International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 8, Issue 2, 2016

Original Article

ANTIMICROBIAL SCREENING OF THE LEAF EXTRACTS OF *BACOPA MONNIERI* (L) PENNELL

MAYA VERMA

Department of Botany, University of Rajasthan, Jaipur
Email: maya25verma@yahoo.com

Received: 20 Jan 2016, Revised and Accepted: 14 Mar 2016

ABSTRACT

Objective: To investigate the antibacterial efficacy of leaves of *Bacopa monnieri* (L.) Pennell

Methods: The present study evaluates the anti-microbial activity of *Bacopa monnieri* against selected bacterial strains (*E. coli, E. coli* K 88,*Klebsiella pneumoniae*, *Proteus vulgaris, Bacillus subtilis* UC 564,*B. pumilus* 8241,*B. licheniformis, Staphylococcus aureus* ATCC 6571,*Streptococcus faecalis* 52,*Enterococcus faecalis* ATCC 29212,*Pseudomonas aeruginosa, Salmonella typhii* 62,*Shigella dysenteriae* 3,*S. flexneri* E0 3429,*S. sonnei* E0 8869) and fungal cultures of *Aspergillus niger* and *candida albicans* using disc diffusion assay.

Results: Methanol extract has maximum (24±0.5) inhibitory effect against *Staphylococcus aureus* ATCC 6571 at a concentration of 15 mg/ml,which is followed by *Streptococcus faecalis* 52 (22±0.0) and *Klebsiella pneumoniae* (22±0.2) at the same concentration.

Conclusion: The results showed maximum antifungal activity against *C. albicans* which was followed by *A. niger*.

Keywords: Bacopa, Antimicrobial activity, Disc diffusion

INTRODUCTION

The phytochemical research based ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) reports that about 80 % of the world's population depends on traditional medicines to meet at least some of their primary healthcare [1]. Plants produce an incredible array of secondary metabolites, and many of these have been developed into economically important products including oils, gum, resins, tannins, rubber, waxes, pigments, flavors, fragrances, surfactants, preservatives, pesticides, and pharmaceuticals [2]. Historically plants have provided a good source of anti-infective agent. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements [3]. Infectious diseases account for approximately one-half of death in tropical countries. The incidence of epidemics due to drug resistant microorganisms and emergence of hitherto unknown pathogenic microbes pose enormous public health concerns. Since the 1990s there has been a growing shift in interest towards plants as a significant source for new Pharmaceuticals [4]. 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 (WHO) report, 80 % of the world population presently use herbal medicine for some aspect of primary health care [5].

With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity. 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. 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 [6].

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 are 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.

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 a thimble and extracted successively with methanol solvent in a soxhlet extractor for 48hr. The crude extracts were concentrated using vacuum evaporator.

Antimicrobial screening

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

Antibacterial activity

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method [7]. Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O. D. 600 = 0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of

Mueller hinton agar 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 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.

Table I: Inhibitory effect of methanol extract of *B. monnieri* against pathogenic micro-organisms

	Zone of inhibition in dif. concentrations (mm)				
				Strepto.	Keto.
micro-organism	**5 mg/ml**	**10 mg/ml**	**15 mg/ml**	**20µg/ml**	**20µg/ml**
Bacillus Licheniformis	12±0.1	13±0.5	15±0.5	17±0.5	-
B. pumilus 8241	12±1.1	13±0.5	16±0.5	18±0.2	-
B. subtilis UC 564	10.5±0.5	12±0.5	13±0.5	16±0.4	-
E. coli	13±0.4	15±0.5	17±0.2	19±0.5	-
E. coli K 88	10±0.2	12±0.5	13±0.4	15±0.1	-
Enterococcus faecalis ATCC 29212	16±0.5	17±0.1	19±0.5	22±0.5	-
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 ATCC 6571	21±0.5	22±0.5	*24±0.5*	26±0.2	-
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	-
S. flexneri E0 3429	12±0.2	13.5±0.2	15±0.2	16±0.2	-
S. sonnei E0 8869	18±0.1	19±0.1	20±0.5	22±0.4	-
A. 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

Determination of minimum inhibitory concentration (MICs)

A minimum inhibitory concentration (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 minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution of Mueller-Hinton broth to obtain concentrations from 100 mg/ml 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 was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration.

Table II: MICs of selected tested micro-organisms against *B. monnieri* leaf extract using disc diffusion method

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

Preparation of the inoculums

Stock cultures were maintained at 4 ℃ on nutrient broth. Active cultures for the experiment were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton agar (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 potato dextrose broth to achieve optical densities corresponding to 2.0. 10^6 colony forming units (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±SEM (n= 3)

RESULTS AND DISCUSSION

Table 1 summarizes the microbial growth inhibition of methanol extract of *B. monnieri* the selected plant species. Fifteen bacterial strains were targeted for the 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 2. Methanol extract has maximum (24±0.5) inhibitory effect against *Staphylococcus aureus* ATCC 6571 at a concentration of 15 mg/ml, which is followed by *Streptococcus faecalis* 52 (22±0.0) and *Klebsiella 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. Antimicrobial activity from plant source can be assumed to be useful. The extract produces anti-infective agent which could be active against human pathogens [8]. 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 [9]. Several flavonoids and phenolic acids may present which exhibit interesting antimicrobial properties. Aqueous extract of different concentrations shows no inhibitory effects on the tested micro-organisms due to loss of some active compounds during extraction processes of the sample. Despite many published reports dealing with

treatment for neurological disorders little is known about antimicrobial activity of *Bacopa monnieri* prior to our investigation. Further studies on the activity directed fractionation for isolation of respective pure compounds results in interesting results.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. World Health Organisation. Traditional medicine strategy, WHO, Geneva Switzerland; 2004.
2. Raven PH, RF Evert, SE Eichhorn. Biology of plants. W. H. Freeman and company: New York; 2005.
3. Hussain J, Khan AL, Rehman N, Zainullah, Hussain ST, Khan F, *et al*. Proximate and nutrient analysis of selected medicinal plant species of Pakistan. Pakistan J Nutr 2009;8:620-4.
4. Balasundaram N, Sundaram, Samman S. Phenolic compounds in plant and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 2006;99:199-203.
5. Uniyal MR. Medicinal plants of bhagirathi valley are lying in Uttarkashi forest division. Indian Forester 1968;94:407-20.
6. Yadav SK, AK Jain, SN Tripathi, JP Gupta. Irritable bowel syndrome: therapeutic evaluation of Indigenous drugs. Indian J Med Res 1989;90:496-503.
7. Rios JL, MC Recio, A Villar. Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol 1988;23:127-49.
8. Gupta MP, PN Solis, AL Calderon, F Guionneau-Sinclair, M Corsea, C Galdemos, *et al*. Medical ethnobotany of the tribes of bocas del toro, panama. J Ethnopharmacol 2005;96:389-401.
9. Mota MLR, G Thomas, JM Barbosa Filho. Anti-inflammatory actions of tannins isolated from the bark of Anacardium occidentale (L). J Ethnopharmacol 1985;13:289-300.