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COMPARATIVE STUDY OF THE ANTIBACTERIAL, ANTI-INFLAMMATORY, AND ANTIOXIDANT EFFECTS OF HONEY, PROPOLIS, AND ROYAL JELLY PRODUCED BY THE APIS MELLIFERA BEE

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Honey, propolis, and royal jelly are natural products of bees that have been used for medicinal purposes for centuries. The aim of this study was to investigate and compare the therapeutic effects of two samples of honey, two of propolis, and two of royal jelly. All hive products were produced by the *Apis mellifera* bee and collected from two regions of Algeria. The study evaluated the antibacterial activity of hive products using agar well-diffusion and microbroth dilution assays. The agar well-diffusion assay involved inoculating agar plates with ten different bacterial strains, while the micro-broth dilution assay involved serial dilutions of hive products and bacterial cultures. The antioxidant potential was assessed using scavenging assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays, which measured the product's ability to donate electrons and neutralize DPPH free radicals. The albumin denaturation method evaluated the anti-inflammatory properties of the hive products by measuring the extent of inhibition of protein denaturation under heat stress. The results demonstrated significant differences in the antibacterial effects of the tested products; honey samples showed greater antibacterial activity than propolis and royal jelly samples. Gram-positive bacteria were more susceptible to the antibacterial effects than Gram-negative bacteria. Regarding antioxidant and anti-inflammatory properties, honey samples were more effective than propolis and royal jelly samples. Propolis sample 1 had the highest concentration of polyphenols and flavonoids. All hive products exhibited potent antibacterial, antioxidant, and anti-inflammatory effects, making them a promising natural supplement for combating various health issues associated with bacterial infections, oxidative stress, and inflammation.

Keywords: *Apis mellifera*, honey, propolis, royal jelly, therapeutic properties

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INTRODUCTION

The *Apis mellifera* bee is a social insect belonging to the genus *Apis* and the family *Apidae*. It is of considerable economic and cultural importance for human society as its role in crop pollination is essential for global food production (1). In addition, the *Apis mellifera* bee provides natural products such as propolis, pollen, venom, royal jelly, and honey with many potential healthy qualities, including antimicrobial (2,3), antimutagenic (4), antioxidant (5), and antitumoral effects (6). Commercialization and export of *Apis mellifera*'s products are a source of income for several countries worldwide, especially for honey production (1,7). Moreover, due to their composition of several bioactive substances, natural bee products are increasingly used in research to develop new treatment alternatives for several diseases (8).

Several properties of honey contribute to its antimicrobial effects. It has a pH between 3.2 and 4.5, which inhibits the growth of most pathogenic microorganisms (9,10). It contains a very high concentration of sugar (glucose and fructose), which dehydrates bacteria and other microorganisms. It also contains glucose oxidase, an enzyme that produces hydrogen peroxide (H_2O_2), which is responsible for the inhibition of bacterial growth. Other factors contribute to the antibacterial effect of honey, such as lysozymes, flavonoids, polyphenols, and other aromatic substances derived from foraged plants (11). In addition, honey has antioxidant activity, preventing oxidation by neutralizing free radicals in the body, which can damage cells, including DNA being also responsible for cell aging. The antioxidant properties of honey are attributed to many biological substances, such as phenolic acids, flavonoids, vitamins, enzymes, and minerals (12). Moreover, honey could also have anti-inflammatory and antioxidant properties, which are associated with each other through multiple cellular signaling pathways (13). Honey has been found to inhibit inflammation, oxidative stress, and gene expression pathways. Inflammation is a crucial part of the body's immune response, but chronic inflammation can lead to diseases like arthritis and cardiovascular disorders. Honey's polyphenolic and flavonoid content can modulate these processes by acting as agonists or inhibitors of key pro-inflammatory cytokines. Honey's antioxidants help counteract oxidative stress by scavenging free radicals and enhancing the body's antioxidant defenses. By addressing both inflammation and oxidative stress, honey not only addresses individual issues but also disrupts the cycle that perpetuates their coexistence, contributing to its therapeutic potential in preventing and managing chronic inflammatory and oxidative stress-related diseases (5,6,13).

Royal jelly is the most elaborate substance of the hive. It is intended for the feeding of queens. It is a creamy, whitish-to-yellowish secretion composed of water (about 66%), carbohydrates (about 14.5%), fatty acids (about 4.5%), including essential fatty acids, and proteins (about 13%), as well as essential amino acids. The antibacterial activity of royal jelly is mainly due to its acidic pH, trans-10-hydroxy-2-decenoic acid, and proteins with glucose-oxidase activity, such as major royal jelly proteins, defensin, royalisin, jelleins, and apisimin (14,15).

Propolis, a resinous substance collected by bees from various botanical sources, is used as a building material to fortify hives and protect them from environmental threats. It fills gaps, seals cracks, and minimizes heat loss during cold days. Propolis also exhibits antimicrobial properties, reducing infection risk and inhibiting the growth of fungi and bacteria, thereby creating a hygienic environment for bees (16). Propolis contains polyphenols, terpenoids, flavonoids, and aromatic acids, which are responsible for its strong antioxidant activity and help prevent the development of bacterial resistance (15,16). In addition to reducing bacterial mobility, propolis may disturb membrane potential, interfere with permeability of the microbe's cell membrane, and inhibit ATP generation (16).

Few studies were conducted to compare the therapeutic effects of beehive products; therefore, in this study, we aimed to evaluate the antibacterial, antioxidant, and anti-inflammatory effects of beehive products (honey, propolis, and royal jelly) from two regions of Algeria.

METHODS

The study analyzed two samples each of honey, propolis, and royal jelly produced by *Apis mellifera* bees from a semi-arid region in central Algeria and a humid region in eastern Algeria. The floral origin of the samples was determined based on the plant species present in the vicinity of each hive. All samples were kept at 6°C until their analysis. Honey and royal jelly were diluted using distilled water. For propolis, 1 g of propolis powder was homogenized and mixed with 10 mL of 80% (v/v) ethanol/water solution for 72 hours and kept at 70 °C for 1 hour. The resulting mixtures were filtered and then evaporated. Subsequently, all samples were passed through 0.22 µm microfilters to eliminate bacterial contamination. A series of dilutions was then prepared at

Table 1. *Color, pH value, and floral and geographical origin of hive bee products*

Samples	Color	pH	Floral origin	Region	Climate
Honey 1	Dark brown	3.15 ± 0.02	*Ziziphus sp, Brassica sp, Erica arborea, Carduus type, Lotus sp, Xanthium sp.*	Djelfa	Semi-arid
Propolis 1	Dark brown	4.19 ± 0.02			
Royal jelly 1	Cream	3.91 ± 0.02			
Honey 2	Brown	3.47 ± 0.01	*Trifolium sp, Eucalyptus sp, Rosmarinus sp, Hedera helix, Convolvulacea, Chrozophora tinctoria*	Annaba	Humid
Propolis 2	Dark brown	5.26 ± 0.03			
Royal jelly 2	Cream	3.24 ± 0.03			

concentrations of 2.5%, 5%, 10%, 20%, 40%, 60%, 80%, and 100% (v/v).

Antibacterial effect assays

The evaluation of the antibacterial activity of the samples was carried out according to the methods described by Bouacha et al., against ten multidrug-resistant bacteria isolated from infected wounds (8). These included Gram-negative bacteria: *Escherichia coli, Enterobacter aerogenes, Citrobacter koseri, Proteus mirabilis, Klebsiella pneumoniae, K. oxytocae, and Pseudomonas aeruginosa,* and Gram-positive bacteria: *Staphylococcus aureus., S. saprophyticus, and Enterobacter faecalis*. An inoculum of each bacterium was prepared by transferring one to two well-isolated, morphologically identical colonies into nutrient broth (Difco, MD, USA). The optical density was measured and adjusted to 0.08–0.10 at 625 nm.

Agar diffusion assay

The study used a standard technique to evaluate the efficacy of antimicrobial agents, specifically honey. Mueller Hinton agar (Difco, MD, USA) was prepared and poured into sterile Petri dishes, and wells of 6 mm in diameter were created as reservoirs for honey samples. Each plate was inoculated with a bacterial suspension standardized to an appropriate concentration. After inoculation, 50 µL of honey dilution was dispensed into each well, and the plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of clear zones of inhibition around the wells. The size of these zones correlated with the antibacterial potency of a honey sample, with larger zones indicating stronger antibacterial activity.

Microdilution assay

Ninety-six-well microplates (Fisher Scientific, UK) were used to determine the minimum inhibitory concentration (MIC) of honey against antibacterial activity. For this test, 100 µL of bacterial inoculum was mixed in each well with 100 µL of honey at various dilutions. Wells containing inoculum without honey served as positive controls, while broth alone served as the negative control. The microplates were incubated at 37°C for 24 hours to cultivate bacteria.

Bacterial growth was evaluated by absorbance at 620 nm to derive MIC values. Formula for calculating growth inhibition: Inhibition (%) = $\left(\frac{A\ test - A\ control}{A\ test}\right) \times 100$, where A control and A test were the absorbance values of the control and test samples, respectively. The lowest honey dilution that inhibited bacterial growth 100%, showing no well growth, was the MIC.

To determine the minimal bactericidal concentration (MBC), 10 µL from wells without growth was plated onto nutrient agar plates. These plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest honey dilution at which no bacterial growth occurred, demonstrating its bactericidal properties. To compare the honey's bacteriostatic (growth-inhibiting) and bactericidal (bacteria-killing) activities, the MBC/MIC ratio was calculated.

Antioxidant effect

Total polyphenolic content determination

The quantification of total polyphenols was carried out using the Folin-Ciocalteu assay (17). A 5 mL aliquot of a methanolic hive product solution (1g in 5 mL) was mixed with 2.4 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. After a 3-minute reaction period, 0.6 mL of a 20% sodium carbonate solution was added to the mixture. The samples were incubated in the dark at 25 °C for two hours. The absorbance of the reaction was measured at 725 nm using a spectrophotometer. Calibration was performed using various concentrations of gallic acid, and results were expressed in milligrams of gallic acid equivalents per 100 grams of honey (mg GAE/100g).

Flavonoids determination

Flavonoid content was determined using a colorimetric method involving aluminum chloride (18). A 1 mL sample of a 2% $AlCl_3$ solution was mixed with 1 mL of a methanolic solution of hive product (1 mg/mL). The mixture was allowed to react at 24 °C for 40 minutes. Absorbance readings were taken at 430 nm. A calibration curve was constructed using quercetin standards at concentrations of 20, 40, 60, 80, and 100 mg/L. Results were reported as milligrams of quercetin equivalents per gram of hive product (mg QE/g).

Ferric reducing/antioxidant power assay

The Ferric Reducing Antioxidant Power (FRAP) assay was performed following the method outlined by Beretta et al. (17). This colorimetric assay measures the change in absorbance at 593 nm, reflecting the reduction of colorless Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) to blue Fe^{2+}-TPTZ by electron-donating antioxidants. Hive products were prepared at a concentration of 1 g/mL in double-distilled water. A 200 µL aliquot of the honey solution was combined with 1.8 mL of FRAP reagent, consisting of 10 mmol/L TPTZ in 40 mmol/L HCl, 20 mmol/L $FeCl_3$, and 0.3 mol/L acetate buffer at pH 3.6. After incubating at 37 °C for 10 minutes, absorbance was measured at 593 nm. The antioxidant capacity was quantified in µmol/L of ascorbic acid equivalents per gram of honey (µmol/L AAE/g), using a standard curve prepared with ascorbic acid concentrations from 20 to 700 µmol/L.

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) assay

The DPPH radical scavenging capacity was assessed using a modified method. A 2.7 mL solution of methanolic DPPH (6.10^{-5} mol/L) was mixed with 0.3 mL of honey. After vortexing the mixture at 2500 rpm for 1 minute, the solution was incubated in the dark for one hour. Following this incubation, the absorbance was measured at 517 nm after an additional 15-minute incubation at 25°C. Ascorbic acid served as the positive control. The DPPH radical scavenging activity (%) was calculated by taking the difference between the absorbance of the control and the sample, dividing it by the absorbance of the test, and then multiplying the result by 100. Using a calibration curve ranging from 0 to 10 mg/L of ascorbic acid, the IC50 value, which represents the honey concentration required to scavenge 50% of DPPH radicals, was determined.

Anti-inflammatory effect

The albumin denaturation method, with a slight modification, was used to evaluate the anti-inflammatory activity in vitro, as described by Ali et al. (19). At varying concentrations, the samples were combined with a 1% water-based bovine serum albumin fraction in test tubes. Following a 15-minute incubation period at 37°C, the tubes were subjected to a 10-minute heating cycle at 70°C. Absorbance was measured spectrophotometrically at 660 nm. Distilled water was used as the negative control, while aspirin at the same concentrations (2.5%, 5%, 10%, 20%, 40%, 50%, 80%, and 100%) served as the positive control. The following formula was used to compute the percentage of inhibition of albumin denaturation = $\left(\frac{A2-A1}{A2}\right) \times 100$.

In this case, A1 represents honey's absorbance, and A2 represents the control's absorbance (distilled water).

Data analysis

Results were expressed as mean values ± standard deviation (SD), and the study used triple analyses to guarantee accuracy and reliability. The GraphPad Prism software was used to conduct the statistical analysis. To differentiate between treatments, a one-way analysis of variance (ANOVA) was employed. When comparing two sets of data, Tukey's post hoc test was used. Statistical analysis was performed with significance set at $p < 0.05$, supporting the reliability of the observed differences.

RESULTS

Antibacterial activity

As shown in Figure 1 and Table 2, the antibacterial activities of hive products have been thoroughly investigated. The results showed that the tested products varied significantly; honey samples outperformed royal jelly and propolis. Furthermore, the hive products had a greater impact on Gram-positive bacteria compared to Gram-negative bacteria. According to the MBC/MIC ratios, none of the bee products were bacteriostatic; rather, they were bactericidal. This indicates that the hive products could kill the bacteria and not just inhibit their growth. These findings indicate that honey, royal jelly, and propolis all exhibit antibacterial activity and may serve as natural antibacterial agents, with honey showing the highest efficacy.

Antioxidant effect

The determination of total phenolic content, flavonoid content, and antioxidant effects of hive products is reported in Figure 2. The total polyphenolic content of honey sample 1 was found to be relatively high in comparison with those of propolis and royal jelly. Flavonoid content also varied significantly; propolis collected from the Djelfa region has the highest concentration. Honey samples exhibited the highest antioxidant activity, followed by propolis and royal jelly samples.

Anti-inflammatory effect

The results of the anti-inflammatory activities of hive products determined by the membrane stabilization method are presented in Figure 3. The hive products showed a good anti-inflammatory activity. Honey and royal jelly samples were more effective than propolis.

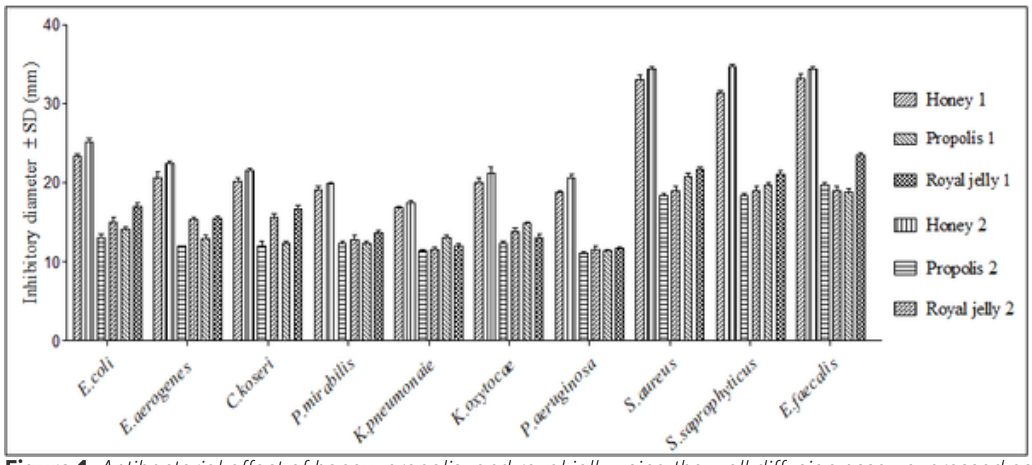

Figure 1. *Antibacterial effect of honey, propolis, and royal jelly using the well diffusion assay expressed as inhibitory diameters ±SD (mm)*

Table 2. *MIC, MBC, and MBC/MIC ratio of honey, propolis, and royal jelly*

	Honey 1		Honey 2		Propolis 1		Propolis 2		Royal jelly 1		Royal jelly 2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	20.0	40	20	20	40	60	40	80	40	60	40	80
E. aerogenes	20.0	20	20	20	40	40	40	80	40	60	20	60
C. koseri	20.0	20	20	20	40	40	40	80	60	60	40	80
P. mirabilis	40.0	80	40	60	40	60	40	80	40	60	40	80
K. pneumoniae	40.0	100	40	60	40	100	40	100	40	100	40	100
K. oxytocae	40.0	60	40	80	40	80	40	80	40	100	40	100
P. aeruginosa	40.0	60	40	80	40	60	40	100	60	100	40	100
S. aureus	02.5	05.0	02.5	05.0	10	20	10	20	10	20	20	40
S. saprophyticus	02.5	05.0	02.5	05.0	5	10	10	20	10	20	20	40
E. faecalis	05.0	05.0	05.0	10.0	10	20	10	20	10	20	20	40
MBC/MIC ratio	01.0-02.5		01.0-02.0		01.0-2.0		01.0-02.5		01.0-02.5		01.0-03.0	

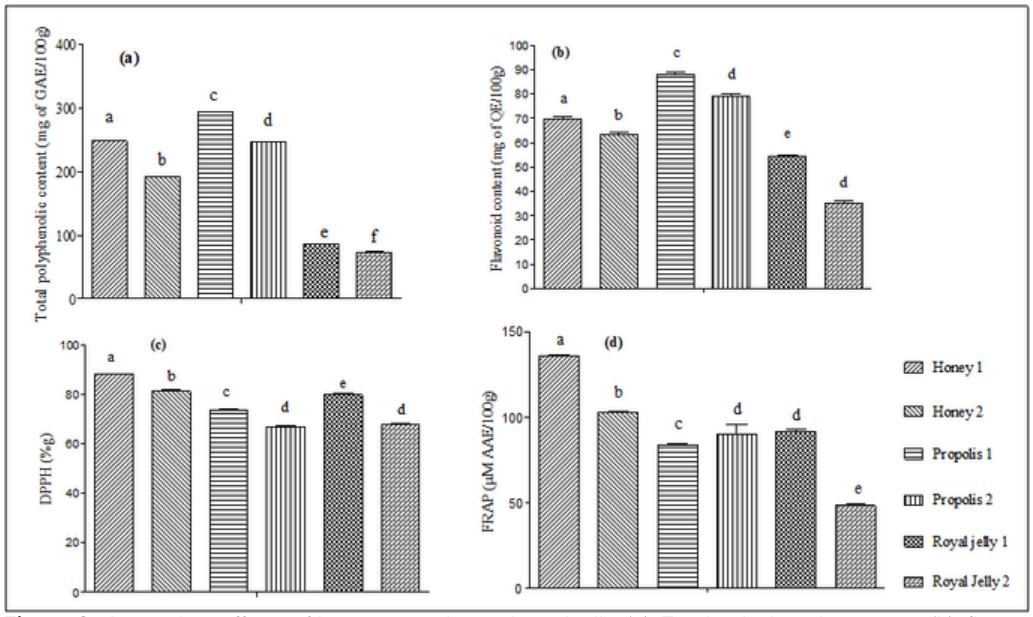

Figure 2. *Antioxidant effects of honey, propolis, and royal jelly. (a): Total polyphenolic content, (b): flavonoid content, (c): DPPH, (d): FRAP. There are significant differences between samples with different letters.*

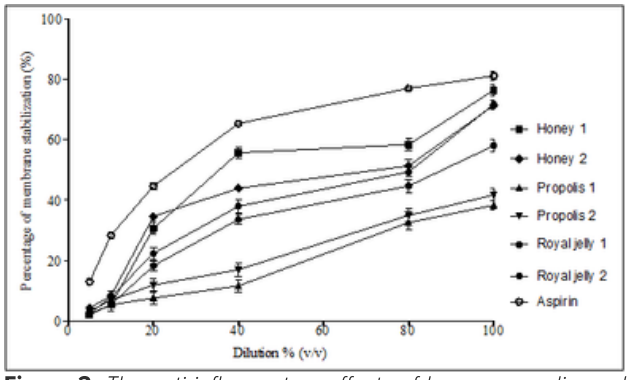

Figure 3. *The anti-inflammatory effects of honey, propolis, and royal jelly.*

DISCUSSION

Hive products showed diversity in their bioactive components, contributing to their effectiveness against a variety of microorganisms, including multidrug-resistant bacteria. As reported in Figure 1 and Table 2, the antibacterial effect of honeybee products varied significantly. Honey samples showed greater efficiency than the other products. Similarly, (20) have also shown that honey exhibited the highest antibacterial activity against *E. coli* and *S. typhimurium* strains. The effectiveness of honey as an antibacterial agent can be attributed to several factors, such as acidity, glucose oxidase, and lysosomes, which may act in a synergetic manner to inhibit the growth and viability of pathogenic microorganisms (10). Similar findings have been previously reported by many authors (2,3,21). The mechanism of the antibacterial action of hive products is not well known. However, some authors suggest that they cause a disruption of membrane potential and permeability, which may contribute significantly to overall cytotoxicity, affecting bacterial viability. In addition, Gram-positive bacteria were more susceptible to hive products than Gram-negative bacteria. This is due to the structure of the outer membrane of Gram-negative bacteria. Indeed, Gram-positive and Gram-negative bacteria have a cell wall that protects the cytoplasmic membrane. In addition, Gram-negative bacteria have additional protection offered by the polysaccharide-rich outer membrane (11). Moreover, the tested hive products exhibit a bactericidal effect on both Gram-negative and Gram-positive bacteria, as their MBC/MIC ratios are between 1 and 3. This ratio is essential for differentiating between a bacteriostatic agent, which prevents bacterial proliferation without killing the bacterium, and a bactericidal agent, which destroys and kills the bacterial cell. According to O'Neill and Chopra (22) an antimicrobial agent is bactericidal when the MBC/MIC ratio is less than or equal to 4; therefore, the tested hive products exhibited a bactericidal effect against multidrug-resistant bacteria (22). Many authors previously reported that honey (3,11,23,24) and propolis (25–27) displayed a bactericidal effect. However, García et al. (14) reported that two royal jelly samples had a bacteriostatic effect.

The determination of the total polyphenolic content, flavonoid content, and antioxidant effects of hive product samples (Figure 2) showed significant differences among them. Propolis had the highest TPC and TFC content when compared with honey and royal jelly. However, honey had the highest antioxidant activity, suggesting that it may contain other compounds with antioxidant effects that can protect against oxidative stress. Similarly, propolis and royal jelly have also been found to have antioxidant properties due to their high phenolic and flavonoid content. Several mechanisms contribute to the antioxidant effects of hive products, including free-radical scavenging, hydrogen donation, metal ion chelation, quenching of singlet oxygen, and acting as a substrate for superoxide and hydroxyl radicals. Many authors report a strong correlation between phenolic compounds and the antioxidant activity of bee products, which is influenced by botanical source, geographic and entomological origin, and climatic conditions. (3,11,28,29). However, hive products also contain other substances with antioxidant effects, such as minerals, amino acids, peptides, proteins, organic acids, and enzymes (15,30). Similar results were previously reported by Nagai et al. (31). In this study, commercial honey, royal jelly, and propolis were found to have a significant antioxidant activity, as measured by their ability to scavenge free radicals and inhibit lipid peroxidation (32). Mouhoubi-Tafinine et al. also found that honey samples had a higher antioxidant activity than propolis samples (12). In contrast, Postali et al. reported that propolis showed the highest antioxidant activity (33). Buratti et al. also found that propolis samples had a higher antioxidant capacity than honey (34). Indeed, many factors could affect the similarity or divergence in the antioxidant effect of hive products, including bee species, geographic region, plant species, forage, harvest, and storage conditions. Additionally, the methods used to evaluate antioxidant activity can significantly impact results. Variations in sample dilution, extraction methods, and other conditions can affect the concentration of bioactive compounds and their potency. Criteria for reporting findings can also cause discrepancies in results.

Therefore, a standardized approach is crucial for reliable and comparable results in antioxidant activity evaluations.
Based on the results in Figure 3, all hive products exhibited the anti-inflammatory effect; however, honey samples were found to have the most potent anti-inflammatory effects, followed by royal jelly and propolis. The anti-inflammatory activity of honey is attributed to its capacity to block the synthesis of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) (35). The antioxidant and anti-inflammatory properties of hive products are closely connected and often interdependent. These effects are largely attributed to the presence of polyphenols and flavonoids, which can modify the immune response, decrease pro-inflammatory cytokine production, scavenge free radicals, and alleviate oxidative stress (15,21).
The study demonstrated that honey, propolis, and royal jelly have significant bactericidal effects against a variety of microorganisms, including multidrug-resistant bacteria. All hive products showed good antioxidant and anti-inflammatory properties due to their phenolic and flavonoid content. Honey samples exhibited the strongest antibacterial, antioxidant, and anti-inflammatory properties. Overall, bee products show potential as natural therapeutic alternatives for various health conditions. However, further research is needed to better understand their mechanisms of action and to establish optimal dosages and formulations for effective use.

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Authors' Contributions

Conceptualization, M.B. and I.B.; Methodology, M.B. and I.B.; Investigation, M.B.; Formal Analysis, I.B.; Writing – original draft, M.B.; Writing – review & editing, M.B. and I.B. Both authors have read and approved the published version of the manuscript.

Statement of Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author.

Statement of Generative AI Use

No generative AI was used.

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