A Preliminary *in vitro* Investigation on the Crude Methanol Extract of *Bacopa monnieri* (L.) Pennell. An Important Multi Medicinal Plant

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Abstract
The present investigation is to study the B. monnieri crude methanol extract antibacterial and anticancer activities. Crude methanol extract showed very positive antibacterial activity on 6 different bacterial strains. Staphylococcus aureus has been identified as more sensitive than the other strains (Staphylococcus aureus>Enterococcus faecalis>Bacillus subtilis> Escherichia coli> Pseudomonas aeruginosa>Proteus vulgaris).

Further, the crude methanol extract exhibited a promising anticancer activity and expressed considerable IC_{50} values (108.6 and 86.4µg/ml for MCF-7 and MDA-MB-231). These observations suggest that the medicinal plant B. monnieri can be used in antibacterial and anticancer studies.

Keywords: Brahmi, cancer cell line, ciprofloxacin, DMSO, inhibition zone, MTT.

Introduction
Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine[3]. About 80% of the people in developing countries use traditional medicines for their health care[14]. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds many of which have been the basis for the development of new lead chemicals for pharmaceuticals. To date, many synthetic antimicrobial and anticancer drugs have been available against various pathogenic microorganism flora and different cancer therapies.

Present modes of treatment based on synthetic drugs have limited potential since, they are toxic, expensive and also alter the cell signalling pathways. However, the plant derived medicines are relatively safer than synthetic drugs which offer very good therapeutic benefits and affordable treatment[8,24]. *Bacopa monnieri* (L.) Pennell belongs to the family Scrophulariaceae, in India it is popularly known as 'Brahmi' and is used in Ayurveda for the preparation of medhyarasayan, a drug used to improve intelligence and memory since it contains secondary metabolite bacosides which are popularly known as memory enhancers[23,29]. Brahmi is the second most important medicinal plant among the list of the most important Indian medicinal plants assessed on the basis of medicinal importance and commercial value for future potential research and development.

Bacopa monnieri has a great commercial market value due to high pharmacological activities[11,26,28]. Antimicrobial activity of various extracts of *B. monnieri* against a diverse microbial flora has been reported earlier[1,6,9,10,12,13,15,17,19,21,25,30-32].

However, the studies related to the anticancer activity of *B. monnieri* are scanty[15,16,20,22]. Therefore, an attempt has been made to study the antibacterial and anticancer activities using leaf crude methanol extract of *B. monnieri* against six bacterial strains and two breast cancer cell lines.

Material and Methods
The *B. monnieri* plant material was collected from the Herbal Garden of Department of Botany, Andhra University, India. The leaf material was separated and washed thoroughly and kept in shade for drying. The resulting air dried material was crushed to form leaf powder using mortar and pestle.

Preparation of methanol extract: The leaf powder of *B. monnieri* was taken and weighed up to 10g. It was packed in Soxhlet apparatus. The solvent methanol was added in a round bottom flask and was heated over a heating mantle using the adjustable rheostat and the extraction was done up to 72 hours. Than the extract was collected and concentrated by evaporating the methanol.

Antimicrobial activity
Nutrient agar medium: All the medium components i.e. peptone (0.5g), beef extract (0.3g), sodium chloride (0.5g) were taken into a conical flask and made up to 100ml with distilled water. Adjust the pH to 7.2 and add 1.5g of agar agar and sterilize in an autoclave at 121ºC under 15lbs pressure for15-20 minutes.

Microbial cultures: Six bacterial strains i.e. three Gram(+) viz. *Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram(-) viz., *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* have been collected from the Microbial Type Culture Collection

(MTCC) of IMTECH, Chandigarh, India for antibacterial activity.

Pure cultures: All the six bacterial strain pure cultures on nutrient agar medium were prepared by taking a loop full of culture from the respective starter cultures and were streaked on nutrient agar plates and incubated at 37ºC for overnight. All the bacterial cultures were maintained in nutrient agar plates and stored at 4°C.

Antimicrobial assay: The crude methanol extract of *B. monnieri* was screened for its antibacterial activity via agar well diffusion method[4]. The nutrient agar medium plates were seeded with different strains of test bacteria. In each of those plates, three wells of 6mm were made using a sterile cork borer. Methanol extract of different concentrations i.e. (100, 200 and 300µg/ml) was added to the respective wells and separate plates were prepared with 10µg/ml ciprofloxacin and 0.1%µg/ml dimethyl sulfoxide (DMSO) as positive and negative controls respectively. All the plates were incubated at room temperature for 15-20mins for the diffusion of extracts into the medium, then incubated at 37ºC for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

Cell lines and Culture medium: Two breast cancer cell lines viz. MCF-7 and MDA-MB-231 were procured from National Centre for Cell Sciences (NCCS), Pune, India, then the stock cells were cultured in DMEM medium supplemented with 10% inactivated fetal bovine serum, penicillin (100units/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC.

MTT assay: Cell viability was measured using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test[18]. Approximately 5X10 cell/well in a fresh medium were incubated under normal growth condition for approximately 24hours before treating with test sample to allow the cells to attach to the wall of the plate. 200µl of DMSO containing with various crude methanol extract i.e. 10,20,40,80,160 and 320µl/ml were added to the plates and were incubated for 24 at 37ºC in a humidified incubator containing 5% CO_2. Control cells were treated with DMSO alone. After an appropriate incubation period, the MTT solution was added to the plates (at a final concentration of 5mg/ml) and was incubated for 4hours in dark at 37ºC. The resultant MTT products were dissolved in DMSO. The viability was calculated by measuring optical density at 570nm using the reference wavelength of 650nm in Elisa reader. From the resultant optical densities, the percentage growth was calculated using the following formula:

% of cell viability = 100X (Sample Absorbance/Control Absorbance)

Statistical Analysis: All the experiments were repeated for 3 times and the data were expressed as mean ± standard deviation.

Results and Discussion

In the present investigation, the crude methanolic extract of *B. monnieri* was screened for antibacterial activity against three Gram(+) and three Gram(-) bacterial strains via agar well diffusion method and anticancer activity with two human breast cancer cell lines viz. MCF-7 and MDA-MB-231 using MTT assay. Cowan[5] stated that for the extraction of phytochemicals from the plant materials, the solvent methanol was found to be a better solvent than other polar and nonpolar solvents, hence in the present study only methanol solvent extract has been utilized. The results of antibacterial activity of *B. monnieri* leaf extract are presented in table 1. Among the results, the zones of inhibition from the six bacterial strains range from 5.40 to 22.00mm. The maximum antibacterial activity i.e. (22.00±0.33mm) at an extract concentration 300µg/ml was noticed against *Staphylococcus aureus* while the minimum i.e. (5.40±0.36mm) at 100µg/ml extract concentration was recorded against *Proteus vulgaris*.

Further, the antibacterial activity against the tested bacterial strains was found to be higher at a concentration of 300µg/ml followed by 200µg/ml and 100µg/ml respectively. On the other hand, the negative control (DMSO) did not show any inhibitory effect while the positive control (ciprofloxacin) exhibited the widest zones of inhibition against all the bacterial strains. However, in the present findings, the Gram positive bacteria are more sensitive than the Gram negative bacteria at all concentrations of *B. monnieri*. These results agree with the earlier researchers who worked on *Bacopa* using crude methanol extract against different Gram-positive and gram-negative bacteria[1,13,17,32]. This may be due to the differences available in the cell wall composition in both types of bacteria[2,27].

The MTT assay results of anticancer activity of different concentrations of the crude methanolic extract of *B. monnieri* against two breast cancer cell lines viz. MCF-7 and MDA-MB-231 are presented in table 2. The highest percent inhibition of cancer cell growth with minimal viability i.e. (82.71% and 75.27% for MCF-7 and MDA-MB-231) respectively was recorded at concentration 320µg/ml. The IC_{50} was found to be 108.6 and 86.4µg/ml against MCF-7 and MDA-MB-231 respectively and these results are in agreement with the earlier observations[16,20].

The present results indicated that the crude methanol extract of *B. monnieri* showed promising anticancer activity, this may probably be due to the phytochemicals present in the methanol extract which inhibits the DNA replication in cancer cell lines[7].

As a whole, the present investigation indicated that the leaf crude methanolic extract of *B. monnieri* might be beneficial for the prevention of pathogenic bacteria growth and treatment of various cancers. Further studies are needed for identification and characterization of the active principle compounds available in *B. monnieri*.

Table 1
Antibacterial activity of *B. monneiri* leaf crude methanol extract

S.N.	Microorganism	Inhibition zone (mm)				
		Concentration of crude methanol extract			Negative Control	Positive Control
		100µg /ml	200µg/ml	300µg/ml		
1.	*Bacillus subtilis*	7.00±0.21	14.50±0.16	19.12±0.26	0.00±0.00	35.00±0.29
2.	*Staphylococcus aureus*	8.90±0.14	16.20±0.18	22.00±0.33	0.00±0.00	36.00±0.32
3.	*Enterococcus faecalis*	7.50±0.20	15.30±0.19	20.20±0.30	0.00±0.00	35.80±0.30
4.	*Escherichia coli*	6.50±0.21	12.40±0.22	18.15±0.42	0.00±0.00	25.00±0.20
5.	*Pseudomonas aeruginosa*	6.20±0.36	11.60±0.41	18.00±0.28	0.00±0.00	24.50±0.22
6.	*Proteus vulgaris*	5.40±0.36	10.90±0.34	17.50±0.29	0.00±0.00	24.00±0.26

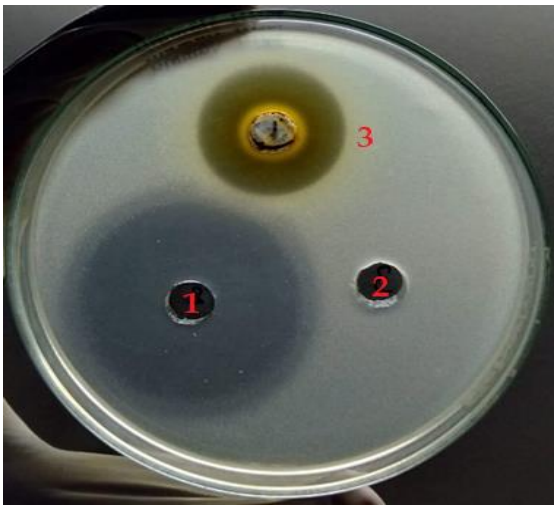

Fig. 1: Antibacterial activity of *B. monneiri* leaf crude methanol on *Staphylococcus aureus*; 1: Positive control; 2: Negative control; 3. Leaf crude extract (300µg/ml)

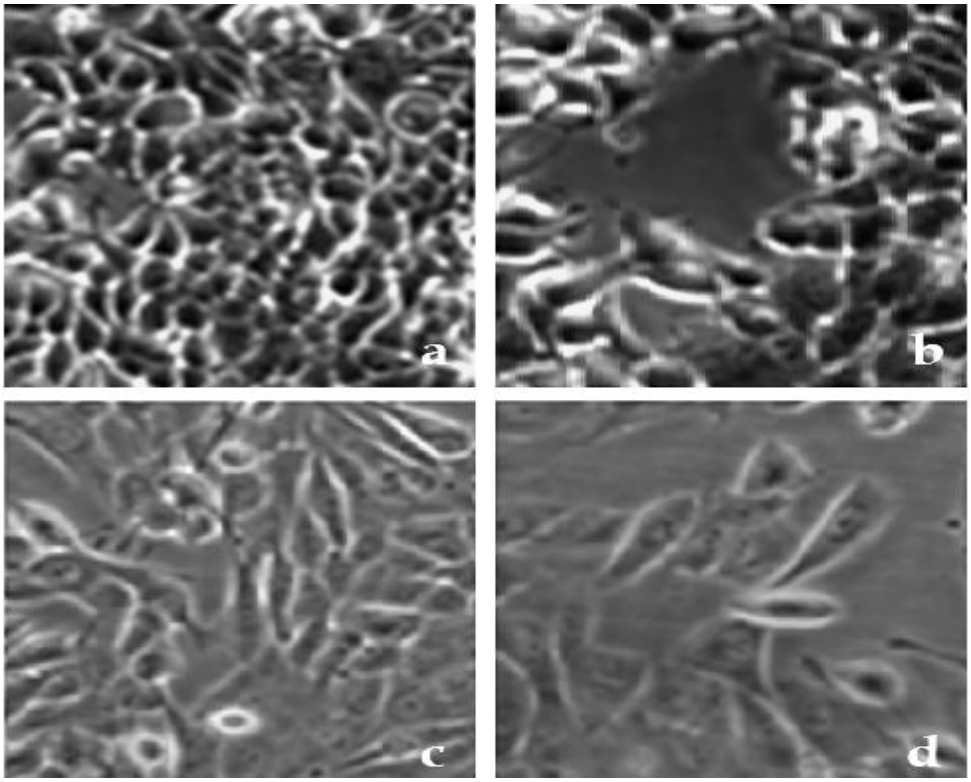

Fig. 2: Anti-cancerous of *B. monneiri* leaf crude methanol extract: a) Breast Cancer cell line MCF-7 control; b) Declined growth of MCF-7 cells after 24 hours; c) Breast Cancer cell line MDA-MB-231 control; d) Declined growth of MDA-MB-231 cells after 24 hours

Table 2
Antigrowth effect of *B. monneiri* leaf crude methanol extract on breast cancer cell lines

S.N.	Con. of extract (µg/ml)	% inhibition	
		MCF-7	MDA-MB-231
1.	Control	0.00±0.00	0.00±0.00
2.	10	4.03±0.22	5.05±0.25
3.	20	11.09±0.18	10.22±0.20
4.	40	32.87±0.24	29.01±0.28
5.	80	49.96±0.20	41.28±0.19
6.	160	66.37±0.31	62.96±0.26
7.	320	82.71±0.27	75.27±0.21
8.	IC_{50}	108.6	86.4

Conclusion

The output of the present research suggests that the leaf crude methanol extract of *B. monnieri* exhibited a promising antibacterial and anticancer activity. Further, the results of the present study can be helpful in future for the identification and characterization of pharmacological compounds of *B. monnieri*.

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