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8**Phytochemical screening and evaluation of antibacterial activity of *Croton bonplandianum* leaves****Bimala Tripathy^{1*}, S. Satyanarayana², K. Abedulla Khan³, K. Raja⁴, Shyamalendu Tripathy⁵**¹Dept. of Pharmacology, St. Mary's Pharmacy College, Deshmukhi (V), Hyderabad- 508284, Telengana, India.²Dept. of Pharmacology, Avanthi Institute of Pharmaceutical Sciences, Vizianagaram, A.P., India.³Department of Clinical Pharmacy & Pharmacology, IBN Sina National College for Medical studies, Al Mahjar, Jeddah, KSA.⁴Executive, Microbiology-QC, M/s Jodas Expoin, Private Ltd., Hyderabad, Telengana, India.⁵Sri Vasavi institute of Pharmaceutical Sciences, Tadepaligudem, West Godavari.

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ABSTRACT: Background: There is a real perceived need for the discovery of new compounds that are endowed with antibacterial activities, possibly acting through mechanism of actions, which are distinct from those of well known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. *Croton bonplandianum* is a species in the genus *Croton*, belongs to the family *Euphorbiaceae* (Spurge Family). **Aim:** The aim of Study to screen the phytochemical constituents and evaluate antibacterial activity of ethanol and petroleum ether leaf extracts of *C. bonplandianum* against bacteria *Streptococcus mutans* and *Proteus vulgaris*. **Method:** The leaf extracts were screened for their antibacterial activity in comparison with standard antibiotic Penicillin (1mg/ml) *in vitro* by well diffusion method. Lawn culture was used by using the test organism on Nutrient Agar (NA). **Result:** The extracts were found to be significantly controlling the test bacteria. Data revealed that plants possessing higher amount of glycoside, flavonoids, tannins which show antibacterial activity. **Conclusion:** The results indicated that the Ethanol and Petroleum ether extracts showed a varying degree of inhibition of the growth against tested organisms.

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Mob. No. 9848245865.**Key words:** *In-vitro* antibacterial activity, bioactive constituents, diffusion, Medicinal plant; Antibacterial.**INTRODUCTION:**Normally Crotons are popular foliage plants. This is a wild species of croton. Due to the resemblance of the leaves and flower cymes to that of Tulsi, this plant is often called *Ban Tulsi* (jungle tulsi) ^[1, 2]. Despite of the availability of a number of antimicrobial agents the main matter of concern in the treatment of microbial

infections is the limited number of efficacious antimicrobial drugs [3]. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic but not bactericidal or lead to the development of resistance due in part to the prolonged periods of administration [4]. The parts of plant are used as tonic, against flat worm [5], dysmenorrhoea, purgative, dyspepsia, chronic enlargement of the liver and remittent fever [6, 7], dysentery, chronic hepatitis, gastric ulcers and gastric cancers, against snake poisoning [7] and to treat infertility [8], fever and wounds healing, antihelminthic, dermatological problem, antitumors and cancerous sores [9, 10]. The results obtained from this work revealed that the plants contained bioactive agents which are related with antibacterial properties in plants because of presence of many phytoconstituents like flavonoids, alkaloids, tannins, saponins and glycosides are producing a better opportunity for testing wide range of microorganism.

MATERIALS AND METHODS:

Materials:

The standard drug Penicillin was procured as gift sample from National Institute of Immunology, New Delhi. All other chemicals used are of analytical grades and procured from a authorized dealer.

Collection of plant material:

C. bonplandianum leaves were collected from Bobbili region, Vizianagaram district, Andhra Pradesh, India and used for this study. The laboratory works are done in Bhaskara Institute of Pharmacy, Komatipalli, Bobbili.

Extraction of plant material:

The leaves were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried. The dried leaves were powdered into fine form. The leaves powder samples (100g/500ml) were extracted by Soxhlation using ethanol and petroleum ether as solvents for 48 h 45°C. The extract from these solvents are soaked and evaporated under reduced pressure. The leaf extracts were concentrated at 50°C and the residue obtained was stored at 4°C for further study [11].

Antibacterial activity assay by well diffusion method:

Microorganisms used:

Two human pathogenic microorganisms, such as *S. mutans* (Gram +ve) and *P. vulgaris* (Gram -ve) strains were used in the study for the evaluation of the

antibacterial activity. Both strains are collected from Department of Microbiology, Bhaskara Institute of Pharmacy, Komatipalli, Bobbili, Andhra Pradesh, India.



Fig 1. Leaves extracts of various solvents of *Croton bonplandianum*.

Experimental Method:

The leaf extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Penicillin (1mg/ml) by *in vitro* well diffusion method. Lawn culture was used by using the test organism on nutrient agar (NA) [12]. The inoculated plates were kept aside for few min using well cutter. Four wells were made in those plates at required distance [13]. In each step of well cutting the well cutter was thoroughly wiped with alcohol. A fixed volume (0.1ml) of the *C. bonplandianum* extract was then introduced into the wells in the increasing concentration. The plates with bacteria were incubated at 37°C for 24 h. The activity of the extract was determined by measuring the diameters of zone of inhibition [14]. The selection of medium depends on the type of organism and nature of compound to be tested. For antibacterial sensitivity, nutrient agar/broth (pH 7.4) was used aseptically by following the pour plate technique [15]. Wells of approximately 4 mm in diameter and 2.5 mm deep were made on the surface of the solid medium using a sterile borer. The plates were turned upside down and the wells labeled with a marker. After 24 h, the plates were removed and zone of inhibition was measured by zone reader and the results were tabulated. Isolated compound with zones of inhibition greater or equal to 6 mm in diameter regarded as positive.

RESULTS AND DISCUSSIONS:

Phytochemical analysis of bioactive compound in different solvent extracts of *C. bonplandianum* is presented in Fig 1. The leaf extracts in different solvents were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of Cardiac Glycosides, Saponins, Alkaloid, Flavonoids, Steroids, and Terpenoids in most prominent amount while alkaloids and tannins in fewer amounts (Table 1). Resins absent in organic solvents [16].

Table 1. Qualitative analysis of *Croton bonplandianum* leaf extract.

Sl. No	Phytochemical Constituents	Ethanol extract	Petroleum ether extract
1	Alkaloids	+	+
2	Flavonoids	+++	+++
3	Saponins	+	+
4	Tannins	++	++
5	Phlobatannins	=	=
6	Glycosides	+++	+++
7	Sterols	+	++
8	Resins	+	-
9	Phenols	+	++
10	Anthraquinones	=	=
11	Terpenoids	=	=
12	Cardiac glycosides	++	++

+ represent – Present and – represent absent.

The extract was tested against infectious diseases causing bacterial pathogens such as *S. mutans* and *P. vulgaris* using the agar well diffusion method [17]. The ethanol extract of *C. bonplandianum* showed more activity against bacteria like *S. mutans* and showed zone of inhibition of diameters 2.65, 2.70 and 2.85 mm and *P. vulgaris* zone of diameter 2.7, 2.85 and 3.15 mm for three different concentrations, when compared to petroleum-ether solvent extract (Fig 2). The petroleum ether extract of *C. bonplandianum* showed the better activity against the bacteria like, *Streptococcus mutans*, possess zone of inhibition diameter is 3.45 mm and *P. vulgaris*, showed the zone of inhibition diameter 3.2 mm. The ethanol extract gives 3.15 mm and 2.85 mm of zone of inhibition against the bacteria *P. vulgaris* and *S. mutans* respectively (Fig 3). In the present study, both bacteria are sensitive to ethanol and petroleum ether extracts showed a varying degree of inhibition of the growth against the tested organisms. The results confirmed that extract of *C. bonplandianum* showed antibacterial activity against the human pathogenic organisms as evident from Fig 4 [18,19]. The leaves extracts in different solvents were screened for the

Table 2. Effect of different solvent extracts of *Croton bonplandianum* leaf on selected bacteria.

Solvents	Conc. (mg/ml)	Test Micro-organisms	
		<i>S. mutans</i> ZD (mm)	<i>P. vulgaris</i> ZD (mm)
Ethanol	25	-	2.05
	50	2.65	2.7
	75	2.70	2.85
	100	2.85	3.15
Petroleum ether	25	-	-
	50	2.4	2.45
	75	2.9	2.9
	100	3.45	3.2
Penicillin	5	3.9	-
Doxycyclin	5	-	3.05
Control	Ethanol 100µl	2.25	4.65
	Pet. Ether 100µl	-	-

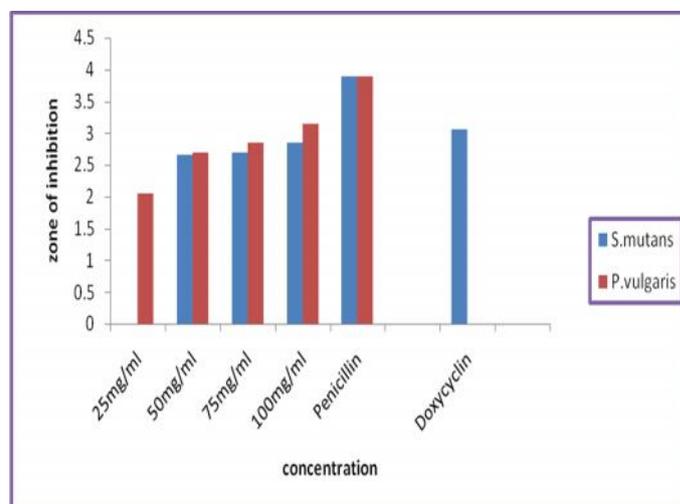


Fig 2. Effect of Ethanol leaves extract of *Croton bonplandianum* on Bacteria.

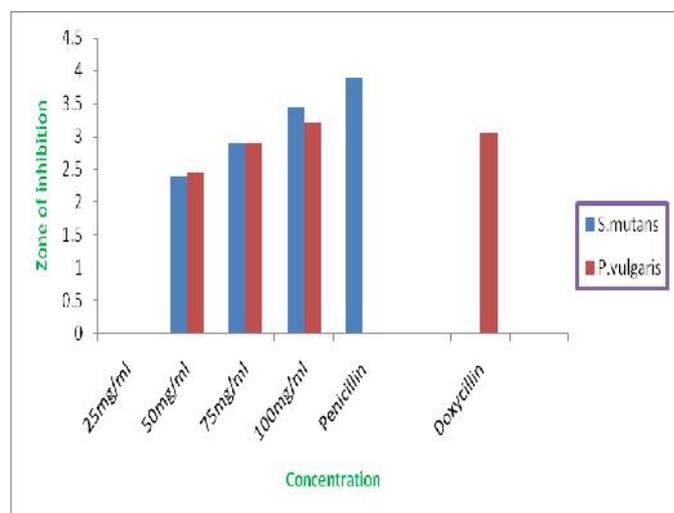


Fig 3. Effect of Petroleum ether leaves extract of *Croton banplandianum* on bacteria.

presence of various bioactive phytochemical compounds like Cardiac Glycosides, Saponins, Alkaloid, Flavonoids, Steroids, and Terpenoids which may responsible for antibacterial activity^[20, 21].

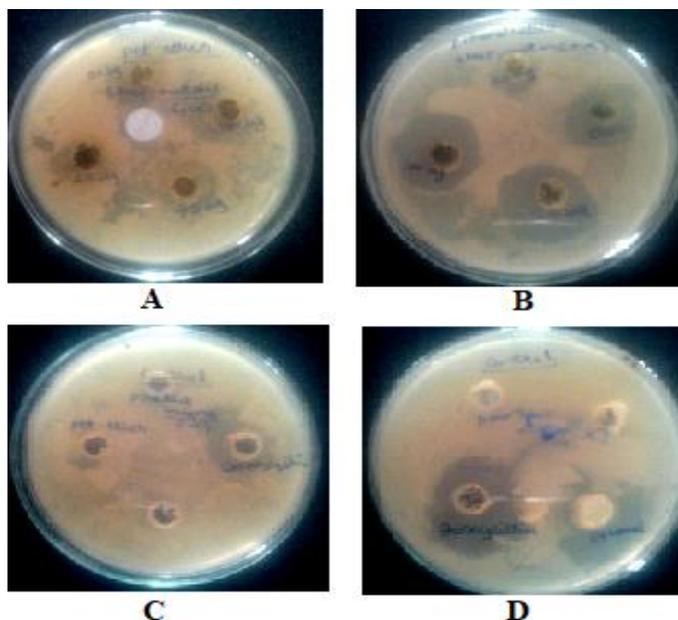


Fig 4. Zone of inhibition by using standard and *C. bonplandianum* extracts against bacteria.

A - Pet. Ether extracts against *S. mutans*, B- Ethanol extracts against *S. mutans*, C - Pet. Ether extracts against *P. vulgaris* and D - Ethanol extracts against *P. vulgaris*.

CONCLUSION:

From the obtained results it could be concluded that although ethanol itself has antibacterial activity, ethanolic extract of *C. bonplandianum* has a synergistic activity. Since *C. bonplandianum* is easily available and well-tolerated, it can be incorporated into medications for bacterial infection. However, further studies for its incorporation into oral preparations, safety and cost-effectiveness has to be conducted.

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