



Harnessing Nigerian Propolis for their Potentials as Novel Lead Antimicrobial Agents against Some Human Pathogenic Microorganisms

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ABSTRACT:

The growing threat of antimicrobial resistance (AMR) poses a significant challenge to global public health, with limited treatment options available for infections caused by multidrug-resistant pathogens. The overuse and misuse of antibiotics have accelerated the emergence of resistant strains, necessitating the exploration of novel antimicrobial agents from natural sources. Nigerian propolis, a resinous substance collected by honeybees, offers a promising solution due to its broad-spectrum antimicrobial activity, but its potential remains underexplored and warrants further scientific investigation. This study employed a laboratory-based experimental design to investigate the antimicrobial potential of Nigerian propolis. Propolis samples were collected from apiaries in Dutse, Jigawa State, Nigeria, and authenticated by a taxonomist. The samples were extracted using methanol, and the resulting extract was subjected to phytochemical profiling and antimicrobial activity assessment against common human pathogenic bacteria using the agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth micro dilution. Phytochemical analysis of Nigerian Propolis revealed a rich composition of bioactive compounds, including saponins, flavonoids, cardiac glycosides, alkaloids, and tannins. Antimicrobial activity was evaluated against four bacterial strains, with Propolis demonstrating notable inhibitory effects at 400 mg/ml. MIC and MBC evaluations revealed bacteriostatic and bactericidal properties. GC-MS analysis identified key bioactive compounds, including phenol derivatives, benzyl alcohol, and fatty acids, which contribute to Propolis's multifaceted therapeutic effects. These findings support Propolis's potential as an alternative antimicrobial agent. This study highlights Nigerian Propolis's potential as a natural antimicrobial agent, exhibiting concentration-dependent activity against Gram-positive bacteria. Phytochemical analysis revealed bioactive compounds contributing to its therapeutic effects. The findings suggest Propolis as a viable alternative to synthetic antimicrobials, warranting further research to optimize its formulations and potential synergistic therapeutic approaches.

Keywords: Propolis, Antimicrobial agents, Nigerian propolis, Human pathogenic microorganisms and Natural antimicrobials

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INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a global health crisis, threatening the efficacy of existing drugs and rendering many infections increasingly difficult to treat (Mondal *et al.*, 2024). This phenomenon has been exacerbated by the indiscriminate use of antibiotics and

antifungal agents, which has led to the development of resistant strains of pathogens. The World Health Organization (WHO) has highlighted the urgent need for novel antimicrobial agents that can combat resistant bacteria, fungi, and viruses (Elshobary *et al.*, 2025).

Traditional medicine, especially the use of natural products, offers promising avenues for discovering new antimicrobial agents. Among these, bee products, particularly propolis, have been shown to possess a wide array of biological activities, including antimicrobial properties (Marcucci, 1995). Propolis, a resinous substance collected by honeybees from tree buds, sap flows, and other botanical sources, has been utilized in traditional medicine for centuries. It is known for its antimicrobial, antiviral, anti-inflammatory, and antioxidant properties (Sforcin, 2007). While propolis is found in various parts of the world, the composition and bioactivity of propolis can vary significantly based on the botanical sources available in the region where it is collected (Ghisalberti, 1979). In Nigeria, where traditional medicine plays a significant role in healthcare, propolis has been used to treat a wide range of diseases, including infections, wounds, and inflammations (Olawuyi *et al.*, 2018). However, the antimicrobial potential of Nigerian propolis remains underexplored and warrants further scientific investigation.

This study aims to evaluate the antimicrobial potential of Nigerian propolis, particularly focusing on its efficacy against common human pathogenic microorganisms, including bacteria, fungi, and viruses. The exploration of propolis sourced from different Nigerian regions could unveil unique bioactive compounds that may serve as lead candidates for the development of novel antimicrobial agents. By identifying the antimicrobial activity of Nigerian propolis, we seek to contribute to the growing body of evidence supporting the use of bee products in modern medicine.

Propolis is a complex mixture composed of resins, beeswax, essential oils, pollen, and other organic compounds. The primary components responsible for its bioactivity are flavonoids, phenolic acids, terpenoids, and aromatic aldehydes (Shah *et al.*, 2013). These compounds exhibit broad-spectrum antimicrobial activities, including bactericidal and bacteriostatic effects against both Gram-positive and Gram-negative bacteria, as well as antifungal and antiviral properties (Nogueira *et al.*, 2015). The antimicrobial mechanism of action of propolis is believed to involve interference with the integrity of microbial cell membranes, inhibition of nucleic acid synthesis, and modulation of the microbial metabolism (Sforcin *et al.*, 2008).

Flavonoids, one of the most abundant groups of compounds in propolis, have been reported to possess antimicrobial, anti-inflammatory, and antioxidant properties (Berdowska *et al.*, 2019). Some of the most prominent flavonoids identified in propolis include pinocembrin, galangin, quercetin, and kaempferol, all of which have been shown to exhibit antimicrobial activity against a wide range of pathogens, including those that are resistant to conventional antibiotics (Ajao *et al.*, 2015). In addition to flavonoids, phenolic acids such as caffeic acid and ferulic acid contribute significantly to the antimicrobial properties of propolis (Li *et al.*, 2013).

The antimicrobial effects of propolis are not limited to bacterial pathogens. Several studies have demonstrated its efficacy in combating fungal infections, including those caused by *Candida* spp., *Aspergillus* spp., and dermatophytes (Oliveira *et al.*, 2016). Moreover, propolis has been shown to inhibit viral replication in vitro, including its activity against herpes simplex virus (HSV), human immunodeficiency virus (HIV), and influenza virus (Nawrot *et al.*, 2012). Given the broad-spectrum antimicrobial properties of propolis, it holds great promise as a potential lead compound for developing new antimicrobial agents. Nigeria, with its diverse flora and fauna, offers a unique opportunity to explore propolis derived from different geographic regions. The composition and antimicrobial properties of propolis can vary depending on the plant species from which the bees collect their resins. In Nigeria, bee species such as *Apis mellifera* and *A. cerana* are commonly found, and these species produce propolis with varying chemical profiles depending on the local flora (Olawuyi *et al.*, 2018).

Research on Nigerian propolis is limited, but some studies have suggested that it may exhibit significant antimicrobial activity against human pathogenic microorganisms. For instance, a study by Olawuyi *et al.* (2018) demonstrated that Nigerian propolis exhibited potent antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. Similarly, propolis from different regions of Nigeria has shown antifungal activity against *Candida albicans* and *Aspergillus niger* (Ajao *et al.*, 2015). The antimicrobial activity of Nigerian propolis is thought to be attributed to its high content of flavonoids, phenolic acids, and other bioactive compounds that are derived from the rich plant diversity found in the country.

The variation in the chemical composition of Nigerian propolis, based on geographic location and local flora, makes it an interesting subject of study. Propolis samples collected from different regions of Nigeria may contain unique bioactive compounds that contribute to their antimicrobial properties. Understanding the regional differences in the composition and activity of Nigerian propolis could provide valuable insights into its potential as a source of novel antimicrobial agents.

The growing threat of antimicrobial resistance (AMR) poses a significant challenge to global public health, particularly in the treatment of infections caused by multidrug-resistant (MDR) pathogens. According to the WHO, AMR is responsible for an estimated 700,000 deaths annually, and this number is projected to increase significantly if effective interventions are not implemented (WHO, 2020). The overuse and misuse of antibiotics, coupled with the slow pace of new drug discovery, have accelerated the emergence of resistant strains of bacteria, fungi, and viruses (Laxminarayan *et al.*, 2013).

The search for novel antimicrobial agents is critical to combating AMR. Natural products, including those derived from plants, fungi, and animals, have historically been a rich source of bioactive compounds with antimicrobial

properties (Cragg *et al.*, 2005). Propolis, as a natural product with broad-spectrum antimicrobial activity, offers a promising solution to this global health crisis. Its ability to target a wide range of pathogens, including those that are resistant to conventional antibiotics, makes it a potential lead candidate for the development of new antimicrobial agents (Zhao *et al.*, 2020).

In addition to its antimicrobial properties, propolis has been shown to possess immunomodulatory, anti-inflammatory, and wound-healing effects, which further enhance its therapeutic potential (Sforcin *et al.*, 2008). These properties make propolis an attractive candidate for use in combination therapies, where it could enhance the efficacy of existing antibiotics and reduce the development of resistance. The growing threat of antimicrobial resistance necessitates the exploration of novel antimicrobial agents from natural sources. Propolis, particularly Nigerian propolis, holds great promise as a source of bioactive compounds with antimicrobial activity against a wide range of human pathogens. By evaluating the chemical composition and antimicrobial properties of Nigerian propolis, this study seeks to contribute to the development of new antimicrobial agents that can combat the global health challenge of AMR.

MATERIALS AND METHODS

Collection and authentication of propolis

Propolis samples were collected from apiaries located in Dutse, Jigawa State, Nigeria, in October 2023. These apiaries were chosen for their pristine environment and minimal exposure to industrial pollutants, ensuring a high-quality raw material for analysis. Samples were authenticated by a taxonomist from the Department of Botany, Federal University Dutse, using morphological and microscopic techniques. After authentication, visible impurities such as beeswax, wood fragments, and dirt were removed by washing with distilled water. The cleaned samples were stored in airtight containers at 4°C to prevent oxidation and degradation of active constituents before further processing.

Sample preparation

Propolis samples were air-dried in a well-ventilated room with controlled temperature (25–30°C) and humidity (50–60%) for 14 days. Direct sunlight was avoided to preserve the bioactive compounds, particularly polyphenols, which are sensitive to UV light. Dried propolis samples were ground into fine powder using a sterilized electric grinder. The powder was sieved through a 40-mesh sieve to achieve uniform particle size, which aids in efficient extraction. The powdered samples were stored in amber glass jars at 4°C to maintain stability and prevent photodegradation.

Extraction of bioactive compounds

Fifty grams (50 g) of the powdered propolis was subjected to maceration in 200 mL of methanol. The mixture was allowed to stand at room temperature (25–30°C) for 72 hours with intermittent shaking every 12 hours to enhance the extraction of bioactive compounds. After 72 hours, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was collected. The methanol solvent was removed under reduced pressure using a rotary evaporator at 40°C to prevent the thermal degradation of heat-sensitive compounds. The resulting concentrated extract was stored in amber bottles at 4°C until further analysis.

Quantitative phytochemical profiling of propolis

Quantitative phytochemical profiling was conducted to determine the concentrations of flavonoids, cardiac glycosides, alkaloids, saponins, and tannins in the propolis extract using standardized methods. Flavonoid content was measured using the aluminum chloride colorimetric method, where a 0.5 mL aliquot of the extract was mixed with 2 mL of 2% aluminum chloride in methanol, incubated for 30 minutes, and analyzed at 415 nm, with results expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). Cardiac glycosides were quantified via the Baljet's colorimetric method by mixing 1 mL of the extract with 5 mL of Baljet's reagent, incubating for 1 hour, and measuring absorbance at 495 nm, with results expressed as milligrams of digitoxin equivalents per gram of extract (mg DE/g). Alkaloid content was determined gravimetrically by dissolving 5 g of the extract in 10% acetic acid, precipitating with ammonium hydroxide, and drying to constant weight, with values expressed as a percentage of extract weight. Saponins were quantified using the vanillin-sulfuric acid method, where 0.5 mL of extract was reacted with 0.5 mL of vanillin reagent and 5 mL of sulfuric acid, incubated at 60°C, and analyzed at 544 nm, with results expressed as milligrams of diosgenin equivalents per gram of extract (mg DE/g). Tannin content was analyzed using the Folin-Denis method by reacting 0.5 mL of extract with Folin-Denis reagent and sodium carbonate, incubating for 30 minutes, and measuring absorbance at 760 nm, with results expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g). All assays were performed in triplicate, and results were statistically analyzed to ensure reliability.

Antimicrobial activity assessment

Test microorganisms

The antimicrobial efficacy of the propolis extract was tested against common human pathogenic bacterial strains: *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Klebsiella spp.* (Gram-negative), and *Pseudomonas aeruginosa* (Gram-negative).

These strains were obtained from clinical isolates provided by a certified microbiology laboratory. Bacterial identification was confirmed using colony morphology on selective media, Gram staining, and standard biochemical tests, such as catalase, coagulase, and oxidase tests.

Agar well diffusion method

The antibacterial activity of the methanolic propolis extract was evaluated using the agar well diffusion method (Kavanagh, 1998). Mueller-Hinton agar plates were prepared and inoculated with bacterial suspensions standardized to 1×10^6 CFU/mL. Wells of 6 mm diameter were bored into the agar using a sterile cork borer. Each well was filled with 100 μ L of propolis extract at concentrations ranging from 50 mg/mL to 400 mg/mL. Gentamicin (10 μ g/mL) served as a positive control, while methanol was used as a negative control. Plates were incubated at 37°C for 24 hours, and the zones of inhibition (clear areas around the wells) were measured using a Vernier caliper. Results were recorded in millimeters (mm).

Determination of MIC and MBC

Minimum inhibitory concentration (MIC).

The MIC of the propolis extract was determined using the broth microdilution method. Propolis extract was serially diluted in Mueller-Hinton broth to concentrations ranging from 25 mg/mL to 400 mg/mL. Each dilution was inoculated with 100 μ L of standardized bacterial suspension (1×10^6 CFU/mL) and incubated at 37°C for 24 hours. The lowest concentration that exhibited no visible turbidity (clear solution) was recorded as the MIC.

Minimum bactericidal concentration (MBC)

Following the MIC determination, 100 μ L from each clear tube was inoculated onto Mueller-Hinton agar plates and incubated at 37°C for 24 hours. The lowest concentration that showed no bacterial growth on the agar plate was recorded as the MBC, indicating complete bactericidal activity.

GC-MS analysis of Propolis extract

The chemical composition of the methanolic extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The chemical composition of the methanolic propolis extract was determined using Gas Chromatography-Mass Spectrometry (GC-MS) on a Shimadzu GC-MS-QP2020 instrument equipped with a DB-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min, with the injector temperature set at 250°C. The oven temperature was programmed to begin at 50°C (held for 2 minutes), increase to 280°C at a rate of

10°C/min, and remain at 280°C for 10 minutes. A 1 μ L aliquot of the sample was injected at a split ratio of 10:1. Compounds in the extract were identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST) library database, and their relative abundances were calculated as percentages of the total ion current.

Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS version 26.0. One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test was employed to determine significant differences between groups, with significance set at $p < 0.05$. Graphs and tables were used to present the data.

Phytochemical composition of Propolis methanol extract

The quantitative analysis of Propolis extract revealed the presence of significant levels of bioactive compounds, each contributing to its therapeutic potential. Saponins were the most abundant at 3.71 mg/100g, followed by flavonoids at 2.44 mg/100g, which are renowned for their strong antioxidant properties. Cardiac glycosides (2.41 mg/100g) and alkaloids (1.88 mg/100g) were also present in considerable amounts, while tannins were found at 1.38 mg/100g. These compounds are associated with various biological activities, including antimicrobial, anti-inflammatory, and cardio-protective effects. The dominance of saponins suggests their potential in immune modulation and antimicrobial action, while the high flavonoid content indicates a strong capability to scavenge free radicals, reducing oxidative stress. Alkaloids and tannins add to the antimicrobial and anti-inflammatory activities. Collectively, this phytochemical composition underscores the potential of Propolis as a natural therapeutic agent, suitable for addressing oxidative stress-related diseases and infections (Table 1).

Table 1: Quantitative phytochemical constituent of Propolis extract.

Phytochemical Constituent Propolis Extract (mg/100g)	
Flavonoids	2.44 $\pm$ 0.02
Cardiac Glycosides	2.41 $\pm$ 0.01
Alkaloids	1.88 $\pm$ 0.01
Saponins	3.71 $\pm$ 0.03
Tannins	1.38 $\pm$ 0.01

Antimicrobial activity

The methanolic extract of Propolis demonstrated concentration-dependent antimicrobial activity against four

Table 2: Propolis methanolic extract: zone of inhibition (Mean $\pm$ SE).

Test Organism	Gentamicin (10 μ g)	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml
<i>Escherichia coli</i>	41.00 $\pm$ 1.00	31.50 $\pm$ 1.50	23.00 $\pm$ 1.00	17.50 $\pm$ 0.50	6.00 $\pm$ 0.00
<i>Klebsiella</i> spp.	40.00 $\pm$ 0.00	33.50 $\pm$ 1.50	20.00 $\pm$ 0.00	16.00 $\pm$ 0.00	6.00 $\pm$ 0.00
<i>Pseudomonas</i> spp.	38.00 $\pm$ 0.00	33.50 $\pm$ 3.50	25.50 $\pm$ 0.50	19.00 $\pm$ 0.00	6.50 $\pm$ 0.50
<i>Staphylococcus aureus</i>	40.00 $\pm$ 0.00	35.00 $\pm$ 2.00	24.50 $\pm$ 0.50	17.00 $\pm$ 0.00	6.00 $\pm$ 0.00

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Propolis Methanolic Extract.

Test Organism	MIC (mg/ml)	MBC (mg/ml)
<i>Escherichia coli</i>	400	>400
<i>Klebsiella</i> spp.	300	400
<i>Pseudomonas</i> spp.	400	>400
<i>Staphylococcus aureus</i>	300	400

Table 4: Sensitivity pattern of Propolis against test organisms.

Test Organism	Gentamicin (10 μ g)	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml
<i>Escherichia coli</i>	S	S	S	R	R
<i>Klebsiella</i> spp.	S	S	S	R	R
<i>Pseudomonas</i> spp.	S	S	S	R	R
<i>Staphylococcus aureus</i>	S	S	S	R	R

- S = Sensitive
- R = Resistant

bacterial pathogens: *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus aureus*. At 400 mg/ml, Propolis showed the highest inhibition zones, with *Staphylococcus aureus* exhibiting the largest zone at 35.00 $\pm$ 2.00 mm, followed by *Pseudomonas* spp. and *Klebsiella* spp. (33.50 $\pm$ 3.50 mm and 33.50 $\pm$ 1.50 mm, respectively). *Escherichia coli* showed an inhibition zone of 31.50 $\pm$ 1.50 mm. The antibacterial activity reduced as the concentration decreased, with moderate zones of inhibition at 200 mg/ml and minimal effects at 50 mg/ml. This indicates that Propolis is most effective at higher concentrations, emphasizing its potential for therapeutic use as a natural antimicrobial agent (Table 2).

MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results highlighted the varying degrees of susceptibility of bacterial pathogens to Propolis methanolic extract. The MIC values ranged from 300 mg/ml for *Klebsiella* spp. and *Staphylococcus aureus* to 400 mg/ml for *Escherichia coli* and *Pseudomonas* spp. The MBC values were 400 mg/ml for *Klebsiella* spp. and *Staphylococcus aureus*, indicating that Propolis was bactericidal at this concentration. However, for *Escherichia coli* and *Pseudomonas* spp., bactericidal activity was not achieved even at concentrations exceeding 400 mg/ml, suggesting that Propolis primarily acts as a growth inhibitor for these organisms. These results underline the concentration-dependent efficacy of Propolis and its potential as a selective bactericidal and bacteriostatic agent (Table 3).

Sensitivity Pattern of Propolis against Test Organisms)

The sensitivity pattern of Propolis methanolic extract against *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus aureus* demonstrates its concentration-dependent antimicrobial efficacy. At the highest concentration (400 mg/ml), all test organisms showed sensitivity, with inhibition zones comparable to the positive control (Gentamicin). Sensitivity was maintained at 200 mg/ml, indicating the consistent effectiveness of Propolis in suppressing bacterial growth at moderate concentrations. However, resistance was observed at lower concentrations (100 mg/ml and 50 mg/ml) across all organisms. This suggests that Propolis's antimicrobial activity diminishes as its concentration decreases, likely due to insufficient levels of bioactive compounds to inhibit or kill the bacteria effectively (Table 4).

GC-MS analysis

The GC-MS analysis of Propolis identified several bioactive compounds responsible for its antimicrobial efficacy. The most abundant compound was Phenol, 2, 4-bis (1, 1-dimethylethyl) - (23.45%), known for its antioxidant and antimicrobial properties. Benzyl Alcohol (15.32%) exhibited both antibacterial and antifungal activities. Other compounds included 1, 2-Benzenedicarboxylic acid, diethyl ester (12.67%), Octadecanoic acid (9.54%), and Hexadecanoic acid, ethyl ester (7.45%). These compounds contribute synergistically to Propolis ability to inhibit and kill

Table 5: GC-MS results of Nigerian Propolis.

Compound	Retention Time (min)	Concentration (%)	Known Activities
Phenol, 2,4-bis(1,1-dimethylethyl)-	12.5	23.45	Antioxidant, Antimicrobial
Benzyl Alcohol	8.7	15.32	Antibacterial, Antifungal
1,2-Benzenedicarboxylic acid, diethyl ester	14.3	12.67	Antimicrobial, Anti-inflammatory
Octadecanoic acid	20.1	9.54	Antibacterial, Anti-inflammatory
Hexadecanoic acid, ethyl ester	18.8	7.45	Antioxidant, Antimicrobial

bacterial pathogens. Their antimicrobial, anti-inflammatory, and antioxidant activities suggest potential applications in pharmaceutical and nutraceutical formulations, further supporting Propolis's use as a natural antimicrobial agent against human pathogenic microorganisms (Table 5).

DISCUSSION

The phytochemical analysis of Nigerian Propolis unveiled a rich presence of bioactive compounds, including saponins, flavonoids, cardiac glycosides, alkaloids, and tannins. These compounds collectively contribute to the therapeutic potential of Propolis. Saponins, which were the most abundant (3.71 mg/100g), play a pivotal role in modulating immune responses and demonstrating antimicrobial properties. Flavonoids, present at 2.44 mg/100g, are well-known for their antioxidant capacity, protecting against oxidative stress and inflammation by scavenging free radicals. Cardiac glycosides (2.41 mg/100g) and alkaloids (1.88 mg/100g) add to the antimicrobial and anti-inflammatory effects, while tannins (1.38 mg/100g), though present in lower concentrations, contribute to antimicrobial properties by forming complexes with bacterial proteins. This composition mirrors findings from Oliveira *et al.* (2020) and Khan *et al.* (2023), reinforcing Propolis's therapeutic applications. Importantly, the synergistic interactions between these bioactive compounds enhance the pharmacological activity of Propolis, suggesting its potential for addressing oxidative stress-related diseases and microbial infections. Recent studies also highlight that the bioavailability of these compounds amplifies their therapeutic effects, positioning Propolis as a promising candidate for pharmaceutical and nutraceutical applications. As Martins *et al.* (2023) and Ahmed *et al.* (2023) have noted, the combined action of these compounds reduces reliance on synthetic antibiotics, promoting natural solutions for microbial infections.

The antimicrobial activity of Nigerian Propolis was evaluated against *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus aureus*. At a concentration of 400 mg/ml, Propolis demonstrated notable inhibitory effects, with *Staphylococcus aureus* exhibiting the largest zone of inhibition (35.00 ± 2.00 mm), followed by *Pseudomonas* spp. and *Klebsiella* spp. (33.50 ± 3.50 mm and 33.50 ± 1.50 mm, respectively). *Escherichia coli* showed moderate inhibition (31.50 ± 1.50

mm). These findings align with Zhang *et al.* (2023), who highlighted the enhanced antimicrobial activity of Propolis, particularly against Gram-positive bacteria, due to its phenolic and flavonoid content. The concentration-dependent antimicrobial activity of Propolis, with diminishing effects at lower concentrations (200 mg/ml and 50 mg/ml), emphasizes the need for optimal dosing to achieve therapeutic efficacy. Studies by Silva *et al.* (2021) and Brown *et al.* (2021) have shown that flavonoids and phenolic acids disrupt bacterial cell walls, inhibiting microbial growth. The pronounced effectiveness of Propolis against *Staphylococcus aureus* highlights its potential in treating resistant Gram-positive infections, although its reduced activity at lower concentrations warrants further research into formulation strategies that enhance its efficacy.

MIC and MBC evaluations revealed that Propolis exhibits bacteriostatic and bactericidal properties in a concentration-dependent manner. *Klebsiella* spp. and *Staphylococcus aureus* were the most susceptible, with MIC values of 300 mg/ml and MBC values of 400 mg/ml. For *Escherichia coli* and *Pseudomonas* spp., MIC values were higher at 400 mg/ml, with no bactericidal activity beyond this concentration. This indicates that Propolis primarily inhibits bacterial growth rather than inducing cell death in these organisms. Ahmed *et al.* (2021) and Singh *et al.* (2022) attributed these bacteriostatic effects to Propolis's interaction with bacterial enzymes, disrupting key metabolic pathways. The higher susceptibility of *Staphylococcus aureus*, a Gram-positive bacterium, may be due to the thicker peptidoglycan layer in its cell wall, making it more vulnerable to phenolic acids, as suggested by Wang *et al.* (2020). These findings support the use of Propolis as a selective antimicrobial agent, especially against drug-resistant pathogens. However, the lack of bactericidal activity against Gram-negative bacteria at higher concentrations highlights the need for combination therapies to augment its effectiveness. The sensitivity pattern demonstrated that Propolis was highly effective at concentrations of 400 mg/ml and 200 mg/ml, showing comparable antimicrobial activity to **Gentamicin** against ***Escherichia coli*, *Klebsiella* spp.,**

***Pseudomonas* spp., and *Staphylococcus aureus*.**

However, resistance emerged at lower concentrations (100 mg/ml and 50 mg/ml) across all tested organisms. This aligns with studies by El-Sayed *et al.* (2021) and Zhao

et al. (2021), which indicated that Propolis requires higher concentrations to achieve optimal antimicrobial effects. The reduced efficacy at lower concentrations underscores the importance of adequate dosing to realize its full therapeutic potential. Furthermore, the sensitivity of *Staphylococcus aureus* to Propolis reinforces its potential as an effective agent against resistant Gram-positive infections. To optimize the use of Propolis, its incorporation into synergistic formulations with conventional antibiotics could enhance its antimicrobial spectrum, as proposed by Rodriguez *et al.* (2022). These findings underline the significance of selecting appropriate concentrations for clinical applications and emphasize the need for careful formulation of Propolis-based treatments. The GC-MS analysis of Nigerian Propolis identified key bioactive compounds, including Phenol, 2, 4-bis (1, 1-dimethylethyl) - (23.45%), Benzyl Alcohol (15.32%), and 1, 2-Benzenedicarboxylic acid, diethyl ester (12.67%). These compounds exhibit antimicrobial, antioxidant, and anti-inflammatory activities. Phenol derivatives are known to disrupt bacterial membranes, increasing permeability and inducing cell death, as observed by Chen *et al.* (2023). Benzyl Alcohol has broad-spectrum antimicrobial properties, effective against both bacterial and fungal pathogens (Singh *et al.*, 2022), while fatty acids like Octadecanoic acid (9.54%) and Hexadecanoic acid, ethyl ester (7.45%) inhibit bacterial lipid synthesis, enhancing Propolis's antimicrobial profile (Luo *et al.*, 2023).

The combination of these bioactive compounds contributes to the multifaceted therapeutic effects of Propolis. This diversity of compounds highlights its broad spectrum of activity, supporting Propolis as a potent agent for antimicrobial, antioxidant, and anti-inflammatory treatments. The findings are consistent with earlier studies that underscore the role of bioactive compounds in amplifying the pharmacological potential of natural products (Ahmed *et al.*, 2023).

Future research should focus on isolating and characterizing these bioactive compounds further, potentially paving the way for drug development aimed at combating antibiotic-resistant infections. The identified metabolites in Nigerian Propolis—phenols, flavonoids, fatty acids, and benzyl alcohol—play integral roles in its antimicrobial efficacy. Phenol derivatives, such as 2,4-bis(1,1-dimethylethyl)-phenol, are potent disruptors of bacterial cell membranes, leading to cell lysis and inhibition of microbial growth, particularly against Gram-positive bacteria like *Staphylococcus aureus*. The presence of Benzyl Alcohol supports broad-spectrum antimicrobial activity, targeting both bacterial and fungal pathogens. Fatty acids, such as Octadecanoic acid, interfere with bacterial lipid synthesis, further enhancing the antimicrobial profile.

This combination of metabolites works synergistically to inhibit bacterial growth, showcasing the multifaceted antimicrobial potential of Propolis.

The concentration-dependent antimicrobial activity, especially the pronounced effect at higher concentrations,

is likely due to the accumulation of these bioactive compounds at higher doses, which collectively enhance Propolis's ability to target various microbial pathogens.

The relationship between the metabolite composition and antimicrobial efficacy highlights the significance of optimizing Propolis formulation for therapeutic use. Further studies into the mechanisms of action and bioavailability of these compounds will provide deeper insights into the potential of Propolis as an alternative antimicrobial agent.

Conclusion and recommendation

The study highlights the significant potential of Nigerian Propolis as a natural antimicrobial agent. The phytochemical analysis demonstrated the presence of bioactive compounds, including saponins, flavonoids, cardiac glycosides, alkaloids, and tannins, all contributing to its therapeutic effects. Propolis exhibited concentration-dependent antimicrobial activity, particularly against Gram-positive bacteria like *Staphylococcus aureus*, with inhibition zones comparable to Gentamicin at higher concentrations. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values confirmed its selective bacteriostatic and bactericidal properties, further supporting its potential in treating microbial infections. The GC-MS analysis identified key bioactive compounds such as Phenol and Benzyl Alcohol, which are responsible for its antimicrobial and antioxidant effects. Overall, the results suggest that Nigerian Propolis could serve as a viable alternative to synthetic antimicrobial agents, especially in addressing antibiotic-resistant infections. Given the concentration-dependent antimicrobial activity, further research should focus on optimizing Propolis concentrations in topical or oral formulations to enhance its efficacy, particularly against Gram-negative bacteria. Since Propolis showed reduced efficacy at lower concentrations, combining it with conventional antibiotics may improve its spectrum of activity, especially against resistant strains, and offer a potential strategy for synergistic therapeutic approaches.

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