



Antibacterial activity of bee propolis against multidrug-resistant *Staphylococcus aureus*: in vitro evaluation and HPLC characterization

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Submitted: 03/03/2026; Accepted: 04/23/2026; Published: 05/06/2026.

ABSTRACT: Antibiotic-resistant *Staphylococcus aureus* is one of the most important pathogens associated with hospital-acquired infections, which calls for the search for effective treatment alternatives from natural sources. This study aimed to evaluate the antibacterial activity of propolis extract against antibiotic-resistant *Staphylococcus aureus* isolates isolated from different clinical samples, while identifying the active compounds responsible for this activity. 200 clinical specimens were collected, including urine, burns, sputum, skin infections, nasal swabs, blood and genital swabs. Bacterial isolates were diagnosed using standard implant and chemochemical tests, and antibiotic susceptibility testing was performed by tablet diffusion. Antipropolis efficacy was evaluated using measurement of inhibition zones at different concentrations, as well as the determination of the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC). High-performance chromatography analysis (HPLC) was also performed for the qualitative and quantitative identification of phenolic compounds in propolis extract. The results showed that *Staphylococcus aureus* was 30% of the total samples, with high rates of resistance to some antibiotics. Propolis extract showed clear inhibitory activity against most of the isolates tested, with MIC values ranging from 1.56 to 25 $\mu\text{g mL}^{-1}$, and MBC values between 3.125 and 50 $\mu\text{g mL}^{-1}$. HPLC analysis also revealed active phenolic compounds, including gallic acid (632.40 ppm), caffeic acid (27.45 ppm), and catechin (98.0 ppm). These results suggest that propolis has a remarkable antibiotic efficacy against antibiotic-resistant isolates of *Staphylococcus aureus*, due to its richness in bioactive phenolic compounds, which supports the potential for its use as a promising natural resource in the development of therapeutic alternatives or adjuvant agents to conventional antibiotics.
Keywords: antibiotic resistance; HPLC analysis; phenolic compounds; MIC and MBC.

Atividade antibacteriana da própolis de abelha contra *Staphylococcus aureus* multirresistente: avaliação in vitro e caracterização por HPLC

RESUMO: A *Staphylococcus aureus* resistente a antibióticos é um dos patógenos mais importantes associados a infecções hospitalares, o que exige a busca por alternativas de tratamento eficazes a partir de fontes naturais. Este estudo teve como objetivo avaliar a atividade antibacteriana do extrato de própolis contra isolados de *Staphylococcus aureus* resistentes a antibióticos, obtidos de diferentes amostras clínicas, e identificar os compostos ativos responsáveis por essa atividade. Foram coletadas 200 amostras clínicas, incluindo urina, queimaduras, escarro, infecções de pele, swabs nasais, sangue e swabs genitais. Os isolados bacterianos foram diagnosticados por meio de testes padrão de imunoabsorção enzimática e de química, e o teste de suscetibilidade a antibióticos foi realizado por difusão em ágar. A eficácia antiprópolis foi avaliada pela mensuração dos halos de inibição em diferentes concentrações, bem como pela determinação da concentração inibitória mínima (CIM) e da concentração letal mínima (CLM). A análise por cromatografia líquida de alta eficiência (CLAE) também foi realizada para a identificação qualitativa e quantitativa de compostos fenólicos no extrato de própolis. Os resultados mostraram que *Staphylococcus aureus* representava 30% do total de amostras, com altas taxas de resistência a determinados antibióticos. O extrato de própolis apresentou clara atividade inibitória contra a maioria dos isolados testados, com valores de MIC entre 1,56 e 25 $\mu\text{g mL}^{-1}$ e de MBC entre 3,125 e 50 $\mu\text{g mL}^{-1}$. A análise por HPLC também revelou compostos fenólicos ativos, incluindo ácido gálico (632,40 ppm), ácido cafeico (27,45 ppm) e catequina (98,0 ppm). Esses resultados sugerem que a própolis possui notável eficácia antibiótica contra isolados de *Staphylococcus aureus* resistentes a antibióticos, devido à sua riqueza em compostos fenólicos bioativos, o que reforça seu potencial de uso como recurso natural promissor no desenvolvimento de alternativas terapêuticas ou de agentes adjuvantes aos antibióticos convencionais.

Palavras-chave: resistência a antibióticos; análise por HPLC; compostos fenólicos; MIC e MBC.

1. INTRODUCTION

Bacterial infections are a growing global health problem, particularly those caused by antibiotic-resistant bacteria,

which is a major challenge for modern health systems. *Staphylococcus aureus* is one of the most common pathogens. It is responsible for a wide range of infections,

ranging from skin and soft tissue infections to respiratory infections, urinary tract infections, bacteremia, and serious nosocomial infections (TONG et al., 2015; KHDHAIR et al., 2024).

These bacteria become more dangerous when they acquire multiple resistance to antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA), limiting available treatment options and increasing morbidity and mortality (TONG et al., 2015). Excessive and bacterial resistance mechanisms, such as the production of antigen-inactivating enzymes, modification of binding sites, activation of ejection pumps and the formation of biofilms that enhance the ability of bacteria to survive in hostile environments. Therefore, it has become necessary to look for new therapeutic alternatives or natural substances with antibacterial effectiveness that can contribute to reducing dependence on conventional antibiotics (ALARJANI et al., 2023).

Propolis is a natural resin that honeybees collect from plant and bud secretions, mix with their own enzymes and beeswax, and use it to protect the hive from microorganisms (ALQAHTANI et al., 2024). Propolis has a complex chemical composition that includes flavonoids, phenolic acids, esters, volatile oils, and other compounds known for their antibacterial, fungal, and anti-viral properties, as well as antioxidant and anti-inflammatory properties (AKSOY et al., 2020). Propolis is not only important as a natural substance of bee origin, but its bioactivity is due to its complex chemical composition, rich in bioactive phenolic compounds.

Studies have shown that propolis contains a wide spectrum of phenolic acids and flavonoids, such as gallic acid, caffeic acid, and catechin, which are key compounds responsible for its antibacterial properties (AL-MAMOORI et al., 2025). These compounds disrupt the integrity of the bacterial cell wall and membrane, inhibit basic enzymatic systems, and affect gene expression and bacterial virulence factors (AL-MAMOORI et al., 2025). HPLC is one of the most accurate and reliable techniques in the analysis of phenolic compounds, as it enables the qualitative and quantitative identification of active ingredients in natural extracts (WANG et al., 2022). Recent HPLC analyses have shown that the presence of elevated concentrations of gallic acid, along with caffeic acid and catechin, is associated with increased antibacterial efficacy of propolis, particularly against Gram-positive bacteria such as *Staphylococcus aureus* (SANG et al., 2025).

Several recent studies have shown that propolis has significant inhibitory activity against different types of Gram-positive and Gram-negative bacteria, with often higher efficacy against positive bacteria such as *Staphylococcus aureus* (KARAGUL et al., 2024). This effect is due to the ability of propolis components to disrupt the bacterial cell wall, inhibit essential enzymes, affect cell membrane permeability, and impede the formation of biofilms (KARAGUL et al., 2024). This study aims to evaluate the antibacterial effect of honey bee-derived propolis against antibiotic-resistant *Staphylococcus aureus* isolates isolated from different clinical samples, while studying the sensitivity of these isolates to common antibiotics, and determining the effectiveness of propolis by measuring inhibition zones and determining the minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC), thus contributing to shedding light on the future therapeutic potential of propolis as a promising natural source in the face of bacterial resistance (ZHOU et al., 2024).

2. MATERIAL AND METHODS

2.1. Clinical specimen collection

This study was conducted on a total of 200 clinical samples collected from patients who visited a number of hospitals and health centers, and included urine samples, burns, sputum, skin infections, nasal swabs, blood, and genital swabs. Samples were collected under sterile conditions and transported to the laboratory within a short period of time to ensure their safety and prevent contamination.

2.1.1. Isolation and diagnosis of *Staphylococcus aureus*

Clinical specimens were cultured on Blood agar medium and Mannitol Salt Agar (MSA) media, and then incubated at 37°C for 24 hours. The diagnosis of isolates was based on the cultivation characteristics, as the colonies of *S. aureus* appeared in a round, smooth, elevated, golden-colored form with the ability to induce hemolysis on the blood agar medium, in addition to the fermentation of mannitol and the turning of the medium to yellow on the MSA. The diagnosis was confirmed using a set of standard chemical tests, including catalysis test, coagulase test, mannitol fermentation, DNase test, phosphatase test, gelatin decomposition test, VITEK2 system and nitrate reduction test, according to the standard approved methods (KANDASWAMY et al., 2024).

2.2. Antibiotic susceptibility test

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on the Mueller-Hinton agar medium, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). A range of common antibiotics have been used, including: Levofloxacin (LEV), azithromycin (AZM), gentamicin (CN), tetracycline (TE), ofloxacin (OFX), chloramphenicol (C), tobramycin (TOB), cefoxetina (FOX), trimethoprim (TMP), and rifampicin (RF). The results of sensitivity were interpreted by classifying isolates as sensitive, medium, or resistant based on the diameters of the inhibition zones (KANDASWAMY et al., 2024).

2.3. Preparation of propolis extract

The raw propolis was obtained from the hives of the honey bees, then cleaned of impurities and ground into a fine powder. Use alcoholic extract to prepare the extract, where the propolis is soaked in ethanol in an appropriate concentration with constant stirring, then the extract is filtered and kept at a low temperature until use (ANIZ, and JASSIM 2024).

2.3.1. High Performance Chromatography Analysis

High Performance Liquid Chromatography (HPLC) was performed for qualitative and quantitative identification of the active phenolic compounds in propolis extract. Use an appropriate stationary phase and a progressive polarity system for the moving phase to allow optimal separation of the target vehicles. Propolis extract was injected into an HPLC device, and retention time and peak area were compared with the standard criteria for caffeic acid, gallic acid, and catechin. Vehicle concentrations were determined based on the calibration curves of standard materials, and results were recorded in parts per million (ppm) (CUSHNIE; LAMB, 2005; DAGLIA, 2012).

2.4. Antibacterial Efficacy Test for Propolis

The antibacterial activity of propolis extract was evaluated using the agar diffusion method, where different concentrations of the extract (12.5, 25, and 50 µg mL⁻¹) were present. *S. aureus* isolates were planted on the Mueller-Hinton agar medium, then propolis concentrations were added, and the dishes were incubated at 37°C for 24 hours. Next, the diameter of the inhibition zones was measured, and the results were recorded in millimeters with standard deviation calculated (JASSIM et al., 2023).

2.4.1. Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC).

MIC and MBC values were determined using the liquid medium sequential dilution method. Sequential concentrations of propolis extract were prepared, then inoculated with the tested bacterial isolates and incubated at 37°C for 24 hours. MIC was defined as the lowest concentration that inhibited visible bacterial growth. In contrast, MBC was defined by sampling growth-free tubules and implanting them on solid food media to determine the lowest concentration capable of killing bacteria completely (COTTICA; BANKOVA, 2021).

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation, and the data were presented using tables to illustrate the isolation ratios, resistance patterns, and efficacy of propolis against different bacterial isolates.

3. RESULTS

During the study, 200 clinical samples were collected and analyzed from various sources, including urine, burns, sputum, skin infections, nasal swabs, blood and genital swabs. The results showed that the percentage of positive isolates for *Staphylococcus aureus* reached 30% of the total samples, with a difference in isolation rates according to the source of the sample (SILVA et al., 2008), as the highest isolation rates were recorded in nasal swabs, followed by sputum and urine samples, while lower percentages were recorded in blood samples and genital swabs, as in Table 1.

The isolates of *S. aureus* were diagnosed based on the implantable traits and standard chemical tests, as the isolates showed positive results for catalysis, coagulation, mannitol fermentation, DNase, blood hemolysis and gelatin degradation, with negative results of the urease test, which confirmed the identity of the bacterial isolates, as shown in Tables 2 and 3 and Figure 1.

Table 1. Distribution of clinical specimens.

Tabela 1. Distribuição das amostras clínicas.

Source	Total Sample (%)	Positive for <i>S. aureus</i> (%)	Another <i>Staphylococcus</i> species (%)	Another bacterial (%)	No Growth (%)
Urine	60	15(25%)	5(8.3%)	15(25%)	25(41.7%)
Burns	25	5(20%)	4(16%)	10(40%)	6(24%)
Sputum	25	7(28%)	5(20%)	10(40%)	3(12%)
Skin infection	25	5(20%)	9(36%)	6(24%)	5(20%)
Nasal swab	35	20(57.1%)	10(28.5%)	5(14.2%)	0
Blood	20	5(25%)	3(15%)	12(60%)	0
Genital swab	10	3(30%)	0	3(30%)	4(40%)
Totally	200	60(30%)	36(18%)	61(30.5%)	43(21.5%)

Table 2. Biochemical test to identify *S. aureus*.

Tabela 2. Teste bioquímico para identificar *S. aureus*.

Diagnostic importance	Result of <i>S. aureus</i>	Biochemical test
Distinguished from <i>Streptococcus</i> bacteria	+	Catalase test
Standard and gold test for <i>S. aureus</i> type confirmation	+	Gouglase test
Its ability to ferment mannitol on an MSA medium distinguishes it from most other species	+	Mannitol Fermentation
It produces an enzyme that breaks down DNA, which is a powerful confirmation test	+	DNase test
It helps to distinguish between some negative types of coagulation accurately	+	Phosphatase test
helps distinguish them from species such as <i>Streptococcus</i> sap	-	Urease test
It has the ability to dilute gelatin	+	Gelatinase test
Its ability to reduce nitrates to nitrite	+	Nitrate reduction test

Table 3. Culture Characteristics of *S. aureus*.

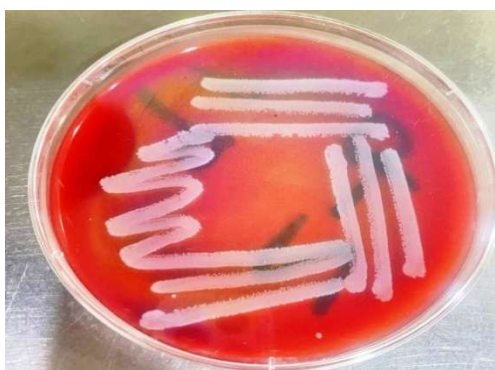
Tabela 3. Características da cultura de *S. aureus*.

No.	Media	Results
1	Blood Agar	Small, round, smooth, golden yellow, raised ,glistening, hemolysis
2	Mannitol salts agar	Yellow colonies

Antibiotic susceptibility test results showed high rates of resistance to some antibiotics, especially tobramycin and cefoxetina, against high levels of sensitivity to other antibiotics, indicating the prevalence of multiple types of

resistance among the isolates studied, as shown in Table 4. The results also showed that resistant isolates were distributed to several different clinical sources, reflecting their prevalence in multiple clinical settings, as shown in Table 5 and Figure 2.

High-performance chromatography analysis (HPLC) of propolis extract showed the presence of active phenolic compounds, including gallic acid, caffeic acid, and catechin, with different concentrations, which supports the vital role of these compounds in the antibacterial activity of propolis, as shown in Figures 3, 4 and 5.



S. aureus on blood agar



S. aureus on Mannitol salts agar

Figure 1. Culture Characteristics of *S. aureus*.

Figura 1. Características do cultivo de *S. aureus*.



Figure 2. Antibiotic sensitivity test (AST).

Figura 2. Teste de sensibilidade a antibióticos (TSA).

Table 4. Antibiotic sensitivity test (AST).

Tabela 4. Teste de sensibilidade a antibióticos (AST).

Antibiotics (n)	R(%)	I(%)	(%)
LEV (50mg)	2	2	96
AZM (50mg)	30	16	54
CN (50g)	6	0	94
TE (50)	36	14	50
OFX (50)	2	2	96
C (50)	4	6	90
TOB (50)	4	0	96
FOX (50)	100	0	0
TMP (50)	22	4	74
RF (50)	20	10	70

Table 5. Source of resistance *S. aureus* isolates.

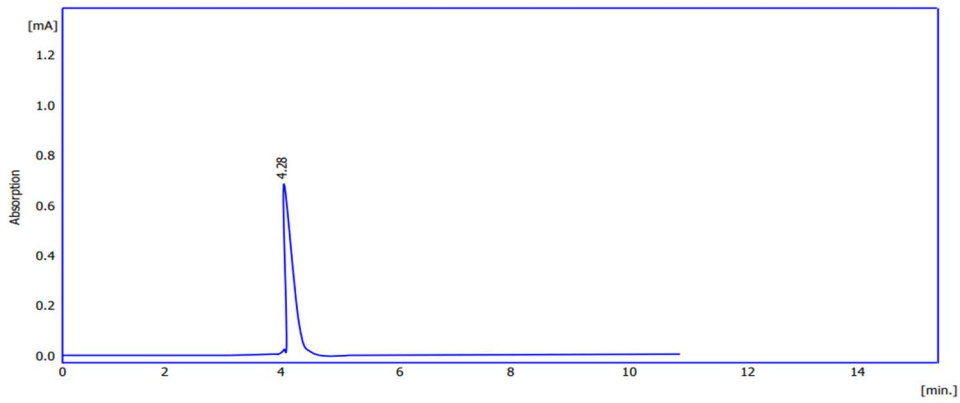
Tabela 5. Fonte de resistência em isolados de *S. aureus*.

Resistance	Isolate	Source of Isolate
Sa 1		Urine
Sa 2		Urine
Sa 3		Sputum
Sa 4		Sputum
Sa 5		Urine
Sa 6		Urine
Sa 7		Urine
Sa8		Urine
Sa 9		Skin swab
Sa 10		Skin swab
Sa 11		Nasal swab
Sa 12		Nasal swab
Sa 13		Nasal swab
Sa 14		Nasal swab
Sa 15		Nasal swab
Sa 16		Nasal swab
Sa 17		Skin swab

HPLC analysis of propolis extract showed the presence of a number of biologically significant active phenolic compounds. Caffeic acid was identified with a retention time of 4.20 min, at a concentration of 27.45 ppm, by conforming to the standard that showed a close retention time (4.28 minutes). The analysis also showed the presence of gallic acid with a retention time of 3.91 min and a high concentration of 632.40 ppm compared to the standard (3.95 min). In addition, Catechin was detected with a retention time of 7.08 min at a concentration of 98.0 ppm, confirming the richness of propolis extract in bioactive phenolic compounds.

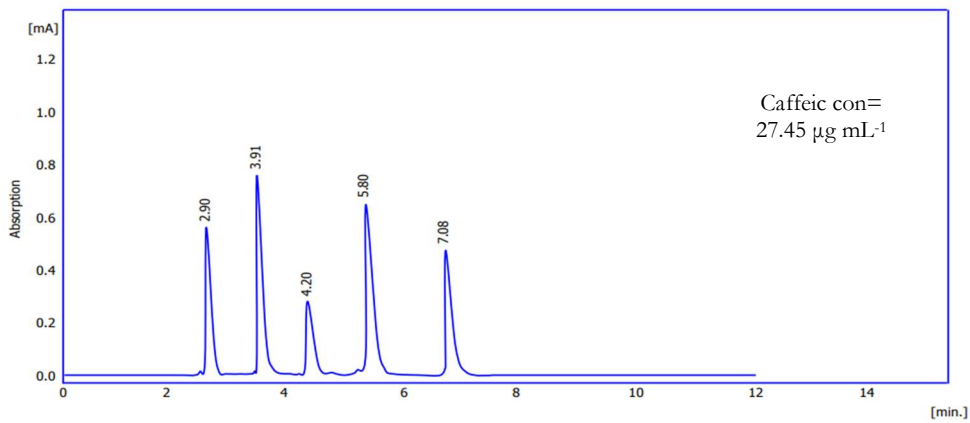
The results of the antibacterial efficacy test of propolis extract showed a clear inhibitory effect against most isolates of *Staphylococcus aureus*, as the diameters of the inhibition zones increased with increasing concentration of the extract, with a variation in the response of different isolates, as shown in Table 6 and Figure 6.

The results of the determination of the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) also showed that propolis extract has a high efficacy at relatively low concentrations, where the MIC values ranged between 1.56 and 25 µg/ml. In contrast, the MBC values ranged between 3.125 and 50 µg/ml, indicating its ability to inhibit and kill bacterial cells, as shown in Table 7.



Caffeic acid stander

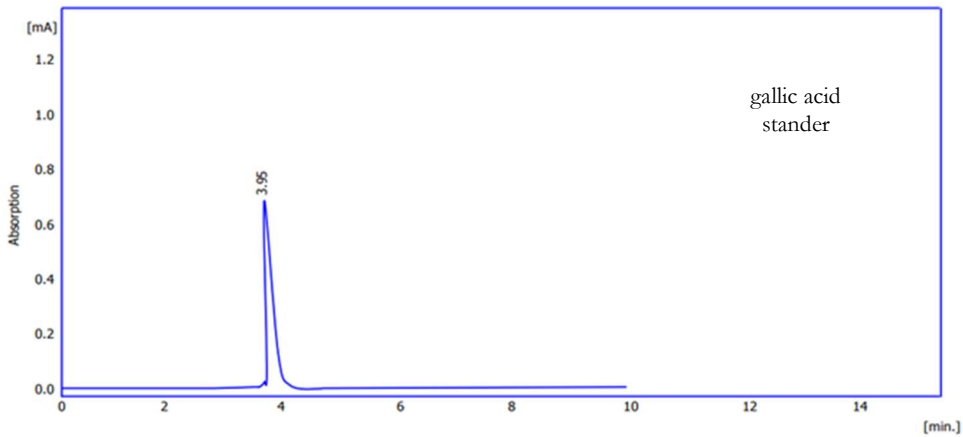
Caffeic acid stander	No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
	1	4.28	2047.98	640.58	100.00	100.00	0.25	
		Total	2047.98	640.58	100.00	100.00		



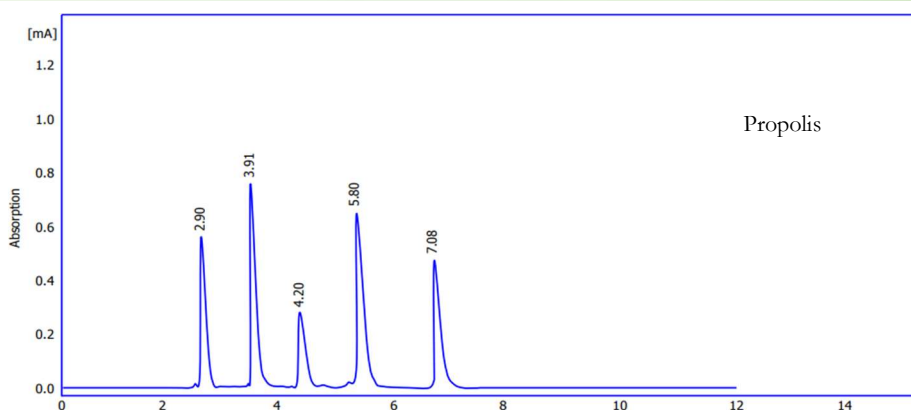
Propolis

Propolis extract	No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
	1	2.90	85462.08	580.98	20.00	20.00	0.15	
	2	3.91	92145.98	785.98	25.00	25.00	0.20	
	3	4.20	56234.00	350.64	15.00	15.00	0.10	
	4	5.80	65874.19	590.11	20.00	20.00	0.20	
	5	7.08	58842.65	460.59	20.00	20.00	0.20	
		Total	358558.35	2768.59	100.00	100.00		

Figure 3. HPLC analysis for caffeic acid in propolis.
 Figura 3. Análise por HPLC do ácido cafeico no própolis.



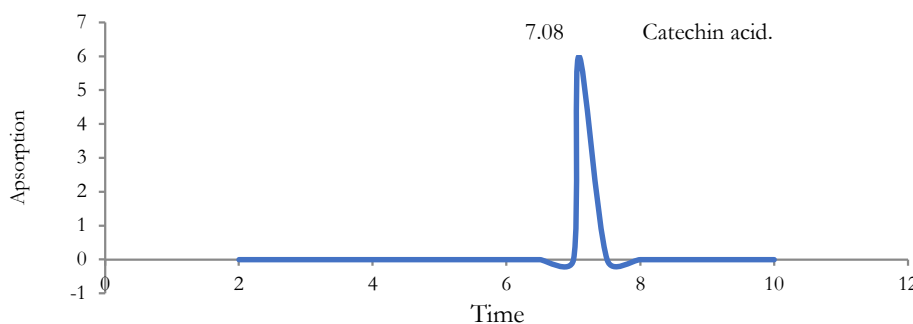
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	3.95	1457.08	701.45	100.00	100.00	0.25	
	Total	1457.08	701.45	100.00	100.00		



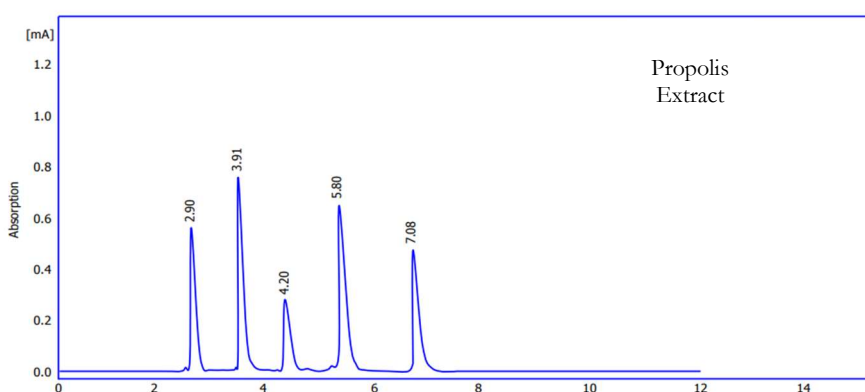
Con. of gallic acid = 632.40 $\mu\text{g mL}^{-1}$

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.90	85462.08	580.98	20.00	20.00	0.15	
2	3.91	92145.98	785.98	25.00	25.00	0.20	
3	4.20	56234.00	350.64	15.00	15.00	0.10	
4	5.80	65874.19	590.11	20.00	20.00	0.20	
5	7.08	58842.65	460.59	20.00	20.00	0.20	
Total		358558.35	2768.59	100.00	100.00		

Figure 4. HPLC detection of gallic acid.
 Figura 4. Detecção de ácido gálico por HPLC.



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]
1	7.08	1697.84	605.25	100.00	100.00	0.25
Total		1697.84	605.25	100.00	100.00	



Catechin Concentration (con.) = 98.0 ppm

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.90	85462.08	580.98	20.00	20.00	0.15	
2	3.91	92145.98	785.98	25.00	25.00	0.20	
3	4.20	56234.00	350.64	15.00	15.00	0.10	
4	5.80	65874.19	590.11	20.00	20.00	0.20	
5	7.08	58842.65	460.59	20.00	20.00	0.20	
Total		358558.35	2768.59	100.00	100.00		

Figure 5. HPLC detection of Catechin acid.
 Figura 5. Detecção de ácido gálico por HPLC.

Table 6. Antimicrobial effect of bee propolis.
Tabela 6. Efeito antimicrobiano da própolis de abelha.

Bacterial isolates	Concentration $\mu\text{g mL}^{-1} \pm \text{SD}$		
	12.5	25	50
Sa 1	15± 3.2	18±2.3	17±3.1
Sa 2	17±	19±2.3	20±4.2
Sa 3	19±3.1	15±4.2	12±1.5
Sa 4	16±	17±1.7	15±3.8
Sa 5	15±3.1	16±3.5	17±3.1
Sa 6	0	0	0
Sa 7	26±3.1	25±	24±0.9
Sa 8	14±	15±2.2	15±3.1
Sa 9	16±2.6	14±	15±2.6
Sa 10	15±	17±0.9	15±0.4
Sa 11	17 ±	15±3.1	24±2.5
Sa 12	20± 2.5	20±2.5	20±3.1
Sa 13	16± 2.6	16±3.1	15±3.1
Sa 14	18±2.2	16±0.4	15±2.2
Sa 15	18±0.4	18±2.5	17±0.4
Sa 16	18±2.2	15±0.4	19±2.2
Sa 17	15±2.5	15±2.6	18±2.6

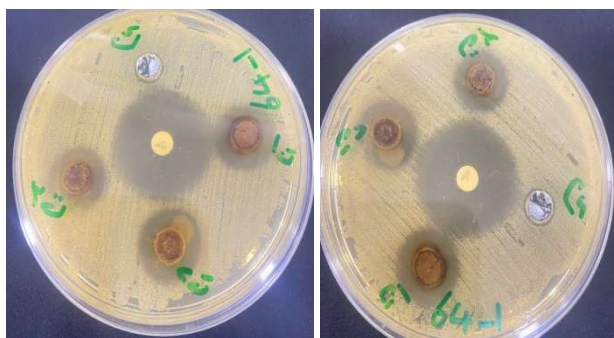


Figure 6. Antimicrobial effect of bee propolis.
Figura 6. Efeito antimicrobiano da própolis de abelha.

4. DISCUSSION

The results of the current study showed that *Staphylococcus aureus* was one of the most isolated bacterial species from

various clinical samples, with a percentage of 30% of the total samples, which is consistent with several studies that indicated the widespread prevalence of this bacterium in community-acquired infections and hospitals. This is due to their high ability to colonize, especially in nasal and dermal swabs, as well as their possession of a wide range of virulence factors (ANDREWS, 2021; CLSI, 2023).

Antibiotic susceptibility test results showed high levels of resistance to certain antibiotics, particularly topramycin and cefoxetine, suggesting the presence of multi-resistant isolates and possibly methicillin-resistant strains (MRSA). This finding is clinically alarming, as it limits available treatment options and underscores the urgent need for new treatment alternatives or support materials to conventional antibiotics (OTTO, 2018; ANDREWS, 2021; CLSI, 2023).

Propolis extract showed clear anti-inflammatory activity against most tested *S. aureus* isolates, with the diameters of the inhibition zones recording marked increases as the propolis concentration increased, demonstrating a direct relationship between concentration and antibacterial efficacy. However, variation in the response of different isolates has been observed, which may be due to differences in genetic structure and resistance mechanisms between bacterial isolates (TONG et al., 2015; CHAMBERS; DELEO, 2021).

The results of the determination of the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) also showed that propolis is highly effective at relatively low concentrations, with MIC values ranging from 1.56 to 25 $\mu\text{g mL}^{-1}$. In contrast, MBC values ranged from 3.125 to 50 $\mu\text{g mL}^{-1}$. These values indicate that the ability of propolis to inhibit is due to its rich chemical composition of phenolic compounds and flavonoids, which disrupt the bacterial cell wall and membrane, inhibit bioenzymes, affect cell membrane permeability and inhibit the formation of biofilms. Previous studies have shown that propolis has a higher activity against Gram-positive bacteria, including *S. aureus*, than Gram-negative bacteria, due to the simplicity of the cell wall structure in Gram-positive bacteria. Not only inhibit bacterial growth, but also kill bacterial cells at higher concentrations (WHO, 2014).

Table 7. MIC and MBC concentration of Bee Propolis.
Tabela 7. Concentração de CIM e CBM da própolis de abelha.

Isolate code	MBC ($\mu\text{g mL}^{-1}$)	MIC ($\mu\text{g mL}^{-1}$)	1.56 $\mu\text{g mL}^{-1}$	3.125 $\mu\text{g mL}^{-1}$	6.25 $\mu\text{g mL}^{-1}$	12.5 $\mu\text{g mL}^{-1}$	25 $\mu\text{g mL}^{-1}$	50 $\mu\text{g mL}^{-1}$
Sa 1	3.125	1.56	-	+	+	+	+	+
Sa 2	6.25	3.125	+	-	+	+	+	+
Sa 3	6.25	3.125	+	-	+	+	+	+
Sa 4	12.5	6.25	+	+	-	+	+	+
Sa 5	6.25	3.125	+	-	+	+	+	+
Sa 6	12.5	6.25	+	+	-	+	+	+
Sa 7	50	25	+	+	+	+	-	+
Sa 8	50	25	+	+	+	+	-	+
Sa 9	25	12.5	+	+	+	-	+	+
Sa 10	3.125	1.56	-	+	+	+	+	+
Sa 11	50	25	+	+	+	+	-	+
Sa 12	12.5	6.25	+	+	-	+	+	+
Sa 13	3.125	1.56	-	+	+	+	+	+
Sa 14	50	25	+	+	+	+	-	+
Sa 15	25	12.5	+	+	+	-	+	+
Sa 16	25	12.5	+	+	+	-	+	+
Sa 17	50	25	+	+	+	+	-	+

Table 8. Pearson correlation analysis between antibacterial parameters.

Tabela 8. Análise de correlação de Pearson entre parâmetros antibacterianos.

Correlated Parameters	Pearson's r	p-value	Interpretation
Propolis concentration vs. Inhibition zone diameter	+0.72	< 0.05	Significant positive correlation
Inhibition zone diameter vs. MIC	-0.68	< 0.05	Significant negative correlation
Inhibition zone diameter vs. MBC	-0.61	< 0.05	Significant negative correlation
MIC vs. MBC	0.83+	0.01 >	Highly significant positive

The antibacterial efficacy shown by propolis extract in this study is due to the fact that it contains active phenolic compounds, in particular gallic acid, caffeic acid, and catechin, which have been confirmed and their concentrations have been confirmed using HPLC technology. These compounds are compounds known to inhibit the growth of Gram-positive bacteria, including *S. aureus*.

Gallic acid is known for its powerful antibacterial and antioxidant properties, as it disrupts the permeability of the bacterial cell membrane and inhibits the enzymatic systems necessary for cell survival. Caffeic acid is also known for its ability to inhibit gene transcription and bacterial cell growth, as well as its role in attenuating virulence factors. Catechins disrupt the bacterial cell wall and affect the formation of biofilms, an important resistance mechanism in *S. aureus* (ALQAHTANI et al 2024).

Accordingly, the presence of these compounds in significant concentrations in propolis extract explains the low values of minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) recorded in this study, and supports the potential for use of propolis as a promising natural source of antibacterial compounds, especially against antibiotic-resistant strains. The strong antibacterial activity of propolis may be attributed to its high gallic acid content (KARAGUL et al., 2024).

Accordingly, the results of this study confirm that propolis is a promising natural substance that can contribute to reducing the problem of bacterial resistance, whether by using it as an adjuvant therapeutic compound or as a starting point for the development of new pharmaceutical compounds of natural origin.

5. CONCLUSIONS

This study confirms that *Staphylococcus aureus* isolated from various clinical samples exhibits high levels of resistance to several commonly used antibiotics, emphasizing the urgent need for alternative antimicrobial strategies.

Bee propolis extract demonstrated significant antibacterial activity against most tested *S. aureus* isolates, as reflected by clear inhibition zones and low MIC and MBC values. HPLC analysis revealed that this activity is strongly associated with the presence of bioactive phenolic compounds, particularly gallic acid, caffeic acid, and catechin.

These findings suggest that bee propolis represents a promising natural antimicrobial agent that may serve as an effective alternative or adjunct to conventional antibiotics in combating antibiotic-resistant *S. aureus*.

6. REFERENCES

AKSOY, C. S.; AVCI, F. G.; UGUREL, O. M.; ATAS, B.; SAYAR, N. A.; AKBULUT, B. S. Potentiating the activity of berberine for *Staphylococcus aureus* in a

- combinatorial treatment with thymol. **Microbial Pathogenesis**, v. 149, e104542, 2020. <https://doi.org/10.1016/j.micpath.2020.104542>
- ALARJANI, K. M.; YEHA, H. M.; BADR, A. N.; SALMA ALI, H.; AL-MASOUD, A. H.; ALHAQBANI, S. M.; ALKHATIB, S. A.; RADY, A. M. Anti-MRSA and biological activities of propolis concentrations loaded to chitosan nanoemulsion for pharmaceutical applications. **Pharmaceutics**, v. 15, n. 10, e2386, 2023. <https://doi.org/10.3390/pharmaceutics15102386>
- AL-MAMOORI, M. H.; ALQARAWEE, H. S.; OKBAGABIR, S. G.; PAKINA, E. N.; ZARGAR, M.; CHUDINOVA, E. Green synthesis of nano-propolis extract and its biological effectiveness in inhibitory growth of phytopathogenic fungi. **Basrah Journal of Agricultural Sciences**, v. 38, n. 2, p. 277-290, 2025. <https://doi.org/10.37077/25200860.2025.38.2.20>
- ALQAHTANI, M.; ALMUKAINZI, M.; ALGHORIBI, M. F.; EL-MAHDY, A. M. Antivirulence effects of trans-resveratrol and curcumin on methicillin-resistant *Staphylococcus aureus* (MRSA) from Saudi Arabia: **Life**, v. 14, n. 4, e491, 2024. <https://doi.org/10.3390/life14040491>
- ANDREWS, J. M. Determination of minimum inhibitory concentrations. **Journal of Antimicrobial Chemotherapy**, v. 76, n. suppl_1, p. i5-i16, 2021. https://doi.org/10.1093/jac/48.suppl_1.5
- ANIZ, E.H.S. JASSIM Y.A. . Anticancer activity of pyoverdine (PVD) producing by antibiotic-resistant *Pseudomonas aeruginosa* isolated from burn and wound infections. **Journal of Applied and Natural Science**, 16(2), 777 - 785.2024 <https://doi.org/10.31018/jans.v16i2.5506>
- CHAMBERS, H. F.; DELEO, F. R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. **Nature Reviews Microbiology**, v. 19, n. 3, p. 629-649, 2021. <https://doi.org/10.1038/nrmicro2200>
- CLSI_Clinical and Laboratory Standards Institute. **Performance standards for antimicrobial susceptibility testing - M100**. 33rd ed. Malvern, PA: CLSI, 2023. 436p.
- COTTICA, D. L.; BANKOVA, V. Propolis chemical diversity and standardization challenges. **Phytotherapy Research**, v. 35, n. 6, p. 3105-3124, 2021. <https://doi.org/10.1007/s11101-022-09816-1>
- CUSHNIE, T. P. T.; LAMB, A. J. Antimicrobial activity of flavonoids. **International Journal of Antimicrobial Agents**, v. 26, n. 5, p. 343-356, 2005. <https://doi.org/10.1016/j.ijantimicag.2005.09.002>
- DAGLIA, M. Polyphenols as antimicrobial agents. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 174-181, 2012. <https://doi.org/10.1016/j.copbio.2011.08.007>
- JASSIM, Y. A. AL-YASSIRY Z.A., AWADH E.F.A. . Study of antibacterial activity and cytotoxicity of the bioactive

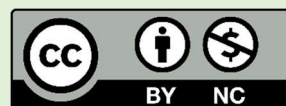
- compound of *Bacillus megaterium* L2 strains isolated from the oral cavity of hospital workers and visitors at Dental Health Centre, Babylon, Iraq. **Journal of Applied and Natural Science**, 15(1), 371 - 378. 2023. <https://doi.org/10.31018/jans.v15i1.4313>
- KANDASWAMY, K.; PANDA, S. P.; SUBRAMANIAN, R.; KHAN, H.; SHAIK, M. R.; HUSSAIN, S. A.; GURU, A.; AROCKIARAJ, J. Synergistic berberine chloride and curcumin-loaded nanofiber therapies against methicillin-resistant *Staphylococcus aureus* infection: Augmented immune and inflammatory responses in zebrafish wound healing. **International Immunopharmacology**, v. 140, e112856, 2024. <https://doi.org/10.1016/j.intimp.2024.112856>
- KARAGUL, B.; UGRAS, S.; KARAGUL, P.; MEHTAP, U.; UGRAS, H. I. Analysis and evaluation of quality parameters of commercial propolis products using a new high-performance liquid chromatography (HPLC) method and comparison of antimicrobial properties. **Microbiological Research**, v. 204, e111023, 2024. <https://doi.org/10.1016/j.microc.2024.111023>
- KHDHAIR, O. A.; JASSIM, Y. A.; ALKREMY, N. A. A.; AL-AMEEDY, T. H. Antibacterial activity of the alcoholic extract of berberine against *Staphylococcus aureus* isolated from burn and wound infections. **Review of Clinical Pharmacology and Pharmacokinetics**, v. 38, n. Sup2, p. 137-139, 2024. <https://doi.org/10.61873/FMPT5825>
- OTTO, M. Staphylococcal biofilms: Mechanisms and traits. **Microbiology Spectrum**, v. 6, n. 4, p. 1-17, 2018. <https://doi.org/10.1128/microbiolspec.gpp3-0023-2018>
- SANG, H.; FENG, K.; JU, Y.; SUN, Y.; ZHANG, Y.; XUAN, H.; WANG, F. Propolis exerts antibiofilm activity against methicillin-resistant *Staphylococcus aureus* by modulating gene expression to suppress adhesion. **Microorganisms**, v. 13, n. 12, e2810, 2025. <https://doi.org/10.3390/microorganisms13122810>
- SILVA, B. B.; ROSALEN, P. L.; CURY, J. A.; IKEGAKI, M.; SOUZA, V. C.; ESTEVES, A.; ALENCAR, S. M. Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. **Evidence-Based Complementary and Alternative Medicine**, v. 5, e380385, 2008. <https://doi.org/10.1093/ecam/nem059>
- TONG, S. Y. C.; DAVIS, J. S.; EICHENBERGER, E.; HOLLAND, T. L.; FOWLER, V. G. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. **Clinical Microbiology Reviews**, v. 28, n. 3, p. 23-28, 2015. <https://doi.org/10.1128/cmr.00134-14>
- WANG, F.; YUAN, J.; LI, J.; LIU, H.; WEI, F.; XUAN, H. Antibacterial activity of Chinese propolis and its synergy with β -lactams against methicillin-resistant *Staphylococcus aureus*. **Brazilian Journal of Microbiology**, v. 53, n. 4, p. 1789-1797, 2022. <https://doi.org/10.1007/s42770-022-00807-0>
- WHO World Health Organization. **Antimicrobial resistance: global report on surveillance**. Geneva: WHO, 2014. 256p. Available online: <https://iris.who.int/server/api/core/bitstreams/a04b4607-0445-4aed-975b-6c31c8a3077e/content>. Accessed on 27 Apr. 2026.
- ZHOU, F.; GU, X.; WANG, W.; LIN, M.; WANG, L. Advancements in MRSA treatment: The role of berberine in enhancing antibiotic therapy. **BMC Microbiology**, v. 24, e540, 2024. <https://doi.org/10.1186/s12866-024-03692-9>

Acknowledgments: Despite its promising in vitro antibacterial activity, further in vivo studies are required to evaluate the safety, efficacy, and pharmacological properties of bee propolis. Additional research is also needed to elucidate the molecular mechanisms of action of its phenolic compounds, assess extract standardization, and explore potential synergistic effects with conventional antibiotics to enhance therapeutic outcomes and reduce antimicrobial resistance.

Authors' contributions: Y.A.J.: responsible for the ideas, investigation or data collection, statistical analysis, administration or supervision, writing (original draft) and writing (review and editing). : is the contribution responsible for the critical review of the work, before and after publication, in addition to preparing the presentation of the work for the original research group. Z.G.Y.: acquisition of financing and investigation or data collection. All authors read and agreed to the published version of the manuscript.

Data availability: Dataset available on request from the corresponding authors.

Conflict of interest: The authors declare that they have no conflict of interest.



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