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SCIENTIFIC ARTICLE

Determination of phenolic profile by HPLC, antioxidant and antibacterial activities of geopropolis from the native stingless bee (*Melipona beecheii* Bennett) from the Yucatan Peninsula

Determinación del perfil fenólico por HPLC, actividades antioxidantes y antibacterianas de geopropóleos de la abeja nativa sin aguijón (*Melipona beecheii* Bennett) de la Península de Yucatán

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ABSTRACT

Melipona beecheii Bennett produces geopropolis rich in phenolic compounds with potential biological activity. In this study, geopropolis samples from the Yucatan Peninsula were analyzed for their chemical composition, antioxidant activity, and antibacterial activities. Phenolic and flavonoid contents were determined spectrophotometrically, and individual compounds were identified by HPLC. Antioxidant activity was evaluated using DPPH and FRAP assays, while antibacterial activity was assessed by agar diffusion. Extracts from Chetumal and Felipe Carrillo Puerto showed high apigenin and pinocembrin contents (5.11 and 4.12 mg/100 g extract, respectively). The San Antonio Yaxche exhibited the highest antioxidant activity. *Escherichia coli* and *Staphylococcus aureu*s were more susceptible to extracts from Santa Cruz Pueblo and Chetumal. Results indicate that the chemical composition and bioactivity of *M. beecheii* geopropolis vary according to geographic location.

KEYWORDS

Antibacterial activity, geopropolis, stingless bees, phenolic compounds.

RESUMEN

Melipona beecheii Bennett produce geoprópolis rico en compuestos fenólicos con potencial actividad biológica. En este estudio, se analizaron muestras de geoprópolis de la Península de Yucatán para determinar su composición química y evaluar sus actividades antioxidante y antibacteriana. El contenido de compuestos fenólicos y flavonoides se determinó mediante ensayos espectrofotométricos, mientras que los compuestos individuales fueron identificados por HPLC. La actividad antioxidante se evaluó mediante los ensayos DPPH y FRAP, y la actividad antibacteriana mediante el método de difusión en agar. Los extractos de Chetumal y Felipe Carrillo Puerto presentaron altos contenidos de apigenina y pinocembrina (5.11 y 4.12 mg/100 g de extracto, respectivamente). El extracto de San Antonio Yaxché mostró la mayor actividad antioxidante. *Escherichia coli* y *Staphylococcus aureu*s fueron más susceptibles a los extractos de Santa Cruz Pueblo y Chetumal. Los resultados indican que la composición química y la bioactividad del geoprópolis de *M. beecheii* varían según el origen geográfico.

PALABRAS CLAVE

Actividad antibacterial, geoprópolis, abejas sin aguijón, compuestos fenólicos.

Introduction

Geopropolis is a resinous material produced by stingless bee species belonging to the tribe Meliponini. In Mexico, a total of 46 Meliponini species have been reported, 16 of which inhabit the Yucatan Peninsula, including *Melipona beecheii* Bennett (Góngora Ovado et al., 2025). The main difference between propolis produced by species of the tribe Apini and geopropolis produced by Meliponini species is that the latter incorporates a mixture of plant resins, waxes, and soil into its composition (Chuttong et al., 2023). Geopropolis has been shown to exhibit anticancer, antioxidant (AOX), and *in vitro* antibacterial properties, mainly due to its high content of phenolic compounds (PCs), which neutralize free radicals and help prevent oxidative stress, a key factor in the development of various chronic diseases (Ferreira et al., 2022).

Traditionally used as a natural antibacterial remedy by indigenous communities, geopropolis has gained increasing attention of its antibacterial effects, especially since the excessive and prolonged use of synthetic antibiotics can disrupt the gut microbiota and contribute to metabolic, cardiovascular, gastrointestinal, and neurological disorders, including anxiety and depression, as well as promote the emergence of resistant bacterial strains (Ferdinand et al., 2024; Socala et al., 2021). These pharmacological properties of geopropolis, which are closely linked to its phytochemical composition, depend on the natural resources available, as well as the geographical and climatic characteristics of the region where the beehives are located. In the case of *M. beecheii*, the volatile compounds identified in its geopropolis have primarily been terpenoids (Torres-González et al., 2016). Additionally, pentacyclic triterpenes have been isolated from chloroform-methanol extracts, while anthraquinones and flavonoids have been identified in methanol and ethanol extracts (Yam-Puc et al., 2019). Despite the biological significance of geopropolis and other stingless bee products, studies specifically evaluating the biological properties of *M. beecheii* geopropolis remain scarce. Therefore, in this study, we report the AOX and *in vitro* antibacterial activities of ethanolic extracts of geopropolis (EEG) produced by *M. beecheii* from the Yucatan Peninsula, as well as their associated chemical composition.

Material and methods

Chemical reagents

All reagents used were analytical grade except for the mobile phase for HPLC analysis, which was of chromatographic grade and purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Geopropolis samples

Seven raw geopropolis samples from *Melipona beecheii* were collected from seven domestic meliponaries located in various rural localities across Yucatan, Campeche and Quintana Roo in the Yucatan Peninsula, Mexico (Figure 1). The geopropolis was obtained from hives between January and June 2020. All samples were stored in amber bottles at -20 °C until analysis.

Preparation of the Ethanolic Extract of Geopropolis (EEG)

The EEG was prepared by mixing 7 g of pulverized geopropolis with 50 mL of 96 % ethanol. The mixture was kept under mechanical agitation in a closed container for 24 h at room temperature and protected from light. It was then filtered using Whatman No. 4 filter paper to separate the inorganic material (soil) and concentrated in a rotary evaporator at 45 °C to obtain the dry extract.

Determination of total phenolic compounds (TPC) and total flavonoids (TF)

TPC content was determined using the azo coupling reaction with diazotized sulfanilic acid salt (4SFD) (Piña Betancourt et al., 2024). A volume of 0.5 mL of EEG (100 μg/mL) was mixed with 2.5 mL of methanol:water solution (1:1 v/v). Subsequently, 0.3 mL of an aqueous solution of diazotized sulfanilic acid salt (1 mg:1 mL) was added, followed by 0.3 mL of 5 % w/v NaOH. The mixture was homogenized using a vortex mixer and allowed to react at room temperature for 90 min. Absorbance was measured at 420 nm using a spectrophotometer (Agilent Technologies, Cary 60 Uv-Vis, USA). The concentration was calculated using a calibration curve prepared with gallic acid in the range

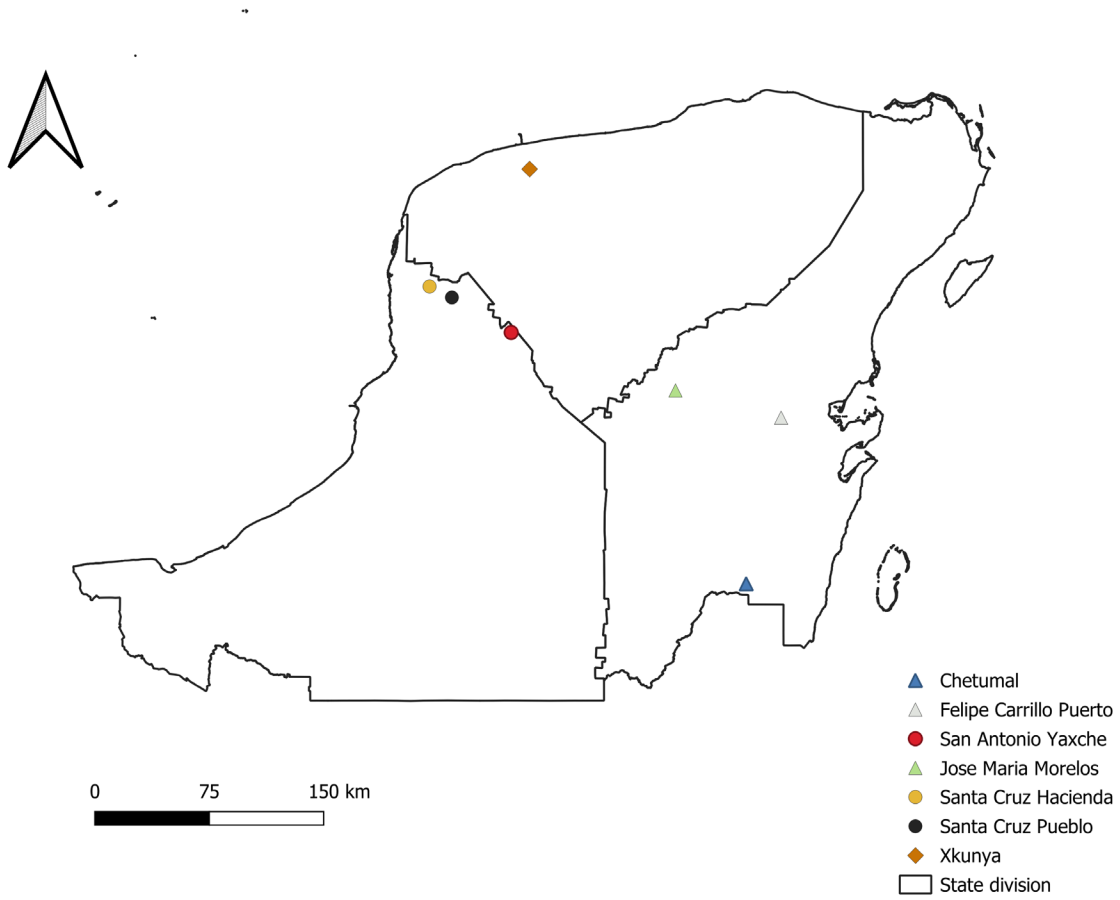

Figure 1. Geopropolis collection sites in the Yucatan Peninsula.

of 40 to 400 mg L^{-1}. TPC was expressed as mg GAE/100 g^{-1} extract. Flavonoid content was determined using the aluminum chloride colorimetric method as described by Chang et al. (2002). Briefly, 0.5 mL of EEG (100 µg/mL) was mixed with 4.5 mL of methanolic solution of aluminum chloride hexahydrate 2 % ($AlCl_3 \cdot 6H_2O$) and incubated for 30 min at room temperature in the dark. After incubation, absorbance was measured at 415 nm. A calibration curve was prepared using quercetin (0.4-16 µg/mL^{-1}) as a standard. Results were expressed as g QE/100 g^{-1} extract.

Analyses of phenolic compounds by HPLC

The identification and quantification of TPC and TF were performed using an HPLC-1220 Infinity system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Nucleosil C18 column (250 mm x 4.6 mm i.d., particle size 5 µm) (Tumbarski et al., 2025). Detection conditions were as follows: injection volume, 20 µL; detection wavelength, 280 nm; and column temperature, 25 °C. The mobile phase consisted of water/formic acid (99:1, v/v; solvent A) and HPLC-grade acetonitrile (solvent B). A linear gradient elution was applied: 2-100 % (B) over 70 min at a flow rate of 0.5 mL/min. Individual phenolic compounds were identified by comparing retention times with those of standards. Quantification was performed using calibration curves (0.001-0.02 mg/ml; $R^2 = 0.99$), and results were expressed as mg/100 g of extract.

Antioxidant Activity Assays

DPPH Free radical-scavenging activity: AOX activity was evaluated using the DPPH radical scavenging assay according to Brand-Williams et al. (1995). Briefly, 3.9 mL of DPPH solution (60 µMol din methanol) was mixed with 100 µL of EEG. Absorbance was measured at 515 nm using with a spectrophotometer (Agilent Technologies, Cary 60 Uv-Vis, USA) after 30 min of reaction. Results were expressed as mg TE/100 g extract

using a calibration curve prepared with Trolox solutions at concentrations from 20 to 320 mg L^{-1}.

Ferric reducing antioxidant power assay (FRAP): Ferric reducing antioxidant power ($Fe^{3+} \rightarrow Fe^{2+}$) was determined following the method described by Can-Cauich et al. (2017). Results were expressed as mg TE/100 g of extract, based on a calibration curve prepared with Trolox solutions at concentrations of 20-320 mg L^{-1}.

***In vitro* antibacterial activity**: *In vitro* antibacterial activity was evaluated using the agar disc diffusion method against two reference strains: *Escherichia coli* ATCC 10536 (Gram-negative) and *Staphylococcus aureus* ATCC 25923 (Gram-positive). The bacteria were cultured in Brain Heart Infusion broth (BHI) at 37 °C for 24 h. Microbial cultures were spread on the surface of Mueller-Hinton agar plates, using a bacterial suspension adjusted to the turbidity of McFarland standard 0.5 (1×10^8 CFU/mL) (Mráz et al., 2025). The EEGs were diluted in 0.5 mL of DMSO and 50 µL of each solution was applied to three sterile paper discs (6 mm diameter), which were subsequently placed on the inoculated Petri dishes. The plates were incubated at 37 ± 0.1 °C for 24 h. *In vitro* antibacterial activity was evaluated by measuring the inhibition zones (mm), excluding the disc diameter. Streptomycin sulfate (10 µg/disc) and DMSO were used as positive and negative controls, respectively. The effect of the concentration of the most active EEG was evaluated using concentrations ranging from 5 to 50 µL (equivalents to 1-17 µg extract/disc) applied to 6 mm discs, following the same agar diffusion method. Results were expressed as inhibition halo diameter (in mm) excluding the disc diameter for each concentration evaluated. The localized inhibition zone corresponds to the area limited to the diameter of the disc.

Statistical Analysis: Analysis of variance (ANOVA) was performed using Statgraphics 1 Plus software, version 2.1 (Manugistic, Inc., Rockville, MD, USA). Means were compared using the Tukey's test, and statistical differences were considered significant at $p < 0.05$. Pearson's correlation coefficient was applied to evaluate relationships among phenolic compounds, AOX and, *in vitro* antibacterial activities (Montgomery, 2017). All assays were analyzed in triplicate.

Results and discussion

Determination of TPC and TF

The chemical composition of geopropolis produced by stingless bees is remarkably diverse and chemically intricate, and several studies have reported its broad spectrum of biological and pharmacological effects. According to Ruiz Ruiz et al. (2023), its phytochemical profile is shaped by various ecological and biological factors, including the predominant flora, geographical location, bee species, the timing of exudate and wax collection, as well as the extraction system with different solvents to isolate bioactive compounds (Ferreira et al., 2022). In this regard, polyphenols, flavonoids, aromatic acids, and diterpenes are recognized as the principal phytoconstituents contributing to its bioactivity (Surek et al., 2021).

Determination of the TPC and TF content in geopropolis is essential, given their well-established AOX and antibacterial roles (Chuttong et al., 2023). The TPC and TF values of EEG produced by *M. beecheii* are summarized in Table 1. The EEG from San Antonio Yaxche exhibited the highest TPC concentration with 31.82 mg GAE/100 g extract and TF of 78.33 g QE/100 g extract, respectively. These values were significantly higher ($p < 0.05$) than those observed in extracts from other locations within the Yucatan Peninsula (Table 1). This suggests that the bee species and/or its habitat or environment may influence the chemical composition and the concentration of phenolic and flavonoid compounds in geopropolis (dos Santos et al., 2017). Such elevated levels may be related to the diversity and abundance of resinous plants in the local flora. The Yucatan Peninsula, characterized by low deciduous forest and exposed limestone rock, creates numerous micro-niches that promote high vascular plant diversity, which stingless bees may exploit as resin sources (Carnevali et al., 2022). In contrast, the EEG from Sta. Cruz Hacienda displayed the lowest concentrations, with TPC of 5.2 mg GAE/100 g extract and TF of 8.63 g QE/100 g extract, indicating a less favorable phytochemical profile. This variation highlights the profound impact of microenvironmental differences, even within the same geographical region, on the biosynthesis and accumulation of bioactive metabolites in geopropolis (Ruiz Ruiz et al., 2023). Interestingly, the TF value of the

Table 1. TPC, TF and AOX activity of geopropolis of *Melipona beecheii*.

Sample	Coupling compounds as actual phenol content (4SFD) in mg GAE/100 g^{-1} dry extract	Total flavonoid content (Aluminum trichloride method) in mg QE/100 g^{-1} dry extract	DPPH radical inhibition in mg TE/100 g extract	FRAP in mg TE/100 g extract
Felipe Carrillo Puerto, Quintana Roo	$17.39^{c} \pm 0.72$	$47.65^{c} \pm 0.75$	$11.49^{c} \pm 0.21$	$26.76^{d} \pm 0.19$
Xkunya, Yucatan	$11.79^{c} \pm 0.06$	$61.19^{b} \pm 2.16$	$56.12^{b} \pm 3.98$	$75.69^{b} \pm 2.36$
Chetumal, Quintana Roo	$23.86^{b} \pm 3.32$	$75.58^{a} \pm 1.99$	$11.29^{c} \pm 0.52$	$68.89^{c} \pm 0.4$
San Antonio Yaxche, Campeche	$31.82^{a} \pm 1.01$	$78.33^{a} \pm 2.15$	$97.37^{a} \pm 8.26$	$101.03^{a} \pm 0.97$
Jose Maria Morelos, Quintana Roo	$5.23^{c} \pm 0.01$	$10.24^{d} \pm 0.48$	$2.21^{d} \pm 0.17$	$6.61^{e} \pm 0.03$
Santa Cruz Pueblo, Campeche	$7.38^{c} \pm 0.32$	$10.84^{d} \pm 0.31$	$2.43^{d} \pm 0.09$	$7.59^{e} \pm 0.03$
Santa Cruz Ex-Hacienda, Campeche	$5.2^{d} \pm 0.24$	$8.63^{d} \pm 0.21$	$1.42^{d} \pm 0.02$	$4.78^{f} \pm 0.05$

Values are expressed as mean ± standard deviation (n = 7). Different superscript letters within a column indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Chetumal extract (75.58 g QE/100 g extract) was statistically comparable to that obtained from the San Antonio Yaxche extract, despite presenting a lower TPC content. This observation suggests that the biosynthetic pathways regulating flavonoid production may be more strongly expressed in response to botanical sources in the Chetumal locality. These findings suggest a non-linear relationship between TPC and total TF contents among the evaluated geopropolis samples, reflecting the influence of local ecological conditions and specialized plant species associated with these environments (Carnevali et al., 2022). Interestingly, the higher TF values relative to TPC observed in some extracts require further interpretation. In propolis research, the observation that TF content appears higher than TPC does not necessarily indicate a compositional inconsistency, even though flavonoids are a subclass of phenolic compounds. This result can be explained by differences in the analytical methods used and their selectivity toward specific phenolic structures. The diazotization-coupling method used for TPC determination responds selectively to certain phenolic hydroxyl groups and may underestimate phenolics with lower reactivity or steric hindrance (Azeem et al., 2020). In contrast, the aluminum chloride ($AlCl_3$) method forms strong complexes with flavonoids containing specific keto and hydroxyl groups, particularly flavones and flavonols, which can lead to higher apparent TF values when these compounds dominate the phenolic profile. Therefore, the higher TF observed likely reflects methodological selectivity and the predominance of $AlCl_3$-reactive flavonoids in the extract, rather than a true contradiction in the phenolic composition. Similar discrepancies between spectrophotometric assays have been reported for complex natural matrices, where results depend strongly on assay chemistry and sample composition (Domínguez-López et al., 2024; Zugazua-Ganado et al., 2024).

In this regard, in a study conducted by dos Santos et al. (2017) of propolis from *M. orbignyi* (Guérin-Méneville), TPC was 12.1 ± 0.6 mg of GAE/100 g of hydroalcoholic extract of geopropolis, while the TF content was 1.99 mg of QE/100 g of hydroalcoholic extract of geopropolis. These differences likely reflect interspecies variations in bee foraging behavior and resin selection, as well as differences in plant biodiversity and climatic conditions across regions, in addition to the analytical techniques used for TPC and TF extraction. Overall, these results suggest that the chemical composition of phenolic compounds, and consequently the biological activities of geopropolis, may be influenced by local ecological dynamics (Ruiz Ruiz et al., 2023). One-way ANOVA and Pearson's correlation analyses between TPC and TF concentrations demonstrated

significant differences among the propolis samples from the different sampling regions (Table 1), as well as a significant positive correlation (r = 0.915) between TPC and TF values. However, although this study revealed variation in TPC and TF contents among locations, it is necessary to consider the solubility and chemical stability of geopropolis extracts, since the analyses were performed using ethanol as the extraction solvent. Therefore, understanding these complex interactions is essential for identifying geopropolis samples with the highest therapeutic potential and for promoting their use in functional food and pharmaceutical applications (Dos Santos et al., 2024).

Analysis of Phenolic Compounds and Total Flavonoids identified by HPLC

The TPC and TF analysis of *M. beecheii* geopropolis from the Yucatan Peninsula revealed high variability with significant pharmacological implications (Table 2). Among the identified hydroxybenzoic acids, the concentration of gallic acid reached 0.26 mg/100 g in Felipe Carrillo Puerto, but was absent in Xkunya and Santa Cruz Pueblo. Likewise, the concentration of 4-hydroxybenzoic acid peaked at 0.16 mg/100 g in San Antonio Yaxche. Both compounds are recognized for their AOX and antibacterial properties, comparable to levels reported in Brazilian and European propolis (Kurek-Górecka et al., 2020). Within the hydroxycinnamic acids, vanillic acid was highest at Chetumal (2.10 mg/100 g), and ferulic acid reached 3.17 mg/g in Felipe Carrillo Puerto, values equal to or higher than those reported for Turkish propolis, whose compounds are recognized for their AOX and antibacterial potential against *Staphylococcus aureu*s and *Escherichia coli* (Hernández-Jaime et al., 2025; Özkök et al., 2021).

Additionally, 2-hydroxycinnamic acid (2.17 mg/100 g in Felipe Carrillo Puerto) and trans-cinnamic acid (0.08 mg/100 g in Felipe Carrillo Puerto) exhibited site-specific distribution, suggesting that botanical origin and environmental conditions as determining factors (Bankova et al., 2018). For flavanols, catechin was predominant in Felipe Carrillo Puerto (0.95 mg/100 g) and Santa Cruz Ex-Hacienda (0.83 mg/100 g), while epicatechin showed a more uniform distribution across zones (0.21 mg/100 g). Both are AOX and neuroprotective compounds whose variability is linked to local resinous flora (Kurek-Górecka et al., 2020; Özkök et al., 2021). In the flavone and flavonol group, apigenin, a flavonoid associated with antibacterial effects against fluoroquinolone-resistant *S. aureu*s (Morimoto et al., 2023) was most abundant in Chetumal (5.11 mg/100 g), while chrysin reached a concentration of 1.45 mg/g at the same site. Furthermore, Felipe Carrillo Puerto samples exclusively contained pinocembrin (4.12 mg/100 g) and galangin (2.27 mg/100 g), both with strong antibacterial potential (Wu et al., 2022).

Particularly, Felipe Carrillo Puerto and Chetumal extracts exhibited high concentrations of apigenin and pinocembrin, compounds widely associated with AOX and antibacterial activities (Morimoto et al., 2023; Wu et al., 2022). These profiles are comparable to those described for propolis of established biological value (Kurek-Górecka et al., 2020), indicating potential functional applications".

Antioxidant activity

Two different methods were used to determine the AOX activities of the EEG: the DPPH free radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay (Table 1). The EEG from San Antonio Yaxche was the most effective in reducing DPPH radicals (97.37 g Trolox equivalents/100 g extract), followed by the extract from Xkunya (56.12 g Trolox equivalents/100 g extract). Similarly, the EEG from San Antonio Yaxche showed the highest ferric reducing capacity (101.03 g Trolox equivalents/100 g extract), followed by the extract from Xkunya (75.69 g Trolox equivalents/100 g extract). Although the extract from Xkunya exhibited lower TPC and TF contents than that from San Antonio Yaxche, it still showed considerable AOX activity, suggesting that additional bioactive constituents may also contribute to its reducing capacity. The AOX activity of the San Antonio Yaxche EEG in both assays underscores the richness of this sample in electron-donating TPC and TF capable of neutralizing free radicals and reducing ferric ions. In a comparative context, the AOX activity observed in the present study is consistent with previous reports, such as the work of Dos Santos et al. (2017), in which geopropolis from *M. beecheii* and *M. mondury* Smith exhibited strong AOX activity. Together, these findings further support the idea that reducing agents, such as TPC and TF, are

Table 2. Contents of individuals phenolic compounds in geopropolis of *M. beecheii* (mg/100 g extract) identified by HPLC.

Phenolic compound	Felipe Carrillo Puerto, Quintana Roo	Chetumal, Quintana Roo	Xkunya, Yucatan	San Antonio Yaxche, Campeche	Santa Cruz Pueblo, Campeche	Jose Maria Morelos, Quintana Roo	Santa Cruz Ex-Hacienda, Campeche
Gallic acid	$0.26^{d} \pm 0.05$	$0.19^{c} \pm 0.03$	ND	$0.19^{b} \pm 0.00$	ND	$0.18^{b} \pm 0.00$	$0.16^{b} \pm 0.00$
4-Hydroxybenzoic acid	$0.12^{d} \pm 0.00$	ND	ND	$0.16^{b} \pm 0.02$	$0.15^{b} \pm 0.04$	$0.12^{b} \pm 0.05$	ND
Vanilic acid	$0.10^{d} \pm 0.02$	$2.10^{b} \pm 0.19$	$0.08^{b} \pm 0.00$	$0.07^{b} \pm 0.00$	$0.05^{b} \pm 0.00$	$0.10^{b} \pm 0.00$	$0.09^{b} \pm 0.00$
Ferulic acid	$3.17^{b} \pm 0.25$	$1.10^{b} \pm 0.19$	ND	$1.12^{a} \pm 0.21$	$1.80^{a} \pm 0.22$	$1.66^{a} \pm 0.10$	$1.58^{a} \pm 0.20$
2-Hydroxycinnamic acid	$2.17^{c} \pm 0.12$	ND	ND	$1.23^{a} \pm 0.02$	ND	ND	ND
Trans-cinnamic acid	$0.08^{d} \pm 0.01$	$0.05^{c} \pm 0.01$	ND	ND	ND	ND	ND
Catechin	$0.95^{d} \pm 0.00$	$0.63^{c} \pm 0.00$	ND	ND	$0.73^{b} \pm 0.01$	ND	$0.83^{b} \pm 0.01$
Epicatechin	$0.33^{d} \pm 0.10$	$0.21^{c} \pm 0.11$	$0.23^{b} \pm 0.00$	$0.23^{b} \pm 0.11$	$0.21^{b} \pm 0.11$	ND	ND
Apigenin	$2.10^{c} \pm 0.10$	$5.11^{a} \pm 0.15$	$2.0^{a} \pm 0.00$	ND	$1.11^{a} \pm 0.00$	$1.08^{a} \pm 0.00$	ND
Chrysin	$1.25^{c} \pm 0.12$	$1.45^{b} \pm 0.11$	ND	ND	ND	ND	ND
Pinocembrin	$4.12^{a} \pm 0.15$	ND	ND	ND	ND	ND	ND
Galangin	$2.27^{c} \pm 0.25$	ND	ND	ND	ND	ND	ND
Vanillin	$0.11^{d} \pm 0.00$	$0.13^{c} \pm 0.00$	ND	$0.08^{b} \pm 0.00$	$0.09^{b} \pm 0.00$	ND	$0.09^{b} \pm 0.00$

Values are expressed as mean ± standard deviation (n = 7). Different superscript letters within a column indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). ND = No detected.

important contributors to the AOX capacity of geopropolis (De Souza et al., 2013; Dutra et al., 2014). Pearson's correlation coefficients (r) revealed significant positive correlations between TPC and TF concentrations and AOX activities assessed by DPPH (0.70 and 0.70, respectively) and FRAP (0.85 and 0.94, respectively), confirming that high levels of these compounds may be associated with greater AOX capacity (Hafshejani et al., 2023; Tumbarski et al., 2025). The strongest correlation was found between flavonoid concentration and AOX activity assessed using FRAP. In both cases, the degree of correlation was higher when AOX activity was evaluated using FRAP. Flavonoid concentration was the variable that explains most of the AOX activity measured by FRAP (88.88 %). Additionally, ANOVA indicated highly significant differences among the seven samples evaluated ($p < 0.05$), reflecting that the chemical composition of geopropolis varies according to the region of origin and directly influences its AOX potential (Hafshejani et al., 2023; Tumbarski et al., 2025). Thus, the high AOX activity observed in the EEG from San Antonio Yaxche may be attributed to its notably elevated TPC and TF contents. This highlights the potential of geopropolis from specific regions not only as a natural source of AOX, but also as a promising candidate for the development of nutraceutical formulations.

In vitro antibacterial activity

The antibacterial activity of the EEG is presented in Table 3. *Escherichia coli* (Gram-negative) displayed variable sensitivity to the extracts. Localized inhibition zones were observed for extracts from Xkunya, Felipe Carrillo Puerto, and Santa Cruz Ex-Hacienda, whereas more pronounced inhibition halos were recorded for the EEG from Santa Cruz Pueblo (3.5 mm) and Chetumal (2.5 mm), indicating antibacterial activity in these samples. In contrast, *Staphylococcus aureus* (Gram-positive) was sensitive to all EEG samples. Overall, the EEGs exhibited moderate antibacterial activity rather than a strong inhibition. Extracts from Felipe Carrillo Puerto, Santa Cruz Ex-Hacienda, and Jose Maria Morelos produced localized inhibition, while the extract from Chetumal showed the most pronounced antibacterial activity against *S. aureus*, with a 6 mm inhibition halo comparable to that of the positive control, streptomycin (7.5 mm). Notably, the EEGs from Santa Cruz Pueblo and Chetumal were active against both bacterial strains. However, despite the observed *in vitro* antibacterial effect, statistical analysis ($p < 0.05$) indicated that both bacteria, particularly *E. coli*, were less sensitive to the extracts than to the positive control (Streptomycin, 16 mm). This finding confirms that the antibacterial activity of the geopropolis extracts was lower than that of the control antibiotic and lower than that reported for some geopropolis samples in previous studies (Fernández-León et al., 2022). These differences may be related to the stingless bee species, resin collection period, and qualitative differences in chemical composition rather, than solely to the overall abundance of TPC or TF. No consistent correlation was observed between TPC or TF content and *E. coli* inhibition, as the extracts from Chetumal and Santa Cruz Pueblo demonstrated comparable *in vitro* antibacte-

Table 3. In vitro antibacterial activity of geopropolis extracts from *Melipona beecheii* from the Yucatan Peninsula.

	Inhibition zone (mm)	
Geopropolis extract	***Escherichia coli* ATCC 10536**	***Staphylococcus aureus* ATCC 25923**
Xkunya, Yucatan	$0.00^{c*} \pm 0.00$	$2.30^{c} \pm 0.58$
Felipe Carrillo Puerto, Quintana Roo	$0.00^{c*} \pm 0.00$	$0.00^{d*} \pm 0.00$
Santa Cruz Ex-Hacienda, Campeche	$0.00^{c*} \pm 0.00$	$0.00^{d*} \pm 0.00$
San Antonio Yaxche, Campeche	$0.00^{c} \pm 0.00$	$4.50^{b} \pm 0.71$
Santa Cruz Pueblo, Campeche	$3.50^{a} \pm 0.71$	$2.00^{c} \pm 0.00$
Jose Maria Morelos, Quintana Roo	$0.00^{c} \pm 0.00$	$0.00^{d*} \pm 0.00$
Chetumal, Quintana Roo	$2.5^{b} \pm 0.71$	$6.00^{a} \pm 0.00$
Positive control (Streptomycin)	16.00 ± 0.00	7.50 ± 0.71
Negative control (DMSO)	0.00 ± 0.00	0.00 ± 0.00

*Localized inhibition. Values are expressed as mean ± SD (n = 7). Different superscript letters within a column indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

rial activity despite their differing chemical profiles (Table 1). However, all EEGs inhibited *S. aureus*, suggesting a possible association between TF content and activity against Gram-positive bacteria. This observation is supported by the extracts from Chetumal and San Antonio Yaxche, which exhibited the highest TF levels, and also produced the greatest inhibition zones against *S. aureus* (6 mm and 4.5 mm, respectively). These findings are consistent with previous studies reporting inhibition of *S. aureus* by geopropolis-derived extracts (Bucio-Villalobos & Martínez-Jaime, 2017; Portela-Márquez et al., 2022). The EEG from Chetumal contained chrysin, a compound well documented for its antibacterial activity, along with other bioactive materials such as apigenin and ferulic acid. Despite having a lower concentration of TPC and TF, the extract from Santa Cruz Pueblo exhibited considerable antibacterial efficacy, requiring only 6 µg to inhibit *E. coli* and 4 µg for *S. aureus*, with the largest inhibition halo observed at 10 µg (Table 4). In contrast, the EEG from Chetumal exhibited antibacterial activity only at the highest tested dose (16.95 µg) against both bacteria, suggesting differences in compound potency or possible synergistic effects among constituents.

The higher resistance of *E. coli* is likely related to its complex outer membrane containing lipopolysaccharides, which restrict the permeability of antibacterial agents. In contrast, the single peptidoglycan-rich cell wall of *S. aureus* renders it more susceptible to phenolic and flavonoid compounds (Coutinho et al., 2023). Phenolic compounds and flavonoids are known to exert antibacterial effects through mechanisms such as disruption of bacterial membranes, inhibition of enzymatic activity, and interference with nucleic acid synthesis. Overall, the results indicates that, although phenolics and flavonoids contribute to antibacterial activity, other bioactive constituents and synergistic interactions may also play important role.

Table 4. *In vitro* antibacterial activity of different concentrations of geopropolis extracts from *Melipona beecheii* from the Yucatan Peninsula.

		Inhibition zone (mm)	
Geopropolis extract	**Extract concentration (µg/disc)**	***Escherichia coli* ATCC 10536**	***Staphylococcus aureus* ATCC 25923**
Santa Cruz Pueblo, Campeche	10.10	$3.50^{a} \pm 0.71$	4.00 ± 0.00
	8.08	$2.00^{b} \pm 0.00$	3.00 ± 0.00
	6.06	$2.00^{b} \pm 0.00$	3.00 ± 0.00
	4.04	$0.00^{c*} \pm 0.00$	1.50 ± 0.71
	2.02	$0.00^{c*} \pm 0.00$	$0.00^{*} \pm 0.00$
	1.01	$0.00^{c} \pm 0.00$	0.00 ± 0.00
Chetumal, Quintana Roo	16.95	$4.00^{a} \pm 0.00$	$4.00^{a} \pm 0.00$
	13.56	$4.00^{a} \pm 0.00$	$0.00^{b} \pm 0.00$
	10.17	$0.00^{b*} \pm 0.00$	$0.00^{b} \pm 0.00$
	6.78	$0.00^{b*} \pm 0.00$	$0.00^{b} \pm 0.00$
	3.39	$0.00^{b*} \pm 0.00$	$0.00^{b} \pm 0.00$
	1.70	$0.00^{b*} \pm 0.00$	$0.00^{b} \pm 0.00$
Positive control (Streptomycin)		16.00 ± 0.00	7.50 ± 0.71
Negative Control (DMSO)		0.00 ± 0.00	0 ± 0.00

*Localized inhibition. Values are expressed as mean ± SD (n = 7). Different superscript letters within a column indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

Conclusions

Geopropolis from *M. beecheii* is a complex natural product rich in bioactive compounds that contribute to its AOX and antimicrobial activities. The phenolic and flavonoid composition of the evaluated extracts varied significantly among locations, highlighting the influence of local flora and environmental conditions. The EEGs from San Antonio Yaxche and Chetumal exhibited the highest TPC and TF values, which correlated with greater AOX activity in the DPPH and FRAP assays. *Staphylococcus aureu*s was the most sensitive bacterium to all extracts, whereas only selected samples, particularly those from Chetumal and Santa Cruz Pueblo, showed inhibitory activity against *Escherichia coli*. These biological activities may be associated with the presence of specific phenolic compounds, including apigenin, chrysin, and ferulic acid. Overall, this study expands current knowledge of the phenolic composition of *M. beecheii* geopropolis from the Yucatan Peninsula and demonstrates its potential as a natural source of AOX and antibacterial compounds.

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