



IN VITRO PHYTOCHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF CRUDE EXTRACT OF *BACOPAMONNIERA*

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Article Info:

Received: 18/07/2013

Revised on : 25/08/2013

Accepted on: 29/08/2013

ABSTRACT

The antibacterial activity of *Bacopa monniera* was screened for different bacterial strains using methanol, ethanol, chloroform and petroleum ether. The phytochemical screening was carried out to know the compounds responsible for these activities. Methanol, ethanol and chloroform extract was tested against *Bacillus amyloliquefaciens* (MTCC 1270), *Streptococcus pyogenes* (MTCC 1923), *Vulgarica*, *Bacillus megaterium* (MTCC 3353), *Aspergillus niger* (MTCC 281), *Bacillus pumilis*, *Salmonella typh*e, *Bacillus subtilis*, *Micrococcus luteus*. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to microorganism and extracting solvent. In most of the above mentioned plants, the methanol extract produced the highest activity. On the basis of the results obtained, it could be concluded that methanol could be used for extracting antimicrobial compounds from Leaves.

Key words: anti-bacterial activity, photochemical screening, *Bacopa monniera*

INTRODUCTION

Nature has been a supply of medicinal agents since thousands of years and providing various drugs to the modern world. It is estimated that 80% of the global population rely on plant derived medicines to address their health care needs [1]. Of the 250,000 to 500,000 known plant species, very few have been investigated for their pharmacological qualities, and compounds of significant medicinal value may still remain undiscovered in many plant species [2]. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [3]. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a

short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains [4]. Herbal medicine is still the main stay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [5]. *Bacopa monnieri* (L) Penn. (family: Scrophulariaceae) commonly known in both India and Bangladesh as 'Brahmi' is an ancient and renowned medicinal plant with legendary reputation as a memory vitalizer [6], in the traditional system of medicine (Ayurveda). In India, infectious diseases accounts for high

proportion of health problems. Morbidity and mortality due to these infections continues to be a major problem, especially amongst children. Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Shigella* sp., *Salmonella* sp., *Enterobacter* sp. are most common [7]. In Ayurveda, *B. monnieri* have been used in the treatment of insanity, epilepsy, hysteria and skin diseases. In this plant possess various biologically active compounds besides alkaloids, saponins and sterols. As well as possess various biological activities such as antioxidant, anticancer and cytotoxic activity, antidepressant activity, anti ulcerogenic, anxiolytic activity, anticonvulsive action, hepatoprotective, anti allergic conditions, neuropharmacological activity and antimicrobial [8,9] The aim of present study is to investigate the antimicrobial activity of *Baccopa monnieri* extracts in order to use it as a possible source for new antimicrobial substances against important human pathogens

MATERIALS AND METHOD

Authenticated roots of *Baccopa monnieri* were collected from Anand Agriculture University, Anand, Gujarat.

Preparation of *Baccopa monnieri* extracts:

Collected roots were firstly washed properly, chopped and dried under hot air oven at 40°C-50°C. dried roots were pulverized by a mechanical grinder. 10g dried root powder successively dissolved in petroleum ether (20 ml)/ chloroform (20ml) /95% ethanol (20 ml)/methanol (20 ml) respectively and kept under shaking condition for 24 hrs [10].The filtrate obtained was used for further study.

Primary Phytochemical screening of crude extract of *Baccopa monnieri*:

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites is carried out. After obtaining the crude extract or active fraction from plant material, phytochemical screening was performed with the appropriate tests to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction [11].

Test microorganisms: In present studies strains used were *Bacillus amyloliquefaciens* (MTCC 1270), *Streptococcus pyogenes* (MTCC 1923), *vulgarica*, *Bacillus megaterium*(MTCC 3353), *Aspergillus niger*(MTCC 281), *Bacillus pumilis*, *Salmonella typh*e, *Bacillus subtilis*, *Micrococcus luteus*, collected from the department of Microbiology, ARIBAS college.

Culture medium:

For antibacterial activity the culture medium used was Muller- Hinton agar medium (himedia), where Sabraudus-dextrose agar medium (himedia) was used for the antifungal activity. All the test samples were sterilized by autoclaving them at 121°C, 15 psi for 30 min.

Anti bacterial and anti fungal activity of *Baccopa monnieri* extract:

Considering the increased incidence of severe opportunistic fungal and bacterial infections in immunologically deficient patients together with the development of resistance among pathogenic gram positive, gram negative bacteria, there is great need in finding new classes of natural products that may be effective against antibiotic-resistant bacteria and fungi. Natural products or their semi synthetic derivatives provide novel examples of such anti-infective drugs. Because of the resistance against antibiotics, there is a great interest in search of new antimicrobial agents from nature.

Antibacterial and antifungal activity of different fractions of *Baccopa monnieri* was carried out by Agar well diffusion method over different test organisms. Serial dilution of the test fractions, isolated compounds and reference drugs were prepared in DMSO to attain a final concentration of 1mg/ml [10]. To make sure that whether solvent had an effect on the microbial growth, a respective parallel control was performed. Streptomycin and Zentamycin were used as standards to compare the antibacterial activity of different plant fractions. These plates were incubated at 37°C for 24hrs and Zone of inhibition was determined. The experiment was performed in triplicates. The relative percentage inhibition of the test extracts with respect to positive control and standard drug was calculated by using previously published protocol [12].

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of *Bacopamonnieri* Secondary metabolites

Secondary metabolites	Name of test	Ethanol	Methanol	Chloroform	Petroleum ether	Ethyl acetate
Alkaloids	Dragendroff test	+++	++++	++	++	+
Anthraquinone	Bomtrager's test	++++	+++	++	+++	++
Cardiac glycolyside	Kellar-Kiliani test	+++	++++	+++	++	++
Flavanoids	NaOH test	++++	+++	++	+	++
Phenol	Phenol test	++	++++	++++	++	+
Phlobatanin	-	++++	+++	++	+	++
Reducing sugar	Fehling test	+++	++++	++	++	+
Saponin	Foam test	+++	++++	+++	++	+
Steroids	Liebermann-Burchardt test	++++	+++	++	++	+
Steroids	Liebermann-Burchardt test	++++	+++	+++	++	+
Tannins	Braemer's test	+++	++++	++	++	++
Pseudotannins	Braemer's test	++++	+++	++	+++	++
Terpenoids	Liebermann-Burchardt test	+++	++++	++	+++	+
Volatile oils	-	++++	+++	+++	+++	++
Pyrolizidine alkaloids	-	++++	+++	+++	++	+
Resins		++	++++	+++	++	+
Proteins		++	++++	+++	+	+
Carbohydrates		++++	+++	++	+++	++
Starch		++++	+++	+++	++	++

Table 2: Minimum Inhibitory Concentration results of *Bacopamonnieri*. (Streptomycin is taken as standard antibiotic)

Test organism	MIC(μ g/ml)		
	Methanol extract	Ethanol extract	Chloroform extract
<i>B.megaterium</i>	45.82	52.42	32.72
<i>B.amymyloliquefaciens</i>	295	198.3	174.23
<i>Streptococcus pyogens</i>	112	98.26	58.23
<i>S.typhe</i>	52.3	65.32	44.02
<i>B.pumilis</i>	135.21	127.45	122
<i>Micrococcus luteus</i>	65.25	69.25	52.14
<i>A.niger</i>	-	-	-
<i>Vulgarea</i>	67.35	48.8	36.56

Infectious diseases are a major cause of morbidity and mortality in India. The number of multiple drug resistant strains and the appearance of the strains with reduced

susceptibility to antibiotics are continuously increasing. This situation provided the impetus to the search for new antimicrobial substances from various sources like

medicinal plants. It is important to investigate scientifically these plants which have been used in traditional medicines as potential source of novel antimicrobial compounds. The first step towards this goal is the *in-vitro* antibacterial activity assay. In the present study, the leaf extracts of *Baccopamonnier* was tested against microbial species like *Bacillus amyloliquefaciens*, *Streptococcus pyogenes*, *Vulgarica*, *Bacillus megaterium*, *Aspergillus niger*, *Bacillus pumilis*, *Salmonella typh*e, *Bacillus subtilis*, *Micrococcus luteus*. Among these species *B.pumilis* was found to be highly sensitive in all the extracts whereas *S.typh*e was found to be highly resistant.

From the present work, it is estimated that these extracts contains alkaloids, tannins, glycosides, etc and the antimicrobial activity may be a result of individual or combination of these bioactive compounds. It can be a source of newer useful drugs and of greater pharmacological importance. Despite many published reports dealing with treatment for neurological disorders, little was known about antimicrobial activity of *Bacopa monnieri* prior to this study. Methanolic extracts of *Bacopa monnieri* was found to possess maximum inhibitory effects against both Gram positive and Gram negative organisms tested compared to chloroform and ethanolic extract. The result agreed with literature cited [13]. The antibacterial activity may be due to the presence of phytochemicals such as alkaloids, phytosterols, proteins, etc., which warrants *Bacopa monnieri* could be subjected to extensive experimental studies in future to treat certain diseases caused by studied bacteria. The results of the study also support the traditional application of the plants and suggest that these plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs.

ACKNOWLEDGEMENT

Authors are grateful to Charutar Vidya Mandal (CVM) Vallabh Vidyanagar, Gujarat for providing platform for this research work. We are also thankful to Dr.Nilanjan Roy Director, Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS) New Vallabh Vidya Nagar, for providing the facilities and his valuable suggestions during our research work. Authors are also thankful to Anand Agriculture University, Anand, Gujarat for providing plant material for this research work.

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