Research Article

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Medicinal value of *Croton sparsiflorus* fresh leaf and fruit extracts as shown by antibacterial activity

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ABSTRACT

Knowledge on plants by the Indigenous people provides an insight to the researchers to screen traditionally used plants for active principles against various human pathogens. Antibacterial study o*n Croton sparsiflorus* fresh leaf and fresh fruit extracts obtained using petroleum ether showed more medicinal value on *Staphylococcus aureus* and less on *Serratia marcescens*. In general, Gram-positive bacteria were more susceptible than Gram-negative bacteria. Leaf extract had profound killing effects on bacteria.

Key words: *Croton sparsiflorus*, leaf extract, fruit extract, petroleum ether, antibacterial activity.

INTRODUCTION

Traditional uses of plants for treating various human diseases by indigenous people have provided an insight to the researchers to search for the presence of active metabolites. One such plant weed which is under scrutiny is *Croton sparsiflorus* Morong. It is present on barren lands and roadsides [1] of South India, especially Tamil Nadu and Puducherry Union Territory. This plant possesses alkaloids- crotsparine, N-methyl crotsparine and N,O-dimethyl crotsparine, **crotsparinine, N-mehtyl crotsparinine, sparsiforine** [2]; amides-crotamides A and B [3]; crotosparsamide [4]. Scientific study on the medicinal properties of this plant has been emerging now. Hypotensive activity [5], anti-inflammatory and anti-pyretic activity [6], antibacterial activity [7] [8] [9] [10] [11] and antifungal activity [9] [12] have been studied using various solvent extracts of *C. sparsiflorus*. Allelopathic effects of various extracts [1] and genotoxic effects of this plant were reported earlier [11].

Petroleum ether was not used for antibacterial activity of fresh leaves and fresh fruits of this plant so far. We were interested to validate the antibacterial potential of petroleum ether extracts obtained from fresh leaves and fresh fruits of this plant.

MATERIALS AND METHODS

Fresh leaves and fresh fruits of *Croton sparsiflorus* Morong were collected from Ariyur, Puducherry, India (Figure 1 A and B).

Extraction of the phytochemicals

Leaves were processed for phytochemical extraction as described earlier [7] using petroleum ether (PE). Briefly, 10 g of fresh leaves were ground and added with 100 ml of PE. They were incubated for 2 d at room temperature. Then they were filtered and the solvent was evaporated in hot-air oven at 80º C. The compounds were dissolved in dimethyl sulphoxide (DMSO) and stored at 4º C until use. The working concentrations (500 µg ml^{-1}, 1000 µg ml^{-1} and 2000 µg ml^{-1}) were prepared by diluting the extract stock using DMSO. Crude extract was also prepared from 10 g of fresh fruits of *C. sparsiflorus* as in the case of fresh leaves.

Culture of bacteria and antibacterial study

The antibacterial efficacy of leaves and fruits of *C. sparsiflorus* was tested against three Gram-positive bacteria: *Bacillus subtilis*, *Streptococcus* sp., *Staphyloccocus aureus* and two Gram-negative bacteria: *Serratia marcescens* and *Salmonella typhi*. Bacteria were grown in nutrient broth (LB broth) and nutrient agar [7] [9]. Nutrient agar plates were made with 20 ml of medium and one day grown bacterial inoculum (100 µl) from nutrient broth was added and spread uniformly with sterile cotton swabs which were already dipped in fresh sterile nutrient broth. Three wells (8 mm diameter) were prepared in each nutrient agar plate. The plates were labelled as control, 500 µg ml^{-1}, 1000 µg ml^{-1} and 2000 µg ml^{-1}. Each well was added with 100 µl of control or extract at different concentrations. To one well of first plate 100 µl of DMSO (as negative control) was added and to the second and third well Erythromycin (2000 µg ml^{-1} each) was added. To the second plate leaf extract at the concentration of 500 µg ml^{-1} was added to each well. Likewise third nutrient agar plate was added with 1000 µg ml^{-1} of leaf extract and the fourth plate wells at the concentration of 2000 µg ml^{-1}.

The bacterial cultures were incubated at room temperature (32º C) for 24 h. Bacterial growth inhibition was considered as antibacterial activity of leaf extract and it was measured as zone of inhibition (ZOI) in mm diameter. Same method was followed for antibacterial study for fruit extracts. The results were analyzed for one-way ANOVA (at the significance level P?0.05) with the open source statistical software, PSPP (GNU PSPP) version 0.7.8. Minimum inhibitory concentrations

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(MICs) for leaf and fruit extracts were determined with decreasing concentrations. The experiments were repeated twice.

RESULTS

Leaf and fruit extracts of *Croton sparsiflorus* was green and non-viscous. Both the extracts showed similar time requirement for the evaporation of the solvent in hot-air oven. Two extracts showed antibacterial activity against five all the bacteria tested.

Antibacterial Potential of Leaf Extract

Leaf extract at 500 µg ml^{-1} showed low antibacterial activity as evident from ZOI on growth of *S. typhi* and higher activity on *S. aureus* (Figure 1C & E). Growth inhibition of *B. subtilis, Streptococcus* and *S. marcescens* was between the values of these two bacteria (Table 1).

Table 1: Antibacterial activity of fresh leaf petroleum ether extract of *Croton sparsiflorus* on bacteria. Results are mean±SD of 2 independent experiments (n= 6). Results were significantly different at P<0.05.

Bacteria	Zone of inhibition (mm)		
	500 µg ml^{-1}	1000 µg ml^{-1}	2000 µg ml^{-1}
Bacillus subtilis	16.50±2.07	17.17±0.75	17.17±0.41
Streptococcus sp.	15.33±1.86	17.17±2.41	18.17±2.14
Staphylococcus aureus	16.83±1.60	18.50±2.26	18.67±1.51
Salmonella typhi	14.67±3.01	16.67±2.34	16.83±2.23
Serratia marcescens	15.00±1.55	16.00±1.90	16.17±1.72

Serratia showed minimum growth inhibition at the concentration of 1 mg ml^{-1}. Maximum growth inhibition was observed on *S. aureus*. High concentration of leaf extract (2 mg ml^{-1}) had more killing ability on *S. aureus* than other bacteria. *S. marcescens* had more resistance towards the leaf extract and showed intense reddish colour on them around the zone of inhibition.

Antibacterial Potential of Fruit Extract

Fruit extract at all concentrations resulted maximum growth inhibition in *S. aureus* when compared to rest of the bacteria (Table 2; Figure 1F). Altogether growth inhibition of *S. typhi* and *S. marcescens* was less by fruit extract. The most resistant bacterium among five selected bacteria was *S. marcescens* (Figure 1D). The growth inhibition by the control DMSO was less (13 mm) in *B. subtilis* when compared to leaf and fruit extracts dissolved in DMSO. The standard antibiotic erythromycin (2 mg ml^{-1}) showed more growth inhibition in *S. typhi* (26 mm) and less (19 mm) in *S. marcescens*. The ZOIs evinced by antibiotic were very clear and high when compared to fresh leaf and fresh fruit extracts on all bacterial strains.

Table 2: Antibacterial activity of fresh fruit petroleum ether extract of *Croton sparsiflorus* on bacteria. Results are mean±SD of 2 independent experiments (n= 6). Results were significantly different at P<0.05.

Bacteria	Zone of inhibition (mm)		
	500 µg ml^{-1}	1000 µg ml^{-1}	2000 µg ml^{-1}
Bacillus subtilis	16.17±1.72	16.50±1.76	17.83±1.47
Streptococcus sp.	15.50±2.07	16.67±1.97	17.17±2.40
Staphylococcus aureus	16.83±2.79	16.83±3.18	18.67±1.86
Salmonella typhi	14.64±2.16	15.67±2.94	16.50±2.18
Serratia marcescens	14.33±1.03	16.50±0.55	16.50±0.84

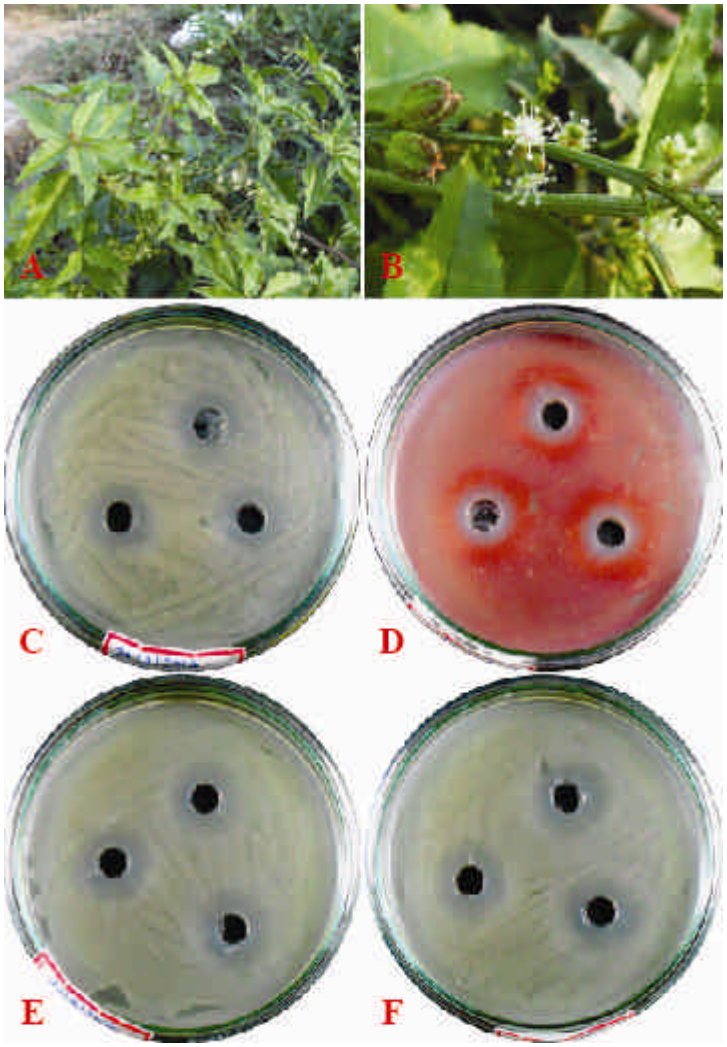

Figure 1. *Croton sparsiflorus*: A) Habit, B) Fruits and flowers. Antibacterial activity of fresh leaf extract on: C) *Salmonella typhi* and E) *Staphylococcus aureus*. Antibacterial activity of fresh fruit extract on: D) *Serratia marcescens* and F) *S. aureus*.

MIC of Leaf and Fruit Extracts

As the lowest concentration (500 µg ml^{-1}) of leaf and fruit extracts could kill all bacteria to some extent it was decided to reduce the concentration of extracts and test the killing efficiency. The MIC for *B. subtilis*, *S.aureus*, and *S. typhi* was 350 µg ml^{-1}. *Streptococcus* had 250 µg ml^{-1} and *S. marcescens* had 100 µg ml^{-1} as MICs. Fruit extracts of *C. sparsiflorus* had 100 µg ml^{-1} as MIC for all the bacteria studied.

DISCUSSION

Petroleum ether extracts obtained from leaves and fruits of *C. sparsiflorus* were not viscous. Our previous study involved *iso*-propanol, ethanol and *n*-butanol extract of fresh leaves of this plant was viscous [7] [8]. The reason for non-viscous nature of PE extract could be absence of any mucilage substances. The PE extract of fresh leaves had higher growth inhibition on *S. aureus* even at low concentration. Singh *et al*. reported that no growth inhibition of *S. aureus* by PE extract even at 125 mg ml^{-1} concentration [9]. This might be due to the

Enhanced reddish colour of *S. marcescens* around the inhibition zone by extracts was due to the elevated level of prodigiosin synthesis [13]. This pigment has been reported to have an antibiotic property [14]. Prodigiosin synthesis upon extract addition is an indication that the phytochemicals found in *C. sparsiflorus* has bioactive property. In general, Gram-positive bacteria were more susceptible than Gram-negative bacteria. Though, leaf extracts had relatively more inhibitory activity than fruit extracts, low MIC of fruit extract directed us to come to a conclusion that fruit extract is needed in low concentration to kill the bacteria.

CONCLUSION

The present study confirms that the fresh leaf extract of *Croton sparsiflorus* is effective as antibacterial agent with more inhibition on *Staphylococcus aureus* and less inhibition on *Serratia marcescens*. Fresh fruit extract had similar antibacterial activity like leaf extract. We propose that fresh leaves are suitable storage organ for secondary metabolites which possess antibacterial property.

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