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The Sensitivity of Gram +ve, Gram –ve Bacterial Pathogens to the Antibacterial Activity of Cultured Tissues *Croton Bonpladianum* Baill and their Phytochemical Studies

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ABSTRACT

The different fractions of methanolic extracts of cultured tissues of *Croton bonplandianum* Bail were subjected to preliminary phytochemical and *in-vitro* anti – bacterial studies. The different fractions revealed the presence of steroids, alkaloids, flavonoids and saponins. The antibacterial activity of the plant (Callus Cultures) different fractions of methanolic extracts was assayed by the agar plate disc diffusion technique. Three gram positive, four gram negative bacterial species were screened for the anti-bacterial investigations. The fraction II of the methanolic extract inhibited the growth of all the test bacterial species whereas fraction III and fraction IV have shown weak antibacterial activity.

Keywords: Antibacterial, Cultured tissues, *Croton bonplandianum* Baill and Phytochemical.

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INTRODUCTION

The use of plants as medicines is as old as human civilization it self. Many of the existing medicinal systems such as ayurveda, unani, homeopathy, naturopathy, siddha and other alternative medicinal systems have been utilizing plants as effective medicines to cure many harmful diseases. *Croton bonplandianum* Bail belonging to (Syn. *C. sparsiflorus* Morong) Euphorbiaceae is common weed throughout the plain of India. It is being used as antiseptic to treat Scabies and other ailments[1]. It is reported to be hypotensive and leaves are analgesics[2]. Earlier photochemical work indicates that benzene soluble fraction of ethanol extracts of leaves and steam contains β sitosterol, teraxerol[3], and neutral fraction contains Vomifoliol[4]. The leaves of *C.bonplandianum* contain flavonoids Quercetin – 3 – rhamnoglucoside[5]. The ethanolic extract of the plant reported to contain Proaporphine alkaloidal bases like Crotosporin, N-methyl crotosporin, N, O- dimethyl crotosporin[6]. Antibacterial spectrum of several *Croton* species has been reported[7-9]. The latex of shoots and leaves of *Croton bonplandianum* is used to cure cuts, wounds, cough and headache[10]. Although phytochemical and antibacterial screening of the plant has been carried out, no extensive pharmacoloigcal and antibacterial activities have been reported on callus extracts. In the present study different fractions of the methanolic extracts of callus cultures of the plant have been investigated.

MATERIALS AND METHOD

Plant material

The *Croton bonplandianum* weed was collected from the Gorantla, Guntur district of Andhra Pradesh state, India. The taxonomical identification was done Dr. T. Pullaiah, Department of Botany, Sri Krishna Devaraya University, Ananthapuram, Ananthapur District, and Andhra Pradesh, India. The voucher specimen was deposited at the Department of Pharmacognosy, SIMS College of Pharmacy, Guntur, Andhra Pradesh, India for future reference.

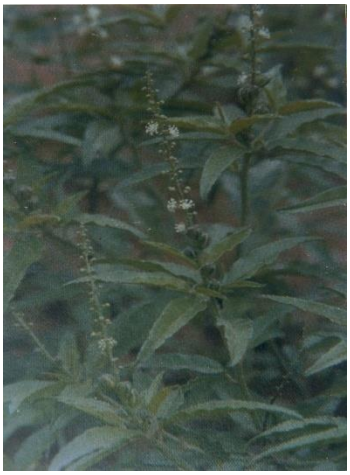


Figure 1A: Twig of *Croton bonplandianum*

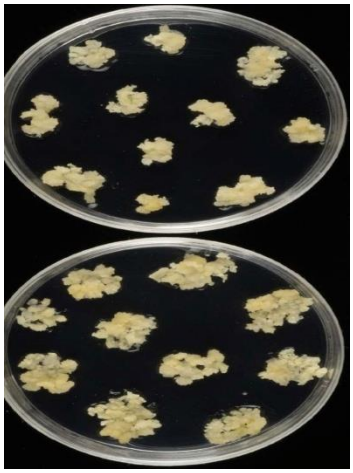


Figure 1B: Cultured tissues of *Croton bonplandianum*

Preparation of callus extracts

Callus cultures were prepared from the collected plant by following the standard steps in tissue culture technique[11] and subjected to extraction as per the standard scheme for preparation of plant extracts for biological screening[12]. To the callus material add 80% methanol and the solution was concentrated under vacuum at 40 ° C. The residue was partitioned between chloroform and 10% citric acid solution. Chloroform layer was separated and evaporated in vacuum at 40 ° C. The residue was partitioned between 90% methanol and petroleum ether (1:1). The petroleum ether layer (Fraction I) and the methanolic layer (Fraction II) were separated and concentrated under vacuum at 40° C. The aqueous citric acid layer was evaporated and concentrated. To the residue, ammonium hydroxide (P^H 9) was added and further extracted with chloroform. Chloroform layer - (Fraction III) and aqueous layer - (Fraction IV) were separated and concentrated under vacuum at 40°C.

Phytochemical studies

All the fractions were subjected to preliminary phytochemical investigations for the presence of secondary metabolites such as steroids, triterpenoids, flavonoids, alkaloids, tannins, saponins and resins utilizing standard methods of analysis[13, 14].

Bacterial strains

Tests were performed on three gram positive bacteria (Table1) - (*Bacillus megaterium* – ATCC 23564, *Bacillus subtilis*- ATCC 6633 and *Staphylococcus aureus* ATCC 25923) and four gram negative bacteria (Table 2) (*Escherichia coli* – ATCC 25922, *Enterobacter faecalis* –ATCC 35550, *Proteus vulgaris* – ATCC 6380 and *Pseudomonas aeruginosa* – ATCC 27853) obtained from Department of Microbiology and Biochemistry, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India.

Table 1 List of Gram Positive Bacteria

S. No	Name of the Microorganisms	Diseases caused by the organisms
1	*Bacillus megaterium* – ATCC 23564	Intestinal disturbances.
2	*Bacillus subtilis*- ATCC 6633	Food poisoning, Oppurtunistic Pathogen.
3	*Staphylococcus aureus* - ATCC 25923	Chronic osteomyletis, Meningitis, endocarditis.

Table 2 List of Gram Negative Bacteria

S. No	Name of the Microorganisms	Diseases caused by the organisms
1	*Escherichia coli* – ATCC 25922	Gasteroenteritis, urinary tract disease.
2	*Enterobacter faecalis* –ATCC 35550	Oppurtunistic human pathogen.
3	*Proteus vulgaris* – ATCC 6380	Urinary tract infections.
4	*Pseudomonas aeruginosa* – ATCC 27853	Wounds and urinary tract infections.

Antibacterial screening

Working bacterial inocula suspensions were obtained from 18h stock culture on nutrient broth at 37 ° C. The inoculum size of each test strain was standardized at 5 x 10^5 CFU/ml, according to the national committee for clinical laboratory standards[15, 16]. A 5 ml volume of the bacterial suspension was evenly mixed with sterile nutrient agar medium and poured into the sterile Petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each fraction was checked for antibacterial activity by introducing 40 µl of a 50 mg/ml concentration into wells. The method was repeated in five plates. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar media and then incubated at 37° C for 18 h in BOD incubator (SM industries, New Delhi). Subsequently, diameter of zone of inhibition was measured. A broad spectrum antibiotic ampicillin (40µg/ml) was used as the reference standard in each plate.

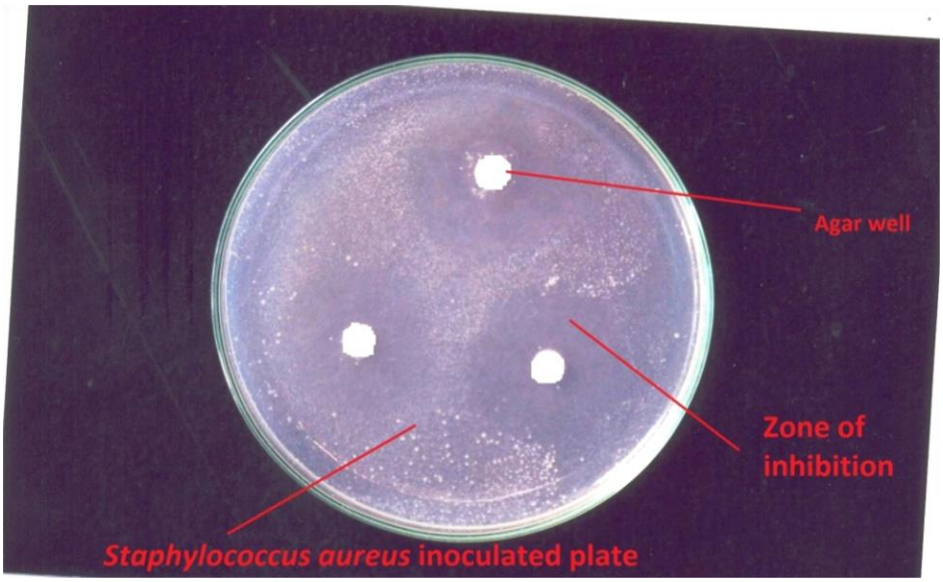


Figure 2 Zone of inhibition of methanolic fraction of cultured tissues of *Croton bonpladinum* on *Staphylococcus aureus*

RESULTS AND DISCUSSION

Antibacterial activity

As table 3 and 4 indicates fraction I has shown no activity against bacteria. Fraction II has shown activity against *Bacillus megaterium, Bacillus subtilis, Escherichia coli, Enterobactor faecalis, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Zone of inhibition 16mm, 15 mm, 10 mm, 13mm, 11mm, 11mm and 17mm respectively). Fraction III has shown activity against *Bacillus megaterium, B. subtilis, Enterobacter faecalis* and *Staphyloccus aureus* (Zone of inhibition 11 mm, 10mm, 09mm and 10mm respectively). Fraction IV has shown the inhibitory action against *Bacillus megaterium, B. subtilis* and *Staphylococcus aureus* (Zone of inhibition 10mm, 09mm, and 09mm respectively).

Preliminary phytochemical studies

The fraction I has revealed the presence of sterols and triterpenoids, fraction II has tested positive for the presence of flavonoids, fraction III has shown the presence of alkaloids and saponins have been detected in the fraction IV as shown in the table 5.

Table 3 Zone of inhibition of different fractions of methanolic extracts of callus cultures of *Croton bonplandianum* on Gram Positive Bacteria [a]

S. No	Microorganism	Inhibition Zone (mm)[b]				Ampicillin (40 µg/ml)
		Frac I	Frac II	Frac III	Frac IV	
1	*Bacillus megaterium* (ATCC 23564)	-	16	11	10	21
2	*Bacillus subtilis* (ATCC 6633)	-	15	10	09	21
3	*Staphylococcus aureus* (ATCC 25923)	-	17	10	09	22

[a] 40 µl of solution (50 mg/ml) was applied to each well

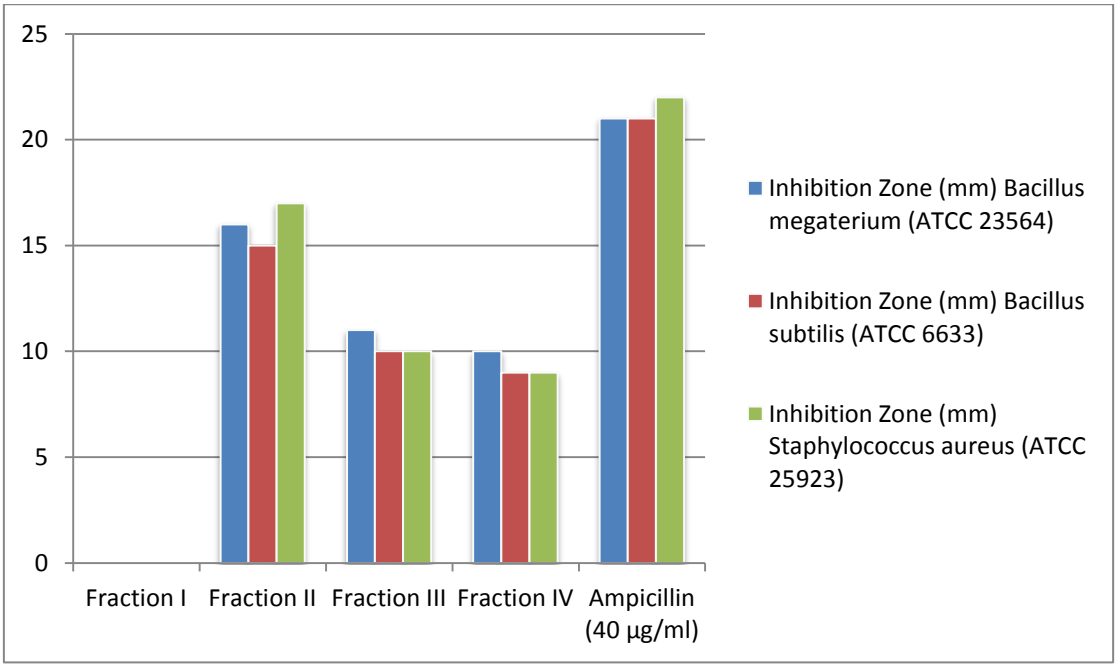
[b] Values are mean of five replicates.

Table 4 Zone of inhibition of different fractions of methanolic extracts of callus cultures of *Croton bonplandianum* on Gram Negative Bacteria [a]

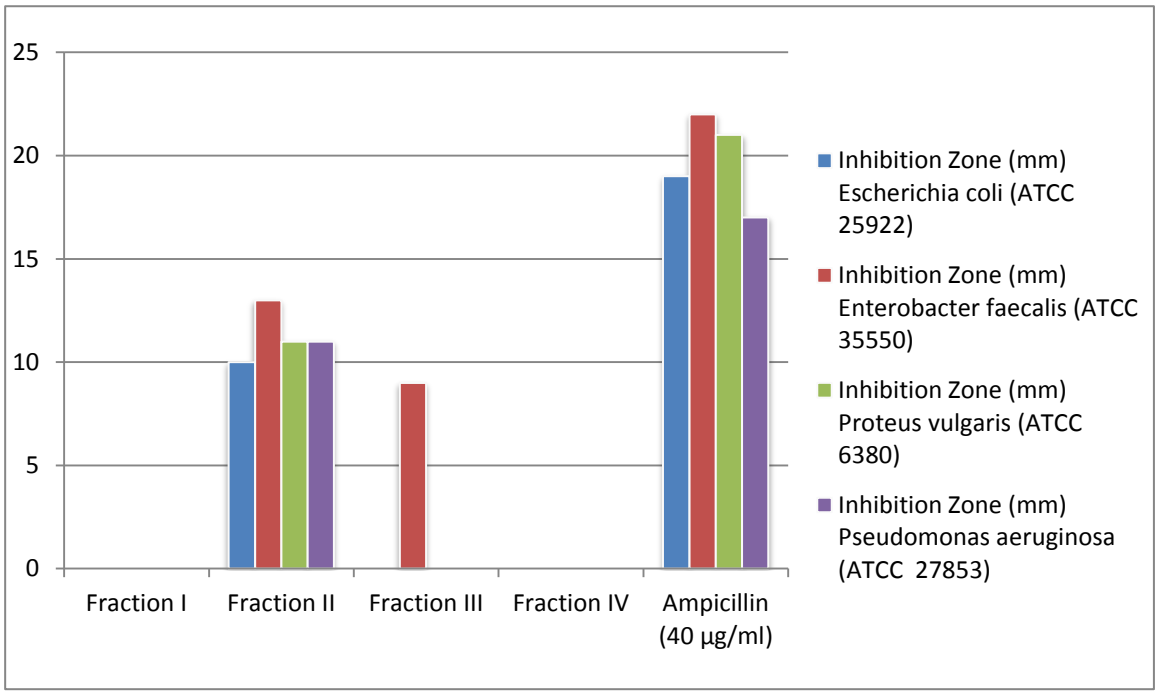
S. No	Microorganism	Inhibition Zone (mm)[b]				Ampicillin (40 µg/ml)
		Frac I	Frac II	Frac III	Frac IV	
1	*Escherichia coli* (ATCC 25922)	-	10	-	-	19
2	*Enterobacter faecalis* (ATCC 35550)	-	13	09	-	22
3	*Proteus vulgaris* (ATCC 6380)	-	11	-	-	21
4	*Pseudomonas aeruginosa* (ATCC 27853)	-	11	-	-	17

[a] 40 µl of solution (50 mg/ml) was applied to each well

[b] Values are mean of five replicates.



Graph 1 Zone of inhibition of different fractions of methanolic extracts of callus cultures of *Croton bonplandianum* on Gram Positive Bacteria



Graph 2 Zone of inhibition of different fractions of methanolic extracts of callus cultures of *Croton bonplandianum* on Gram Negative Bacteria

Table 5: Phytochemical screening of different fractions of methanolic extract of callus cultures of *Croton bonplandianum*

Secondary metabolites	Fraction I	Fraction II	Fraction III	Fraction IV
Alkaloids	-	-	+	-
Flavonoids	-	+	-	-
Resins	-	-	-	-
Saponins	-	-	-	+
Sterols	+	-	-	-
Tannins	-	-	-	-
Triterpenoids	+	-	-	-

+ Present; - Not detected.

CONCLUSION

Phytomedicines are effective in treating most of the infectious diseases mainly skin infections. Most of the secondary metabolites, serve as plant defense mechanisms against microorganisms, insects and herbivores[17, 18]. The different fractions of methanolic extract of callus cultures of *Croton bonplandianum* found to contain alkaloids, flavonoids, saponins, sterols and triterpenoids. The antibacterial activity of tested medicinal plants can be attributed to any of these constituents[19]. However there was a marked difference in level of activity among these fractions. The results have clearly indicated that fraction II of the methanolic extract of callus cultures of *Croton bonplandianum* has shown the better antibacterial activity than other fractions and also exhibited high zone of inhibition on Gram +ve bacteria than Gram-ve bacteria. This may be due to the presence of flavonoids in the

fraction II of the methanolic extract. Previous reports have indicated that these compounds have shown antibacterial activity[20]. The antibacterial activity of fraction three may be attributed to the presence of alkaloids. Earlier reports have suggested the antibacterial activity of alkaloids[21]. Saponins, which are known to have cytotoxic properties, may be responsible for antibacterial activity of fraction four[22, 23]. Hence the detailed phytochemical investigation and antibacterial screening of secondary metabolites from these plant cultures may yield promising anti bacterial agents.

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