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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF WHOLE PLANT EXTRACTS OF *BACOPA MONNIERI* (L.) PENNELL

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ABSTRACT: The aim of this study was to screen various solvent extracts of whole plant of *Bacopa monnieri* to investigate potent antioxidant activity in vitro, antimicrobial activity against selected microorganisms and total phenolic contents..Sevan bacterial strains (*E.coli* K 88, *Staphylococcus aureus* ATCC 6571, *Streptococcus faecalis* 52, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa*, *Salmonella typhii* 62, *Shigelladysenteriae* 3 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).The highest zone of inhibition against *Pseudomonas aeruginosa* (22.5±0.02) followed by *Shigella* (21.4±0.01), *E.coli K* 88 (20.1±0.12) noted in the case of methanolic extracts. Minimum zone of inhibition was seen in *E.feacalis* ATCC 29212 (16.2±0.01).The IC_{50} value of methanol extract was 46.3 µg/ml, n-hexane and aqueous extracts with value of 76.0 µg/ml and that of aqueous extract was 82.0 µg/ml. The total phenolic contents of all the fractions were also determined. The total phenolic content 120.7 mg GAE/g extract was found significantly higher as compared to other solvent fractions. Data from present studies results revealed that *B.monnieri* act as an antioxidant agent due to its free radical scavenging activity.

Key words: DPPH, Antioxidant, antimicrobial activity

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INTRODUCTION

Oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Young and Woodside, 2001). A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh et al., 1999). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sudarajan et al., 2006). The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao et al., 1996).

Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, carcinoma and parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources (Halliwell and Gutteridge,1990). Some of the in vivo free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be damaging. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources (Rimbach et. Al,2005). Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals (Kahkonen et.,al,1999). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Proestos,2006).

Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases (Lai et.al.,2001). There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants. Hence, the present study was aimed for screening of antimicrobial and antioxidant activity *Bacopa monnieri*.The*Bacopa monnieri* (L.)Scrophulariaceae, is a creeping, glabrous, succulent herb, rooting at nodes whose habitat include 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 spathulate, entire, nerves obscure and lower surface dotted, flowers blue or white with purple veins, axillary and solitary on long pedicles and capsule ovoid glabrous, upto 5 mm long..

Brahmi, is an important Medhyarasayan (Brain Tonic) in Ayurveda for improvement of intelligence, memory & revitalization of sense organs. It is also capable of imparting youthful vitality and longevity. The drug is reported to be used in cold, sweat, cooling, laxative, intellect promoting, carminative, digestive, anti-inflammatory, anticonvulsant, cardiotonic, bronchodialator and tonic. It is useful in epilepsy, amentia, ulcer, constipation, asthma, sterility, fever and general debility. It helps to regain general mental health through its rejuvenative effect (Bone,1996; Anonymous,1997; Anonymous,1999). In India and the tropics. *B. monnieri* grows naturally in wet soil, shallow water and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet and is easily cultivated adequate water is available. The entire plant is mainly used. The pharmacological studies showed that *Bacopa monnieri* possessed many pharmacological effects included central nervous effects (memory enhancement , antidepressant , anxiolytic , anticonvulsant and antiparkinsonian) , antioxidant , gastrointestinal , endocrine , antimicrobial , anti-inflammatory , analgesic , cardiovascular and smooth muscle relaxant effects. The present work focused on antimicrobial and antioxidant effects of *Bacopa monnieri*.

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 soxhlet extractor for 48hr.The crude extracts were concentrated using vacuum evaporator.

Antimicrobial screening

All bacterial strains of (*E.coli* K 88,*Staphylococcus aureus* ATCC 6571,*Streptococcus faecalis* 52,*Enterococcus faecalis* ATCC 29212,*Pseudomonas aeruginosa*, *Salmonella typhii* 62,*Shigella dysenteriae* 3) 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 37º C.

Antibacterial activity

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method .Briefly overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600 = 0.08) to obtain a bacterial suspension of 108 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 (6mm diameter) were impregnated with 50µl,100µl and 150µl 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 37º C for 24 hrs.. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicates.

Determination of minimum inhibitory concentration (MICs)

A minimum inhibitory concentration (MICs) is the lowest concentration of an antimicrobial that inhibits growth of a microorganism after 18-24 hrs. 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 a concentrations from 100mg/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 37º C for 18 hrs. MICs was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration.

Preparation of the inoculums

Stock cultures were maintained at 4ºC on nutrient broth. Active cultures for experiment were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton agar (MHA) for bacteria that were incubated without for agitation for 24 hrs at 37º C and 25º C respectively. The cultures were diluted with fresh Mueller-Hinton to achieve optical densities corresponding to 2.0· 106 colony forming units (CFU/ml) for bacteria strains.

Antioxidant assay

Each sample was dissolved in 95% methanol to make a concentration of 1 mg/ml and then diluted to prepare the series concentrations for assays. Reference chemicals were used for comparison in all assays

DPPH radical scavenging activity assay

The free radical scavenging activity of the fractions was measured in vitro by 2,2'- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier (Williams et al,1995;Bursal and Gulcin,2011).The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20 C until required. The working solution was prepared by diluting DPPH solution with methanol to attain an absorbance of about 0.89±0.01 at 517 nm using the spectrophotometer. A 3 ml of aliquot of this solution was mixed with 100 µl of the sample at various concentrations (10 – 500 µg/ml).The reaction mixture was shaken well incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517nm.The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = [(control absorbance – sample absorbance)/(control absorbance)] × 100

Estimation of total phenolic content

The total phenolic content was determined by the spectrophotometric method (Kim et al,2003).According to this 1 ml of sample (1mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes,10 ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min.at 23º C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing bu gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligram of gallic acid equivalent (GAE) per g of dried sample.

Statistical analysis

Data are expressed as mean SD from three separate observations. For antioxidant assays one way ANNOVA test followed by Tukey's test ($P < 0.05$) was used to analyse the differences among EC50 of various fractions for different antioxidant assays. The EC_{50} values were determined using the graph pad prism 5 software. Data on biochemical investigations of in vivo experiments were analysed by one way ANNOVA and the group means were compared by Dunnet's multiple range test. A probability of $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Results obtained in the present study revealed that the tested medicinal plant extracts possess potential antimicrobial activity against tested micro-organisms (Table-1).In vitro antimicrobial screening using gentamycin as a positive control clearly indicated that extracts of *B.monnieri* show promising antimicrobial activity against all the selected microorganisms.Table-1 summarizes the microbial growth inhibition of different extracts of *B.monnieri* against the selected bacterial species.Sevan 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 has been represented in the Table 2 .The highest zone of inhibition against *Pseudomonas aeruginosa* (22.5±0.02) followed by *Shigella* (21.4±0.01), *E.coli* K 88 (20.1±0.12) noted in the case of methanolic extracts. Minimum zone of inhibition was seen in *E.feacalis* ATCC 29212 (16.2±0.01).Moderate zone of inhibition was noted in n-hexane and aqueous extracts of plant respectively.

The maximum and minimum zone of inhibition values were observed against *P.aeruginosa* (18.3±0.02) and *S.typhii* 62 (11.4±0.01) respectively in n-hexane extract observations.Active methanolic extract were further evaluated for their MICs .The lowest MICs against *E.coli* K 88 (0.12 mg/ml), *S.faecalis* (0.03 mg/ml) and *S.dysenteriae* 3 (0.5 mg/ml) were recorded. were recorded.Figure.1 shows that the scavenging effects of samples on DPPH radical. The percentage inhibition of DPPH by the various solvent extracts and the standard was recorded in decreasing order. Ascorbic acid>rutin> methanol > n-hexane>aqueous extract. The IC_{50} value of methanol extract was 46.3 µg/ml, n-hexane and aqueous extracts with value of 76.0 µg/ml and that of aqueous extract was 82.0 µg/ml (Table-3).Though the antioxidental potential of fractions was found to be low (P> 0.05) than those of ascorbic acid , the study revealed that methanol and n-hexane have prominent antioxidant activity; the presence of phenolic compounds (containing phenolic hydroxyls) are mainly found in these two fractions and could be attributable to the observed high anti-radical properties of these fractions. Total phenolic contents was estimated by using Folin-Ciocalteureagent. Total phenolic content of the different fractions of *B.monnieri* was solvent dependent and expressed as milligrams of gallic acid equivalents (GAE) equivalent.Table-4 summarizes that total phenolic compounds in fractions varied widely, ranging from 42.0 to 120.7 mg/g expressed as gallic acid equivalents (GAE) .Methanol extract exhibited the highest total phenolic content.

Table-1 : Inhibitory effect of different plant extracts of *B.monnieri*against pathogenic microorganisms

	Zone of inhibition in dif. Concentrations (mm)			
Micro-organism	**methanol**	**n-hexane**	**aqueous**	**Streptomycin**
E.coli K 88	20.1	12.5± 0.5	16.2± 0.4	23.1± 0.1
Enterococcus faecalis	16.2	15± 0.1	14± 0.5	22.2± 0.5
ATCC 29212				
Pseudomonas aeruginosa	22.5	18.3± 0.0	14.2± 0.2	29.2± 0.5
Staphylococcus aureus	19±0.1	16.0± 0.5	15.1± 0.2	23.4± 0.2
ATCC 6571				
Streptococcus faecalis 52	17.4	13.2± 0.2	16.0± 0.0	20.2± 0.1
Salmonella typhii 62	19.6	11.4± 0.5	13± 0.2	22.0± 0.2
Shigelladysenteriae 3	21.4	12.6± 0.5	16.4± 0.5	24.3± 0.5

Table 2-MICs of selected tested micro-organisms against *B.monnieri* leaf extract using disc diffusion method

	MIC (mg/ml)
Micro-organism	
E.coli K 88	0.12
Enterococcus faecalis	>2
ATCC 29212	
Pseudomonas aeruginosa	0.5
Staphylococcus aureus	0.25
ATCC 6571	
Streptococcus faecalis 52	0.03
Salmonella typhii 62	0.35
Shigelladysenteriae 3	0.5

Table-3. DPPH radical scavenging activity of *B.monnieri* extracts

Plant extracts	IC_{50} (µg/ml)
Methanol extract	46.3±0.13
n-hexane fraction	76±0.04
Residual aqueous fraction	82±0.04
Ascorbic acid	17±0.03
Rutin	32±0.01

Table - 4.Total phenolic content of methanol extract and soluble fractions of *B.monnieri*

Plant extracts	Total phenolics
	(mg gallic acid equivalent / g)
Methanol extract	120.7±0.12
n-hexane fraction	54.7±0.31
Residual aqueous fraction	42.0±0.02

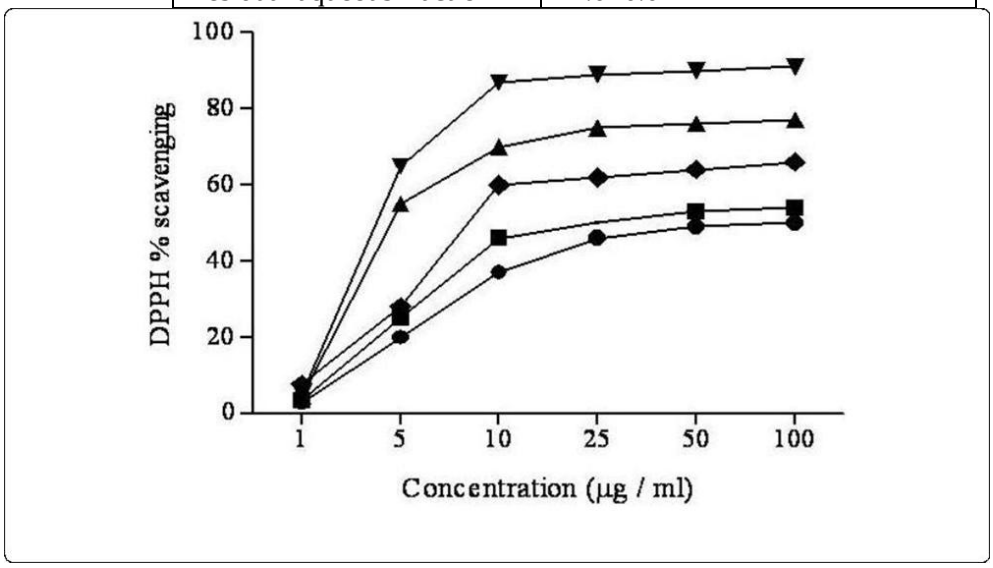

Figure 1. DPPH radical scavenging activities of different plant extracts of *B.monnieri*. Each value represents a mean ± SD (n = 3). Methanol extract (▲), n-hexane(◆), aqueous (▼), Ascorbic acid (●) and rutin (■).

DISCUSSION

Several techniques have been used to determine the antioxidant activity in vitro in order to allow rapid screening of substances since substances that have low antioxidant activity in vitro,will probably show little activity in vivo. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect Us from many diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms.DPPH antioxidant assay is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Krishnaiahet al,1999). In the present study among all the fractions tested, n-butanol, chloroform and ethyl acetate showed significantly higher inhibition percentage and positively correlated with total phenolic content. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Kahkonen et al, 1999). Phenolic compounds are considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified (Naczk and Shahidi,2004).The methanol extract exhibited the highest total phenolics content, whereas the contents obtained with residual aqueous fraction were much smaller that is in agreement with other reports (Aoc et al, 2008). Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability. The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. In the present study analysis of free radical scavenging activity and total phenolic content showed that methanol extract of *B.monnieri* can be the potent source of natural antioxidants.

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