



Phytochemical analysis and anti microbial activity of *Cleistanthus collinus* (ROXB.) Benth. & Hook

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ABSTRACT

Cleistanthus collinus is known for being toxic and frequently used for homicidal and suicidal purposes contains three identical compounds, viz. Cleistanthin A, B and C (diphyllin glycoside) in addition to other secondary metabolites. This species has a restricted global distribution occurring in India and Sri Lanka. In India, it has been recorded in hills of Himachal Pradesh to Bihar, Andhra Pradesh, Karnataka and Kerala. This is very common in north eastern districts of Andhra Pradesh. The aerial parts of the tree are investigated for phytochemicals and its antimicrobial properties for value addition of this important non wood forest product (NWFP). The bark and leaf powder of *Cleistanthus collinus* were extracted with different solvents and quantified with a view to isolate natural compounds with high value and novel properties for better applications. Methanol and ethyl acetate extracts of leaf and bark were investigated for anti microbial activity on four species of bacteria *Escherichia coli*, *Bacillus*, *Staphylococcus*, *Pseudomonas*. The percentage of growth inhibition of the four bacterial species to various concentrations of extracts was calculated and tabulated. The methanol extract as well as ethyl acetate extracts of both bark and leaf were found to inhibit all four species of bacteria. On broader comparison, the methanol extract of leaf was found to be more effective in inhibiting all the four bacterial species compared to methanol extract of bark and ethyl extract of both leaf and bark extracts. Thus it was concluded that the methanol extract possessed the compounds which were capable of inhibiting the growth of human pathogen at very low concentrations.

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KEYWORDS

Cleistanthus collinus;
Leaf;
Bark;
Extracts;
NWFP;
Anti microbial.

INTRODUCTION

India is a country of vast diversity with diversified climate with different physiographic factors and is blessed with all types of vegetation. Of the nearly 425 families of flowering plants in the world, 328 families with 21,000 species occur in India. These plants pro-

duce highly diverse secondary metabolites for its defense and survival mechanism and invariably these phytochemicals have varied uses to mankind and the demand has steadily increased for their search in numerous beneficial purposes worldwide in recent years.

The genus *Cleistanthus* of the family Euphorbiaceae, comprises 140 species of which

Full Paper

Cleistanthus collinus is known for being toxic and frequently used for homicidal or suicidal purposes^[5]. This species has a restricted global distribution occurring in India and Sri Lanka. Within India, it has been recorded in dry hills of various parts of India, very common in most parts of the Northern and eastern Districts of Andhra Pradesh.

It contains three identical compounds, namely *Cleistanthin A*, *Cleistanthin B* and *Cleistanthin C* in addition to other secondary metabolites^[4]. Various extracts of this plant yield a multitude of compounds that include glycosides, arylmethoxyphenol lignan lactones such as cleistanthin A and B, collinusin and oduvin found to have anti-microbial activities. Cleistanthin A and B were extracted from *C. collinus* leaves by alcoholic soxhalation.

The phytochemical analysis of parts of *Cleistanthus collinus* to identify the potential bioactive component as well as look into the *in vitro* antifungal activity of this plant against wood degrading fungi which would aid in the development of a potential wood preservative and also *in vitro* antibacterial activity of the plant against human pathogens to throw light on its pharmacological benefits.

MATERIALS AND METHODS

The plant materials were collected from Vishakapatnam forest area in Andhra Pradesh. The collected bark and leaf materials were powdered and extracted sequentially with organic solvents varying from non polar to polar and the extracts were dried and quantified and used in further experimentation.

The small portion of the extracts were taken for preliminary phytochemical analysis as per standard procedure to test for alkaloids, terpenoids, steroids, coumarins, tannins, flavanoids, phenols, volatile oils, quinones and cardiac glycosides.

Methanol and ethyl acetate extracts of bark and leaf were taken for its evaluation of antibacterial activity. The extracts were dissolved in Dimethyl sulfoxide (DMS) solvent and three different concentrations (0.05, % 0.1% 0.25%) were taken for further studies.

The bacterial isolates like *Escherichia coli*, *Bacillus*, *Staphylococcus*, *Pseudomonas* were taken for evaluating its potency against the extract by sub-

culturing first in nutrient broth (*Hi Media Ltd.*) and incubating at 28°C for 24 hours by modified agar well diffusion method^[2]. A separate plate was maintained as control. 10 µl of the extracts were added using sterile micropipettes and allowed to diffuse into the wells. The diameter of the zone of inhibition was recorded. The percentage growth inhibition of the four bacterial species to various concentrations of the extracts was calculated and plotted. The significance of the experiment conducted was subjected to ANOVA and the critical difference was studied.

RESULTS AND DISCUSSION

The phytochemical analysis of the various extracts of parts of *C. collinus* revealed the presence of cardiac glycosides in all the extracts of bark and leaf. Also tannins were present in all the extracts except the water extract of leaf. The terpenoids and flavanoids which were present in the methanol extracts of bark and leaf were found to be absent in their corresponding water extracts (TABLE 1). These phytochemical compounds are known to play important roles determining the bioactivity of the plant. Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure^[6]. This compound has been reported to be a novel cancer therapeutic agent^[3]. All the parts of *C. collinus* extracts contain cardiac glycosides, which can be explored further for pharmacological aspect. Tannins (tannic acid) are water-soluble polyphenols that are present in many

TABLE 1 : Qualitative phytochemical analysis

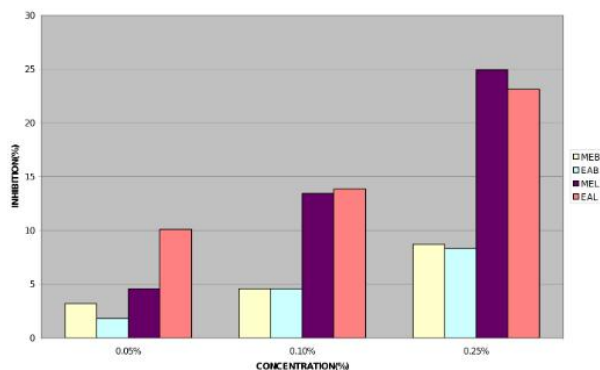
S. N.	EXTRACTS	AL	TE	ST	CO	TA	FL	PH	VO	QU	CG		
		B	L	B	L	B	L	B	L	B	L	B	L
1	Petroleum ether	-	-	+	-	+	+	+	-	+	+	+	+
2	Benzene	-	-	+	-	+	+	-	-	-	-	-	+
3	Chloroform	-	-	+	-	+	+	-	-	-	-	-	+
4	Ethyl acetate	-	-	+	-	+	+	-	-	+	+	-	+
5	Methanol	-	-	+	+	-	+	+	+	+	+	-	+
6	Water	-	-	-	-	-	+	+	-	-	-	-	+

AL = ALKALOIDS FL = FLAVANOIDS
 + = PRESENCE - = ABSENCE
 TE = TERPENOIDS PH = PHENOLS
 B = BARK L = LEAF
 ST = STEROIDS VO = VOLATILE OILS
 CO = COUMARINS QU = QUINONES
 TA = TANNINS CG = CARDIAC GLYCOSIDES

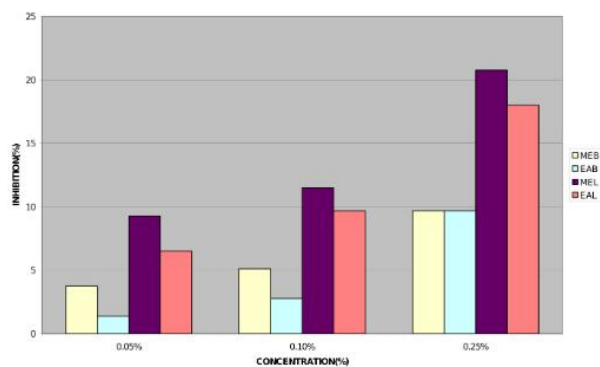
plants. The growth of many fungi, yeasts, bacteria and viruses were inhibited by tannins. Terpenoids are known to be very active against bacteria. Flavonoids are known to be synthesized by plants in response to microbial infection and are known to be active against microorganism^[1].

The antimicrobial investigations carried out on *C.collinus* extract revealed that the plant possess remarkable antimicrobial activity against the tested organisms. The methanol extract as well as the ethyl acetate extracts of both bark and leaf were found to inhibit the growth of all the four species of bacteria. In the case of *E.coli* the percentage of inhibition was found to be more in the methanol extracts of bark and leaf. The percentage growth inhibition was found to increase with increase in concentration. Similarly in the *Bacillus*, *Staphylococcus*, and *Pseudomonas* also the percentage growth inhibition calculated from the diameter of inhibition zones formed was found to increase with increase in concentration and more for the methanol extracts of bark and leaf compared to the ethyl acetate extracts. On a broader comparison, the methanol extract of leaf was found to be more effective in inhibiting all the four

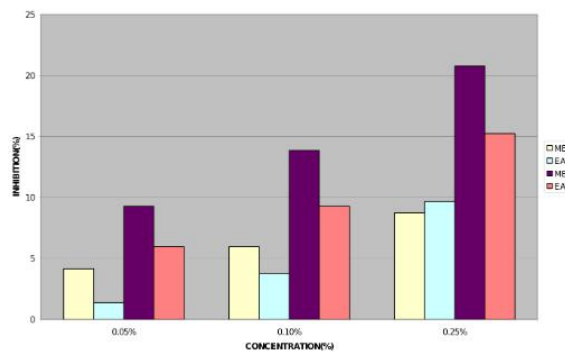
bacterial species compared to the methanol extract of bark and the ethyl acetate extracts. Thus it was concluded that the methanol extracts possessed compounds which were capable of inhibiting the growth of four major human pathogens even at very low concentrations. The methanol extract of bark and leaf was found to be very effective and efficient in inhibiting the growth of both bacteria and fungi. (Graph 1, 2, 3, 4)



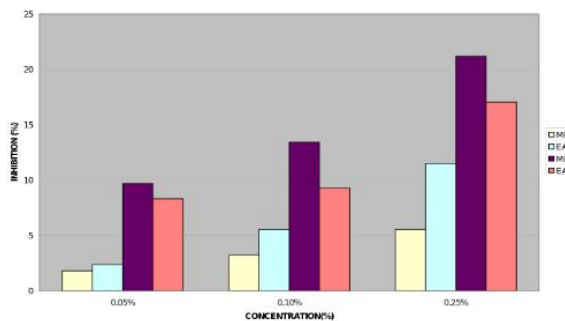
Graph 1 : Percentage growth inhibition of bark and leaf extracts to *E.coli*



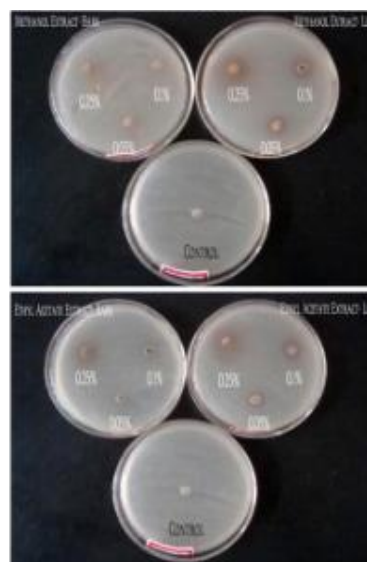
Graph 2 : Percentage growth inhibition of bark and leaf extract to *pseudomonas*



Graph 3 : Percentage growth inhibition of bark and leaf extract to *bacillus*

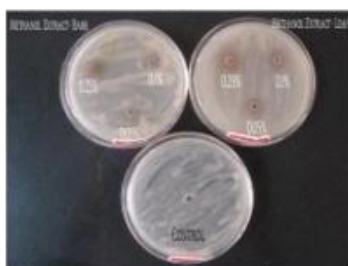


Graph 4 : Percentage growth inhibition of bark and leaf extracts to *staphylococcus*

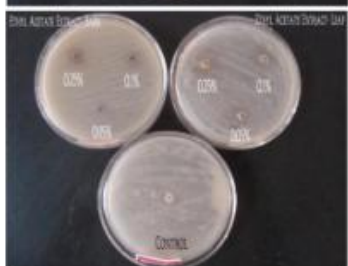
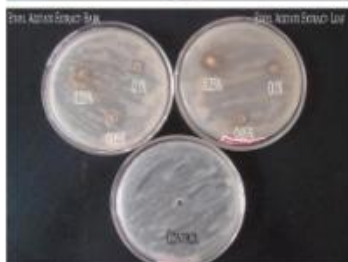


STAPHYLOCOCCUS

Full Paper



BACILLUS



E.COLI



PSEUDOMONAS

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