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Research Article

Evaluation of Antimicrobial Efficacy and Phenolic Compound Profiles in Geopropolis Samples from Bolivia and Venezuela

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Abstract

In this study, we investigated the balsamic contents, total phenolic contents, flavone-flavonol contents, and flavanones-dihydroflavonols contents, and antimicrobial effects of seven geopropolis samples produced by four neotropical stingless bee species: Venezuelan *Melipona favosa*, and Bolivian *M. grandis*, *Scaptotrigona depilis*, *S. polysticta*. The balsamic content value was found highest in the sample of *S. polysticta* (sample 7) collected from Bolivia, highest total phenolic content in *M. favosa* geopropolis (sample 1) from Venezuela, highest flavone-flavonol content in *M. favosa* geopropolis (sample 2) collected from Venezuela and highest flavones-dihydroflavonols content in S. *polystica* (sample 7) from Bolivia. As a result, except for balsamic content values, other investigated values were lower compare to the previous researches about different stingless bee species geopropolis. This is proved that climatic conditions, bee species and collecting area affected the chemical content of geopropolis significantly. The antimicrobial findings indicated that the examined geopropolis extracts displayed different degrees of inhibition against the growth of Gram-positive bacteria and fungi, which correlated with their phenolic contents. Nevertheless, these extracts did not demonstrate a comprehensive inhibitory effect on Gram-negative bacteria. Standardized geopropolis samples, rich in phenolic content, can complement antibiotics naturally for preventing and treating infections from Gram-positive bacteria and *Candida albicans*. However, further studies are still needed regarding the clinical applications of geopropolis in various infections.

Keywords: Geopropolis, *Melipona favosa, Melipona grandis, Scaptotrigona depilis, Scaptotrigona polysticta,* Antimicrobial activity, Phytochemicals

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Introduction

Plants produce resinous secretions from various parts such as buds, bark, flowers, and fruits, to protect against microbial clay and provide thermal isolation. This resinous substance collected by honey bees and processed with digestive enzymes and beeswaxes is called propolis. It is used for various functions in the stingless bee nest [1,2].

Geopropolis, generated by some species of stingless bees belonging to the *Meliponini tribe,* represents a distinct variety of propolis. Stingless bees produce geopropolis by combining the plant resinous substances with wax, digestive enzymes, and soil. On the other hand, there is no soil in the propolis produced by *Apis mellifera* [3,4].

In tropical and subtropical regions worldwide, there are 56 genera and 605 species of stingless bees [5,6]. It was found that more than some 500 species are in the Neotropical region and 259 species in Brazil [7].

The stingless bee species *Melipona favosa* is an important species for Venezuela, living in the plains and along the coastlines of the country. This gentle stingless bee species is locally known as "erica" and "maba." These bees typically construct their nests in trees, on walls, and on fence posts, and are managed in hives by traditional stingless beekeepers [8].

Stingless bees are social insects like honey bees, they also produce honey, pollen, beeswax, and cerumen besides

geopropolis. Furthermore, they are very important pollinators of many crops like, lychee, avocado, macadamia, mango, coffee [9-12]. For these reasons stingless bees are used locally and beekeping with stingless bees is called meliponiculture [5].

Meliponiculture is an industry that develops in tropical countries and holds high economic value due to the increased productivity of agricultural products through pollination services. It also allows for the extraction of commercially valuable products like beeswax, pollen, propolis, and royal jelly. Therefore, meliponiculture is a valid activity that can generate income for local communities in the Amazon region [6,13].

Propolis has many functions in the beehive, such as thermal isolation, closing the holes crevices, and disinfection of the hive with its antimicrobial effects [1]. Also, the geopropolis is using for has similar functions in the hive by stingless bee nest.

Geopropolis, similar to propolis, possesses a highly complex and variable chemical composition, which is influenced by factors like flora, climate, and the species of bees [14]. Studies have shown that geopropolis is rich in many components such as phenolic acids, flavanoids, terpenes, fatty acids and steroids, organic acids and alcohols [7,14-16]. Thanks to these compounds, antibacterial, antifungal, cytotoxic, antioxidant effects have been reported to have a very therapeutic effect [7,16].

By this study, we aimed to investigate the balsamic, total phenolic, flavone-flavonol, flavanone-dihydroflavonol contents and antimicrobial effects of geopropolis collected from Venezuela and Bolivia that are collected by *M. favosa, M. grandis, Scaptotrigona depilis, S. polysticta*.

Material and Methods

Geopropolis Samples

Several geopropolis samples from various stingless bee species were gathered in different regions from Bolivia and Venezuela *(Table 1)*.

Table 1. *Countries where samples collected and Stingless bee species and countries of origin of propolis samples*

Sample No	Stingless Bee Species	Country
1	*Melipona favosa*	Venezuela
2	*Melipona favosa*	Venezuela
3	*Melipona favosa*	Venezuela
4	*Melipona grandis*	Bolivia
5	*Scaptotrigona depilis*	Bolivia
6	*Scaptotrigona depilis*	Bolivia
7	*Scaptotrigona polysticta*	Bolivia

Stingless bee samples were collected using isopropyl alcohol, dried at ambient temperature, prepared using tissues in rigid plastic boxes, and entomological identifications were conducted.

The propolis samples were obtained through collaboration with Apitherapy and Bioactivity (APIBA), University of the Andes, Mérida, Venezuela. *M. favosa* propolis was collected from the Paraguaná Peninsula. Paraguaná Peninsula is located in the north of Falcón State, alongside the Caribbean Sea. It is one of the driest areas in Venezuela, situated within an arid and semi-arid bioclimatic environment, characterized by strong winds and an average annual precipitation of about 340 mm. It is a xerophytic area of about 3.405 km² with almost entirely arid soil. Cerro de Santa Ana 830 masl is surrounded by predominant plains with savannah xerophytic vegetation. The geopropolis samples of *M. favosa* were collected at Cerro Santa Ana piedmont from the meliponary of the Agenda Petroleo Project.

M. grandis, *S. depilis*, and *S. polysticta* propolis were collected from the Amboró National Park in Bolivia at the beginning of the winter season. Amboró National Park covers 636.000 hectares from 320 to 3.300 masl, located in the eastren lowlands of Bolivia, near to San Carlos, with high biodiversity of the Bolivian Amazonian Forest. Environmental problems are timber trafficking and illegal coca cultivation. Geopropolis samples were collected near to Pirai river, from the stingless bee keepers of the sustainable meliponiculture project promoted by the Ecological Association of the East (ASEO), and the Association of Native Honey Producers (APROMIN). The geographical localization of the stingless bee nests producing the collected geopropolis samples is shown in *Fig. 1*.

Propolis Extraction

The extraction of propolis was conducted following the method outlined by Popova et al.[17]. Initially, propolis was shredded, and a 1-gram sample of propolis powder was combined with a 70% ethanol solution (Merck, Germany). After the dilution process, an ultrasound bath treatment

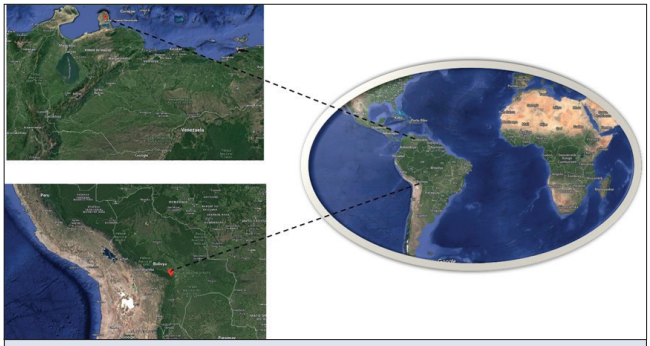

Fig 1. The geographical localization of the collected geopropolis samples

was conducted with a 300 W ultrasonic bath (ISOLAB, Germany, model: 621.05.022s). Subsequently, The blend was filtrated using Whatman No. 4 filter paper (Millipore, USA), and the remaining solid residue underwent a second extraction. After this step, the ethanol extracts were combined and diluted to a final volume of 100 mL using 70% ethanol.

Balsamic Content

Three parallel extracts were arranged for each crude sample using 70% ethanol. Two milliliters from each extracts were evaporated until they reached a consistent weight after drying. The ethanol-soluble fraction was used to calculate the proportions of balsam in the extracts. The mean of these values was determined [17] and expressed as a percentage (%).

Estimation of Total Polyphenol Content by Folin-Ciocalteu Colorimetric Method

The Folin-Ciocalteu colorimetric method, as outlined by Slinkard and Singleton [18], was employed to determine the total polyphenol content in the ethanolic extract of propolis (EEP). Absorbance measurements were recorded at 760 nm utilizing a UV-VIS spectrophotometer (Genesys 10S UV-VIS Spectrophotometer). For the calibration curve, gallic acid served as the standard. The total content of the extracts was quantified by comparing the results with a calibration curve established with gallic acid as the standard (r^2=0.997) and stated as milligrams of gallic acid equivalents (GAE) per gram of propolis extract (mgGAE/g). Each extract was measured three times for accuracy.

Flavone and Flavonol Content

The analysis of flavone and flavonol content followed the procedure outlined by Popova et al.[17]. Absorbance measurements were recorded at 425 nm using a UV-VIS spectrophotometer (Genesys 10S UV-VIS Spectrophotometer). Calibration was carried out with quercetin as the reference compound (r^2=0.999). Three measurements were conducted for each extract, and the results were expressed as a percentage (%).

Flavanone and Dihydroflavonol Content

For the analysis, 1 mL of the extract was combined with 2 mL of a DNP (2,4-dinitrophenylhydrazine) solution. The DNP solution was rearranged by dissolving 1 g of DNP in 2 mL of 96% sulfuric acid, consequently, the mixture was diluted to a final volume of 100 mL with methanol using a volumetric flask. The blend was then subjected to heating at 50°C during 50 min. Upon reaching room temperature, the solution was further diluted to 10% KOH in methanol. Afterward, 0.5 mL of the resultant solution was introduced into 10 mL of methanol, followed by additional dilution to achieve a final volume of 25 mL with methanol. The absorbance was measured at 486 nm employing a UV-VIS spectrophotometer (Genesys 10S UV-VIS Spectrophotometer). Calibration was conducted with naringenin serving as the reference compound. Three measurements were taken for each extract [19] and expressed as a percentage (%).

***Table 2**. Bacterial and fungal strains tested for propolis bioactivity*

Agent	Microorganisms
Gram positive bacteria	*Staphylococcus aureus* ATCC 29213
	Methicillin-Resistant *Staphylococcus aureus* (MRSA) NCTC 10442
	Enterococcus faecalis ATCC 29212
Gram negative bacteria	*Escherichia coli* ATCC 25922
	Klebsiella pneumoniae ATCC 13883
	Pseudomonas aeruginosa ATCC 27853
	Acinetobacter baumannii ATCC 19606
Fungi	*Candida albicans* ATCC 10231

Antimicrobial Activity Assay

The test organisms employed in the study are detailed in *Table 2*. Before the research commenced, all strains were stored and preserved through cryopreservation at -86°C in the Microbiology Laboratories of Gülhane Faculty of Medicine. Bacterial strains were cultivated on 5% Sheep Blood Agar (RTA Labs, Gebze, Türkiye) at 37°C for 24 h, whereas yeasts were cultured in RPMI-1640 medium (Thermo Fisher Scientific, USA) at 25°C for 48 h. Propolis extracts, diluted in 70% ethanol, were subjected to broth microdilution assays to ascertain their Minimal Inhibitory Concentration (MIC) values, in accordance with the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) standart M7-A9 and M27-A3.

Briefly, the ethanolic extracts of propolis (EEP) were prepared with two-fold serial dilutions in Mueller Hinton Broth (RTA Labs, Gebze, Türkiye), and then transferred to the wells of U-bottom microdilution plates. Bacterial and fungal inoculum suspensions were adjusted to final concentrations of 10^5 CFU/mL and 10^4 CFU/mL, respectively, and dispensed into microdilution wells containing various concentrations of EEP in 20 µL. Afterward, microdilution plates inoculated with bacteria were incubated in a 37°C incubator for 24 h whereas those inoculated with *Candida albicans* were kept at 25°C for 48 h. Positive controls were established using Meropenem (Sigma-Aldrich, USA) for bacteria and Fluconazole (Sigma-Aldrich, USA) for fungal strains. In each experiment, a positive control and a negative control and ethanol control (2% ethanol) were used. The Minimal Inhibitory Concentration (MIC) was determined as the lowest concentration of each extract that inhibited visible

growth of microorganisms. The Tetrazolium/formazan test (TTC) was used to evaluate the viability of the tested microorganisms [20]. The experiments were conducted in triplicate, and the outcomes were reported in mg/mL.

Statistical Analysis

The statistical analysis was conducted with Statistical Package for Social Sciences (SPSS) Version 25 (SPSS, Inc.). The normal distribution suitability of variables was assessed through the Shapiro-Wilk and Kolmogorov-Smirnov tests, skewness and kurtosis values, histogram graphs, and decisions were made based on mean ± standard deviation and median values. If the p-value was below 0.05, it was interpreted as "significant". Spearman correlation analysis was performed to assess the correlation of numerical data that does not conform to normal distribution. Likewise, the Mann-Whitney U test was employed to compare numerical data between groups that do not conform to normal distribution.

Results

In this study, balsamic contents, total phenolic contents, flavone-flavonol contents, flavanone-dihydroflavonol contents, and antimicrobial effects of seven geopropolis samples belonging to four stingless bee species *(M. favosa*, *M. grandis*, *S. depilis*, *S. polysticta)* collected from Bolivia and Venezuela were analyzed.

According to our results, the balsamic content of geopropolis samples ranged from 3.33% to 30.15% *(Table 3)*. The maximum value was found in geopropolis of *S. polysticta* stingless bee species collected from Bolivia.

While the maximum flavone and flavonol content (0.89±0.01) was detected in the geopropolis (sample 2) belonging to the *M. favosa* stingless bee species collected from Venezuela, no flavone and flavonol content was found in samples 4 *M. grandis* and 7 *S. polysticta (Table 3)*.

Although the flavone-dihydroflavonol contents of geopropolis collected from Bolivia varied between 2.02±0.01 and 7.43±0.01, no flavanone and dihydroflavonol contents were detected in geopropolis collected from Venezuela *(Table 3)*.

In our study, we determined total polyphenol content according to Folin-Ciocalteu colourimetric method Slinkard and Singleton [18] and we found values between 1.66±0.00 and 16.73±0.00 mgGAE/g. The highest total phenolic value was determined to be 16.73 mgGAE/g, and it is associated with sample number 1 derived from *M. favosa*. The lowest total total phenolic content value was found to be in 1.66 mgGAE/g belong to the sample 3 collected by *M. favosa*.

The antimicrobial effect of seven geopropolis extracts obtained from four stingless bee species was tested against Gram-negative bacteria *(Escherichia coli, Klebsiella*

Table 3. *Bee species, and analytical results*

Sample No	Bee Species	Balsamic Content%	Total Phenolic Content (mgGAE/g)	Flavone and Flavonol Content (%)	Flavanones and Dihydroflavonols Content (%)
1	*Melipona favosa*	4.33	16.73±1.36	0.62±0.01	-
2	*Melipona favosa*	3.50	6.93±0.01	0.89±0.01	-
3	*Melipona favosa*	6.16	1.66±0.01	0.49±0.01	-
4	*Melipona grandis*	15.66	12.44±0.01	-	2.02±0.01
5	*Scaptotrigona depilis*	3.33	2.13±0.009	0.12±0.06	3.07±0.01
6	*Scaptotrigona depilis*	22.99	8.59±0.01	0.25±0.02	4.25±0.01
7	*Scaptotrigona polysticta*	30.15	5.91±0.01	-	7.43±0.01

Table 4. *Minimum inhibitory concentration (mg/mL) for the studied microorganisms*

Sample	*E. coli* ATCC 25922	*K. pneumonia* ATCC 13883	*P. aeruginosa* ATCC 27853	*A. baumannii* ATCC 19606	*S. aureus* ATCC 29213	*MRSA* NCTC 10442	*E. faecalis* ATCC 29212	*C. albicans* ATCC 10231
PROPOLIS 1	>41	>41	>41	>41	5.125	5.125	5.125	5.125
PROPOLIS 2	>41	>41	>41	>41	5.125	5.125	10.25	5.125
PROPOLIS 3	>41	>41	>41	>41	10.25	10.25	20.5	20.5
PROPOLIS 4	>41	>41	>41	>41	5.125	5.125	10.25	10.25
PROPOLIS 5	>41	>41	>41	>41	20.5	20.5	20.5	20.5
PROPOLIS 6	>41	>41	>41	>41	5.125	5.125	5.125	10.25
PROPOLIS 7	>41	>41	>41	>41	10.25	10.25	10.25	5.125

Table 5. *Statistical analysis between geographical region and balsamic content, total phenolic content and antibacterial effect*

Variables	Venezuela n=3 (42.9%)	Bolivia n=4 (57.1%)	P-Value*
Balsamic content (%)	4.33 (3.50-6.16)	19.33 (3.33-30.15)	0.400
Total phenolic content, (mgGAE/g)	6.93 (1.66-16.73)	7.25 (2.13-12.44)	1.000
S. aureus (ATCC 29213) MIC (mg/mL)	5.13 (5.13-10.25)	7.69 (5.13-20.50)	0.629
MRSA (NCTC 10442) MIC (mg/mL)	5.13 (5.13-10.25)	7.69 (5.13-20.50)	0.629
E. faecalis (ATCC 29212) MIC (mg/mL)	10.25 (5.13-20.50)	10.25 (5.13-20.50)	1.000
C. albicans (ATCC 10231) MIC (mg/mL)	5.13 (5.13-20.50)	10.25 (5.13-20.50)	0.629

**Mann-Whitney U test, Data are expressed as median (min-max)*
Since no antibacterial effect was detected on Gram-negative bacteria, it was not included in the analysis

pneumoniae, Pseudomonas aeruginosa, and *Acinetobacter baumannii),* Gram-positive bacteria *(Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus faecalis),* and the fungus *C. albicans*. The MIC values for the tested microorganisms ranged from 5.125 to >41.0 mg/mL. Detailed findings are provided in *Table 4*. The propolis sample obtained from the stingless bee *M. favosa* (sample 1) exhibited significant and broad-spectrum antimicrobial activity, primarily attributed to its high phenolic content. Remarkably, geopropolis extracts demonstrated increased sensitivity against Gram-positive bacteria and fungi when compared to Gram-negative bacteria. The strains *S. aureus* ATCC 29213 and MRSA NCTC 10442 proved to be the most susceptible microorganisms, with minimum inhibitory concentrations ranging from 5.125 to 20.5 mg/mL.

The Spearman correlation analysis revealed a statistically significant high degree of negative correlation between the total phenolic content and MIC values of *S. aureus*, MRSA, and *E. faecalis* strains (r=-0.837, P=0.019; r=-0.837, P=0.019; r=-0.850, P=0.015, respectively). Simultaneously, a statistically significant high degree of positive correlation was found between the MIC value of *E. faecalis* and the MIC values of *S. aureus* and MRSA strains (r=0.791, P=0.034; r=0.791, P=0.034, respectively). No statistically significant correlation was detected between balsamic content, total phenolic content, and MIC values of the strains. In the regional comparison conducted, no statistically significant difference was observed concerning balsamic content, total phenolic content, and MIC values *(Table 5)*.

Discussion

Propolis, also known as bee glue, is a sticky substance used by bees as a hygienic building material in their hives, derived from a mixture of insect secretions and plant resins. Numerous studies have demonstrated various pharmacological activities of propolis, including antimicrobial, anti-inflammatory, anticancer, antioxidant, hepatoprotective, cytotoxic, and immunomodulatory properties. The majority of these pharmacological activities are generally attributed to propolis produced by *A. mellifera*, the most widespread bee species worldwide [21,22].

Nevertheless, the chemical composition and pharmacological activities of geopropolis, produced by stingless bees, commonly referred to as meliponines, have been inadequately explored in existing research. Unlike honeybees, some stingless bees mix their propolis with clay or soil, resulting in a softer resinous material known as geopropolis. Despite the compositional differences, geopropolis exhibits similar effects to *A. mellifera* propolis [23].

The geographical features and bee species significantly influence the chemical and biological characteristics of geopropolis. Geopropolis contains fatty acids, organic acids, sugars, alcohols, steroids, as well as polyphenolic compounds, triterpenes, and saponins [3].

In this study, seven geopropolis samples from four bee species collected from Venezuela and Bolivia were examined for their balsamic, total phenolic, flavone-flavonol, flavone-dihydroflavonol contents, and antimicrobial effects.

In our study, the balsamic content of geopropolis collected from Venezuela was generally found to be low. The balsamic content of all geopropolis samples ranged from 3.33% to 30.15%. In the literature, there is no available data regarding the balsamic content of geopropolis. When comparing our results with *A. mellifera* propolis, it is observed that the geopropolis fall within the lower range of balsamic content seen in *A. mellifera* propolis but do not reach the higher levels. In a study on poplar-type propolis from different geographic regions, 114 samples were investigated, and the minimum value was reported as 18%, the maximum value as 82%, and the mean value as 57% [17]. In contrast, our mean value is only 11.6%. It is evident that the balsamic content of geopropolis is significantly lower than that of propolis caused by the admixture with clay, a material without balsamics.

The reason of this diferrences is caused probably from

the source of geopropolis. While the source of propolis is resins of plants, the source of geopropolis is both resin and soil. Owing to this feature, geopropolis can include resin in lower ratios compare to the propolis of *A. mellifera*. As a result of lower resin, it has lower balsamic content than propolis of *A. mellifera*.

The total phenolic content of geopropolis samples ranged from 1.66±0.00 to 16.73±0.00 mgGAE/g. At in previous studies, total phenolic content of geopropolis samples belong to the *M. fasciculata* geopropolis was found between126.60±0.84 and 631.26±4.22 mgGAE/g, geopropolis of *M. orbigny* between 211.0±7.5 and 23±1.0 QE/100 g, and geopropolis of *M. scutellaris* as 620.01±6.45 mgGAE/g [15,16,24].

Compared to the previous researches, our results for total phenolic contents are so lower. This can be sourced from bee species, climatic conditions, flora of the location geopropolis where plant resins were collected.

As a result of literature scanning search, we can say that researchers invetigated the total phenolic contents of geopropopolis collected by *M. fasciculata*, *M. orbigny*, *M. fasciolata*, *M. scutellaris* but there is no any data about the total phenolic content of *M. favosa*, *M. grandis*, *S. depilis*, *S. polysticta* geopropolis. In this case our results will be the first data for these stingless bees species.

Similar to the balsamic content, there is no any information available about flavone-flavonol and flavanones-dihydro-flavonols contents of geopropolis. The previous researches about geopropolis were mostly based on its total phenolic content. Geopropolis has a very rich chemical content, especially phenolic content affected the antioxidant capacity in a positive way.

Researches about biological activities of geopropolis evaluated that geopropolis has also so many bioactivities as propolis, due to its phytochemical content (benzoic acids, dihydrocinnamic acids, cinnamic acids prenylated coumaric acids, diterpenic acids, aliphatic acid and esters, alcohols, aromatic acids, hydrocarbons, terpenes triterpenic alcohols, and sugars). The presence of these chemical compounds shows differences between samples, and this variability is probably caused by different from bee species [7,25]. It was shown that *M. fasciculata* geopropolis, contains fatty acids, organic acids, sugars, alcohols, steroids gallic acid, elagic acid and hydrolyzable tannins and due to these compound it has antiviral, anticarcinogenic, anti-inflammatory and antioxidant properties [14,24,26]. The absence of detailed phytochemical analyses in our study, which would have provided a comprehensive demonstration of the components responsible for biological activity in geopropolis samples collected from two different regions, can be considered a limitation of the study.

Geopropolis, which differs structurally from propolis with no soil, shows physicochemical differences too. Compared to chemical content, it is known that geopropolis contains much lesser phenolic acid than propolis [27]. This explains that geopropolis has lower antioxidant and antimicrobial activity than propolis. There is a need for comprehensive research comparing the antioxidant and antimicrobial activities of propolis and geopropolis, as well as investigating their anticarcinogenic, anti-inflammatory, analgesic, antidepressant, anxiolytic, and immunomodulatory effects. Such studies would help identify the specific components responsible for these effects.

In our research, we evaluated the antimicrobial efficacy of ethanol extracts derived from seven geopropolis samples collected from four stingless bee species. The testing included Gram-negative and Gram-positive bacteria, along with reference strains of *C. albicans*. Specifically, it was noted that geopropolis extracts displayed increased sensitivity against Gram-positive bacteria and fungi compared to Gram-negative bacteria. The antibacterial effect of geopropolis samples on Gram-positive bacteria was found to be dependent on phenolic contents. However, it was determined that there was no positive correlation between antibacterial effect, balsamic content, and the geographical region and bee species from which they were collected.

Previous studies have consistently noted the resistance of Gram-negative bacteria to ethanol extracts of propolis compared to Gram-positive bacteria, attributed to differences in their cell wall structures [21,22,28-32]. The antimicrobial activity studies have demonstrated that the antibacterial effect of propolis types on Gram-positive bacteria is strongly linked to the content of propolis, and it can vary widely with a broad range of MIC values (between 6 and 20000 µg/mL) [29,33]. However, the MIC values we determined for the geopropolis extracts tested in our study may be found to be relatively high when compared with other research on the antimicrobial activity of geopropolis. This inconsistency could be attributed to the relatively low phenolic content present in our geopropolis samples, which plays a significant role in antimicrobial activity.

In our prior study investigating the antimicrobial activity of pollen samples, we observed that pollen extracts exhibited no activity against the *C. albicans* ATCC 10231 strain [34]. Interestingly, conversely, geopropolis extracts exhibited the capacity to hinder the growth of *C. albicans*, with MIC values ranging from 5.125 to 20.5 mg/mL. Previous research has indicated that geopropolis extracts possess antifungal activity at MIC values ranging from 1 to 3.4 mg/mL [22,29,32,35-38]. However, a more comprehensive phytochemical analysis is warranted to identify the active

metabolites responsible for the antifungal effects of geopropolis.

Completely natural and non-toxic propolis is not only used in traditional and complementary medicine but is also widely utilized in various applications such as food, functional foods, pharmaceuticals, livestock, cosmetics, and particularly in oral health practices in dentistry, thanks to its effective antibacterial properties [22,39,40]. However, the low water solubility of propolis limits its use in several other areas. It has been shown that propolis has the capacity to enhance the effectiveness of antibacterial agents, exhibiting a synergistic effect with antibiotics, allowing for high antimicrobial efficacy at low doses [41].

Based on these findings, we believe that standardized geopropolis samples with high phenolic content could serve as a natural complement to antibiotics in the prevention and/or treatment of infections caused by Gram-positive bacteria and *C. albicans*. Additionally, the statistically significant positive correlation found among the MIC values of Gram-positive bacterial standard strains in our study suggests that geopropolis samples could be used in the treatment of mixed infections involving Gram-positive bacteria. Nevertheless, additional research is required to investigate the clinical applications of geopropolis in various infections. In particular, evaluating clinical strains with different sensitivity patterns in vitro alongside standard strains in studies would provide more accurate results. Additionally, there is a need for comprehensive geopropolis and antibiotic synergy studies to ensure the control of resistant pathogenic bacterial species, such as MRSA.

Unfortunately, despite the natural, non-toxic, and highly effective nature of this product that God has bestowed upon humans, there are some challenges in its clinical use. Similar to propolis produced by *A. mellifera*, the inability to establish a standard for the chemical characterization of geopropolis due to its different chemical compositions based on geographical regions and botanical sources, and the lack of standardization in the extraction procedure, can be emphasized as significant challenges in clinical applications. Overcoming these challenges in the clinical use of this product requires advanced laboratory analyses, comprehensive pharmacodynamic and pharmacokinetic studies, laboratory animal research, and clinical trials.

Declarations

Availability of Data and Materials: Datasets analyzed during the current study are available with the corresponding author (Ö. Kuru) on reasonable request.

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