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Chemical profile, botanical origin, and antioxidant activity of stingless bees propolis from Konawe Island, Southeast Sulawesi, Indonesia

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Abstract. *Pujirahayu N, Uslinawaty Z, Ritonga H, Hadjar N, Prasetya D, Dirvin, Gari A. 2025. Chemical profile, botanical origin, and antioxidant activity of stingless bees propolis from Konawe Island, Southeast Sulawesi, Indonesia. Biodiversitas 26: 2495-2507.* This study aimed to determine the chemical profile, plant sources, and antioxidant activity of stingless bee propolis from Konawe Islands Regency, Southeast Sulawesi, Indonesia. Gas Chromatography-Mas Spectrometry (GC-MS) was used to determine the chemical composition of propolis. The antioxidant activity of propolis ethanol extract was then tested using the DPPH method. The results showed that 2 species of stingless bees, *Tetragonula* aff. *biroi* and *Tetragonula sapiens* exhibited chemical compounds with similarities and differences. These compounds consisted of triterpenes and diterpenes, such as 9,19-cyclolanost-24-en-3-ol, (3β.), 9,19-cyclolanost-24-en-3-ol acetate, lupenone, solanesol, norolean-12-ene, beta-amyrone, methyl commate b, ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl-,(3β.,4α,5α), α-amyrenone, and tricyclo[20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy. Others included phenolics, such as 3-[pentadecylphenol and 5-heptylresorcinol. A comparison of GC-MS analysis of propolis extract and plant exudates showed that the botanical origin of propolis was *Anacardium occidentale* (cashew), *Mangifera indica* (mango), and *Artocarpus heterophyllus* (jackfruit). Propolis ethanol extract from *T.* aff. *biroi* and *T. sapiens* had strong antioxidant activity with IC_{50} of 96.8 4±2.35 µg/mL and 49.5±0.42 µg/mL, respectively. Based on the results, the extract from two species of bees could be a potential source of antioxidant agents as medicinal ingredients and immune supplements in the food and pharmaceutical industry.

Keywords: Antioxidant activity, chemical profile, GC-MS, Konawe Island, stingless bee propolis

INTRODUCTION

A tropical rainforest is an area with a high biodiversity of phytopharmaceutical sources. At present, more than 40,000 plant species have been reported around the world, with approximately 6,000 having bioactive components used in traditional healing processes (Elfahmi et al. 2014; Suwardi et al. 2021). These bioactive components are obtained from various parts of plants, such as stems, leaves, roots, fruit, seeds, bark, and sap. Natural bioactive compounds are not only important for plants but are also used by humans as medicine and by animals for protection.

Honey bees are insects that use the resin and exudate of plant buds to build and defend nests from predators and other microbes. The resinous exudate and buds are collected by the bees and then mixed with salivary enzymes, leading to their conversion into propolis (Przybyłek and Karpiński 2019). Several studies have shown that propolis has various biological functions, including anti-microbial (Boisard et al. 2015), anti-cancer (Elnakady et al. 2017), anti-inflammatory (Rimbach et al. 2017), antioxidant, and α-glucosidase inhibitor (Pujirahayu et al. 2019a; Kyomya et al. 2023). Propolis consistently exhibits biological activity despite variations in its composition (Miguel 2013). Several studies have shown that its nutritional and medicinal properties may equal or exceed those found in commonly used bee products, particularly those from the genus *Apis* (Al-Hatamleh et al. 2020).

The chemical composition of propolis varies significantly depending on geographic location, the specific plant sources available, and the bee species (Torres et al. 2018). Countries and regions may also have unique stingless bee species adapted to their local ecosystems (Abdullah et al. 2020). These adaptations encompass climate, vegetation, predators, and competitors, impacting nest structures and bee foraging habits. This subsequently influences the types of products produced, including propolis. In Europe, North America, and non-tropical regions of Asia, the primary source of propolis is poplar tree buds (Salatino et al. 2021) Russian propolis originates from *Betula verrucosa* (Popova et al. 2013), Argentinian propolis from *Zuccagnia punctata* and *Larrea nitida* resin (Agüero et al. 2011; Salas et al. 2016), Nepalese propolis from *Dalbergia* sp., and Iranian propolis from *Ferula* sp. (Salas et al. 2016; Salatino et al. 2021). Australia produces propolis from *Acacia paradoxa* and *Lepidosperma* sp. (Duke et al. 2017), Okinawa and Taiwan from *Macaranga tanarius*, and Brazil from *Baccharis dracunculifolia* and *Dalbergia ecastophyllum* (Bobiş 2022).

Stingless bees are a group of small to medium-sized animals with vestigial (non-functional) stings, living in colonies (social) and collecting pollen (Vollet-Neto et al. 2017). Recent studies conducted on Sulawesi Island reported

that 10 species of Sulawesi stingless bees were found, including *Tetragonula biroi* (Friese, 1898), *Tetragonula clypearis* (Friese, 1908), *Tetragonula fuscobalteata* (Cameron, 1908), *Tetragonula laeviceps* (Smith, 1857), *Tetragonula pagdeni* (Schwarz, 1939), *Tetragonula sapiens* (Cockerell, 1911), *Heterotrigona itama* (Cockerell, 1918), *Homotrigona canifrons* (Smith, 1857), *Lepidotrigona terminata* (Smith, 1878), and *Wallacetrigona incisa* (Sakagami and Inoue 1989). The different body sizes of worker bees are generally considered a form of adaptation in foraging activities and exploiting flower resources (Trianto et al. 2024). However, reports of stingless bee species from Southeast Sulawesi are still limited. At present, only one type of stingless bee propolis has been reported from Kendari and South Konawe, namely *T. sapiens* propolis, which primarily sources its resin from *Mangifera indica*. Propolis from these bees contains cycloartan-triterpene compounds, which have strong antioxidant activity and α-glucosidase inhibitors (Pujirahayu et al. 2019a). Findings show that propolis from other types of stingless bees from various regions in Southeast Sulawesi has never been reported, as well as information about the chemical compounds, plant sources, and biological activities. Therefore, this study aimed to determine the chemical profile, plant source, and antioxidant activity of propolis from 2 stingless bee species from the Konawe Islands, Southeast Sulawesi, Indonesia.

MATERIALS AND METHODS

Materials

Propolis samples were taken from the natural nests of *Tetragonula* aff. *biroi* and *T. sapiens*, which came from the Konawe Kepulan region, and resin samples were taken from *M. indica*, *Anacardium occidentale*, and *Artocarpus heterophyllus* trees growing around the nests. Stingless bees were identified by the Study Center for Biology, LIPI, Bogor, Indonesia, based on bee specimens, nest structure, and entrance shape.

Propolis and resin extraction

Extraction of propolis samples follows Pujirahayu et al. (2014) with several modifications, namely refined crude propolis was extracted with ethanol at a temperature of 40°C in a shaker. Then 25 g of propolis (after grinding in a mixer) was extracted with 200 mL of 70% ethanol at 40°C in a shaker (Stuart GFL 1086) in a closed room for 6 days. The suspension was filtered using filter paper (Whatman no 41), the filtrate was taken, and the residue was macerated again. Furthermore, the filtrate was taken every 2 days for 6 days with a total of 3 repetitions. The maceration results were then filtered using a Buchner filter, and the filtrate was evaporated using a rotary evaporator (Laborota 4002) at a temperature of 30°C-40°C until a concentrated extract was formed (ethanol extract of propolis of *T. sapiens* or EEPTs and ethanol extract of propolis of *T. biroi* or EEPTb). *M. indica*, *A. heterophyllus*, and *A. occidentale* resin samples were extracted following Pujirahayu et al. (2019b). A total of 2 g of the resin extract was ground into a fine powder and then extracted 3 times with 20 mL of 70% ethanol in a shaker at room temperature for 24 hours after filtering using the same method as propolis to get Ethanol Extract of Resin (EER).

Gas Chromatography-Mas Spectrometry (GC-MS) analysis

Gas Chromatography-Mas Spectrometry (GC-MS) analysis was carried out as followed, 0.1 g of sample was added with 5 mL of methanol and chloroform in a ratio (1:1). Extraction was performed using a sonicator for 20 minutes at 40°C, and the extract was pipetted into a vial and analyzed using the GC-MS QP-2010 Plus Autosampler AOC-20i, replications were three times. The Gas Chromatography-Mass Spectrometry (GC-MS) conditions were set as follows: the injector temperature was 250°C with split-less mode, the pressure was 76.9 kPa, and the flow rate was 14 mL/min, with a split ratio of 1:10, using helium as the carrier gas. The ion source and interface temperatures were maintained at 200°C and 280°C, respectively, and the solvent cut time was set to 3 minutes with a mass-to-charge ratio range of 400-700 m/z. The column used was an SH-Rxi-5Sil MS column, measuring 30 m in length and 0.25 mm in inner diameter. The initial column temperature was 70°C with a hold time of 2 minutes. It was then increased to 200°C at a rate of 10°C/min, followed by a final temperature of 280°C with a hold time of 9 minutes, increasing at rate of 5°C/min. Furthermore, the chromatogram data obtained were analyzed using the NIST and Wiley 9 libraries (Bozkuş et al. 2021).

Antioxidant activity

The antioxidative activities of the propolis extract were assayed for the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Darmstadt, Germany). Free radicals were assayed using a previously reported method (Pujirahayu et al. 2019a) with some modifications. A total of 1 mL of 0.1 mM DPPH (HIMEDIA) solution in methanol was mixed with 2 mL of the sample solution in different concentrations (5-80 mg/L). The mixture was then incubated at room temperature for 30 minutes in a dark place. The absorbance was measured against methanol as a blank at 517 nm using a UV-Vis spectrophotometer (Genesys 150 Thermo Scientific, US). Methanol was blank because it was a solvent used to dissolve DPPH and samples. A decrease in the absorbance value of the reaction mixture indicated higher DPPH free radical inhibition activity, and the positive control used was vitamin C (Sigma-Aldrich®). The percentage of DPPH radical inhibitory activity of each compound was calculated as % DPPH inhibition using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100\% \quad (1)$$

Where:

Ao : Absorption of the control

As : Absorption of the sample solution under testing

The IC_{50} value of each sample was determined by (a) Inhibitory activity (y) plotted against concentration (x) at 5 points (80, 40, 20, 10, and 5 g/mL), (b) the equation of the regression line was determined (y = ax+b), and (c) sample concentration (x) was calculated by substituting y=50 into the regression equation (b).

Data analysis

Data were normalized to the maximum concentration per species prior to analysis. Heatmap visualizations were generated using Python 3.9 with Seaborn 0.11.2 and Scikit-learn 1.0.2. with automatic text contrast optimization. Compound concentrations were normalized to 0-1 scale per species to enable cross-comparison while preserving original percentage values in annotations.

RESULTS AND DISCUSSION

Chemical profile of *Tetragonula* aff. *biroi* and *T. sapiens* propolis

Chemical profiling of propolis ethanol extract was carried out using GC-MS analysis. The compounds identified in *T. biroi* propolis were depicted in Table 1, while the compounds identified in *T. sapiens* propolis were described in Table 2.

The results of the GC-MS analysis showed that the ethanol extract of *T. biroi* propolis was dominated by triterpenes, diterpenes, pentadecylphenol. The predominant triterpenes such as 9,19-cyclolanost-24-en-3-ol and lupenone. Besides triterpenes, *T. biroi* propolis was also dominated by 3-pentadecylphenol and 5-pentadecylresorcinol.

Although there were similarities in the components contained in both propolis samples, there were also compounds that were only found in *T. biroi* propolis and not found in *T. sapiens* propolis such as norolean-12-ene, androstan-3-ol, 9-methyl-, acetate, (3β,5α.), naphthalene, decahydro-1,6-dimethyl-4-(1-methylethyl), and lupenone. In contrast, ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl-, 3.β, 4α, 5α, solanesol, tricyclo [20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy-, β-amyrenone, pregn-4-ene-3,20-dione, methanopicene, and methyl commate B were only found in *T. sapiens* propolis from the Konawe Islands (Figure 1).

Based on the component groups contained in both propolis, there are six main compound groups, namely fatty acids, phenols, diterpenes, triterpenes, steroids, and other groups. Other groups include several compounds that are present in smaller amounts. As shown in Figure 2, the primary components of propolis are triterpenoids, which make up more than 50% of its composition, followed by the phenol group.

Botanical origin of *Tetragonula* aff. *biroi* and *T. sapiens* propolis from Konawe Island

The chemical composition of propolis was highly dependent on the plant source. The comparison between the main propolis components of GC-MS chromatogram of Ethanol Extract of Propolis (EEP) of *T.* aff. *biroi* and the main components of the resin of *A. occidentale* could be seen in Figures 3 and 4.

The chromatogram pattern of propolis *T. sapiens* was also similar to that of cashew nut exudates (Figure 5 and Figure 6). Several components in both *T.* aff. *biroi* and *T. sapiens* propolis were also thought to originate from *A. heterophyllus* and mango exudates (*M. indica*) (Figures 3, 4 and 5) such as 9-octadecenoic acid (Z)-, methyl ester, 5-heptylresorcinol, and 3-pentadecylphenol.

The main components of the ethanol extract of *T. biroi* propolis and *T. sapiens* propolis were identified by comparing the spectra with those of known compounds stored in the NIST and Wiley 9 library (Figures S1-S8).

The phytochemical composition of propolis is directly related to the botanical sources of resin surrounding the nest. Figure 7 illustrates the three main botanical sources for propolis from *T. biroi* and *T. sapiens: A.occidentale*, *Artocarpus heterophylla*, and *M.indica*.

Antioxidant activity

The antioxidant activity test of the ethanol extract-propolis of *T.* aff. *biroi* and *T. sapiens* using the DPPH method was shown in Table 3. The antioxidant value expressed in IC_{50} (ug/mL) with ascorbic acid (Vitamin C) was seen as a control, and the smaller the IC_{50} value, the stronger the antioxidant activity.

Table 1. Major compounds identified in the ethanol extract of *Tetragonula biroi* propolis from Southeast Sulawesi through GC-MS analysis

Retention time	Compound name	Formula	Area (%)	Group of compounds
18.39	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.45	Fatty acid
21.28	9,12-octadecadienoic acid (z,z)-	$C_{18}H_{36}O_3$	0.35	Fatty acid
21.42	9-octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	0.97	Fatty acid
28.55	norolean-12-ene	$C_{29}H_{44}O_3$	3.74	Triterpenoid
28.75	β-amyrone	$C_{30}H_{48}O$	2.24	Triterpenoid
28.92	α.-amyrenone	$C_{30}H_{48}O$	2.24	Triterpenoid
29.79	lup-20(29)-en-3-ol, acetate, (3. β .)-	$C_{32}H_{52}O_2$	4.01	Triterpenoid
29.86	lanosterol	$C_{30}H_{50}O$	2.64	Triterpenoid
31.04	androstan-3-ol, 9-methyl-, acetate, (3β,5α.)	$C_{22}H_{36}O_2$	2.65	Steroid
32.05	lupenone	$C_{30}H_{48}O$	10.73	Triterpenoid
32.51	9,19-cyclolanost-23-ene-3,25-diol, (3. β.,23e)-	$C_{30}H_{50}O_2$	9.88	Triterpenoid
33.00	5 pentadecylresorcinol	$C_{21}H_{36}O_2$	10.64	Phenol
33.57	9,19-cyclolanost-24-en-3-ol, (3. β)- (cycloartenol)	$C_{30}H_{50}O$	13.55	Triterpenoid
33.68	9,19-cyclo-9. β.-lanostane-3. β .,25-diol	$C_{30}H_{52}O_2$	4.10	Triterpenoid
34.52	3-pentadecylphenol	$C_{21}H_{36}O$	12.65	Phenol
36.15	5-heptylresorcinol	$C_{13}H_{20}O_2$	3.07	Phenol
37.81	andrographolide	$C_{21}H_{36}O_5$	1.98	Diterpenoid
39.15	naphthalene, decahydro-1,6-dimethyl-4-(1-methylethyl)	$C_{20}H_{36}O_2$	1.32	Diterpenoid
39.89	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	$C_{30}H_{50}O_2$	1.28	Triterpenoid

Table 2. Major compounds identified in ethanol extract of *Tetragonula sapiens* propolis from Southeast Sulawesi through GC-MS analysis

Retention time	Compound name	Formula	Area (%)	Group of compounds
18.38	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.92	Fatty acid
21.27	9,12-octadecadienoic acid (z,z)-	$C_{18}H_{36}O3$	0.67	Fatty acid
21.41	9-octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O2$	1.97	Fatty acid
22.57	9-octadecenoic acid (z)-, ethyl ester	$C_{20}H_{38}O_2$	1.57	Fatty acid
29.78	methanopicene	$C_{30}H_{50}O$	1.92	Triterpenoid
30.36	β-amyrone	$C_{30}H_{48}O$	1.21	Triterpeoid
30.99	tricyclo[20.8.0.0 (7,16)]triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	2.90	Triterpenoid
31.37	3-pentadecylphenol	$C_{21}H_{36}O$	7.07	Phenol
31.78	9,19-cyclo-9. β.-lanostane-3. β.,25-diol	$C_{32}H_{52}O_2$	4.57	Triterpenoid
31.98	9,19-cyclolanost-23-ene-3,25-diol, (3 β.,23e)-	$C_{30}H_{50}O2$	4.01	Triterpenoid
32.66	methyl commate b	$C_{31}H_{50}O_3$	3.83	Triterpenoid
32.95	solanesol	$C_{45}H_{74}O$	7.18	Politerpenoid
33.12	9,19-cyclolanost-24-en-3-ol, (3 β.)- (cycloartenol)	$C_{30}H_{50}O$	13.44	Triterpenoid
33.30	9,19-cyclolanost-24-en-3-ol acetate	$C_{32}H_{52}O3$	10.97	Triterpenoid
33.62	ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl-, (3β ,4α 5α)	$C_{30}H_{50}O$	4.28	Triterpenoid
34.05	lup-20(29)-en-3-ol, acetate, (3. β.)-	$C_{32}H_{52}O_2$	1.25	Triterpenoid
36.09	5-heptylresorcinol	$C_{13}H_{20}O_2$	2.14	Phenol
36.64	pregn-4-ene-3,20-dione	$C_{21}H_{30}O_2$	4.4	Steroid
37.72	andrographolide	$C_{21}H_{36}O_5$	1.98	Diterpenoid

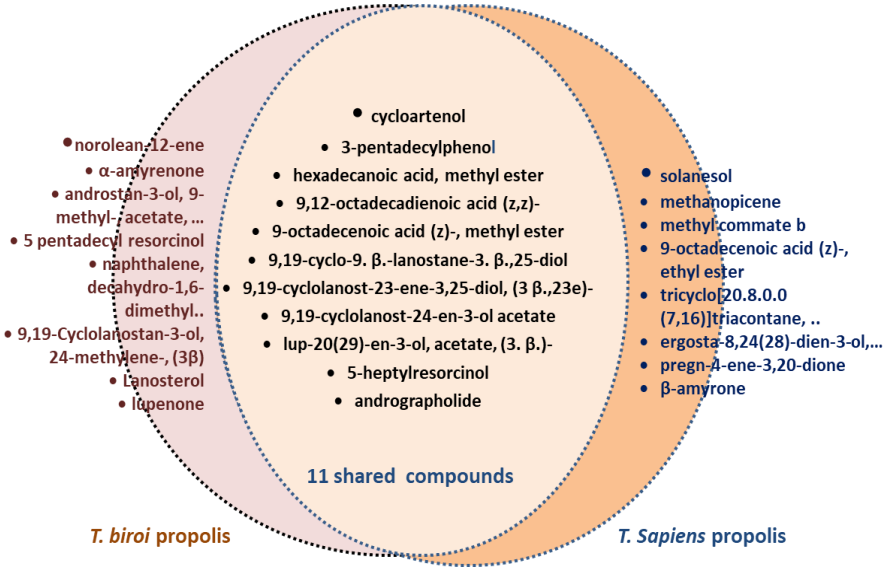

Figure 1. Venn diagram correlation of compounds found in both propolis and unique compounds in each *Tetragonula biroi* and *T. sapiens* propolis from Konawe Islands, Southeast Sulawesi, Indonesia

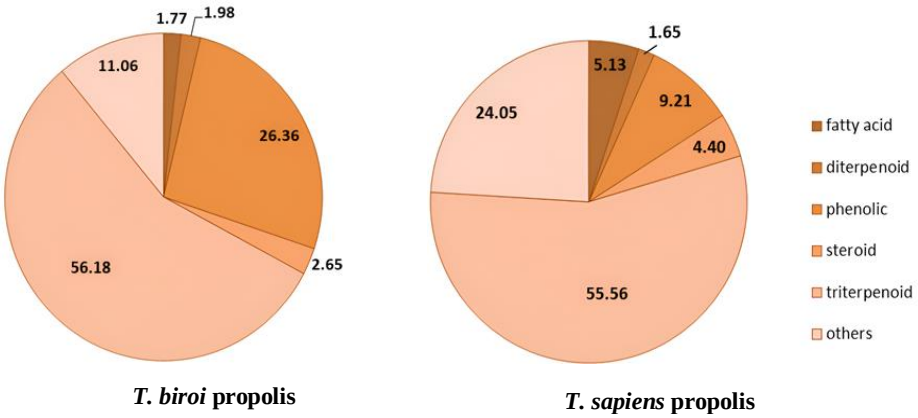

Figure 2. Component groups contained in *Tetragonula biroi* and *T. sapiens* propolis from the Konawe Islands, Southeast Sulawesi, Indonesia

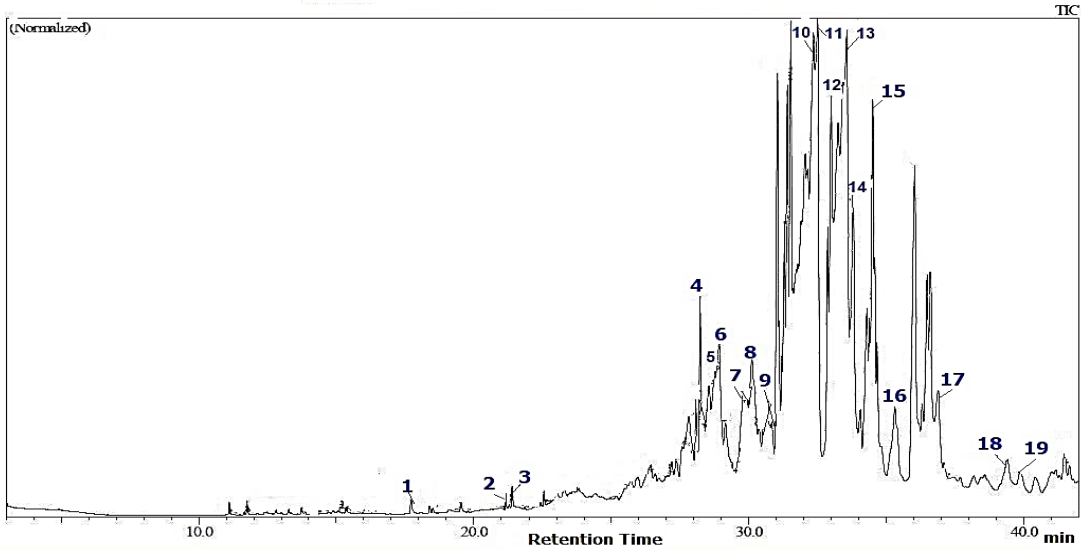

Figure 3. GC-MS chromatogram of Ethanol Extract of Propolis (EEP) of *Tetragonula* aff. *biroi*. The x-axis of the chromatogram shows the retention time in minutes; the numbered peaks represent the compounds detected in the *T. biroi* propolis sample, as detailed in Table 1

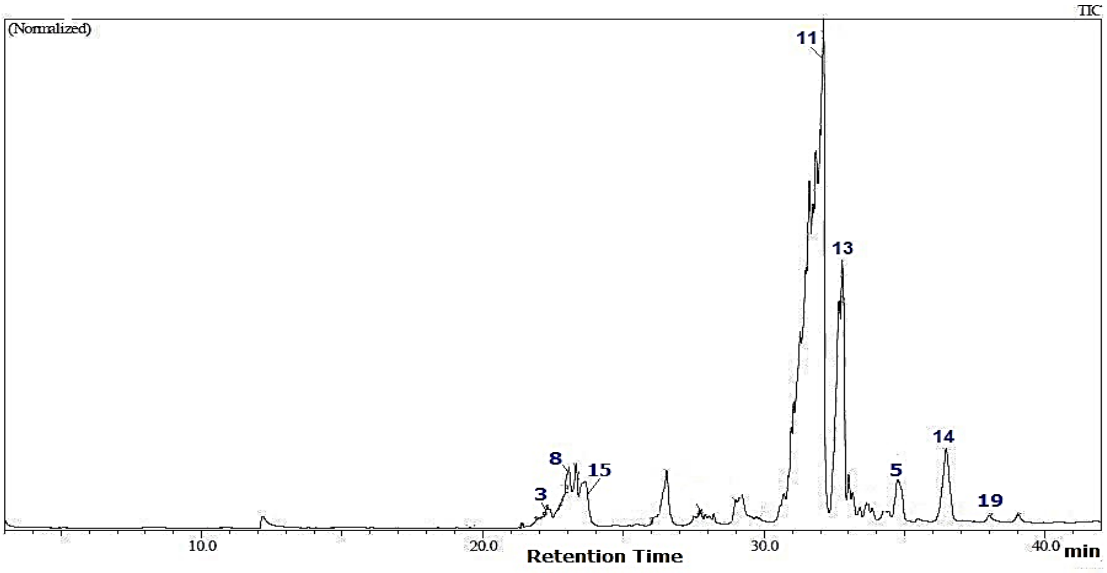

Figure 4. GC-MS chromatogram of *Anacardium occidentale* exudates, the x-axis of the chromatogram shows the retention time, with peak number: 9-octadecenoic acid (z)-, methyl ester (3); methanopicene (5); 3-pentadecylphenol (8); methyl commate b (11); cycloartenol (13); 9,19-cyclolanost-24-en-3-ol acetate (14) ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl-, (3β ,4α ,5α) (15); 9,19-cyclolanostan-3-ol, 24-methylene-, (3β)-(19)

Discussion

Chemical profile and botanical origin of propolis

The compounds in both propolis samples showed that there were 11 shared compounds, and 8 unique components for each propolis (Figure 1). The components in the propolis samples collected by *T.* aff. *biroi* and *T. sapiens* bees have an abundance of triterpene compound groups. *T. biroi* propolis consists of 56.18% triterpenes, 26.36% phenolics, and 2.65% diterpenes, while *T. sapiens* consists of 55.56% triterpenes, 9.21% phenolics, and 5.13% fatty acids (Figure 2). The triterpene component consisted of 9,19-cyclolanost-24-en-3-ol, (3.beta.) or cycloartenol as much as 13.55 % area, followed by lupenone (10.73%) and 19-cyclolanost-23 -ene-3, 25-diol (9.85%). Both propolis sample also contained 3-pentadecylphenol, which was the phenolic compound (12.65 %), and 5-heptylresorcinol. Although Both propolis samples share some common main components from the cycloartene terpenoid group, including cycloartenol and 9,19-cyclo-9β-lanostane-3β,25-diol, as well as the phenolic compound pentadecylphenol. However there are unique compounds found in each sample, as seen in Figure 1. For instance, androstan-3-ol, 9-methyl-, acetate, (3β.,5α.) and 1-naphthalenepropanol, α.-ethenyldecahydro-2-hydroxy-. α.,2,5,5,8a-pentamethyare only present in *T. biroi* propolis samples. In contrast, methanopicene, tricycle [20.8.0.0 (7,16)] triacontane, solanesol, and pregn-4-ene-3,20-dione are only present in *T. sapiens* propolis samples.

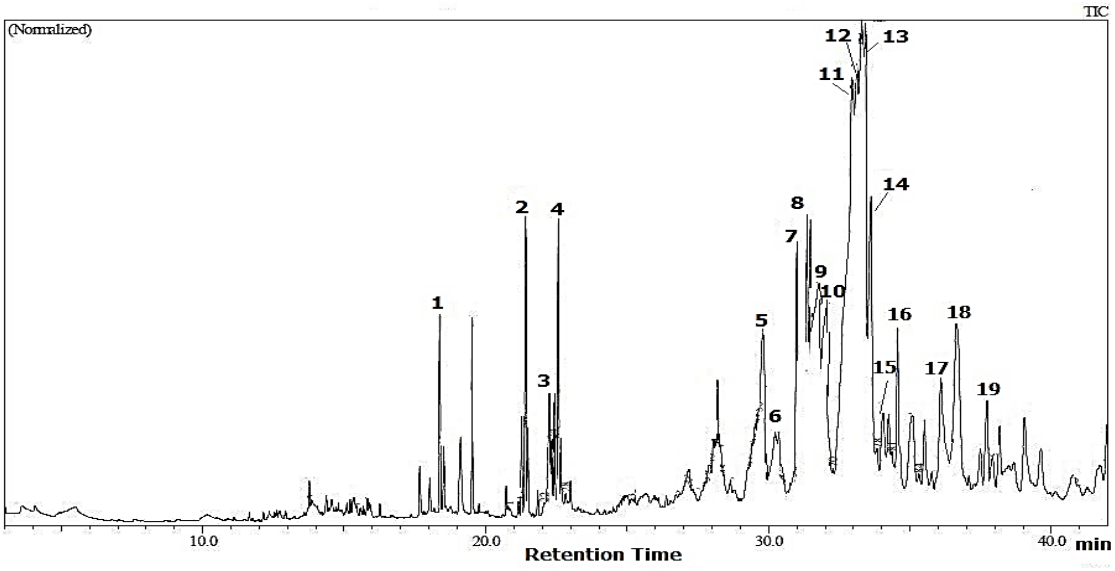

Figure 5. GC-MS chromatogram of Ethanol Extract of Propolis (EEP) of *Tetragonula sapien.* The x-axis of the chromatogram shows the retention time; the numbered peaks represent the compounds detected in the *T. sapiens* propolis sample, as detailed in Table 2

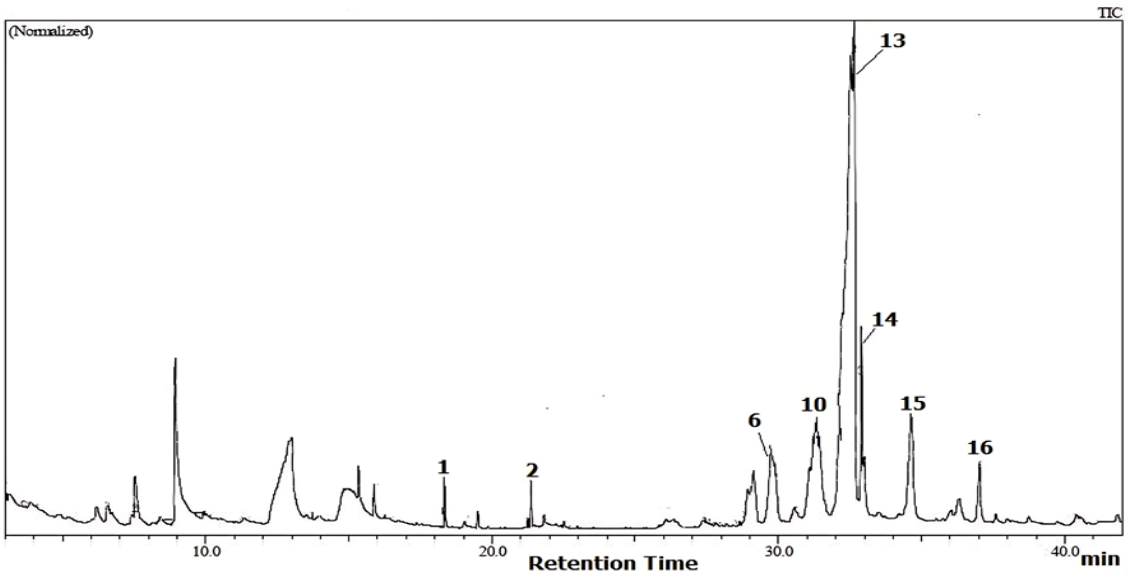

Figure 6. GC-MS chromatogram of *Artocarpus heterophyllus* exudates, the x-axis of the chromatogram shows the retention time in minutes, with peak number: hexadecanoic acid, methyl ester (1); 9,12-octadecadienoic acid (z,z)-(2); beta-amyrone (6); 9,19-cyclolanost-23-ene-3,25-diol, (3 β.,23e)-(10); cycloarteol (13); 9,19-cyclolanost-24-en-3-ol acetate (14); 9,19-Cyclolanostan-3-ol, 24-methylene-, (3β) (15); Solanesol (16)

Cycloartenol, triterpenoid compound, was a significant phytochemical found in various plant species and was recognized for its diverse biological activities. Cycloartenol was the compound detected in both propolis samples and in all three resins observed (Figure 8). This was structurally similar to lanosterol, which was a precursor in the synthesis of cholesterol in animals and fungi, indicating its crucial role in the sterol biosynthesis pathway in plants (Cheek et al. 2018). Lupenone, another triterpene compound found in propolis, possessed multiple biological activities and was widely distributed in many species of plants and herbs as a therapeutic candidate for the management of diabetes (Yin et al. 2017; Xu et al. 2022). This was a common polar-type triterpenoid with a ketone group at position 3 of the core ring. Lupenone had attracted the attention of medical professionals and investigators due to its wide range of pharmacological activities, one of which was its promising antidiabetic activity (Durgam et al. 2023).

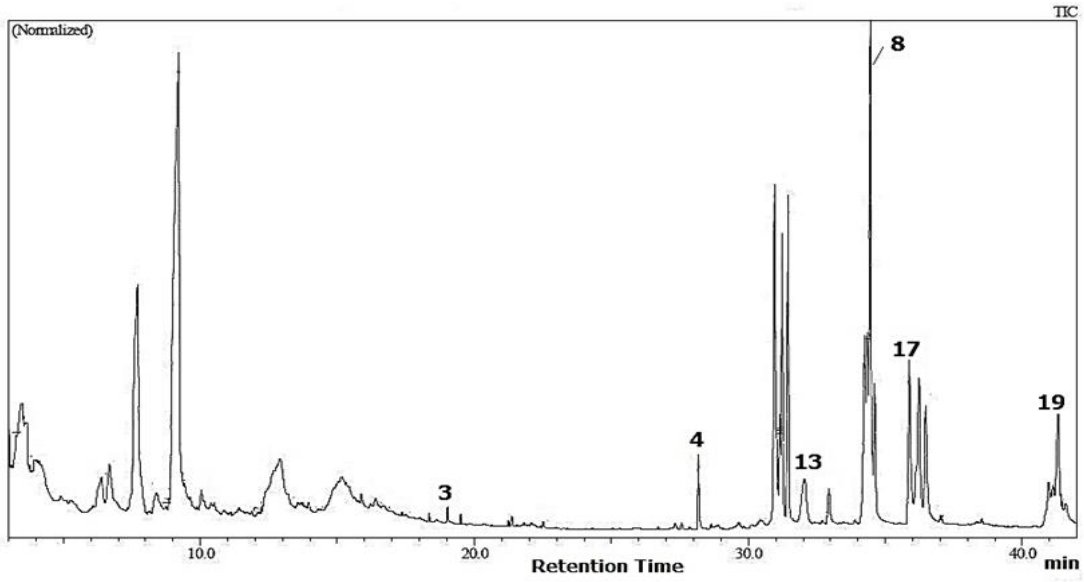

Figure 7. GC-MS chromatogram of *Mangifera indica* exudates, the x-axis of the chromatogram shows the retention time in minutes, with peak number: 9-octadecenoic acid (z)-, methyl ester (3), 9-octadecenoic acid (z)-, ethyl ester (4), 3-pentadecylphenol (8), cycloartenol (13) and 5-heptylresorcinol (17), 5-Pentadecylresorcinol (19)

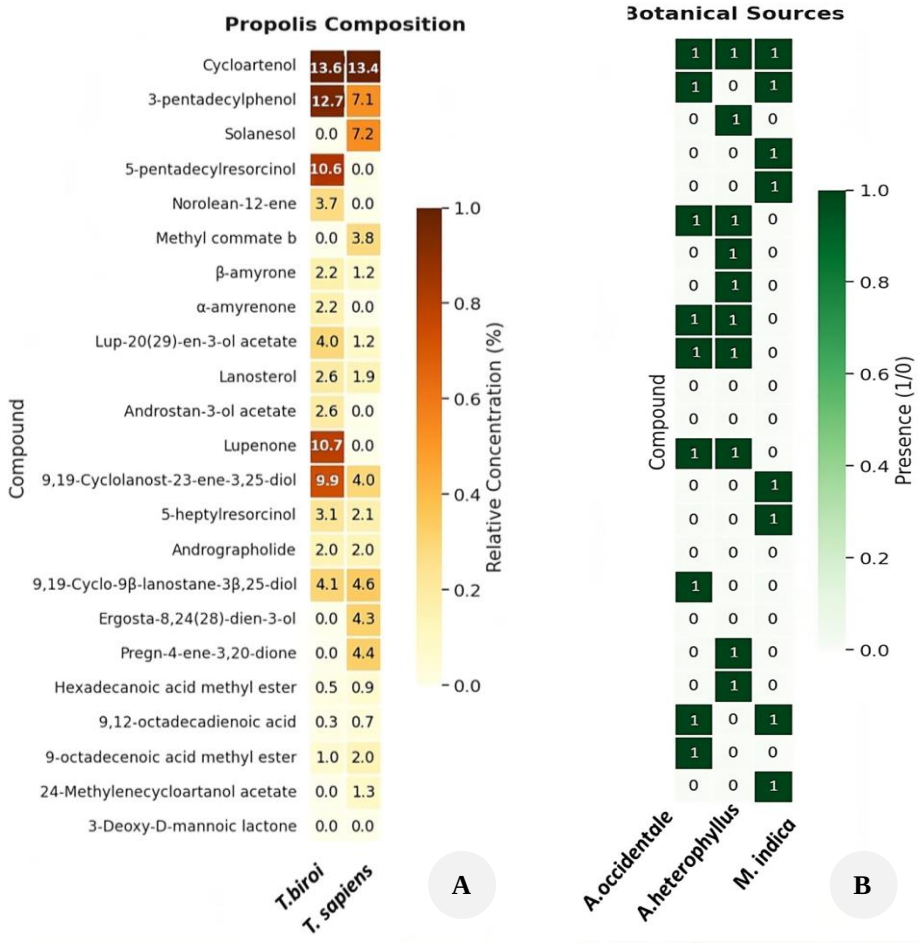

Figure 8. Comparative heatmap of chemical composition in *Tetragonula biroi* and *T. sapiens* propolis with botanical resin sources. The heatmap integrated both quantitative propolis composition data (normalized to 0-1 scale) and binary plant resin occurrence data using dual color mapping. A. Left panel: Relative concentrations (%) of key compounds in propolis (YlOrBr color scale); B. Right panel: Presence/absence (1/0) of compounds in resin sources (Green color scale). Compound names are displayed only on the left for clarity. The analysis revealed species-specific chemical profiles linked to distinct botanical origins, with *T. biroi* showing higher phenolic content from *Mangifera indica*, while *T. sapiens* contains unique triterpenoids from *Artocarpus heterophyllus*

Table 3. Antioxidant activity of the ethanol extract propolis of *Tetragonula* aff. *biroi* and *T. sapiens* from Konawe Island, Southeast Sulawesi, Indonesia

Propolis	IC_{50} (µg/mL) *)	Antioxidant class **)
Tetragonula aff. *biroi*	96.84±2.35	Strong
T. sapiens	49.5±0.42	Very strong
Ascorbic acid (Vit. C)	10.91±0.99	Very strong

Note: *: Data are the mean±SD (n=3); **: According to the classification made by Blois, the extracts with an IC_{50}<50 µg/mL are very strong antioxidants, IC_{50}, values of 50-100 µg/mL belong to strong antioxidants, 101-150 µg/mL denote medium antioxidants, and an IC_{50}>150 µg/mL belongs to weak antioxidants (Zongo et al. 2023).

The chromatogram profiles of the resin from *M. indica*, *A. heterophyllus*, and *A. occidentale* showed similarities in several compounds with the 2 types of propolis analyzed. The chromatograms for the 3 resins revealed the presence of triterpene compounds with retention times between 25 and 37 minutes, as well as hexadecanoic acid and 9-octadecanoic acid, which appeared at retention times between 18 and 23 minutes (Table 1 and Table 2). Notably, 19-cyclolanost-23-ena-3,25-diol was identified as the main component of *A. heterophyllus* (Figure 6). The compounds 3-pentadecylphenol and 5-heptylresorcinol were primarily derived from the exudates of mango *M. indica* (Figure 7). The heatmap visualization (Figure 8) employs a dual-color system to simultaneously display quantitative propolis composition and binary botanical source data. This integrated approach reveals previously unrecognized patterns of resin selectivity between *Trigona* species, particularly the strong association between *T. biroi* propolis and phenolic compounds from *Mangifera* and *T. sapiens* propolis contains unique triterpenoids (solanesl) from *Artocarpus*. It can be said that *T. biroi* tends to utilize *Mangifera* resin for phenolic compounds, while. *T. sapiens* relies more on *A. heterophyllus* and *A. occidentale* for complex triterpenoids

Several studies had reported that triterpene compounds and 3-pentadecylphenol were key components of *M. indica* bark resin (Sellés et al. 2021), while 5-heptylresorcinol was recognized as one of the compounds derived from *A. occidentale* seed oil (Rengga et al. 2019). Both types of propolis contained fatty acid methyl esters, specifically hexadecanoic acid methyl ester, 9-octadecenoic acid, and 9,12-octadecadienoic Acid (Z, Z). These compounds were detected between retention times of 18.38 and 22.57 minutes and accounted for 1.77% of *T. biroi* propolis and 3.56% of *T. sapiens* propolis. Hexadecanoic acid, also known as methyl palmitate, 9-octadecenoic acid or oleic acid methyl ester, and 9,12-octadecadienoic acid (Z, Z), also referred to as linoleic acid, were compounds that were recognized for their various biological activities. These included hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, antioxidant, and anticancer effects (Ibrahim et al. 2018; Ayoola et al. 2020).

Previous studies conducted by Halim et al. (2013) and Sativa and Agustin (2018) identified the chemical components of stingless bee propolis using GC-MS. Both studies found that the propolis samples contained terpenoids, and Halim's study utilized propolis obtained from Wonosobo, East Java, which was primarily composed of cyclolanost compounds, followed by α-amyrin and 5-heptylresorcinol. In contrast, Sativa and Agustin analyzed solid stingless bee propolis samples from Maribaya, Bandung, and West Java, with the major chemical components having been ethyl oleate, hexadecanoic acid, and ethyl ester.

The results of this study aligned with previous studies on the components of Malaysian stingless bee propolis, specifically from 2 species namely *Heterotrigona itama* and *Geniotrigona thoracica* (Smith, 1857). In both propolis extracts, the triterpene compound 9,19-cyclolanost-24-en-3-ol, also known as cycloartenol, was found to be the most abundant. This accounted for 14.11% of the methanol extract and 11.63% of the ethanol extract. Additionally, other triterpenes such as α-amyrine, β-amyrenonen, and lupenone were identified, along with lanosterol and 5-heptylresorcinol, which were part of the resorcinol group (Ibrahim et al. 2018). Another study found that propolis from Meliponula ferruginea in Tanzania primarily contained terpenoids (both di- and triterpenes), including α-amyrine, β-amyrenonen, and lupenone (Popova et al. 2021). These compounds were also present in the propolis studied from *T. sapiens* and *T. biroi* propolis. The terpene compound tricyclo[20.8.0.0(7,16) 1(22),7(16)-diepoxy-, was identified at a retention time of 30.99 minutes, with an area of 2.90%, and this compound was previously reported in *Cyperus rotundus* (Yagi et al. 2016). Stingless bees, which were small in size, could access tiny flowers from shrubs and grasses, such as nutsedge (*C. rotundus*).

Exudates plants as a source of propolis were found around the hive, but the bees only chose a few plants as a source of propolis. Therefore, propolis from a single beehive usually consisted of exudates only from 1, 2, and rarely 3 or more dominant plant species (propolis plant precursors) (Isidorov et al. 2016). This relationship between bee species, botanical source, and chemical composition of propolis was strengthened by numerous studies that had found that different stingless bee species showed different preferences for resin sources, leading to variations in the chemical composition of the resulting propolis (Pujirahayu et al. 2019a; Herrera-López et al. 2023).

Due to the high diversity of flora worldwide and other factors, the chemical composition of propolis as a substance was unstable and not uniform. The results of the GC-MS analysis showed that several main components of propolis α-amyrenol, lupenone, and amyrin were presented in *A. heterophyllus*. Like a previous study conducted by (Pujirahayu et al. 2019a, 2019b). This reported that the main exudate collected by *T. sapiens* bees from South Konawe, Indonesia was *M. indica* with an abundance of triterpene-type cycloartan compounds such as cycloartenol, ambonic acid, ambolic acid, mangiferolic acid, and mangiferonic acid.

In addition to the triterpene group of compounds, the 3 plant exudates also contained steroid group pregn-4-ene-3, 20-dione, or progesterone. These results also showed that terpenes were important components of stingless bee propolis. The compound 3-pentadecylphenol was one of the phenol

components found in both propolis sample observed. In addition, the results of GC-MS analysis showed that this compound was suspected to be collected by bees from the *A. occidentale* and *M. indica* resin. Comparison of GC-MS chromatograms of *T. sapiens* and *T. biroi* propolis with chromatograms of mango resin, *A. heterophyllus*, and *A. occidentale* resin showed similarities in the main components, such as 9,19-cyclolanost-24-en-3-ol,(3β.), 9,19-cyclolanost-23-ene-3,25-diol,(3β.,23e), 3-pentadecylphenol and 5-heptylresorcinol. Previous studies had also reported that 3-pentadecylphenol could be derived from Anacardiaceae *Spondias tuberosa* (Guimarães et al. 2018) and *M. indica* bark (Sellés et al. 2021). From the heatmap analysis, the characteristics of the botanical source of propolis were obtained, namely *A. occidentale* is the main source of Methyl commate b and cycloartenol acetate (only found in *T. sapiens* propolis). *A. heterophyllus* is an exclusive source of solanesol and α-amyrenone, while *M. indica* is a strong marker of 5-pentadecylresorcinol and norolean-12-ene (only found in *T. biroi* propolis).

Antioxidant activity

The antioxidant activity of propolis from 2 species, *T.* aff. *biroi* and *T. sapiens* were evaluated using the IC_{50} value. The results showed that the IC_{50} value of *T. sapiens* propolis was higher than that of *T. biroi* propolis (Table 2). IC_{50} was the concentration of a substance required to inhibit 50% of certain biological activity, such as antioxidant activity. A lower IC_{50} value indicates higher antioxidant activity, as it requires a lower concentration of propolis to achieve the desired inhibition. For *T.* aff. *biroi* propolis, the IC_{50} value was determined to be 96.84±2.35 µg/mL, while *T. sapiens* was 49.5±0.42 µg/mL. Based on the control IC_{50} value of 10.91±0.99 µg/mL, the IC_{50} value of *T. sapiens* propolis was classified as a very strong antioxidant. At the same time, *T. biroi* was included in the strong antioxidant group. This value indicated that a lower concentration of *T. sapiens* propolis was needed to achieve the same level of antioxidant activity as *T.* aff. *biroi* propolis.

These results indicated that both propolis samples had antioxidant activity. However, *T. sapiens* propolis from the Konawe Islands showed a higher potential in counteracting free radicals or inhibiting oxidative stress. This value could be attributed to the presence of specific compounds unique to *T. sapiens* propolis, such as ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl-, 3β, 4α, 5α, solanesol, tricyclo [20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy-, α amyrenone, pregn-4-ene-3,20-dione, and methyl comate B were not found in *T.* aff. *biroi* propolis.

The stronger antioxidant activity of *T. sapiens* is thought to be due to the presence of oxygenated terpenoids with multi-mechanism radical stabilization capabilities, and the complementary secondary metabolic profile between phenolic and terpenoid compounds, as well as evolutionary adaptation to the *Artocarpus* resin source, which is rich in antioxidant precursors.

The antioxidant properties of both propolis are greatly influenced by the dominant compounds that characterize each propolis (Tables 1 and 2). *Trigona biroi* propolis is dominated by long-chain phenolics 3-pentadecylphenol and 5-pentadecylresorcinol, while *T. sapiens* is dominated by modified terpenoid compounds solanesol (7.18%) and 9,19-cyclolanost-24-en-3-ol acetate (10.97%).

Solanesol is a long-chain triterpenoid alcohol classified as a nonaprenol, mainly found in plants of the Solanaceae family, such as tobacco, tomato, and eggplant, and has a unique molecular structure that facilitates effective electron delocalization. The unique structure of solanesol, consisting of nine isoprene units, supports its diverse bioactivities. This property is very important for providing stability to radicals, thereby increasing substantial antioxidant capacity. The extended isoprene chain in solanesol supports extensive π electron delocalization, which is important for stabilizing radical intermediates—a vital mechanism in its ability to scavenge free radicals. This molecular structure is reinforced by several unconjugated double bonds, which provide several potential sites for interaction with radical species, enhancing the overall antioxidant efficacy of solanesol (Yan et al. 2019; Ma et al. 2025). It is thought that solanesol contributes to the very strong antioxidant properties of *T. sapiens* propolis

Pentadecylresorcinol is characterized by the phenolic group -OH, which can act as a hydrogen donor. This function is very important in the formation of hydrogen bonds, which are important in various biological and chemical phenomena. Hydrogen bonds can determine solubility and reactivity in solvent chemistry and molecular interactions (Hou et al. 2018). However, the long alkyl chain associated with pentadecylresorcinol reduces its solubility in water. The long hydrophobic chain usually inhibits the ability of molecules to interact favorably with polar solvents such as water due to the hydrophobic effect, which inhibits the accessibility of the -OH group for hydrogen bonding (Yao et al. 2018).

The differences in antioxidant potential of the two propolis are mainly due to: electronic effects, conjugated system of solanesol vs. isolated -OH groups on phenolics; bioavailability, better solubility of acetylated compounds (9,19-cyclolanost-24-en-3-ol acetate in *T. sapiens* propolis); synergistic effects, and unique interactions between polyterpenoids-triterpenoids in *T. sapiens* propolis. Although the phenolic -OH groups of pentadecylresorcinol can serve as effective hydrogen donors, the presence of long alkyl chains presents significant challenges in achieving optimal water solubility and efficient molecular interactions. This is considered as one of the reasons for the lower antioxidant activity of *T. biroi* compared to *T. sapiens* propolis.

Antioxidants play a crucial role in protecting our cells from damage caused by free radicals. These compounds worked by donating electrons to free radicals that had unpaired electrons. This process helps stabilize the free radical and prevents the chain reaction of highly reactive and harmful molecules that cause damage to cells and tissues. Oxidative stress occurred when there was an imbalance between the levels of free radicals and antioxidants in the body. This condition could lead to oxidative damage affecting everything from cells and tissues to entire organs (Sukweenadhi et al. 2020). Antioxidants were implicated in the treatment of various chronic diseases like cardiovascular disease, eye defect, cancer, diabetes, liver disease, and

neurodegenerative disorders (Ayoola et al. 2020). The antioxidant activity of propolis was influenced by its rich composition of bioactive compounds derived from its plant sources, phenolics, flavonoids, terpenes, steroids, alkaloids, fatty acids, and other polyphenols. These compounds were shown to have strong antioxidant properties and could ward off free radicals, therefore protecting cells from oxidative damage. In addition, it must be noted that propolis, regardless of species, contained other beneficial compounds, such as triterpenes and diterpenes, which contributed to its antioxidant activity. These findings suggested that *T. sapiens* propolis from the Konawe Islands had the potential to be used as a source of powerful natural antioxidants in various applications, including pharmaceuticals, cosmetics, and functional foods.

In conclusion, the primary compounds found in the propolis from *T. sapiens* and *T. biroi* bees on Konawe Island, Southeast Sulawesi, Indonesia included triterpenes (cycloartenol, lupenone, solanesol and lanosterol), phenolics (3-pentadecylphenol and 5-heptylresorcinol), steroids (pregn-4-ene-3,20-dione, ergosta-8,24(28)-dien-3-ol, 4,14-dimethyl), and fatty acids (hexadecanoic acid methyl ester and (Z)-9-octadecenoic acid methyl ester). The botanical origins of *T. biroi* and *T. sapiens* propolis were *A. occidentale*, *M. indica* (mango), and *A. heterophyllus* (jackfruit). *T. biroi* tends to utilize *Mangifera* resin for phenolic compounds, while. *T. sapiens* relies more on *A. heterophyllus* and *A. occidentale* for complex triterpenoids Propolis ethanol extract from *T.* aff. *biroi* and *T. sapiens* showed strong to very strong antioxidant activity with IC_{50} 96.8 4±2.35 µg/mL and 49.5±0.42 µg/mL.

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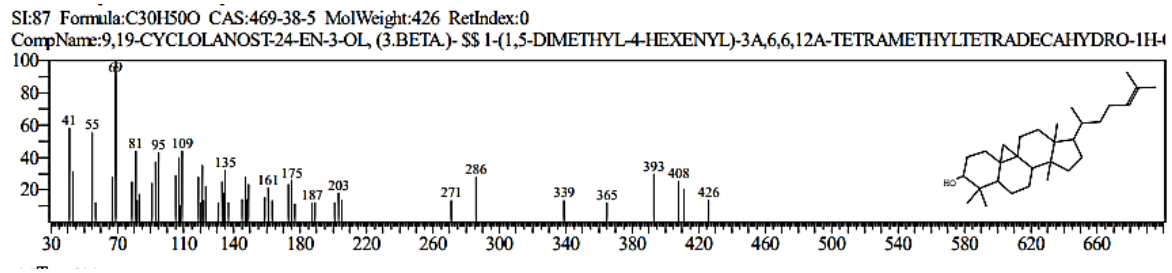

Figure S1. A typical gas chromatogram of cycloartenol

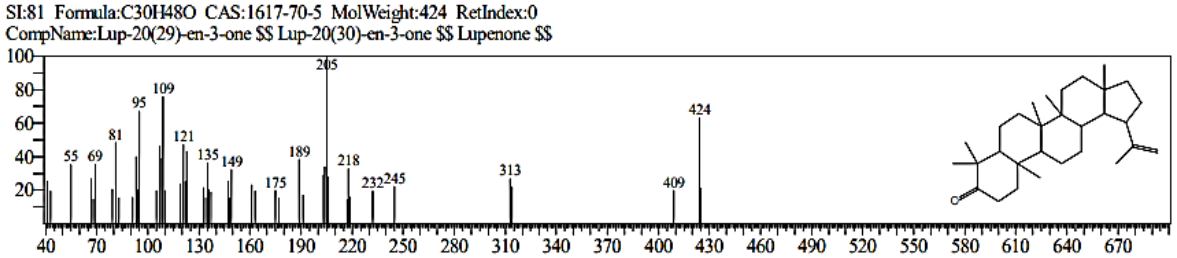

Figure S2. A typical gas chromatogram of Lupenone

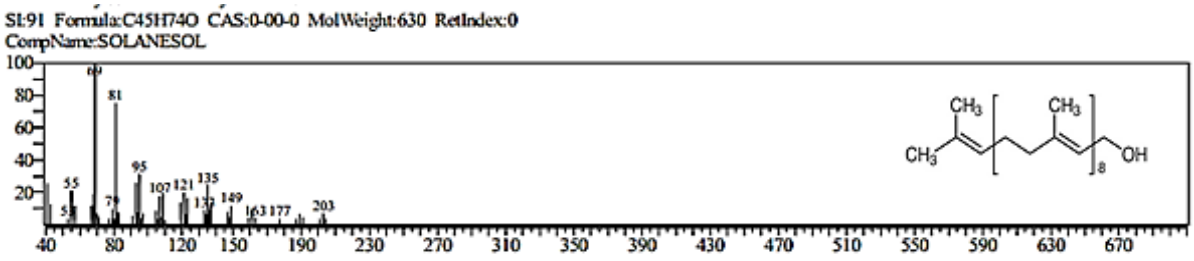

Figure S3. A typical gas chromatogram of solanesol

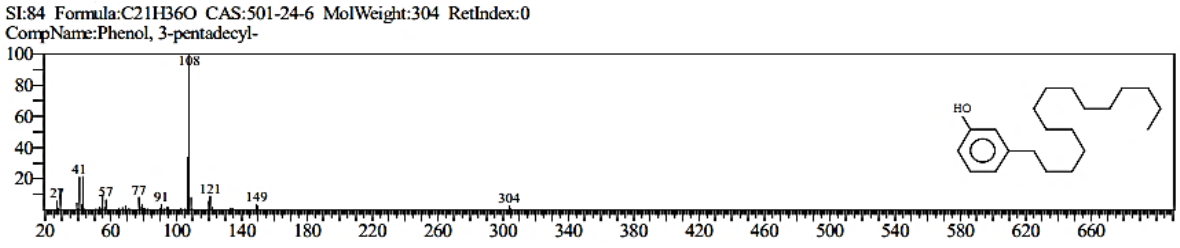

Figure S4. A typical gas chromatogram of 3-pentadecyphenol

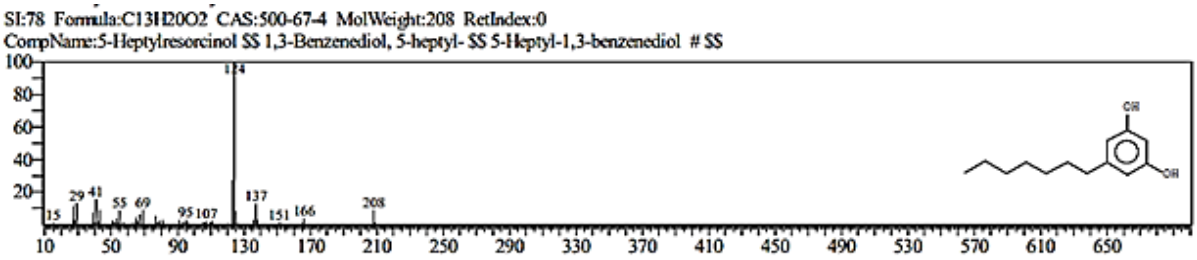

Figure S5. A typical gas chromatogram of 5-heptyl resorcinol

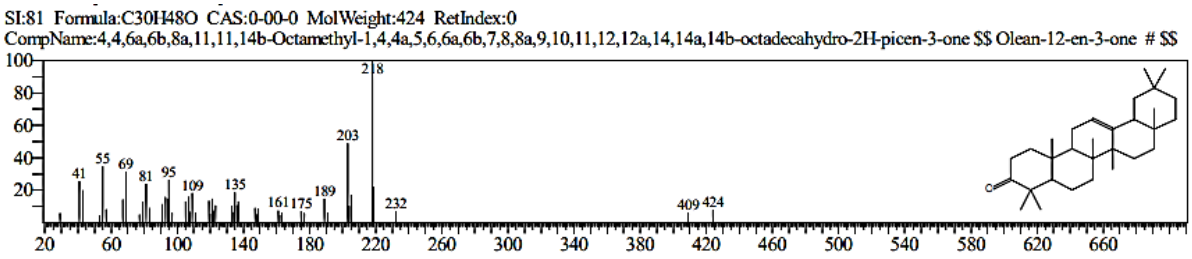

Figure S6. A typical gas chromatogram of β-amyrone (4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,14, 14a, 14b ..

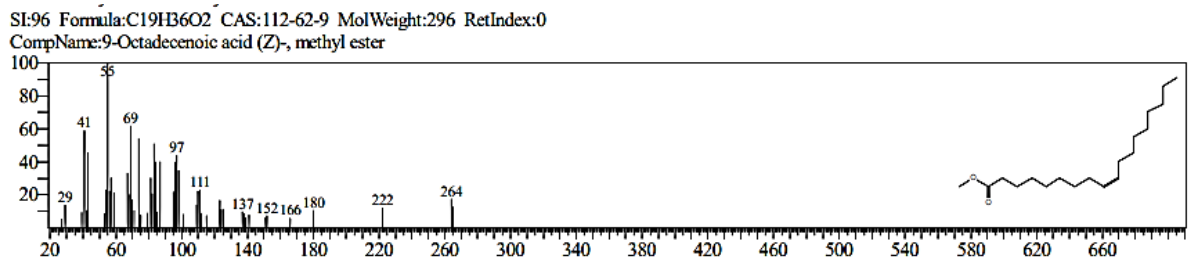

Figure S7. A typical gas chromatogram of 9-octadecenoic acid, methyl ester

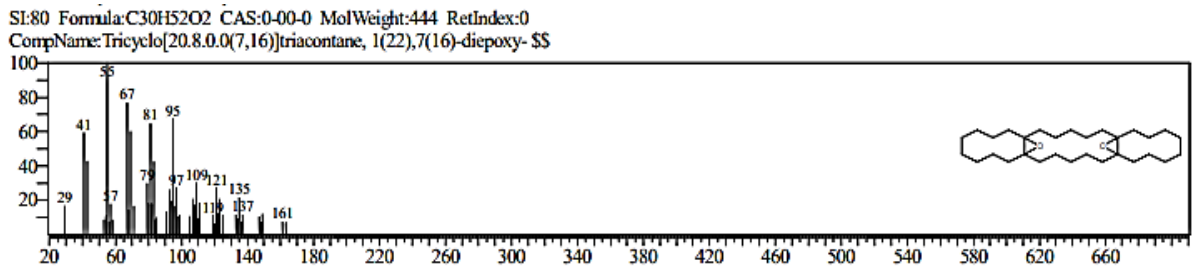

Figure S8. A typical gas chromatogram of tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy