Research Article

In vitro Assessment of Antibacterial Potential of *Chrozophora rottleri* Fruit Different Extracts against Common Pathogens

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Submission Date: 22-09-2024; Revision Date: 08-10-2024; Accepted Date: 24-11-2024.

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ABSTRACT

Aim: To assess the antibacterial properties of *Chrozophora rottleri* fruit extracts against three common pathogenic bacteria, *Bacillus subtilis, Enterococcus faecalis* and *Serratia marcescens*. **Materials and Methods:** *Chrozophora rottleri* fruit was extracted using petroleum ether, chloroform and ethanol solvents. Minimum Inhibitory Concentration (MIC) experiment was done to identify the minimum inhibitory concentration of each extract against bacterial growth. Zone of Inhibition (ZOI) experiment was done to evaluate the clear area around each extract on agar plates, suggesting its antibacterial activity. **Results:** All samples exhibited antibacterial activity against a minimum of one bacterial strain. Ethanolic extract displayed the maximum antibacterial activity with MIC values ranging from 0.05-50 µg/mL and ZOI values reaching 18.66 mm. Chloroform extract demonstrated modest efficacy against certain bacteria, with MIC values up to 50 µg/mL and ZOI values up to 7.33 mm. Petroleum ether extract demonstrated the least effectiveness, with no inhibition against E. faecalis and little action against other strains. **Conclusion:** The ethanolic extract of *Chrozophora rottleri* fruit has demonstrated considerable antibacterial activities against several pathogenic bacteria, demonstrating its potential as a substitute or additional therapy to traditional antibiotics. Further study is needed to discover the particular bioactive components.

Keywords: Antibacterial activity, Antibiotic resistance, *Chrozophora rottleri*, Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI).

INTRODUCTION

Antibiotic resistance is a serious worldwide health issue.[1] Misuse of antibiotics promotes resistance, making treatments less effective.[2] According to world health organization's antimicrobial resistance poses an increasing danger to worldwide public health, undermines antibiotic effectiveness and increases morbidity and mortality.[3] Antimicrobial resistance caused 4.95 million global fatalities in 2019, with projections of 10 million deaths by 2050.[4] Antibiotic-resistant medical therapies face hurdles from both gram-positive and gram-negative bacteria, including members of the ESKAPE group.[5,6] Antimicrobial Resistance (AMR) affects cancer therapy since patients frequently require extended antibiotic regimens, which increases their vulnerability to infections. Addressing AMR in cancer care is critical for ensuring successful therapy and reducing the risk of fatal bloodstream infections.[7] To combat medicine resistance, antibiotics are frequently administered at high dosages, resulting in adverse effects such as ototoxicity and nephrotoxicity. Economic difficulties and legislation have slowed the development of new antibiotics, new antibiotics confront challenges such as lengthy RandD cycles and careful usage to prevent resistance.[8,9] Antibiotic resistance in bacteria is a growing concern, prompting the search for alternative antibacterial agents from natural sources. According

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DOI: 10.5530/ajbls.2024.13.75

to the WHO, 80% of the developing world uses traditional medicines derived from medicinal plants, with over 20,000 species identified as possible sources. Over 100 nations control medicinal plants and more than 1,340 species have antibacterial properties, with 30,000 chemicals identified, such as spermidine, rutin, quercetin, tocopherol, carotenoids, polyphenols and alkaloids, which have antimicrobial, antioxidant, anti-inflammatory and antiviral properties.[10]

Previously, different medicinal plants were studied, such as bearberry, cranberry juice, lemon balm, garlic and tea tree, which have potential antimicrobial agents for urinary tract infections and skin infections.[11] Cameroonian plants demonstrate considerable antibacterial effects.[12] Plant extracts like *Myrtus communis, Verbena officinalis*, carrot seed oil and tea tree oil have antibacterial properties, offering potential treatments for bacterial infections.[10]

Chrozophora rottleri A. Juss, also known as Suryavarti, is a blooming plant that comes from the *Euphorbiaceae* family and has high therapeutic potential. The species is abundantly spread in Malaysia, India andaman, Thailand and Myanmar.[13,14] The plant is known for its caustic, toxic, emetic, cathartic and emetic properties, aiding in wound healing in Sudan, treating jaundice in Saudi Arabia and India and purifying blood in India, while seeds and leaves are used as laxatives in Ethiopia and Senegal.[15] *Chrozophora* rattleri (*C. rottleri*) is a plant with a variety of phytochemicals, including alkaloids, sugars, glycosides, tannins, steroids, flavonoids and saponins, which contribute to its medicinal effects. Notable components include quercetin 3-o-rutinoside, acacetin 7-o-rutinoside and chrozophorin. The seed oil contains linoleate, enhancing its nutritional and therapeutic value. Roots and leaves contain xanthone and chromone glycosides, enhancing their chemical profile and potential health benefits.[16,17] *C. rottleri*, recognized for its powerful antioxidant capabilities, efficiently combats oxidative stress by neutralizing free radicals.[12,18] Its anti-inflammatory qualities give relief from inflammatory illnesses.[19] Additionally, research reveals its extraordinary antibacterial powers against numerous pathogens and its promise in treating parasitic worm diseases.[20,21] Moreover, studies stress its anti-mutagenic characteristics, avoiding DNA damage and mutations.[14,22] Displaying the many therapeutic effects of *C. rottleri*.

This study intends to evaluate the antibacterial properties of *C. rottleri* fruit extracts against the major human pathogens Bacillus subtilis, Enterococcus faecalis, and *Serratia* marcescens. It employs Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) methodologies to assess the antibacterial activity of petroleum extract, chloroform extract and ethanolic extracts of *C. rottleri* fruit, offering new insights for a full understanding.

MATERIALS AND METHODS

Plant collection and extraction

The disease-free *C. rottleri* plant was collected from Seernakatte, in a Chitradurga wasteland. The plant was identified by taxonomist Dr. V. Krishna in July 2021. Both fruits and stems underwent drying, crushing and soxhlet extraction. The powdered *C. rottleri* in different parts, approximately 50 g, was employed for crude extracts prepared utilizing solvents of decreasing polarity, such as petroleum ether (pet-ether), chloroform and ethanol, using a soxhlet apparatus. The method involves cycling the solvent through the sample several times, with each solvent cycled for 12 cycles. After that, the solvent was recovered from the rotary evaporator. The resulting crude dried extracts from various solvents were collected in sterile petri plates, labeled and preserved at the desiccator for further use.[22]

Test Bacterial strains

Three therapeutically relevant bacterial strains of *Bacillus subtilis* (*B. subtilis*, MTCC 1133), Enterococcus faecalis (E. faecalis, MTCC 439) and *Serratia marcescens* (*S. marcescens*, MTCC 86) were used in this study. E. faecalis is a facultative anaerobic gram-positive bacterium that is a major pathogen found in humans and animals gastrointestinal tracts, soil, water and food products. Although harmless in the gut, it can cause infections, especially in those with compromised immune systems or underlying health issues.[23] *B. subtilis* is a rod-shaped, gram-positive and less pathogenic to humans and animals, reliable probiotic strain with high antibacterial activity and thermal stability, producing bacteriocin protein, a potential alternative to antimicrobial drugs.[24] *S. marcescens* is a rod-shaped, gram-negative bacterial component of the Entero bacteriaceae family. *S. marcescens* is commonly found in soil, water and in various environments, including hospitals. It can also be an opportunistic pathogen, causing infections like urinary tract, respiratory, wound and bloodstream infections. In healthcare, it can cause outbreaks in intensive care units and immune-compromised patients.[25]

IN VITRO ANTIBACTERIAL ACTIVITIES

Minimum Inhibitory Concentration (MIC) Assay

The MIC of *C. rottleri* fruit extracts was determined using the broth microdilution method.[26] 0.5 Mcfarland

Standard dilution of microbes to be used for the study. 100 µL diluted log cultures of bacteria *B. subtilis, E. faecalis* and *S. marcescens* were added to the micro centrifuge tube and 5 µL of prepared treatment dilutions of different concentrations (0 to 1000 µg) were added to the defined tubes and incubated for 24 hr. After incubation, the content was transferred to the 96-well plates and turbidity readings were taken by the Elisa Plate Reader (iMarkBiorad) at 630 nm. Ciprofloxacin (10 µg) was used as a positive control. These were performed in triplicate and given as mean±SD.

Zone of Inhibition (ZOI) Assay

The Kirby-Bauer method was used to determine the ZOI of *C. rottleri* fruit extracts.[27] Mueller-Hinton Agar (MHA) plates were inoculated with bacterial cultures (100 µL) of *S. marcescens, B. subtilis* and *E. faecalis* and discs containing 0 to 1000 mg/mL concentrations were placed. A vehicle control was loaded with solvent alone, while a Ciprofloxacin disc (50 µg) was used as a positive control. Bacterial plates were cultured at 37°C for 24 hr and the extract's inhibition zones against each test organism were measured using a calliper. The data was presented as the mean±standard deviation of three replicates.

Statistical analysis

The experimental data, including mean and standard deviation, were analyzed using GraphPad Prism version 8.

RESULTS

Minimum Inhibitory Concentration

Based on the study, the efficacy of various samples against different test organisms was evaluated. When exposed to E. faecalis, the sample of chloroform extract demonstrated a Minimum Inhibitory Concentration (MIC) of 100 µg/mL, while the ethanol extract exhibited a lower MIC of 0.5 µg/mL. However, neither of the extracts showed any significant activity against E. faecalis. Conversely, B. subtilis was inhibited by all samples, with chloroform, pet ether and ethanol displaying an MIC of approximately 50 µg/mL. Similarly, *S. marcescens* was susceptible to chloroform, pet ether and ethanolic extracts, while ehanolic extracts exhibiting the highest activity with an MIC of approximately 0.05 µg/mL, followed by pet-ether with an MIC of approximately 1 µg/mL and chloroform with 50 µg/mL shown in Table 1. In each case the MIC was determined by experiments analyzing the concentrations inhibiting at least 20% of bacterial growth. These findings suggest that ethanolic extracts generally possess greater antimicrobial activity compared to chloroform and pet ether extracts against the tested organisms displayed in Figure 1.

Zone of Inhibition

Based on the results obtained it is observed that different samples exhibited varying degrees of antibacterial activity against the respective test organisms when treated with different concentrations on agar plates. For instance, in the case of E. faecalis, sample pet ether extract displayed a maximum inhibition zone of 6.08 mm at a dose of 1000 µg, while chloroform extract showed a maximum inhibition zone of 7.33 mm at the same dose. Notably, sample ethanolic extract showed the highest activity against E. faecalis, with a maximum zone of inhibition of 18.66 mm observed at a dose of 125 µg, surpassing even the positive control, which exhibited a maximum zone of inhibition of 28.33 mm at a dose of 50 µg. Similarly, against *S. marcescens*, sample pet-ether and ethanolic both extracts displayed antibacterial activity, with maximum zones of inhibition of 7.33 mm and 16.33 mm, respectively, at a dose of 1000 µg, while chloroform showed no activity compared to the positive control, which exhibited a maximum zone of inhibition of 35.33 mm at a dose of 50 µg. Furthermore, against *B. subtilis*, sample pet ether, ethanol and chloroform extracts demonstrated antibacterial activity with maximal zones of inhibition of 15.3 µg/mL, 16.3 µg/mL and 16.6 µg/mL, respectively, at various doses, compared to the positive control, which exhibited a maximum zone of inhibition of 32 mm at a dose of 50 µg displayed in Table 2. These findings highlight the effectiveness of the samples in inhibiting bacterial growth, as evidenced by the zones of inhibition observed on the agar plates shown in Figure 2.

Table 1: MIC values of *C. rottleri* fruit different extracts tested against microorganisms.

Bacteria	Extracts	MIC (µg/mL)
B. subtilis	Pet-ether	50
	Chloroform	50
	Ethanolic	50
E. faecalis	Pet-ether	N/A
	Chloroform	100
	Ethanolic	0.05
S. marcescens	Pet-ether	1
	Chloroform	50
	Ethanolic	0.05

Note: Mean±SD (n=3).

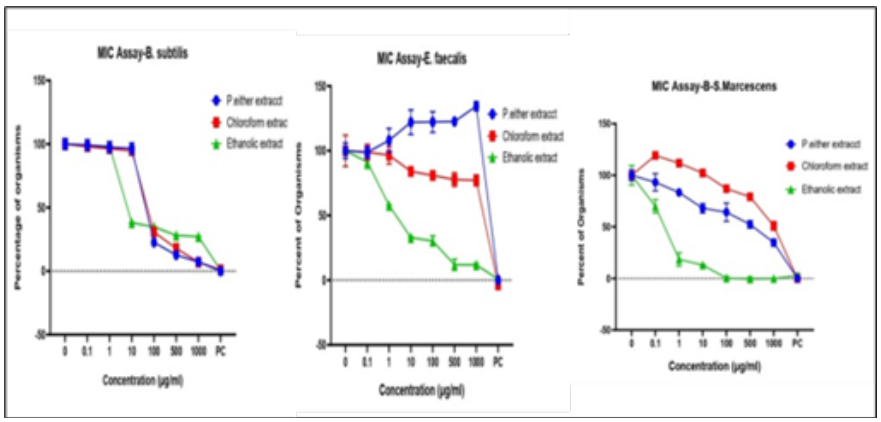

Figure 1: Effects of *C. rottleri* different extracts against bacterial strains.

Table 2: Antibacterial activity of *C. rottleri* different extracts ZOI values.

Bacteria	Extracts	ZOI (mm)
B. subtilis	Pet-ether	15.3 at 1000 µg dose.
	Chloroform	16.6 at 50 µg dose.
	Ethanolic	16.3 at 1000 µg dose.
	Ciprofloxacin	32.0 at 50 µg dose.
E. faecalis	Pet-ether	7.33 at 1000 µg dose.
	Chloroform	6.08 at 1000µg dose.
	Ethanolic	18.66 at 125 µg dose.
	Ciprofloxacin	28.33 at 50 µg dose.
S. marcescens	Pet-ether	7.33 at 1000 µg dose.
	Chloroform	N/A
	Ethanolic	16.33 at 1000 µg dose.
	Ciprofloxacin	35.33 at 50 µg dose.

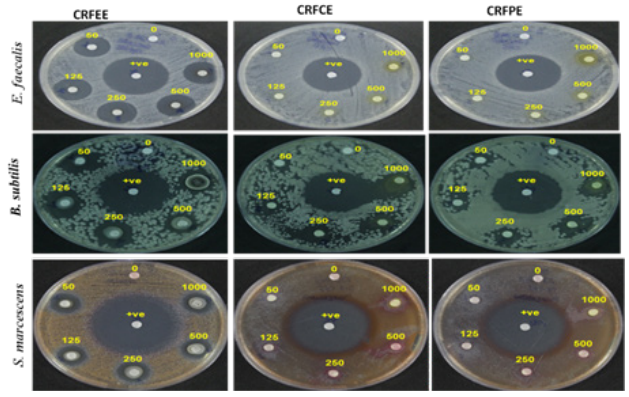

Figure 2: *C. rottleri* different extracts ZOI against pathogens.

DISCUSSION

Nowadays plant extracts, functioning as antimicrobials, provide eco-friendly alternatives to synthetic medications. By using natural molecules, they successfully manage infections, reducing dependency on synthetics and increasing sustainability in healthcare.[28] The absence of adverse effects in natural drugs, unlike their synthetic counterparts, stands as a fundamental reason for their rising demand as control agents. However, as combinations of numerous chemicals with distinct modes of action, natural drugs may contribute to resistance development, as revealed in research.[29,30] The ethanolic extract of neem leaf has been found to exhibit antibacterial properties.[31] The essential oil extracted from lemongrass leaves (*Cymbopogon*) has also shown considerable antibacterial activity against numerous microbes.[32] Traditional Indian medicine has used crude extracts from herbs like cinnamon, garlic, basil, curry, ginger, sage and mustard for centuries to treat and prevent infections. These plants have antibacterial properties against various bacteria, making them promising alternatives to conventional antibiotics and reducing antimicrobial resistance risks.[33] Plants include natural compounds, including phenolics, terpenoids and alkaloids, that may kill or weaken microorganisms and possess antimicrobial properties by disrupting microbial membranes, inhibiting cellular metabolism and controlling biofilm formation. These compounds are under investigation as Resistance-Modifying Agents (RMAs), potentially enhancing the therapeutic effects of antibiotics.[34] In this research, the antibacterial activity of *Chrozophora rottleri* extracts was investigated against *Bacillus subtilis, Enterococcus faecalis* and *Serratia marcescens*, due to their link with various ailments as revealed by previous studies.[23–25] The ethanolic extract showed the highest activity, notably against *E. faecalis* (18.66 mm ZOI at 125 µg) and *S. marcescens* (16.33 mm ZOI at 1000 µg), whereas the chloroform extract demonstrated the maximum action against *B. subtilis* (16.6 mm ZOI at 50 µg). Pet-ether extract revealed minimal antibacterial activity across all tested microorganisms. Standard Ciprofloxacin, the synthetic antibiotic, consistently displayed the highest

zones of inhibition, particularly 32.0 mm against *B. subtilis* at 50 µg and 35.33 mm against *S. marcescens* at 50 µg. While the ethanolic extract demonstrated promise action, ciprofloxacin was significantly more effective, indicating the potential but limited effectiveness of natural extracts compared to synthetic antibiotics. These findings are compatible with the results of previously reported a greater antibacterial action of *Chrozophora tinctoria* extract against bacteria such *Bacillus subtilis, Micrococcus leuteus, Staphylococcus aureus*. Additionally, it has been established that *Chrozophora rottleri* leaves displayed antibacterial effect against *Aeromona shydrophila, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes*. These studies exhibited comparable inhibitory effects, indicating the potential of *Chrozophora rottleri* extracts as an efficient natural antibacterial agent against the pathogens.[20,35]

Furthermore, the Minimum Inhibitory Concentration (MIC) values of *Chrozophora rottleri* fruit extracts differed across the tested microorganisms. For *Bacillus subtilis*, all extracts (pet-ether, chloroform, ethanolic) had a MIC of 50 µg/mL, exhibiting similar antibacterial activity. Against *Enterococcus faecalis*, the ethanolic extract was the most potent with a MIC of 0.05 µg/mL, while the chloroform extract had a MIC of 100 µg/mL and the pet-ether extract showed no effect. For *Serratia marcescens*, the ethanolic extract again had the lowest MIC (0.05 µg/mL), followed by the pet-ether (1 µg/mL) and chloroform (50 µg/mL) extracts. The ethanolic extract consistently demonstrated the maximum antibacterial efficacy.

However, in contrast to our results, previously reported that *chrozophora* distinct extracts exhibited similar activity against both Gram-positive and Gram-negative bacteria.[20] This discrepancy may be due to differences in the extraction methodologies or variations in the phytochemical composition of *Chrozophora rottleri* across geographical regions, as suggested by.[36,37] The robust activity against pathogens suggests that *Chrozophora rottleri* extract may be abundant in active compounds like Apigenin- 7-O-methyl ether and Narigenin, which have been documented for their antibacterial properties. These compounds might interact with bacterial cell walls, leading to cell lysis or inhibition of essential bacterial enzymes, as suggested by previous research.[17,38,39]

The findings of this study suggest that *Chrozophora rottleri* fruit ethanol extract holds promise as a natural antibacterial agent, particularly against Gram-positive bacteria. Further research is vital to isolate and characterize the bioactive compounds responsible for this activity. Additionally, investigating various extraction methods and varying concentrations of the extract might optimize its antibacterial potential, particularly against Gram-negative bacteria. It would be advantageous to conduct *in vivo* investigations to assess the therapeutic potential of *Chrozophora rottleri* extract in treating bacterial infections. Furthermore, research against a wider range of bacterial species would provide more comprehensive data on its antibacterial spectrum.

CONCLUSION

The study reveals the powerful antibacterial activities of *Chrozophora rottleri* fruit extracts, notably the ethanolic extract, against a range of bacterial pathogens. These findings, as evidenced by *in vitro* experiments, confirm the plant's traditional therapeutic usage and underline its potential as a source of new antibacterial drugs. The approach of plant-derived alternatives offers a sustainable method for combating diseases while lowering dependency on synthetic pharmaceuticals, harmonizing with the global push for eco-friendly healthcare solutions. Nevertheless, the study highlights the possibility of resistance development inherent in natural medications, encouraging continued care and research into this field. Moving forward, further research is needed to validate the efficacy, safety and mechanism of action of *C. rottleri* extracts, identify bioactive compounds and develop targeted therapies for antibiotic resistance.

AUTHOR CONTRIBUTIONS

The experimental design and research were conducted by Yadaladaku Rajanna Nagesh. Data analysis, article preparation, were done by Vinaykumar Nagenahalli Manjunathand and Ravishankara Burladinni and Ravi Kumar Shivakumar. Final editing and manuscript review were done by Riaz Mahmood.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Department of Biotechnology at Kuvempu University in Shankaraghatta, Shimoga, Karnataka, India, for providing the necessary research facilities. We extend our profound appreciation to Dr. V. Krishna for their exemplary laboratory help, coordination and excellent support in the execution of this work. All people recognized have provided their express approval to be identified.

CONFLICT OF INTERESTS

The authors disclose no conflicting interests.

ABBREVIATIONS

CRFEE: *Chrozophora rottleri* fruit ethanol extract; **CRFPE:** *Chrozophora rottleri* fruit pet-ether extract; **CRFCF:** Chrozophora rottleri fruit chlroform extract; **ESKAPE:** *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp; **RandD:** Research and development; **SD:** Standard deviation; **N/A:** nill activity.

SUMMARY

Plant extracts provide eco-friendly alternatives to synthetic medicines, preventing antimicrobial resistance. Neem leaf, lemongrass, cinnamon and garlic extracts offer high antibacterial capabilities owing to their metabolites.[40] Similarly, *Chrozophora rottleri* fruit, from the Euphorbiaceae family, demonstrates promising antibacterial activities, indicating its medicinal potential. The investigation on the antibacterial activity of *Chrozophora rottleri* fruit ethanol extract exhibited substantial inhibitory effects against *Bacillus subtilis, Enterococcus faecalis* and *Serratia marcescens*, with increased effectiveness (MIC: 50 µg/mL). Strengths of the research include the use of the usual broth dilution procedure and the wide variety of phytochemicals maintained in the ethanol extract. However, the studies limitations include examining just three bacterial strains and the absence of *in vivo* trials. When compared with other research, the results match with prior studies indicating greater antibacterial efficacy against Gram-positive bacteria. These results imply possibilities for creating plant-based antibacterial medicines, especially against resistant Gram-positive infections, but more investigations on a broader variety of pathogens and *in vivo* models are required to generalize these findings for practical usage or policy creation. Further study is required to evaluate effectiveness, safety and discover active molecules for future pharmaceutical development against common illnesses.

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Cite this article: Nagesh YR, Mahmood R, Burladinni R, Manjunath VN, Shivakumar RK. In vitro Assessment of Antibacterial Potential of Chrozophora rottleri Fruit Different Extracts against Common Pathogens. Asian J Biol Life Sci. 2024;13(3):608-12.