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INVITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL PROFILE ANALYSIS BY TLC AND GCMS FROM THE CHLOROFORM EXTRACT OF *CHROZOPHORA ROTTLERI*

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ABSTRACT

The aim of this study was to bearing the phytochemical 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 and GCMS. 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. The GCMS analysis revealed quercetin and kaempferol as major ingredients in the screening chloroform extract of *Ch. rottleri*. Interestingly, the content of quercetin and kaempferol varied with the concentration of elution ethanol. In conclusion, the chloroform extract of *Ch. 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.

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INTRODUCTION

Herbal plants are fit reflected in the essential sources of natural bioactive compounds. It has calculated that about 60% of the treatment products in Asia, USA and Europe countries are mostly from natural or their relative derivatives products (Newman *et al*., 2003). Beyond the 250,000-500,000 lower and higher plant species in the earth, just 1-10% have been scrutinized biologically and chemically for their possible pharmaceutical value (Verpoorte, 2000). The employing of traditional medicine has histrionically increased in India along with USA, in the last two decades (Marsland, 2007) Approximately 30% of antibacterial compounds are obtained from herbal plants and other natural resources; though, there are still a many of herbal plants that have an antibacterial probable but they have not yet been fully investigated (Cragg, 2005).

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Thus, the other solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines as very few studies have been reported on the use of herbal medicine in management of bacterial infection (Rao *et al*., 2004). This microbial property provides a persistent need to develop novel therapeutical formulations with resistance-combating capacity. Plants signify a major reservoir a gathering of diverse secondary or natural products that are of incredible medicinal value and that can be exploited in the search for new medications (Pan *et al*., 2009). *C. rottleri* is an erect, branched annual or perennial herb, up to 50 cm. Most parts densely covered in greyish stellate hairs. Leaves are rhombic-ovate, up to 7 × 5 cm with a long petiole, plicate-undulate, especially when young, 3-5-veined from the base with 2 dark purple glands at the base; margin more or less entire or obscurely toothed. Flowers are born on leaf-opposed or pseudo-axillary inflorescences, covered in stellate hairs, unisexual. Male flowers orange-yellow or pinkish; female flowers, crimson-red, Fruit up to 5 × 9 mm, 3-lobed, densely covered in satellite hairs,

reddish or bluish-purple when ripe. *C. rottleri* is traditionally used by the tribes and traditional practitioners for the treatment of various diseases. In traditional healers of Sudan, powdered whole plants are useful to wounds for improve healing. In Ethiopia, an infusion of the seeds and leaves is taken as a laxative. The plant is also used medicinally in Saudi Arabia, Pakistan and India (e.g. against jaundice and purifying blood). In Senegal, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhoea, where as in Kenya, camels graze it. Leaf and roots of *C. rottleri* contain xanthone glycosides and a chromone glycoside. Oil extracted from seeds was rich in linoleate and the whole plant contains tannin. The scopoletin, coumarin and alkaloid ricinine, flavonoids, xanthones and chromones have been reported that Abdel-Sattar, 1985; Hashim *et al*., 1990; Agrawal and Singh, 1988). *C.rottleri* extract have exhibited the highest antioxidant capacity which is also able to modulate hydroxyl radical formation more efficiently than other compounds acting as direct hydroxyl radical scavengers and chelating iron (Dipankar *et al*., 2011). The flavonoid compounds of *C. rottleri* were known to show curative activity against several pathogens (Hassan *et al*., 2004) and therefore it can be used traditionally for the treatment of wide array of illnesses. The antimicrobial screening of other species of *C. senegalensis* leaf extracts showed considerable amount of inhibition against *Bacillus subtilis, S. aureus, E. coli* and *P. aeruginosa*; with much activity on *S. typhi* (Usman *et al*., 2007).

MATERIALS 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 Tamil nadu 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.

Phytochemical analysis of *C. rottleri:* The chloroform 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 0f 33% 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,

$$Rf = \frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent}}$$

Culture collection and maintenance: Bacteria used for the determination of antibacterial activities were *S. aureus* (MTCC 29213) and *Proteus vulgaris* (MTCC 1771), Gram negative; *E. 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.

Gas Chromatography–Mass Spectroscopy: GC–MS analysis was performed using the chloroform extract of *C. rottleri*. GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan) equipped with 30- mX0.25 mm DB-5MS column (Agilent Technologies, J & W Scientific Products, Folsom, CA).QP-2010, Shimadzu Co – 5 capillary column with a dimension of 30m × 0.25mm, 0.25 mm film width, and composed of 95% dimethyl polysiloxane was used in the study. Helium was the carrier gas, with 0.5mL/min flow rate. Volume of the injection sample was determined as 1µL. Inlet temperature of about 250°C was maintained. At the start of the experimentation, the oven temperature was set for the first 4 minutes at 110°C and then gradually raised to 240°C. The temperature was set to rise at a rate of 20°C for every 5 minutes; this continued until a temperature of 280°C was reached. Overall run time was about 90 minutes. At 200°C temperature, the MS transfer line was continued. The source temperature was set at 180°C. Electron impact ionization at 70Ev was used to evaluate GC-MS. Using total ion count (TIC), compounds were identified and quantified. The spectrum of the components was compared with the database of spectrum of known components stored in the GC–MS library.

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

Table 1. Phyto chemical screenings of chloroform extract of *Chrozophora rottleri* leaves

Sl. No.	Phytochemical Constituents	Observation	Methanol leaves extract of *W. trilobata*
1	**Alkaloids** Dragendorff's test Mayers test	Orange /red precipitate Cream pie ppt	+ +
2.	**Flavonoids** Alkalai Reagent Lead aceate test	Intense yellow colour Precipitate formed	+ +
3.	**Glycosides** Keller-Killiani test	Pink colour (Ammonia layers)	-
4.	**Tannin** $FeCl_3$ test	Blue-blackcolour	+
5.	**Saponins** Frothing test	Foam	+
6.	**Terpenoids** Salkowski test	Reddish brown colour ring formed in interface	+
7.	**Polyphenols** Ferrozine test	Raddish blue	+
8.	**Anthocyanin** Ammonia test	Pink color in ammonia layer	+

+ Positive result; - Negative result

Table 2. The Partial characterization of chloroform extract of *Chrozophora rottleri* leaves by TLC

S.No.	Chloroform extractof *Chrozophora rottleri* leaves		
	UV Light 360nm	**UV light 240nm**	**Normal Light**
1	0.83	0.83	0.83
2	0.74	0.74	0.74
3	0.62	0.62	0.62
4	0.55	0.55	0.55
5	0.49	-	-
6	0.34	-	-
7	0.28	-	-

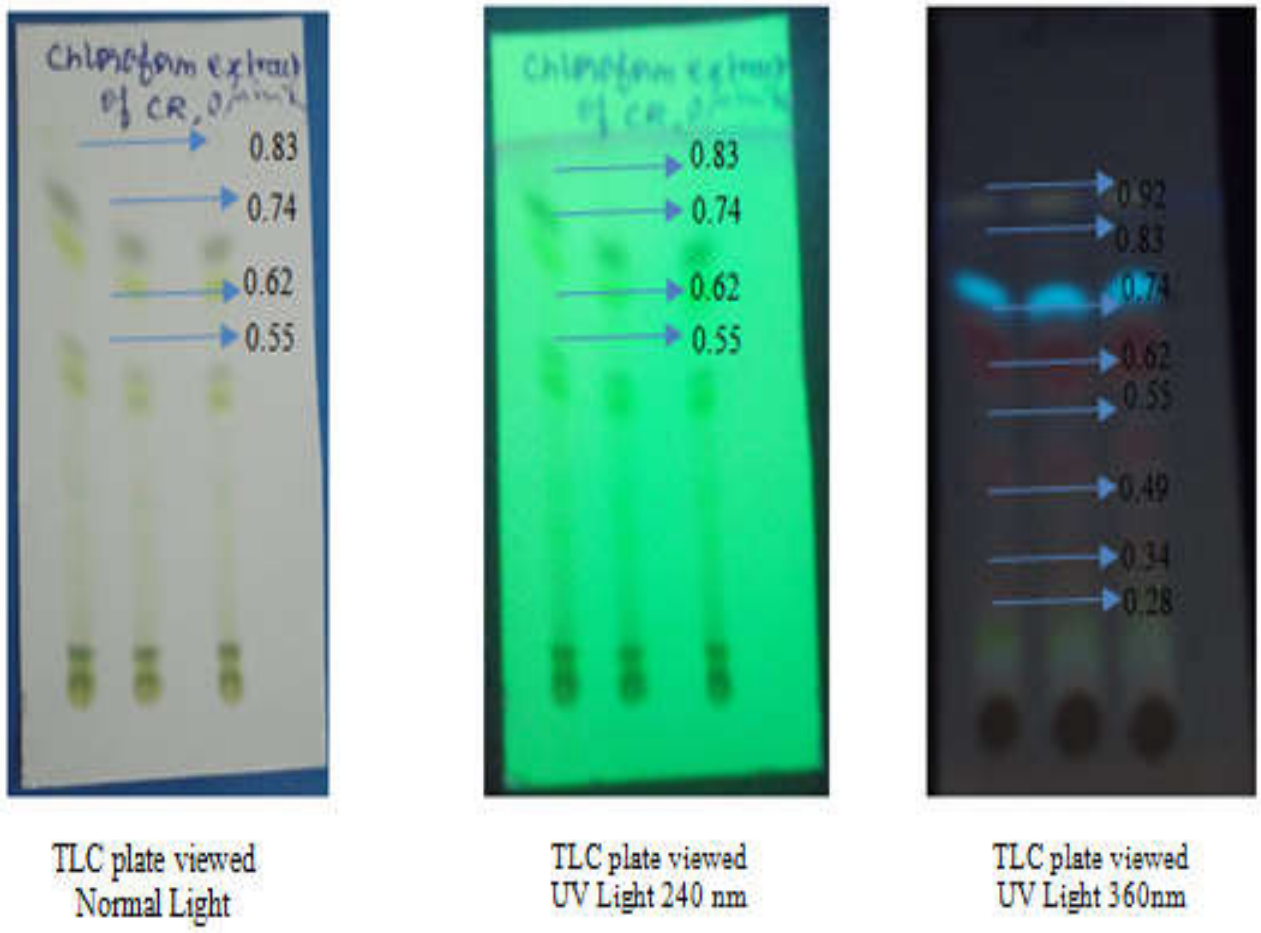

Fig. 1. The Partial characterization of chloroform extract from the leaf of *Chrozophora rottleri* by TLC

Table 3. Phyto chemical profile analysis of chloroform extract of *Chrozophora rottleri* by GC–MS

S.No	Compound	Retention Time(min)	Molecular weight	Major peaks
1	2-propenol	10.28	290	113.1, 99, 85.01
2	N-cyclooct-4-enylacetamide	11.03	154	154, 140, 130, 126
3	N- methyl-2-hydroxy-4-phenyl-3-butanamide	12.08	198	155, 151, 141, 127
4	Phenol, 2, 4ibis (1,1dimethylethyl)	12.52	136	136, 121, 107, 99
5	Nathalene, octahydro-4a-methyl-7	13.7	152	152, 122, 106, 81
6	1,5 dihydroxy-2,6,6-trimethyl cyclohex	14.32	168	168, 153, 125, 97
7	Isoaromadendrene epoxide	15.02	154	154, 137, 109, 81
8	Spiro (4,5) decan-7-one	16.1	236	235.5, 208.6, 178.7
9	10-Octadecenoic acid methyl ester	18.8	296	295.7, 263.7, 245.7
10	Strichinidin-10-one	21.15	410	409.5, 394.5, 353.6

Table 4. The antibacterial activity of the acetone extract from the leaf of *Chrozophora rottleri* 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	14.3±1.6
Proteus vulgaris	10±1.3	12±1.7	14±1.3	16.6±1.4

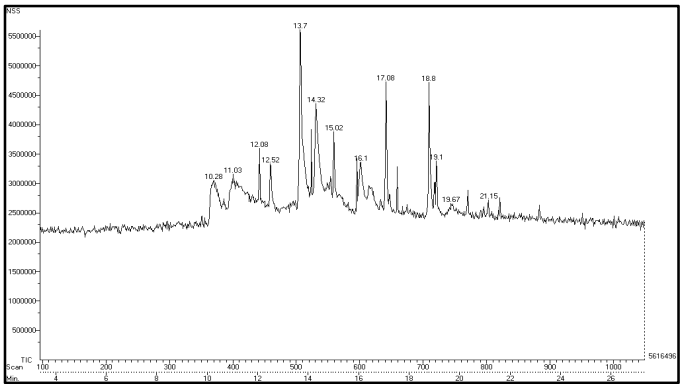

Fig. 2. Consolidated major phytochemical peak of Chloroform extract of *Chrozophora rottleri*

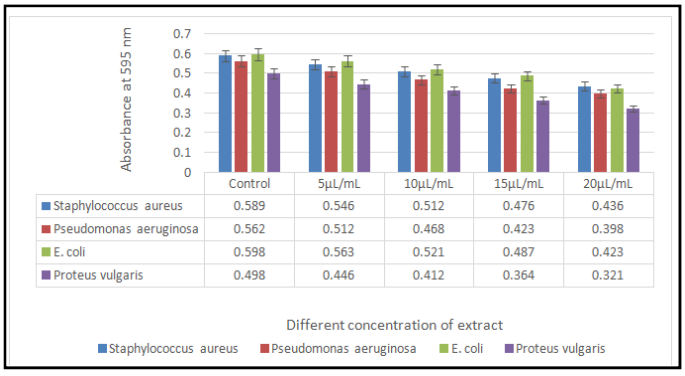

Fig. 3. Minimum Inhibitory Concentration of chloroform extract of *Chrozophora rottleri*

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% chloroform. 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 $A560$ 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 4.5ml of bacterial inoculates and 0.5 ml of 7% chloroform used as bacterial control, 4.5 ml of uninoculated 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).

RESULT 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 chloroform extract of *Chrozophora rottleri* leaves and absence of cardiac glycosides (Table-1). The present study agree with previous reported bioactive compounds xanthone glycosides and a chromone glycoside, flavonoids, tannin, coumarin, scopoletin, the alkaloid ricinine (Abdel-Sattar, 1985).

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 (Table 2 and Fig.1). Chloroform extract of *C. 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).

Gas Chromatography–Mass Spectroscopy profile of *Chrozophora rottleri* chloroform extract: The chloroform extractof *Chrozophora rottleri* had the highest phenolic and flavonoid compound and there by showed the strongest antioxidant activity (Table-3). It was therefore analyzed by GC-MS to determine its chemical composition that may contribute to this activity. The GC-MS analysis showed a variety of phenolic compounds (Fig. 2).

Antibacterial activity: The Chloroform extract of *C. rottleri* screened for potential antibacterial activity against *S.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 for several biological activities in living systems. Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites (Jaiganeshand Arunachalam, 2011). The results from Chloroform extract of *C. rottleri* show that mostly more effective against the Gram-negative inhibition zones were (*Proteus vulgaris* 16.6mm*, E. coli,* 14.3 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 (Barnabas and Nagarajan, 1988).

Minimum Inhibitory Concentration: The MIC values of the Chloroform extract of *C. rottleri* ranged from5 µL/mL to 20µL/mL after 18 h of incubation. The average MIC values varied for the different bacterial species with the lowest value (5 µL/mL) against *S. aureus, P. aeruginosa, E. coli, P. vulgaris* (Fig. 2). The crude Chloroform extracts evaluated, considerable antibacterial activities with MICs between 5 µL/mLto20µL/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 of action.

Conclusion

This study showed that the chloroform extract of *Chrozophora rottleri* have potential as antibacterial agents. This gives scientific confidence to the use of these plants traditional leaders. Also, the TLC and GCMS are useful in assay-guided isolation of active compounds. Based on current results it may be useful to isolate and characterize the compounds present in chloroform extract of *Chrozophora rottleri*.

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