Original Article

Journal of Phytology 2014, 6: 1-6
http: //scienceflora.org/journals/index.php/jp/

Ethnomedicinal and antimicrobial screening of *Bacopa monnieri* (L.) pennell

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Received: 10.10.2011
Revised: 12.02.2012
Accepted: 25.03.2012

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ABSTRACT

The present study evaluates the ethnomedicinal importance and anti-microbial activity of *Bacopa monnieri* (L.), a creeping herb, belonging to *Scrophulariaceae* against selected bacterial strains (*Escherichia coli, E. coli* K88, *Klebsiella pneumoniae*, *Proteus vulgaris, Bacillus subtilis* UC 564, *Bacillus pumilus* 8241, *Bacillus licheniformis, Staphylococcus aureus* ATCC 6571, *Streptococcus faecalis* 52, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa*, *Salmonella typhii* 62, *Shigella dysenteriae* 3, *Shigella flexneri* E0 3429, and *Shigella sonnei* E0 8869) and fungal cultures of *Aspergillus niger* and *Candida albicans*. Methanol extract has maximum (24 ± 0.5) inhibitory effect against *S. aureus* ATCC 6571 at a concentration of 15 mg/ml, which is followed by *S. faecalis* 52 (22 ± 0.0) and *K. pneumoniae* (22 ± 0.2) at the same concentration.

KEY WORDS: Antimicrobial activity, Bacopa, traditional use

INTRODUCTION

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Sujatha, 2005). The phytochemical research based ethnopharmacological information is, generally, considered an effective approach to the discovery of new anti-infective agents from higher plants. Since 1990s, there has been a growing shift in interest toward plants as a significant source for new Pharmaceuticals (Dymock *et al.*, 1986). Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs, which have adverse side effects. As per the World Health Organization report, 80% of the world population presently uses herbal medicine for some aspect of primary health care.

With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity (Colombo *et al.*, 1996). Many herbs contain dozens of active constituents that combine to give the plant its therapeutic value. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.*, 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannin, and phenolic compounds (Edeoga *et al.*, 2005).

Rural communities or tribes depend on plant resources mainly for herbal medicine, food, forage, making household implements, and for fire and shade. The use of medicinal plants as traditional medicines is well known in the rural areas of many developing countries (Gupta *et al.*, 2005). Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas *et al.*, 2006).

One of the important medicinal plants is *Bacopa monnieri* (Brahmi) belonging to family Scrophulariaceae, a well-known nootropic herb. Bacopa is a creeping, glabrous, succulent herb rooting at nodes whose habitat includes wetlands and muddy shores. Stem 10-30 cm long, 1-2 mm thick, soft, glabrous; branches ascending. Leaves 0.6-2.5 cm long and 3-8 mm broad, sessile, obovate-oblong or spatulate, entire, nerves obscure and lower surface dotted, flowers blue or white with purple veins, axillary and solitary on long pedicles and capsule ovoid

glabrous, up to 5 mm long. The plant being traditional Ayurvedic medicine used for centuries as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative, and antiepileptic agent.

Its pharmacological activities have been documented by many workers as anti-oxidant action in frontal cortex, striatum and hippocampus on the cognition-facilitating action (Tripathi *et al.,* 1996; Bhattacharya *et al*., 2000), cardiotonic properties (Jain *et al*., 1994), anti-cancer properties against walker carcinosarcoma 256 intramuscular in rat (Bhukani *et al*., 1969) and anti-inflammatory and analgesic activities in rodents (Jain *et al.,* 1994). The herb contains the alkaloids brahmine, herpestine ($C_{34}H_{46}N_2O_6$ mp 116-17°), and a mixture of three bases. The herb also contains the saponins, monnierin ($C_{51}H_{82}O_{213}H_2O$, mp 262-63°); hersaponin (mp 232-34° [decomp]), bacoside A ($C_{41}H_{68}O_{13}.5H_2O$, mp 250° [decomp]) and bacoside B ($C_{41}H_{68}O_{13}.5H_2O$, mp 203° [decomp]). Monnierin, on hydrolysis, gave glucose, arabinose and an aglycone ($C_{30}H_{48}O_4$, mp 235-37°) whereas, bacosides A and B gave glucose, arabinose and bacogenins A_1, A_2, A_3, and A_4; bacogenins A_1 and A_2 are epimers, and A_4 is an ebelin lactone. Other constituents present in the plant are D-mannitol, betulic acid, B-sitosterol, stigmasterol and its esters, heptacosane, octacosane, nonacosane, triacontane, hentriacontane, dotriacontane, nicotine, 3-formyl-4- hydroxy-2H-pyran ($C_6H_6O_3$), luteolin and its 7-glucoside. The presence of a-alanine, aspartic acid, glutamic acid and serine is also reported (Rastogi *et al*., 1994).

A new triterpenoid saponin, bacoside A_3, a constituent of bacosides, the saponin mixture of Brahmi was isolated and characterized. Its structure was established as 3-beta-{O-beta-D- glucopyranosy1 (1 to 3)-O-{alpha-L arabinofuranosy1 (1 to 2)} –O-beta-D-glucopyranosy1) oxy} jujubogenin by chemical and spectral analysis. The cis-isomer of ebelin lactone was also obtained as one of the artifacts of the aglycone.

Three new dammarane-type triterpenoid saponins, bacosaponins A, B and C, of biological interest have been isolated from *B. monnieri* and identified as 3-O-alpha-L- arabinopyranosyl-20-O-alpha-L-arabinopyranosyl-jujubogenin, 3-O-{alpha-arabinofuranosyl (1 to 2) alpha-L-arabinopyranosy1} Pseudojujubogenin and 3-O-{beta-D-glucopyranosy1 (1 to 3)-{alpha-L-arabinofuranosy1 (1 to 2)} alpha-L-arabinopyranosy1} pseudojujubogenin by spectroscopic methods and some chemical transformations.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plants were collected from regional areas of Jaipur and authenticated by taxonomist. The leaves were shade dried then coarsely powdered.

Solvent Extraction

The dried leaves were powdered with the help of waring blender then powder was filled in thimble and extracted successively with methanol solvent in a soxhlet extractor for 48 h. The crude extracts were concentrated using vacuum evaporator.

Antimicrobial Screening

All bacterial strains of (*Escherichia coli, E. coli* K 88, *Klebsiella pneumoniae*, *Proteus vulgaris, Bacillus subtilis* UC 564, *Bacillus pumilus* 8241, *Bacillus licheniformis, Staphylococcus aureus* ATCC 6571, *Streptococcus faecalis* 52, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa, Salmonella typhi* 62, *Shigella dysenteriae* 3, *Shigella flexneri* E0 3429, *Shigella sonnei* E0 8869) and fungal cultures of *Aspergillus niger* and *Candida albicans* were obtained from S.M.S. Medical College and Microbiology Laboratory, Department of botany, university of Rajasthan, Jaipur respectively. The bacteria were maintained on nutrient broth at 37°C, and fungus was maintained on potato dextrose agar (PDA) at 28°C.

Ethnobotanical Survey

The ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through questionnaire, interviews, and discussions among the tribal practitioners in their local language (Table 1).

Antibacterial Activity

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method (Rios *et al*., 1988). Briefly overnight bacterial cultures were diluted in the Mueller-Hinton broth (OD 600 = 0.08) to obtain a bacterial suspension of 10^8 colony forming units (CFU)/ml. Petri plates containing 20 ml of Mueller-Hinton agar (MHA) were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Filter paper discs of Whatman no. 1 (6 mm diameter) were impregnated with 50 µl, 100 µl, and 150 µl of the extract which is equivalent of 5, 10, and 15 mg/ml, were placed on the inoculated agar surface and allowed to dry completely. Standard antibiotic

Table 1: Ethno-medicinal importance of *B. monnieri* in Rajasthan

Part used	Mode of preparation	Disease treated
Whole plant	Plant paste used three times daily in treatment of blisters	Blisters
	Plant juice along with ginger, sugar and bark extract of *Moringa oleifera* given to children in stomach disorders	Stomach disorders
	Joyawake tea (combination of *B. monnieri* and *Camellia sinensis*) is considered as main rejuvenating herb for nerve and brain cells	Nervine tonic
	Plant juice (8 ml) or 1/2 g of plant powder once a day has been traditionally used to Increase the speed of learning and extent of memory power	Memory enhancement
Leaves	Eaten for intellect promoting	Memory enhancement
	Fried in ghee, taken to get rid of hoarseness of vic of voice	Hoarseness of voice
	Its Ghrita or medicated ghee is given with Pushkar Amul (*Sauseria* lappa's root)	Memory enhancement
	Powdered leaves about 5 g with 2 or 3 black pepper are given in a single dose in bone fracture	Bone fracture
	Leaf paste used 3 times daily in swelling in legs of animals for one week	Swelling of legs
	Leaves and stem are boiled in water, filtered about 100 ml filtrate taken orally twice a day for 5-10 days as an anti-asthmatic drug	Asthma
	Warmed paste applied on abdomen to cure abdominal pain, besides this used for curing urinary duct inflammations	Urinary duct inflammations
	Juice of leaves conjoined with petroleum used as a local application in rheumatism	Rheumatism
	Leaves of brahmi and seeds of *Piper longum*	Memory
	Together with almond are ground in water and sugar taken orally for memory enhancement	Enhancement
Root	Fresh root decoction used as antidote in snake-bite	Snake-bite
	Scorpion stings	
	Root juice mixed with milk is given 3 times daily in weakness and rheumatism	Rheumatism
	Dried root and fruit powder burnt and inhaled as smoke 3 times daily in bronchitis	Bronchitis

B. monnieri: Bacopa monnieri

Streptomycin (20 µg) placed as controls. Plates were incubated at 37°C for 24 h. The same procedure was followed for the fungus also. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicates.

Determination of Minimum Inhibitory Concentration (MICs)

A MICs is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their MIC. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution of Mueller-Hinton broth to obtain concentrations from 100 to 0.19 mg/ml. Streptomycin was placed as control-A 10 µl of 10^7 (CFU) bacterial cultures were added to the tubes and were incubated at 37°C for 18 h. MICs were determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the MIC.

Preparation of the Inoculums

Stock cultures were maintained at 4°C on nutrient broth. Active cultures for experiment were prepared by transferring a loopful of cells from the stock cultures to the test tubes of MHA for bacteria and potato dextrose broth (PDB) for fungi that were incubated without for agitation for 24 h at 37°C and 25°C, respectively. The cultures were diluted with fresh Mueller-Hinton and PDB to achieve optical densities corresponding to 0.2 × 102 CFU/ml for bacteria and 2.0×10^5 spore/ml for fungal strains.

Statistical Analysis

The data of all the parameters were statistically analyzed (Statistical Software used Minitab 14-state College, PA, USA) and zone of inhibition diameter values are expressed as mean diameter ± standard error mean ($n = 3$).

RESULTS AND DISCUSSION

In Rajasthan, various ethnic groups are widely distributed throughout the state and have considerable communication with each other. As a result, most of the ethnobotanical information is passed by one group to others. Tribals possess a great deal of knowledge of these medicinal plants about their identifications, characteristics, habitat and uses and can be of great help, if involved, for further studies about their research. These tribal are not only engaged in collection, processing and marketing of medicinal herbs to augment their exiguous income but use them to cure a number of disease in interior villages where high standard medical facilities are not available (Bhattacharya *et al.*, 2000). The knowledge of indigenous people is invaluable in the ethnobotanical data, so there is urgent need to inventories and records all ethno-biological information among the diverse ethnic communities before these are completely lost.

In Rajasthan, ethnobotanical work has been done immensely by many workers: Ethnobotanical survey of the Bhils (Joshi, 1982); Medicinal plants of Ajmer forest division (Dixit

and Mishra, 1976); Medico-ethnobotany of Mount-Abu (Sebastian and Bhandari, 1984); Medicinal plantlore of the tribals of eastern Rajasthan (Singh and Pandey, 1982); Ethnobotanical uses of plant species used by Kathodias a monkey eating tribe (Joshi, 1993). Some plants in folk medicine of Udaipur district (Katewa and Arora, 1997); Ethnobotany of primitive tribes of Rajasthan (Joshi,1995); Medicinal plants for skin and hair care (Sharma *et al*., 2003); Ethnobotany of Rajasthan (Singh and Pandey, 1998).

Bacopa forms an important ingredient of a number of Ayurvedic preparations such as Brahmighritam, Brahmirasayanam, etc (Iyer, 1983). The juice of leaves is given to children for relief in bronchitis and diarrhoea (Malhotra and Moorthy, 1973). An oil is also prepared with this drug, used in habitual headaches, to relieve brain-fag. The whole plant is used for therapeutic and medicinal purposes to treat various diseases and disorders (Table 1). Brahmi is considered by Hindu physicians a nervine tonic useful in insanity, epilepsy and hoarseness (Shah *et al*., 1983). In Pondicherry, Brahmi is considered as an aphrodisiac while in Shrilanka, under the name of Loonooweela, prescribed in fever, used as mild purgative for children and in curing erysipeales and elephantiasis. In the Phillipines, it is used as a diuretic (Uphof, 1968). The juice of fresh stem and leaves is given internally for snake-bite (Kirtikar and Basu, 2001).

In the recent years, Memory plus, a product that contains the standardized extract of bacosides from Brahmi, has been marketed in India. Brahmi has been used in Ayurvedic formulations for conditions ranging from catarrhal complaints, gastrointestinal disturbances due to excessive tobacco use, habitual abortions and high blood sugar due to anxiety disorders and epilepsy (Chopra *et al.,* 1994; Kirtikar and Basu, 2001; Nadkarni, 1954; Satyawati *et al*., 1987). In Ayurveda, *B. monnieri* is described as medhya rasayan or brain tonic with the ability to promote mental functioning along with providing general rejuvenative effects (Martis *et al*., 1992). Therapeutic usage of Bacopa is mentioned in Ayurveda as it cures skin disorders including leprosy, anemia, urinary disorders, bronchitis, edema and fever (Dash, 1991). According to Atharvaveda, it strengths body and improves quality of semen. In Charak Samhita, it is mentioned for invigorating and life-sustaining tonic (uniyal, 1968). According to Ayurvedic concepts, plant is bitter, diuretic, blood purifier and invigorates sex. Extract of Bacopa is useful in purification of blood, boils and epilepsy (Uniyal and Chauhan, 1973) rheumatism (Shah *et al*., 1983), stomach complaints (Shah and Menon, 1981), cough and cold (Malhotra and Moorthy, 1973), insanity (Tiwari and Majumdar, 1979), eczema and expelling of ringworm (Saxena and Vyas, 1983), liver complaints (Bhatt and Sabnis, 1987), mental disorders (Shah *et al*., 1983) and abdominal pain (Joshi, 1982) neuralgia (Warrier *et al*., 1993), tumors, makes the voice clear (Aiyer and Kolammal, 1964; Kurup *et al*., 1979) and promotes sleep (Anonymous, 1990; Kurup *et al*., 1979).

In Agnipurana, Bacopa is mentioned as poison killer and its juice is effective against epilepsy (Uniyal and Chauhan, 1973). In Unani system of medicine, this plant is used in the treatment of cold and cough (Malhotra and Moorthy, 1973). It is digestive and useful in splenomegaly, skin diseases, fever and cures convulsions (Warrier *et al*., 1993; Aiyer and Kolammal, 1964). It is anodyne, carminative, anti-inflammatory, depurative, diuretic, emmenagogue, sudorific, febrifuge and tonic and used in biliousness, amentia, dyspepsia, flatulence, constipation, asthma, leucoderma, erysipeales, syphilis, hoarseness, dysmenorrhea, sterility and general debility (Warrier *et al*., 1993; Kirtikar and Basu, 2001). Bacopa is also used for enhancing the power of speech, arresting process of ageing and overcoming condition of stress (Sarin, 1996).

Table 2 summarizes the microbial growth inhibition of methanol extract of *B. monnieri* the selected plant species. Fifteen bacterial strains were targeted for screening of antibacterial properties. Various bacterial strains produced different zone diameter (mm) in their respective MIC in comparison with streptomycin (reference drug). MIC values have been represented in Table 3. Methanol extract has maximum (24 ± 0.5) inhibitory effect against *S. aureus* ATCC 6571 at a concentration of 15 mg/ml, which is followed by *S. faecalis* 52 (22 ± 0.0) and *K. pneumoniae* (22 ± 0.2) at the same concentration.

The results also showed maximum antifungal activity against *C. albicans* with an inhibition zone of 15 ± 0.5 mm diameter which was followed by *A. niger* at inhibition zone of 14 ± 0.1 mm diameter at extract concentration of 15 mg/ml. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Yadav *et al*., 1976). Antimicrobial activity from plant source can be assumed to be useful. The extract produce anti-infective agent, which could be active against human pathogens (Kumar *et al*., 2006). Apart from antimicrobial activity exhibited by tannins, they also lead with proteins to provide the typical turning effect. Medicinally, this is important for the treatment of inflamed tissues (Mota *et al*., 1985). Several flavonoids and phenolic

Table 2: Inhibitory effect of methanol extract of *B. monnieri* against pathogenic

Micro-organisms	Zone of inhibition in difference concentrations (mm)				
	5 mg/ml	10 mg/ml	15 mg/ml	20 μg/ml	
				Strepto.	Keto.
Bacillus	12±0.1	13±0.5	15±0.5	17±0.5	-
Licheniformis					
Bacillus pumilus 8241	12±1.1	13±0.5	16±0.5	18±0.2	-
Bacillus subtilis UC 564	10.5±0.5	12±0.5	13±0.5	16±0.4	-
Escherichia coli	13±0.4	15±0.5	17±0.2	19±0.5	-
Escherichia coli K 88	10±0.2	12±0.5	13±0.4	15±0.1	-
Enterococcus faecalis	16±0.5	17±0.1	19±0.5	22±0.5	-
ATCC 29212					
Klebsiella pneumoniae	19±0.5	20±0.5	22±0.2	24±0.2	-
Proteus vulgaris	12±1.1	13±0.1	15±0.5	16±0.3	-
Pseudomonas aeruginosa	10.5±0.5	12±0.0	13±0.2	15±0.5	-
Staphylococcus aureus	21±0.5	22±0.5	24±0.5	26±0.2	-
ATCC 6571					
Streptococcus faecalis 52	19±0.5	20±0.2	22±0.0	24±0.1	-
Salmonella typhii 62	12±0.2	13±0.5	13±0.2	15±0.2	-
Shigella dysenteriae 3	11±0.1	12±0.5	16±0.5	18±0.5	-
Shigella flexneri E0 3429	12±0.2	13.5±0.2	15±0.2	16±0.2	-
Shigella sonnei E0 8869	18±0.1	19±0.1	20±0.5	22±0.4	-
Aspergillus niger	9±0.2	11±0.5	14±0.1	-	18±0.2
Candida albicans	11±0.5	12±0.2	15±0.5	-	20±0.5

B. monnieri: Bacopa monnieri

Table 3: MICs of selected tested micro-organisms against *B. monnieri*

Leaf extract using disc diffusion method	
Microorganism	MIC (mg/ml)
Bacillus	>2
Licheniformis	
Bacillus pumilus 8241	0.25
Bacillus subtilis UC 564	0.8
Escherichia coli	0.06
Escherichia coli K 88	0.12
Enterococcus faecalis	>2
ATCC 29212	
Klebsiella pneumoniae	0.12
Proteus vulgaris	0.5
Pseudomonas aeruginosa	0.5
Staphylococcus aureus	0.25
ATCC 6571	
Streptococcus faecalis 52	0.03
Salmonella typhii 62	0.35
Shigella dysenteriae 3	0.5
Shigella flexneri E0 3429	0.06
Shigella sonnei E0 8869	0.15
Aspergillus niger	0.5
Candida albicans	0.62

B. monnieri: Bacopa monnieri, MIC: Minimum inhibitory concentration

acids may present which exhibit interesting antimicrobial properties. Aqueous extract of different concentrations shows no inhibitory effects on the tested microorganisms due to loss of some active compounds during extraction processes of the sample (Iwu *et al*., 1999). Despite many published reports dealing with treatment for neurological disorders little is known about antimicrobial activity of *B. monnieri* prior to our investigation. Further studies on the activity directed fractionation for isolation of respective pure compounds results in interesting results.

ACKNOWLEDGMENTS

The authors are thankful to the tribal people for sharing their knowledge on medicinal plant.

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