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Evaluation of Antibacterial Properties of *Chrozophora rottleri* Leaves in Two Different Solvents.

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ABSTRACT

The antimicrobial activity of the methanol and benzene extract was tested against four pathogenic human microbes by the disc diffusion method. The methanol extract of the *Chrozophora rotleri* leaf showed a zone of inhibition of 15.0-19.0 mm at a concentration of 20 µl. *Klebsiella pneumoniae* proved to be very susceptible, forming a very high zone of inhibition which constitutes a strong barrier against this microorganism. This pathogen was susceptible to benzene extract, which produced an inhibitory zone of 14.0-17.0 mm, indicating that the methanolic extract of *Chrozophora rotleri* was more effective than benzene extract against most of the pathogens studied. A minimum inhibitory concentration (MIC) test was also performed to determine the antibacterial activity of the alkaloid extracts during the 18 hours of incubation. Methanol and benzene extracts of *Chrozophora rotleri* inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* to a greater extent than *Bacillus subtilis* and *Proteus vulgaris*.

Keywords: Antimicrobial activity, Minimum Inhibitory Concentration, *Chrozophora rottleri,* disc diffusion, MIC.

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INTRODUCTION

Natural products are defined as substances obtained from natural sources that have biological activity. Natural products are used as alternative medicine and in the development of modern medicine. One of the primary goals of natural product chemistry is drug design and discovery. Medicinal plants refer to a class of plants used for therapeutic or pharmacological activities in humans and animals. Morphologically, medicinal plants are no different from other plants, however, they differ in several plant properties that indicate their medicinal properties. The area of Indonesia is about 110 million hectares, where about 80% of the world's medicinal plants are grown. There are about 28,000 plant species in Indonesian forests. Of these, 7,000 are medicinal plants, representing 90% of Asian medicinal plants. Until now, 1000 species are known and used in traditional medicine [1].

Chrozophora rottleri is a member of the Suryavarti-related Euphorbiaceae family. In India, Myanmar, Thailand, the Andaman Islands, and Malaysia's Central Java region, the plant grows naturally. *C. rottleri*, an upright hairy annual common wasteland, blooms profusely from January to April. It is an upright herb with silvery hairs; the stem's upper section is hairy and has a thin tap-root. The lower part of the stem is hairless. The alternate, thick, and rugose three-lobed leaves are. The flowers are produced by monoecious plants and are borne in sessile auxiliary racemes with staminate flowers at the top and pistillate blooms at the bottom [2].

Local tribes and healers have historically utilized *Chrozophora rottleri* to cure a variety of illnesses. The entire plant or powdered stems are given to wounds in Sudan to speed up healing. An infusion of seeds and leaves is consumed in Ethiopia as a laxative. Additionally, Saudi Arabia, Pakistan, and India use this plant medicinally (for example, to treat jaundice and purify the blood). While sheep and goats occasionally consume the plant in Senegal because it induces vomiting and diarrhea, camels in Kenya can see it. In East Africa, carpets are dyed using a blue-violet dye made from fruits [3]. Fruit juice, which contains a laxative (seeds) and a cleanser (leaves) as well as physiologically active ingredients, is suggested in Nepal for coughs and colds [4-5]. The leaves are also used as a depurative and are quite helpful in treating skin conditions [6]. The seeds are thought to have purgative qualities [7] and are used as a cathartic like Ghodtapde [8]. According to Priyanka *et al* [9], this plant's leaves show substantial anti-helminthic properties against *Pheritima posthuma* (Indian Earthworm). This plant's aqueous extract had phytotoxic effects on rice, wheat, and mustard. The leaf extracts showed greater inhibition of shoot, root, and radial elongation than the stem and root in an experimental investigation by Suparna and Tapaswi [10].

MATERIALS AND METHODS

Plant material collection

The leaves of *Chrozophora rottleri* were collected from near the Kalaignar Karunanidhi Government Arts College, Tiruvannamalai, Tamilnadu. The plant authenticated identification was done by Dr. S. Sankaranarayanan, Head, Department of Medical Botany, Sairam Siddha Medical College, Tambaram. The voucher number was P.5125 (Fig. 1).

Figure 1: Habit of *Chrozophora rottleri*

Culture collection and maintenance

The bacterial strains *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis* and *Proteus vulgaris* were used for the experiment. The above strains were obtained from the Microbial Culture Type Collection and Gene Bank (MTCC), Institute of Microbiological Technology, Chandigarh, India. Stock cultures were maintained at 4°C on Mueller-Hinton agar.

Phytochemical screening of *Chrozophora rottleri* leaves

The aqueous extract of *Chrozophora rottleri* was freshly prepared and various chemical constituents were analyzed according to methods described by Harbone (1976) [11]. The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, polyphenols and flavonoids.

Preparation of extracts

Organic solvents (methanol and benzene) extract of the *Chrozophora rottleri* leaves were prepared according to the method described by Boaky- Yiadon [12] with little modifications.

The Partial Characterization of Thin Layer Chromatography in *Chrozophora rottleri* leaves

The *Chrozophora rottleri* leaves alkaloid and flavonoid extract were taken from the leaves and loaded onto pre-coated TLC (60 F2 54). It was developed using a solvent system in the ratio of 1:0.5:0.1 (Hexane, Chloroform, and Methanol), and it is fluorescent with UV light at 360 nm.

Antibacterial activity of *Chrozophora rottleri* leaves

The antibacterial activities of the crude alkaloid extracts were assayed using the disc diffusion method [13].

Minimum inhibitory concentrations (MICs) extract of *Chrozophora rottleri* leaves

The minimum inhibitory concentrations of the isolated compounds were determined by the dilution method [14].

Effect of antibacterial activity of *Chrozophora rottleri* leaves on leakage of the membrane in pathogenic bacteria

Different quantities of MH medium, antibacterial substance, and pathogenic bacteria cells were put into 10 ml cultures with a final concentration of 100 g/ml antibacterial compound and 109cfu/ml pathogenic bacteria to identify the leakage of reducing sugars and proteins via the membrane. [15-16].

Effect of *Chrozophora rottleri* on the respiratory chain dehydrogenase enzyme activity in pathogenic bacteria

The enzyme activity was determined by the Iodonitrotetrazolium Chloride method with slight modifications [17].

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous leaves extract of *Chrozophora rottleri*

The phytochemical analysis of the aqueous extract in *Chrozophora rottleri* studied showed the presence of alkaloids, flavonoids, polyphenols, saponins, glycosides, terpenoids and tannin (Table-1).

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

S.No.	Phytoconstituents	Leaf extract of *Chrozophora rottleri*
1.	**Alkaloids - Dragendroffs reagent** **- Mayer's test**	- -
2.	**Flavonoids - Alkali test** **- Leadacetate test**	+ +
3.	**Polyphenols - Ferrozine test**	+
4.	**Terpenoids - Salkowski test**	+
5.	**Saponins - Froth test**	-
6.	**Tannin - $Fecl_3$ test**	+
7.	**Glycosides - Keller-Kilani test**	-

The Partial characterization of methanol and benzene extract of *Chrozophora rottleri* by TLC

The methanol and benzene extract of *Chrozophora rottleri* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of hexane, chloroform and methanol in the ratio of 1:0.5:0.1 for alkaloid. The developed plate was viewed under UV 240nm and 360nm (Table-2 and Fig-2& 3).

Table 2: Partial characterization of Methanol and Benzene extract from the *Chrozophora rottleri* leaf by TLC

S.No.	**Methanol and Benzene extract of *Chrozophora rottleri* leaf**		
	UV 240 nm Rf value	UV 360 nm Rf value	Visible Rf value
Methanol extract			
1.	0.27	-	-
2.	0.59	-	-
3.	0.76	0.76	-
4.	-	0.88	-
5.	-	0.91	-
Benzene extract			
1.	0.36	-	-
2.	0.41	-	-
3.	0.53	0.53	-
4.	0.79	0.79	-

Figure 2: Partial characterization of Methanol extract from the *Chrozophora rottleri* leaf by TLC

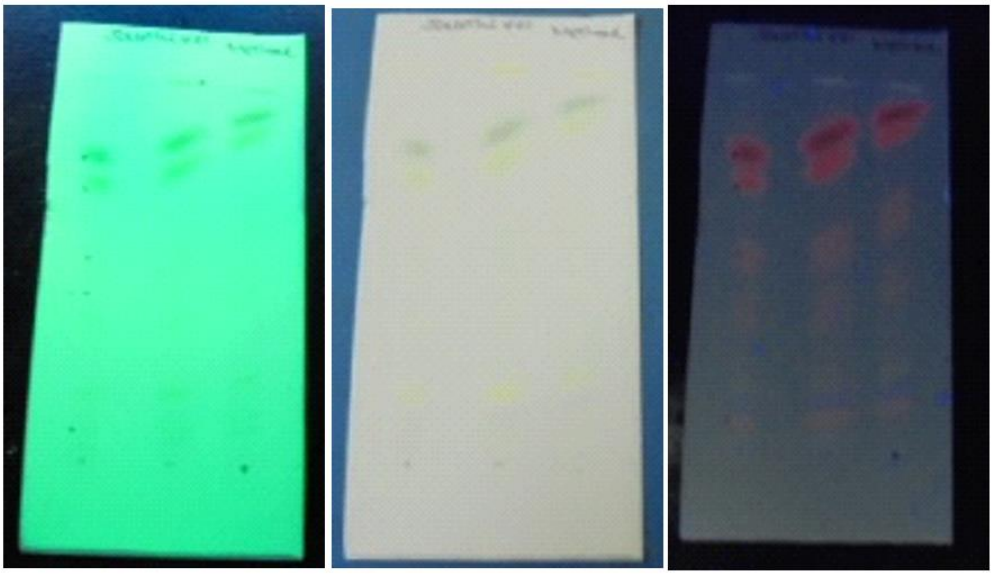

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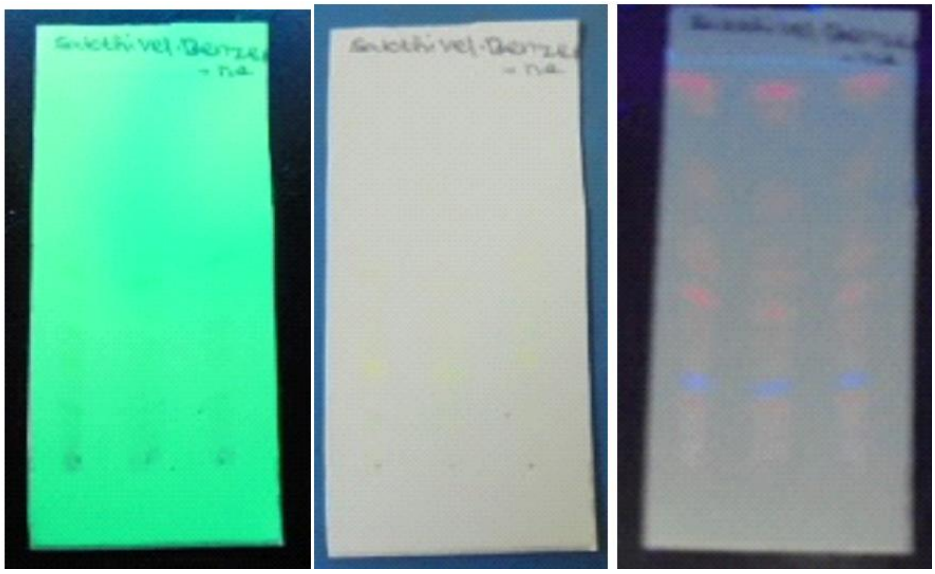
Figure 3: Partial characterization of Benzene extract from the *Chrozophora rottleri* leaf by TLC



Effect of methanol and benzene extract from the leaf of *Chrozophora rottleri* on the growth of Pathogenic bacteria by disc diffusion method

The methanol and benzene extract of *Chrozophora rottleri* at different concentrations (5, 10, 15 and 20µl/ml) was tested against *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonia* and *Bacillus subtilis.* The methanol and benzene extract of *Chrozophora rottleri* exhibited more bactericidal action against *Staphylococcus aureus* and *Klebsiella pneumonia* than *Proteus vulgaris* and *Bacillus subtilis* with a higher inhibition zone was found at 20µl/ml concentration (Table-3 and Fig-4, 5). Three factors may account for the limited range of antimicrobial activity in the Benzene extract: first, antibacterial compounds' polarity makes them easier to extract using organic solvents than methanol; second, the active compound may not be present in sufficient amounts in the crude extract to demonstrate activity at the dose level used; and third, even if the active principle is present in large amounts, there may be other constituents in the extract [18].

Table 3: The antibacterial activity of the methanol and benzene extract from the leaf of *Chrozophora rottleri* by disc diffusion method

Pathogenic bacteria	Methanol extract exhibited the Zone of inhibition (mm)[a]				
	Positive control 10µl Ampicillin	Different concentrations of Crude extract (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	15.0	10.0	13.0	17.0	18.0
Klebsiella pneumonia	13.0	11.0	14.0	16.0	19.0
Proteus vulgaris	14.0	9.0	11.0	14.0	15.0
Bacillus subtilis	16.0	10.0	12.0	15.0	17.0
	Benzene extract exhibited the Zone of inhibition (mm)[a]				
Staphylococcus aureus	15.0	9.0	11.0	14.0	16.0
Klebsiella pneumonia	13.0	8.0	12.0	14.0	17.0
Proteus vulgaris	14.0	8.0	10.0	12.0	15.0
Bacillus subtilis	16.0	7.0	10.0	12.0	14.0

[a]The inhibitory diameter was measured using calipers. All the assays were duplicated, and the mean values were recorded.

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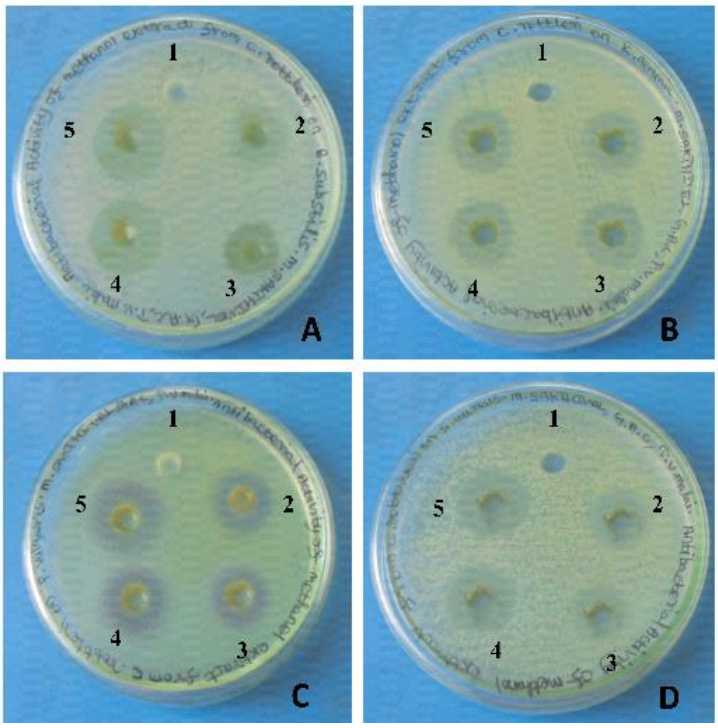
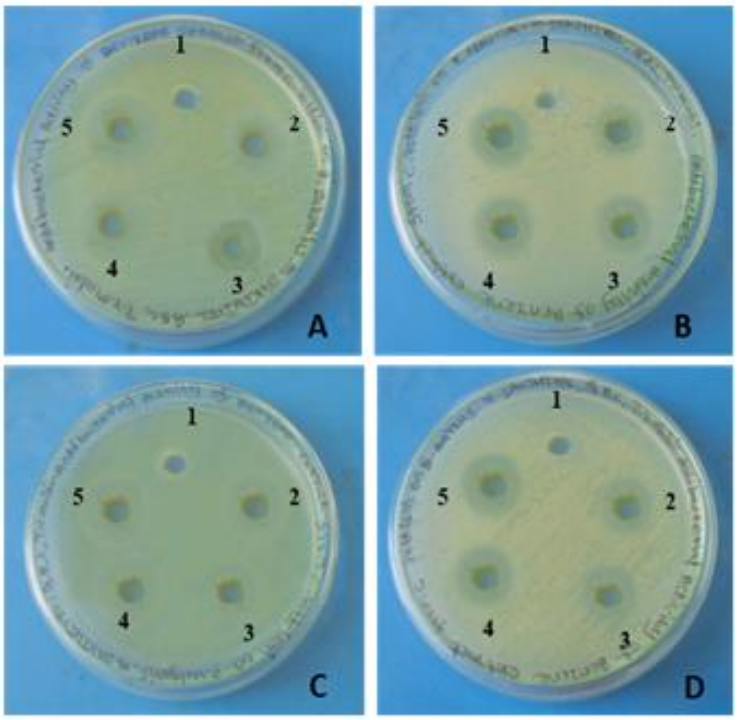
Figure 4: Antibacterial activity of the methanol extract of *Chrozophora rottleri* by disc diffusion method



A - *Bacillus subtilis,* B - *Klebsiella pneumonia,* C - *Proteus vulgaris,* D - *Staphylococcus aureus*
1 - Control; 2 - 5 µl; 3 - 10 µl; 4 - 15 µl; 5 - 20 µl

Figure 5: Antibacterial activity of the benzene extract of *Chrozophora rottleri* by disc diffusion method

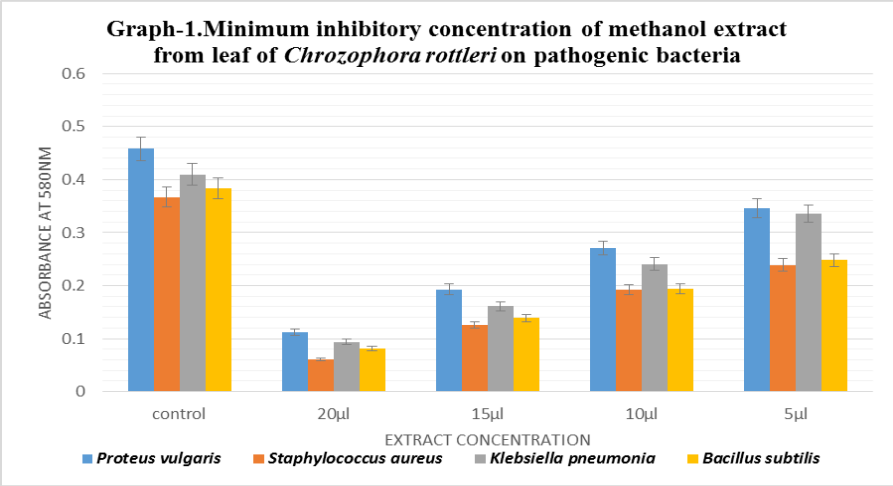


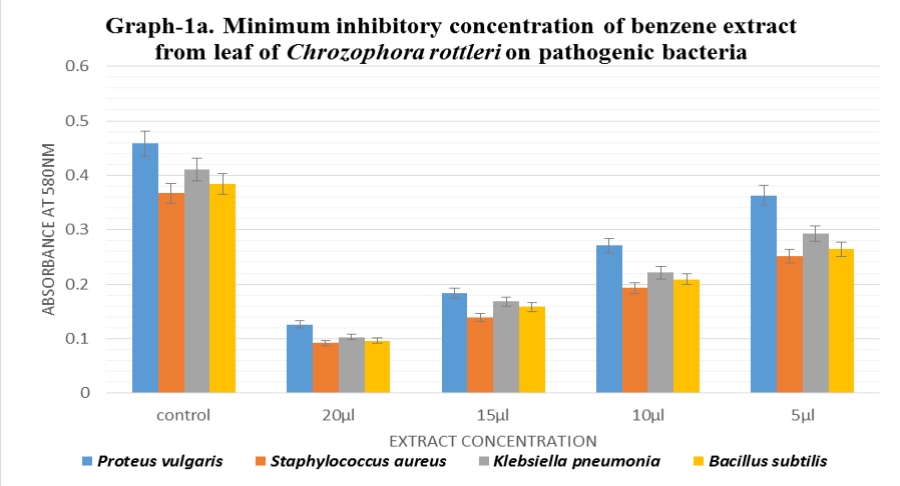
A - *Bacillus subtilis,* B - *Klebsiella pneumonia,* C - *Proteus vulgaris,* D - *Staphylococcus aureus*
1 - Control; 2 - 5 µl; 3 - 10 µl; 4 - 15 µl; 5 - 20 µl

ISSN: 0975-8585

Effect of methanol and benzene extract from the leaf of *Chrozophora rottleri* on the growth of antibacterial activity by Minimum inhibitory concentration.

Minimum Inhibitory Concentration (MIC) assays were also performed to determine the antibacterial activities of alkaloid extract at 18 hours of incubation. *Chrozophora rottleri* methanol and benzene extract inhibited the growth of *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonia* and *Bacillus subtilis* at a minimum concentration of 5µl/ml. *Chrozophora rottleri* methanol and benzene extract inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumonia* more than *Bacillus subtilis* and *Proteus vulgaris* (Graph- 1&1a).





Effect of methanol and benzene extract from leaves of *Chrozophora rottleri* on leakage of the membrane of pathogenic bacteria.

The leakage of the membrane was noticed by estimating the reducing sugar in the bacterial cultures treated with *Chrozophora rottleri* methanol and benzene extract. The values ranged from 23.17 to 87.91µg/mg and 23.17 to 83.96µg/mg of bacterial dry weight of *Staphylococcus aureus,* 20.43 to 81.36µg/mg and 20.43 to 79.16µg/mg in *Klebsiella pneumonia,* 21.23 to 84.66 µg/mg and 21.23 to 76.99 µg/mg and 18.98 to 76.99µg/mg in *Bacillus subtilis* and 18.98 to 77.48µg/mg in *Proteus vulgaris* of *Chrozophora rottleri* methanol and benzene treated of pathogenic bacteria cultures (Graph-2 & 3).

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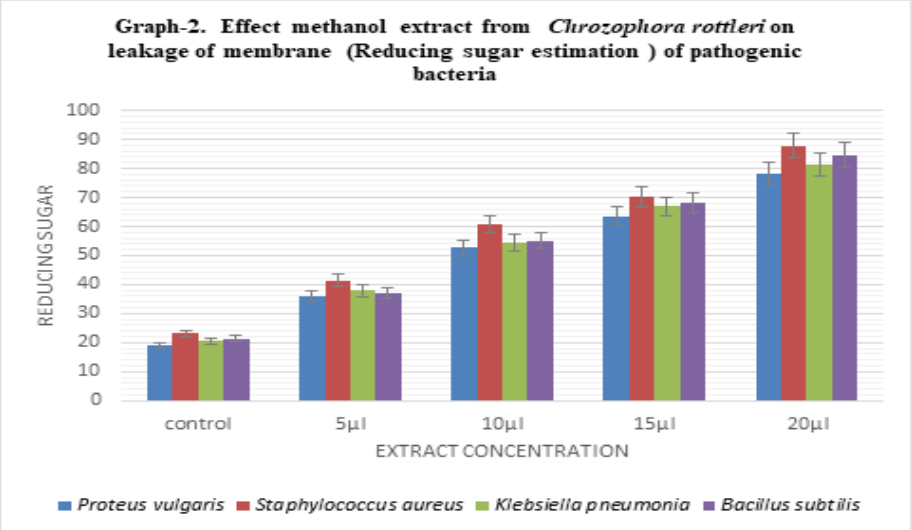
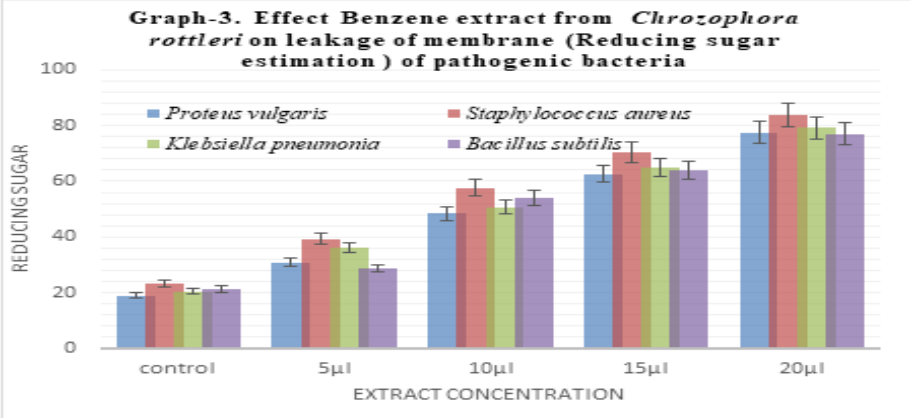
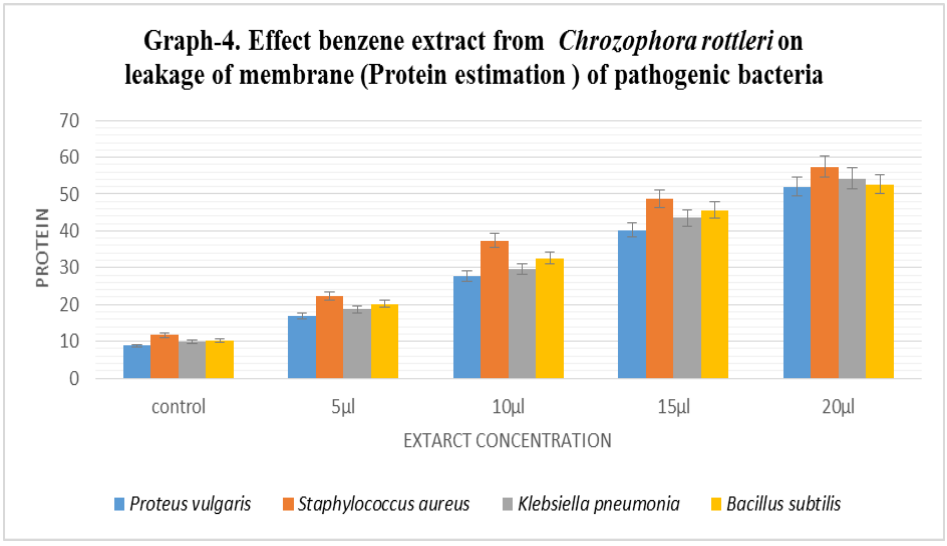




The amount of protein in the methanol and benzene fraction of *Chrozophora rottleri* treated in both cultures was estimated and the OD value was referred to with a standard graph of BSA. The estimation of protein yielded a greater value than the control, indicating that *Chrozophora rottleri* methanol and benzene extract was effective against the pathogen even in its early stages. The amount of protein estimated at the 18th hour ranged from 11.67 to 60.34µg/mg and 11.67 to 57.34 µg/mg in *Staphylococcus aureus*, 9.92 to 57.76µg/mg and 9.92 to 54.29 µg/mg in *Klebsiella pneumonia,*10.31 to 61.45 and 10.31 to 52.67 in *Bacillus subtilis* and 8.76 to 55.12µg/mg and 8.76 to 51.98µg/mg in *Proteus vulgaris* of *Chrozophora rottleri* methanol and benzene extract treated cultures (Graph-4 & 5).



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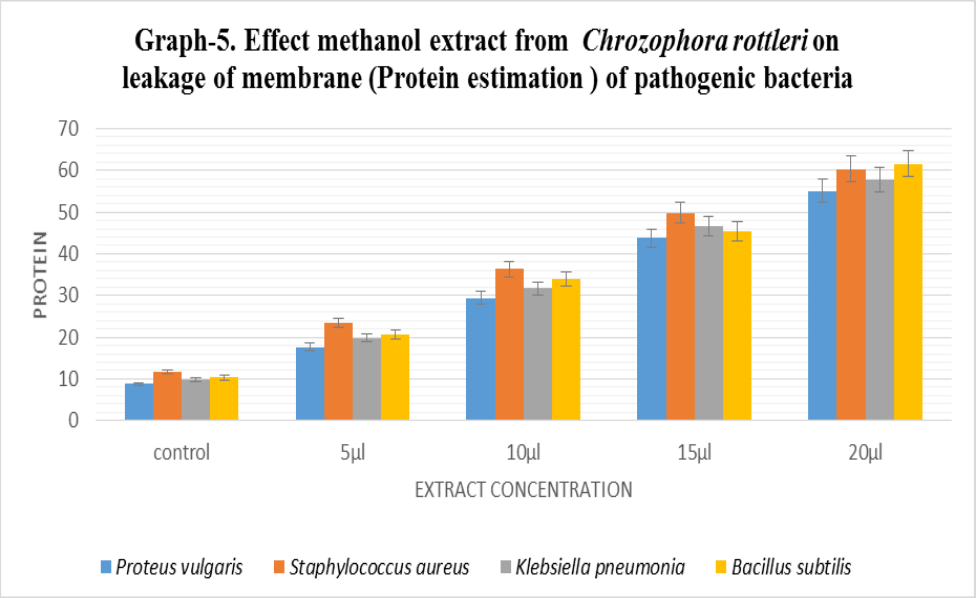
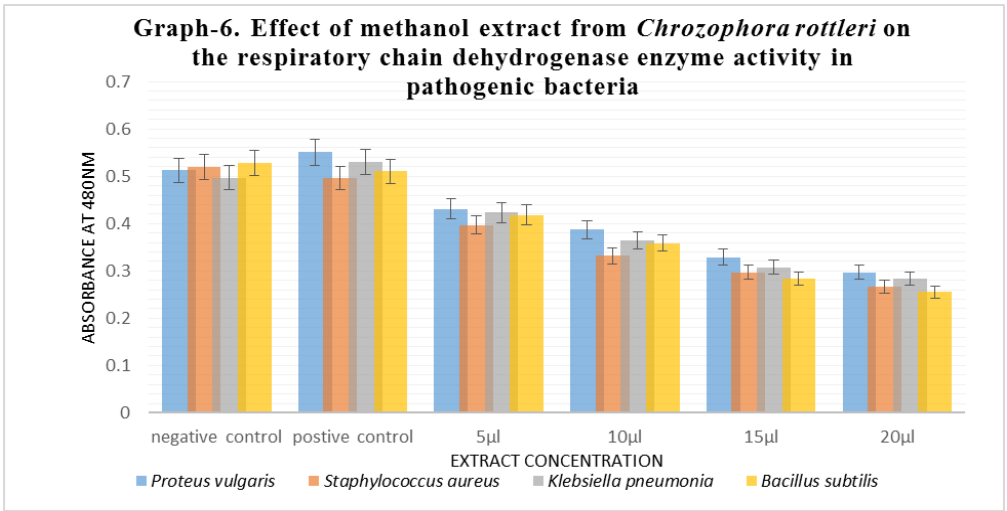


Effect of methanol and benzene extract from *Chrozophora rottleri* on the respiratory chain dehydrogenase enzyme activity in pathogenic bacteria

The effect of methanol and benzene extract from *Chrozophora rottleri* leaves on respiratory chain dehydrogenases of the four bacteria where shown in Graph-6 &6a. The activity of the enzyme in all three bacteria increased in the positive control with increasing time, but there was no change in the negative control. Initially, the enzymatic activity of the cell treated with 5µl/ml of methanol and benzene was even higher than the positive control, but the activity fell with increasing incubation time. The activity of the enzyme decreased with increasing concentration of methanol and benzene extract. After being treated for 30 minutes the enzymatic activity was almost feeble. The result revealed that the activity of respiratory chain dehydrogenases of the bacterial pathogen would be inhibited by the methanol and benzene of *Chrozophora rottleri*. It also explained that the higher the concentrations of methanol extract the lower the activity of the enzyme.

Wen *et al*.'s work [19] on the antibacterial activity and mechanism of silver nanoparticles on *E. coli* provided support for the finding. Additionally, the research supported Holt and Bard's [20] discovery that Ag+ decreased *E. coli* respiration by monitoring changes in the amount of oxygen dissolved in the culture fluid.



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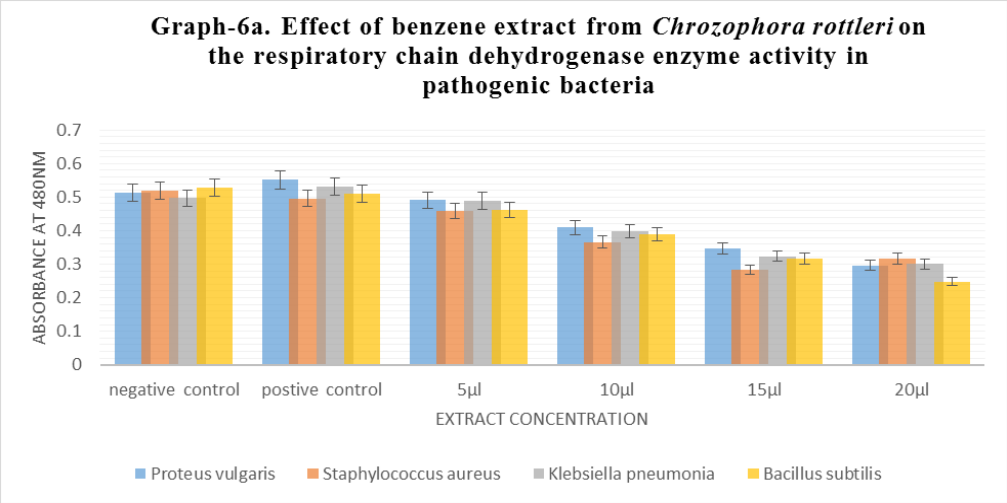

CONCLUSIONS

The results of the current study allow us to draw the conclusion that all of the *Chrozophora rottleri* extracts examined had good efficacy against both Gram-positive and Gram-negative pathogens, with Gram-positive bacteria being more inhibited than Gram-negative bacteria. This likely explains why the native people have used this plant for many generations to treat various illnesses. In-depth research is required, including in vivo studies on this plant to determine the toxicity of the active constituents, their side effects, and pharmacokinetics properties to exploit the bioactive principles, for therapeutic utility in treating ear infections. As of now, little has been done on the antimicrobial activity and plausible medicinal applications of the phytochemical compounds. If the active ingredients are purified and the right dosage is established for delivery, the antibacterial activity can be improved. The development of an efficient phytocompound into a marketable herbal product that has no side effects and no issue with medication resistance is finally what is required at this point.

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