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ANALYSIS OF BACOPA MONNIERI LEAF EXTRACTS ANTIBACTERIAL EFFICACY

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ABSTRACT
Background: Bacopa monnieri (Linn) Pennell (Scrophulariaceae) is widely distributed in tropical regions of Asia, and used in the treatment of cough or as an antiseptic. The traditional use of this plant suggests its possible antibacterial properties, but its efficacy has not been examined yet.
Objective: Evaluate the antibacterial efficacy against pathogenic bacteria using the disk diffusion method.
Materials and methods: Five different concentrations (500 µg, 1, 2, 5, 10, and 15 mg/mL) of crude leaf extracts of Bacopa monnieri (L.) Pennell were tested for antibacterial efficacy against seven Gram-positive and 11 Gramnegative bacteria. The sensitivity of plant fractions was tested using the disk diffusion method.
Results: Maximum activity was revealed by ethyl acetate and methanol extracts, followed by aqueous, benzene, and petrol extracts. Phyto-chemical analysis of the plant leaf showed the presence of alkaloids, flavonoids, and saponins.
Conclusion: This plant may be effective for treatment of different pathogenic diseases.
Keywords: Antibacterial efficacy, Bacopa monnieri (L.) Pennell, crude extracts, traditional use

INTRODUCTION

Although large numbers of plant products are used to treat human diseases worldwide, information on the effectiveness of most plant species is either insufficient or lacking[1, 2]. India has great potential for discovery of novel plant-derived-drugs needed to combat various human diseases[3, 4]. Many medicinal plants are used in traditional phyto-therapy for centuries[5, 6].

Bacopa monnieri (Linn) Pennell (Scrophulariaceae) (B. monnieri) is growing widely in tropical regions of Rajasthan , and used in the treatment of cough or as an antiseptic). The traditional use of this plant suggests possible antibacterial properties. However, its efficacy has not been examined fully yet. This study was aimed to evaluate the antibacterial efficacy of B. monnieri. Antibacterial screening of the crude leaf extracts was made using seven Gram-positive (G+ve) and 11 Gram negative (G-ve) bacteria.

MATERIALS AND METHODS

Plant material

Fresh plant of B. monnieri was collected after the September rainy season. The specimen were collected in the wild, where they grow naturally, in the marshy location at the Herbarium, Department of Botany of Rajasthan University, Jaipur India. They were authenticated by Taxonomist of University of Rajasthan, Jaipur, India.

Preparation of extracts and fractions

Plant extracts of B. monnieri were prepared according to the following procedure[7, 8].

1) Freshly dried and healthy plant material was pulverized into fine powdered form by an electric grinder and was stored in a dessicator.
2) Five hundred gram plant powder was refluxed with 95% methyl alcohol (MeOH) on a water bath for 10 hours. Mother liquor (crude MeOH extract) was filtered, residual plant material was refluxed with 95% MeOH for 10 hours. The process was repeated four times for maximum yield of MeOH extract. The extract was evaporated to dryness at 35°C under reduced pressure using a rotary evaporator.
3) Dried MeOH extract was refluxed with petroleum ether (60-80°C) for five hours. After filtration, the residual MeOH extract was refluxed with petroleumether for five hours. It was then tiltered and the process was repeated five times. Petrol was evaporated under a reduced pressure to obtain petroleum ether-soluble extract.
4) Petroleum ether insoluble fraction of MeOH extract obtained in step 3, was refluxed with benzene for five hours. Then, it was refluxed with benzene for five hours and filtered. The process was repeated five times. Benzene was evaporated under reduced pressure to obtain benzene soluble extract.
5) Benzene insoluble fraction obtained in step 4 was refluxed with ethyl acetate (EtOAc) for five hours. Then, it was refluxed with EtOAc for five hours and filtered. The whole process was repeated five times to ensure a maximum yield. EtOAc was evaporated under reduced pressure to obtain EtOAc-soluble extract.
6) EtOAc-insoluble fraction obtained in step 5 was refluxed with MeOH (95%) for five hours, filtered and was repeatedly refluxed five times with methanol. The MeOH-soluble fraction was evaporated under reduced pressure to obtain MeOH extract, while MeOH-insoluble residue was discarded.

Preparation of aqueous extract

Five hundred gram plant powder was poured with double distilled water, and left for 72 hours at room temperature. Then, the flask was refluxed over hot water bath for 10 hours, and the mother liquor was filtered, process was repeated for four times to obtain the maximum yield. The filtrate obtained was evaporated to complete dryness under reduced pressure using a rotary evaporator. The aqueous extract thus obtained was kept in a closed bottle at low temperature until further use.

Dried plant extract were stored in sterilized screwcapped bottles at -20oC in a deep freezer.

Microorganisms test

The leaf extracts of B. monnieri were tested for possible antibacterial activity in the disk assay using seven G+ve bacteria (Staphylococcus aureus, Staphylococcus aureus ATCC 25953, Staphylococcus albus, Streptococcus haemolyticus Group-A, Streptococcus

haemolyticus Group-B, Streptococcus faecalis, and Bacillus subtilis) and 11 G-ve bacteria (Escherichia coli, Edwardsiella tarda, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Shigella flexneri, and Plesiomonas shigelloides). The bacterial strains were obtained from the Bacterial Stock of the Department of Microbiology, Suresh Gyan Vihar University, Jaipur India. The bacterial cultures were maintained at 4oC on nutrient agar.

ANTIMICROBIAL ASSAY

Agar plates were inoculated using a sterile swab dipped into culture inoculums adjusted to 1.5x108 bacterial / mL. The agar was streaked in three directions turning the plates by 60 degree for each streak. All the extracts were sterilized by filtration thorough membrane filter (diameter: 0.045 mm), and autoclaved at 122oC. The paper disk (Whatman filter paper) impregnated with 1mg/mL, 2 mg/mL, 5 mg/mL, 10 mg/mL, and 15 mg/mL plant extracts were dried and placed on the agar surface. The sensitivity disks were placed to make complete contact with the surface of the medium on the plate. Plates were kept at room temperature for 30 minutes (pre-diffusion time). Inoculated petri dishes were incubated at 37oC overnight and at the end of the period. Inhibition zones formed on the medium were evaluated in millimeter, and experiments were repeated three times.

STUDIED ACTIVITY

Antibacterial activity was tested using the disc diffusion method [9]. Diameters of petri dish and disk were 9.0 cm and 0.6 cm, respectively.

Phyto-chemical analysis

Binary and tertiary solvent systems were used to detect the presence of alkaloids, flavonoids, triterpenes, anthraquinones, coumarins, saponins, and glycosides in non-polar and polar extracts of plant on the thin layer chromatography plates. Reagents used were dragendorff reagent, mayers reagent, potassium hydroxide, alcoholic ferric chloride, Vaniline + supfuric acid, and liebermann-burchard reagent[10]. Solvent system with different ratios for non-polar extracts included toluene-acetone, toluene-chloroform-acetone for semi-polar extracts, and n-butanol-glacial acetic acid-water for polar extracts.

Statistical analysis

All values are expressed as mean ±standard error of mean (SEM). Linear regression analyses were made to determine correlation between two variables using MS-DOS software (GraphPad InStat statistical program).

RESULTS

Antibacterial efficacy of B. monnieri leaf extracts using different solvents (Petroleum ether, Benzene, EtOAc, MeOH, and aqueous) against seven G+ive and 11 G-ve bacteria is shown in Table 1. Apparently, EtOAc and MeOH extracts exhibited strong antibacterial activity against maximum number of bacterial species (15 each). Both extracts were sensitive against all G+ve bacterial strains. Aqueous extract also demonstrated biological activity against all G+ve bacteria strains, but it could only resist the growth of five of the G-ve pathogens.

Table 1. Antibacterial efficacy of B. monnieri leaf extracts against 18 pathogenic bacteria-Inhibition zone (mm)

	Gram positive (G+ve) bacteria							Gram negative (G+ve) bacteria										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Petrol																		
500 µg	-	2	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-
1 mg	-	2	-	-	2	-	6	-	-	-	-	-	-	-	-	-	-	-
2 mg	3	5	-	-	6	-	7	-	-	-	-	-	-	-	-	-	-	-
5 mg	5	8	-	-	9	-	10	-	-	-	-	-	-	-	-	-	-	-
10 mg	7	10	-	-	12	-	12	-	-	-	-	-	-	-	-	-	-	-
15 mg	10	16	-	-	14	-	16	-	-	-	-	-	-	-	-	-	-	-
Benzene																		
500 µg	-	2	-	4	-	-	4	-	-	-	4	-	-	3	-	-	-	-
1 mg	-	3	-	5	-	-	4	-	-	-	4	-	-	4	-	-	-	-
2 mg	-	6	-	7	-	-	7	-	-	-	6	-	-	7	-	5	-	-
5 mg	-	11	-	9	-	-	10	-	-	-	9	-	-	10	-	7	-	-
10 mg	-	15	-	13	-	-	14	-	-	-	12	-	-	15	-	10	-	-
15 mg	-	17	-	15	-	-	16	-	-	-	15	-	-	19	-	12	-	-
Ethylacetate																		
500 µg	4	4	4	5	1	3	4	-	4	4	-	4	-	1	3	2	2	3
1 mg	4	5	5	5	2	4	5	-	4	5	-	4	-	2	5	3	3	4
2 mg	8	8	7	8	4	6	9	-	8	7	-	6	-	4	9	6	6	7
5 mg	14	14	12	14	7	10	15	-	10	10	-	9	-	6	14	9	9	10
10 mg	17	16	17	19	9	12	19	-	13	13	-	14	-	9	17	11	11	12
15 mg	21	20	21	21	12	18	21	-	17	18	-	19	-	11	21	13	15	17
Methanol																		
500 µg	3	4	4	-	-	2	4	5	-	-	3	-	3	-	-	2	2	4
1 mg	4	5	4	2	-	3	5	5	-	2	4	-	4	-	-	2	5	5
2 mg	8	9	8	6	4	6	8	8	-	6	8	-	6	-	4	5	6	7
5 mg	14	15	12	9	7	10	14	14	-	9	11	-	9	-	6	9	9	12
10 mg	17	17	17	13	9	12	17	17	-	12	13	-	12	-	9	11	11	14
15 mg	21	21	20	19	12	16	20	20	-	16	18	-	16	-	11	15	14	16
Aqueous																		
500 µg	-	-	-	2	-	-	-	-	-	1	-	1	-	-	-	-	-	-
1 mg	-	3	2	3	-	-	-	-	-	2	-	2	-	-	-	-	-	-
2 mg	5	6	5	6	-	5	3	-	2	5	-	5	-	-	4	-	5	-
5 mg	10	11	10	10	3	7	8	-	5	8	-	7	-	-	6	-	7	-
10 mg	13	13	13	14	5	10	11	-	8	10	-	9	-	-	8	-	10	-
15 mg	16	17	15	16	8	12	15	-	10	13	-	11	-	-	11	-	12	-
Chloramphenicol																		
10 µg/disc	18	18	16	-	-	-	16	18	16	-	16	18	-	16	17	19	18	20

G+ve bacteria: 1. Staphylococcus aureus, 2. Staphylococcus aureus ATCC 25953, 3. Staphylococcus albus, 4. Streptococcus haemolyticus Group-A, 5. Streptococcus haemolyticus

Group-B, 6. Streptococcus faecalis, 7. Bacillus subtilis. G-ve bacteria:
1. Escherichia coli, 2. Edwardsiella tarda, 3. Klebsiella pneumoniae, 4. Proteus mirabilis, 5. Proteus vulgaris, 6. Pseudomonas aeruginosa, 7. Salmonella typhi, 8. Shigella boydii, 9. Shigella dysenteriae, 10. Shigella flexneri, 11. Plesiomonas shigelloides.
aValues are expressed as mean of replication of three values. (-): no inhibition.

Table 2 shows results by phyto-chemical analysis of B. monnieri leaf. Interestingly, the plant leaf has alkaloids, flavonoids, and saponins.

Table 2. Phtotochemical analysis of B. monnieri leaf.

Plant part	Alkaloids	Anthraquinones	Coumarins	Flavonoids	Saponins	Polyphenols	Cardiac glycoside
Leaf	++++	-	-	++++	++	-	-

DISCUSSION

The present result showed that the maximum activity is revealed by B. monnieri EtOAc extracts and MeOH, followed by aqueous, benzene, and petrol extracts (Table 1). 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 novel antibacterial drugs.

Pathogenic bacteria are known to develop resistance for antibiotics, thus search for new antibiotics is a never-ending process[11]. Crude extracts of B. monnieri, especially EtOAc and MeOH 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

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