JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2010. Vol. 4(1), p. 397-400

Morphogenetic Studies and Antibacterial Potentialities of *Andrographis echioides* (L.) Nees

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(Received: 11 September 2009; accepted: 25 October 2010)

***Andrographis echioides* (L.) Nees is an erect annual herb under the family Acanthaceae. The plant is seen as an invasive weed in tropical areas. The whole plant has medicinal properties hence widely exploited that creates threat to its existence. Only a few studies were done on this plant. This work is an attempt to reveal the advantages of callus cultures in the propogation and to evaluate the antibacterial activity of both the leaf and the callus extract. For tissue culture studies different hormone combinations were used to observe the profuse callus growth. NAA/ BA combinations showed profuse callus growth. For antibacterial studies three bacterial strains were used. Only the leaf extracts showed the inhibition where as no response was noticed in the case of the callus extracts. Based on the present studies, it is concluded that tissue culture is an easy method to protect and conserve the plant from extinction and the callus culture extracts are less efficient as an antibacterial agent.**

Key words: *Andrographis echioides*, tissue culture, callus, leaf extract, antibacterial activities.

Andrographis echioides belongs to the family Acanthaceae, is an erect annual herb, rarely branched. The stem is covered with whitish hairs. In the systems of medicine it is predominantly used against blood cancer, liver troubles, jaundice, diabetes, cholera, dysentery , fever etc. The water extract of the leaves is used orally in natural medicines. Traditionally the plant has been used as febrifuge, bitter tonic, astringent and also for dysentery and fever[1-5]. Much scientific studies were not done on the plant parts. This is an attempt to study the morphogenetic and antimicrobial potentialities of this plant. The aerial parts were documented at the herbarium of University college (Voucher No.10010).

MATERIAL AND METHODS

The leaves are sessile, obtuse and sparsely hairy with cuneate base of 2.5 inch length and 0.5 inch broad. The fresh leaves were used to make crude extract. The collected leaves were cleaned and weighed 100g and shade dried for 30 days. Then the dried leaves were finely powdered. 20g of the dried powder were weighed

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and active ingredients from the fine powder of leaves were extracted by distillation process using Soxlet apparatus. The solvent used was methanol.. 200ml of methanol was taken into the round bottomed flask. The apparatus was kept over a heating mantle. Then the distillation by using soxhlet apparatus was carried out continuously for 7-8 hours at 65 – 70 ^{0}C. The extract was collected from the round bottom flask for further condensation by distillation. Then the extract was kept open for 2 days for the evaporation of methanol, thus only crude extract remained in the beaker. Then it was kept in the refrigerator for further experiment.

MS medium was selected for the study as it had proved successful in tissue culture studies in almost all families [6]. Internodal explants were used as materials suitable for callus induction. The range of auxin - cytokinin combination was determined based on the general observation [7]. Callus started developing about 3 days after inoculation. The growth continued for 4 weeks after which the growth rate retarded and medium became gradually dried and depleted. Subculture was done after 4 weeks intervals. Callus characteristics are summarized in Table I. All of the auxins used were successful in inducing calluses in combination with cytokinins. However, highest callus growth was produced by NAA/BA combinations and 2,4 D – Kinetin combinations. The calluses induced by the different hormone combinations varied in their morphology. The callus growths were collected dried and powdered and methanol extract was prepared. The extract was used for the antibacterial studies.

Table 1. Callus characteristics of *Andrographis echioides* stem explants grown in MS Medium with different phytohormone combinations

Auxin(mg/l)	Cytokinin(mg/l)	Callus growth	Remarks
IAA0.1	BA0.0	++	Greenish white
0.05	0.001	+	Callus
0.1	0.01	++	
0.2	0.05	Nil	
0.5	0.1	+	
IBA	BA		
0.1	0.0	++	Yellowishwhite
0.05	0.001	+	Friable callus
0.1	0.01	+++	
0.2	0.05	++	
0.5	0.1	Nil	
NAA	BA		
0.1	0.0	+++	Shoot formation
0.05	0.001	++	Compact
0.1	0.01	+++	Yellowishwhite
0.2	0.05	+++	callus
0.5	0.1	++	
2,4 D	KIN		
0.1	0.0	+++	Shoot formation
0.05	0.001	++	Compact
0.1	0.01	+	Greenishwhite
0.2	0.05	++	callus
0.5	0.1	+	

(+) Meagre callus growth (callus fresh wt.0.5g after 4 weeks)
(++)Average callus growth (callus fresh wt. 0.5g to 1g after 4weeks)
(+++) Profuse callus growth (callus fresh wt. 1g to1.5 g after 4 weeks)
(++++) Maximum callus growth (callus fresh wt. 1.5g after 4 weeks. This is considered as 100% growth

Antibacterial studies were done with disc diffusion method [8]. All the glasswares used was washed and autoclaved at 121°C for 15 minutes. Pure cultures of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* obtained from Kerala Agricultural University, Trivandrum were inoculated into the peptone water. It was prepared by adding 13.6g of peptone in 1000ml distilled water. The medium used for culturing bacteria was prepared by adding (28g/ L) nutrient agar in distilled water. Then it was boiled and autoclaved at 121°C for 15 minutes. Required volume of the molten medium was poured into the sterile petridishes under aseptic conditions.

The pure cultures of bacteria from the peptone water were transferred into the petridishes containing nutrient agar medium. Discs of different concentrations of leaf and callus extracts were placed into the petridishes. Control disc with methanol and a standard disc with Streptomycin were also placed on the petridishes. It was kept for incubation.

RESULTS AND DISCUSSION

In the present investigations rate of sensibility to the leaf extract and callus extract with methanol was recorded from the measurement of zone of inhibition and it was compared with that of the control and standard. The zone of inhibition showed by each bacterium were summarized in Table 2.

The leaf extract in methanol would inhibit the growth of bacteria reveals that it is responsible for bringing about the lysis of the cellwall materials. It is also possible that the phytochemicals might have brought about the inhibition of some of the enzymes involved in the metabolism or growth and development. The bacterial cell wall possess proteins which are the antigens. The secondary metabolites can build with these antigens and bring about immune precipitations which arrest further growth of bacteria and which may ultimately bring about a clear zone of inhibition around the disc[9,10]. In the case of the callus extract in methanol wouldn't inhibit the growth of microbes. This may be due the presence of low rate of secondary metabolites or due to their absence in the callus tissue. From the present study it was made clear that the callus tissue during the young stages do not possess sufficient quantity of phytochemicals which can inhibit the growth of microbes.

Table 2. Antimicrobial activities of various concentrations of Leaf and Callus extracts of *Andrographis echioides*

Test Organism	Disc No.	Concentration of leaf extract (In mg/ml)	Concentration of leaf extract (In mg/ml)	Zone of Inhibition In leaf extract (In mm)	Zone of Inhibition In callus extract (In mm)	Control Standard (Streptomycin In mm)
	1	1	1	10	-	14
E.coli	2	2	2	16	-	17
	3	3	3	15	-	18
Pseudomonas	1	1	1	12	-	14
aeruginosa	2	2	2	15	-	17
	3	3	3	18	-	18
Staphylococcus	1	1	1	14	-	14
aureus	2	2	2	16	-	17
	3	3	3	15	-	18

CONCLUSION

The methanolic leaf extract of *Andrographis echioides* showed a remarkable inhibition of growth in all the three bacterial cultures studied and the inhibition rate is almost same as that of the standard used. These extract

can be used as an alternative antibiotic in medicinal preparation after a thorough evaluation. On the otherhand a comparison of the antimicrobial activity using the callus extract showed very poor result that none of the combination showed an inhibition on the bacterial cultures. This proved that the callus during their young stage do not possess sufficient amount of phytochemicals to inhibit the growth of bacterial colonies. The morphogenetic techniques are very efficient and easy method to propogate and conserve the medicinal plants like *Andrographis echioides* which is on the verge of threat.

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