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SCREENING OF ANTI-BACTERIAL ACTIVITY OF TWO DIFFERENT SOLVENT EXTRACT FROM THE LEAVES OF *CHROZOPHORA ROTTLERI*AGAINST PATHOGENIC BACTERIA

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ABSTRACT

The aim of this study was to bearing the phyto chemical profiles from the chloroform extract of *Chrozophora rottleri* leaves and it's evaluate antibacterial activity. The chloroform extract of *Chrozophora rottleri*leaves were prepared by ethanol gradient elution orderly and analyzed by TLC. The bacterial strains were used to evaluate the antibacterial activities by the disc diffusion and MIC method. Results showed that the disc diffusion against bacteria ranged from 5 µL/mL to 20 µL/mL of *Escherichia coli*,*Staphylococcus aureus* and *Proteus vulgaris*). Alsochloroform extract exhibited MIC values ranging 5 µL/mL against both gram positive and negative bacteria. Remarkable antibacterial potential was noticeable with higher inhibition zone recorded in*Escherichia coli*,*Staphylococcus aureus* than other organism. The TLC fingerprint profiles demonstrated the presence of various phytochemicals in leaf extract. In conclusion, the chloroform extract of *C. rottleri* possessed the property like antibiotics against bacteria. These results support an individual phytochemical profile further investigation for the isolation of novel compounds with antimicrobial bioactivity and also afford hypothetical supporting as natural food preservatives and medicinal plant.

Key words:*Chrozophora rottleri*, chloroform and methanol extract, inhibition of bacterial

INTRODUCTION

Medicinal plants are the "backbone" of traditional medicine, which suggests quite 3.3 billion people within the less developed countries utilize medicinal plants on a daily basis. These medicinal plants consider as an upscale resources of ingredients which may be utilized in drug development and synthesis. Besides that these plants play a critical role within the development of human cultures round the whole world (Davidson-Hunt, 2001).Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant [Harborne, 1993; Marasini, 2015]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties [Singh *et al*., 2012; UNESCO, 1996]. A number of phyto therapy manuals have mentioned various medicinal plants for treating infectious diseases. The Indian sub-continent features a very rich diversity of plant species during a wide selection of ecosystems.

There are about 17.000 species of upper plants, of which approximately 8.000 species, are considered medicinal and employed by village communities, particularly tribal communities, or in traditional medicinal systems, like the Ayurveda. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the upkeep of excellent health, has been widely observed by UNESCO, 1996. Furthermore, an increasing reliance on the utilization of medicinal plants within the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants also as from traditionally used rural herbal remedies (UNESCO, 1998). Antimicrobial bioactive chemicals are essentially important in reducing the global burden of infectious diseases (Bhatia and Narain, 2010). However, occurrence and distribution of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even occasionally no, effective antimicrobial agents accessible for the infection caused by pathogenic bacteria (Giamarellou, 2010).Thus, in the light of the evidence of the rapid global spread of unaffected clinical isolates, the need to find new antimicrobial agents is of dominant importance. However, the past record of rapid, widespread appearance of resistance to newly make known to antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectation (Marasini *et al*., 2015). *Chrozophora rottleri* is traditionally used by the tribes and native medical practitioners for the treatment of various diseases, in India, powdered stems or whole plant are applied to wounds to improve healing, an infusion of the seeds and leaves is taken as a laxative. The plant is also used medicinally against jaundice and purifying blood, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhea. A vast number of therapeutic plants have been documented as valuable properties of natural antimicrobial compounds as a substitute that can possibly be effective in the handling of these problematic bacterial infections (Singh *et al*., 2012). Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate *in vitro* antibacterial activity of acetone extract of *Chrozophora rottleri.*

MATERIAL AND METHODS

Plant Samples / Sources: Leaves of *Chrozophora rottleri* were collected from Medicinal Plant Garden at Government Siddha Medical College, Arumbakkam, Chennai-600 106, a recognized institution of Government of Tamilnadu and the Department of AYUSH, Government of India. This plant identified and authenticated by Dr. S. Sankaranarayanan, Head of the Department, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Culture Collection and Maintenance: Bacteria used for the determination of antibacterial activities were *Staphylococcus aureus* MTCC 29213 and *Proteus vulgaris* MTCC 1771, Gram negative; *Escherichia coli* MTCC 25922, *Pseudomonas aeruginosa* MTCC 2488. These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. The stock culture was maintained on Mueller Hinton agar medium at 4 ◦C.

Antibacterial Activity by Disc Diffusion: The antibacterial activities of the chloroform extract of *Chrozophora rottleri* were assayed using the disc diffusion method. Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90º to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 6 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% acetone. The different concentration of bioactive compound was introduced on to each disc and the control disc received only 7% chloroform. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

Minimum Inhibitory Concentrations (MIC): The minimum inhibitory concentrations of the isolated compounds were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the chloroform extract of *Chrozophora rottleri* were prepared to give solutions of 5, 10, 15, and 20 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MHbroth inoculated with 0.5 ml bacterial suspension at a final concentration of 106 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% chloroform used as bacterial control, 4.5 ml of un inoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 ◦C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

Data analysis: All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Tests using SPSS vers. 15 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Two different solvent chloroform and methanol extract from the leaves of *Chrozophora rottleri* were assessed for their anti-bacterial possessions. Antibacterial movement was confirmed in contradiction of standard strains of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*and *Proteus vulgaris* (Table-1 and 2)**.** *Chrozophora rottleri* chloroform extracts were the most effective against all of the microorganisms tested. Methanol extracts from the same plant had antimicrobial activity against the same microorganism in some situations. *Chrozophora rottleri* chloroform extracts, for example, were active against *S. aureus, Proteus vulgaris,* and *E. coli.* This difference in outcome may be due to the use of a solvent system of different polarity. The therapeutic value of flora lies in the bioactive metabolites present in plants, which have the ability to melt in various solvent systems, according to comprehensive research.

Table-1. The antibacterial activity of the chloroform extract from the leaf of *Chrozophora rottleri* by disc diffusion method

Pathogenic organism	**Different concentrations chloroform extract (µl/ml)**			
	5 µl/ml	**10** µl/ml	**15** µl/ml	**20** µl/ml
Staphylococcus aureus	10.2±1	12.3±1.4	13.9±0.7	16.3±1.5
Pseudomonas aeruginosa	8.5±1.3	10.3±1.5	12.6±1.3	14.6±2.1
E. coli	8.1±0.4	10.6±0.9	13.4±1.1	14.3±1.6
Proteus vulgaris	8.4±1.6	11.2±1.4	13.4±1.3	15.7±0.3

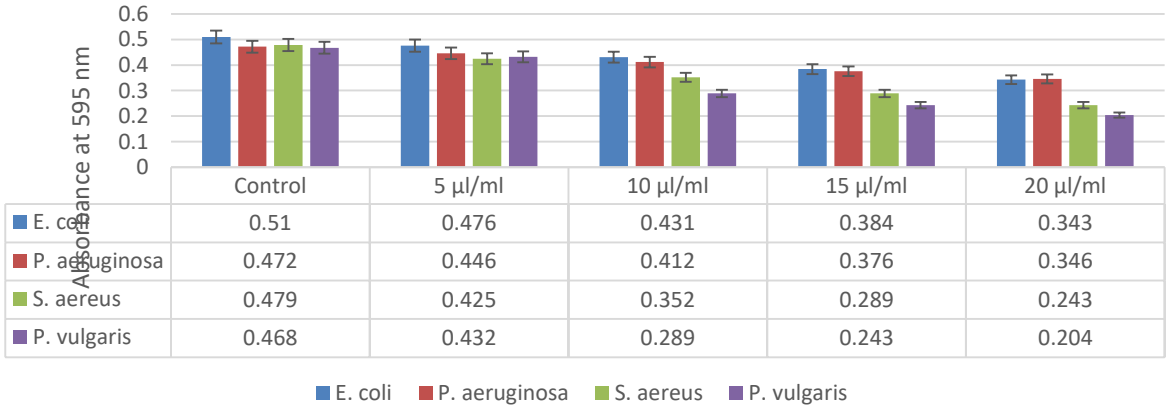
Table-2. The antibacterial activity of the methanol extractfrom the leaf of *Chrozophora rottleri* by disc diffusion method

Pathogenic organism	**Different concentrations Acetone extract (µl/ml)**			
	5 µl/ml	**10** µl/ml	**15** µl/ml	**20** µl/ml
Staphylococcus aureus	8.5±1	10.7±1.4	12.9±0.7	13.8±1.5
Pseudomonas aeruginosa	7.5±1.6	9.6±1.2	13±1.3	15.7±2.1
E. coli	7.6±0.5	9.4±0.9	11.2±1.1	12.3±1.6
Proteus vulgaris	10±1.3	12±1.7	14±1.3	16.6±1.4

The antibacterial activity of the chloroform and methanol extractfrom the leaf of C*hrozophora rottleri* by minimum inhibitory concentration method

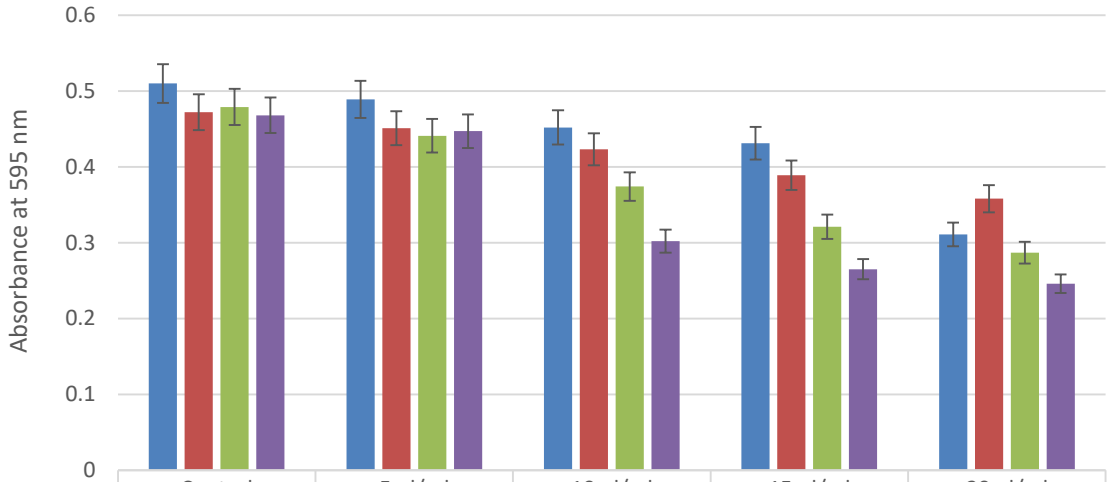
Results for the methanol and chloroform extracts of *Chrozophora rottleri*are displayed in Graph-1 and 2, Fig-3 and 4. These extract with greatest movement was selected and fractional process wascomplete. The plantsexcerptcan be recognizedfor example the maximumdynamicexcerptthrough MICs extendingbeginning25-100 µg/ml on four microbial strains investigated *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*and *Proteus vulgaris*. The leaves chloroform extract of *Chrozophora rottleri*also confirmed highest antibacterial activity against *Staphylococcus aureus*and *Proteus vulgaris* with MIC of 25µg/ml. The methanol extract of *Chrozophora rottleri*plantscorrespondingly ensued through the MIC of 25µg/ml compared tofour different bacteria. Upon small process of the methanol extract of plantswas the less active fraction inhibiting fourmicroorganismsverified with MICs reaching from 25µg/ml to 100µg/ml. The chloroform section exhibited strong antibacterial activity on fourmicroorganismsthan standard streptomycin with MIC of 25µg/ml.The chloroform extracts also have shown to comprise alkaloid and terpenoids from previous phytochemical examination. Alkaloid and terpenoid, have imperative uncontaminatedaction. The antibacterial action of alkaloid is payable to their capability to compositethrough extracellular alsosolvable protein then to multifacetedbymicrobial cell wall although that of terpenoidmight be connected to their capacity to deactivatebacterialbonds, enzymes and cell enclose proteins(Rajkumari et al., 2019).

GRAPH-1.THE ANTIBACTERIAL ACTIVITY OF THE CHLOROFORM EXTRACT FROM THE LEAF OF *CHROZOPHORA ROTTLERI* BY MINIMUM INHIBITORY CONCENTRATION METHOD



	Control	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
E. coli	0.51	0.476	0.431	0.384	0.343
P. aeruginosa	0.472	0.446	0.412	0.376	0.346
S. aereus	0.479	0.425	0.352	0.289	0.243
P. vulgaris	0.468	0.432	0.289	0.243	0.204

GRAPH-2. THE ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT FROM THE LEAF OF *CHROZOPHORA ROTTLERI* BY MINIMUM INHIBITORY CONCENTRATION METHOD



	Control	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
E. coli	0.51	0.489	0.452	0.431	0.311
P. aeruginosa	0.472	0.451	0.423	0.389	0.358
S. aereus	0.479	0.441	0.374	0.321	0.287
P. vulgaris	0.468	0.447	0.302	0.265	0.246

E. coli P. aeruginosa S. aereus P. vulgaris

Overall, gram-negative bacteria showed the least kindness against solvent extracts, and most plant extracts showed decreased and unvarying activity except for *Escherichia coli*, which represents the bacteria's encounter with the medicinal plant extract. This was to be anticipated, as gram-negative microbes indicate a far more complex protective mechanism toward foreign material overload (example antibacterial agent).This is attributed to the unique cell wall structure and, in particular, the presence of the travel packages, which results in the water resistance of these microbes to plant novel compounds and other enzymes, as well as the regulation and restriction of their passageway to the board region (Wink, 2012).As a result, gram-negative organisms show resistance to plant substances to a far higher degree than gram-positive microbes (Peters and Pasvol, 2007). The lipophilic or hydrophilic existence of molecules also influences their antimicrobial activity (or lack of same).Additives that are thought to be more protective towards gram-negative organisms have a lower lipophilicity. This is due to the gram-negative cell wall's composition, although it has a larger enzyme activity (Diniz *et al.*, 2009).Throughout earlier phytochemical analysis, chloroform samples of *Chrozophora rottleri* also contained polyphenols including tannins and flavonoids. Polyphenols including tannins and flavonoids have strong antibacterial properties. The highest antibacterial activity was observed with chloroform extract of *Chrozophora rottleri* against *E. coli, P. aeruginosa,P. vulgaris,S. aureus* respectively while minimum activity was observed with methanol extract of *Chrozophora rottleri* against *E. coli* and *P. aeruginosa.*

CONCLUSION

Based on the results, it can be concluded that using mathanol and chloroform extraction effectively improve the extraction yield. Overall, chloroform extracts from the leaves of *Chrozophora rottleri* possess antimicrobial activity as they could inhibit the growth of tested general pathogens microorganisms. The chloroform extracts from the leaves of *Chrozophora rottleri* had antimicrobial activity against all tested microorganisms. A decrease in cytoplasmic pH (pH_{int}) and cell wall disruption was observed in cells treated with plant extracts, suggesting a possible mechanism of antibacterial action. These findings indicate that the plant extracts tested in this study could be used as natural preservative agents in contamination to eliminate or control the growth of spoilage and pathogenic microorganisms.

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