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Full Length Research Paper

Enhanced antibacterial activity in leaf-callus extracts of *Alophyllus cobbe* L.

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Antimicrobial activity of crude extracts from the leaves and leaf-callus of *Alopyllus cobbe* L. (Sapindaceae) was studied. Chloroform, acetone, ethanol and water extracts were tested in three different concentrations (250, 200 and 150 µg/disk) against two gram (+)ve and two gram (-)ve strains of bacteria namely *Bacillus subtilis, Streptococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*. Streptomycin was the control, while disk impregnated with DMF was used as blank. Acetone extract showed maximum activity in both leaf and leaf-callus extracts. It is also found that the zone of inhibition was much higher in callus extracts, when compared to that of leaf extracts.

Key words: *Alophyllus cobbe*, antimicrobial activity, callus extract, leaf extract

INTRODUCTION

The familiarity of humankind with plant species producing food, medicine, essential oil etc. dates back to beginning of civilization. Today, apart from the tremendous advancement in the field of modern medicine, the age-old knowledge of healing herbs is popular in many parts of the world, especially in rural folk. Further, about 80% of the drugs used in modern medicine are the products of plant origin; their sales exceed US$ 65 billion in 2003 (Patwardhan et al., 2004).

This clearly indicates the revitalization of herbal drugs in modern medical system. But there is a large difference between the demand and production of many of the healing herbs. Generally raw herbal drugs are collected from wild, often in a destructive manner. This may lead to extinction of many plant species with potential benefits to mankind even before they are studied completely. Thus in recent days, apart from other conservational measures, major efforts have been directed towards callus and cell suspension cultures for the production of secondary metabolites of pharmacological and pharmaceutical interests. The present work is an attempt for the pharmacological screening of the leaf of medicinal plant *Alophyllus cobbe* Bl. and its calli.

A. cobbe Bl. (Sapindaceae) is extensively used for the treatment of diarrhoea, elephantasis, cuts and wounds. The leaves are also used as wormifuge and colic (Jain, 1991; Yoganarasimhan, 1996). *A. cobbe* is an erect or scandent shrub with trifoliate leaves (Figure 1). The plant is common in moist forests and along banks of rivers and streams. In the region, it is widely distributed in dry deciduous to evergreen forests of the Western Ghats (Saldanha, 1996). In India, it is reported from Plains of Eastern India, Western Ghats and Deccan plateau, up to 600 m elevation (Pant, 2000). *A. cobbe* is widely spread in Archipelago, extending to Africa, America and Australia (Hooker, 2007); also reported from Myanmar, Bangladesh, Sri Lanka and Philippines (Pant, 2000).

EXPERIMENTAL

Plant material

The plant materials of *A. cobbe* were collected from the botanical gardens of Karnatak University Campus, Dharwad. The plant was authenticated and voucher specimen was deposited in the herbarium at Department of Botany, Karnatak University, Dharwad (KUD/Ang/810). The leaves were dried in an oven at 40°C for 72 h

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Figure 1. Habit of *A. cobbe* L.

and powdered in an electric grinder.

Callus induction

The healthy leaves of *A. cobbe* were sterilized in Laboline (1%; Qualigens, India) for 15 min and washed in 1% $NaOCl_2$ (Ranbaxy, India) for 10 - 15 min and rinsed with 0.2% $HgCl_2$ for 8 - 10 min followed by 4 - 5 times rinse in sterile distilled water. Explants were inoculated in MS basal medium, supplemented with 15 µM 2,4-D (2, 4-Dichlorophenoxyacetic acid) (Murashige and Skoog, 1962). The cultures were maintained at 24 ± 2°C in 16 h photoperiod of 40 µmol m^{-2} s^{-1} light provided by cool white fluorescent tubes with 70% relative humidity. The callus was harvested after 60 days and was dried at 40°C for 72 h in an oven. This dried callus powder was taken for further extraction procedures.

Extraction

Ten grams of the powdered material was subjected to continuous extraction in Soxhlet apparatus. The solvents used were chloroform, acetone, ethanol and water in order. The extracts were evaporated to dryness and were re-dissolved in aliquots of Di Methyl Formamide (DMF) to obtain the test solutions of the concentrations 25, 20 and 15 mg/ml. Further, the effective concentrations of 250, 200 and 150 µg/disk were obtained by impregnating the sterile disks by 10 µL of each test solutions.

Antimicrobial assay

All the extracts were tested against four bacterial strains. *Bacillus subtilis* (ATTC 6633) and *Streptococcus faecalis* (ATTC 29212) were the Gram (+)ve strains, while *Escherichia coli* (ATTC 8739) and *Pseudomonas aeruginosa* (ATTC 9027) were the Gram (-)ve strains. The bacterial strains were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune.

The antimicrobial activity was assayed by standard disk diffusion method (Bauer et al., 1966). Streptomycin (SD 031, Hi-media) was the standard antibiotic used in the assay, while a disk impregnated with DMF served as blank.

The plates were incubated at 37°C for 24 h and the zone diameters (including the 6 mm disk) were recorded. Sterile conditions were maintained throughout the experiment.

The experiment was carried out in triplicates and the mean readings were tabulated with standard errors (Table 1). Further, the activity index of each extract was calculated by taking the ratio of inhibition zone of extract to that of standard. The percent increase in the Activity Index of the respective callus extracts, when compared to that of leaf extracts, are presented in Table 2.

RESULTS AND DISCUSSIONS

In the present investigation, all the extracts have shown antibacterial activity, even though their range of activity varied and likewise the sensitivity of the microbes to different extracts (Table 1). Significant enhancement has been observed in the corresponding callus extracts when compared to the leaf extracts (Table 2). 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 µg/disk concentration, against all the tested microbial strains. Highest increase in the percent activity index was found again in acetone extract at its highest tested concentration. All the strains were sensitive to

Table 1. Antimicrobial activities of leaf and leaf-callus extracts of *A. cobbe* L.

Extract and concentration[b]		Zone of inhibition (mm.)[a]							
		P. aeruginosa		*B. subtilis*		*S. faecalis*		*E. coli*	
		Leaf	Callus	Leaf	Callus	Leaf	Callus	Leaf	Callus
Chloroform	250	8.50 ± 0.07	18.40 ± 0.09	9.30 ± 0.9	13.86 ± 0.09	9.20 ± 0.07	13.50 ± 0.07	9.10 ± 0.05	10.50 ± 0.07
	200	8.10 ± 0.07	17.30 ± 0.08	8.50 ±0.09	12.50 ± 0.07	8.50 ± 0.9	12.10 ± 0.08	8.00 ± 0.09	9.30 ±0.08
	150	8.10 ± 0.09	15.20 ± 0.09	7.60 ± 0.09	10.40 ± 0.08	7.20 ± 0.07	8.40 ± 0.06	7.86 ± 0.07	8.40 ± 0.09
Acetone	250	10.20 ± 0.09	20.53 ± 0.15	12.10 ± 0.08	20.40 ± .07	9.60 ± 0.09	17.20 ± 0.09	11.40 ± 0.08	14.20 ± 0.09
	200	9.40 ± 0.07	18.20 ± 0.08	10.50 ± 0.08	17.20 ± 0.06	9.10 ± 0.05	15.30 ± 0.07	10.40 ± 0.05	11.80 ± 0.07
	150	8.40 ± 0.08	15.40 ± 0.08	9.50 ±0.09	13.20 ± 0.07	8.30 ± 0.09	11.00 ± 0.05	8.50 ± 0.08	9.50 ± 0.08
Ethanol	250	8.20 ± 0.09	12.16 ± 0.12	8.56 ±0.04	12.20 ± 0.08	10.10 ± 0.09	14.20 ± 0.08	9.20 ± 0.07	11.30 ± 0.09
	200	7.50 ± 0.09	11.20 ± 0.09	8.00 ± 0.08	10.20 ± 0.07	9.10 ± 0.11	12.40 ± 0.08	8.60 ± 0.07	10.20 ± 0.07
	150	7.20 ± 0.08	9.50 ± 0.07	7.20 ± 0.07	9.30 ± 0.08	7.50 ± 0.07	10.50 ± 0.07	7.50 ± .08	8.63 ± 0.08
Aqueous	250	9.10 ± 0.08	12.80 ± 0.08	10.50 ± 0.05	12.96 ± 0.09	9.50 ± 0.08	10.63 ± 0.18	9.50 ± 0.09	12.40 ± 0.07
	200	8.10 ± 0.08	10.6 ± 0.09	10.10 ± 0.08	11.30 ± 0.09	8.56 ± 0.04	9.20 ± 0.08	8.50 ± 0.09	11.10 ± 0.09
	150	7.50 ± 0.08	8.50 ± 0.05	9.10 ± 0.08	9.50 ± 0.08	7.86 ± 0.09	8.30 ± 0.09	8.10 ± 0.09	8.63 ± 0.09
Control (DMF)[c]		NI	NI	NI	NI	NI	NI	NI	NI
Standard[d]		25.20 ± 0.07	25.06 ± 0.05	24.40 ± 0.05	24.16 ± 0.11	22.40 ± 0.08	22.40 ± 0.09	24.30 ± 0.05	24.40 ± 0.07

[a] Mean values of triplicates; [b] µg/disk; [c] 10 µL/disk; [d] Streptomycin (10 µg/disk) NI = No inhibition.

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. Eloff (1998), thus rightly suggested that acetone is a most suited extractant for antimicrobial screening as it dissolves many hydrophyllic and lipophyllic components from the plants.

Tannin is reported from the root of the plant, while β-sitosterol and benzylamide are reported in stem and leaves respectively, along with the traces of alkaloids (Yoganarasimhan, 1996; Rastogi and Mehrotra, 1999). However, no reports are available on biological activity of the plant.

The enhancement in the antimicrobial activity of callus extracts was observed in all the cases (Table 2). This enhancement in the activity might 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 *Heterostemma tanjorense*, *Eclipta alba*, *Bixa orellana* and in some halophytes by earlier workers (Kathiresan and Ravikumar, 1997; Lakshmi et al., 1999; Sagar et al., 2000; Castello et al., 2002). However, the extent of enhancement depends on many factors like type and composition of media, plant growth regulators and culture conditions; among which plant growth regulators play vital role in the production of the active compounds (Morris et al., 1985). However, there is no unanimous opinion about the relation between growth regulators in the media and production of active principle(s) (Nezbedová et al., 1999; Kathiresan and Ravikumar, 1997; Morris et al., 1985). The results of the present study revealed the positive relation between production of active metabolites in the callus and growth regulator (2,4-D) in the media (Table 1 and 2). Further comparative studies involving different phytoharmones will only help to standerdize protocol for maximum production of bioactive compounds from the leaf- callus of *A. cobbe*.

Table 2. Percentage increase in activity index of the callus extracts.

Extract and concentration[a]		Percent increase in activity index[b] of the callus extract			
		P. aeruginosa	*B. subtilis*	*S. faecalis*	*E. coli*
Chloroform	250	39.69	19.25	19.19	5.59
	200	36.89	16.90	16.07	5.19
	150	27.92	11.90	5.36	2.08
Acetone	250	51.45	34.85	33.93	11.28
	200	35.32	36.16	27.68	5.57
	150	28.12	15.7	12.05	3.96
Ethanol	250	15.99	15.41	18.31	8.45
	200	14.93	9.43	14.73	6.41
	150	9.33	8.99	13.39	4.50
Aqueous	250	14.96	10.61	5.04	11.72
	200	8.00	5.38	2.86	10.52
	150	4.21	2.03	1.97	2.03

[a] µg/disk; [b] $\text{Activity Index} = \frac{\text{Inhibition zone of extract}}{\text{Inhibition zone of standard}}$

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