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ANTIMICROBIAL POTENTIAL OF LEAF EXTRACT OF NORMAL AND TISSUE CULTURED PLANTS OF *ANDROGRAPHIS PANICULATA* NEES

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ABSTRACT

Various extracts of root, stem and leaf of normal and tissue cultured plants of *Andrographis paniculata* were tested against five pathogenic bacteria. Four months old field grown and tissue cultured plants raised on MS+BAP (8.86 µM) were used as source plants. *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris* were pathogenic bacteria tested by Agar well diffusion method. The effect of various extracts measured by zone of inhibition varies with pathogens and also the source. Comparatively, methanolic leaf extracts of tissue cultured plants showed better zone of inhibition than the normal plants against all pathogens. Enhanced antibacterial activity of extracts of tissue cultured plants is related to the better growth performance and high content of secondary metabolites than the normal plants.

KEY WORDS: Tissue cultured plants, Andrographolide, Antimicrobial activity, Agar well diffusion method, *Andrographis paniculata*.

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INTRODUCTION

Andrographis paniculata Nees, one of the most widely used multipurpose medicinal herb commonly called as Kalmegh, belongs to the family *Acanthaceae*. The main active constituents are diterpene lactones, Andrographolides. The crude extract of the whole plant is used as a popular remedy for the treatment of various disorders in different health care systems especially as antitumoural [1], anti inflammatory [2] and anti viral [3]. Diterpenoids and flavonoids are the main constituents of *Andrographis paniculata* which are believed to be responsible for the most biological activities [4]. Wide medicinal value of this taxon has led to the overexploitation. Plant tissue culture technique has now established as an alternate strategy to conserve the elite varieties of medicinal plants by micropropogation. Several *in vitro* studies in the medicinal plants have shown that tissue cultured plants perform better than the normal plants both in morphology and is having better biosynthetic machinery [5,6,7]. Previous studies in this plant have shown the enhanced primary and secondary metabolism in tissue cultured plants [5]. Hence, the present investigation is an attempt to correlate the enhanced production of secondary metabolites with the antibacterial activity of the extracts of normal and tissue cultured plants against selected pathogenic bacteria.

MATERIALS AND METHODS

(i) Source of material

Roots, stems and leaves of 4 months old tissue cultured plants raised on Murashige and Skoog's medium [8] supplemented with BAP (4.43 µm and 8.86 µm) [9] and normal field grown plants of the same age were used as source plants. Both tissue cultured and normal plants are maintained in the Department of Botany, Bangalore University, Bangalore. The samples were cut into small pieces and shade dried for 15 days. They were ground into coarsely powdered form with the help of mortar and pestle**.**

(ii) Preparation of the crude extract

Dry powder of 100 g of roots, shoots and leaves of normal (*in vivo)* and tissue cultured *(in vitro*) plants of *Andrographis paniculata* were sequentially extracted with polar to non-polar solvents such as Petroleum ether (PE), Ethanol (95% V/V) Methanol (ME) and aqueous extract using soxhlet extractor on the water bath for 12 hrs each[10] . Each of the mixtures was carefully filtered using filter paper (Whatman No. A-3) and the extracts were concentrated to dryness in vacuum. The powdered extracts were dissolved in Di methyl formamide (DMF) in the concentrations of 1, 2, and 4 mg/l. These extracts were assessed for the antimicrobial activity against the selected pathogenic bacteria by Agar well diffusion method [11].

(iii)Pathogens used for antibacterial activity

Five bacterial strains were used in the present investigations. The selected bacteria were procured from the Department of Microbiology, University of Agricultural Sciences, GKVK, and Bangalore.

1. *Escherichia coli* Castellania and Chalmers: It is a gram negative, rod shaped bacteria living as a parasite in human and animal intestine (enteropathogenic). It causes enteric diseases, haemorrhage colitis (bloody haemorrhage), urinary tract infections and septicaemia [12].
2. *Bacillus subtilis* Frankland and Frankland: It is a gram positive, sporogenous rod shaped bacteria dwelling in water, dust, air and soil. *Bacillus* species other than *Anthrax bacillus* have been implicated in serious infections associated with immunosuppression, traumatic wounds and burns, operative procedures, haemodialysis, parenteral drug abuse and food poisioning. *Bacillus* species are also causative agents of meningitis, pneumonia,urinary tract infections and ocular infections [13,14] .
3. *Pseudomonas aeruginosa* (Schroeter) Migul**:** It is a gram negative, aerobic, non-sporic, motile bacteria present in soil, water, sewage, mammalian gut and plants.

It causes nasocomical infections including metabolic, haematological and malignant diseases. Several epidemic diarrhoea of infants, ocular infections, burn infections, cystic fibrosis, hot tub and whirlpool-associated folliculitis and osteomyelitis are caused by *P. aeruginosa* [15].

4. *Staphylococcus aureus* Sir Alexander Ogston**:** It is a gram positive, non motile and round bacteria which causes infections after injury and surgery. It is also known to cause boils, skin infections, pimples, impetigo, cellulitis, folliculitis, carbuncles, lung, heart, brain infections (meningitis) and bone inflammations.
5. *Proteus vulgaris* Hauser**:** It is a gram negative, rod shaped motile enterobacteria, present in alimentary canal of humans and animals. It also inhabits soil, water and faecal matter. It invariably causes urinary tract infections, wounds and nausocomial infections.

(iv) Culture media

Bacteria were cultured in nutrient agar broth (Himedia, India) and incubated at 37°C for 4h in BOD incubator and the suspension was checked to provide approximately 10^5 cells/ml. Preparation of nutrient agar media. [16]

Composition:

Bacteriological peptone	: 5g
Beef extract	: 3g
Sodium chloride	: 5g
Agar	: 15g

The above constituents were dissolved in 1000 ml of distilled water and pH was adjusted to 7.2. Agar was added to the medium and sterilized in an autoclave at 121^0 for 15 min at 15 lbs pressure.

(v) Agar well diffusion method [11]

In vitro antibacterial assay was carried out by Agar well diffusion method[11]. 200 µl of suspended inoculum was evenly spread on solidified nutrient agar in a petriplates using a sterilized spreader to get a uniform lawn of bacteria. With the help of sterile cork borer, four wells of 0.5 mm diameter were made. Where in 20 µl of each concentration of the extracts were filled using sterile syringes. Streptomycin was used as a positive control for antibacterial activity. The petriplates were incubated at 37±2°C. The zone of inhibition was recorded by measuring the diameter at the end of 24 hrs. As negative control, DMF in which the extracts were dissolved was added in separate petriplates. Each experiment was triplicated and average values are tabulated.

RESULTS AND DISCUSSION

The successive extracts of roots, stems and leaves in Petroleum ether, ethanol, methanol and aqueous extracts in normal and regenerated plants were investigated for *in vitro* antibacterial activity against a few pathogenic bacteria by agar well diffusion method by measuring the diameter of the growth of inhibition zone. Our observations revealed amongst leaf, stem and root extracts, the leaf extracts exhibited significant antibacterial activity [17, 18]. Among the different concentrations tested from 1, 2, and 4 mg/l, the best effective concentration was proved to be 4 mg/l against all the tested pathogenic organisms. Of all the extracts tested methanol leaf extracts proved to be the best for *E.coli* with 14.8±0.86 mm zone of inhibition in normal plants and in regenerated plants the zone of inhibition was 15.8±0.56 mm in ethanolic extract as compared to Streptomycin (ref compound) wherein the zone of inhibition recorded as 22.8±0.75 mm. (Table 1 and 2).

Table 1
Antimicrobial activity of normal plants of Andrographis paniculata Nees (4 mg/l)

Sl. No.	Microorganisms	Leaf (mm)				Stem (mm)				Root (mm)				Strepto mycin
		PE	EE	ME	AE	PE	EE	ME	AE	PE	EE	ME	AE	
1.	*Escherichia coli*	11.5± 0.75	14.6± 0.75	14.8± 0.86	11± 0.56	10± 0.23	11± 0.24	10± 0.25	07± 0.26	09± 0.76	10± 0.85	08± 0.76	07± 0.30	22.8± 0.75
2.	*Bacillus subtilis*	12± 0.65	15± 0.86	14.8± 0.68	10± 0.75	10± 0.54	10± 0.26	11± 0.23	12± 0.78	08± 0.56	09± 0.76	07± 0.86	08± 0.78	24.8± 0.65
3.	*Pseudomonas aeruginosa*	11.8± 0.75	15.6± 0.25	14.6± 0.78	11.5± 0.85	09± 0.49	08± 0.32	09± 0.28	08± 0.56	09± 0.56	08± 0.58	06± 0.34	05± 0.56	25.0± 0.75
4.	*Staphylococcus aureus*	15.2± 0.85	15.8± 0.65	14.7± 0.85	12± 0.75	08± 0.48	07± 0.45	08± 0.413	09± 0.46	08± 0.34	07± 0.37	06± 0.56	08± 0.87	24.8± 0.65
5.	*Proteus vulgaris*	14± 0.85	15.6± 0.56	15.6± 0.86	12.2± 0.85	08.5± 0.49	7.84± 0.56	08± 0.45	9.8± 0.46	08± 0.46	09± 0.78	07± 0.56	08± 0.56	25.2± 0.65

PE – Petroleum ether extract; EE – Ethanolic extract; ME – Methanolic extract; AE – Aqueous extract.

Table 2
Antimicrobial activity of regenerated plants of Andrographis paniculata (4mg/l)

Sl. No.	Microorga1nisms	Leaf (mm)				Stem (mm)				Root (mm)				Strepto mycin
		PE	EE	ME	AE	PE	EE	ME	AE	PE	EE	ME	AE	
1.	*Escherichia coli*	10.5± 0.23	15.8± 0.56	15.8± 0.34	9.8± 0.56	9.7± 0.65	8.5± 0.72	9.9± 0.62	8.9± 0.23	9.6± 0.26	11.5± 0.63	11.8± 0.45	9.8± 0.54	22.8± 0.75
2.	*Bacillus subtilis*	10.2± 0.52	16.2± 0.63	16.2± 0.72	10.2± 0.58	9.8± 0.62	10.1± 0.23	10.8± 0.36	9.8± 0.45	8.9± 0.58	11.6± 0.37	11.5± 0.36	9.6± 0.54	24.8± 0.65
3.	*Pseudomonas aeruginosa*	9.8± 0.48	15.2± 0.62	16.3± 0.54	9.9± 0.42	8.9± 0.48	9.8± 0.62	11.5± 0.56	9.3± 0.34	9.2± 0.43	10.8± 0.38	12.5± 0.62	10.2± 0.45	25.0± 0.75
4.	*Staphylococcus aureus*	8.9± 0.52	14.8± 0.38	15.9± 0.45	10.1± 0.65	9.2± 0.45	10.5± 0.52	10.5± 0.45	10.8± 0.23	8.9± 0.34	10.2± 0.42	12.8± 0.35	11.0± 0.46	24.8± 0.65
5.	*Proteus vulgaris*	9.4± 0.25	15.2± 0.56	15.8± 0.25	10.5± 0.24	9.5± 0.42	9.8± 0.53	10.2± 0.62	9.2± 0.46	9.5± 0.37	9.8± 0.28	12.6± 0.42	10.6± 0.36	25.2± 0.65

PE – Petroleum ether extract; EE – Ethanolic extract; ME – Methanolic extract; AE – Aqueous extract.

Ethanol and methanolic extracts were more effective with 15±0.86 mm and 14.8±0.68 mm zones of inhibition against *B.subtilis* in normal plants and 16.2±0.63 mm &16.2±0.72 mm zones of inhibition in regenerated plants. The same extracts also proved to be significant against *P.aeruginosa* wherein in normal plants the zone of inhibition were 15.6±0.25 mm & 14.6±0.78 mm and in regenerated plants the zone of inhibition was 16.3±0.54 mm for methanolic extract. The zones of inhibition exhibited against *S.aureus* in ethanolic and petroleum ether extracts in normal plants were 15.8±0.65 mm & 15.2±0.85 mm, whereas in methanolic extract of regenerated plants it was 15.9±0.45 mm as compared to normal which measured 14.7±0.85 mm against *S.aureus*. Ethanolic and methanolic extracts were more effective in P.vulgaris and almost similar zones of inhibition were exhibited both in normal and regenerated plants. Petroleum ether and aqueous extracts exhibited least activity compared to ethanolic and methanolic extracts for all the pathogenic bacteria in the present studies which is in agreement with the report of Aniel Kumar et al.[18]. Alpana *et al*.,[19] and Saxena *et al.* [20] found that the methonal is the best solvent for exhaustive extraction of andrographolide and its derivatives. The enhanced antibacterial activity of methanolic extracts found in the present studies may be due to the presence of these compounds which are known to exhibit antimicrobial activity [21]. Ram *et al.,*[22] had found significant antimicrobial activity of ethanolic extracts of crude drug samples of *Andrographis paniculata* against several pathogenic microorganisms. The aforesaid data indicate the wide range of spectrum of antibacterial activities of leaf extracts of regenerated plants as compared to normal plants. Tejavathi et al. [17] have reported the enhanced antimicrobial activity of the extracts of the regenerated plants of *Agave vera-cruz* than the normal plants. Preliminary phytochemical studies conducted in normal (*in vivo*) and regenerated plants (*in vitro*) have shown that *in vitro* plants contain more quantities of primary and

secondary metabolites as compared to *in vivo* plants. This perhaps is related to higher content of secondary metabolites particularly, Andrographolide, in regenerated plants compared to normal plants. [5]. Similar results were reported in *Bacopa monnieri,* where the tissue cultured plants were found to be superior than normal plants in the contents of both primary and secondary metabolites [23]. Phytochemical and HPLC analysis in *Andrographis paniculata* have shown that flavonoids, alkaloids, triterpenes, glycosides and particularly andrographolides and its derivatives are the main constituents of *Andrographis paniculata* [20, 5].

CONCLUSION

The plant extracts of both normal and tissue cultured plants of *Andrographis paniculata* tested against different bacteria showed inhibitory effect but varied with the organisms. Ethanolic and methanolic leaf extracts of both samples exhibited significant antibacterial activity against the tested bacteria. The presence of compounds like phenols, tannins, flavonoids, alkaloids, glycosides and triterpenes in the extracts might be responsible for the antimicrobial activity. Tissue cultured plants proved to be superior to the normal in their antibacterial activity, since they are having more of these compounds than the normal plants. From the present study, it appears that although *Andrographis paniculata* and other herbs are not absolute substitutes for antibiotics, these plants could have a complementary effect when used along with antibiotics. In fact in the recent years it has become a practice to combine natural remedies with synthetic medications in therapies that are more effective and safer than individual applications.

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