Evaluation of antioxidant and antimicrobial potential of *Bacopa monnieri* L.

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Abstract: Medicinal plants have tremendous potential as therapeutic agents. Their uses have been described in ancient Indian manuscripts and are also part of several alternative therapies. *Bacopa monnieri* L. commonly known as *brahmi* has been used in Indian system of medicine as brain tonic. In the present work antioxidant and antimicrobial activity of *brahmi* was evaluated. The total phenolic content and total flavonoid content was found to be 1.59% ± 0.24 GAE and 0.4% ± 0.1, respectively. The plant extract showed significant antioxidant activity when tested through DPPH, FRAP and NOSA assays. The test plant extract showed potential antimicrobial activity against test gram positive and gram negative bacteria. The result showed that *brahmi,* apart from being a brain tonic, can be used as antioxidant and antimicrobial agent, which will be useful in alleviating several pathological conditions.

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1. Introduction

All known cultures of past Egyptian, Babylonian, Jewish, Chienese, Indus Valley etc. have their own glorious system of medicine and health care. Plants have been the traditional source of raw material and finished medicine since the dawn of civilization. The earliest written record of Chinese, Hindu, and Egyptian revealed that plants were integral part of day to day life of human beings.

Various systems of medicine viz. Ayurveda, Siddha, Unani and Homeopathic predominantly use plant based raw materials in their preparation or formulations. With the advent of synthetic medicines these systems of medicines were not given much attention earlier but knowing the complications associated with these drugs people has now started to show interest in alternative and complementary medicines. Pharmaceutical companies and scientists are now devoting time and money to explore various plants and plant based products for their therapeutic potential and this has reestablished the era of *phytoceuticals*. There are several reports which ascertain the immense potential of plants(Mathur et al. 2007; Bhatia et al. 2008a; Bhatia et al. 2008b; Vats, 2012; Vats and Alam, 2013a; Vats and Alam, 2013 b; Vats and Kamal, 2013; Vats and Kamal, 2014a; Vats and Kamal, 2014b).

Free radicals are generated in the human body through natural processes. However, stress, disease and improper lifestyle lead to excessive generation of free radical which initiates a chain reaction and adversely affects the cellular metabolism. Antioxidants are substances, which scavenge the free radicals and hinder the oxidation process in the body. There is presence of antioxidants in the cell but need of external supplementation of the same in always necessary. Some natural antioxidants are phenolics, flavonoids, carotenoids, Vitamins, anthocyanin etc.

On the other hand need of new antimicrobial agents has intensified nowadays. This is mainly due to the occurrence of new infectious agents and indiscriminate use of synthetic antimicrobials. *Bacopa monnieri* L. (Family: Scrophulariaceae) commonly called as *Brahmi* has been used in traditional and Ayurvedic medicine for many years. Generally formulations used to enhance memory and to cure brain related complications essentially have *Brahmi* as an important ingredient. The plant is rich in metabolites having therapeutic value. Alkaloids like brahmine, nicotine, herpestine have been identified. Various saponins, sapogenins, bacosides, monnierasides have also been reported (Gohil and Patel, 2010). Thus, the present study was undertaken to evaluate the antioxidant and antimicrobial potential of *B. monnieri.*

2. Materials and Methods

2.1 Collection of plant material

Healthy, disease free, mature leaves of *B. monnieri* were collected and air dried for 3-4 days. The leaves were then finely powered.

2.2 Preparation of extract

10g of leaf powder was extracted in 80% methanol in a rotary shaker for 24 hours at 110rpm at 37°C. Thereafter, the solution was filtered and filtrate was defatted with petroleum ether thrice. The ethyl

acetate fraction was separated out from the defatted extract and dried. The extract was dissolved in methanol for further analysis.

2.3 Antioxidant activity

2.3.1 Total Phenolic Content (TPC)

The TPC was determined spectrophotometrically using Folin-Ciocalteaeu method (Sharma et al. 2009). Briefly, 0.5 mL of water and 0.125 mL of the methanolic extract of neem leaves were mixed. Folin-Ciocalteu reagent (0.125 mL), 1.25 mL of the sodium carbonate solution and 3 mL of water was added successively and allowed to stand for 90 minutes. The absorbance was measured at 760nm. TPC was expressed as gallic acid equivalents (GAE).

2.3.2 Total flavonoid Content (TFC)

Total flavonoid content was estimated using the method of Vats et al. (2012) and expressed as quercetin equivalent. Quercetin was used to make the calibration curve. Plant extract (0.5 mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.

2.3.3 DPPH radical scavenging assay

Different fractions (1ml) were mixed with 1ml of 0.3mM. DPPH reagent and allowed to stand at room temperature for 30 minutes in dark. The absorbance was taken at 517nm. Radical scavenging activity was calculated according to the following formula and IC50 value was evaluated (Vats, 2012).

2.3.4 FRAP assay

FRAP assay was done according to the method of Vats (2012). Briefly, the stock solution included 300 mM acetate buffer, 10mM TPTZ and 20mM FeCl3.6H2O. The fresh working solution was made by mixing acetate buffer, TPTZ and $FeCl_3.6H_2O$. 50µl of plant extract and 1.5 ml of FRAP reagent was mixed and incubated for 10 min. The absorbance was taken at 593 nm. Aqueous solution of known Fe (II) concentration was used for calibration.

2.3.5 Nitric oxide radical scavenging activity (NOSA)

1 ml of Sodium Nitroprusside (5mM) in Phosphate buffer saline was mixed with different concentration of extract. The mixture was incubated for 150 min at 25 ºC. The solution was later treated with Griess Reagent. The absorbance was taken at 546 nm and % inhibition was calculated (Vats and Alam, 2013b).

2.4 Antimicrobial Assay

2.4.1 Sources and maintenance of organisms

Gram-positive organisms- *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 121) and Gram-Negative organisms- *Proteus vulgaris* (MTCC 1771), *Salmonella typhi* (MTCC 537), *Pseudomonas aeruginosa* (MTCC 429), *Escherichia coli* (MTCC 443) were maintained on Mueller- Hinton Agar medium (MHAM).

2.4.2 Antimicrobial Bioassay

Microbial suspension was prepared in sterile normal saline and adjusted to 0.5 Macfarland standards (10^8Cfu/ml). Test organisms were inoculated uniformly on labeled plates. Wells were made using sterile cork borer (5mm diameter) and poured with 100 µl (1000 µg/ml) of extract. Ethyl acetate was used as negative control. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation (NCCLS, 2000).

2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

To measure the MIC values, stock of 1500 µg/ml extract was serially diluted to 25, 50, 100, 200, 400, 800, 1000 µg/ml (NCCLS, 2000) and mixed with equal volume of MHAM. A loopfull of microbial suspension (10^8Cfu/ml) was inoculated in different test tubes. The tubes were incubated at 37°C for 24-48 h. Two control tubes, tube containing the growth medium, saline and the inoculum, were also prepared. The lowest concentration of the extract that produced no visible microbial growth when compared with the control tubes was regarded as MIC (Vats et al. 2012).

2.4.4 Determination of Minimum Bactericidal Concentration (MBC)

MHAM plates were categorized into different sections and labeled with the different concentrations on the base of the plates. The contents of MIC tubes were plated in the respective sections of the plates. The plates were incubated for 18-24 h at 37 °C. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal Concentration (Vats et al. 2012).

3. Statistical analysis

The experiments were repeated five times and the results were expressed as mean ?standard deviation.

4. Results and Discussions

Phenolics compounds have been known to combat various diseases and dietary use of the same has been advocated. In the present investigation the phenolic content of *brahmi* in terms of gallic acid equivalent was estimated to be 1.59% ± 0.24GAE. The TFC was found to be 0.4% ± 0.1. There are other reports which suggest differential phenolic and flavonoid content in the test plant (Shah et al. 2012; Mohan et al. 2011), which might be due to the

topographical and environmental effect which leads to variation in metabolite content.

The 50% inhibition of DPPH was achieved at 35 µg/ml of the extract. Lower IC_{50} value means better antioxidant potential. The reducing ability of the test plant extract was judged by FRAP assay in terms of $FeSO_4$ (550 µM/L). The *brahmi* extract was found to have good nitric oxide scavenging potential with 50 µg/ml IC_{50} value but the activity was less than that of DPPH assay. This might be due to individual phenolic acids also their amount showing varies activity. Reactive oxygen species (ROS) play an important role in various pathological events. ROS together with nitric oxide are involved in inflammation and cancer. Oxidative damage also results in oxidation of tyrosine residue of protein, peroxidation of lipids, and DNA damage. NO can lead to generation of hydroxyl radical which intensifies the pathological conditions (Vats and Alam, 2013a). The antioxidant activity of the extract is mainly due to good phenolic and flavonoid content. These phytochemicals have been known to possess antioxidant potential are in use to treat many disorders and diseases (Ross and Kasum, 2002).

Microbial infections pose a health problem throughout the World, and plants are a possible source of antimicrobial agents (Burapadaja and Bunchoo, 1995; Adenisa et al. 2000). Medicinal plants contain active principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections.

The antimicrobial efficacy of the *brahmi* extract (1000 µg/mL) was assessed against certain gram positive and gram negative pathogens. The inhibition zone (IZ) was maximum against *P. vulgaris* (15±1mm) and minimum against *P. aeruginosa* (8mm±1mm). The IZ was in the range of 9-14mm in other test microbes (Fig. 1). In terms of MIC the highest activity was found to be against *P. vulgaris* (45±1.6 µg/mL) and lowest against *P.aeruginosa* (358±1.2 µg/mL). In other organism the MIC was in the range of 60-235 µg/mL. The results were almost at par for MBC (Fig. 2). The plants produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants. Metabolites namely flavonoids, alkaloids and triterpenoids offer a better opportunity for testing against a wide range of microorganism. The above effects might be due to phenolic content. However, effect of other phytochemicals cannot be undermined.

5. Conclusion

The study reveals that *brahmi* apart from being a brain tonic can also serve as potent antioxidant. Moreover, the range of antimicrobial activity showed by the plant extract presents it as a potential antimicrobial agent having a broad spectrum activity.

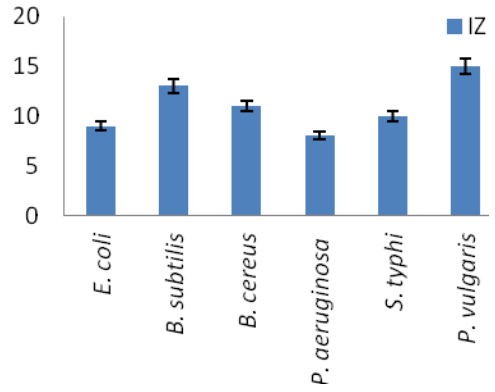

Fig. 1: Antimicrobial activity (IZ in mm) of *B. monnieri* (1000 µg/ml)

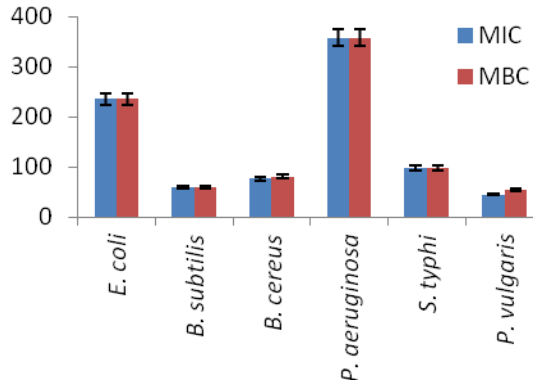

Fig. 2: Antimicrobial activity (µg/ml) in terms of MIC and MBC

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