European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied Website:
https://farmclin.com/

In Vitro Assessment of the Antibacterial Potential of a Curcuma longa and Acacia Extracts Combination Against Staphylococcus aureus and pseudomonas aeruginosa

Israa Ali Shihab[1], Rasha Kareem Mohammed[2]

[1]Biology Department, College of Science, Baghdad University, Baghdad, Iraq.
Email: israa.ali2402@sc.uobaghdad.edu.iq

ABSTRACT

Background: Interest in plant-derived agents as alternative therapies has grown due to the rise in antimicrobial resistance. Pseudomonas aeruginosa and Staphylococcus aureus are significant pathogens linked to biofilm formation and infections that are challenging to treat.

Aim of this study: To assess the combined ethanolic extracts of Curcuma longa and Acacia's antibacterial and antibiofilm activity in vitro against clinical isolates of S. aureus and P. aeruginosa.

Methods: 80% ethanol was used in a Soxhlet apparatus to extract the bark of Acacia and the rhizomes of C. longa. Two isolates of S. aureus and P. aeruginosa were used to test various extract combinations. Agar well diffusion, broth microdilution, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were used to measure antibacterial activity. The microtiter plate assay with crystal violet staining was used to assess biofilm formation and eradication. The main phytochemical components were identified using GC-MS analysis.

Results: Both bacteria were significantly inhibited by the extract combinations. The best effect against P. aeruginosa isolate 1 was seen at 0.5:1.5 (3.99 µg/mL), while the lowest MIC was found against S. aureus isolate 2 at the 1.5:0.5 ratio (1.95 µg/mL). Bactericidal action was indicated by MIC/MBC ratios. The combinations reduced preformed biofilms to some extent, but they did not clearly prevent biofilm formation.

Conclusion: the combined extracts of C. longa and Acacia showed limited antibiofilm efficacy but encouraging antibacterial activity, especially against planktonic cells.

KEYWORDS: Curcuma longa; Acacia; antibacterial activity; biofilm; Staphylococcus aureus; Pseudomonas aeruginosa.

How to Cite: Israa Ali Shihab, Rasha Kareem Mohammed, (2026) In Vitro Assessment of the Antibacterial Potential of a Curcuma longa and Acacia Extracts Combination Against Staphy lococcus aureus and pseudomonas aeruginosa., European Journal of Clinical Pharmacy, Vol.8, No.1, pp. 2652-2659

INTRODUCTION

Antibiotic resistance is now a major global public health issue because antibiotics are being used too much and in the wrong ways. Staphylococcus aureus and Pseudomonas aeruginosa are two types of pathogenic bacteria that cause a lot of infections and are becoming less responsive to standard antimicrobial treatments. This situation has prompted the quest for alternative antimicrobial agents sourced from natural origins (1, 2).

Medicinal plants are abundant in bioactive compounds such as alkaloids, flavonoids, phenolics, and terpenoids that possess antimicrobial properties. Curcuma longa has attracted significant attention among these plants due to the presence of curcumin and other phenolic compounds that exhibit substantial antibacterial activity against various pathogens (3, 4).

Curcumin, the main bioactive compound in turmeric, has been shown to stop bacteria from growing and mess with the ways that bacteria become virulent. Recent research has demonstrated that curcumin can impede secretion systems in Pseudomonas aeruginosa, which are essential to bacterial pathogenicity (5, 6).

Plants in the Acacia genus are also used a lot in traditional medicine. They have tannins, flavonoids, and phenolic compounds that are known to kill germs. Acacia species extracts have exhibited antibacterial efficacy against multidrug-resistant strains of Staphylococcus aureus and Pseudomonas aeruginosa (7).

The significance of molecular biomarkers in comprehending disease mechanisms and enhancing diagnostic precision has been emphasized by recent biomedical researches. The increasing interest in biological markers and encourage the investigation of new therapeutic strategies, such as the study of bioactive compounds derived from plants that may have therapeutic uses (8, 9).

Combining plant extracts may create synergistic antibacterial effects, making them more effective against bacteria and less likely to become resistant. Consequently, this study sought to assess the antibacterial efficacy of combined extracts of Curcuma longa and Acacia against Staphylococcus aureus and Pseudomonas aeruginosa utilizing in vitro techniques..

MATERIALS AND METHODS

Plant Extraction Procedure

Rhizomes of Curcuma longa were obtained from local markets in Al-Ghazalia, Baghdad, Iraq, while the bark of Acacia species was collected from the Plant Research Garden at the Department of Biology, University of Baghdad. The plant materials were taxonomically authenticated by a plant taxonomist at the same department.

The samples were washed with distilled water to get rid of any dirt, and then they were left to air dry in a shaded, well-ventilated area at room temperature (25–30 °C) for 7–10 days. Then, the dried materials were ground into a fine powder and kept in airtight containers until they were ready to be extracted.

Soxhlet apparatus (Schott Duran, Germany) was used to do the extraction (10). About 25 g of plant powder was put into a cellulose extraction thimble and mixed with 500 mL of 80% ethanol. The extraction took about 8 hours at the boiling point of ethanol (about 78 °C) until the solvent in the siphon tube was almost clear.

By evaporating the solvent in a hot air oven at 38–40 °C, the resulting extract was concentrated. The extraction yield was calculated by weighing the dried crude extract, which was then kept in sterile screw-capped tubes at 4 °C until needed. Curcuma longa and Acacia sp. extracts were dissolved in dimethyl sulfoxide (DMSO) to create stock solutions (500 µg/mL). Curcuma longa to Acacia ratios of 1.75:0.25, 1.5:0.5, 1.25:0.75, 1:1, 0.75:1.25, 0.5:1.5, and 0.25:1.75 were used to prepare combined extract preparations. Before being used in the experiment, each solution was freshly made.

Bacterial Isolates

Pseudomonas aeruginosa and Staphylococcus aureus clinical isolates were acquired from the microbiology lab at the University of Baghdad's College of Science. The study included four isolates: two S. aureus and two P. aeruginosa. 16S rRNA molecular analysis verified the isolates' prior identification using the VITEK-2 system. Bacterial cultures were subcultured on selective media after being activated in nutrient broth at 37 °C for 24 hours. While P. aeruginosa was cultivated on Cetrimide agar, which produced distinctive greenish pigmentation, S. aureus was cultivated on Mannitol Salt Agar (MSA), where yellow colonies indicated mannitol fermentation. Before being used, pure cultures were kept at 4 °C on nutrient agar.

Antibacterial Activity and Biofilm Inhibition Assays

Fresh cultures were used to create bacterial suspensions, which were then adjusted to the 0.5 McFarland standard ($\approx 1.5 \times 10^2$ CFU/mL). Agar well diffusion and broth microdilution techniques were used to assess the plant extracts' antibacterial activity. Equal volumes of bacterial inoculum and plant extract were combined in 96-well microplates to determine the minimum inhibitory concentration (MIC) (11, 12). The mixture was then incubated at 37 °C for 24 hours, and bacterial growth was identified using resazurin dye. Samples from wells with no discernible growth were subcultured onto Mueller-Hinton agar plates to determine MBC. In the agar well diffusion assay, inhibition zones were measured after 50 µL of plant extracts were added to wells on Mueller-Hinton agar plates that had been inoculated (11). Biofilm inhibition and eradication were assessed using the microtiter plate method with crystal violet staining, and optical density was measured at 630 nm to determine biofilm reduction compared with the control (13).

RESULTS

Antibacterial activity (Agar well diffusion assay)

Determination of MIC and MBC for extraction on bacteria (*Staphylococcus aureus, Psuedomonas aeruginosa*) by using microplate 96-well and resazurin day (table 1).

Number of well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**G**	**10**
Concentration of extraction	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.62 µg/ml	7.81 µg/ml	3.GG µg/ml	1.G5 µg/ml	0.G7 µg/ml

Table 1: show raw of microplate 96-well contain serial dilution of extraction (1-10 column).

Determination of MIC and MBC of *Curcuma longa* and *Acacia* Extract Combination Against Staphylococcus aureus

Table 2 shows the majority of extract combinations showed inhibitory activity against S. aureus, according to the results. The MBC values were between 7.81 and 31.25 µg/ml, and the MIC values were between 3.99 and 15.62 µg/ml.

Several combinations, including 0.25:1.75, 0.5:1.5, 1:1, and 1.5:0.5, showed the lowest MIC value (3.99 µg/ml) for isolate 1, indicating strong antibacterial activity at these ratios. However, at ratios 1.25:0.75 and 1.75:0.25, higher MIC values (15.25–15.62 µg/ml) were found, indicating decreased inhibitory activity when the proportion of *Curcuma* extract increased excessively.

MIC values for isolate 2 varied from 1.95 to 7.81 µg/ml, with the 1.5:0.5 ratio exhibiting the lowest MIC (1.95 µg/ml), suggesting that this combination has increased antibacterial potency.

Combination (*Curcuma*:*Acacia*)	***Pseudo. aeruginosa* 1 MIC µg/ml**	***Pseudo. aeruginosa* 1 MBC µg/ml**	**MIC/MBC ratio**	***Pseudo. aeruginosa* 2 MIC µg/ml**	***Pseudo. aeruginosa* 2 MBC µg/ml**	**MIC/MBC ratio**
0.25:1.75	7.81	15.62	0.5	7.81	15.62	0.5
0.5:1.5	3.99	7.81	0.51	7.81	15.62	0.5
1:1	7.81	15.62	0.5	7.81	15.62	0.5
1.25:0.75	15.62	31.25	0.49	7.81	15.62	0.5
1.5:0.5	15.62	31.25	0.49	7.81	15.62	0.5
1.75:0.25	15.62	31.25	0.49	7.81	15.62	0.5

Table2: MIC and MBC Determination of *Curcuma* and *Acacia* extracts combination against *Staphylococcus aurreus*

Determination of MIC and MBC of *Curcuma longa* and *Acacia* Extract Combination Against *Pseudomonas aeruginosa*

In table 3 the MBC values ranged from 7.81 to 31.25 µg/ml and MIC values ranged from 3.99 to 15.62 µg/ml.

The lowest MIC value (3.99 µg/ml) for P. aeruginosa isolate 1 was found at the 0.5:1.5 ratio, suggesting that increased *Acacia* extract proportions may enhance antibacterial activity against this organism.

The MIC values for isolate 2 were comparatively constant (7.81 µg/ml) in the majority of combinations, indicating a moderate level of bacterial susceptibility to the extract mixtures.

The extracts also had bactericidal effects against P. aeruginosa, as evidenced by the MIC/MBC ratios for all combinations ranging from 0.49 to 0.51.

Combination (*Curcuma*:*Acacia*)	***Staph.aureus* 1 MIC µg/ml**	***Staph.aureus* 1 MBC µg/ml**	**MIC/MBC ratio**	***Staph.aureus* 2 MIC µg/ml**	***Staph.aureus* 2 MBC µg/ml**	**MIC/MBC ratio**
0.25:1.75	3.99	7.81	0.51	7.81	15.62	0.512
0.5:1.5	3.99	7.81	0.51	7.81	15.62	0.512
1:1	3.99	7.81	0.51	3.99	7.81	0.51
1.25:0.75	15.25	31.25	0.48	3.99	7.81	0.51
1.5:0.5	3.99	7.81	0.51	1.95	3.99	0.488
1.75:0.25	15.62	31.25	0.48	3.99	7.81	0.51

Table 3: MIC and MBC Determination of *Curcuma* and *Acacia* extracts combination against Pseudomonas aeruginosa

Inhibition of Biofilm Formation

The microtiter plate assay was used to assess the combined extracts' capacity to prevent biofilm formation, and optical density was measured at 360 nm. The following were the control values:

M1 is equal to 0.057
M2 is equal to 0.053
M3 = 0.054

Despite variations in extract ratios, Table 4 results demonstrated that all tested combinations raised optical density values when compared to the negative control, suggesting the presence of biofilm formation under treated conditions.

The 0.5:1.50 combination yielded the highest OD values among the tested ratios, suggesting a stronger interaction with bacterial cells during biofilm formation.

OD values varied from 0.142 to 0.187 for *Staphylococcus aureus* and from 0.137 to 0.188 for *Pseudomonas aeruginosa.*

Table 4: Optical Density (OD360 nm) Measurement of Biofilm Formation

Combination (*Curcuma*:*Acacia*)	***S. aureus* 1**	***S. aureus* 2**	***P. aeruginosa* 1**	***P. aeruginosa* 2**
0.25:1.75	0.144	0.124	0.143	0.139
0.75:1.25	0.149	0.151	0.154	0.156
0.5:1.50	0.175	0.176	0.187	0.182

(values represent mean of M1–M3)

Biofilm Eradication Activity

The plant extract combinations' capacity to eliminate pre-formed biofilms was assessed. Table 5 displays the control biofilm values prior to treatment.

Bacteria	OD (mean)
S. aureus 1	0.245
S. aureus 2	0.256
P. aeruginosa 1	0.245
P. aeruginosa 2	0.349

Table 5: Control OD Values Before Treatment

Biofilm Removal After Treatment

As in table 6, a discernible decrease in optical density values was seen following treatment with the combined extracts when compared to the untreated control, suggesting that the established biofilm had been partially eliminated.

The strongest biofilm removal effect was demonstrated by the 0.5:1.50 combination, especially against *Staphylococcus aureus* isolate 1, where OD values dropped to 0.142–0.144 from the control value of 0.245.

Pseudomonas aeruginosa biofilms also showed moderate reductions, though they were less noticeable than those of S. aureus. This could be because of the bacterium's potent biofilm-forming capacity and resistance mechanisms.

Table 6: Optical Density (OD360 nm) Measurement of Biofilm Eradication

Combination (*Curcuma*:*Acacia*)	*S. aureus* 1	*S. aureus* 2	*P. aeruginosa* 1	*P. aeruginosa* 2
0.25:1.75	**0.201**	**0.181**	**0.173**	**0.175**
0.75:1.25	**0.213**	**0.201**	**0.204**	**0.204**
0.5:1.50	**0.143**	**0.176**	**0.208**	**0.144**

GC-MS analysis of *Acacia* bark

The chromatogram shows that the major constituents in the ethanol extract of *Acacia* bark are fatty acids and their methyl esters, with a dominant presence of hexadecanoic acid (palmitic acid) and methyl stearate.This indicates that the extract contains significant lipid-related components, which are common in plant tissues and may contribute to antimicrobial, antioxidant, and anti-inflammatory properties. Table (7) shows Below is a clear scientific interpretation of GC-MS results for *Acacia* bark extract (80% ethanol, Soxhlet) based on the compounds identified, their retention times, and relative peak areas

	RT (min)	Area%	Name	Quality	CAS Number
1	24.914	17.63	Hexadecanoic acid, methyl ester	99	000112-39-0
2	26.128	46.68	n-Hexadecanoic acid	98	000057-10-3
3	27.529	4.90	9,12-Octadecadienoic acid, methyl ester	99	002462-85-3
4	27.628	3.76	cis-13-Octadecenoic acid, methyl ester	97	1000333-58-3
5	28.032	20.92	Methyl stearate	99	000112-61-8
6	43.712	6.10	Tris(tert-butyldimethylsilyloxy)arsane	59	1000366-57-5

Table 7: GC-MS analysis of the ethanoloic (80%) extraction of *Acacia* bark using Soxhlet apparatus

GC-MS analysis *Curcuma longa* rhizobium

Table 8 The main ingredient (Peak 1) was quantified to be around 1800 mg / 2 g extract, or 72 mg/g of raw rhizome (roughly 7.2% w/w of the crude rhizome). Peak 2, the second main component, was found to be 199 mg/ 2 g extract, or 7.96 mg/g raw rhizome (≈ 0.796% w/w). Additionally found were trace nitrogen-containing compounds (e.g., 1-methyl-1,2,4-triazole and 3-aminopyrazole), Curlone, and Perilla alcohol tiglate, among other minor ingredients. Peaks 3 and 4 showed very low concentrations of these chemicals, each of which was about 0.4 mg per 2 g extract, or 0.016 mg/g raw rhizome (≈ 0.0016% w/w). Their existence could be a sign of secondary metabolites or potential artifacts connected to extraction.

Table 8: GC-MS analysis of the ethanoloic (80%) extraction of *Curcuma longa* rhizobium using Soxhlet apparatus

Peak No.	Retention time (min)	Tentative identification	Area (%)	Weight in 2 g extract (mg)	(mg/g) ethanolic extract	(mg/g) raw dry rhizobium[1]	W/W (%) in dry rhizobium[1]
1	14.424	Turmerone isomers (α/β/ar) / γ-Atlantone	90	1800	900	72	7.2
2	14.854	Curlone (β-Turmerone) / γ-Atlantone	9.95	199	99.5	7.96	0.796
3	15.544	Trace N-containing compound(s)	0.02	0.4	0.2	0.016	0.0016
4	15.639	Trace N-containing compound(s)	0.02	0.4	0.2	0.016	0.0016

DISCUSSION

The findings demonstrated that a number of Curcuma longa and Acacia extract combinations had significant inhibitory effects on Staphylococcus aureus. For isolate 2, the lowest MIC value found was 1.95 µg/ml at the 1.5:0.5 ratio, indicating a potent antibacterial effect of this combination. On the other hand, when the proportion of Curcuma longa increased excessively (e.g., 1.75:0.25), higher MIC values were seen, suggesting that the balance between the two extracts may be important for the antibacterial activity.

These results corroborate earlier research indicating that Curcuma longa exhibits significant antimicrobial properties attributed to its bioactive constituents, including curcumin and turmerones, which compromise bacterial membranes and disrupt cellular metabolism. Recent studies have shown that curcumin can stop Pseudomonas aeruginosa from being virulent by stopping the type III secretion system, which is very important for bacterial pathogenicity (5). Similarly, a research reported that Curcuma longa extracts show potent antibacterial activity against multidrug-resistant bacteria (1).

The antimicrobial activity of Acacia species has also been widely reported. Extracts of Acacia contain tannins, flavonoids, phenolic acids, and fatty acids, which are known to disrupt microbial cell walls, inhibit enzymes, and interfere with bacterial protein synthesis, a study reported that Acacia extracts demonstrated inhibitory activity against both Staphylococcus aureus and Pseudomonas aeruginosa, supporting the findings of the present study (7).

Pseudomonas aeruginosa is a Gram-negative bacterium recognized for its inherent resistance to numerous antimicrobial agents, attributed to its outer membrane barrier, efflux pumps, and biofilm-forming capability. Consequently, plant extracts frequently exhibit diminished efficacy against Gram-negative bacteria in contrast to Gram-positive organisms (14). Even though there was resistance, the extract combinations in this study showed measurable antibacterial effects. This suggests that the phytochemicals in Curcuma and Acacia may work through more than one antibacterial mechanism. Another significant finding in this study is that the MIC/MBC ratios varied from 0.48 to 0.51, suggesting that the extracts demonstrated bactericidal rather than bacteriostatic properties. An MIC/MBC ratio of 4 or less usually means that the extracts were bactericidal, which means they could kill the bacteria instead of just stopping their growth. This finding shows that these plant extracts could be useful as alternative antimicrobial agents in medicine.

The augmented antibacterial efficacy noted in specific combinations may be ascribed to synergistic interactions among the phytochemicals inherent in both plants. Previous study have demonstrated that combinations of plant extracts can yield enhanced antimicrobial effects compared to individual extracts, attributable to synergistic mechanisms of action, including membrane disruption, enzyme inhibition, and the induction of oxidative stress (15). Therefore, combining Curcuma longa and Acacia extracts may provide a promising strategy for developing plant-based antimicrobial formulations.

The biofilm assay demonstrated that all evaluated combinations of Curcuma longa and Acacia extracts yielded OD values exceeding the negative control mean (M1–M3 ≈ 0.055). According to these results, the combination did not clearly stop biofilm formation in the conditions that were tested. Biofilm biomass was still present in all treated wells, with OD values between 0.124 and 0.176 for S. aureus and between 0.139 and 0.187 for P. aeruginosa. The 0.5:1.50 combination had the highest values, which means that this ratio was the least effective at stopping early biofilm growth and may have even let attached biomass stay attached (16).

The current study investigated the impact of the combined extracts of Curcuma longa and Acacia on biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa utilizing the microtiter plate assay. All of the combinations that were tested had optical density (OD) values that were higher than the negative control. This means that there was still biofilm biomass in the treated wells, so the extract combinations didn't clearly stop biofilm from forming in the conditions used in this study. Biofilm formation is a significant virulence factor in both S. aureus and P. aeruginosa. When bacteria stick to a surface and start making extracellular polymeric substances, they are harder to get rid of. The biofilm matrix shields bacterial cells from antimicrobial substances, environmental stressors, and host immune responses. Consequently, substances that are efficacious against free bacterial cells may exhibit diminished activity against biofilm-associated cells. This may elucidate why the extract combinations in the current study exhibited promising antibacterial activity in MIC/MBC assays yet did not demonstrate significant inhibition in the biofilm assay (17, 18).

The results of this study may also be connected to the characteristics of the microtiter plate method itself. This test looks at the total amount of attached biomass, which includes living cells, dead cells, and extracellular matrix material, not just living cells. So, a higher OD value means that biofilm biomass is still present in the well. This study indicates that all treated groups yielded values exceeding those of the control, leading to the conclusion that the extracts were ineffective in inhibiting biofilm formation at the tested concentrations and ratios. This interpretation is more precise than asserting that biofilm inhibition took place.

Curcumin, the principal bioactive component of Curcuma longa, is extensively documented for its antimicrobial and antibiofilm properties. Earlier research has demonstrated that curcumin can disrupt bacterial adhesion, quorum sensing, and the expression of genes associated with biofilms. But its usefulness in practice may be limited by its low stability, poor water solubility, and inability to penetrate the biofilm matrix well. These factors may have diminished its antibiofilm efficacy in the present study, especially when utilized as part of a crude extract mixture rather than in a purified or nanoformulated state (19, 20).

Acacia species also contain tannins, flavonoids, and other phenolic compounds that are known to kill bacteria. These compounds may function by compromising microbial membranes, deactivating enzymes, or disrupting bacterial metabolism. Acacia extracts have also been shown to have effects against biofilms in some studies. The efficacy of these compounds is contingent upon various factors, including plant species, extraction method, concentration, and the specific microorganism targeted. In the present study, it is conceivable that the concentration of active compounds in the tested combinations was inadequate to inhibit biofilm formation, despite the observation of antibacterial activity against planktonic cells (21, 22).

The higher OD values seen with the 0.5:1.50 ratio may mean that this combination did not have a synergistic antibiofilm effect. The phytochemical balance at this ratio may not have been good enough to stop bacteria from sticking to things or forming a matrix. This is an important point because plant extract combinations don't always work together in every test. A combination that exhibits efficacy in growth inhibition assays may not be effective in preventing biofilm formation. Consequently, the current findings indicate that antibacterial and antibiofilm activities should be assessed independently when evaluating the therapeutic potential of medicinal plant extracts.

The current study demonstrated that the amalgamation of Curcuma longa and Acacia extracts effectively diminished pre-formed biofilms of both Staphylococcus aureus and Pseudomonas aeruginosa, as evidenced by the reduction in optical density post-treatment. The 0.5:1.50 combination had the biggest effect, especially on S. aureus isolate 1, where the OD went down from 0.245 to 0.143. This indicates that the combined extracts possess partial biofilm eradication efficacy, although total elimination of the mature biofilm was not accomplished.

Recent study show that curcumin is an important antibiofilm agent against S. aureus because it stops adhesion, matrix formation, and biofilm-related regulation. This could explain the decrease seen in this study. These reviews also stress that it is hard to get rid of biofilms completely because they are very well-structured and protected (23). Review on natural antibiofilm compounds have noted that curcumin and related phytochemicals may reduce P. aeruginosa biofilm formation and virulence, but their activity is often moderate unless enhanced formulations are used. This supports the present result, where biofilm reduction in P. aeruginosa was less pronounced than in S. aureus (20). The contribution of Acacia extract may also be linked to the activity observed in this study. Tannins, flavonoids, and other phenolic compounds are primarily responsible for the antibacterial and antibiofilm properties reported in studies on Acacia species. Acacia may enhance the antibiofilm effect of the combination used in this work, according to a recent study on Acacia extracts that demonstrated activity against both S. aureus and P. aeruginosa (24). The current results, however, deviate from some report that found that purified compounds or sophisticated formulations produced stronger antibiofilm effects. According to recent research, curcumin-loaded or functionalized systems frequently outperform crude extracts due to curcumin's low solubility and restricted biofilm penetration. This could be the reason why biofilm biomass was decreased but not entirely eliminated by the extracts used in this investigation (20).

The main components of the ethanolic extract of Acacia bark were n-hexadecanoic acid, hexadecanoic acid methyl ester, and methyl stearate, according to GC–MS analysis, suggesting that the extract is rich in fatty acids and their esters. This result is consistent with earlier research demonstrating the presence of lipid-derived components in addition to tannins, flavonoids, and other antimicrobial phytochemicals in Acacia species. Although GC-MS may not accurately reflect the polar phenolic fraction of the bark, these compounds may have contributed to the observed antibacterial activity in the current study (25, 26).

Turmerone isomers and curlone dominated the chromatogram in Curcuma longa rhizome, which is in line with recent findings that turmerones are the main volatile components of turmeric rhizome. The strong biological activity of the turmeric extract in this study may be explained by these sesquiterpenes, which are commonly linked to antimicrobial and anti-inflammatory properties. Overall, the GC-MS results are consistent with the theory that the presence of turmerone-rich volatile compounds from Curcuma longa and fatty acid derivatives from Acacia may contribute to the antibacterial effect of the combined extracts (27).

LIMITATIONS OF THE STUDY

First, only four clinical isolates were tested, resulting in a small sample size. Second, the results cannot be directly applied to in vivo or clinical settings because the entire study was done in vitro. Third, consistency and biofilm activity may have been impacted by the study's evaluation of crude extracts rather than purified active compounds. Fourth, no standard antibiotic control was used to compare efficacy directly. Furthermore, optical density measurements, which represent total biomass rather than the viability of biofilm cells, were the primary basis for the antibiofilm assessment. Lastly, a thorough investigation of the two extracts' mechanisms of action and potential synergistic interactions was lacking.

CONCLUSION

The current study demonstrated the promising in vitro antibacterial activity of Curcuma longa and Acacia extracts against Pseudomonas aeruginosa and Staphylococcus aureus. Strong bactericidal effects were demonstrated by a number of extract ratios that produced low MIC and MBC values; depending on the bacterial species, certain combinations performed better. The findings imply that phytochemicals from both plants may interact to improve antibacterial efficacy. Nevertheless, under the tested conditions, the combined extracts did not clearly inhibit biofilm formation, despite being able to partially reduce preformed biofilms. Overall, these results indicate that Curcuma longa and Acacia have the potential to be natural sources of antibacterial compounds and that their combination could be helpful in creating plant-based antimicrobial formulations.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the study. Material preparation, plant extraction, laboratory experiments, and data collection were performed by the authors. Data analysis and interpretation were carried out collaboratively by all authors. The first draft of the manuscript was written by the corresponding author, and all authors reviewed, revised, and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Financial Disclosure

The authors received no specific financial support for the research, authorship, or publication of this article.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the Department of Biology, College of Science, University of Baghdad, for providing laboratory facilities and technical support. The authors also thank the staff of the microbiology laboratory for their assistance in handling and identifying the bacterial isolates, and the plant taxonomy specialists for authenticating the plant materials used in this study.

REFERENCES:

1. Kirmani F, Saddiqe Z, Saleem S, Ali F, Haq F-u. Phytochemical investigation and antibacterial activity of Curcuma longa against multi-drug resistant bacteria. South African Journal of Botany. 2024;164:137-45.
2. Jaafer DR, Mohamed MBM. Curcumin-Loaded Organogel for The Topical Skin Disorder Based on 12 Hydroxystearic Acid. Iraqi Journal of Pharmaceutical Sciences. 2024;33(3):171-86.
3. Syarifah A, Azizah W, Prasuma GS. FORMULATION AND ACTIVITY TESTS OF NANOEMULSION OF TURMERIC RHIZOME (CURCUMA LONGA L) EXTRACT FOR METHICILLIN-RESISTANT

STAPHYLOCOCCUS AUREUS (MRSA) BACTERIA. Jurnal Farmasi Sains dan Praktis. 2024:205-14.

4. Al-Obaidi OH. Isolation of some active materials and aqueous, alcoholic and oil seed extracts of the plant (Curcuma longa) and study Antibacterial and anticancer activity. Baghdad Science Journal. 2013;10(3):32.
5. Diaz-Guerrero M, López-Jácome LE, Franco-Cendejas R, Coria-Jiménez R, Martínez-Zavaleta MG, González-Pedrajo B, et al. Curcumin inhibits type III secretion of Pseudomonas aeruginosa. PeerJ. 2025;13:e19725.
6. Salim FD, Ahmed SH, Abd AM, Skben SZ, Ramadhan NA. Determination of total phenol, antioxidant and antimicrobial activities of Avena sativa and Ocimum basilicum. Baghdad Science Journal. 2014;11(2):110.
7. Hassan A, Garba S, Appah J, Uo O, Egbe N. Antibacterial activity of Acacia nilotica and Jatropha curcas plants leave extracts against multi-drug resistant strains of Pseudomonas aeruginosa and Staphylococcus aureus. Int J Med Body Health Res. 2023;4:34-46.
8. Waly AHT, Baraaj AH. Estimation of Nestin and AMACR markers expression in Iraqi patients with prostate tumor: histopathological and immunohistochemical study. Passer Journal of Basic and Applied Sciences. 2025;7(2):929-39. doi: 10.24271/psr.2025.531205.2211.
9. Waly AHT, Baraaj AH. The Indispensable Role of Immunohistochemistry in Differentiating Prostate Cancer from Benign Prostatic Hyperplasia. Journal of the Faculty of Medicine Baghdad. 2025;67(4):464-74. doi: 10.32007/jfacmedbaghdad3185.
10. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med aromat plants. 2015;4(196):2167-0412.
11. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.
12. Moosdeen F, Williams J, Secker A. Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. Journal of Antimicrobial Chemotherapy. 1988;21(4):439-43.
13. Peeters E, Nelis HJ, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of microbiological methods. 2008;72(2):157-65.
14. Breijyeh Z, Jubeh B, Karaman R. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 2020;25(6):1340.
15. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future microbiology. 2012;7(8):979-90.
16. Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. Journal of global infectious diseases. 2020;12(3):129-34.
17. Idrees M, Sawant S, Karodia N, Rahman A. Staphylococcus aureus biofilm: morphology, genetics, pathogenesis and treatment strategies. International Journal of Environmental Research and Public Health. 2021;18(14):7602.
18. Han A, Lee S-Y. An overview of various methods for in vitro biofilm formation: a review. Food Science and Biotechnology. 2023;32(12):1617-29.
19. Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, et al. The natural product curcumin as an antibacterial agent: Current achievements and problems. Antioxidants. 2022;11(3):459.
20. Shariati A, Noei M, Askarinia M, Khoshbayan A, Farahani A, Chegini Z. Inhibitory effect of natural compounds on quorum sensing system in Pseudomonas aeruginosa: a helpful promise for managing biofilm community. Frontiers in pharmacology. 2024;15:1350391.
21. Elamary RB, Albarakaty FM, Salem WM. Efficacy of Acacia nilotica aqueous extract in treating biofilm-forming and multidrug resistant uropathogens isolated from patients with UTI syndrome. Scientific Reports. 2020;10(1):11125.
22. Villanueva X, Zhen L, Ares JN, Vackier T, Lange H, Crestini C, et al. Effect of chemical modifications of tannins on their antimicrobial and antibiofilm effect against Gram-negative and Gram-positive bacteria. Frontiers in Microbiology. 2023;13:987164.
23. Wu X, Wang H, Xiong J, Yang G-X, Hu J-F, Zhu Q, et al. Staphylococcus aureus biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. Biofilm. 2024;7:100175.
24. Hammad AA, Abdelgadir AA, Yassin S, Alzain AA, Ahmed EM. GC-MS, antibacterial and in silico studies of sudanese Acacia polyacantha Stem bark alcoholic extract. Journal of Experimental Pharmacology. 2024:365-76.
25. Hafez LO, Brito-Casillas Y, Abdelmageed N, Alemán-Cabrera IM, Morad SA, Abdel-Raheem MH, et al. The Acacia (Vachellia nilotica (L.) PJH Hurter & Mabb.): traditional uses and recent advances on its pharmacological attributes and potential activities. Nutrients. 2024;16(24):4278.
26. Hasan MR, Haque MM, Hoque MA, Sultana S, Rahman MM, Shaikh MAA, et al. Antioxidant activity study and GC-MS profiling of Camellia sinensis Linn. Heliyon. 2024;10(1).
27. Orellana-Paucar AM. Turmeric essential oil constituents as potential drug candidates: a comprehensive overview of their individual bioactivities. Molecules. 2024;29(17):4210.