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Antioxidant Testing and Identification of Bioactive Compounds in Ethanol Extract of Propolis from Various Locations in Indonesia using LCMS-QTOF

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Abstract: Propolis produced by *Trigona sp*. stingless bees contains various bioactive substances including alkaloids, flavonoids, polyphenols, saponins, steroids, and terpenoids. The geographical origin of propolis production can influence its composition of secondary metabolites. This study aimed to qualitatively analyze ethanol extracts of propolis from Bintan, Lampung, and Makassar, and evaluate their phenolic and flavonoid contents, as well as antioxidant activities. The extraction method employed kinetic maceration with continuous stirring over 24 hours, using 70% ethanol as the solvent. Additionally, antioxidant activity was assessed using the DPPH method. The qualitative phytochemical analysis revealed that all three ethanol extracts contained alkaloids, flavonoids, and polyphenols. Saponin compounds were uniquely identified in the Makassar ethanol extract. Furthermore, antioxidant activity tests indicated significant potential in all three propolis extracts. These findings highlight the potential of *Trigona sp*. Stingless bee propolis as a valuable source of bioactive compounds beneficial for human health.

Keywords: antioxidant, ethanol extract, phytochemical analysis, Trigona sp stingless bee.

***Abstrak*:** *Propolis yang dihasilkan oleh lebah Trigona sp. tanpa sengat mengandung berbagai zat bioaktif termasuk alkaloid, flavonoid, polifenol, saponin, steroid, dan terpenoid. Asal geografis produksi propolis dapat memengaruhi komposisi metabolit sekundernya. Penelitian ini bertujuan untuk menganalisis secara kualitatif ekstrak etanol propolis dari Bintan, Lampung, dan Makassar, dan mengevaluasi kandungan fenolik dan flavonoidnya, serta aktivitas antioksidannya. Metode ekstraksi menggunakan maserasi kinetik dengan pengadukan terus menerus selama 24 jam, menggunakan etanol 70% sebagai pelarut. Selain itu, aktivitas antioksidan dinilai menggunakan metode DPPH. Analisis fitokimia kualitatif menunjukkan bahwa ketiga ekstrak etanol mengandung alkaloid, flavonoid, dan polifenol. Senyawa saponin diidentifikasi terdapat di dalam ekstrak etanol Makassar. Hasil uji aktivitas antioksidan menunjukkan potensi yang signifikan dalam ketiga ekstrak propolis. Penelitian ini menyoroti potensi propolis lebah Trigona sp. tanpa sengat sebagai sumber senyawa bioaktif yang berharga yang bermanfaat bagi kesehatan manusia.*

Kata kunci: *antioksidan, ekstrak etanol, analisis fitokimia, lebah tanpa sengat Trigona sp*

INTRODUCTION

Indonesia, a tropical country rich in natural resources, hosts diverse flora and fauna, including stingless bees, which are valuable for their production of propolis, a bioactive substance with potential health benefits. Among these stingless bees, Trigona sp. is notable for its prolific propolis production, yielding up to three kilograms annually compared to other species like Apis sp., which produce significantly less (Maroof & Gan 2020; Rosyidi *et al*. 2018). According to Stojanović *et al.* (2020), propolis has garnered attention due to its chemical constituents, which are explored for alternative therapeutic applications. Extensive research has highlighted its diverse biological activities, such as antibacterial, anticancer, anti-inflammatory, and antioxidant effects (Stojanović *et al*. 2020).

The composition of propolis compounds varies based on geographical location and bee species, influencing its quality and bioactivity. Indonesia's expansive and biodiverse regions offer ample opportunities to harness its natural wealth, particularly in honey bee products like propolis (Abu *et al*. 2012; Bankova *et al.* 2000; Wagh 2013; Wang 2018). Rosyidi *et al*. (2018) investigated the total phenolic, total flavonoid, and antioxidant activity (IC_{50}) of *Trigona* sp. propolis from Batu and

Mojokerto. The maceration process was used for extraction, with a solvent of 70% ethanol (Sinaga *et al*. 2023). The results showed that the total phenolic and flavonoid content of Batu propolis was higher than Mojokerto propolis, and the best antioxidant activity (IC_{50}) was observed in Batu's *Trigona sp* propolis with an IC_{50} value of 166.25 µg (Adawiah *et al*. 2015).

Currently, the market is dominated by imported products with high prices. The potential of propolis in Indonesia presents a significant opportunity that requires further research, from identifying bioactive compounds to testing its potential and utilizing it to create various products for the benefit of society. Several cities in Indonesia, such as Lampung, Bintan, and Makassar, are known for producing significant amounts of propolis (Odden *et al*. 2013). Previous studies have reported that propolis from Lampung and South Sulawesi has shown potential in affecting levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in the body (Contini & Costabile 2020; Rosyidi *et al*. 2018). Apart from differences in production volume, each region can also have significant variations in the phytochemical composition of their propolis. This research provided context on the contribution of propolis from different regions in Indonesia, highlighting specific benefits observed in Lampung and South Sulawesi, and indicating the study's focus on exploring the phytochemical variations among propolis from Lampung, Bintan, and Makassar through phytochemical testing, antioxidant activity, phenolic and flavonoid content, and identifying bioactive compounds using LCMS QTOF.

MATERIALS AND METHODS

The Instruments and Materials

The instruments used in this study were stirrer, rotary evaporator, dark-colored bottles, whatman 41 filter paper, maceration apparatus, weighing balance, UV-Vis Spectrophotometry, LCMS QTOF (Liquid Chromatography Mass Spectrometry Quadrupole Time-of-Flight), volumetric flask, sonicator. The materials used in this study were raw propolis from Bintan, Lampung and Makassar, ethanol 70%, Folin-Ciocalteu reagent, sodium carbonate 7%, hydrochloric acid 2%, ammonia 10%, chloroform, Mayer's reagent, Dragendorff's reagent, ether, anhydrous acetic acid, concentrated sulfuric acid, magnesium powder, DPPH (1,1-diphenyl-2-picrylhydrazyl) solution 0.4 mM, positive control solution (ascorbic acid), gallic acid solution (standard solution), ferric chloride, quercetin, aluminum chloride 10%, potassium acetate, aquabidest, methanol (Widiastuti *et al*. 2023)

Making Propolis Ethanol Extract (PEE)

Sample raw propolis was obtained from three provinces, namely South Sulawesi, Riau Islands, and Lampung. Knife was used to cut the samples into smaller pieces (0.5 - 1 cm). A modified maceration approach, known as kinetic maceration, was then used for the extraction because constant stirring was done seven hours a day for 24 hours at room temperature and shielded from light. Propolis extraction was carried out using 70% ethanol solvent with a ratio of 1:10 (100 grams prolonged in a 1000 mL 70% ethanol solution). The extraction was conducted in a dark-colored bottle using a stirrer for 48 hours. After that, the mixture was filtered through Whatman 41 filter paper. Following filtering, a thick propolis residue was obtained by centrifuging the propolis ethanol extract (PEE) at 40°C in a rotary evaporator. The weight of this residue was then calculated to estimate the extract's percentage yield (Mulyati *et al*. 2021; Zarate *et al*. 2018).

Phytochemical Test of Propolis Ethanol Extract

Identification of Active Compounds in Ethanol Extract of Propolis (Noer & Pratiwi 2016).

Active compound groups in the ethanol extract of propolis were identified through qualitative phytochemical screening. Firstly, 0.5 g of propolis ethanol extract was mixed with 5 mL of 10% hydrochloric acid, shaken, and then combined with 5 mL of 10% ammonia solution. The mixture was extracted with chloroform and subsequently evaporated. The remaining residue was treated with 1.5 mL of 2% hydrochloric acid and divided into two

(a) (b) (c)

Figure 1. Raw propolis from (a) Bintan, (b) Lampung, and (c) Makasar**.**

tubes. Mayer's reagent was added to one tube, and Dragendorff's reagent to the other, resulting in the detection of alkaloids by the formation of yellowish-white and brick-red precipitates, respectively.

For the steroid and terpenoid test, the extract was macerated with ether, spotted on a TLC plate, and developed. The residue was hydrolyzed with 2N HCl over a water bath and retested using Liebermann Bouchard reagent, identifying steroids by the appearance of blue or green colors, and terpenoids by red coloration. Additionally, up to 0.5 g of the extract was heated in 10 mL of water over a water bath, and divided into two tubes for the flavonoid and saponin test. Magnesium powder, strong hydrochloric acid, and amyl alcohol were added to one tube, with the presence of flavonoids indicated by coloration in the amyl alcohol layer. The second tube exhibited froth formation, persisting upon addition of 1% hydrochloric acid, confirming the presence of saponins. Finally, the presence of polyphenols was determined by adding a few drops of $FeCl_3$ to 2 mL of the extract solution, resulting in the formation of a dark blue color indicative of a positive result.

Antioxidant Test using The DPPH Method

Antioxidant efficacy was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay with a UV-Vis spectrophotometer. Ascorbic acid (vitamin C) was prepared at 500 ppm in ethanol. A calibration series ranging from 1 to 5 ppm was established. Each propolis extract (2.5 mg) was dissolved in 5 mL of ethanol to yield a 500 ppm solution. Concentrations of 5, 10, 25, 50, and 100 ppm were prepared from this stock solution. To each concentration, 0.6 mL of 0.4 mM DPPH solution was added, followed by ethanol to a final volume of 3 mL. After 30 minutes of incubation at 37°C, absorbance was measured at the maximum wavelength using UV-Vis spectrophotometry. Antioxidant activity was determined by comparing absorbance values of test, blank, and positive control solutions. Percentage inhibition of free radicals was calculated using the formula (Martiani *et al*. 2024):

$$\%\text{free radical inhibition} = \frac{\text{blank absorption - sample absorption}}{\text{blank absorption}} x\ 100\%$$

Next, using the following procedure to find the slope and intercept values, the IC_{50} value of each extract is calculated by finding the equation of the line where the concentration is the x-axis and the percent inhibition is the y-axis:

IC_{50}= (50-Intercept)/slope

Total Phenol Analysis

The total phenol content was determined using a colorimetric method modified from Anh-Dao *et al*. (2023), with gallic acid (GAE) as the standard solution. Initially, 10 mg of gallic acid was dissolved in 10 mL of analytical-grade ethanol to achieve a concentration of 1000 ppm. From this stock solution, 2.5 mL was taken and diluted to 25 mL with ethanol to obtain a concentration of 100 ppm. The 100 ppm gallic acid solution was further diluted to prepare concentrations ranging from 5 to 50 ppm in ethanol. For the analysis, 1 mL of the propolis extract (dissolved in 10 mL of analytical-grade ethanol) was mixed with 0.4 mL of Folin-Ciocalteau reagent. After an incubation period of 4-8 minutes, 4.0 mL of 7% Na_2CO_3 solution was added and vortexed until homogeneous. Distilled water was added to bring the total volume to 10 mL, and the mixture stood for 2 hours at room temperature. Absorbance was measured at a wavelength of 744.8 nm. A calibration curve was constructed using different concentrations of gallic acid to correlate absorbance with phenol concentration (µg/mL).

Flavonoid Analysis

Quercetin (10 mg) was dissolved in ethanol p.a. to a volume of 10 mL, resulting in a concentration of 1000 ppm. Subsequently, 1 mL of the 1000 ppm quercetin standard solution was drawn and dissolved in ethanol p.a. to a volume of 10 mL, achieving a concentration of 100 ppm. Several concentrations, namely 5, 10, 15, 20, and 25 ppm, were generated. For each quercetin standard solution concentration, 3 mL of ethanol p.a., 0.2 mL of 10% AlCl3, and 0.2 mL of 1 M potassium acetate were appended, followed by the addition of distilled water to reach a total volume of 10 mL. The mixture was incubated for 30 minutes at room temperature, and the absorbance was gauged using UV-Vis spectrophotometry at a wavelength of 431 nm. For each propolis extract, 100 mg was assessed and dissolved in ethanol p.a. to a volume of 10 mL. One milliliter of the propolis extract solution was drawn, and 3 mL of ethanol, 0.2 mL of 10% AlCl3, and 0.2 mL of potassium acetate were incorporated. Distilled water was then introduced to reach a total volume of 10 mL. The mixture was incubated at room temperature for 30 minutes, and the absorption was assessed at a wavelength of 431 nm (Ahmad *et al*. 2015; Hanifa 2015).

Identification of Active Compounds with LCMS-QTOF

The determination of bioactive compounds in propolis, particularly the group of flavonoid compounds, is conducted utilizing the LCMS QTOF method. Propolis samples, cut into small fragments, are quantified to approximately ± 0.1 gram and deposited into a 10 mL volumetric flask. Methanol is introduced, and the mixture is subjected to sonication for 5-10 minutes, then compressed. Dilution ensues, succeeded by filtration employing a GHP 0.2 µm filter, and the solution is introduced into the LCMS QTOF system. The process of screening for active natural substances via LC-MS/MS is executed utilizing UNIFI software, which encompasses a spectrum library of mass spectra of active natural

substances sourced from the Waters database. UNIFI software has the capability to recognize the mass spectrum of compounds within the sample, subsequently correlating it with the mass spectrum accessible in the library.

RESULTS AND DISCUSSION

Propolis contains secondary metabolite components in the form of phenolic and flavonoid compounds, making it potentially toxic, particularly as a natural antioxidant. The differences in the locations where *Trigona sp* produces propolis from the resin of various plants can influence the content of secondary metabolite components it possesses. Raw propolis from Bintan, Lampung, and Makasar respectively were extracted with 70% ethanol solvent (Figure 1).

The Results of Propolis Extraction

Extraction of *Trigona sp* propolis was carried out through kinetic maceration. Maceration aims to avoid damage to the organic compound components within it. Raw propolis in the form of chunks is reduced in size to expand the surface area of propolis, allowing the solvent to easily extract active compounds. A 70% ethanol solvent was chosen for extraction because propolis extracts used for medicinal purposes generally use ethanol as a solvent. Ethanol is a polar organic solvent that can easily dissolve phenolic and flavonoid components with a lower cytotoxicity level compared to other organic solvents (Mazumder *et al*. 2023). Maceration or concentrated propolis extract was performed in 9 repetitions, and the yield of each extract displayed in Table 1. The maximum yield is found in the ethanol extract of propolis from Bintan, as indicated by the yield results in Table 1.

The differences in propolis ethanol extract yields among Bintan, Lampung, and Makasar were explained by several factors evident from Table 1. Bintan consistently showed higher yields, ranging from 10.77% to 25.00%. This was likely due to a combination of favorable climatic conditions and rich botanical diversity that supported abundant resin production by local bees. Lampung exhibited intermediate yields between 8.15% and 16.77%, possibly influenced by moderate environmental conditions and diverse but perhaps less abundant plant sources compared to Bintan. Makasar, with yields ranging from 7.61% to 21.83%, showed variability possibly due to its environmental conditions and specific flora, which may have varied in resin productivity across different seasons or years. These differences underscored the impact of local environmental factors, botanical diversity, and possibly bee species behavior on propolis yield variations observed among the three regions.

The Phytochemical Test's Findings

The Table 2 presented the findings of phytochemical screening conducted on ethanol extracts of *Trigona sp*. propolis collected from different regions: Bintan, Lampung, and Makassar.

Based on the phytochemical screening test conducted to determine the presence of active compounds in crude propolis extracts, the results revealed positive indications of alkaloid, flavonoid, and polyphenol compounds in the ethanol extracts of *Trigona sp* propolis from three different regions. Specifically, all three extracts showed positive results for alkaloids, flavonoids, and polyphenols, whereas saponin components were only found in the Makassar ethanol extract. Analysis of the ethanol extract from Makassar showed the formation of foam in the test results, indicating the existence of glycosides that may produce stable foam in water for 15 minutes. Glycosides act as polar members, whereas steroids and terpenoids behave as nonpolar members. Substances containing both polar and nonpolar members have surface-active characteristics, allowing the saponins to develop into micelles when stirred using water.

Table 1. Yield of propolis ethanol extract

Origin of Propolis	Yield (%)									Average
	1	2	3	4	5	6	7	8	9	
Bintan	10.77	19.20	14.55	18,92	25.00	16.85	12.91	13.69	16.69	16.51
Lampung	9.73	14.59	16.77	8,15	9.30	11.78	10.78	8.96	10.19	11.14
Makasar	7.79	7.61	13.40	12,00	21.83	18.83	17.23	15.18	17.32	14.58

Table 2. The results of phytochemical profiles of *Trigona sp*. Propolis

Regional sources	Alkaloid		Flavonoid	Polyphenol	Saponin
	Dragendorff's reagent	Mayer's reagent			
Bintan	+	+	+	+	
Lampung	+		+	+	
Makassar	+		+	+	+

In this study, alkaloid compounds were positively identified through the formation of brick-red precipitates upon the addition of Dragendorff's reagent. Meanwhile, Mayer's reagent showed positive results for the ethanol extract of propolis from Bintan, indicating a yellowish-white sedimentation. This analytical approach is based on the deposition caused by ligand substitution. Nitrogen atoms with free electron pairs in alkaloid compounds can be used to replace Bi ions in Mayer's assay. The alkaloid assay with Mayer's reagent causes an interaction between nitrogen and potassium ions, resulting in potassium alkaloid complexes that precipitate (Ramonah *et al*. 2023). Flavonoid compounds were positively identified through the formation of an orange color in the amyloidalcohol layer. Polyphenol compounds, according to the Harborne method (1993), showed positive results with a dark blue color after the addition of a few drops of $FeCl_3$ to the propolis extract.

Results of Total Phenol and Flavonoid Contents

The total phenolic and flavonoid levels in each extract differ, according to the calculations shown in Table 3. The total phenolic level is higher than the total flavonoid level, indicating that a high content of phenolic components in propolis does not always result in high flavonoid values. Therefore, the presence of flavonoids in propolis is only a contribution. The findings of the determination of total phenolic and flavonoid levels show that the ethanol extract of propolis from Lampung has the greatest values when compared to propolis from Bintan and Makassar.

The comparison of total phenol and flavonoid contents in ethanol extracts of propolis from Bintan, Lampung, and Makassar revealed notable variations in chemical composition among these regions. Lampung propolis stood out with significantly higher levels of total phenols (327.86 mg GAE/g) and flavonoids (17.08 mg QE/g) compared to Bintan (phenols: 158.81 mg GAE/g, flavonoids: 6.16 mg QE/g) and Makassar (phenols: 152.46 mg GAE/g, flavonoids: 10.64 mg QE/g). These findings suggested that propolis from Lampung offered superior antioxidant potential due to its richer phenolic and flavonoid profiles. Such variability underscored the influence of geographical factors on propolis composition and highlighted Lampung propolis as a promising source of natural antioxidants with potential health benefits.

Antioxidant Activity of Ethanol Extract of Propolis

The results of antioxidant activity tests on an ethanol extract of propolis utilizing DPPH as the free radical scavenging approach, with ascorbic acid as the control compound were presented in Figure 2.

Table 3. Preference level test

Propolis	Total Phenol Content (mgGAE/g)	Flavonoid Content (mg QE/g)
Bintan	158.81	6.16
Lampung	327.86	17.08
Makassar	152.46	10.64

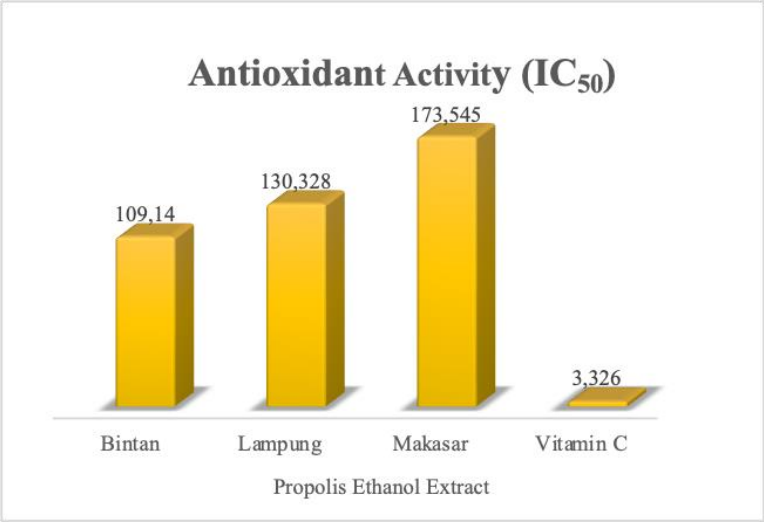


Figure 2. IC_{50} values diagram for vitamin C, ethanol extract of propolis from Bintan, Lampung, and Makassar. Please compare the findings to other results!

Ethanol extracts of propolis from Bintan and Lampung showed moderate antioxidant potential with IC_{50} values of 109.14 ppm and 130.328 ppm, respectively. Meanwhile, the propolis ethanol extract from Makassar was categorized as a weak antioxidant, as it had an IC_{50} value of 173.545 ppm.

The differences in IC_{50} values observed among propolis extracts from Bintan, Lampung, and Makassar can be attributed to variations in their chemical composition due to geographical factors, extraction methods, storage conditions, and assay techniques. Understanding these factors is crucial for interpreting antioxidant activity data and optimizing the use of propolis in functional foods, dietary supplements, and pharmaceutical applications

Chemical Compound Identification of Propolis Ethanol Extract Employing Liquid Chromatography-Mass Spectrometry Quadropole Time of Flight (LC-MS QTF).

Analysis with Liquid Chromatography-Mass Spectrometry (LC-MS) Quadropole Time of Flight to identify chemical compounds was conducted in duplicate, considering their molecular weight and fragmentation patterns present in the ethanol extracts from Bintan, Lampung, and Makassar. The results of LC-MS are presented in Figures 3, 4, and 5.

According to the fragmentation spectrum of ethanol extract of propolis from Bintan, compounds were identified at the peaks of the chromatography spectrum. These peaks reflect the numerous chemical components present in the ethanol extract of Bintan propolis, as presented in Table 4.

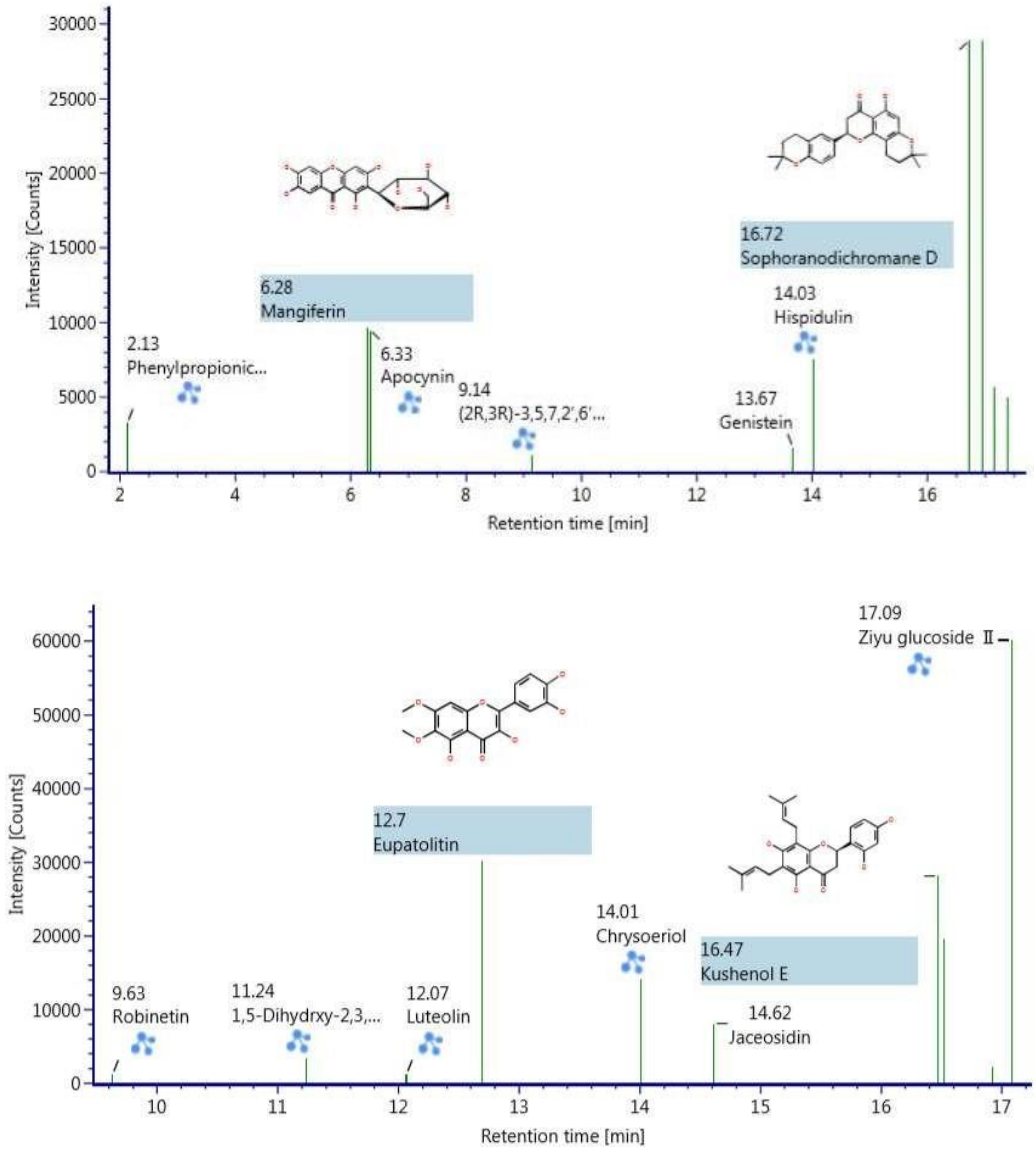


Figure 3. Fragmentation spectrum LCMS of Bintan propolis ethanol extract; ESI- (a) and ESI+ (b).

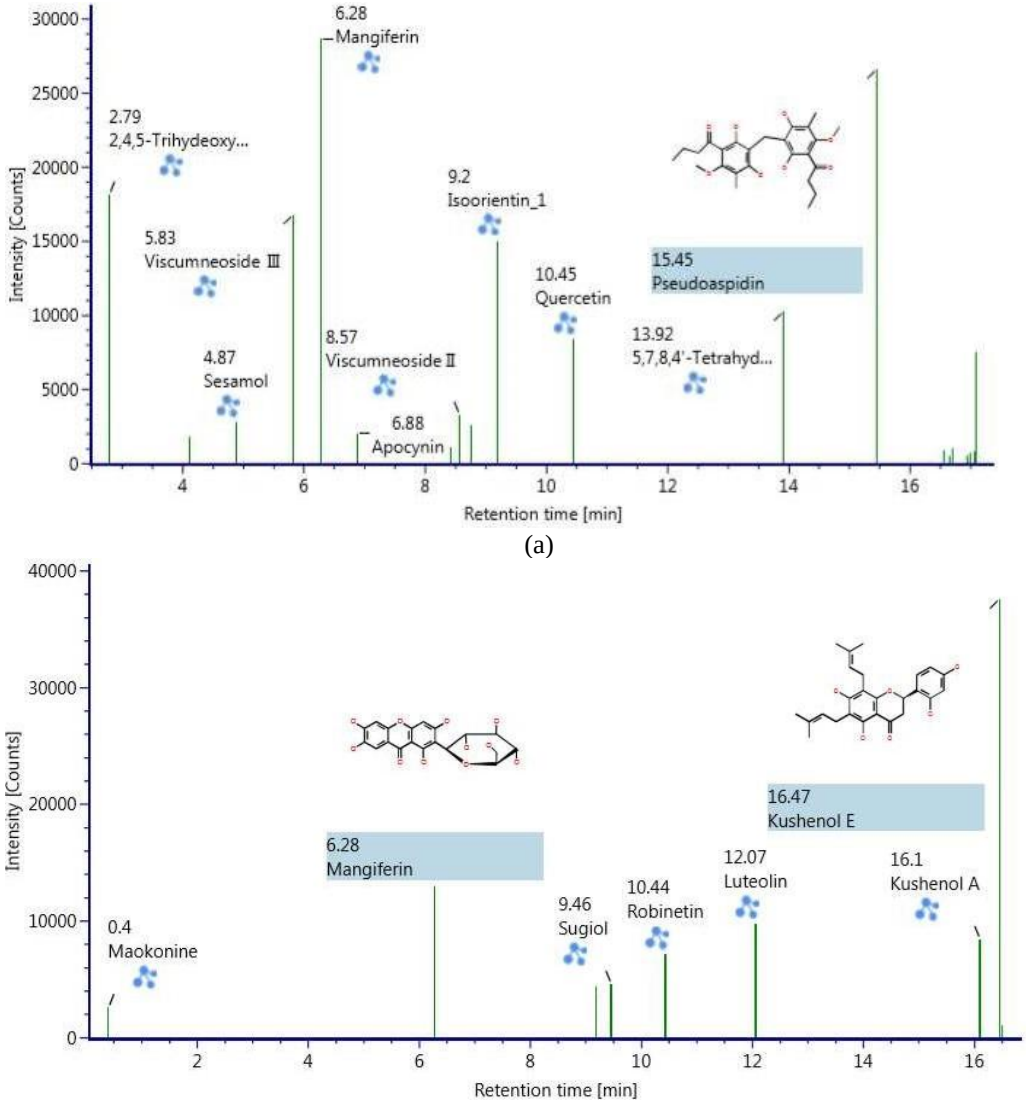
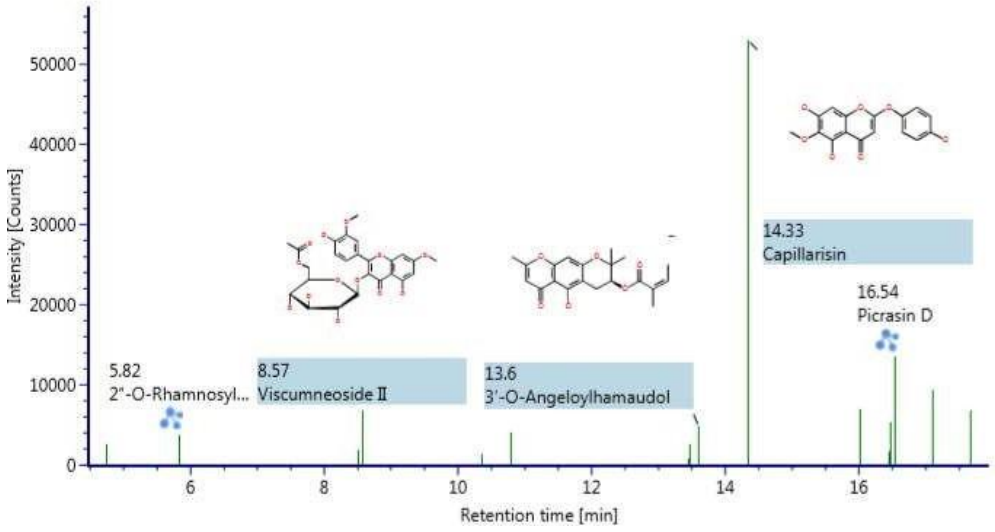
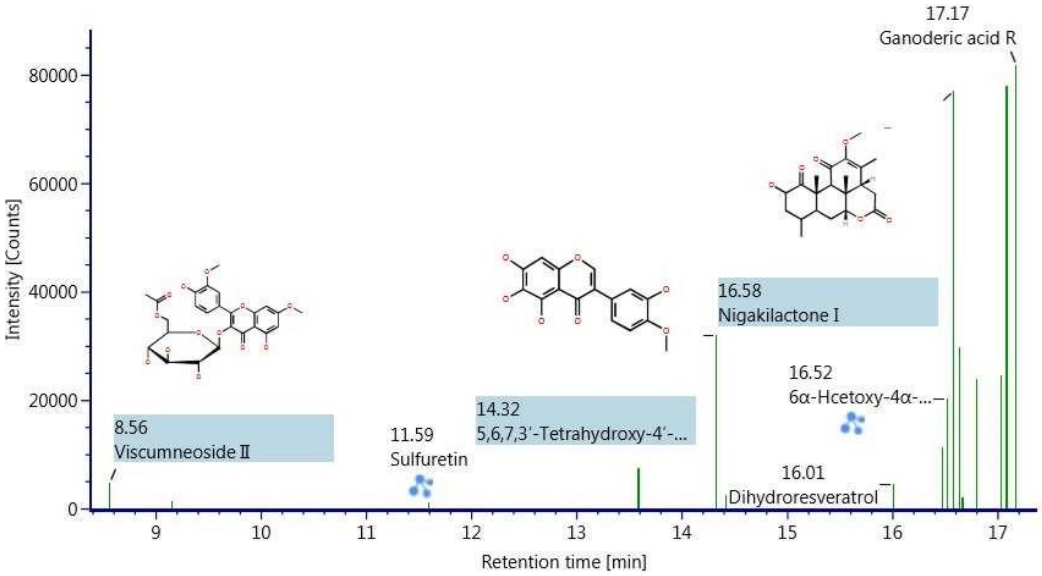


Figure 4. Fragmentation Spectrum LCMS of Lampung Propolis Ethanol Extract; ESI- (a) and ESI+ (b).



(a)



(b)

Figure 5. Fragmentation Spectrum LCMS of Makassar Propolis Ethanol Extract; ESI- (a) and ESI+ (b).

Table 4. Chemical compound of Bintan propolis ethanol extract

No	Compound Name	Retention Time (minutes)	Molecular Weight (m/z)	Compound Classes
1	Robinetin	9.63	302.043	Flavonol
2	Luteolin	12.07	286.048	Flavone
3	Eupatolitin	12.7	346.069	Flavone
4	Chrysoeriol	14.01	300.063	Flavone
7	Genistein	13.67	270.053	Isoflavone
8	Hispidulin	14.03	300.063	Monomethoxyflavone
9	Mangiferin	6.28	422.085	C-glycosyl (Xanthone)

Based on the LC-MS analysis, luteolin was identified as one of the compounds present in the ethanol extract of Bintan propolis. According to Deng *et al*. (2024), luteolin has been implicated in antioxidant activity. The study on *Thymus sipyleus* reported that luteolin exhibited significant free radical scavenging activity, specifically showing an inhibition percentage of 86.4% against DPPH radicals. This indicates strong potential for antioxidant properties. Luteolin belongs to the flavone class of compounds, which are well-known for their antioxidant capabilities. Flavonoids, including flavones like luteolin, often possess antioxidant and free radical scavenging properties due to their molecular structure and ability to donate hydrogen atoms or electrons to neutralize free radicals. Literature references (Ndhlala *et al*. 2024) further support the antioxidant potential of luteolin. Studies on different plants have consistently shown that luteolin exhibits significant antioxidant activity, making it a promising candidate for such properties in natural extracts like propolis. While luteolin has been highlighted as a suspected antioxidant compound in the Bintan propolis extract based on LC-MS analysis and its documented antioxidant activity in various studies, eupatolitin provides an additional candidate with similar potential. The compound eupatolitin was reported (Hussien *et al*. 2016) to have potential antioxidant activity, having an IC_{50} of 27.6 µg/mL, and was successfully isolated from *Pulicaria undulata* (Malarz *et al*. 2023).

Based on the fragmentation spectrum of ethanol extract of propolis from Lampung, the compounds identified at the peak of the chromatography spectrum were observed in Table 5.

Quercetin was identified as one of the compounds through LC-MS analysis in ethanol extracts of propolis from Lampung. According to (Meemongkolkiat *et al*. 2023) quercetin exhibits significant antioxidant activity. This activity was demonstrated through the DPPH free radical scavenging method, where quercetin showed effective inhibition of free radicals. The study also correlates this antioxidant potential with the phenolic content found in the 70% ethanol extracts of propolis. Antioxidants like quercetin play a crucial role in

Table 5. Chemical compound content of Lampung propolis ethanol extract

No	Compound Name	Retention Time (minutes)	Molecular Weight (m/z)	Compound Classes
1	Luteolin-7-O-β-D-glucopyranoside	9.19	448.101	Flavone
2	Sesamol	4.87	138.032	Benzodioxoles
3	Patuletin	6.29	332.053	Trimethoxyflavone
4	Apocynin	6.88	166.063	Aromatic ketone (1-phenyletanone)
5	Isoorientin_1	9.2	448.101	Flavonoid
6	Quercetin	10.45	302.043	Flavonol
7	Sugiol	9.46	300.209	Diterpenoid
8	4',5,6,7-Tetramethoxy-flavone	16.53	342.11	Tetramethoxy-flavone

Table 6. Chemical compound content of Makassar propolis ethanol extract

No	Compound Name	Retention Time (minutes)	Molecular Weight (m/z)	Compound Classes
1	Sulfuretin	11.59	270.053	1-Benzofuran
2	Dihydroresveratrol	16.01	230.094	Stilbenol
3	Skullcapflavone II	16.48	374.1002	Tetramethoxy Flavone
4	Nigakilactone I	16.58	376.189	Triterpenoid
5	Picrasin D	16.63	390.204	Triterpenoid
6	4,4′-Dihydroxy-3,5-dimethoxybibenzyl	17.02	274.121	Phenols and Stilbenoid
7	Genistein	13.47	270.053	Isoflavone
8	Capillarisin	14.34	316.058	Coumarins
9	Dihydroresveratrol	16.02	230.094	Stilbenol
31	Chrysosplenetin B	16.47	374.1	Tetramethoxy Flavone

neutralizing oxidative stress, which is linked to various health benefits. Several literatures supported the notion that quercetin contributes to antioxidant activity in propolis. Phenolic compounds, including quercetin, are known for their antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals, thereby protecting cells from oxidative damage. Quercetin has been reported to possess antibacterial properties against both gram-positive (e.g., *S. aureus*) and gram-negative bacteria (e.g., *E. coli*, *P. aeruginosa*, *S. enteritidis*), further highlighting its potential health benefits beyond its antioxidant activity.

Based on the fragmentation spectrum of ethanol extract of propolis from Makassar, the compounds that were identified at the peak of the chromatography spectrum can be seen in Table 6.

CONCLUSION

The qualitative phytochemical test on the groups of alkaloid, flavonoid, and polyphenol compounds showed positive results in propolis ethanol extract samples from Bintan, Lampung, and Makasar. Meanwhile, positive results for the saponin compound group were observed in propolis ethanol extract from Makasar. Saponins are not typically known for their antioxidant properties. In fact, they can sometimes interfere with antioxidant assays by forming complexes or interacting with assay components, potentially leading to misleading results in antioxidant activity tests. This could explain why the extracts from Makassar show weaker antioxidant activity compared to those from Bintan and Lampung, where saponins were absent or present in lower concentrations. Propolis ethanol extract from Lampung had the highest total phenolic content and

flavonoid values, measuring 327.86 mgGAE/g for total phenol and 17.08 mg QE/g for flavonoids. The lowest IC_{50} is propolis ethanol extract samples from Bintan and it showed that this extract has the highest antioxidant activity.

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