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Enhanced Antibacterial Activity in Leaf Callus Extracts of Andrographis Paniculata Wall

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Abstract: ***Antimicrobial activity of leaf and leaf callus extract of Andrographis paniculata Wall., was studied using different solvent like chloroform, acetone, ethanol and water against two - gram (+) ve and two - gram (+) ve bacterial strains like Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa, and Escherichia coli. The antibacterial activity was determined by disc diffusion method. Out of the four - extract used, acetone and ethanol extracts were found to be highly active in both leaf and leaf extracts. It is also found that the zone of inhibition was much higher in callus extracts, when compared to that of leaf extracts.***

Keywords: Andrographis paniculata, callus extract, Pseudomonus aeruginosa Bacillus subtilis, Staphylococcus aureus and Escherichia coli

1. Introduction

Andrographis paniculata Wall., of the family *Acanthaceae*, the much - branched annual herb known as "King of bitters". The plant is distributed in Assam, Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Tamil Nadu and Kerala in India. Traditional practitioners use *Andrographis paniculate* in the treatment of hyperdipsia, wounds, ulcers, chronic fever, malarial and cough, bronchitis, skin diseases, leprosy, flatulence, colic, diarrhea and dysentery. "Alui" is prepared by mixing the powder of cumin (Cuminium cyminum) and large cardamom (Amomum subulatum) in the juice of this plant and administered for the treatment of malaria (Warrier et al, 1993 and Thakur et al, 1989). It has andrographolidic properties and the higher plants produce a diverse range of organic chemicals called secondary metabolites and these secondary metabolites are produced for self - defense, which are active against disease causing microbes. Most of these chemicals could be of significant interest in the treatment of human diseases. But there is a need of scientific approaches to study their safety and efficacy. The pharmacological screening of the plant wealth would probably, contribute to development of some more new drugs. Thus, several plant species have been documented for antimicrobial activities, still the search for antimicrobial chemicals from new plant sources to control common diseases continues on an increasing scale. At the same time priority should be given to proper utilization and conservation of healing herbs. The development of tissue culture technology helps in the conservation of plant diversity up to certain extent. Apart from the innovation aspects, major efforts have been directed towards callus and cell suspension cultures for the secondary metabolite biosynthesis of pharmacological and pharmaceutical interests. The present work is an attempt for the pharmacological screening of the leaf of medicinal plant *Andrographis paniculata* wall. and its calli.

2. Materials and Methods

Plant Material: *Andrographis paniculata* Wall., is an erect branched annual 1 - 3 ft. high; branches are quadrangular and narrowly winged in the upper part. Leaves are lanceolate, acute, glabrous, slightly undulate, and pale beneath; base tapering; main nerves 4 - 6 pairs, slender; petioles long. Flowers are small, solitary, distant, spreading axillary and terminal racemes, the whole forming a large pyramidal paniculate inflorescence. Calyx long sepals equal, linear - lanceolate, glandular - pubescent. Corolla rose colored, long, and hairy outside. Filaments flattened, hairy in the upper part and anthers bearded at the base. Ovary is glabrous and style slightly pubescent. Capsules are linear - oblong, acute at both ends. Seeds are numerous, subquadrate, glabrous and yellowish - brown.

Extraction Procedure: The leaves of *Andrographis paniculata* Wall., were collected from the botanical garden of Bangurnagar Arts, Science and Commerce College Dandeli, Uttara Kannada District, Karnataka State, Southern India. The leaves were dried under shade and prepared coarse powder using an electrical grinder. Callus induction by inoculating young leaf segments in MS medium supplemented with 20 µm 2, 4 –D by standard tissue culture technique. Callus harvested after two months, dried at 40 ^{0}C in oven for 24 hrs. and made in to fine powder. The powder was subjected for successive extraction with ethanol, chloroform, acetone and water using Soxhlet apparatus separately. The extracts were dried and dissolved in DMF (Dimethyl formamide) solution and screened for antimicrobial activity.

Preliminary Phytochemical Screening: The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis (Kokate 1993) was carried out by following procedures:

Test for Alkaloids: A small portion of the extract is stirred with few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

Test for Sapohins: One ml of extract is diluted with 20ml of distilled water and shaken vigorously for 15 min formation of stable foam indicates the presence of saponin

Test for Tannins: Development of blue green color in the extrac6t when treated with ferric chloride indicates the presence of tannins.

Test for Phenols: Phenol test Small quantity of extract is diluted with 5% ferric chloride solution. Development of intense color indicates the presence of phenols.

Test for Steroids and Triterpenes

Leibermann - Burchards test - The extract is treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of reddish - brown ring indicates the presence of steroids.

Salkowskis test - A few drops of chloroform and few drops of concentrated sulphuric acid was added to the extract. Appearance of yellow color in the lower portion indicates the presence of triterpenes

Test for Flavonoids

Ferric chloride test - The extract is treated with few drops of 5% ferric chloride. The appearance of blackish green color indicates the presence of flavonoids.

Antimicrobial assay: The antimicrobial screening was done by using bacterial strains like *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa* and *Escherichia coli.* All the bacterial strains were obtained from the stock culture Department of Botany Bangurnagar Degree College Dandeli. The antimicrobial activity was determined by disc diffusion method (Bauer et al 1966). Three different concentrations of 25mg/ml, 20mg/ml and 15mg/ml respectively were prepared. Each sterile disc was loaded with 10µl of test extract to obtain effective concentrations as 250, 200 and 150 µg/disc and placed on the agar plates inoculated with respective microorganisms. The plates were kept for half an hour for pre - incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hrs for bacteria. The incubation zones around the discs were measured in mm using Hi Antibiotic Zone scale. The study was performed in triplicate. Streptomycin disc was used as standard for bacteria.

Determination of Minimum concentration: The minimum inhibitory concentration was determined by serial dilution method (Rollins and Joseph 2000). Serial dilution of the extract was prepared in the test tubes containing peptone water as diluent. Fifty mg of the extract was dissolved in one ml of DMF which is further subjected for two - fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37°C for 24hrs. The tubes were then examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

3. Results and Discussion

Table 1 comprises the phytochemical analysis of the leaf and leaf callus extract of *Andrographis paniculata* and the presence of flavonoids and phenolic compounds. The antibacterial activity of *Andrographis paniculata* leaf and leaf callus extract and the zone of inhibition in comparison with the standard used. In the present investigation, all the extracts have shown antibacterial activity, even though their range of activity varied and the sensitivity of the microbes to different extracts (Table 2). Significant enhancement has been observed in the callus extracts when compared to the leaf extracts. The activity can be positively correlated to the dose, as there is an increase in the zone of inhibition with increased dose. Highest zone of inhibition was observed in acetone extract at 250 ug/disk concentration, against all the tested microbial strains. Highest increase in the percent activity index was found in acetone extract at its highest tested concentration. All the strains were sensitive to streptomycin, while control (DMF) did not show any activity. In all, four different solvent extracts were tested each with three different concentrations. Among them, acetone extract is most active against all the tested microorganisms when compared to other extracts. The enhancement in the antimicrobial activity of callus extracts was observed in all the cases (Table 3). This enhancement in the activity could be due to the accumulation of active metabolites in the cell lines of callus cultures (Nezbedová et al., 1999). Similar enhanced activity of the callus extracts have been reported in *Bacopa monnier, Eclipta alba, Bixa Orellana, Solanum trilobatum* and *Alophyllus cobbe* by earlier workers (Tejavathi *et al*., 1996 Kathiresan and Ravikumar, 1997; Lakshmi *et al*., 1999; Sagar *et al*., 2000; Castello *et al*., 2002; Nagarajan *et al*., 2009).

4. Conclusion

The *Andrographis paniculata* extracts of leaf and leaf callus extract tested against different bacteria showed inhibitory effect but varied with the organisms. Acetone extracts of leaf and leaf callus exhibited significant antibacterial activity against the tested bacteria. The compounds like phenols, tannins, flavonoids, alkaloids, glycosides and triterpenes in the extracts might be responsible for the antimicrobial activity. Leaf callus extract proved to be superior to the antibacterial activity, since they are having more of these compounds than the normal plants. The present study opens a new era in correlating the Ayurveda and Siddha with modern microbiology. The promising result obtained in this study may lead to the development of a potential antibiotic from the leaf callus extract of *Andrographis paniculata* against bacterial strains. Further it also encourages the young researchers to test other medicinal plants for their biological activities.

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Table 1: Phytoconstituents of *Andrographis paniculata* Wall., leaf and callus extract

	Successive extracts							
Phytoconstituents	Chloroform		Acetone		Ethanol		Water	
	Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus
Alkaloids	+	+	-	+	+	+	+	-
Saponins	-	-	-	-	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Phenolic compounds	-	-	+	-	+	+	+	+
Steroids/Triterpenes	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	-

Table 2: Percentage inhibition of extracts of *Andrographis paniculate* Wall., when compared to control.

Extract	CONC.[a]	Percentage Inhibition compared to control[b]							
		Bacillus subtilis		Pseudomonas aeruginosa		Staphylococcus aureus		Escherichia coli	
		Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus
Chloroform	250	9.36 ±0.08	14.76 ±0.08	8.68 ±0.06	18.80 ±0.10	9.60±0.06	13.80±0.08	9.10 ±0.08	11.50±0.06
	200	8.62 ±0.10	12.80 ±0.06	8.60 ±0.04	17.60 ±0.08	8.80 ±0.08	12.60±0.06	8.00 ±0.08	10.40 ±0.08
	150	7.20 ±0.06	10.60 ±0.08	7.10 ±0.08	16.20 ±0.08	7.30 ±0.09	8.60 ±0.04	7.68 ±0.06	8.60 ±0.04
Acetone	250	13.32 ± 0.06	20.86 ±0.06	10.68±0.09	20.64±0.09	9.20±0.08	16.30±0.09	11.46 ±0.06	14.50±0.06
	200	12.40 ±0.08	18.40 ±0.08	9.60 ±0.06	18.60 ±0.08	9.40 ±0.06	15.60±0.06	10.48 ±0.08	12.80±0.08
	150	10.40 ±0.09	14.20 ±0.07	7.40 ±0.06	15.36 ±0.08	8.60 ±0.08	11.80±0.06	9.68 ±0.06	10.50 ±0.08
Alcohol	250	8.56 ±0.06	12.26 ±0.08	8.32±0.09	12.14±0.12	10.20±0.08	14.30±0.06	9.22 ±0.05	11.50±0.09
	200	8.10 ±0.08	10.40 ±0.07	7.10 ±0.08	11.20 ±0.09	9.10 ±0.10	12.40±0.08	8.60 ±0.07	10.80±0.07
	150	7.40 ±0.07	9.20 ±0.08	7.20 ±0.08	9.46 ±0.08	7.30 ±0.07	10.50±0.06	7.58 ±0.07	8.64 ±0.08
Aqueous	250	10.50 ±0.06	12.96 ±0.08	9.22±0.09	12.80±0.12	9.560±0.08	10.60±0.16	9.52 ±0.08	12.50±0.09
	200	10.10 ±0.08	11.40 ±0.09	8.10 ±0.08	10.20 ±0.09	8.46 ±0.04	9.40±0.08	8.50 ±0.08	11.80±0.08
	150	9.10 ±0.07	9.40 ±0.08	7.50 ±0.08	8.46 ±0.05	7.80 ±0.08	8.40±0.06	8.18 ±0.08	8.63 ±0.08

[a] µg/disk [b]Values are mean of triplicates