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RESEARCH ARTICLE

IN VITRO ANTIMICROBIAL ACTIVITY OF ACETONE EXTRACT FROM THE LEAVES OF *CHROZOPHORA ROTTLERI* AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The aim of this study was to bearing the phyto chemical profiles from the acetone extract of *Chrozophora rottleri* leaves and it's evaluate antibacterial activity. The acetone 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*). Also chloroform 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 phyto chemicals in leaf extract. In conclusion, the chloroform extract of *Ch. rottleri* possessed the property like antibiotics against bacteria. These results support an individual phyto chemical profile further investigation for the isolation of novel compounds with antimicrobial bioactivity and also afford hypothetical supporting as natural food preservatives and medicinal plant.

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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 phytotherapy 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.

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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 ofdominant 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.

Phyto chemical Analysis of *Chrozophora rottleri:* The acetone extract of *Chrozophora rottleri* were freshly prepared and various chemical constituents were analysed according to methods described by Allen (1974) and Harbone (1976). The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, anthocynin, polyphenol and flavonoids.

Determination of Total Phenolic Content: The total phenolic content (TPC) of *Chrozophora rottleri* leaves extract was determined using the method by Gutfinger (1981). The chloroform extract (1 mL, 1 mg/mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400X*g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (ELICO (SL150) UV–Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a tannic acid equivalent.

Estimation of Flavonoid: A 1ml aliquot of chloroform extract of *Chrozophora rottleri* was placed into a 25 ml measuring flask. To this sample,1ml of 2% aluminium chloride and 0.5 ml 0f33% acetic acid was added, after which the flask is filled with 90% methanol to the mark and the content is thoroughly stirred. The obtained solution is allowed to stand for 30 minutes and the absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Rutin was used as a standard.

Thin Layer Chromatography Profile of Chloroform Extract of *Chrozophora rottleri:* Thin layer chromatography of chloroform extract of *Chrozophora rottleri* was performed using standard procedures (Harborne 1973).The chloroform extract of *Chrozophora rottleri* was placed carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1) or solvents of ethyl acetate-glacial acetic acid-formic acid-water (100:11:11:26 v/v/v/v). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer. The Rf value of the spots was calculated by using the standard formula,

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 ofun 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

Phytochemical Analysis: In the present study, efforts were made to qualitatively assessment the various medicinally active constituents such as flavonoids, saponins, tannins, steroids, alkaloids and terpenoids present in leaves acetone extract of *Chrozophora rottleri* leaves and absence of cardiac glycosides. The present study agree with previous reported bioactive compounds xanthone glycosides and a chromone glycoside, flavonoids, tannin, coumarin, scopoletin, the alkaloid ricinine (Abdel,2001).

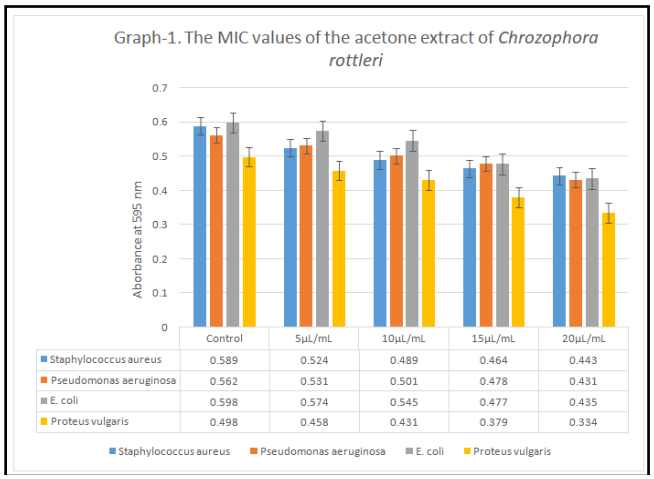
Thin layer chromatography profiles: The TLC plates were viewed under UV light 240 and 360 nm to develop coloured bands. The plates showing proper separation were observed and their Rf value was calculated. Acetone extract of *Chrozophora rottleri* showed the maximum number of separation followed by acetone, petroleum ether, and methanol. The formation of coloured bands was attributed to different phyto chemical groups (Wagner and Bladt, 1996).

Antibacterial activity: The acetone extract of *Chrozophora rottleri* screened for potential antibacterial activity against *Staphylococcus aureus, Proteus vulgaris*, *E. coli, Pseudomonas aeruginosa,* (Table-3). Results showed that the most susceptible organism was *P. vulgaris,* which inhibition zone was recorded 16.6 mm. Secondary metabolites in plant products are responsible forseveral biological activities in living systems. Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites (Al Maqtari*et al*., 2014). The results from acetone extract of *Chrozophora rottleri* show that mostly more effective against the Gram-negative inhibition zones were (*Proteus vulgaris* 16.6 mm,*E. coli*12.3 mm and *P. aeruginosa* 15.7 mm)compared to the Gram-positive bacteria (*S. aureus*). These observations may be attributed to the nature of biological active components whose activity can be increased in the presence of ethanol. Numerous types of alkaloids, glycosides, terpenoids and flavonoids have been reported to have the antibacterial activity (Al-Daihan *et al*., 2013).

Minimum Inhibitory Concentration: The MIC values of the acetone extract of *Chrozophora rottleri* ranged from5 µL/mL to 20µL/mLafter 18 h of incubation. Theaverage MIC values varied for the different bacterial species with the lowest value (5 µL/mL) against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Proteus vulgaris* (Fig-2). The crude Chloroform extractevaluated, considerable antibacterial activities with MICs between 5 µL/mL to 20µL/mL. The MIC values obtained were comparable to that of the reference antibiotic (Streptomycin). Previous reported bioactive compounds in *Chrozophora rottleri* such as xanthone glycosides and a chromone glycoside, flavonoids, tannin, coumarin, scopoletin, ricinine. The presence of these phyto chemical supports the significant bioactivity exhibited by the crude extracts against the microorganisms tested. According to Wink, (2015) plant bioactive metabolites are associated with many active molecules including inhibitory properties against a wide range of pathogens. Among these secondary metabolites, alkaloids, flavonoid and terpenoid have been studied extensively in terms of their antimicrobial activities and mechanism ofaction.

Table-1. The antibacterial activity of the acetone extractfrom the leaf of *Chrozophorarottleri* by disc diffusion method

Pathogenic organism	Different concentrations Acetone extract (µl/ml)			
	5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	8.5±1	10.7±1.4	12.9±0.7	13.8±1.5
Pseudomonas aeruginosa	9.4±1.6	11.5±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



Conclusion

Based on the results, it can be concluded that using acetone extraction effectively improve the extraction yield. Overall, acetone extracts from the leaves of *Chrozophora rottleri* possess antimicrobial activity as they could inhibit the growth of tested general pathogens microorganisms. The acetone 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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