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Phytochemical and Antibacterial Activity on Various Extracts of *In Vivo* and *In Vitro* of *Baliospermum montanum* (Willd.)Muell. Arg.

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ABSTRACT

The present study was aimedto investigate the phytochemical and antibacterial activity of *in vivo*fresh leaf and *in vitro*derivedleafcallus of *Baliospermummontanum*(Willd.)Muell.Arg. An *in vitro*regeneration protocol was developed for induction of callus from leaf segments cultured on Murashige and Skoog (MS) medium supplemented with α-naphthaleneacetic acid(NAA) and 6-benzylaminopurine(BAP).Phytochemical screening of major bioactive constituents of leaf and leaf derived callus of different solvents extractwere analyzed. The antibacterial activitywas performed by agar well diffusion method. The maximum callus induction (82.66±2.51) was observed in leaf segments on MS medium supplemented with combination of BAP (2.22µM&4.44µM/l) + NAA(2.69µM/l). The phytochemical studies revealed the presence of alkaloids, flavonoids, proteins, tannins, phenols, steroids and terpenoids, phytosterols, glycosides, coumarins, carbohydrates, betacynin, resins, gums and mucilage etc. The Methanolic extract of leaf exhibited maximum zone of inhibition against *Bacillus subtilis* (21.33±1.52).Whereas acetone extract of the callus showed maximum inhibition against *Pseudomonas fluorescence* (17.0±1.00) compare to other extracts.In conclusion the leaf and the callus extract reveals the presence of major phytochemicals and antimicrobial compounds and further studies has to be carried out to isolate the major bioactive compounds from *Baliospermummontanum*.

KEY WORDS:*Baliospermummontanum*, *in vitro*, callus, phytochemical, antibacterial activity.

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INTRODUCTION

According to world health organisation (WHO) 80% of population in developing countries depend on medicinal plants for their primary health care[1]. India is one of the hotspots of biodiversity for medicinal and aromatic plants in the world. Western Ghats and the North eastern region of India are the verge of extinction due to excessive plundering[2]. Medicinal plants synthesize enormous array of secondary metabolites which are important for human life and it promote positive health and maintain resistance against infection by re-establishing body equilibrium[3]. A number of medicinal plants showed effective antimicrobial activities which were comparable to synthetic standard drugs[4]. Utilization of natural products as an alternative way for the convectional action in healing the different ailments has been increased in few decades[5]. An alternative source for commercial exploitation was plant cell cultures which will produce high yield compare to field grown plants[6].

Baliospermummontanum belongs to the family Euphorbiaceae commonly known as Danti. It is a stout, monoeciousunder shrub with many shoots arising from the base[7].The species is distributed from Kashmir to Arunachal Pradesh and southern peninsular India[8]. The root and leaf are rich source of metabolites like montanin, baliospermin, 12-deoxyphorbol-13-palmate,2-deoxy-16-hydroxyphorbol-13-palmates,12-deoxy-5β-hydroxyphorbol-13-myristate,8-sitosterol, 8-D-glucoside andhexacosanol. The Plant extract are used to treat as anthelmintic, diuretic, skin disease, wounds, piles, jaundice, dropsy, asthma, bronchitis and headache. Stem decoction are used to treat toothache, inflammations, flatulence and snakebite[9-11]. To keep pace with the growing demand of this herb, evaluation and utilization of tissue culture system has an effective and alternative source to fulfill the requirements[11]. Based on the above fact, the present study was aimed to establish effective protocol of callus induction and to investigate the phytochemical and antibacterial activity of*in vivo* fresh leaf and *in vitro* derived leaf callus.

MATERIAL AND METHODS

Collection of Plant material and sterilization:

Plant materialswere collected from Western Ghats, Karnataka and maintained in green house condition. Leaves were washed thoroughly under running tap water followed by teepol and bavistin for 30 m, then in 70% ethanol for 2m. The explants were then treated with 0.1%$HgCl_2$ for 2 m under aseptic condition and then washed with sterile water for 3-5 times to remove the traces of sterilants. Finally surface sterilized explants were cultured on MS medium.

Callus induction:

For callus induction, sterilized leaf segments(0.5 – 1.0cm^2) were cultured on MS medium supplemented with 3% sucrose, 0.8% agar with different concentrationsand combinations of auxin(NAA) and cytokinin (BAP). Cultures were incubated at 25±2ºC in a culture room with 70-80% relative humidity and 16h of photoperiod.

Qualitative analysis:

5gof fresh leaf and callus weresoaked in different solvents namely aqueous, methanol, ethanol, acetone, butanol, chloroform, petroleum ether and hexanefor 3-4 days. The solvent extracts were filtered with Whatman filter paper No.1. The procedure was repeated for another two cycles to ensure complete extraction of phytochemical compounds and the filtrates were stored at 4ºCuntil the further analysis[12].Thelyophilized extracts were dissolved in respective solvents and were screened for the qualitative analysis for the presence of alkaloids, flavonoids, proteins, tannins, phenols, steroids and terpenoids, phytosterols, glycosides, coumarinsetc., by standard methods[13-16].

Antibacterial assay:

Antibacterial activity was performed by well diffusion method following the protocol of Johnson *et al.*[9]. The bacterial strains *Bacillus subtilis*, *Staphylococcusaureus*,*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonasfluorescence*were obtained from Department of Microbiology, Bangalore University, Bengaluru. Nutrient agar was prepared using beef extract 3g/l, peptone 5g/l, sodium chloride 5g/l and agar 15g/l with pH adjusted to 7.2. Fresh leaf and callus were subjected for antibacterial activity against pathogenic bacteria. Petri plates were prepared with nutrient agar media and wells were bored with 8mm diameter sterilized cork borer. Different solvent extractswere used for bacterial sensitivity test. Plates were incubated for 24 h at 37ºC and zone of inhibition were recorded in mm and compared with standard antibiotics(Streptomycin and Tetracyclin).

Statistical Analysis:

The data are expressed as Mean ± S.E. All the experiments were repeated thrice, data were analysed statistically by one way analysis of variance followed by Duncan's multiple range tests using SPSS software. Probability values $P<0.05$ were considered significant.

RESULTS AND DISCUSSION:

Callus induction:

Growth hormones play an important role in tissue culture for the development of cultured cells or tissues[17]. It also regulates the various physiological and morphological processes and enhances metabolites synthesis[18,19]. In the present study,different concentration of cytokinins and auxins were supplemented for the callus induction. The green friable callus was observed at the cut ends of leaf explants on MS medium supplemented with different concentrations and combinations of BAP (2.22µM-17.75µM) and NAA (2.69µM-21.48µM) (Fig.2). Callus induction was maximum in MS medium supplemented with BAP (2.22µM&4.44µM)+NAA(2.69µM/L) after six weeks of inoculation (Fig. 1). The competence of callus induction depends on the type of growth regulators, explants source and culture medium[20]. Previous studies byTeshome*et al*.[21]and Kumari *et al*.[22] reported higher callus induction from leaf explants inoculated on MS medium supplemented with NAAandBAP.

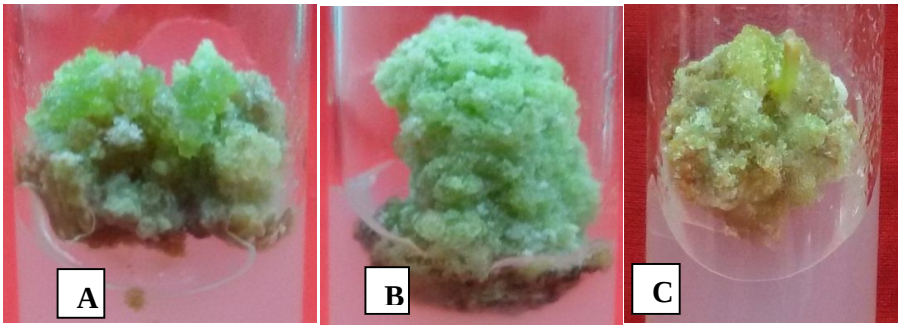

Fig. 1: A-C Induction of semi friable green callus from leaf explants

A: MS+BAP (2.22µM)+NAA (2.69µM), B: MS+BAP (4.44µM) +NAA (2.69µM) and C: MS+BAP (17.75µM) +NAA (2.69µM)

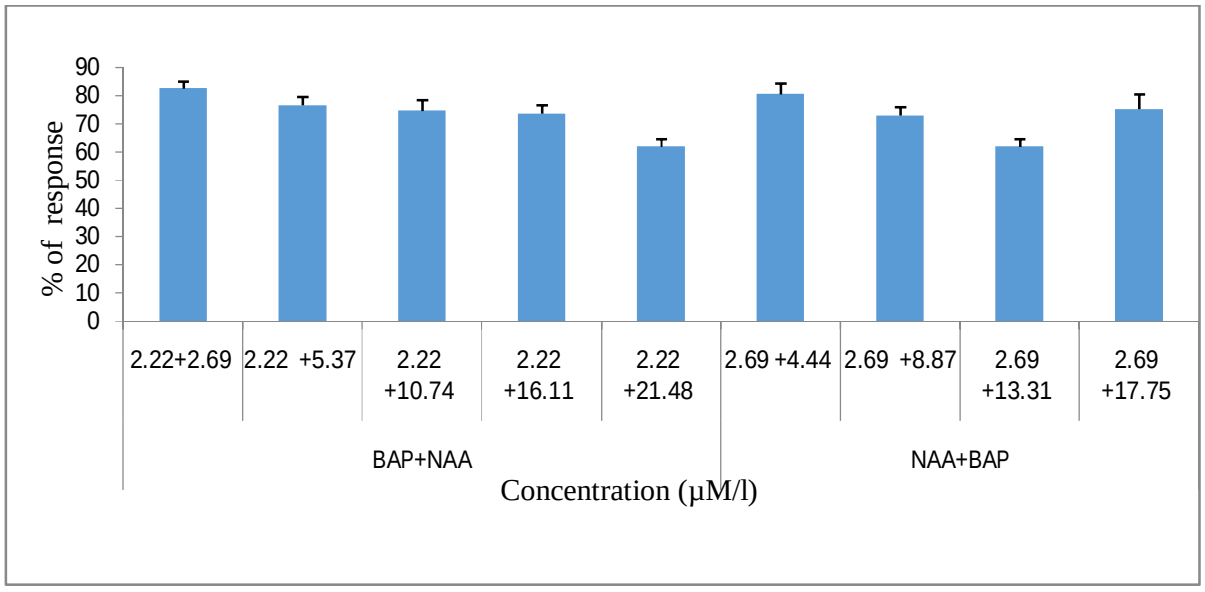

Fig. 2: Percentage of callus induction on different concentrations and combinations of auxin and cytokinin of *B.montanum* from leaf explants.

Phytochemical analysis:

Medicinal plants were found to contain various phytochemical produced at different stage of development under stress condition[23].Secondary metabolites has abiological and therapeutic properties and itserves to protect the plant growth against microbial attacks and from animal predators.Qualitative phytochemical analysis of different solvent extracts were performed in leaf and callus of *B.montanum.* Our results indicated the presenceof alkaloids, flavonoids, proteins, tannins, phenols, steroids and terpenoids, phytosterols, glycosides, carbohydrates, betacyanin, resins, Phlobatannins, volatile oils, and coumarins(Table.1)**.**Methanolic extract proved to be a better solvent compared to other extracts. Sangha*et al.*[23] reported that methanolic extract is a good solvent to extract major phytochemicals from root, stem, leaf, latex and flower extracts in *B.montanum.* Studies by different workers demonstrated that methanolic extract proves to be a better solvent for the isolation of phytochemicals from different plant species[24-26].

TABLE.1: QUALITATIVE ANALYSIS OF *B. MONTANUM* FRESH LEAF AND FRESH CALLUS

TESTS	FRESH LEAF								FRESH CALLUS							
	I	II	III	IV	V	VI	VII	VIII	I	II	III	IV	V	VI	VII	VIII
Alkaloids	-	-	-	+	+	-	+	-	-	-	+	-	+	-	-	+
Flavonoids	+	+	+	-	+	-	+	-	+	+	+	-	-	-	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
Phenols	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	+
Tannins	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	+
Steroids &Terpenoids	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	-
Phytosterols	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	-
Glycosides	+	+	+	-	+	-	+	+	+	+	+	-	-	-	+	+
Coumarins	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	+
Carbohydrates	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+
Betacyanin	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+
Resins	-	+	+	-	-	-	-	-	+	+	+	+	-	+	-	-
Phlobatannins	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Volatile oils	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-	+
Emodols	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Gums and mucilage	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-

I-Aqueous, II-Ethanol, III-Methanol, IV-Petroleum ether, V-Chloroform, VI-Hexane, VII-Butanol, VIII-Acetone

Antibacterial activity:

Plant extracts can be good source of antibiotics against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutically potential and they have no side effect compare to synthetic compounds[27]. The results of antibacterial activity of different solvents extracts of leaf and leaf derived callus were represented (Fig. 3&4). The result revealed that methanolic and aqueous leaf extract showed maximum activity against *Bacillus subtilis* (21.33±1.527) and *Klebsiella pneumoniae*(21.00±1.00). Whereas in callus, acetone and ethanolic extracts showed higher inhibitory activity against *Staphylococcus aureus* (17.00±1.00) and *Escherichia coli*(16.66±1.52).The results are concordance with the report of Johnson *et al.*[9] wherein ethanolic extract of *B. montanum* (leaf, root and callus) showed maximum inhibition against different bacterial pathogen.

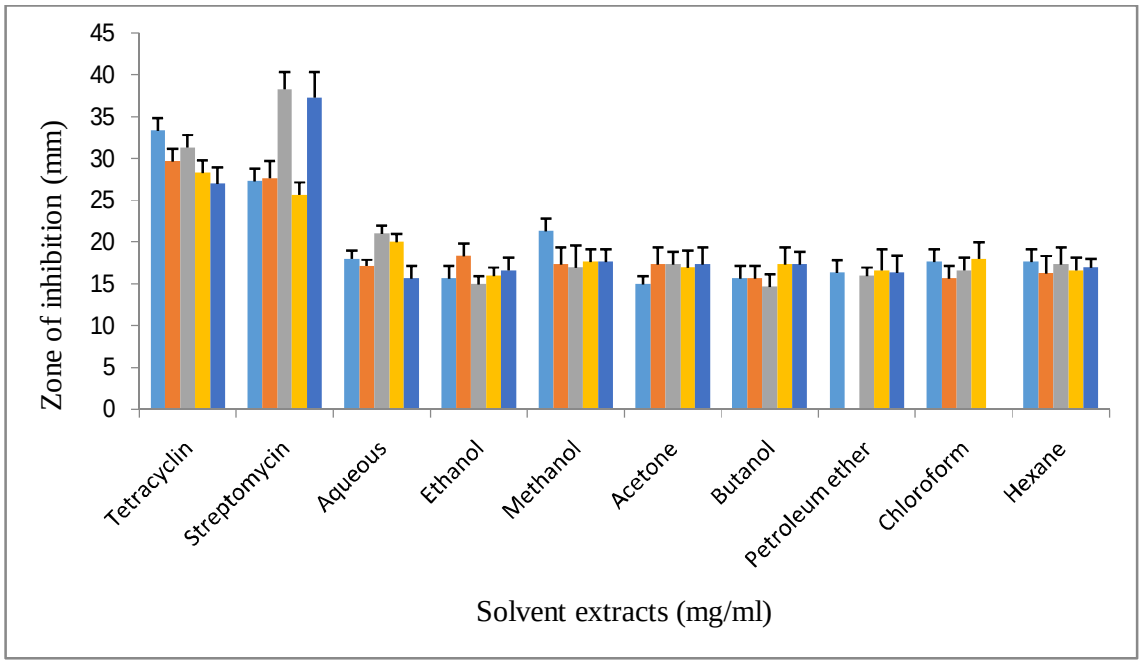

Fig. 3: Zone of inhibition in different solvents of *B.montanum* from fresh leaf

B.SubtilisE.coli K. Pneumonia P.FluorescenceS.aureus

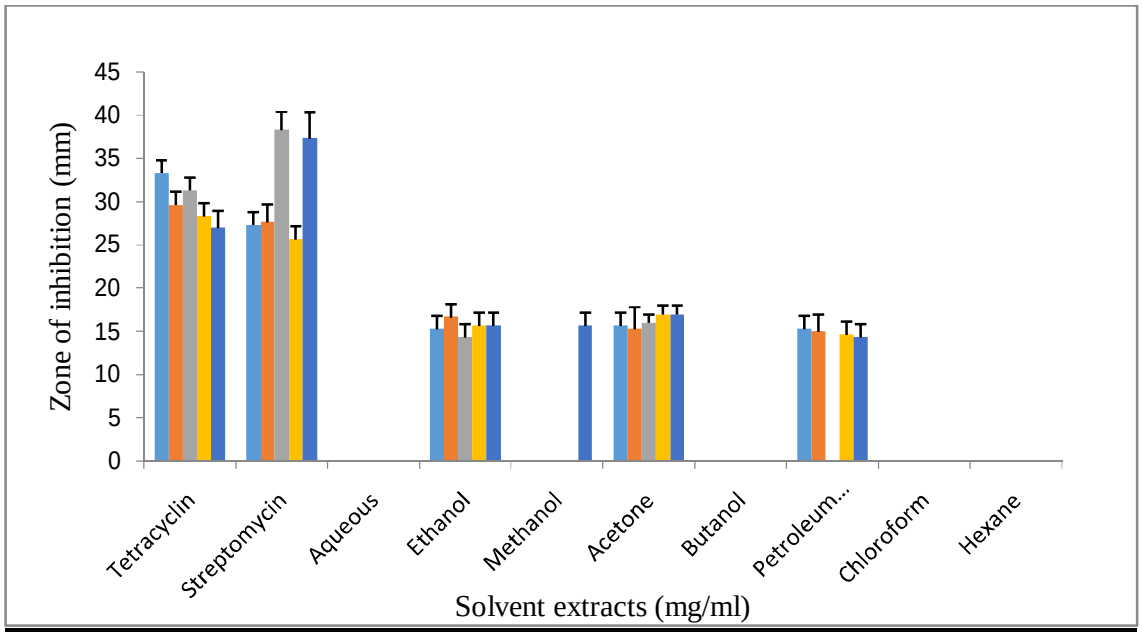

Fig. 4: Zone of inhibition in different solvents of *B.montanum* from fresh callus

B.subtilisE.coli K.pneumoniae P.fluorescenceS.aureus

CONCLUSION:

In conclusion, maximum callus induction was noticed in lower concentration and combination of NAA and BAP. Methanol and acetone are found to be a better solvent for the extraction of major phytochemical from *B. montanum.* The methanol and acetone of leaf and callus were found to be more effective against pathogens compared to other extracts.Further studies have to be carried out on the isolation and identification of antimicrobial compounds.

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