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IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL CONSTITUENTS OF *CROTON SCABIOSUS* BEDD.

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ABSTRACT

In the present study, organic solvents and aqueous extracts of *Croton scabiosus* a rare and endemic species to Kadapa district, Andhra Pradesh, India. Leaves, stem bark and root were screened for their phytochemical composition, and antimicrobial activities. Among the different extracts tested, alcohol, ethyl acetate, n-hexane and water extracts showed significant antimicrobial activities. The most susceptible microorganisms were found to be *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Phytochemical analysis of various test extracts revealed that the phenolic compounds, especially alkaloids are responsible for antimicrobial activities. The results obtained suggest that *Croton scabiosus* could be exploited in the infectious management of various diseases.

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INTRODUCTION

Medicinal plants are widely used for treatment of diseases all over the world. According to WHO report about 80% of the world populations are taking interest in indigenous medicinal plant remedies ^[1]. Therefore, it is a need to investigate traditional medicine with a view to identify and exploit safe and effective remedies for ailments of both microbial and non-microbial origin. Plant derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medicinal practitioners and they form an important part of the health care system in the western world ^[2]. It is estimated that about 75% of the biologically active plant derived compounds, presently in use world wide, have been derived through follow up researchers to verify the authenticity of data from folk and ethno medicinal uses. So, there is a great scope for new drug discoveries based on traditional plant uses ^[3]. There is a need to establish the pharmacological activities for identifying and comparing the various crude drugs for potency. Natural compounds formed the base of modern drugs as we use today. Phytoconstituents are the natural bio active compounds exhibit potential therapeutic properties. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Phytochemicals especially secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. In the present study, we have selected *Croton scabiosus* a rare and endemic species to Kadapa District, Andhra Pradesh, screened against multi-drug resistant bacteria including *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The ethnobotanical information obtained from the traditional healers may

serve as an initial lead for isolation and characterization of bioactive compounds (Cox & Balick, 1994).

MATERIALS AND METHODS

Plant collection:

The plant material was collected from Thummalapalli forests of Kadapa district and identified with the help of regional floras ^[3 & 4]. Interestingly *Croton scabiosus* is seen only in Thummalapalli uranium mining area with monodominant habitat. The ethno medicinal properties of the test species was recorded based on intensive interviews conducted with adivasi communities the sugalis inhabited in and around the forests. The voucher specimens were deposited in Sri Krishnadevaraya University Herbarium, Anantapur (SKU).

Preparation Of extracts:

The plant material was separated into different parts (leaves, stem bark & roots), air dried and ground to moderately fine powder. 30g of each plant part extracted with 200 ml of alcohol, methanol, ethyl acetate, n-hexane and water by using soxhlet unit ^[5]. The excess of solvents was recovered from the extract under reduced pressure with rototflash evaporator.

Qualitative Analysis of Phytoconstituents:

The semidried solvent extracts were subjected to preliminary phytochemical tests, as described by Amarasingham et al., (1964), Gibbs (1974) and Harborne (1984) ^[6,7,8]. The positive reactions were observed for 30 different groups of secondary metabolic compounds. Alkaloids and coumarins were recorded as most predominant chemical derivatives followed by glycosides, proteins, flavonoids, steroids, gallic tannins, etc (Table-1).

Table- 1 Distribution of phytochemical constituents in *Croton scabiosus*

S.No	Compounds	Leaf			Stem bark			Root		
		H	M	AQ	H	M	AQ	H	M	AQ
1	Alkaloids	+	+	+	+	+		+	+	+
2	Anthocyanins									
3	Anthocyanidins									
4	Anthracene glycosides			+		T	T			
5	Anthroquinones	+	+	+						
6	Aucubins									
7	Carbohydrates									
8	Carotenoids									
9	Catecholic compounds		+							
10	Coumarins	+		+	+		+	+		
11	Dihydrochalcones									
12	Emodins									
13	Flavonoids		+			+				
14	Flavonols		+			+				
15	Flavanones									
16	Flavones		+			+				
17	Fatty acids									
18	Gallic tannins					+				
19	Glycosides		+		+	+	+			
20	Iridoids									
21	Lignans									
22	Phenols		+							
23	Proteins				+			+		
24	Polyoses									
25	Reducing compounds									
26	Saponins						+			
27	Steroids	+								
28	Tannins									
29	Triterpenoids		+	+						
30	Volatile oils									

Antibacterial Assay:**Microorganisms used:**

The microbial strains viz., *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC1688), *Klebsiella pneumoniae* (MTCC 109), *Micrococcus luteus*, (MTCC 7256), and *Salmonella typhimurium* (MTCC 98), were used to test the killing activity of the extracts. The organisms were obtained

from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India.

The antimicrobial activity was performed by employing the disc diffusion method adopted by Bauer et al., (1966) and Cruickshank (1968) ^[9, 10]. The semi solid crude extracts of each sample dissolved in same solvent and the concentrations of 25, 50 and 100 mg/ml

were prepared. Previously prepared paper discs containing different concentrations of the extracts were placed on the surface of petriplates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded ^[11]. Three independent trials were conducted for each concentration to confirm the activity.

RESULT & DISCUSSION

Phytochemical analysis of hexane, methanol and aqueous extracts of leaves, stem and roots revealed that alkaloids and coumarins were present in all polar

solvents, while anthroquinones were seen in leaf extracts and flavonoids in methanol extracts of stem bark and leaves.

Data on *in vitro* antimicrobial properties of *Croton scabiosus* was depicted in Table 2, 3 & 4. Present observations revealed that all the crude extracts had significant antimicrobial activity on test pathogens. Root and stem bark shows maximum inhibition activity in ethyl acetate, n-Hexane and aqueous solvents. Root extract of ethyl acetate exhibited maximum inhibition zones (30 mm) when compared that of alcohol, aqueous and n-Hexane. The leaf extracts shows low activity against bacterial strains except alcohol extracts on *Staphylococcus aureus* (MTCC 7443), *Klebsiella pneumoniae* (MTCC 7028) and water extracts on *Staphylococcus aureus* (MTCC 7443).

Table 2: Antimicrobial screening of *Croton scabiosus* stem bark

Organisms	Inhibition Zone (mm)											
	AQ			AL			EA			n-H		
	Concentration (mg/ml)											
	25	50	100	25	50	100	25	50	100	25	50	100
<i>Escherichia coli</i>	-	10	12	-	-	-	-	-	12	-	-	10
<i>Klebsiella pneumoniae</i>	-	-	10	-	-	-	8	10	12	-	8	17
<i>Micrococcus luteus</i>	9	10	13	10	14	15	-	11	18	11	16	18
<i>Pseudomonas aeruginosa</i>	-	13	15	-	-	-	-	-	-	7	12	18
<i>Salmonella typhimurium</i>	-	8	13	-	-	-	-	9	10	-	-	-
<i>Staphylococcus aureus</i>	-	10	12	-	-	-	-	-	-	-	-	-

Table 3: Antimicrobial screening of *Croton scabiosus* root

Organisms	Inhibition Zone (mm)											
	AQ			AL			EA			n-H		
	Concentration (mg/ml)											
	25	50	100	25	50	100	25	50	100	25	50	100
<i>Escherichia coli</i>	-	-	-	-	-	10	-	9	10	-	-	15
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	14	-	-	-
<i>Micrococcus</i>	-	-	10	10	18	22	-	16	18	-	-	-

<i>luteus</i>												
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	20	22	23	-	12	15
<i>Salmonella typhimurium</i>	-	-	-	-	-	-	10	26	30	-	13	19
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	10	13	-	13	14

Table 4: Antimicrobial screening of *Croton scabiosus* leaf

Organisms	Inhibition Zone (mm)											
	AQ			AL			EA			n-H		
	Concentration (mg/ml)											
	25	50	100	25	50	100	25	50	100	25	50	100
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	-	8	-	-	-	-	-	-	-	-	-
<i>Micrococcus luteus</i>	-	-	-	-	13	14	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Salmonella typhimurium</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	9	10	11	-	-	12	-	-	-	-	-	-

AQ: Aqueous, **AL:** Alcohol, **EA:** Ethyl acetate, **n-H:** n- Hexane

CONCLUSION

The current work reveals that *Croton scabiosus* a potential source of antimicrobial agents and its potential inhibition activity against various clinical isolates suggest to perform further studies for isolation and identification of active principles. As the test species is rare and endemic to certain hill tracts in Kadapa district. The molecular studies have been conducted in the laboratory for elucidation of bioactive compounds.

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