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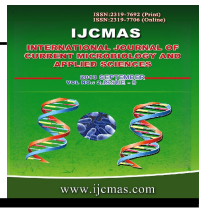

Original Research Article

Comparative assessment on antibacterial activity of bark of *Carallia brachiata* (Lour.) Merr prepared in single and gradient extraction methods

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A B S T R A C T

Bark of *Carallia brachiata* was evaluated for antibacterial activity in gradient extraction and single extraction (95% ethanol) methods. Gradient extraction was conducted using petroleum ether, acetone, ethanol, and water as extracting solvents in the gradation of increasing polarity. Single extraction method is common in homeopathic medicine preparations. We compared antibacterial activity in both methods. Antibacterial activity was tested towards ten pathogenic bacterial strains. The antibacterial activity was observed at its maximum in acetone extract prepared in gradient fashion. All the 10 bacterial strains showed considerable inhibition of growth in acetone extract. Acetone extract of the bark showed like minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 12.5mg/ml and 25mg/ml. towards *Serratia marcescens*. Single ethanolic extraction method is commonly employed for preparing medicines in Homeopathy; our investigation showed that gradient extraction method could give better results compared to single extraction in 95% ethanol.

Keywords

Carallia brachiata; antibacterial activity; bark extracts; gradient extraction; disc diffusion.

Introduction

Carallia brachiata (Lour.) Merr. belongs to the family Rhizophoraceae. Its synonyms are *Diatoma brachiata* Lour, *Carallia integerrima* DC. Local names of the plant are Vallabham, Varrungu and Vankana. The plant is a large tree found in semi-evergreen forests and also in planes. These subcanopy trees are geographically distributed in Indo-Malasia and Australia. (Sasidharan, 2004). Bark of *Carallia brachiata* is traditionally used in wound healing, treating itch, oral ulcer, inflammation of throat and stomatitis Nadkarni and Nadkarni, 1995).

The leaves and bark are used medicinally against itch and septic poisoning. The fruit extracts are medicinally important to treat ulcers (Krishnaveni *et al.*, 2009). *Carallia brachiata* bark extract exhibited hepato-protective activity (Kumari *et al.*, 2012). Reactive radical scavenging and xanthine oxidase inhibition of proanthocyanidins namely carallidin, mahuannin A and para-hydroxy benzoic acid were observed from the bark of *Carallia brachiata* (Phuwapraisirian *et al.*, 2006). The crude extracts of *Carallia brachiata* showed antibacterial and antifungal activity

(Neeharika *et al.,* 2010). The present investigation aims to assess antibacterial activity difference in single ethanolic extract of the bark and gradient extraction of the same.

Materials and Methods

Preparation of plant extracts

Fresh specimen of *Carallia brachiata* (Rizophoraceae) bark was collected from Thodupuzha of Idukki district Kerala in the month of January 2013. . A voucher specimen (AS 1240) was deposited at the herbarium of St. Thomas College Palai. Bark washed properly, air dried and powdered well and used for preparing extracts. About 100gm of powdered material were successively extracted in petroleum ether, chloroform, acetone, ethanol and water in gradation of increasing polarity by Soxhlet apparatus (Raghavendra *et al.,* 2006). The extraction was also done using single solvent (95% ethanol). Homeopathic extracts are usually prepared in 95% ethanol, known as Tinger (Mandal and Mandal, 2004).

Bacterial strains

Test organisms collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh. These include *Bacillus cereus, Klebsiella pneumoniae, Serratia marcescens, Salmonella typhi, Streptococcus haemolyticus, Vibrio cholerae, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Proteus rettgeri*. The bacteria were sub cultured on nutrient agar slants, incubated at 37°C for 5 hours and stored 4°C in the refrigerator to maintain the stock culture. Bacterial strains were maintained in nutrient agar slants and broth.

Antibacterial test (Disc-diffusion method)

The disc diffusion method as described by Bauer et al., 1996 was used to determine the growth inhibition of bacteria by plant extracts. Sterile liquid Muller Hinton Agar Media (pH 7.4 ±2) was transferred into the sterile petridish and after solidification; the bacteria (1ml broth of approximately 10^5 CFU) were swabbed with a sterile swab under aseptic conditions. Sterile discs prepared using Whatman No.4 Filter paper, of 5mm diameter were used in the study. The original solvent in which the extract prepared was used as a control. 20µL of the extract solution was loaded per disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. M.H. agar plates were lawned with inoculums of bacteria using sterile cotton swabs dipped in the nutrient culture. The discs were then applied to the lawned plates. Inhibitory zone around each disc was observed, after overnight incubation at 37°C. Also sterile disc impregnated with solvents alone were used as control. For each organism triplicates were carried out and average inhibitory zone diameter was determined. Discs were introduced onto the surface of the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth was determined by measuring the diameter of zone of inhibition. Experiments were conducted in more than three replicates and average inhibitory zone diameter along with standard deviation.

Minimum inhibitory Concentration (MIC)

The MIC of the extracts was performed by incorporating various amounts (sample concentration of 100mg/ml) of the extract

into sets of test tubes with the culture media (Barry, 1976). 50 □l of the bacterial broth culture was added into each of the test tubes. The bacterial cultures containing the plant extracts were incubated at 37^{o} C for 24 hours. Test tube containing only the growth medium and each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not permit any visible growth when compared to that of the control tubes.

Minimum bactericidal concentration (MBC)

Samples from the tubes used in the MIC assays, which did not show any visible growth after a period of incubation were subcultured onto a freshly prepared nutrient medium (Ratimi *et al.,* 1988). The minimum bactericidal concentration was taken as the lowest concentration of the extract that did not yield a single colony on the nutrient agar plate after 24 hours incubation period.

Results and Discussion

Antibacterial activity analysis of *Carallia brachiata* (Lour.) Merrill were conducted using petroleum ether, chloroform, acetone, ethyl alcohol and water as extracting solvents in the gradation of increasing polarity. The extraction was also done using 95% ethanol. Two different extractions were done in order to compare the antibacterial activity of single extract and various gradient solvent extracts. Petroleum ether extract did not show any antibacterial activity. This indicated that non-polar compounds present in petroleum ether extract did not have any antibacterial potential.. Chloroform extract was highly effective against *Vibrio cholera*. The antibacterial activity observed at its maximum in acetone extract. Antibacterial activity was obtained against ten pathogenic bacterial strains. Maximum activity was shown against *Serratia marcescens* (Table1). Ethyl alcohol extract obtained in gradient extraction showed considerable antibacterial activity against all the above 10 pathogenic bacterial strains except *Serratia marcescens* and *Escherichia coli* (Table1). Single extract showed antibacterial activity against *Bacillus cereus, Vibrio cholerae, Klebsiella pneumoniae, Serratia marcescens* and *Proteus rettgeri* (Table1). Extracts prepared in solvents in the gradation of increasing polarity showed better antibacterial activity than extracts prepared in single solvent (95% ethanol).

In order to evaluate the intensity of antibacterial activity, we evaluated the same by evaluating the activity of strain that gave maximum antibacterial activity. *Serratia marcescens* showed maximum zone of inhibition in acetone extract, therefore its MIC and MBC values were estimated. The MIC value of 12.5mg/ml and the MBC value of 25mg/ml were obtained. Another observation is that non-polar extracts showed lower level of antibacterial activity. The present result supported the medicinal (ethnobotanical) uses of the plant in treating skin diseases, respiratory and urinary infections, cough stomatitis as reported by Nadkarni and Nadkarni (1995). Single ethanolic extraction method is the common procedure of preparing medicines in Homeopathy (Mandal and Mandal, 2004).

Table.1 Antibacterial activity of *Carallia brachiata* towards various bacterial strains

Bacterial strains	95% Ethanol extract	Inhibition zone diameter (in millimeter) and extract used				
		Petroleum ether	Chloroform	Acetone	Ethyl alcohol	Water
Bacillus cereus	10 ± 0.72	----	----	**11±0.2**	10±0.4	10±0.1
Vibrio cholerae	10±0.8	----	20±0.2	**12±40**	10±0.02	9±0.32
Escherichia coli	----	----	----	**10±0.04**	----	11±0.2
Salmonella typhi	-----	----	10±0.06	**15±0.21**	7±0.3	10±0.21
Klebsiella pneumoniae	9±0.03	----	9±0.041	**9±0.6**	10±0.6	10±0.3
Serratia marcescens	10±0.02	----	----	**20±0.43**	----	----
Pseudomonas aeruginosa	----	----	----	**11±0.06**	9±0.3	10±0.32
Proteus vulgaris	----	----	----	**12±0.57**	10±0.2	----
Proteus rettgeri	11±0.7	----	----	**10±0.22**	12±0.4	----
streptococcus haemolyticus	----	----	----	**11±0.7**	10±0.12	----

* Values: Mean ± Standard deviation

Our investigation showed that gradient extraction could give better results compared to single extraction in 95% ethanol. This might be due to the fact that gradient extraction of the plant helped helped to isolate and elute antibacterial compounds in a better fashion than single extraction.

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