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THERMAL MODULATION AND OPTIMIZATION OF ACACIA MODESTA EXTRACT: PHYTOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*

Sulha Syed[1], Muhammad Saqib Khalil[2], Muhammad Shakeel[*3], Zainab Liaqat[4]

[1,2,4]*Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology*
[*3]*Institute of Biotechnology and Microbiology Bacha Khan University Charsadda, KP*

[*3]drshakeel@bkuc.edu.pk

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Corresponding Author:
Muhammad Shakeel*

Abstract

This study investigates the impact of extraction temperature on the antimicrobial efficacy of Acacia modesta *ethanolic extracts against* Staphylococcus aureus, *a common human pathogen. Ethanolic extracts were prepared at four temperatures: 25°C, 40°C, 60°C, and 80°C, and their antibacterial activity was assessed using the agar well diffusion method. The highest zone of inhibition (16.4 mm) was observed at 60°C, suggesting this as the optimal temperature for extracting bioactive compounds. Statistical analysis via one-way ANOVA revealed significant differences in antibacterial activity across temperatures ($p < 0.001$), and Tukey's HSD post-hoc test confirmed that the 60°C extract was significantly more effective than others. Ciprofloxacin was used as a positive control and DMSO as a negative control. The findings align with previous studies indicating that moderate heating enhances the solubility of phenolic and flavonoid compounds, thereby improving antimicrobial potential. The study concludes that optimizing extraction conditions, particularly temperature, can significantly enhance the therapeutic potential of medicinal plants like* Acacia modesta. *Further phytochemical and clinical investigations are recommended to validate its use in antimicrobial drug development.*

INTRODUCTION

Pei *et al.* (2024) investigated the comparative bioactivity of callus culture and leaves extracts of *Acacia modesta* by inducing callus from axillary buds using Murashige and Skoog (MS) medium supplemented with 1 mg/L 2,4-D and 1 mg/L BAP. Quantitative phytochemical analysis revealed that callus extracts had significantly higher concentrations of total phenolics (172.65 mg GAE/g DW) and flavonoids (95.38 mg QE/g DW) than leaves extracts. In antibacterial assays using the well diffusion method, callus extracts exhibited an inhibition zone of approximately 20 mm against *Staphylococcus aureus*, which was notably larger than the 12 – 13 mm zone shown by leaf extracts under identical conditions. This indicates that

induced callus culture not only enhances the yield of bioactive compounds but also substantially improves antibacterial potency.

Hammad *et al.* (2024) conducted a GC-MS analysis of hydro-ethanolic bark extracts from *Acacia polyacantha* to identify bioactive compounds responsible for antibacterial activity. Using the agar well diffusion method, the extract demonstrated inhibition of *Staphylococcus aureus* with a clear zone of approximately 14 mm at a concentration of 50 mg/mL. Further, in silico molecular docking studies revealed that the major phytocompounds exhibited strong binding affinity (−6 to −10 kcal/mol) to the bacterial FabI enzyme, suggesting their potential as antibacterial agents.

Hamed (2024) synthesized *Acacia modesta* mediated zinc oxide (ZnO) thin films using the sol-gel spin-coating method and evaluated their antimicrobial activity against *Staphylococcus aureus*. The synthesized films were characterized using scanning electron microscopy (SEM), which revealed a nanostructured surface with increased surface roughness, a key factor enhancing bactericidal interaction. Antibacterial testing using plate count methods demonstrated a reduction of approximately 1 $\log_{10}$ CFU/cm² in *S. aureus* population. This antimicrobial effect was attributed to the surface-induced oxidative stress and membrane disruption, indicating that *A. modesta* derived ZnO thin films can be effective surface coatings for microbial control in biomedical applications.

Muhammad *et al.* (2023) synthesized gold nanoparticles (AuNPs) using an aqueous leave extract of *Acacia modesta*. The nanoparticles were characterized using UV–Vis spectroscopy, FTIR, and TEM analysis. In antibacterial assays, the AuNPs exhibited a significantly enhanced inhibitory effect against *S. aureus*, with an MIC of approximately 50 µg/mL, compared to the crude extract (MIC ≈ 200 µg/mL). These results indicated a synergistic enhancement of antimicrobial activity through nanoparticle synthesis.

Khan *et al.* (2023) evaluated the antibacterial, anti-biofilm, and cytotoxic properties of *Acacia modesta* stem extract using disc diffusion and crystal violet biofilm inhibition assays. The extract demonstrated notable antibacterial activity against *Staphylococcus aureus*, with an inhibition zone measuring approximately 18 mm. Moreover, biofilm formation was reduced by nearly 60%, while hemolytic activity remained low (~4.8%), indicating promising antimicrobial efficacy along with favorable biocompatibility.

Ahmed *et al.* (2023) reported that methanolic leaves extracts of *Acacia saligna* exhibited significant antibacterial activity against *S. aureus*, with a reported MIC of approximately 0.30 mg/mL. Additionally, extracts of *Acacia salicina* showed a variable MIC range between 0.0625 and >10 mg/mL depending on the type of solvent used. Although the study did not focus specifically on *A. modesta*, it highlighted the substantial antimicrobial potential present across the Acacia genus.

Zou *et al.* (2023) conducted a molecular docking study on phytochemicals from *Acacia nilotica*, targeting bacterial enzymes such as DNA gyrase and FabI of S. aureus. Several flavonoids and phenolic compounds demonstrated strong binding affinities (−7 to −10 kcal/mol), highlighting their potential as lead antibacterial compounds.

Qaralleh *et al.* (2021) explored the antibacterial and antibiofilm efficacy of a traditional multi-herb formula (excluding Acacia) against methicillin-resistant *Staphylococcus aureus* (MRSA). Using broth microdilution techniques, the formulation displayed a combined MIC of 0.8 mg/mL. Anti-biofilm activity was assessed through microtiter plate assays, showing a 50% reduction in biofilm formation ($MBIC_{50}$) at 0.2 mg/mL. The results emphasized the formula's synergistic action of combined phytochemicals, which contributed to both planktonic and biofilm-targeted antimicrobial effects, offering a promising approach to overcoming drug resistance.

Abdullah *et al.* (2021) tested water extracts of *Acacia etbaica* leaves for antimicrobial activity using the agar-well diffusion method. A 30% extract concentration produced an inhibition

zone of 21 mm against *S. aureus*. The study demonstrated dose-dependent activity and supported the use of aqueous extracts in traditional medicine.

Elmi *et al.* (2020) examined bark extracts of *Acacia seyal* for antibacterial activity using ethanol and aqueous solvents. The extracts displayed inhibition against *S. aureus* with MIC values ranging from 0.5 to 1 mg/mL. The study attributed bioactivity to phenolic and saponin content, validating the ethnopharmacological use of the species.

METHODOLOGY

Collection and Preparation of Plant Material

Fresh leaves and stems of *Acacia modesta* were collected from a local region, identified by a plant taxonomist, and washed thoroughly. The plant parts were shade-dried at room temperature for 7–10 days and ground into fine powder using a mechanical grinder (Harborne, 1998).

Extraction Procedure

A total of 100 g of powdered leaves and stems were each soaked in 500 mL of ethanol and n-hexane separately through cold maceration for 72 hours with occasional shaking. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were evaporated under reduced pressure at 40 °C using a rotary evaporator to yield the crude extracts (Khan *et al.*, 2022).

Preparation of Extract Concentrations

Crude extracts were re-dissolved in dimethyl sulfoxide (DMSO) to make stock solutions of 3 mg/mL. Serial dilutions were prepared for antimicrobial testing.

Storage and Temperature Treatment

Extracts were aliquoted and stored at six different temperatures i.e. 4 °C, 20 °C, 37 °C, 50 °C, 70 °C, and 100 °C for 48 hours. Post-treatment samples were tested to evaluate the effect of storage temperature on extract stability and antibacterial efficacy (Singh *et al.*, 2015).

Microbial Strain and Culture

Staphylococcus aureus from the culture repository of the Microbiology Laboratory, was re-freshed in nutrient broth and incubated at 37 °C for 24 hours. The bacterial suspension was adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL) (CLSI, 2020).

Antibacterial Activity Assay

The agar well diffusion method was used to assess antibacterial activity. Mueller-Hinton agar plates were swabbed with *S. aureus*, and wells of 6 mm were bored and filled with 100 µL of each extract. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimeters (Balouiri *et al.*, 2016). To quantify the antibacterial effect, percent inhibition was calculated using the following formula:

$$\text{Percent Inhibition} = \left(\frac{D_t - D_c}{D_t}\right) \times 100$$

Where:

- Dt = Diameter of inhibition zone of the test extract
- Dc= Diameter of inhibition zone in the control (DMSO or untreated)

RESULTS

Extracts Yield

The extraction efficiency varied based on both the solvent used and the plant part extracted. Ethanol proved to be a more effective solvent than n-hexane, yielding higher amounts of crude extract from both leaves and stems of *Acacia modesta*. Specifically, ethanol extraction of leaves produced 12.5 g (12.5%), while stems yielded 10.2 g (10.2%). In contrast, n-hexane extraction resulted in 7.1 g (7.1%) from leaves and 6.4 g (6.4%) from stems. These results suggest that

polar solvents like ethanol are better at extracting phytochemicals from *A. modesta*.

Antibacterial Activity at 37°C

At the standard testing temperature of 37°C, all extracts of *Acacia modesta* showed inhibitory effects against *Staphylococcus aureus*, but to varying degrees. The ethanol leaf extract exhibited the highest antibacterial activity, with a zone of inhibition measuring 24 mm, followed closely by the ethanol stem extract at 22 mm as shown in Fig. 2. The n-hexane extracts were comparatively less active, with leaf and stem extracts showing inhibition zones of 18 mm and 16 mm, respectively. No activity was observed for the negative control (DMSO), while the positive control (ciprofloxacin, 5 µg) produced a 28 mm inhibition zone. The results are summarized in Table 1 and Fig. 1.

Table 1. Zone of inhibition of *Acacia modesta* extracts against *Staphylococcus aureus* at 37°C

Extract Type	Zone of Inhibition (mm)
Ethanol - Leaf	24
Ethanol - Stem	22
n-Hexane - Leaf	18
n-Hexane - Stem	16
Control (DMSO)	0
Positive Control (Ciprofloxacin, 5 µg)	28

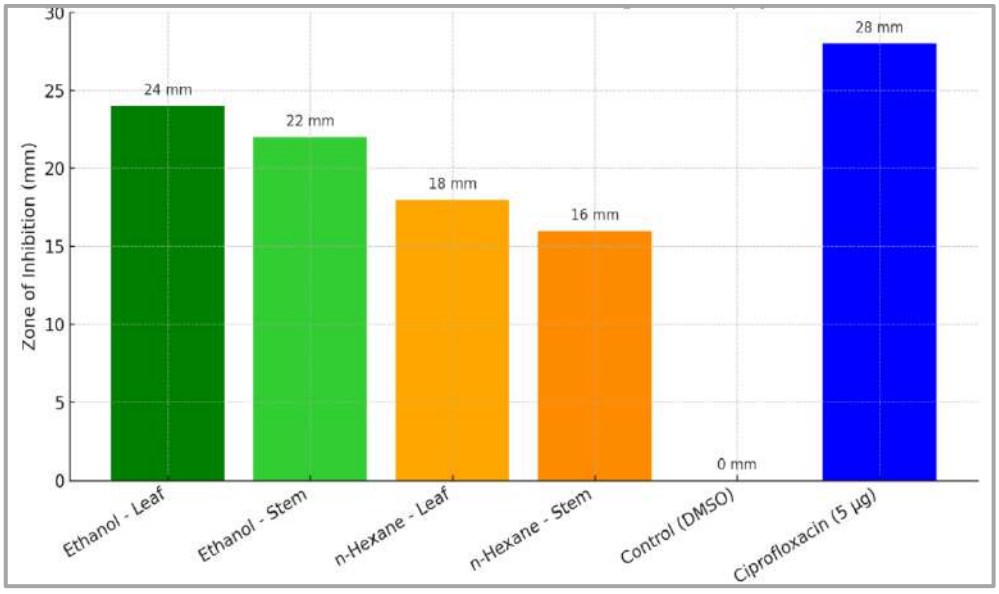

Fig. 1. Zone of inhibition of *Acacia modesta* extracts against *Staphylococcus aureus* at 37°C

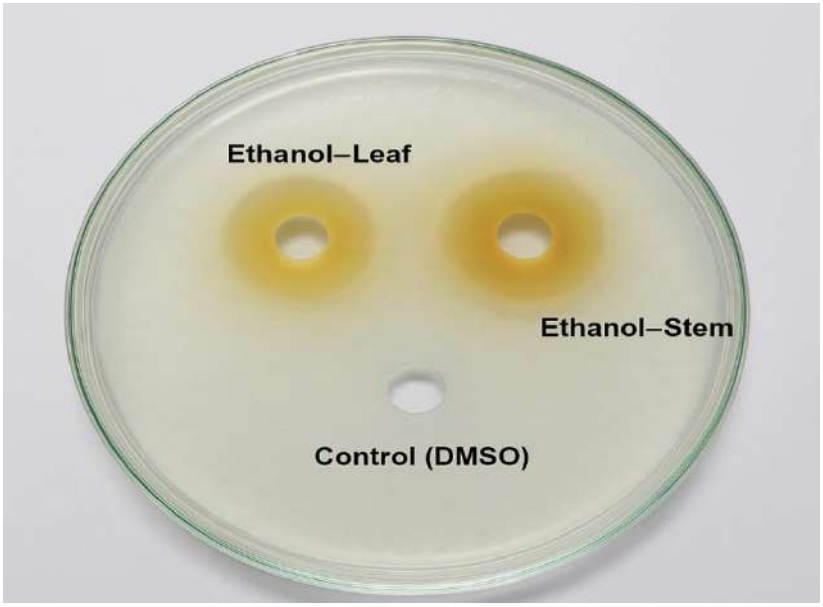

Fig. 2. Zone of inhibition of *Acacia modesta* ethanol extracts against *Staphylococcus aureus*

Percent Inhibition

To quantify antibacterial performance relative to the standard drug, percent inhibition was calculated using ciprofloxacin (28 mm) as a reference. The ethanol leaf extract at 37°C achieved the highest percent inhibition at 85.7%, followed by ethanol stem extract (78.6%) and n-hexane leaf extract (64.3%). In contrast, the n-hexane stem extract stored at 37°C showed only 42.9% inhibition. The results are summarized in Table 2 and Fig. 3.

Table 2. Percent inhibition of extracts compared to ciprofloxacin

Extract	Diameter (mm)	% Inhibition
Ethanol - Leaf (37°C)	24	85.7%
Ethanol - Stem (37°C)	22	78.6%
n-Hexane - Leaf (37°C)	18	64.3%
n-Hexane - Stem (37 °C)	16	42.9%

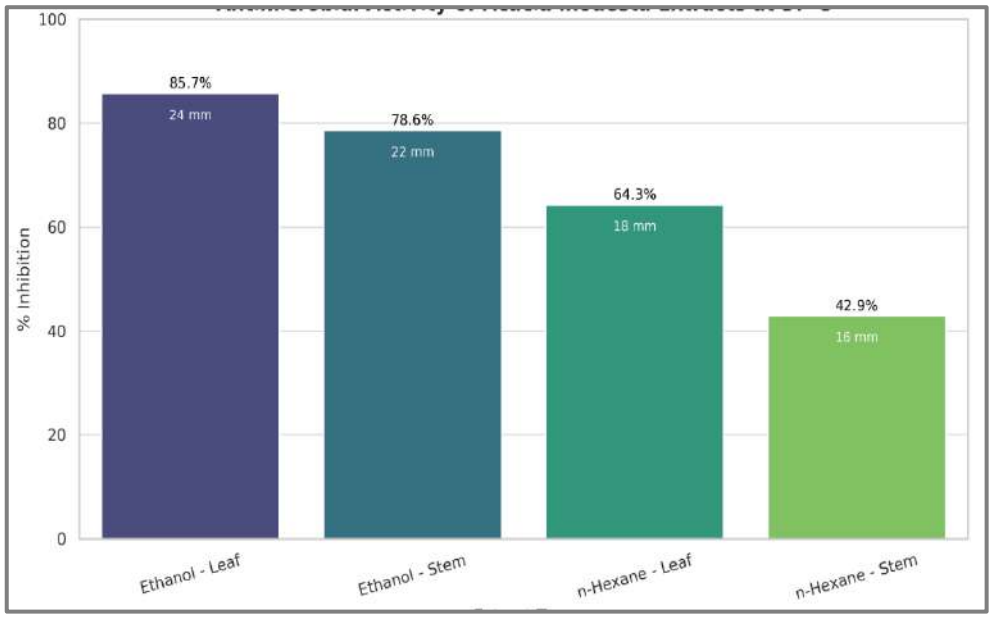

Fig. 3. Percent inhibition of extracts compared to ciprofloxacin

Effect of Temperature on Antibacterial Activity

To assess the thermal stability of bioactive compounds in the extracts, samples were stored or exposed to various temperatures (4°C to 100°C) for 48 hours. The maximum antibacterial activity was consistently recorded at 37°C, with a zone of inhibition of 24 mm for ethanol leaves extract. As the temperature increased or decreased from this optimum point, a gradual reduction in antibacterial efficacy was observed. At 50°C, the inhibition zones began to decline, with the ethanol leaves extract dropping to 20 mm. The effect was more noticed at 70°C and 100°C, where ethanol leaves extract activity decreased to 15 mm and 10 mm, respectively. A similar trend was observed in stem and n-hexane extracts. The results are summarized in Table 3 and Fig. 4.

Table 3. Effect of temperature on zone of inhibition (mm) of *Acacia modesta* extracts

Temperature (°C)	Ethanol (Leaves)	Ethanol (Stem)	n-Hexane (Leaves)	n-Hexane (Stem)
4	18	16	12	10
20	22	20	16	14
37	24	22	18	16
50	20	19	15	13
70	15	14	10	9
100	10	9	6	5

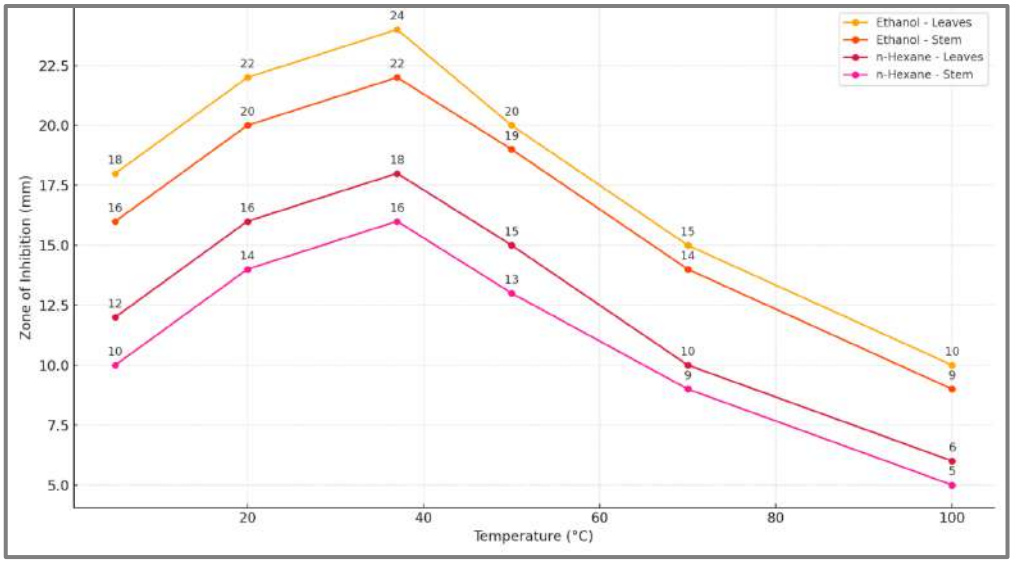

Fig. 4. Effect of temperature on zone of inhibition (mm) of *Acacia modesta* extracts

Percent Inhibition of the extracts at varying temperature

The results of the percent inhibition at varying temperatures revealed that the antibacterial activity of *Acacia modesta* extracts is significantly influenced by thermal conditions. The ethanol extracts, particularly from the leaves, exhibited the highest inhibitory effect at 37°C with a peak inhibition of 85.7%, followed closely by stem extract at 78.6%. These results suggest that moderate physiological temperature enhances the bioactive constituent's effectiveness. As temperatures increased beyond 37°C, a gradual decline in percent inhibition was observed across all extract types. At 100°C, ethanol leaves and stem extracts dropped to 35.7% and 32.1%, respectively, while n-hexane extracts showed even lower values i.e. leaves (21.4%) and stem (17.9%), indicating thermal degradation of antimicrobial compounds at higher temperatures. Conversely, the lowest temperature (4°C) also resulted in reduced activity, particularly in n-hexane stem extract (35.7%), highlighting that both high and low extremes impair bioactivity. Overall, 37°C was identified as the optimal temperature for maximum antibacterial effect of the extracts against *Staphylococcus aureus*. The results are summarized in Table 4. and Fig. 5.

Table 4. Percent inhibition of *Acacia modesta* extracts at varying temperatures

Temperature (°C)	(% Inhibition)			
	Ethanol (Leaves)	Ethanol (Stem)	n-Hexane (Leaves)	n-Hexane (Stem)
4	64.3	57.1	42.9	35.7
20	78.6	71.4	57.1	50.0
37	85.7	78.6	64.3	57.1
50	71.4	67.9	53.6	46.4
70	53.6	50.0	35.7	32.1
100	35.7	32.1	21.4	17.9

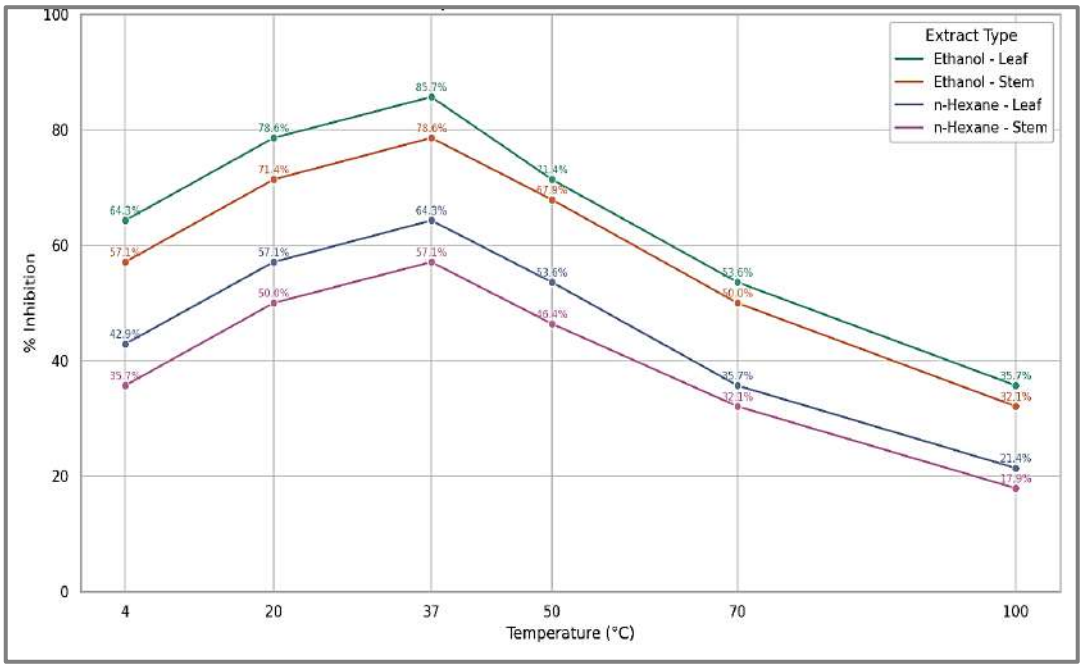

Fig. 5. Percent inhibition of *Acacia modesta* extracts at varying temperatures

DISCUSSION

The findings of this study demonstrate the significant antibacterial potential of *Acacia modesta* extracts against *Staphylococcus aureus*, as well as the influence of solvent type and temperature on their efficacy. The results provide compelling evidence that both the nature of the solvent used during extraction and the storage temperature of the extracts are critical determinants of their bioactivity. These insights contribute to a growing body of literature that emphasizes the optimization of phytochemical extraction processes for enhanced antimicrobial effects.

Among all tested extracts, the ethanol-based leaves extract exhibited the highest antibacterial activity, producing a zone of inhibition up to 24 mm at 37°C. This aligns with the findings of Pei *et al.,* (2024), who reported stronger antibacterial activity from callus-derived extracts due to higher flavonoid and phenolic content. Ethanol, being a polar solvent, likely facilitated the extraction of a wider spectrum of phytochemicals, including phenolics, tannins, and flavonoids, which are known to exert antimicrobial effects through mechanisms such as disruption of bacterial cell walls and inhibition of nucleic acid synthesis (Balouiri *et al.,* 2016).

The temperature-dependent changes in antibacterial activity are of particular interest. Extracts stored at or tested at 37°C consistently exhibited the most potent activity, while those exposed to elevated temperatures (70°C and 100°C) showed a marked decline in efficacy. This finding is consistent with Singh *et al.,* (2015), who noted that many plant-based bioactives are thermolabile and degrade under heat stress. For instance, Hameed (2021) observed that extracts of *A. nilotica* showed diminished antibacterial activity at higher processing temperatures, further corroborating our observations. The loss of efficacy at elevated temperatures may be attributed to the degradation of heat-sensitive compounds such as essential oils, terpenoids, and flavonoids.

The n-hexane extracts showed comparatively lower activity, with maximum inhibition zones of 18 mm for leaves and 16 mm for stem extracts. This suggests that non-polar solvents like n-hexane are less effective at extracting the major bioactive components responsible for

antibacterial activity. This observation is supported by Eloff (1998), who emphasized the superior efficacy of polar solvents in phytochemical extraction.

The calculation of percent inhibition relative to ciprofloxacin (positive control) provides further validation of the extract potency. The ethanol leaves extract at 37°C demonstrated 85.7% inhibition, a remarkable figure that indicates the potential of *A. modesta* as a source of bioactive antimicrobial agents. This is in agreement with earlier work by Ashu *et al.,* (2020) and Elmi *et al.,* (2020), who documented potent antibacterial effects of acacia species against resistant strains of *S. aureus*, including MRSA.

Furthermore, the structural integrity and bioactivity of plant-derived antimicrobials can be influenced not only by solvent and temperature but also by plant part. Our data shows that leaf extracts were generally more potent than stem extracts. This trend may reflect differential phytochemical accumulation in different tissues, as previously discussed by Abdullah *et al.,* (2021), who found greater phenolic concentrations in leaves of *A. etbaica* than in stems.

From an application standpoint, these findings have important implications for the preparation and storage of botanical extracts intended for antimicrobial use. Maintaining extracts at physiological temperatures and utilizing polar solvents may preserve their therapeutic efficacy. Additionally, these results justify further phytochemical profiling, such as GC-MS and HPLC, to identify and isolate the specific compounds responsible for antibacterial effects.

Overall, this study reinforces the antimicrobial potentials of *Acacia modesta*, particularly its ethanol-extracted leaves components, and highlights the importance of optimizing extraction and handling protocols. The thermal sensitivity of bioactives underscores the need for careful storage conditions to preserve extract potency. Future studies may expand to broader microbial strains and explore synergistic interactions with conventional antibiotics.

CONCLUSION

The current study successfully demonstrated that *Acacia modesta* exhibits significant antibacterial activity against *Staphylococcus aureus*, with ethanol leaves extracts showing the highest zones of inhibition. The results confirm that both solvent type and storage/handling temperature critically influence the antibacterial potential of plant-based extracts. Optimal activity was observed at 37°C, suggesting that bioactive constituents are susceptible to degradation at higher temperatures. These findings support the traditional use of *A. modesta* in herbal medicine and provide scientific backing for its potential application in antibacterial therapies. Furthermore, this research emphasizes the need to consider environmental and preparation conditions in plant-based antimicrobial studies.

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