

Phytochemical Constituents and Antimicrobial Studies of the Exotic Plant Species, *Croton bonplandianum* Bail

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ABSTRACT Plants are the rich sources of many natural products. The leaves of the exotic plant species, *Croton bonplandianum* are highly medicinal and used for controlling high blood pressure, and for the treatment of skin diseases and cuts and wounds, because of the presence of active principle, rutin. In the present study, leaf extracts of *Croton bonplandianum* were screened phytochemically to know the presence of secondary metabolites and for the antimicrobial properties. The results of the study revealed the presence of alkaloids, flavanoids, saponins, steroids, resins and phenols. The methanol extracts of the leaf showed effective inhibition against the fungi and bacteria studied. Therefore the leaves of *Croton bonplandianum*, can be considered to be the promising source of antimicrobial compounds.

INTRODUCTION

Plants remain the most common source of antimicrobial agents (Bibitha et al. 2002; Maghrani et al. 2005). Many aromatic plants have been used traditionally in folk medicine as well as to extend the self life of foods, showing inhibition against bacteria, fungi and yeast (Hulin et al. 1998). Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases (Perumal Samy and Ignacimuthu 2000). In recent years, pharmaceutical companies have been involved largely in developing plant based natural products to produce most cost effective remedies that are affordable to the poor people. The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the needs to search for newer antibiotic sources.

The species, *Croton bonplandianum* (Euphorbiaceae), one of the exotic weeds is mostly seen in wastelands and commonly known as 'bantulasi'. Leaves of this plant are highly medicinal and used for controlling high blood pressure and for the treatment of skin diseases and cut and wounds and it is antiseptic and antidote also (Nishanta et al. 2002; Chaudhuri 2007; Bhakat and Sen 2008). *Croton* is rich in

secondary metabolites including alkaloids and terpenoids (Rizk 1987), the latter including irritant co-carcinogenic phorbol esters (Phillipson 1995). Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of this plant.

MATERIALS AND METHODS

Preparation of Extracts

Apparently healthy plant leaves were collected around the Maruthamalai and shade dried in room temperature for 15 days and powdered. Thirty gm leaf was extracted in solvents such as petroleum ether, chloroform, acetone and methanol by using soxhlet apparatus. The extracts were stored at 4° C for phytochemical screening and antimicrobial analysis.

Phytochemical Screening of Leaf Extracts

The presence of various phytochemical compounds in the leaves of *C. bonplandianum* was confirmed by using the methods of Brindha et al. (1977) and Harbone (1988).

Antimicrobial Analysis

Test Organisms

Bacteria and fungal isolates were used for this study which include, *E. coli*, *Pseudomonas* sp., *Salmonella typhi* (gram negative bacteria) and

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Staphylococcus aureus and *Bacillus subtilis* (gram positive bacteria) and the fungal species, *Mucor* sp., *Aspergillus flavus*, *Rhizopus* sp. and *Penicillium* sp. All these clinical isolates were obtained from the Microbiology Laboratory, Tamil Nadu Agricultural University, Coimbatore. All strains were suspended in nutrient broth and incubated at 37° C for 48 hours.

Antimicrobial activity of organic extracts of the plant sample was evaluated by the paper disc diffusion method (Aida et al. 2001). The different extracts of leaf of *C. bonplandianum* at two different concentrations viz., 50 and 100mg/100ml were used for antimicrobial activity. The antibiotic disc tetracycline (30µg) was taken as positive control. Nutrient agar and PDA medium were inoculated respectively with the bacteria and fungi by spreading the microbes on the medium. Sterile filter paper discs impregnated with 50 and 100 mg/100ml extracts and tetracycline were applied over the culture plates. Bacterial cultures were incubated at 37°C for 18 hr and fungal cultures were incubated at room temperature (30-32° C) for 48 hours. Antimicrobial activity was determined by measuring the zone of inhibition around each paper disc. Three separate trials were conducted for each extract against each organism.

Statistical Analysis

The resultant clear zones around the discs were measured in mm. The antimicrobial activity of leaf extracts was indicated by clear zone of growth inhibition. Data of experiments represented by three replicates from each experiment were subjected to New Duncan's Multiple Range Test (Gomez and Gomez 1976).

RESULTS AND DISCUSSION

The results of phytochemical screening of *Croton bonplandianum* showed the presence of various phytochemicals (Table 1). All the four organic solvents such as petroleum ether, acetone, chloroform and methanol showed positive results for the presence of phenols and resins. In addition, the methanol and acetone extracts showed the presence of glycosides and steroids also. Compared to all other extracts, methanol extract has higher number of secondary metabolites with higher contents. However, as in other solvents, low levels of tannins and

resins are present in methanol extract. All these secondary metabolites present in other herbs are known to exhibit medicinal properties (Dosumu et al. 2006; Srinivasan et al. 2007; Pratima and Sundar 2010).

Table 1: Preliminary phytochemical analysis of *Croton bonplandianum* with different solvents

Com-pound	Petroleum ether	Chloro-form	Acetone	Methanol
Alkaloids	+	-	+++	+++
Flavonoids	+	+	+	+++
Glycosides	+	+	++	+++
Steroids	+	++	++	++
Phenols	+	++	+	+++
Tannins	+	+	+	+
Saponins	+	-	++	+++
Resins	+	+	+	+

'+' and '-' indicate the presence and absence of the compounds respectively. '+' indicates moderate degree of presence and '+++' indicates the high degree of the presence.

Results of antibacterial and antifungal activities of the various extracts of leaf part of *C. bonplandianum* are shown in Tables 2 and 3 respectively. The results showed that both the non-polar (petroleum ether) and polar (chloroform, acetone, methanol) solvent extracts were active against both bacteria and fungi. The highest inhibition (diameter of zone of inhibition, 17± 1.15 mm) was observed in the methanol extract at 100mg/100ml against the bacterium *Salmonella typhi*. Compared to all other extracts, 50 and 100 mg/100ml of methanol extracts showed higher activity against the bacteria tested. The highest antifungal activity (diameter of zone of inhibition, 17.33± 1.15 mm) was demonstrated by the 100 mg/100ml methanol extracts of leaf, against the fungus, *Rhizopus* sp. The inhibitory activity of the methanol extract of the leaf part of *C. bonplandianum* was also considerably higher against the other fungi tested in comparison with other extracts (Table 3).

Phytochemical constituents such as alkaloids, flavanoids, glycosides and several other aromatic compounds are secondary metabolites in plants that have alleviated the pathogenic and environmental stress (Lutterrodt et al. 1999; Marjorie 1999; Edreva et al. 2008). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with no or lesser side effects due to an array of secondary metabolites (Lee et al. 1999). In accordance with this fact the anti-

Table 2: Antibacterial activity of the leaf extracts of the exotic plant, *Croton bonplandianum*

Bacteria	Tetracycline (Positive control)	Diameter of zone of inhibition in mm							
		Petroleum ether (mg/ml)		Chloroform (mg/ml)		Acetone (mg/ml)		Methanol (mg/ml)	
		50	100	50	100	50	100	50	100
<i>Staphylococcus aureus</i>	20.67±1.15	-	-	7.33±1.15	12.00±2.00	-	-	11.33±1.15	7.33±1.15
<i>Pseudomonas</i> sp.	20.67±1.15	7.30±1.10	15.33±1.15	-	12.67±1.15	10.00 ±2.00	7.33 ±1.15	9.33±1.15	16.00±2.00
<i>Escherichia coli</i>	22.00±2.00	12.00±2.00	14.32±1.15	-	8.67±1.15	10.00±2.00	10.00±2.00	11.33±1.15	15.33±1.15
<i>Bacillus subtilis</i>	22.00±2.00	-	-	6.66±1.15	8.66±1.15	-	-	9.33±1.15	11.33±1.15
<i>Salmonella typhi</i>	21.33±2.33	-	-	-	-	-	-	15.33±1.15	17.33±1.15

Table 3: Antifungal activity of the leaf extracts of the exotic plant, *Croton bonplandianum*

Bacteria	Tetracycline (Positive control)	Diameter of zone of inhibition in mm							
		Petroleum ether (mg/ml)		Chloroform (mg/ml)		Acetone (mg/ml)		Methanol (mg/ml)	
		50	100	50	100	50	100	50	100
<i>Mucor</i> sp.	36.66±1.15	11.33±1.15	10.66±1.15	8.66±1.15	8.66±1.15	-	9.33±1.15	8.66±1.15	13.33±1.15
<i>Aspergillus flavus</i>	20.66±1.15	7.30±1.15	11.33±1.15	-	8.00±2.00	9.33±1.15	10.66±1.15	11.33±1.15	14.00±3.46
<i>Rhizopus</i> sp.	20.66±1.15	-	-	9.33±1.15	10.66±1.15	-	-	9.33±1.15	17.33±1.15
<i>Penicillium</i> sp.	20.66±1.15	10.67±1.15	7.33±1.15	13.33±1.15	-	-	-	8.00±2.00	16.00±2.00

crobal activities of various crude extracts the study species *C. bonplandianum*, may also be explained due to the presence of many kinds of secondary metabolites or compounds like glycosides, saponins, tannins, flavanoids, terpenoids, alkaloids (Okeke et al. 2001; Shamala Gowri and Vasantha 2009). These antimicrobial activities against bacteria and fungi confirm the presence of broad spectrum antibiotic compounds in the leaves of this species (Srinivasan et al. 2001). Similar trends of antimicrobial activity has been reported in another species, *Croton zambesicus* which exhibited wide spectrum of antibacterial and antifungal effects (Abo et al. 1999). In the same family Euphorbiaceae, various species of *Phyllanthus* also reported to have better inhibitory effect against the bacteria even at low concentrations (Mdlolo et al. 2008).

Of the four solvents used for extraction, the methanol extracts showed the highest activity against both bacterial and fungal test organisms, followed by the chloroform extracts. It may be due to high polarity of these solvents which naturally has the ability of extracting of high quantity of phytochemicals (Marjorie 1999). Hence, it is understood that the methanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity.

The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to provide primary health care in the developing countries and moreover, the use of herbal remedies has risen in the developed countries in the last decade. In this manner, plants continue to be a rich source of therapeutic agents. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial and fungal infections. The need of the hour is to screen a number of plants that are traditionally used and also to evaluate their probable phytoconstituents (Parekh and Chandra 2007; Abdulla et al. 2009).

CONCLUSION

The present findings support the high degree of antimicrobial activity of the exotic plant species, *Croton bolplandianum*. Broad spectrum of antibacterial activity of this species may help the discovery of new classes of antibiotic chemicals that could serve as selective agents against infectious diseases, chemotherapy and control.

This investigation has opened up the possibility of the use of this plant in drug development for human consumption for the treatment of wound infection and typhoid fever etc.

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