



## Effect on antibacterial activity of isolated compound of *Chrozophora rotleri*

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### Abstract

*Chrozophora rotleri* is commonly used in traditional medicine to treat gastrointestinal disorders and diabetes. In this study, isolated compound of *Chrozophora rotleri* were investigated to antibacterial and antioxidant activities. The total phenolic and flavonoid contents and antioxidant capacities in terms of ABTS scavenging, Lipid peroxidation, and superoxide scavenging, nitric oxide scavenging and metal chelating activity were determined, and the correlations among the results were assessed using Pearson's correlation. The antimicrobial activity was assessed through agar disc diffusion and broth micro dilution assays. Phytochemical screening showed that *Z. spinosa* extracts had alkaloids, glycosides, saponins, triterpenoids, phenols, and flavonoids in different abundances. The antibacterial properties of the isolated compound of *Chrozophora rotleri* were analyzed by the disc diffusion method, and the isolated compound had higher antibacterial activities against the four bacterial strains used in the study. This study provides information on the synergistic antioxidant and antibacterial properties of isolated compound of *Chrozophora rotleri*.

**Keywords:** *Chrozophora rotleri*, isolated compound, antibacterial, antioxidant

### Introduction

All people of the world moreover completely or moderately reliant on plants and plant products, together straight and secondarily. For instance, remedial plants are employed in old-style medicine performs international. Numerous people in the India quiet trust that traditional medicines like Siddha and Ayurveda to modern practices in the empire of various treatment. Plant extract, either as unadulterated compounds or as homogenous extracts, deliver limitless chances for new drug detections because of the matchless chemical assortment they can deliver (Wink, 2020) [17]. Conferring to the World Health Organization (WHO), more than 70% of the domains people trusts on old-style medication for their chief healthcare requirements. This has detained the attention of several investigators to discover native curative plants for appreciated therapeutic characters. Some revisions specify that remedial plants comprehend compounds like alkaloids, essential oils, phenols compounds. These complexes are important in beneficial application against human and animal pathogens, with bacteria, fungi and viruses (Pavrez *et al.*, 2016). In current years, many drug struggles in human pathogenic microorganisms have established due to the unselective use of viable antimicrobial drugs normally used in the handling of infectious diseases.

Oxidants are clusters of atoms or a non-charged atom with smallest of one non-paired electron (free radical) in the external record orbit; consequently, they are extremely reactive. In breathing membranes, they foundation cellular fragmentation, which eventually indications to death. They are frequently molded by many chemicals that are current in

the environment contained toxic substance in air, water, and soil etc. and are widespread in large numbers in the blood. To respond the negative properties of these radicals, the living system produces numerous antioxidants. If the living system is incapable to do, additions must be added in the diet. Their mode of action contains polyunsaturated lipids (lipid peroxidation) of cell membranes, sideways with the standard RNA and DNA of the cell. An active antioxidant complex has numerous radical congregation antioxidant locations of diverse nature. These sites seek and terminate free radicals at many cellular sites (Stamp *et al.*, 2012) [15].

*Chrozophora rotleri* belongs to Euphorbiaceae family normally known as Suryavarti. In this plant occurs certainly throughout India, and other Asian country. *C. rotleri* is an upright hairy annual plant which found in common waste lands, blossoms abundantly from January to April. The entire plant cover with silvery hairs; lower part of stem is stripped, upper part hairy and has slight tap-root. The leaves are three-lobed alternative, thick and rugose. The plants are monoecious, the flowers borne in sessile axillary racemes with staminate flowers in upper and pistillate flowers in the lower part of raceme. *C. rotleri* is recommended by the tribes and traditional medical physicians for the handling of numerous diseases. The powdered stems are externally used to wounds for improve healing. Infusion of the seeds and leaves are suggested as a laxative. This plant is likewise used medicinally in India and Saudi Arabia against jaundice and cleansing blood.

*Chrozophora* yielded some flavonoid glycosides, these were quercetin 3-o-rutinoside, acacetin 7-o-rutinoside, apigenin, apigenin 7-o- b-dglucopyranoside and chrozo phorin, the

last one was reported as a new natural product (Radwan *et al.*, 2000) <sup>[12]</sup>. The methanol aqueous extract revealed antimicrobial activity with MIC values 125 µg/mL against Gram-positive bacteria and antioxidant activity, antiseptic for wounds and for hemorrhoids (Mohamed *et al.*, 1995) <sup>[10]</sup>. Fourteen novel dolabellane diterpenoids have been isolated from the aerial part of *Chrozophora* sp. namely acylated at the C-16 hydroxyl group with 3-hydroxy-3-methylglutaric acid (Adam *et al.*, 1999) <sup>[1]</sup>. Even though the extensive healing possible accessible by *C. rottleri*, records on the phytochemical profile, antioxidant, and antimicrobial activities of this plant are still lacking. Subsequently, the current experimental motivated on defining the content of total phenolic and flavonoids and assessing antimicrobial and antioxidant activities of ethyl acetate extracts of the leaf of *C. rottleri*. The present work also aims to provide an overview of this species' chemical and biological properties to develop sufficient background knowledge for continued research into the ethyl acetate extracts of this plant.

## Materials and Method

### 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 *Chrozophora Rottleri*

The aqueous extract of *Chrozophora rottleri* were freshly prepared and various phytochemical constituents were analysed according to methods described by Sofowara (1993) <sup>[14]</sup> and Harbone (1973). The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, anthocynin, polyphenol and flavonoids.

### Pharmacognostic Study

Fresh leaves was taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters. For the microscopical studies, transverse sections of leaves was prepared and stained as per standard procedure (Akbar *et al.*, 2014) <sup>[2]</sup>.

### Bacterial Strains

Gram-positive and gram-negative bacteria were used for testing the antibacterial properties of the selected plant samples. The following is a list of the bacteria selected for the study: *Staphylococcus aureus* MTCC 29213, *Escherichia coli* MTCC 25922, *Proteus vulgaris* MTCC 25840 and *Pseudomonas aeruginosa* MTCC 2488 were gram-negative. These strains were obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh-160 036, India. They were sub cultured on nutrient agar medium, incubated at 37°C for 24 hours, and stored at 4°C in a refrigerator to maintain stock culture.

### Antibacterial Activity of Isolated Compound by DISC Diffusion Method

The antibacterial activity of the isolated compound was determined by the disc-diffusion method (Velickovic and

Smelcerovic, 2003) <sup>[16]</sup>. The bacterial strains namely *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris* were grown overnight on Mueller Hinton agar plates. Different concentrations (5, 10, 15 and 20 µL/mL) of the isolated compound were added to separate, sterile paper disc of 5 mm diameter. Then, the discs were allowed to dry. Each of these disc were tapped gently down onto the agar to provide uniform contact. The plates were incubated at 37 °C for 24 hours. Clear zones of inhibition were measured and calculated.

### Minimum Inhibitory Concentrations (Mics) For Isolated Compound

The minimum inhibitory concentrations of the isolated compound was determined by dilution method. (Eloff, 1998). Different concentrations (5, 10, 15 and 20 µL/mL) of the isolated compound were added to separate test tubes containing 4mL of MH broth inoculated with 0.5 mL bacterial suspension at a final concentration of 10<sup>8</sup> CFU/mL. Subsequently, the aforementioned steps were followed.

### Assay on The Effect of Antibacterial Compound And Its Leakage In The Membrane Of Pathogenic Bacteria

The leakage in the membrane of pathogenic bacteria was estimated using isolated compound of *Chrozophora rottleri* for its protein and carbohydrate content. The experimental test tubes consist of Muller Hinton Broth with different concentrations (5, 10, 15 and 20 µL/mL) of *Chrozophora rottleri* isolated compound. The tubes were inoculated with *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris* at last concentration of 10<sup>9</sup> CFU/mL. The experimental cultures were held in reserve at 37±2 °C in a rotary shaker at 140 rpm. One milliliter culture was collected instantly after inoculation subsequent to 0-8-hours treatment. The treated cultures were centrifuged at 10,000 rpm. The culture filtered was frozen at -30 °C immediately. Then protein and carbohydrate content were quantified according to the technique of Miller, 1959 and Bradford, 1976, respectively.

### Inhibition of Biofilm Formation

The potential of the extracts to prevent initial cell attachment was investigated through the biofilm inhibition assay (Sandasi *et al.*, 2008) <sup>[13]</sup>. Briefly, a 100 µl aliquot of standardised concentration of cultures with OD560=0.02 (1.0 × 10<sup>6</sup> CFU/mL) of *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris* was added into individual flat-bottomed 96-well microtitre plates and incubated at 37 °C for 4 h without shaking. Then the plates were removed from the incubator and 100 µl (2 mg/ml) aliquots of plant extracts were added in twelve replicates into the wells of 96-well microtitre plates to give a final concentration of 1 mg/mL and then incubated further at 37 °C for 24 h without agitation. Gentamicin served as positive control while acetone and sterile distilled water served as negative controls. The biomass was quantified using the modified crystal violet staining method (Djordjevic *et al.*, 2002) <sup>[6]</sup>.

## Result and Discussion

### Phytochemical Screening of Aqueous Leaves Extract of *Chrozophora rottleri*

The phytochemical screening of aqueous leaves extract of *Chrozophora rottleri* leaves studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and Saponin (Table -1 and Fig-2 not Shown).

**Table 1:** Phytochemical screenings of aqueous leaves extract of *Chrozophora rottleri*

Sl. No.	Phytochemical Constituents	Observation	Aqueous leaves extract of <i>Chrozophora rottleri</i>
1	Alkaloids-Dragendorff's test -Mayers test	Orange / red precipitate Creampie ppt	+ +
2.	Flavonoids -Alkalai Reagent-Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	Glycosides-Keller-Killiani test	Pink colour (Ammonia layers)	-
4.	Tannin -FeCl <sub>3</sub> test	Blue-black colour	+
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 activity; - Negative activity

### Total Phenolic and Flavonoid Content of Leaves Extract of *Chrozophora Rottleri*

In this framework, the primary investigates exposed that ethyl acetate extract was the best solvent for the extraction of phenolics from *Chrozophora rottleri* at 60 °C for 60 min.

Meanwhile it valuable for maximum yield of phenolics. The yields dry plant of *Clinacanthus nutans* extracts ranged from 58.12% (w/w). Therefore, the total phenolic and flavonoid contents were reported as catechin and ruti equivalents respectively (Table-2).

**Table 2:** Yield and phenolic content from the leaves of *Chrozophora rottleri*

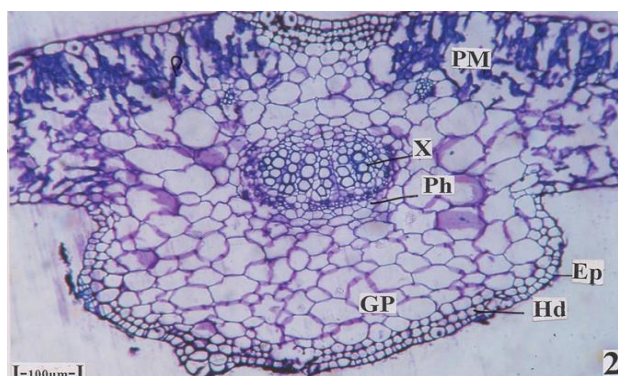
Sample	Yield of extract (g/100 g of defatted Content)	Total phenolic content (mg catechin equivalents per gram ethyl acetate extract)	Total flavonoid content (mg rutin equivalents per gram ethyl acetate extract)
Chloroform leaf extract of <i>Chrozophora rottleri</i>	58.12±2.16 <sup>a</sup>	64.12±1.64 <sup>b</sup>	44.78±2.41 <sup>b</sup>

<sup>a</sup>Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

<sup>b</sup>Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

### Anatomy of The Leaf Of *Chrozophora Rottleri*

The leaf consists of a wrinkled with thick midrib and lamina. The middle vein is Plano convex with flat upper (adaxial) part and thick semicircular lower (abaxial) part (Fig-11a). The midrib in 750 µm thick and 950 µm wide. The epidermal layer on the abaxial side consists of small squash cells. Beneath the epidermal layer these are four layers of sclerenchyma cells of squash shaped cells. The epidermal layer of the abaxial midrib consists of small squash shaped thick walled cells. Inner to the epidermis these are two or three layer of thick small cells. The ground tissue is parenchymatous; the cells are wide thin walled and compact. The vascular bundle of the midrib in small, elliptical is horizontal plant and collateral (Fig. 1). The vascular bundles consist of about light vertical lines of xylem elements which are circular, wide and thick walled. Along the lower end of the xylem elements occurs a thin are of phloem. The lamina has smooth and was surfaces. It is dorsiventral with adaxial land of palisade zone and abaxial zone of spongy parenchyma zone (Fig. 1). The lamina is 200 µm thick. The adaxial epidermis has cylindrical cells and abaxial epidermal cells are small and spindle shaped



Ads: Adaxial sides, ACB – Accessory Bundle, Ep – Epidermis, GP – Ground Parenchyma, Hd – Hypodermis, La – Lamina, MR – Midrib, Mx – Mata xylem, Ph Phloem, PM – Palisade Mesophyll.

**Fig 1:** T.S. Leaf of *Chrozophora rottleri* through midrib

### Isolation of Compounds from Chloroform Extract of *Chrozophora Rottleri* By Silica Gel G-60 Column Chromatography

Chloroform extract of *Chrozophora rottleri* leaves, which was then concentrated to dryness. The residue was dissolved in chloroform, and this chloroform extract was further partitioned successively with n-hexane. For Chloroform extract of *Chrozophora rottleri*, n-Hexane and chloroform were used as eluent. During this process, the flow rate of the eluate was adjusted to 1 ml/ 2 min. The fractions (each 15ml) were collected individually and tested for its purity by TLC (Fig-2). The identical fractions were pooled and concentrated under vacuum. The fractionation and chromatographic isolation were guided by antibacterial activity against four pathogen, and led to the isolation of compound. A correlated finding with present study was found with previous report on isolation of antibacterial compound eluded by dichloromethane of leaves fraction from *Chrozophora rottleri* which exhibited antibacterial activity against Human pathogen.

### Characterization of Isolated Compound From *Chrozophora Rottleri* By Infra Red Spectroscopy

The isolated compound of *Chrozophora rottleri* was engaged to define the arrangement by spectral analysis such as IR. IR has proven to be a respected instrument for the description and documentation of functional groups present in the isolated compound. Numerous IR captivation was recorded. The isolated compound of *Chrozophora rottleri* had IR absorptions at 3492, 3384, 3185 cm<sup>-1</sup> (OH), 2957, 2883cm<sup>-1</sup>(C-H), 1694, 1685 cm<sup>-1</sup>(C=O), 1645 cm<sup>-1</sup> (-C=C-), 1460 cm<sup>-1</sup> (C-H) and 1437cm<sup>-1</sup>(C-C). The peaks revealed the number of functional groups that have a great significant towards medicinal prospects of *Chrozophora rottleri* (Fig-2).

### Antibacterial Activity of Isolated Compound from The Leaves Of *Chrozophora Rottleri* Against Pathogenic Bacteria By Disc Diffusion And Mic Method

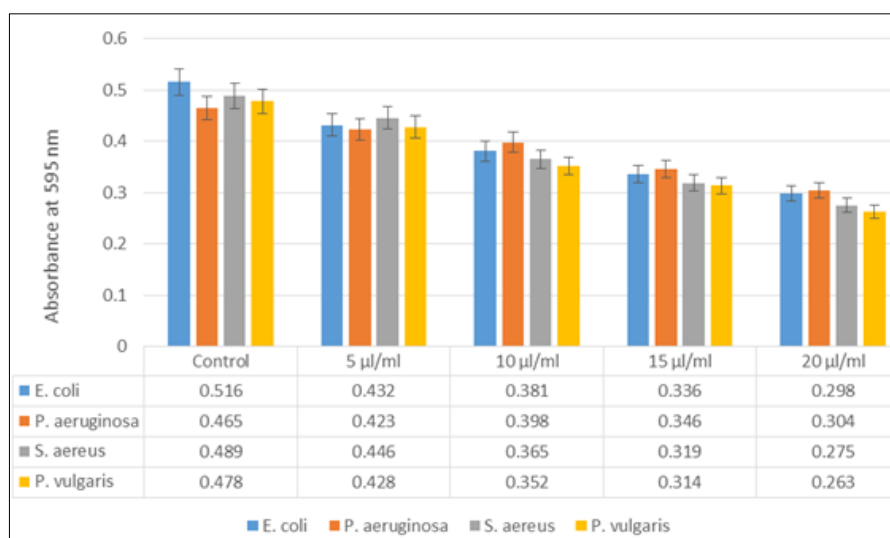
Antibacterial activity of separated compounds of *Chrozophora rottleri* was evaluated against all the four different bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*. The zone of inhibition was marked as a guide for the antibacterial activity of the purified compounds. However the sizes of zones were in accordance to the concentration gradient. Among the pathogens *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris* were found to be more susceptible to isolated compound than other bacteria (Table-3). Correspondingly, the result of MIC examinations presented that the compound identified from

*Chrozophora rottleri* controlled antibacterial activity against the tested organisms (Fig-3). Isolated compounds revealed a broad spectrum of activity, in which the concentration level of 100µg/ml was pointedly inhibitory. Consequently the antibacterial activity experimental in the present examination is recognized to the alkaloid compound which have been widely known to occur in different species of this genus. Olinila and Prakash 2018 reported previously, preliminary antibacterial studies of chloroform extract of *Chrozophora rottleri* with disc diffusion method recommended a remarkable outline of antimicrobial action against gram positive and gram negative bacteria especially that the greatest disposed organism was *P. vulgaris*, which embarrassment zone was noted 16.6 mm.

**Table 3:** The antibacterial activity of isolated compound of *Chrozophora rottleri* by disc diffusion method

Pathogenic bacteria	isolated compound of <i>Chrozophora rottleri</i> Zone of inhibition (mm) <sup>a</sup>				
	Positive control 10 µl Ampicillin	Different concentrations of isolated compound of <i>Chrozophora rottleri</i> (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
<i>Staphylococcus aureus</i>	13.5±1.2	9.6±1.23	12.4±1.46	15.3±0.89	17.36±0.56
<i>Escherichia coli</i>	13.3±0.9	10.4±1.48	13.2±0.78	16.3±0.2	18.34±1.70
<i>Proteus vulgaris</i>	14.4±1.4	10.2±1.47	12.9±1.48	16.7±1.24	18.7±1.49
<i>Pseudomonas aeruginosa</i>	13.8±1.4	9.8±0.24	11.9±1.63	14.3±1.46	16.3±1.34

<sup>a</sup>The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.



**Fig 2:** The antibacterial activity of isolated compound of *Chrozophora rottleri* by minimum inhibitory concentration

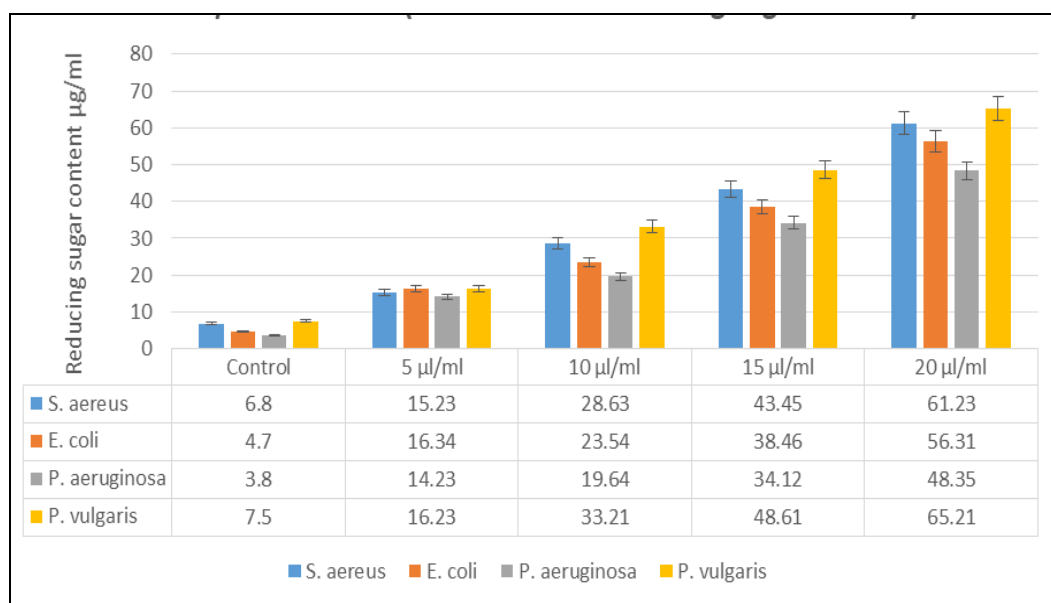
### Effect of Leakage Causing Agent In The Membrane Of Pathogenic Bacteria By Isolated Compound From The Leaves Of *Chrozophora Rottleri*

Several records have described that plant contained chemical based substance possess a membrane-active mechanism that reasons severe membrane destruction through the interruption of the membrane veracity (Bai *et al.*, 2015) [3]. Hence, membrane integrity was selected as another restriction to discover the antibacterial mechanism of isolated compound of *Chrozophora rottleri*. The present results mentioned that isolated compound of *Chrozophora rottleri* increased the proportion of cells in the fluorescent section, representative an increase in the number of membrane-damaged bacterial cells to *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgari*. The values ranged from 15.23 to 56.31 µg/mg of bacterial dry weight of

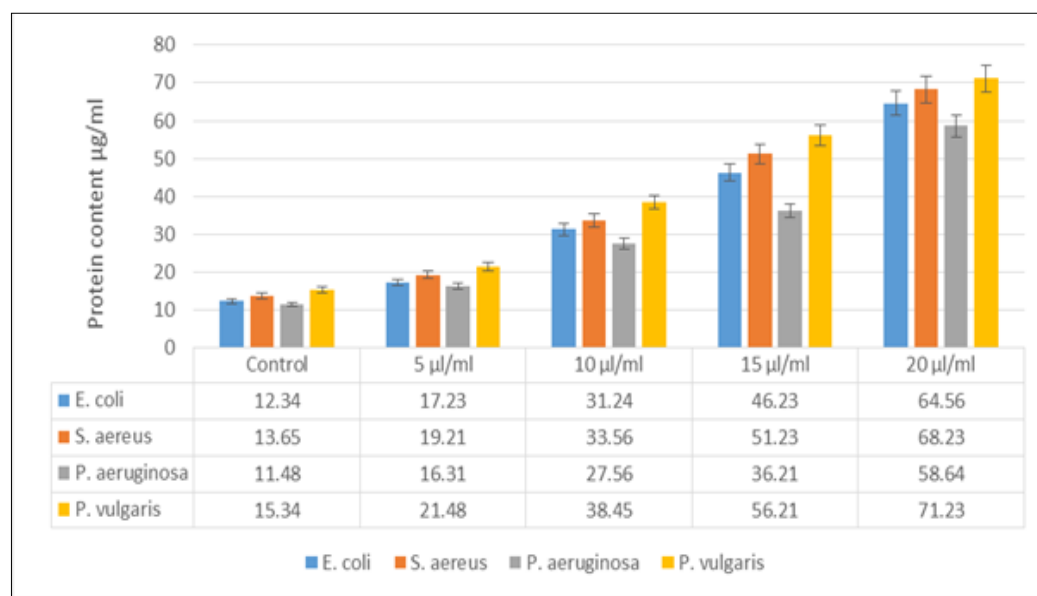
*Staphylococcus aureus*, 16.34 to 61.23 µg/mg in *Escherichia coli*, 14.23 to 52.34 µg/mg in *P. aeruginosa* and 16.32 to 47.43 µg/mg in *Proteus vulgaris* of isolated compound of *Chrozophora rottleri* treated cultures. After 18 hours of incubation/ treatment, the amount of reducing sugar was estimated to be higher than the initial values (Fig-4). Firstly the protein estimated was higher in the all bacterial cultures when compared with control. It sloping that the antibacterial isolated compound from *Chrozophora rottleri* were effective against the pathogen undeviating at the early period. When short-sighted after 18 hours of conduct the amount of protein estimated was much higher than the initial value. The amount of protein estimated at 18<sup>th</sup> hour ranged from 19.21 to 53.47 µg/mg of bacterial dry weight of *Staphylococcus aureus*, 18.65 to 61.23 µg/mg in *Escherichia coli*, 17.23 to 56.31 µg/mg in *P. aeruginosa* and

21.48 to 63.24  $\mu\text{g}/\text{mg}$  in *Proteus vulgaris* treated with isolated compound of *Chrozophora rottleri* treated cultures (Fig-5). This study compromised that the higher the

concentration of the antibacterial compounds and longer the duration of inhibition, the higher might be the leakage of membrane.



**Fig 3:** Effect of leakage causing agent in the membrane of pathogenic bacteria by isolated compound from the leaves of *Chrozophora rottleri* (After 18 Hours Reducing sugar content)

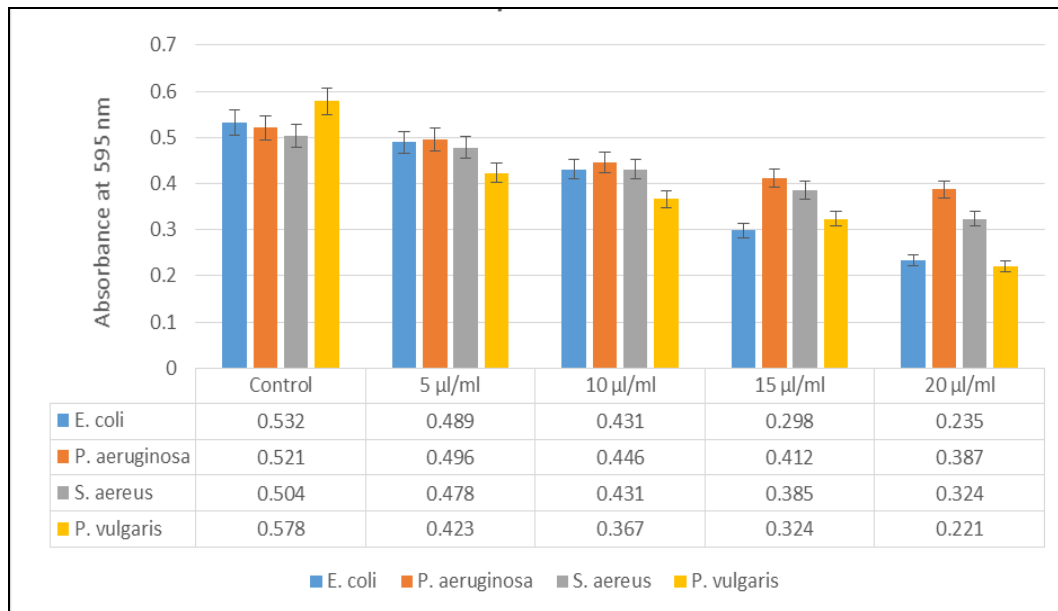


**Fig 4:** Effect of leakage causing agent in the membrane of pathogenic bacteria by isolated compound from the leaves of *Chrozophora foaled* (After 18 hours protien content)

#### Effect of Biofilm Inhibition Against Gram-Negative And Gram-Positive Bacteria After 24 H Isolated Compound From The Leaves Of *Chrozophora Rottleri*

Bacterial biofilm odds and ends a global threat to health due to from top to bottom refractoriness to handling and the capacity to exaggerate nosocomial infections. Hence, search for novel effective molecules to encounter this problem is an urgency. In this study, the activities of the secluded alkaloid compound from the leaves of *Chrozophora rottleri* were tested against the biofilms of the bacteria species were *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus* than *Pseudomonas aeruginosa*. This appears to be the first study to determine the antibiofilm activity of the selected isolated compound. The activities of isolated compound from *Chrozophora rottleri* with good anti-cell accessory

assets on the anticipation or decrease of extra biofilm development in 24 h preformed biofilms are presented in Graph- (Fig-6). Isolated compound of *Chrozophora rottleri* reduced biofilm biomass at 24 h post-development against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgari*. *Chrozophora rottleri* bioactive compound of had poor biofilm inhibiting activity of *P. aeruginosa*. Biofilm development is unique of the confrontation approaches of several pathogens which kinds them extra challenging to control than their planktonic complements (De La *et al.*, 2012). A biofilm is a compound medium of societies some bacterial collected of polysaccharides, proteins and other organic components mainly bind together with cell to form strong attachments to biotic or abiotic surfaces (Mm and Rohloff, 2016) [9].



**Fig 5:** Effect of biofilm inhibition against gram-negative and gram-positive bacteria after 24 h isolated compound from the leaves of *Chrozophora rotleri*

### Conclusion

Numerous of the plants had little harmfulness which kinds them good candidates for imaginable expansion into herbal products or for isolation of novel pure compounds that consider serve as patterns for novel antimicrobial drugs. Although we used bacterial strains suggested to determine the mode of antibacterial action of the isolated compound of *Chrozophora rotleri* and how they act on the different pathogens. To a sensible amount, our conclusions have providing credibility to the value of selected plant for antimicrobial examination on invitro basis. These outcomes may excite additional biological research on these and other under-explored plant species in the Eupharbiaceae family, especially those native to India.

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