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Antimicrobial activity of leaf callus of *Bacopa Monnieri L.*

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ABSTRACT

Bacopa monnieri L. (family Scrophulariaceae) is a traditional medicinal plant in India, known locally as ibrahmi. In the current study, the methanolic extract of the leaf callus of Bacopa monnieri at the concentrations o.25, o.o5, o.o3 mg/disc was investigated for its antimicrobial activity by modified Kirby-Bauer diffusion method using ciprofloxacin and griseofulvin as antibacterial and antifungal reference standard respectively. The finding of this study revealed that the extract of the leaf callus of Bacopa monnieri possessed a dose dependent antimicrobial activity against all the tested bacterial and fungal species indicated by the zones of inhibition of the microbial growth. The antimicrobial effect of the plant callus under investigation was comparable with those of the reference standards.

Key words: Antimicrobial activity, leaf callus, *bacopa monnieri*,

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INTRODUCTION

Plant tissue culture is *in vivo* cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant. This technique affords alternative solutions to problems arising due to current rate of extinction and decimation of flora and ecosystem. The whole process requires a well-equipped culture laboratory and nutrient medium. This process involves various steps viz. preparation of nutrition medium containing organic and inorganic salts, supplement with vitamins, plant growth hormones(s) and amino

acids. Sterilization of explants (source of plant tissue), glass ware and other accessories, inoculation and incubation [1].

Culture of single cells growing under controlled conditions in a liquid medium, or callus culture consisting of undifferentiated masses of cells developing on a semisolid medium, can be initiated from parenchymatous tissues of shoots, roots and other plant part. The maintenance of such cultures depends on an adequate supply of nutrients, including growth hormones (e.g Auxins, Gibberellins and kinetin) and a controlled sterile environment. The cells, although undifferentiated, contain all the genetic information present in the normal plant. By suitable manipulation of the hormone content of the medium, it is possible to initiate the development of roots, shoots and complete plants from the callus cell culture and to encourage the division of cells in a suspension culture [2].

The major advantages expected from plant tissue culture system over conventional technique may be summarized as follows:

1) The process offers the prospect of absolutely uniform biomass obtainable at all times and manageable under regulated and reproducible conditions, rarely possible in working with entire living plant.

2) It is possible to use plant cell culture technique for synthesis of those medicinal compounds which are too difficult or impossible by chemical synthesis.

3) The useful natural compounds could be produced under controlled environmental conditions, independent of soil conditions and change in climatic conditions.

4) The cells of any plant, tropical or temperate could be multiplied to yield specific metabolites produced by them.

5) The cultured cells could be maintained free from any microbial contamination and insect attack.

6) Another important application of plant tissue culture technique is immobilization of cells which could be used for various biotransformation or biochemical reactions. A particular strain of cells obtained from suspension culture is immobilized by suspending it in a sodium alginate solution, precipitate the alginate plus entrapped cells with calcium chloride solution, pelleting and allowing the product to harden.

7) It is possible to attempt biotransformation reactions in plant cell cultures. It is expected that specific modification of chemical structures of certain compounds may be achieved more easily in cultured plant cells rather than by chemical synthesis.

8) The technique could used to study biogenesis of secondary metabolites. It is possible to feed labeled precursors to cell culture and deduce interpretations pertaining to metabolic pathways of desired compound(s) [3].

Bacopa monnieri Linn. (Scrophulariaceae), commonly known as brahmi is used in indigenous system (s) of medicine in India. The plant is a prostate, creeping, juicy, succulent, glabrous herb that branches profusely, found in wet places, damp or marshy areas near the border of the ponds, water cannels, wells, irrigated fields etc [4]. The plant is reported to contain tetracyclic triterpenoids saponins, bacosides A and B [5-7], phytosterols [7], hersaponin [8], flavonoids [9-10] viz. luteolin-7-glucoside, glucoronyl-7-apigenin. It is a valuable nervine tonic for curing memory loss [11], mental stress [12], and anxiety [13]. It is used for controlling asthma, rheumatism, hoarseness and fever [11]. Also it is used in generalized weakness, lethargy, fatigue and exhaustion [14].

Literature survey showed that much work has not been reported from leaf callus. So here in the present study we have initiated and developed the callus on the leaf of plant *Bacopa monnieri* and then evaluated the anti-microbial activity of methanolic extract of leaf callus of *Bacopa monnieri* against various bacterial and fungal strains.

MATERIALS AND METHODS

The plant was authenticated by Dr. M. P. Sharma, Taxonomist, Department of Environmental Botany, Faculty of Science, Jamia Hamdard, New Delhi-62. After authentication, fresh and healthy leaves of *Bacopa monnieri* were collected from the plants grown in herbal garden of Hamdard University. The leaf callus of *Bacopa monnieri* was initiated on M S medium supplemented with IAA + 2, 4-D + Kinetin (1 ppm each); IAA (1ppm) + 2, 4-D (1ppm) + Kinetin (2ppm) and NAA (1ppm) + 6-BA (1ppm) + Kinetin (2ppm). Finally, leaf callus was successfully maintained on M S medium supplemented with IAA (1ppm) + 2, 4-D (2ppm) + Kinetin (4ppm).

Preparation of plant extract

The methanolic extract of the leaf callus was prepared by heating the samples (5g) in methanol at 100°C on water bath. The extract was filtered and the filtrate was evaporated to dryness. The dried extract was stored till the time of the study.

Phytochemical study

Preliminary phytochemical screening was performed [15]. The presence of phytoconstituents such as alkaloids, amino acids, carbohydrates, phenolic compounds, terpenoids, steroids, proteins, saponins, coumarins, ascorbic acid and tannins were confirmed.

Antimicrobial activity

The antimicrobial activity of methanolic extracts of leaf callus was evaluated against four bacterial strains: *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2034) and *Klebsiella pneumoniae* (NCIM 2011) and four fungal strains *Microsporum audouinii* (MUCC 545), *Trichophyton mentagrophytes* (MUCC 665), *Candida albicans* (UCC 29) and *Aspergillus niger* (MUCC 177) at different concentrations (0.25 mg/disc, 0.12 mg/disc 0.05 mg/disc and 0.03 mg/disc in DMSO), according to the modified Kirby-Bauer disk diffusion method [16]. The solvent dimethyl sulphoxide (DMSO) was used as negative control and ciprofloxacin/griseofulvin were used as standard references for antibacterial and antifungal activity respectively. Diameters of the zone of inhibition (in mm) were meseaured

and the average diameters for test sample were calculated for triplicate sets. The diameter obtained for the extract sample was compared with that produced by the standard drug (ciprofloxacin/griseofulvin).

RESULTS AND DISCUSSION

Bacopa maonnieri is a traditional plant in India, known locally as Brahmi and as Medhya Rasayana. It has been used for centuries to increase mental capacity, improve mental and brain functions. It is also used in the treatment of asthma, bronchitis, hoarsness, water retention and diarrhea. The plant was reported to have anti-inflammatory, analgesic, antipyretic, antioxidant and anticancer activities. It was also reported to have antibacterial and antifungal effects. In this study the methanolic extract of the leaf callus was investigated for the antibacterial and antifungal properties in the aim of ensuring the antimicrobial effect of the plant and to assess whether tissue culture of the plant will retain the same activity or not.

Preliminary phytochemical tests on the leaf callus methanolic extract revealed the presence of alkaloids, amino acids, carbohydrates, phenolic compounds, terpenoids, steroids, proteins, saponins, coumarins, ascorbic acid and tannins as a major phytoconstituents. As ahown in table 1 and table 2, the methanolic extract of leaf callus at concentrations of 0.03, 0.05, 0.12 and 0.25 mg/disc exhibited significant and dose dependent inhibition of the bacterial growth indicating antimicrobial activity against all microorganisms investigated. The antimicrobial activities of the Leaf callus extract was almost comparable with those of the standard drugs Ciprofloxacin and Griseofulvin at a concentration of 0.25mg/disc.

Development of resistance by microbes against known antibiotics is a huge concern in medical field, thus searching for new antimicrobial compounds is a never-ending process. From the results obtained in this study, it seems that *Bacopa monnieri* is a promising plant in this context (table I and II). However, further tests against other strains of bacteria and fungi are needed. In addition, the active ingredient(s) should be isolated and its/their mechanism(s) of action(s) should be enlightened by further studies.

Table- 1 Antibacterial activity of extract of leaf callus of *Bacopa monnieri* L.

Leaf callus extract concentrations (mg/disc)	**Diameter of Zone of Inhibition* (mm)**			
	Bacterial strains			
	B. subtilis	***S. aureus***	***P. aeruginosa***	***K. pneumonia***
0.03	5.4	4.1	4.5	6.0
0.05	6.2	5.0	6.0	7.8
0.12	8.1	7.2	7.5	9.0
0.25	10.0	11.5	11.8	10.5
Control (DMSO)	-	-	-	-
Ciprofloxacin (0.25)	9.2	10.5	11.0	

***** *Values are average of three determinations*

Table- 2 Antifungal activity of extract of leaf callus of *Bacopa monnieri* L.

Leaf callus extract Concentrations (mg/disc)	Diameter of Zone of Inhibition* (mm)			
	Fungal strains			
	C. albicans	***M. audo.***	***A. niger***	***T. menta.***
0.03	4.4	5.2	3.8	5.0
0.05	5.8	7.1	5.5	7.3
0.12	10.2	11.5	9.2	10.5
0.25	12.5	13.8	11.2	13.0
Control (DMSO)	-	-	-	-
Griseofulvin (0.25)	11.3	12.0	10.5	11.5

****** *Values are average of three determinations*

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