Antimicrobial Activity of Various Fractions of Ethanol Extract of *Bacopa monnieri* Linn. Aerial Parts

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The ethyl acetate and n-butanol fractions of ethanol extract of *Bacopa monnieri* Linn. aerial parts were screened for antibacterial and antifungal activities by both zone of inhibition study and determination of minimum inhibitory concentration (MIC). The ethyl acetate fraction was found to be more potent than the n-butanol fraction, though both of them were endowed with antimicrobial activity. The present study reveals the potential usefulness of *B. monnieri* aerial parts in the treatment of various pathogenic diseases as mentioned in the Ayurvedic literature.

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Bacopa monnieri Linn. (Family: Scrophulariaceae), is a creeping, glabrous, succulent herb, rooting at nodes. It is distributed throughout India in all plain districts, ascending to an altitude of 1,320m[1]. In Ayurveda, *B. monnieri* have been used in the treatment of insanity, epilepsy, hysteria and skin diseases[2]. The alcoholic extract is reported to increase the learning performance of rats and the activity is attributed to saponin mixtures consisting mainly of bacosides A and B[3,4]. The plant is also reported to show sedative[5], antiepileptic[6], vasoconstrictor[7], antiinflammatory[8] and anthelmintic[9] activities. The plant is reported to contain tetracyclic triterpenoid saponins, bacosides A and B[10,11], hersaponin[12] and some flavonoids[13,14]. The antimicrobial activity of the ethanol extract of the plant has already been reported by the authors[15]. In the present investigation, the antimicrobial activity of ethyl acetate and n-butanol fractions of the ethanolic extract of *B. monnieri* aerial parts were studied in a scientific manner.

The plant material was identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. After authentication, fresh aerial parts were collected in bulk from young matured plants from the rural belts of Salipur during early summer, washed, shade dried and then milled into coarse powder by a mechanical grinder.

The powdered plant material (500 g) was defatted with petroleum ether (60-80°) and then extracted with 1.5 l of ethanol (95%) in a Soxhlet apparatus. The solvent was then removed under reduced pressure, to yield a greenish-black sticky residue (yield: 11.6% w/w with respect to dried plant material). A part of the ethanol extract was kept as such and the remaining part was partitioned successively between ethyl acetate-water system and then between n-butanol-water system. The ethyl acetate (yield: 48.28% w/w with respect to ethanol extract) and n-butanol (yield: 24.74% w/w with respect to ethanol extract) fractions of the ethanolic extract were used in the present study.

The microorganisms used in the present study include *Staphylococcus aureus, Bacillus subtilis, Bacillus polymexia, Streptococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Escherichia coli, Penicillium* notatum, Aspergillus *niger* and *Candida albicans*. Suitable strains of these microorganisms were procured from the microbiology laboratory of our institute, which were originally obtained from Department of Microbiology, Orissa University of Agricultural University, Bhubaneswar.

Antibacterial and antifungal activities were studied by agar disc diffusion method[16] and determination of minimum inhibitory concentration (MIC) by broth dilution method[17]. The zone of inhibition of the fractions was performed at concentrations of 2, 5 and 10 mg/ml of the fractions in dimethyl sulphoxide (DMSO). Ciprofloxacin (5 μg/ml) and clotrimazole (25 μg/ml) were used as reference controls for the antibacterial and antifungal studies, respectively. Solvent control (only DMSO) was also maintained throughout the experiment. MIC was performed at concentrations of the fractions ranging from 25 to 800 μg/ml in DMSO against all the test microorganisms.

The ethyl acetate fraction was found to be more potent than the n-butanol fraction. Zone of inhibition study

TABLE 1: ZONE OF INHIBITION OF VARIOUS FRACTIONS OF ETHANOL EXTRACT OF *B. MONNIERI* AERIAL PARTS

Micro-organisms	EAF (mg/ml)			NBF (mg/ml)			Standards[a]
	2	5	10	2	5	10	
Gram-positive bacteria							
S. aureus ATCC 25923	10.7	15.0	20.7	8.3	15.3	17.7	27.3
B. subtilis UC 564	11.7	18.7	22.0	8.7	14.3	15.7	25.0
B. polymexia 474	8.7	14.0	17.7	10.3	11.7	15.0	22.3
Streptococcus faecalis	9.3	12.7	16.7	7.0	11.3	14.3	26.7
Gram-negative bacteria							
P. aeruginosa 25619	8.0	11.3	15.3	7.3	9.7	12.3	24.3
S. typhi 57	10.7	18.0	23.3	8.7	13.0	17.3	23.3
V. cholerae 824	10.3	12.7	17.0	7.3	10.7	14.3	22.3
S. dysenteriae ATCC C_3	9.3	14.0	17.7	7.0	11.3	13.0	25.3
E. coli NCTC 8196	9.0	13.7	16.3	7.3	10.7	13.0	21.0
Fungi							
P. notatum ATCC 11625	8.3	12.3	15.7	7.3	11.0	14.7	20.3
A. niger AB 41	8.3	13.0	18.0	7.7	11.7	13.7	23.7
C. albicans ATCC 18804	12.3	18.0	22.3	7.7	12.3	15.7	28.3

EAF is ethyl acetate fraction and NBF is n-butanol fraction. [a]Standards: antibacterial studies- ciprofloxacin (5 μg/ml); antifungal studies- clotrimazole (25 μg/ml). Values are zone of inhibition (mm) and mean of 3 readings.

TABLE 2: MIC OF VARIOUS FRACTIONS OF ETHANOL EXTRACT OF *B. MONNIERI* AERIAL PARTS

Micro-organisms	EAF	NBF
Gram-positive bacteria		
S. aureus ATCC 25923	50	100
B. subtilis UC 564	25	200
B. polymexia 474	50	100
Streptococcus faecalis	100	400
Gram-negative bacteria		
P. aeruginosa 25619	200	600
S. typhi 57	25	100
V. cholerae 824	100	200
S. dysenteriae ATCC C_3	50	400
E. coli NCTC 8196	100	400
Fungi		
P. notatum ATCC 11625	200	600
A. niger AB 41	100	400
C. albicans ATCC 18804	50	200

EAF is ethyl acetate fraction and NBF is n-butanol fraction. [a]Values are minimum inhibitory concentration, MIC (µg/ml)

reveals that both the fractions showed antibacterial and antifungal activity in a dose dependant manner and was comparable with the standard drugs (Table 1). The MIC results showed that both the fractions have antibacterial and antifungal activity against the tested strains. MIC value ranges from 25 to 200 µg/ml for ethyl acetate fraction and 100 to 600 µg/ml for n-butanol fraction. The results also reveal that both the fractions were more active against *B. subtilis* and less active against *P. aeruginosa*, which is naturally resistant to antibacterial agents[18] (Table 2).

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