosa*, *Vibrio cholera* and *Staphylococcus aureus* were screened against clinical strains isolated from different wastewater sources discharged to shatt Al-Arab and Garmat Ali River. the results of study showed that effect of *Bacopa monnieri* plant on all bacterial species. The higher Inhibition rate of *Vibrio cholera* is (24) mm at the concentration (200) mg/mL in ethanol extraction while the lower inhibition rate was (6) mm for *Staphylococcus aureus* at concentration (50) mg/ mL in ethanol extraction. The extract has strong antimicrobial effects against the tested microorganisms especially the bacterium *Vibrio cholera*. The findings support the use of this plant in traditional medicine.

Kew words : *Bacopa monnieri* , *Phytoremediation*, *Antibacterial activity* , *wastewater*

Introduction

Bacopa monnieri (Linn) Pennell (family Scrophulariaceae) is a medicinal and aquatic plant in Iraqi water canals, wells, irrigated fields, is a creeping, juicy, succulent, glabrous herb that branches profusely, found in wet places, damp or marshy areas near the border of the rivers[4]. this plant are the main source of many medicinal drugs used for various therapeutic purposes Especially in treating many of the chronic diseases they are preparing A source of active ingredients that go into preparing a lot of (Pharmaceutical compounds in the form of extracts or other forms) (AL-Rawi and Chkravarty, 1998).

Bacopa monnieri also growing widely in tropical regions of Asia, and used in the treatment of cough or as an antiseptic. The leaf of *Bacopa* species has

been used in the Indian medical system of Ayurveda since the 6th century AD. It has been used in treatment of insanity, epilepsy, hysteria and skin diseases (Chopra *et al.*, 1992). The traditional use of this plant suggests possible antibacterial properties. However, its efficacy has not been examined fully yet. Therefore, our aim is to evaluate the antimicrobial efficacy of *B. monnieri*. Therefore, our aim is to determine the antimicrobial effects of the ethanolic and methanolic extracts obtained from this plant against microorganisms.

Although large numbers of plant products are used to treat human diseases worldwide, information on the effectiveness of most plant species is either in sufficient or lacking (Patumraj and Yoisungneon, 2007; Koyama *et al.*, 2008). Many medicinal plants are used in traditional phytoremediation for centuries (Khan *et al.*, 2008; Khan, 2006).

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Materials and Methods

Preparation of tissue culture plant

A rapid micro propagation protocol was established for *Bacopa monnieri* plant, The plants were cultured on MS nutrient medium supplemented with BA and KIN alone or in combination of BA, KIN and NAA according to (Al-Aradi *et al.*, 2017). Plantlet was propagated *in vitro* Fig. (1).



Fig. 1. Growth of *Bacopa monnieri* plant in vitro

Phytoremediation Testing of *Bacopa monnieri*

Samples of wastewater were collected from Shatt Al-Arab and Garmat Ali areas, which are the rural regions in Basrah Government.

The samples of wastewater separated into sterilization glass bottles, Subsequently the multiplication plants from tissue culture were transferred to laminar airflow cabinet and transferred to glass bottles 250mL and cultured singly in containing MS (Murashoge and Skoog, 1962) medium with 30g/L sugar, Also added Nutrient NPK with 1 g/L, and sucrose with 10 g/l to feed the bacteria and not to drain the components of the medium (Fig 2. A & B). The cultures were maintained at 25 ± 2 °C with light intensity varied from 2000–3000 lux. The photoperiod was generally 14 hours light 10 hours dark. After a week of cultivating plants in the wastewater, the bacteriological examination was carried out again.

Plant Material was taken from multiplication shoot growth *in vitro* from tissue culture laboratory in marine science center. The material was air dried in shade, powdered mechanically and stored in air-tight

Preparation of extracts

The preparation of the *Bacopa monnieri* leaves ex-

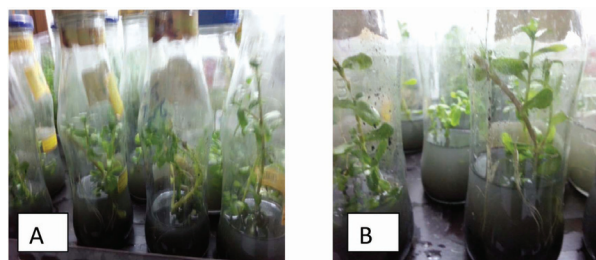


Fig. 2. Showed the *in vitro* cultured of *Bacopa monnieri* growth in wastewater were collected from Shatt Al-Arab (A) and Garmat Ali areas (B).

tracts were performed following the methods described by Emeruwa (Emeruwa, 1982). and Trease and Evans (Trease and Evans, 1996). A sample (50 g) of the dry powdered plant material was extracted with 400 mL of 95 % of solvent (ethanol and methanol) contained in a 500 mL sterile conical flask and covered with cotton wool plug and wrapped with aluminum foil for 24 hours. Extraction was allowed to proceed for 48 h in a shaker water bath maintained at 40 °C. The extract was filtered using a clean muslin cloth and then The extract was filtered using Whatman filter paper no. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C. Dried extract were stored in bottles at - 18 C.

For the preparation of dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by re dissolving in the respective extracting solvents and further diluted to obtain 200, 100 and 50 mg/mL.

Preparation of microbial cultures

Three pathogens namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholera* were selected to know the antagonistic activity. 2.5 g of nutrient broth was dissolved in 100 mL of distilled water and autoclaved at 120 °C, 14 lbs. for 20 min. The broth was cooled down to room temperature and bacterial cultures were inoculated in sterilized nutrient broth and were incubated at 37 °C for 24 hrs.

Studying the effect of plant extracts on the growth of bacteria

The extracts were tested for their anti-bacterial activity by agar well diffusion method (Uddin *et al.*, 2012). In the bacterial sensitivity test for the plant extract, 0.1 mL of bacterial suspension for each microorganism was published on the center of the Muller Hanton Steel. Using the Cork borer and then

worked (5) drills with a diameter of 6 mm per hole and added (0.1) of the plant extract and different concentrations as mentioned in the above paragraph. Distilled water was added to one of the holes as a negative control. The dishes were left for 15 minutes and then incubated at 37 °C for 24 hours. Control experiments comprising streptomycin were set up. Antibacterial activities (Inhibition Zone) were measured by ruler.

Results

Table 1 shows some chemical properties in two filed sites for Wastewater discharged to the Shatt al - Arab and Garmat Ali River. For represent the bacterial pollution, the Samples of sewage water was tested bacteriologically. Several bacteria have been listed: *Esherichia coli*, *Pseudomonas aeruginos*, *Vibrio cholera*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Salmonella typhi*, *Shigella dysentery*

In vitro, After week of grows *B. monnieri* plant in sewage water, Samples was tested bacteriologically, four species of bacteria *Esherichia coli*, *Staphylococcus saprophyticus*, *Salmonella typhi*, *Shigella dysentery* were disappeared from all sample ,this result refers to efficiency of plant to remediation and uses as a model for phytoremediation plant.

Also plant extracts were tested to antimicrobial activity, The ethanol extract showed highest antimicrobial activity of the two solvent extracts. Thus the phytochemical analysis of these extracts of this plant

was showed from the analysis, the presence of alkalooids, Flavonoids and phenols was observed in *B. monnieri* plant extracts (Table 2).

The effect of the extract on the permeability of the cell membrane and the work of the bacterial cell can be increased. The effect of the *B. monnieri* plant extracts is due to the presence of phenols that have inhibitory effect on positive and negative chromosomes.

The antimicrobial activity of ethanol and methanol extracts of medicinal *Bacopa monnieri* plant were investigated against the selected clinical pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio cholera*.

All the examined plant extracts showed varying degrees of antimicrobial activities against the clinical pathogens tested Figure 3-5.



Fig. 3. Antibacterial activity *B. monnieri* plant ethanol and methanol extracts against of *Vibrio cholera*.

Table 1. Some chemical properties of wastewater samples

Parameter Unit	EC mS/m	Sal g/L	COD mg/L	pH IU	Ca	Mg	Na	K	Cl	HCO ₃ mg/L	TDS	SO ₄	TH	TA	PO ₄
Shatt Al-Arab Area	8.6	5.4	200	6.03	320	340	474	48	1981	195	5504	212	2200	320	1.14
Garmat Ali Area	6.44	4.2	100	8.15	320	194	393	62	1483	179	4120	339	1600	290	1.82

Table 2. Several Active material in *Bacopa Monnieri* plant.

Active material	% dry weight	References
Bacoside A and B (bacopaside-I)	with A being up to 8% 1.43%	(Russo and Borrelli, 2005; Shinomol and Muralidhara, 2011) (Shinomol and Muralidhara, 2011)
bacopaside-II	2.74%	(Shinomol and Muralidhara, 2011)
Bacosterols		(Bhandari, 2006)
Bacosine	0.61%	14, (Shinomol and Muralidhara, 2011)
Luteolin	0.26%	(Bhandari, 2006; Shinomol and Muralidhara, 2011)
Apigenin	0.12%	(Bhandari, 2007; Bhandari <i>et al.</i> , 2009)
total phenolic content	3.18mg/mL	(Mukherjee, 2011)
flavonoid content	115mg/mL	(Mukherjee, 2011)

The antimicrobial activity of ethanol extracts of *Bacopa monnieri* plant against tested pathogens was shown in Table 3. The ethanol extract of *Bacopa monnieri* showed maximum zone of inhibition against *V. cholera* (24 mm) while minimum inhibitory zone was observed against *Staphylococcus aureus* (6 mm).

The antimicrobial activity of methanol extracts of *Bacopa monnieri* plant against tested pathogens was shown in Table 4. The methanol extract of *Bacopa monnieri* showed maximum zone of inhibition (20 mm) against *Streptococcus* sp, *Pseudomonas aeruginosa* and *Vibrio cholera*. The minimum inhibitory zone (7 mm) was exhibited against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The results showed that all species of bacteria were sensitive to the ethanol and methanol extracts of the plant through the apparent contrast of the concentration factor used to influence the growth of these bacteria until the maximum effect at the concentration of 200 mg/mL of extracts. It was noticed that the increase in concentration had an effect on the increase of inhibitory effect in the growth of these bacteria. The highest inhibitory effect was observed at the concentration of 200 mg/mL of the ethanol extract of the bacteria in *V. cholera* (24 mm) and the lowest effect was 100 mg/mL of methanol extract in bacteria *S. aureus*. As it reached (6) mm (Fig 4, 5).

Phytochemical analysis

Phytochemical analysis for major constituents of the *Bacopa Monnieri* plant extracts was performed by the



Fig. 4. Antibacterial activity *B. monnieri* plant ethanol and methanol extracts against of *Staphylococcus aureus*



Fig. 5. Antibacterial activity *B. monnieri* plant ethanol and methanol extracts against of *Pseudomonas aeruginosa*.

Table 3. Inhibition diameters growth some species of Bacteria in different concentrations of ethanol extract of *B. monnieri* plant.

Concentrations (mg/mL)	Inhibition diameter (mm)		
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Vibrio cholera</i>
200 mg/mL	18	17	24
100 mg/mL	15	12	16
50 mg/mL	7	6	9

Table 4. Inhibition diameters growth some species of Bacteria in different concentrations of methanol extract of *B. monnieri* plant.

Concentrations (mg/mL)	Inhibition diameter(mm)		
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Vibrio cholera</i>
200 mg/mL	20	20	20
100 mg/mL	12	15	14
50 mg/mL	7	7	9

several researcher in Table 2.

Discussion

The present result showed that the good activity is revealed by *B. monnieri* extracts (Table 3, 4). This suggests that this plant may be of clinical benefit for uses in different pathogenic diseases. Bioactive compounds in these extracts may be used in the development of antibacterial drugs.

Pathogenic bacteria are known to develop resistance for antibiotics, thus search for new antibiotics is a never-ending process (Vanden and Vlietinck, 1991). Crude extracts of *B. monnieri*, especially Ethanol and Methanol extracts, may deserve further investigations to develop a new antibiotic that may help in combating several bacterial infirmities in tropical countries. In conclusion, *B. monnieri* may be effective for treatment in different pathogenic diseases. Our finding may be useful for development of antibacterial drugs from this plant. *B. monnieri* (L.) Pennell.

The antimicrobial activity of *B. monnieri* extracts were evaluated in this study by measuring the diameters of zones of growth inhibition on some pathogenic strains of *P. aeruginosa*, *S. aureus* and *V. cholera* using the agar well diffusion method as detailed in Figs. 3-5. The growth of the entire test organisms were inhibited by the crude leaf extracts though to varying degrees, depending on the extracting solvent and the bacterial species. Other workers have also shown that extracts of some plants inhibited the growth of various microorganisms at different concentrations (Ogbolie *et al.*, 2007; El-Mahmood and Ameh, 2007; El-Mahmood *et al.*, 2008).

In the present study, the leaf extracts of medicinal plant *Bacopa monnieri* were tested against clinical pathogens *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Vibrio cholera*. Despite many published reports dealing with treatment for neurological disorders, little was known about antimicrobial activity of *Bacopa monnieri* prior to this study. ethanolic extract of *Bacopa monnieri* was found to possess inhibitory effects against both Gram positive and Gram negative organisms tested, than methanolic extract. The result agreed with literature cited (Ayyappan *et al.*, 2010). The antibacterial activity may be due to the presence of phytochemicals such as alkaloids, phytosterols, proteins, etc., which warrants *Bacopa monnieri* could be subjected to extensive

experimental studies in future to treat certain diseases caused by studied bacteria.

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