Antibacterial and Antioxidant Activities of Ethanolic and Water Extracts of Stingless Bees
*Tetrigona binghami, Heterotrigona itama,*
and *Geniotrigona thoracica* Propolis Found in Brunei

Nadzirah Zullkiflee¹, Hussein Taha², Nurul Aliah Abdullah¹, Fatimah Hashim¹, and Anwar Usman¹*

¹Department of Chemistry, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410 Brunei Darussalam
²Environmental and Life Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410 Brunei Darussalam
³Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

In the present study, the antibacterial activities of ethanolic and water extracts of propolis produced by stingless bees *Tetrigona binghami, Heterotrigona itama,* and *Geniotrigona thoracica* found in Brunei against four different bacterial strains were quantified. Additionally, spectroscopic and colorimetric methods, including Fourier transform infrared, absorption, fluorescence, aluminum chloride, and Folin-Ciocalteu analyses were used to characterize the propolis extracts. Flavonoid, phenolic, and aromatic acid compounds in the propolis extracts were also quantified, as these compounds are responsible for their antioxidant capacity. The antibacterial activity was determined based on bacterial growth inhibition zones using the disc diffusion, and it was further confirmed by the minimum inhibitory and bactericidal concentrations, which were evaluated using the broth macrodilution method. The propolis extracts exhibited antibacterial activities but were lower compared to streptomycin, which was used as a standard antibiotic. The MIC values of the water extracts were 2500 μg/mL, and those of the ethanolic extracts were in the range of 2500–10000 μg/mL, much higher than other reported propolis from different countries. Their MBC test further suggested that the propolis extracts were bacteriostatic. The overall findings evidenced the quantities of the antibacterial and antioxidant properties of the propolis extracts, although the results suggested low antibacterial activities of the propolis extracts from Brunei stingless bees.

Keywords: antibacterial activity, antioxidant activity, flavonoid content, phenolic content, stingless bee propolis

*Corresponding author: anwar.usman@ubd.edu.bn*
INTRODUCTION

Stingless bees, which are associated with the Meliponini tribe, consist of more than 600 species and are highly distributed in both tropical and subtropical regions all over the globe (Rasmussen and Cameron 2007). The stingless bee propolis, also called bee glue, is a mixture of resinous substances from flowers, buds, leaves of plants, and mandibular secretion of the stingless bees (Abdullah et al. 2019; Bankova et al. 2021). The propolis consists mainly of lipids with a total content of more than 45%, making it waterproof and preventing it from the growth of microbes. Furthermore, the organic compounds present in propolis (Mogana et al. 2020) – including flavonoids, phenolics, and aromatic acids – are responsible for its antimicrobial (Song and Ge 2019), antioxidant (Moreira et al. 2008), and antidiabetic properties (El-Sayed et al. 2009). Due to these bioactivities, propolis has been utilized as an important remedy in traditional medicines, modern biomedicines, and natural products cosmetics, as well as a constituent of health foods (Suran et al. 2021).

Typically, propolis has more than 300 organic compounds and organic acids (Peña 2008; Salleh et al. 2021) – including bioactive compounds such as tectochrysin, galangin, apigenin, pinobanksin 3-acetate, kaempferol, ferulic acid, caffeic acid, coumaric acid, and cinnamic acid (Marcucci and Bankova 1999; Medić-Šarić et al. 2004; Bonamigo et al. 2017). Propolis of different species of stingless bees has different chemical constituents and compositions due to the variation in time of collection, botanical surroundings, and geographical origins (Marcucci 1995; Park et al. 2000; Bankova et al. 2000).

Geniotrigona thoracica, Heterotrigona itama, and Tetrigona binghami are some of the common stingless bee species found in Brunei. They are among the most frequently domesticated since their log hives may be located and collected in natural woods, and they can be easily cultivated in suburban environments. In our recent studies, propolis of stingless bees G. thoracica, H. itama, and T. binghami found at the same botanical and geographical origin in Brunei exhibited different growth inhibition zones of bacterial strains, suggesting that they have different bioactive chemicals (Abdullah et al. 2019, 2020). However, it must be understood whether Brunei propolis is bactericidal (bacteria-killing) or bacteriostatic (bacteria-inhibiting). In this sense, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are important to determine the susceptibility of bacteria and to provide effective treatments for bacterial infections (Salman and Kareem 2021). Therefore, this study focused on the antibacterial properties of the water and ethanolic extracts of T. binghami, H. itama, and G. thoracica propolis from Brunei. The research objectives are as follows: [i] to determine their bacterial growth inhibition, MIC, and MBC; [ii] to evaluate flavonoid, phenolic, and aromatic acid compounds contained in the propolis extracts by the spectroscopic and colorimetric methods – including Fourier transform infrared, absorption, fluorescence, aluminum chloride, and Folin-Ciocalteu analysis; and [iii] to evaluate the antioxidant activities of propolis extracts by measuring their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

MATERIALS AND METHODS

The raw propolis collected from T. binghami, H. itama, and G. thoracica stingless bees were obtained from Tasbee Meliponiculture Farm in Brunei. The samples were collected in July, September, and December of 2020. After each collection, the samples were rinsed with water and dried using a dehumidifier for 2 wk. The dried propolis was then ground into small pieces and soaked in either ultrapure water or ethanol. The ratio of propolis to water/ethanol was 1 g/25 mL. The flask was placed inside a temperature-controlled thermoshaker operating at 37 °C and 150 rpm for 18 h. After filtration, the solution was poured onto watch glasses and dried. Finally, the propolis extracts were scrapped and kept in airtight vials until use. All the data collected were analyzed.

The propolis extracts were characterized by spectroscopic methods. Their vibrational spectra were measured using an FTIR spectrometer (Shimadzu IRPrestige-21, Japan) in the range of 4000–400 cm⁻¹ with a spectral resolution of 4 cm⁻¹. The absorption and fluorescence spectra were recorded by UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) and photoluminescence spectrometer (Shimadzu R6000, Japan), respectively. The absorption spectra of propolis were measured in the spectral region between 200–500 nm.

Antibacterial activity – including inhibition of bacterial growth, MIC, and MBC of the propolis extracts against Gram-positive bacterial strains [Bacillus subtilis (ATCC-11774) and Staphylococcus aureus (ATCC-29213)] and Gram-negative bacterial strains [Pseudomonas aeruginosa (ATCC-27853) and Escherichia coli (ATCC-11775)] – was investigated. Bacterial growth inhibition was performed according to the standard disc-diffusion method (Rekha et al. 2018). In these tests, the positive control was a standard antibiotic – namely, streptomycin – whereas water was used as the negative control. The media in these tests were pre-sterilized distilled water-dissolved Mueller-Hinton agar (MHA) and Oxoid Mueller-Hinton broth (MHB). Briefly, 25 mL of MHA was solidified in a Petri dish at room temperature. In a sterile environment, 200 µL of 0.5 McFarland bacterial culture was uniformly
spread on the agar plate and allowed to dry. A sterile filter paper was soaked in a solution of the propolis extract (10 mg/mL) and put onto the solidified agar, followed by incubation overnight at 37 °C. The same procedure was applied for the different propolis extracts as well as with the antibiotic. The diameters of the bacterial inhibition zone on the agar surface were then measured in micrometers (mm).

MIC of the propolis extracts was quantified using the broth macrodilution method (Salman and Kareem 2021). Here, 0.5 mL of the 0.5 McFarland bacterial culture was diluted with 74.5 mL of MHB, and it was used as a standardized inoculum. A colloidal solution of propolis extracts (20 mg/mL) was prepared in MHB, from which other colloidal solutions with different concentrations of propolis extract between 0.0195–10 mg/mL were prepared by two-fold dilutions. All these colloidal solutions were used to obtain the lowest effective concentration of the bacterial growth inhibition. A 1-mL standardized inoculum was then added to each colloidal solution in a test tube to reach the final concentrations of 5 × 10^5 CFU/mL. The tubes were covered with a sterile aluminum foil and then incubated for 16–20 h at 37 °C.

The MBC was quantified by inoculating the samples onto the sterile MHA plates. Here, a total of 25 mL of MHA was spread over a Petri dish. Once the agar had solidified, the propolis extracts were swabbed using the inoculating loops onto the surface of the solidified agar. After incubation at 37 °C for 24 h, the MBCs of the propolis extracts were determined for those that did not show visible bacterial growth.

Antioxidant activity was associated with DPPH radical scavenging activity. The reactivity of the propolis extracts was estimated based on light absorption of the radical at 517 nm by a solution mixture of 3.5 mL DPPH (50 mg/mL) in ethanol and 0.5 mL aqueous colloidal solution of propolis extract or serial concentration of ascorbic acid as radical scavenger standard. Baseline correction was performed by subtracting the measured absorbance from that of the mixture of 70:10 (v/v) ethanol and water. The inhibition concentration, IC_{50}, of the propolis extracts was then estimated, as previously described in detail (Abdullah et al. 2020).

The total phenolic content of propolis extracts was quantified using the Folin-Ciocalteu methods (El-Sayed et al. 2021). Here, the aqueous solution of propolis extracts of three different stingless bee species was prepared with three different concentrations (0, 0.5, and 1.0). For TFC measurement, 100 μL of each solution of propolis extracts was mixed with 2% aluminum chloride (AlCl₃) (w/v) and poured into a 96-well microplate. The mixture was incubated for 30 min at room temperature. The light absorption of the solutions was measured at 420 nm in the ELISA microplate reader. Quercetin (0–100 mg/L) was used as the standard, and the same procedure was applied. Thus, the final results of TFC were then expressed in terms of mg quercetin equivalents (QE)/g of propolis extract.

All the experiments were performed in four replicates, and all of the collected data were involved in analyses. Statistical analysis was carried out using an unpaired t-test to compare the significant difference between the two means at a significance level of p < 0.05. The data were presented as the mean values and the standard deviation errors.

RESULTS AND DISCUSSION

Figure 1 displays the FTIR spectra of the water and ethanolic extracts of T. binghami, H. itama, and G. thoracica propolis. In general, in the fingerprint region, the main bands were observed at 1043, 1078, 1411, and 1500–1750 cm⁻¹. The bands at 1043 and 1078 cm⁻¹ could be assigned to the vibrations of C–O esters and C–C aromatic rings, whereas those at 1411 cm⁻¹ and at 1500–1750 cm⁻¹ were associated with the vibrations of CH₂, N–O, C–N, and C=O of aromatic rings. In the high-frequency region, the peaks at 2800–2950 cm⁻¹ and 3200–3600 cm⁻¹ were due to C–H, N–H, and O–H vibrations. Overall, the FTIR spectra of the propolis extracts showed similar features to those of Abdullah et al. (2019) and Alvarez et al. (2021), suggesting that the extracts contained similar aromatic flavonoid and phenolic compounds to those of their corresponding raw propolis. Although the FTIR spectra feature of the propolis extracts from the different stingless bee species were similar, their relative peak intensities – which could be related to the compositions of flavonoids, phenolics, and other
chemicals in the propolis extracts – are dependent on the stingless bee species.

As shown in Figure 2, the different propolis extracts almost have similar absorption spectral features, i.e. a clear peak around 272 nm, a shoulder peak at 350 nm, and Rayleigh scattering. The absorbance of propolis extracts can be a marker of their flavonoid and phenolic contents (Márghităș et al. 2013). It is also noteworthy that the relative absorption peak at 272 nm was more clearly observed for the ethanolic extracts compared with the water extracts, indicating that the ethanolic extracts contained a higher amount of aromatic compounds. Upon excitation at 272 nm, the propolis extracts showed a fluorescence band at 290–420 nm with a main peak at 315 nm. The fluorescence spectra of the ethanolic and water extracts were similar, implying that the fluorescent compounds in all the propolis extracts had similar chemical structures.

The propolis extracts are found to form colloidal solutions in water. This notion was revealed by dynamic light scattering measurement, where it was found that the particle sizes of the aqueous extracts of H. itama propolis were within 144–1448 nm (Abdullah et al. 2020), and 219–4783 nm and 113–3113 nm for T. binghami and G. thoracica propolis extracts, respectively. The rough surface of both T. binghami and G. thoracica propolis extracted in an ethanolic solution indicated that grainy texture embedded on the surface and agglomeration of irregularly shaped particles – which could be due to the different nature of raw materials, compositions, surface properties, and structures of propolis of the different stingless bee species.

All antibacterial activities of the water extracts of propolis were compared to those of the ethanolic extracts reported previously by Abdullah et al. (2020). Table 1 summarizes the antibacterial activities of water and ethanolic extracts of stingless bees T. binghami, H. itama, and G. thoracica.
Table 1. The inhibition zones and MIC of four different bacteria strains using water and ethanolic extracts of stingless bees G. thoracica, T. binghami, and H. itama propolis and those of streptomycin as a standard antibiotic

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Bee species</th>
<th>Propolis extract</th>
<th>Water Zone inhibition (mm)</th>
<th>Ethanol Zone inhibition (mm)</th>
<th>Water MIC (μg/mL)</th>
<th>Ethanol MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>T. binghami</td>
<td>9.9 ± 2.5</td>
<td>8.7 ± 0.8</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>T. binghami</td>
<td>7.3 ± 1.4</td>
<td>7.8 ± 0.9</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>H. itama</td>
<td>8.6 ± 2.3</td>
<td>9.3 ± 0.8</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>G. thoracica</td>
<td>8.0 ± 1.7</td>
<td>8.0 ± 0.7</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>H. itama</td>
<td>7.3 ± 1.3</td>
<td>13.0 ± 4.6</td>
<td>2500</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>H. itama</td>
<td>10.5 ± 1.3</td>
<td>9.8 ± 1.3</td>
<td>2500</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>G. thoracica</td>
<td>8.3 ± 2.5</td>
<td>10.8 ± 1.0</td>
<td>2500</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>T. binghami</td>
<td>17.0 ± 2.5</td>
<td>9.7 ± 4.6</td>
<td>2500</td>
<td>2500</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>G. thoracica</td>
<td>8.5 ± 1.5</td>
<td>9.5 ± 0.5</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>G. thoracica</td>
<td>7.8 ± 1.6</td>
<td>9.8 ± 1.7</td>
<td>5000</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>T. binghami</td>
<td>10.7 ± 1.2</td>
<td>10.0 ± 1.9</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>H. itama</td>
<td>10.6 ± 1.0</td>
<td>11.7 ± 1.2</td>
<td>2500</td>
<td>10000</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Streptomyacin</th>
<th>Mic (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>13.7 ± 1.7</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>15.6 ± 1.9</td>
</tr>
<tr>
<td>E. coli</td>
<td>17.4 ± 1.9</td>
</tr>
<tr>
<td>S. aureus</td>
<td>16.3 ± 2.1</td>
</tr>
</tbody>
</table>

*All experiments have been performed in four replicates. All collected data have been analyzed and statistical analysis was performed using an unpaired t-test to compare the significant difference between two means at a significance level of *p* < 0.05, and the results include the standard deviation errors.

Table 2. The mean value of antibacterial inhibition zones and t-test of water and ethanolic extracts of G. thoracica, T. binghami, and H. itama propolis.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Bee species</th>
<th>Propolis extract</th>
<th>t-test</th>
<th>Zone inhibition (mm)</th>
<th>Zone inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>T. binghami</td>
<td>9.9</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. itama</td>
<td>7.3</td>
<td>13</td>
<td>0.23</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>G. thoracica</td>
<td>8.5</td>
<td>9.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>T. binghami</td>
<td>7.3</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. itama</td>
<td>10.5</td>
<td>9.8</td>
<td>0.26</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>G. thoracica</td>
<td>7.8</td>
<td>9.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>T. binghami</td>
<td>8.6</td>
<td>9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. itama</td>
<td>8.3</td>
<td>10.8</td>
<td>0.23</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>G. thoracica</td>
<td>10.7</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>T. binghami</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. itama</td>
<td>17</td>
<td>9.7</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>G. thoracica</td>
<td>10.6</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
propolis against *B. subtilis*, *P. aeruginosa*, *E. coli*, and *S. aureus*. All the propolis extracts obtained from different stingless bee species exhibited antibacterial activity against all of the tested bacteria with different bacterial inhibition zones. The statistical analysis of the inhibition zones using the unpaired t-test revealed that all of the propolis samples extracted in different solvents showed lower results compared to those of the standard. The highest inhibition zones recorded were from *G. thoracica* and *H. itama* propolis extracts against the *S. aureus* bacterial strain, whereas *T. binghami* propolis extracts were most effective against *E. coli*. However, the antibacterial activities of all of the propolis extracts were lower compared with streptomycin, which was used as a standard antibiotic.

The MIC results generally did not show visible bacterial growth at concentrations greater than 2500–5000 µg/mL for water extracts and 2500–10000 µg/mL for ethanolic extracts against all bacterial strains, as listed in Table 1. With the same experimental conditions, the MIC of streptomycin was 19.5–39.1 µg/mL (see Table 1).

In comparison with other reported MICs of propolis extracts against *S. aureus*, a Gram-positive bacterial strain, the MIC values of ethanolic extracts of propolis in this study were 2500 µg/mL – much higher compared to those of propolis from Oman, Taiwan, and Turkey (MIC values 81, 10, and 8 µg/mL, respectively) (Uzel et al. 2005; Popova et al. 2013; Chen et al. 2018). These were also higher compared to the ethanolic extracts of propolis from Chile (MIC value of 1445 µg/mL) (Barrientos et al. 2013), Australia (MIC value 1200 µg/mL) (Massaro et al. 2015) and another propolis from different continents (Przybyłek and Karpiński 2019). The current finding could only be deemed comparable to those of propolis from Brazil (MIC values of 710–2850 µg/mL) (Pamplona-Zomenhan et al. 2011). Against *E. coli*, a Gram-negative bacterial strain, the MIC values of ethanolic extracts of propolis in this study were within 5000–10000 µg/mL – also much higher compared to all reported propolis obtained from different geographical origins such as Germany, Ireland, Slovakia, Oman, and Turkey (MIC values within 116–5000 µg/mL) (Uzel et al. 2005; Mavri et al. 2012; Popova et al. 2013; Al-Ani et al. 2018). This comparison inferred that propolis extracts from Brunei have low antibacterial activities against both Gram-positive and negative bacteria. This conclusion was supported by the MBC tests, where the propolis extracts with concentrations higher than their MIC values confirmed bacterial growth on the agar plates. This finding further suggested that the mechanism of bacterial growth inhibition by the propolis extracts was bacteriostatic, rather than bactericidal (Salman and Kareem 2021).

The water extracts of *T. binghami*, *H. itama*, and *G. thoracica* propolis exhibited antioxidant activity, as summarized in Table 1. All antioxidant, TPC, and TFC analyses plus the results of the water propolis extracts were compared to those of the ethanolic extracts reported previously (Abdullah et al. 2020). All the propolis extracts showed antioxidant activities with IC50 values ranging between 1905–3441 mg/L. Considering that the IC50 value of ascorbic acid was 24.3 mg/L, the total antioxidant capacity (TAC) of the propolis extracts was then estimated in terms of ascorbic acid equivalent (AAE). The TAC value of the *H. itama* propolis extract was found to be 12.8 mg AAE/g, and those of the *G. thoracica* and *T. binghami* propolis extract were 9.3 and 7.1 mg AAE/g, respectively. The different TAC values were attributed to relative chemical compositions of the flavonoid and phenolic compounds contained in the propolis extracts obtained from various species of stingless bee (Oryan et al. 2018). This notion was reflected in the highest TPC and TFC values of *H. itama* propolis extract, followed by *G. thoracica* and *T. binghami* propolis extracts, as listed in Table 3. In addition, the water extracts were surpassed by ethanolic extracts in the TPC and TFC values (Isla et al. 2001; Abdullah et al. 2020), suggesting that the ethanolic fraction extracted more flavonoid and phenolic compounds. Therefore, the TPC and TFC values in the propolis extracts can be related to the antibacterial and antioxidant activities; in this study, it was evidenced that they varied depending on the stingless bee species. The statistical analysis confirmed the antioxidant and antibacterial activities may be associated with phenolic

**Table 3.** The antioxidant activity IC50 (mg/L), TAC, TPC, and TFC values of the water extracts of *T. binghami*, *H. itama*, and *G. thoracica* propolis.

<table>
<thead>
<tr>
<th>Propolis species</th>
<th>Parameters</th>
<th>TAC (mg AAE/g)</th>
<th>TFC (mg QE/g)</th>
<th>TPC (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. binghami</em></td>
<td>1905 ± 31</td>
<td>7.1 ± 0.2</td>
<td>221.8 ± 7.2</td>
<td>1743 ± 15</td>
</tr>
<tr>
<td><em>H. itama</em></td>
<td>3441 ± 73</td>
<td>12.8 ± 0.4</td>
<td>264.1 ± 6.1</td>
<td>2051 ± 25</td>
</tr>
<tr>
<td><em>G. thoracica</em></td>
<td>2611 ± 47</td>
<td>9.3 ± 0.3</td>
<td>189.3 ± 5.7</td>
<td>1985 ± 31</td>
</tr>
</tbody>
</table>

**IC50** was obtained from the linear plot of radical scavenging activity of the propolis extract to scavenge DPPH radical as a function of the propolis concentration. The IC50 is defined as the propolis concentration required to scavenge 50% of the initial DPPH concentration (Abdullah et al. 2019).
and flavonoid contents present in the propolis. It is also noteworthy that flavonoids and phenolic compounds have been revealed to be also responsible for the medicinal, pharmaceutical, and other biological activities of propolis (Mogana et al. 2020; Huang et al. 2014).

CONCLUSION

To conclude, the present study shows that – despite the variations in the chemical compositions and botanical origins – all water and ethanolic extracts of propolis of different species exhibit significant antibacterial and antioxidant activities. This is an expected result as propolis is known for having a defense mechanism against bacterial infections. All the propolis extracts exerted antibacterial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. The antibacterial and antioxidant activities could be related to the flavonoids and phenolic compounds contained in the propolis extracts, as revealed by their TFC and TPC – as well as FTIR, UV-Vis, and fluorescence spectroscopies. Therefore, more comprehensive research in identifying specific components and bioactivities may provide a better understanding of its biological studies. Finally, further research on propolis and its biological studies is needed, as it may benefit from its applications in the medical and pharmaceutical industries.

ACKNOWLEDGMENTS

The authors would like to thank Mitasby H. Mamit (Tasbee Meliponiculture Farm) for supplying the propolis.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


ISLA MI, NIEVA MORENO MI, SAMPIETRO AR, VATTUONE MA. 2001. Antioxidant activity of argen


MARCUCCI MC, BANKOVA V. 1999. Chemical compo-
sition, plant origin, and biological activity of Brazilian propolis. Current Topics in Phytochemistry 2: 115–123.

MĂRGHITAȘ LA, DEZMIREAN DS, BOBIȘ O. 2013. Important developments in Romanian propolis re-
search. Evidence-based Complementary Alternative Medicine [Article ID 159392].

MASSARO CF, SIMPSON JB, POWELL D AND BROOKS P. 2015. Chemical composition and anti-
microbial activity of honeybee (Apis mellifera ligustica) propolis from subtropical. The Science of Nature 102 (11–12): 68.

MAVRI A, ABRAMOVIĆ H, POLAK T, BERTONCELJ J, JAMNIK P, SMOLE MOŽINA S, JERŠEK B. 2012. Chemical properties and antioxidiant and antimicrobial activities of Slovenian propolis. Chemistry & Biodi-
versity 9(8): 1545–1558.

MEDIĆ-ŠARIĆ M, JASPRICA I, MORNAR A, SMOL-


PARK YK, IKEGAKI M, ALEN CAR SM, MOURA FF. 2000. Evaluation of Brazilian propolis by both physi-


POPOVA M, DIMITROVA R, AL-LAWATI H, TSVET-


RASMUSSEN C, CAMERON SA. 2007. A molecular phylogeny of the old world stingless bees (Hymenop-


SALMAN JAS, KAREEM AJ. 2021. Antibacterial and anti-virulence factors of purified dextran from Lac-

