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RESEARCH ARTICLE

Antimicrobial potential of *Rhus coriaria*, *Hibiscus sabdariffa*, *Syzygium aromaticum* and *Punica granatum* plant extracts against *Streptococcus pneumoniae*: An *in vitro* study

Israa Abed Ali Al-Hawani*

Department of Prosthetics and Orthotics Techniques, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq

*Correspondence email - IsraaAbedali@atu.edu.iq

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Abstract

For a long time, traditional treatment has gained approbation of ancient people, elders and people under poverty. The antibiotic-resistant, Gram-positive, virulent bacterium *Streptococcus pneumoniae* is one of the most significant pathogens responsible for a wide range of nasopharyngeal infections. These infections are often persistent and difficult to treat due to the organism's Gram-positive nature and multiple virulence factors, including a polysaccharide capsule that protects the bacterium from host defenses. In addition, *S. pneumoniae* rapidly develops mechanisms of antibiotic resistance, further complicating effective treatment. Finding a sustainable alternative treatment, particularly from plant sources, has gained traction due to the bacterium's increasing antibiotic resistance. Aqueous and alcoholic extracts of *Rhus coriaria* L., *Hibiscus sabdariffa* L., *Syzygium aromaticum* (L.) Merr. & L.M.Perry and *Punica granatum* L., were assayed on *S. pneumoniae*, which was diagnosed by biochemical tests. Sensitivity test was carried out in comparison with standard antibiotic disc. Cytotoxicity test on blood cells were also performed. The antimicrobial susceptibility assays showed that *S. pneumoniae* was moderately sensitive for *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum*. These plant extracts can be suggested as a traditional treatment alternative to antibiotics.

Keywords: *Hibiscus sabdariffa; Punica granatum; Rhus coriaria; Streptococcus pneumoniae*; *Syzygium aromaticum*

Introduction

Pneumococcus is extremely challenging to control because of the increasing use of antibiotics, the spread of many resistant clones, capacity for capsular flipping and serotype replacement and the horizontal transmission of antibiotic resistance genes (1).

Streptococcus pneumoniae, a Gram positive, blood hemolytic and catalase negative bacteria, grows optimally at nasopharyngeal temperatures (~33 °C) and under aerobic conditions with catalase, suggesting that environmental factors significantly influence its growth characteristics and virulence. Colonies are partially hemolytic on blood agar (2–4). *Streptococcus pneumoniae* is a leading cause of invasive pneumococcal disease (IPD), resulting in significant morbidity and mortality worldwide. It can cause severe infections like pneumonia, sepsis and meningitis, as well as less severe infections such as sinusitis and otitis media (5). Pneumococcal infections cause significant clinical and economic impacts, particularly in children and the elderly (6).

The main virulence factors of *S. pneumoniae* include the emergence of novel serotypes due to capsule variation, this makes the design and efficacy of vaccines more difficult (7). Over 95 % of isolates show resistance to commonly used antibiotics, such as erythromycin and azithromycin, mediated by resistance genes (8). The prevalence of penicillin-resistant strains varies, with significant resistance observed in certain serotypes, such as 19F and 23F (9).

Over the years, people have used traditional medicinal plants for the relief of various types of pain. Medicinal plants contain numerous phytochemicals that enable them to combat free radicals, bacteria, fungi and diabetes. The therapeutic properties of medicinal plants are mainly attributed to the active chemical compounds present in them (10).

Sumac or *Rhus coriaria* L., is a plant that belongs to the Anacardiaceae family. It is known for having many phytochemicals, which might affect blood pressure, glycemic indices and body composition, as shown in the systematic review and meta-analysis (11). *Rhus coriaria* is a useful plant that is important for both biological and economic reasons which is known for its ability to fight bacteria and fungi, as well as its antioxidant properties (10).

Hibiscus sabdariffa L., commonly referred to as red sorrel or rosella in English, but is known as karkadeh in Saudi Arabia and Ethiopia, belong to the family Malvaceae, is extensively cultivated in South East Asia, Central and West Africa and other places (12, 13).

The presence many essential oils, such as thymol and carvacrol in *H. sabdariffa* gave it the ability to be antioxidants and antibacterial (14).

Syzygium aromaticum (L.) Merr. & L.M.Perry, commonly known as cloves, contains potent antioxidants that can protect cells from damage. Extracts made from cloves, especially acidic extracts, contain high amounts of phenolic compounds. These compounds are effective at preventing oxidative harm to human red blood cells. They can also neutralize free radicals, according to earlier studies (15). *Syzygium aromaticum* is a spice with strong antibacterial and antioxidant properties. The plant has been used traditionally for medicinal purposes to treat a variety of illnesses. Research shows its ability to act against harmful bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* in individuals suffering from chronic tonsillitis. The bioactive compounds in spices work to stop the spread of infection and reduce inflammation. While ancient healers used cloves for general wellness, modern science continues to reveal this spice's many protective health effects (16).

Punica granatum L., commonly known as pomegranate, is a fruit that is excellent for health and nutrition. It contains many antioxidants, including punicalagin and anthocyanins, which may help with various health issues, such as maintaining heart health, reducing inflammation and potentially preventing cancer (17).

Punica granatum is a powerful antibacterial agent, especially against *S. aureus* and *Escherichia coli*. The peel extract stopped these bacteria from growing when it was tested and it helped rats live longer, which indicates that it could be a useful medicine (18).

During winter, sinusitis infections affect a large proportion of the population in Iraq. The misuse and overuse of antibiotics have raised serious concerns about bacterial antibiotic resistance. To reduce the spread of resistance and minimize the adverse effects associated with conventional antibiotics, the use of alternative, natural, affordable substances with fewer side effects is recommended. The current study focuses on lightening the importance of *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum* plants in combating an antibiotic resistance in *S. Pneumoniae.* There are many studies focused on using *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum* for treatment of different pathogens, but no study was carried out on *S. pneumoniae.* Our study focuses on *S. pneumoniae* as an antibiotic resistance bacterium.

Materials and Methods

Plant extraction

Rhus coriaria, *H. sabdariffa*, *S. aromaticum* and *P. granatum* were procured from local markets, thoroughly washed, dried and sieved prior to extraction. The extraction process took place in the first week of June. *R. coriaria* seed, *H. sabdariffa* flower, *S. aromaticum* flower bud and *P. granatum* peel, were ground to a fine powder. For aqueous extraction, a total of 25 g of each powdered plant was measured and placed into clean containers. Aqueous extracts were prepared by adding 30 mL of distilled water to 25 dried pre-weighed plant parts. The mixtures were stirred on a magnetic stirrer at room temperature for 48 hr. Following this, the mixtures were filtered using Whatman No.1 filter paper and the supernatant from each plant extract was collected and poured into dry Petri dishes to allow for evaporation at room temperature.

The ethyl alcohol extracts of *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum* were prepared following earlier described methods with some modification. 10 g of finely ground plant parts were placed in a Soxhlet apparatus and extracted with 200 mL of ethanol for 24 hr. The resulting filtrate was concentrated using a rotary evaporator at 40–45 °C, with the extraction repeated as necessary to yield an adequate amount. The concentrate was then dried in an electric oven at 40–45 °C. The dried material was transferred to a tightly sealed, pre-weighed glass container and stored at 4 °C in a refrigerator until use (19).

Isolation of *Streptococcus pneumoniae*

A specimen was collected from the nasal swab of the author, diagnosed with sinusitis. The swab was streaked onto various routine bacterial media, including nutrient agar, MacConkey agar, Mannitol salt agar, blood agar and chocolate agar, placed in Petri dishes. These plates were incubated at 37 °C for 24 hr under both aerobic and microaerophilic conditions. Post-incubation, the plates were examined and the morphological characteristics of the colonies were recorded.

Identification of *Streptococcus pneumoniae*

The colonies observed from the culture were subjected to Gram staining to assess their microscopic features. Subsequently, several biochemical tests were performed based on the suspected isolate. The Gram stain procedure followed standard protocols, utilizing fresh colonies not older than 24 hr. Selected biochemical tests, including oxidase, catalase and blood hemolysis assays were conducted. Oxidase assay (filter paper method) was carried out by placing a piece of filter paper in a petri dish and applying 3 drops of freshly prepared oxidase reagent. Using a sterile glass rod, a colony of *S. pneumoniae* was aseptically transferred from a culture plate onto the filter paper and smeared evenly.

The catalase assay (slide-based method) was carried out using a sterile wooden applicator to transfer a small amount of colony material onto a clean, dry glass microscope slide. A drop of 3 % hydrogen peroxide was then added to the slide and the formation of oxygen bubbles was observed as an indicator of enzymatic activity. Blood hemolysis tests were also observed and the results were recorded (20).

Antimicrobial susceptibility testing

The antibacterial susceptibility of the *S. pneumoniae* isolate was determined using the disk diffusion method (Fig. 1). The bacterial isolate was diluted to a concentration of 1.5×10^8 CFU, matching the 0.5 McFarland standard for turbidity. A volume of 0.1 mL of this bacterial suspension was evenly spread onto Muller Hinton Agar (MHA, Merck) using a swab. Six standard antibiotic disks were utilized as controls: Erythromycin, Amoxicillin, Vancomycin, Tetracycline, Streptomycin and Cefoxitin (Table 1). For testing the plant extracts, the well diffusion method was employed. A cork borer was used to create wells with a diameter of 6 mm, which were filled with 0.1 mL of each extract at a concentration of 200 mg/mL. Sterile distilled water and 70 % ethanol served as control solvents. Following a 24 hr incubation at 37 °C, the diameters of the inhibition zones surrounding each well were assessed and documented (21). This experiment was done 3 times and the mean of the inhibition zone was calculated (22).

Minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration was determined using the well diffusion method, as previously described, with five concentrations of both alcoholic and aqueous extracts (200, 100, 50, 25 mg/mL and control) (23).

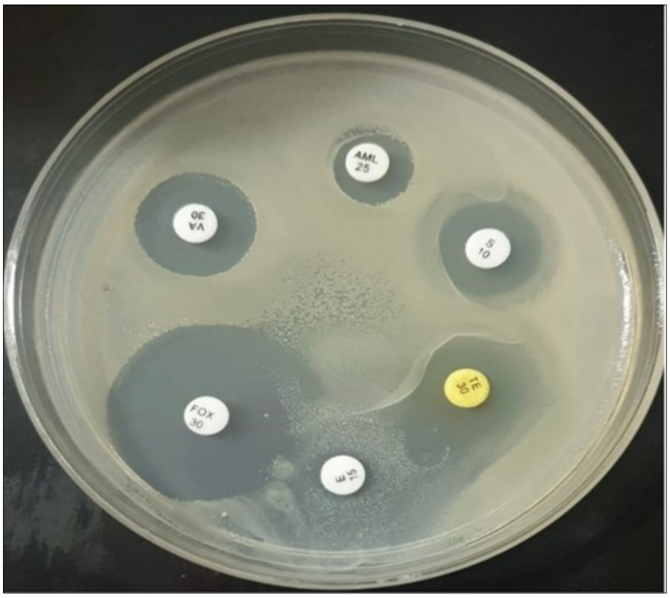

Fig. 1. Effect of standard antibiotics on *Streptococcus pneumoniae*, showing the highest inhibition zone with Cefoxitin (FOX, 30 mm) and the lowest inhibition zone with Erythromycin (E).

Table 1. Antibiotic standard disks used in the study with their concentration per disc

Antibiotic	Symbol	Concentration
Erythromycin	E	15 µg
Amoxicillin	AML	25 µg
Vancomycin	VA	30 µg
Tetracycline	TE	30 µg
Streptomycin	S	10 µg
Cefoxitin	FOX	30 µg

Cytotoxicity testing

Cytotoxicity of the aqueous and alcoholic extracts was assessed on human red blood cells (RBCs) to maintain osmotic pressure. Ringer solution was used for this purpose. Concentrations of 25, 50, 100 and 200 mg/mL of each plant extract were added to 1 mL of RBCs mixed with 19 mL of Ringer solution in serial test tubes. The mixtures were monitored hourly for a duration of 12 hr (24).

Statistical analysis

Statistical analysis were performed using two-way analysis of variance (ANOVA) according to the general linear model as described in previous studies. All analyses were conducted using the stats models package (v0.14.0) in Python (v3.11) (25, 26).

Results

Extraction of *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum* resulted in gaining 2 g of dry extract of each plant for both aqueous and alcoholic extracts. There are no references indicating the effect of the solvent on the amount of extract yield; rather, many factors interfere with the quality of the extract, including solvent type, duration of extraction and temperature (22).

Rhus coriaria (Sumac) alcoholic extract showed an effect on *S. pneumonia* compared to the control. The lowest inhibition zone was shown when the concentration of both alcoholic and aqueous extracts was used (25 mg/mL), with diameter measured 13 and 12 mm respectively. The highest effect was seen when 100 mg/mL of each extract where used (20 mm for alcoholic and 20 mm for aqueous extract). The controls (Absolute ethanol and water) showed no effect on the bacterium and no inhibition zone was seen around any of them (Table 2, 3).

Hibiscus sabdariffa activity on *S. pneumoniae*, comparing to the control, showed that the lowest inhibition zone of both alcoholic and aqueous extracts was 15 and 12 mm respectively, in the 25 mg/mL concentration, while, the highest effect was seen when 100 mg/mL of each extract where used (20 mm for alcoholic and 15 mm for aqueous extract). The controls (Absolute ethanol and water) showed no effect on the bacterium and no inhibition zone was seen around any of them (Table 2, 3).

The antimicrobial effect of *S. aromaticum* alcoholic and aqueous extract on *S. pneumonia* was assessed, the results showed that the lowest inhibition zone was shown when the concentration of both alcoholic and aqueous extracts was 25 mg/mL, the diameter of inhibition was 15 and 12 mm respectively, while, the highest effect was seen when 100 mg/mL of each extract where 20 and 15 mm inhibition diameter were recorded for alcoholic and aqueous extract respectively compared to the control, which showed no inhibition (Table 2, 3).

For *P. granatum* alcoholic and aqueous extract, the results recorded the lowest inhibition zone (25 mg/mL) for both alcoholic and aqueous extracts. The diameter measured 12 and 11 mm respectively, while the highest effect was in 100 mg/mL with 20 and 17 mm respectively. The controls showed no effect since no inhibition zone was observed around bacterial growth (Tables 2, 3).

Table 2 compared the antibacterial activity (mean inhibition zones, mm) of *R. coriaria*, *H. sabdariffa*, *S. aromaticum* and *P. granatum* against *S. pneumoniae*, using alcoholic extract at three concentrations (25, 50 and 100 mg/mL), with a control (C = 0 mg/mL). The results showed a proportional relation between the concentration and inhibition effect. At 100 mg/mL, *R. coriaria, H. sabdariffa, S. aromaticum* showed the greatest inhibition (20 mm). At all concentrations, *P. granatum* consistently exhibits the least amount of inhibition (Fig. 2).

Table 3 evaluated the antibacterial activity (mean inhibition zones, mm) of *R. coriaria, H. sabdariffa, S. aromaticum* and *P. granatum* against *S. pneumoniae* using aqueous extract at three concentrations (25, 50 and 100 mg/mL) in comparison to a control (C = 0 mg/mL). The results showed that the inhibitory effect and concentration were proportionately related. At 100 mg/mL, the highest inhibition (20 mm) was observed in *R. coriaria, H. sabdariffa, S. aromaticum*. At all doses, *P. granatum* consistently exhibits the least amount of inhibition (Fig. 2).

Table 2. Comparison of the inhibitory effect of alcoholic extracts of *Rhus coriaria*, *Hibiscus sabdariffa*, *Syzygium aromaticum* and *Punica granatum* against *Streptococcus pneumoniae*, expressed as mean ± SD for all extracts

Concentration (mg/mL)	*Rhus coriaria*	*Hibiscus sabdariffa*	*Syzygium aromaticum*	*Punica granatum*	Mean ± SD (all extracts)
100	20	20	20	15	18.8 ± 2.5
50	18	18	18	13	16.8 ± 2.5
25	13	15	15	12	13.8 ± 1.3
Control	0	0	0	0	0

Table 3. Comparison of the inhibitory effect of aqueous extracts of Rhus coriaria, Hibiscus sabdariffa, Syzygium aromaticum, and Punica granatum against Streptococcus pneumoniae, expressed as mean ± SD for all extracts.

Concentration (mg/mL)	*Rhus coriaria*	*Hibiscus sabdariffa*	*Syzygium aromaticum*	*Punica granatum*	Mean ± SD (all extracts)
100	20	15	15	17	16.8 ± 2.2
50	15	14	14	13	14.0 ± 0.8
25	12	12	12	11	11.8 ± 0.5
Control	0	0	0	0	0

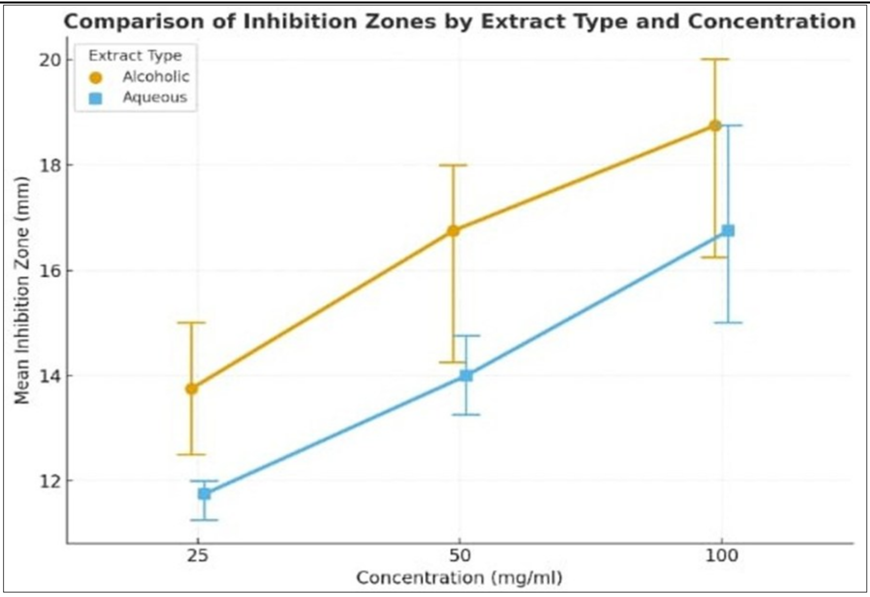

Fig. 2. Mean inhibition zones (mm) against *Streptococcus pneumoniae* at varying concentrations (25, 50 and 100 mg/mL) generated by alcoholic and aqueous extracts of *Rhus coriaria*, *Hibiscus sabdariffa*, *Syzygium aromaticum* and *Punica granatum*. The mean ± standard deviation is used to display the data (n = 4).

When comparing the inhibition effect of alcoholic and aqueous extracts of all plants, the results showed that alcoholic extract has superior effect compared with aqueous extracts (Table 4).

To sum up alcoholic extracts consistently show higher inhibition than aqueous extracts (by about 2 mm) at all doses, suggesting that alcohol is used to extract more powerful phytochemicals with antibacterial qualities, such as flavonoids, tannins and phenols.

Table 4. Comparison of alcoholic vs aqueous extracts, according to their average inhibition at equal concentrations

Concentration	Mean inhibition (Alcoholic)	Mean inhibition (Aqueous)
100 mg/mL	18.8 mm	16.8 mm
50 mg/mL	16.8 mm	14.0 mm
25 mg/mL	13.8 mm	11.8 mm

Statistical analysis

Two-way ANOVA

The results of two-way ANOVA analysis of the effect of alcoholic extracts of *R. coriaria, H. sabdariffa, S. aromaticum, P. granatum* on *S. pneumoniae* (Table 5), revealed that concentration significantly affected the measured inhibition values ($p < 0.001$), while plant species showed no significant effect ($p = 0.999$). Additionally, no significant interaction between concentration and plant species was observed ($p = 0.999$), indicating that the response to concentration was consistent across all tested plant extracts.

The results of two-way ANOVA analysis of the effect of aqueous extracts of *R. coriaria, H. sabdariffa, S. aromaticum, P. granatum* on *S. pneumoniae* demonstrated that inhibition values were significantly influenced by concentration and plant species ($p < 0.001$). A significant interaction between concentration and plant species was also observed ($p < 0.001$), indicating that the effect of concentration varied among the different plant extracts. (Table 6).

Table 5. ANOVA analysis for the effect of alcoholic extracts of *Rhus coriaria, Hibiscus sabdariffa, Syzygium aromaticum, Punica granatum* on *Streptococcus pneumoniae*

Source	df	Sum of Squares	F-value	*p*-value	Significance
Concentration	4	2597.04	174.19	<0.001	Significant
Plant species	3	0.07	0.006	0.999	Not significant
Concentration × Plant	12	4.64	0.10	0.999	Not significant
Error	40	149.09			

Table 6. ANOVA analysis for the effect of aqueous extracts of *Rhus coriaria, Hibiscus sabdariffa, Syzygium aromaticum, Punica granatum* on *Streptococcus pneumoniae*

Source	df	Sum of Squares	F-value	*p*-value	Significance
Concentration	3	1974.8	71810.94	< 0.001	Significant
Plant species	3	21.02	764.45	< 0.001	Significant
Concentration × Plant	9	36.18	438.58	< 0.001	Significant
Error	32	0.29			

Discussion

The intricate combination of phytochemicals amplifies the antimicrobial effect, frequently exceeding that of individual compounds (27). The rising occurrence of multidrug-resistant (MDR) bacteria necessitates the investigation of alternative treatments, including phytochemicals, which may enhance the efficacy of current antibiotics and potentially reverse resistance mechanisms (28).

The antimicrobial activity of the alcoholic extract of *R. coriaria* against multidrug-resistant *S. pneumoniae* has been shown to be moderate, *R. coriaria* exhibits significant antibacterial properties, with studies indicating MIC values ranging from 0.2–3.1 mg/mL against various bacteria (29). The alcoholic extract of sumac fruits has shown efficacy against various antibiotic-resistant strains, surpassing aqueous extracts in antibacterial and anti-biofilm activities (30). Certain fractions of sumac, especially subfraction Rs5, exhibit pronounced antibacterial properties, indicating that extraction techniques may improve effectiveness (29). The research on *R. coriaria* exhibited significant antibacterial properties against multiple bacterial strains, with subfraction Rs5 displaying a MIC of 0.4 mg/mL. The current study reveals that the effect of *R. coriaria* on *S. pneumoniae* was less than other studied bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabiles* and *Klebsiella pneumoniae*. The sumac alcoholic extract showed a moderate activity against *S. pneumoniae*, which means it wasn't as effective. This could be because there are a lot of resistance genes that have been showing up in recent years from *S. pneumoniae* (29) .

Hibiscus sabdariffa extracts, both alcoholic and aqueous, have demonstrated efficacy against MDR bacteria, notably *S. pneumoniae* (Table 2, Panel 1). This is in line with a growing body of research that shows plant-based antimicrobials could be used instead of traditional antibiotics. The antimicrobial efficacy of these extracts varies significantly in terms of their MIC, indicating their potential effectiveness in clinical applications (31). Phytochemicals in *H. sabdariffa* extracts may damage bacterial cell membranes, stop biofilm formation and mess with efflux pumps, which are important for the growth of antibiotic resistance (32, 33). *Hibiscus sabdariffa* ethanolic extract has shown a similar effect on *K. pneumoniae*, it shows the lowest inhibition in 10 mg/mL concentration, which align with current study results (33).

The most important virulence factors are antibiotic resistance; bacterial toxins and a variety of enzymes that help the infection adapt to different environments. The way that pathogenic bacteria impact their hosts is determined by bacterial virulence factors, which enable them to survive and develop virulence. Gram-positive bacteria possess a cytoplasmic membrane surrounded by a thick peptidoglycan layer. In contrast, Gram-negative bacteria have a cell wall consisting of a thin peptidoglycan layer located between the cytoplasmic and outer membranes. Some Gram-positive and Gram-negative bacteria also produce capsules. Because bacterial cell wall structures are rigid and complex, proteins require specialized mechanisms to cross or interact with them (34).

Our study has investigated how well *S. aromaticum* (clove) extracts work against MDR bacteria, especially *S. pneumoniae*. The results indicate that both alcoholic and aqueous extracts have antimicrobial effects (Table 2, Panel 1). This means they could be used as an alternative treatment for infections caused by resistant strains. Clove extracts have demonstrated considerable antimicrobial efficacy against a range of pathogens, including *S. pneumoniae*, although some studies indicate only moderate effectiveness (35, 36). This may be attributable to the virulence factors inherent in *S. pneumoniae*, such as their capsule, which manifests as a formidable barrier. The ethanolic extract of clove was more effective than aqueous one, also, they showed that the lowest concentration of clove that inhibit *S. pyogenes* was 1 % (16, 37).

The MIC for clove extracts was reported as 40 µg/mL, indicating a promising potential for inhibiting bacterial growth (36). Earlier reports indicated that alcoholic extracts (methanolic and ethanolic) generally demonstrated higher antimicrobial activity compared to aqueous extracts, with methanolic extracts showing the most potent effects (36, 38). This suggests that the concentration of our extract was lowest than the effective concentration and it needs to be adjusted.

Aqueous extracts, while less effective, still contributed to the overall antimicrobial profile of clove (35). Cloves contain various bioactive compounds, including eugenol, which is responsible for its antimicrobial properties (16, 36).

Phytochemical analyses revealed the presence of alkaloids, glycosides and saponins, which may enhance the antimicrobial effects of clove extracts (37).

The antimicrobial activity of *P. granatum* extracts against *S. pneumoniae* has been explored in our study, revealing moderate efficacy against this MDR bacterium (Table 2, Panel 1). The extracts, both alcoholic and aqueous extracts of *P. granatum* have shown antimicrobial properties against *S. pneumoniae*, with varying degrees of effectiveness (38). The biological activity of *P. granatum* attributes to the presence of active compounds such as flavonoids and tannins, which contribute to their antibacterial activity by disrupting bacterial cell walls and membranes. Previous reports indicate that while *P. granatum* extracts exhibit moderate activity against *S. pneumoniae*, the combination of *P. granatum* extracts with conventional antibiotics has shown a synergistic effect, enhancing the overall antimicrobial action against resistant strains (39, 40).

Different factors can affect plant extracts concentration that affect microorganisms, these include difference in their chemical makeup, the target microorganism and the extraction technique, thus, the MIC varies greatly (41).

Hemolysis assay is a common method for evaluating the cytotoxic potential of chemicals, drugs and biomaterials. In the assay, red blood cells are exposed to the substance being tested in a controlled environment. The extracts from *R. coriaria*, *H. sabdariffa* species, *S. aromaticum* and *P. granatum* cytotoxicity on RBCs results showed that all the extracts have no effect on red blood cells, which indicate the safety of using those extracts as treatment *in vivo* (42).

Effect depending on concentration

The antibacterial activity of both extract types increases dose-dependently; larger inhibition zones correspond to higher concentrations. This shows that there are active ingredients in the extract and that their impact is proportionate to their concentration.

Differences in extract type

Greater antibacterial activity was demonstrated by alcoholic extracts, indicating that ethanol extracts a wider variety of bioactive compounds than water. This pattern is consistent with other research that reports stronger inhibition from alcoholic extracts of phenolic-rich plants (43).

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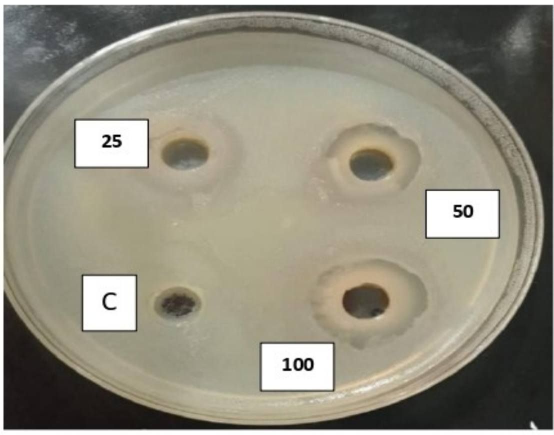
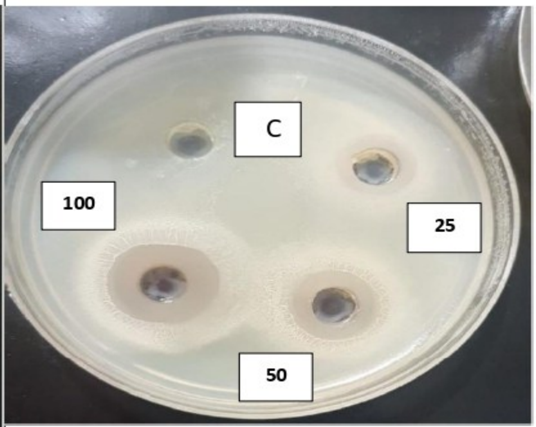
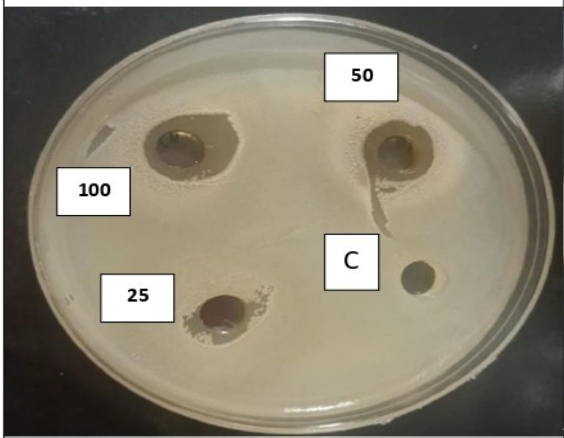
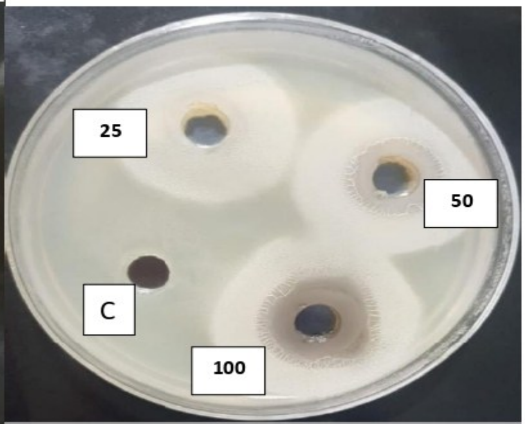
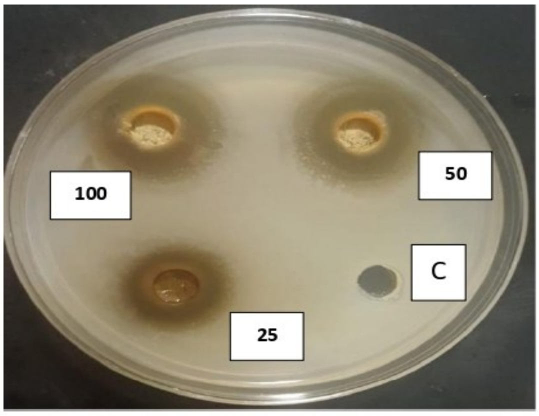
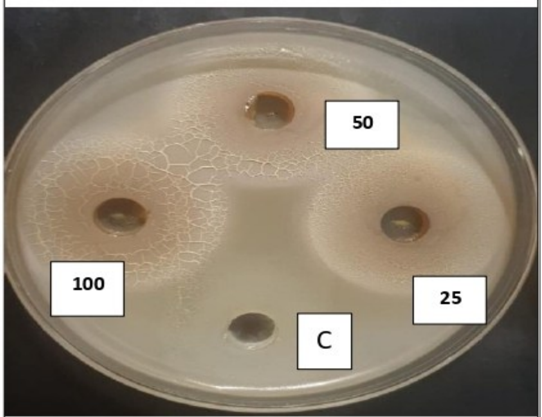
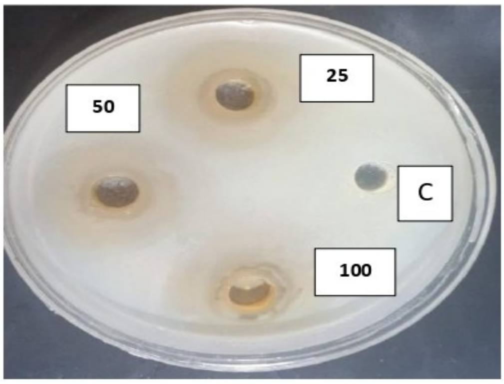
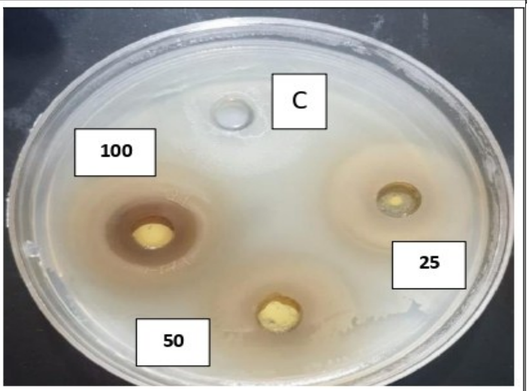
<tr><td>Rhus coriaria</td><td></td><td></td></tr>
<tr><td>Hibiscus sabdariffa</td><td></td><td></td></tr>
<tr><td>Syzygium aromaticum</td><td></td><td></td></tr>
<tr><td>Punica granatum</td><td></td><td></td></tr>
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Panel 1. Inhibition effect of alcoholic and aqueous extracts of *Rhus coriaria*, *Hibiscus sabdariffa*, *Syzygium aromaticum* and *Punica granatum* against *Streptococcus pneumoniae*.

Comparing different plants:

Overall, the highest inhibition was observed in *R. coriaria* and *S. aromaticum. Punica granatum* was consistently lower, indicating that it has fewer active antimicrobial components that are effective against *S. pneumoniae* in these circumstances.

The results indicate that concentration exerts an extremely strong influence on the response variable ($p < 0.001$), highlighting a pronounced dose-dependent effect. The significant plant species effect suggests biological variability among species in terms of uptake, tolerance, or physiological response. Furthermore, the significant interaction effect demonstrates that the impact of concentration differs across plant species, implying species-specific sensitivity to treatment levels. The very low residual error indicates high experimental precision and reliability.

These findings are consistent with prior research demonstrating that concentration gradients strongly modulate biological responses and that species vary in their adaptive or detoxification mechanisms

Table 6 shows that concentration is the only statistically significant factor influencing the response ($p < 0.001$), while plant species and the interaction effect are not significant. This suggests that the response is primarily controlled by treatment intensity rather than biological variation among species. The lack of interaction indicates a uniform response pattern across species.

Similar findings have been reported in controlled dose-response studies where treatment intensity dominates interspecies variability (31, 36, 38).

Conclusion

The efficiency of extracts from *Rhus coriaria*, *Hibiscus sabdariffa* species, *Syzygium aromaticum* and *Punica granatum* varies. It is concentration dependent and can be improved by using higher concentration. It may also be enhanced through synergistic interations with other materials such as nanoparticles to increase the properties and effect of them. To effectively treat infections caused by resistant strains, further study of their mechanisms of action is essential, particularly regarding disrupting biofilms and preventing resistance mechanisms. Investigating active compounds separately is necessary to identify the most effective phytochemical components in the extract. Evaluating interactions between active compounds and different chemicals or nanoparticles, to improve their efficiency is also needed. In addition, expanding the number of specimens and strains tested and using reference strains would improve the robustness of the findings.

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Authors' contributions

IAAA carried out the plant extraction, biological activity studies, and immunoassays; participated in the study design; performed the statistical analysis and reference collection; and drafted, wrote, and revised the manuscript. The author read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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