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Original Research Article

Comparative Evaluation of Bioactive Compounds in Semarang Propolis Extracted with Water and Ethanol: Composition and Efficiency Analysis

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ABSTRACT

Propolis, a resinous substance produced by bees, possesses significant bioactive properties influenced by its chemical composition and extraction method. This study compares the bioactive compounds and osteoblast cell viability effects of Semarang propolis extracted using water and ethanol. Aqueous and ethanol extractions were performed to analyze the chemical composition of propolis. Gas chromatography–mass spectrometry (GC-MS) was used to identify bioactive compounds, while X-ray fluorescence (XRF) determined the mineral content. Flavonoid content was assessed using UV-Vis spectrophotometry, and vitamin levels were quantified. Osteoblast cell viability was evaluated using MTT assays on MC3T3-E1 cells. GC-MS analysis revealed that ethanol extraction yielded a wider range of bioactive compounds, including ethyl oleate (28.0%) and benzene derivatives, whereas aqueous extraction provided n-hexadecanoic acid. XRF analysis showed distinct mineral compositions, with calcium levels of 34.0% in ethanol extracts and 45.0% in aqueous extracts. Flavonoid content was significantly higher in ethanol extracts (36 mgQE/g) compared to aqueous extracts (3.8 mgQE/g). Ethanol extracts also contained higher levels of vitamins C (112.95 µg/g), E (31.39 µg/g), and B12 (0.316 µg/g). MTT assays demonstrated that ethanol extracts enhanced osteoblast viability in a dose-dependent manner, with the highest concentration (P1000, 1000 µg/mL) significantly improving cell viability. Ethanol is a more effective solvent for extracting bioactive compounds from Semarang propolis, yielding superior chemical composition and biological activity. These findings highlight the potential of ethanol-extracted Semarang propolis in pharmacology and bone tissue engineering.]

Keywords: Semarang propolis, aqueous and ethanol extraction, GC-MS, XRF, flavonoid, osteoblast

Introduction

Propolis is a natural material harvested by honeybees from several plants, including poplar, palm, pine, coniferous secretions, gums, resins, mucilage and leaf buds. Honeybees meticulously collect and transport it to seal gaps and fissures in their colonies. Propolis acts as an antiseptic, preventing microbial infections in beehives and hindering the decomposition of intruders. Moreover, propolis has been utilised in traditional medicine for ages. [1] The biological activity of propolis samples varies based on their distinct geographical origin. [2] Furthermore, its chemical content is considerably affected by factors such as vegetation types visited by bees, climatic conditions, bee species and collection techniques. [2, 3] Propolis is a complex natural substance composed of various bioactive compounds and typically comprises 10% volatile substances, 50%–55% resins (predominantly flavonoids, phenolic acids and esters), 30%–40% beeswax and 5%–10% pollen, along with other minor components.

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The specific chemical profile of propolis is determined by multiple factors, such as the botanical origin of its resin sources, environmental conditions, seasonal alterations, bee species and the methods used for its collection. Over 300 chemical constituents have been identified in propolis to date, demonstrating its extensive chemical diversity. [1, 4, 5]
In temperate regions, propolis typically consists of 50%–60% resins and balsams, 30%–40% wax, 5%–10% essential and aromatic oils, and approximately 5% pollen, along with trace amounts of other constituents. It contains a diverse range of bioactive compounds, such as aliphatic acids, aromatic esters and acids, fatty acids, flavonoids, carbohydrates, dihydrochalcones, amino acids, terpenoids, and chalcones, as well as essential vitamins like B1, B2, B6, C, and E. In addition, it is rich in minerals, including calcium, copper, iron, zinc and manganese. This chemical complexity underpins the broad pharmacological potential of propolis. [2, 6, 7]
Tropical propolis, particularly from Southeast Asia, has attracted the attention of researchers owing to its unique and highly diverse chemical composition and biological activities, which remain relatively underexplored. Indonesian propolis, in particular, is a largely untapped resource with immense potential as detailed investigations on its chemical constitution and botanical origin are conspicuously lacking in the current literature. [8]
Classified within the Pacific region propolis, along with those from Japan and Taiwan, Indonesian propolis possesses distinctive properties that are determined by the unique flora and climatic conditions of the region. [2, 9] Indonesian propolis from East Java has been reported to contain 11 distinct compounds, including four alkenylresorcinols, four

prenylflavanones and three cycloartane-type triterpenes. The alkenylresorcinols were identified for the first time in propolis, along with notable findings of plant sources such as *Macaranga tanarius L.* and *Mangifera indica L.* Structural analysis of these compounds was performed using spectral techniques, which revealed their biological activities. The prenylflavanones exhibited strong antioxidant properties, effectively scavenging diphenylpicrylhydrazyl radicals, while one compound showed considerable antibacterial activity against *Staphylococcus aureus*. [8]

The propolis utilized in this study was sourced from Semarang, Central Java, and derived from the *Trigona* bee species. *Trigona* bees, unlike *Apis* species, incorporate plant-derived substances along with tree resin, resulting in a unique chemical composition rich in polyphenols, flavonoids, and antibacterial compounds 10. These bioactive compounds contribute to its superior pharmacological potential, including antimicrobial, anti-inflammatory, and osteogenic properties. [11]

The selection of *Trigona* propolis is further supported by its relevance in biomedical applications, particularly in bone tissue engineering, due to its ability to enhance osteoblast activity and mineralization. [12, 13] Additionally, Semarang's geographical diversity, transitioning from low-lying coastal zones (≤20 meters above sea level) to hilly and mountainous terrains, influences the floral sources available to *Trigona* bees, potentially enriching the bioactive profile of the propolis. [13, 14]

This diversity exacerbates Semarang's distinct environmental and infrastructural constraints, including vulnerability to coastal flooding in the northern regions and land stability concerns in the elevated southern areas. [14] These background factors might greatly influence the composition of propolis and its bioactive components. [2, 9] Comprehending these variations is essential as the study of propolis holds enormous potential for identifying novel bioactive compounds. These compounds could drive pharmacological, nutraceutical and biotechnological advancements. Therefore, comprehensive chemical and biological profiling of propolis is vital to explore and harness its value as a natural resource fully. Hence, this study aimed to investigate the composition of Semarang propolis by evaluating two extraction methods, namely, maceration and freeze-drying, and compare the bioactive contents obtained from these techniques.

Materials and Methods

This study was ethically approved by the Health Research Ethics Commission of the Dental Faculty, Universitas Islam Sultan Agung (Approval No. 572/B.1-KEPK/SA-FKG/VI/2024). Propolis samples were obtained from Universitas Islam Sultan Agung, Semarang City. The research stages are divided into four stages: sample collection and preparation, propolis extraction, chemical analysis, and bioactivity assay (MTT).

Sample Collection and Preparation

Whole propolis samples were immediately transferred to polyethylene bags and stored at 4°C for 20 minutes during transportation. The raw propolis was thoroughly washed with distilled water, air-dried, and crushed into small pieces. The entire 1 kg sample was frozen, finely ground using a laboratory mill, and passed through a 35-mesh sieve. The processed sample was stored at −20°C until extraction. [15]

Propolis Extraction

Aqueous Extraction

For water extraction, 10 g of ground propolis was mixed with 100 mL of distilled water in a 100 mL Erlenmeyer flask. The mixture was continuously stirred at room temperature for 24 hours in the dark. After extraction, the sample was centrifuged at 4,000 × g for 5 minutes to separate solid residues (debris, waxes, and insoluble components) from the liquid extract effectively, and the supernatant was separated and stored at 4°C for further analysis. [15]

Ethanol Extraction

For ethanol extraction using the maceration method, 10 g of ground propolis was mixed with 100 mL of 70% ethanol in a 100 mL Erlenmeyer flask. The mixture was stored in the dark at room temperature for 24, 48, and 72 hours to facilitate the extraction of bioactive compounds. After maceration, the extract was filtered, and the filtrate was concentrated using a rotary evaporator. The concentrated extract was then stored under controlled conditions for the determination of polyphenol and flavonoid content. To ensure reliability, each extraction was performed in triplicate. The final extracts were analysed using validated analytical methods based on the specific compounds under investigation. [15, 16]

Chemical Analysis

Gas Chromatography–Mass Spectrometry (GC–MS)

The analysis was performed using a Fisons GC 8000 gas chromatograph (Yokogawa, India) coupled to a Fisons MD 800 (Yokogawa, India) mass detector with electron impact ionisation at 70 eV. The interface temperature was set at 230°C, and the mass spectrometer scanned a range of 35–450 atomic mass units. A fused silica OV1 capillary column (25 m × 0.25 mm internal diameter) was used for chromatographic separation. Helium served as the carrier gas at a flow rate of 10 mL/min. [17]

The oven temperature for GC–MS analysis was programmed to increase from 100°C to 280°C at a consistent rate of 10°C per minute. Initially, propolis samples were analysed with the column temperature held at 60°C for 2 min. The temperature was then increased to 230°C at a rate of 2°C per minute and maintained for 3 min. Finally, the temperature was increased to 280°C at a rate of 3°C per minute. The sample was injected in split mode at an injector temperature of 220°C. [18]

X-Ray Fluorescence (XRF)

The propolis sample was positioned in the sample holder and analyzed using the Olympus DELTA Professional Handheld XRF Analyzer (Tokyo, Japan) was powered on. After preparing the XRF calibrator chip, the analyser was calibrated. The device was set to mining plus mode to detect elements expected to exceed 1% concentration [19] and includes elements that are often referred to as major elements and positioned over the propolis sample. The XRF trigger button was then pressed, allowing the system to operate for one minute. Once the analysis was complete, the XRF was removed, and the process was considered finished when the red indicator light turned off. [20]

Flavonoid Quantification

The total flavonoid content was determined using a modified aluminium chloride colourimetric method. Quercetin served as the standard for the calibration curve and was prepared by dissolving 10 mg of quercetin in 96% ethanol and diluting to obtain concentrations of 2, 4, 6, 8 and 10 µg/mL. [21, 22] For the analysis, 1 mL of each standard or sample solution was added to 3 mL of 96% ethanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. The resulting mixture was incubated at room temperature for 10 min with intermittent shaking to ensure proper mixing. [22]

The absorbance of the solution was measured at 420 nm using a Cecil CE7410 UV-Vis spectrophotometer (Cambridge, United Kingdom), and a blank solution (without aluminium chloride) was used for the calibration. The total flavonoid content was calculated as the mean ± standard deviation (n = 3) and expressed in terms of quercetin equivalent per 100 mg of the extract. [22, 23]

Vitamin Analysis

Vitamin (C, E and B12) stock solutions (100 ppm) were prepared by dissolving 1 mg each of ascorbic acid, tocopherol and cobalamin in ethanol in a 10 mL volumetric flask. Aliquots of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL and 1.2 mL were taken from this stock solution using a pipette and diluted to 10 mL with ethanol in separate volumetric flasks. This dilution resulted in standard vitamin solutions with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm. These solutions were then analysed using a UV-Vis spectrophotometer at a wavelength of 252 nm. [24]

For the sample analysis, a measured amount of the sample was dissolved in ethanol and made up to 10 mL. The sample was then prepared, and its absorbance was measured using a spectrophotometer

at 252 nm. The vitamin concentration in the sample was calculated in percentage and subsequently converted to ng/mL. This method ensured precise determination of vitamin content in the sample. [24]

Bioactivity Assay (MTT Analysis (3-(4, 5-Dimethylthiazolyl-2)-2, 5-Diphenyltetrazolium Bromide))

Ethanol-based propolis extracts were prepared as stock solutions at concentrations of 10, 100, and 1000 µg/mL. These extracts were used to treat MC3T3-E1 cells, a mouse osteoblastic cell line (Merck, Darmstadt, Germany), which was maintained in Dulbecco's modified eagle medium (Merck) with high glucose, supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours, allowing them to reach approximately 80% confluence before treatment. [25]

After the incubation, the medium was carefully removed and the cells were washed twice with phosphate-buffered saline (PBS). The next step involved adding 110 µL of MTT solution (final concentration 0.5 mg/mL) to each well. The plates were incubated in the dark for 2 h to allow the intracellular reduction of MTT to dark-blue formazan crystals. The MTT solution was then removed, followed by another PBS wash. Finally, 100 µL of DMSO (dimethyl sulfoxide) was added to dissolve the formazan crystals, and the absorbance was measured at 540 nm using a microplate reader. [26]

Results and Discussion

This study investigated the chemical composition of propolis from Semarang using two extraction techniques: maceration with ethanol and freeze-drying with water. This research aimed to assess the efficiency of these methods in preserving or enhancing bioactive components. This investigation intended to determine which technique was more effective by comparing the yields and compositions obtained. The findings were expected to provide valuable insights into the effects of extraction methods on the quality and potential applications of propolis, with pharmacological, nutraceutical and biotechnological implications. The extracted samples were analysed to evaluate the influence of each method on the yield and composition of bioactive compounds, enabling a comprehensive assessment of their efficiency. The findings aided in determining the optimal extraction technique for maximising the therapeutic and commercial potential of propolis.

GC-MS Analysis of Propolis Extracts: Ethanol Extraction Yields a More Diverse Bioactive Profile than Aqueous Extraction

The results indicated that ethyl oleate (retention time: 32.1 min, area: 28.0%) was one of the major compounds in the aqueous extract, which was most abundant and substantially contributed to the sample composition (Table 1, Figure 2A). Another pertinent compound was hexadecanoic acid, ethyl ester (retention time: 28.7 min, area: 16.9%). Benzeneethanol, beta-ethenyl and 1-(3-butenyl) cyclobutabenzene exhibited significant peaks at 30.6 and 31.4 min, respectively. Aromatic compounds, including phenols, were also present. Phenol, 3-pentadecyl (retention time: 38.2 min) is known for its potential antioxidant properties [27], despite its low abundance of 2.0%.

In contrast, the key compound in the ethanol extract of propolis was 2,3-butanediol (Table 2, Figure 2B). This compound was most abundant, suggesting that it may substantially impact the bioactivity of the extract (area: 30.19%). Another noteworthy compound was benzeneethanol, β-ethenyl- (area: 13.35%), which may contribute to its antimicrobial and antioxidant properties. Moderate amounts of heneicosane were present in two locations (areas: 7.12% and 0.13%), which could be ascribed to the presence of distinct isomers or forms. Hexadecanoic acid, ethyl ester (area: 2.38%), is a fatty acid derivative widely recognised for its potential bioactive properties. [28] Minor components, including tetratriacontane and (5-nitrohex-1-enyl) benzene, were found in small amounts but may still confer distinctive properties to the extract. Propenoic acid, 3-(cycloheptatrien-7-yl)-, methyl ester and anthracene were less prevalent but may provide unique characteristics to the chemical profile of Semarang propolis.

Regarding the general composition, the ethanol extract displayed a more diverse array of compounds than the aqueous extract, including a substantial number of hydrocarbons and alcohols (e.g., 2,3-butanediol, 30.19%). Ethyl oleate (28.0%), hexadecanoic acid, ethyl ester (16.9%) and other fatty acid esters were abundant in the aqueous extract. Ethyl oleate was present in both extracts, with the ethanol extract containing 30.19% and the aqueous extract containing 28.0%. A unique compound in the ethanol extract was 2,3-butanediol (30.19%), which contributed significantly to its bioactivity. Phenolic compounds, such as 3-pentadecyl phenol, were present in minor quantities but are known for their potential antimicrobial properties. [29] On the contrary, in the aqueous extract, 9,12-octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester (3.0%) was a unique component likely to possess anti-inflammatory and lipid metabolism-enhancing properties. [30, 31] The bioactive properties of both extracts were determined by their abundance of fatty acid derivatives, including ethyl esters and hexadecanoic acid. Alcohols and phenolic compounds, which are moderately polar, were more effectively extracted with ethanol. [32] Conversely, compounds such as fatty acid esters were more effectively extracted with water as they are slightly less polar. [33] Ethanol enabled superior extraction of phenolics and alcohols, which may contribute to enhanced antioxidant and antimicrobial properties. [34] A greater variety of compounds indicates a superior potential for pharmacological applications. [35] In contrast, the aqueous extract contained ethyl oleates and fatty acid esters, which facilitated its moisturising and anti-inflammatory properties. [36, 37] Simpler compositions may be more appropriate for cosmetic and nutraceutical formulations. Therefore, the extraction method must be selected depending on the intended application. Ethanol extraction is recommended for medicinal and antioxidant-rich applications whereas aqueous extraction is indicated for lipid-based and cosmetic applications. [38]

XRF Analysis of Propolis Extracts: Elemental Composition Comparison of Aqueous and Ethanol Extracts.

The aqueous extract is appropriate for applications in which the mineral content is critical, such as bone health and remineralisation, owing to its abundance in calcium (45.0%), iron (8.56%) and potassium (18.0%), as inferred from the XRF analysis. These findings emphasise its ability to extract water-soluble compounds by detecting specific elements such as magnesium, iron and zinc, which were not present in the ethanol extract (Table 3). In contrast to the aqueous extract, the ethanol extract contained higher levels of phosphorus (20.4%), chlorine (26.3%) and bromine (8.12%), which is indicative of its ability to extract organic and less polar compounds. Hence, this technique is more appropriate for isolating bioactive compounds that are less water-soluble, such as certain flavonoids and volatile components. To substantiate this notion, total flavonoid levels were determined in subsequent experiments.

Both extraction techniques yielded complementary profiles of propolis content. Aqueous extraction was superior for water-soluble minerals and trace elements, whereas ethanol extraction was better at extracting less polar organic molecules. [38] These differences highlight the need to select an extraction process based on the intended use of propolis, whether for mineral supplementation or the isolation of certain bioactive components.

Quantification of Total Flavonoid Content in Aqueous and Ethanol Extracts of Propolis

Flavonoid analysis revealed that ethanol was a more effective solvent for extracting Semarang propolis than water, as inferred from the 9.5-fold higher concentration of flavonoids in the ethanol extract (Figure 1A). This observation emphasises the importance of solvent selection in optimising the bioactive compound yield from natural sources such as propolis. Ethanol appears to be more efficient in extracting flavonoids from propolis than water. This finding agrees with the reports of Pujirahayu et al. (2024) that raw propolis ethanol extract possessed a higher flavonoid content than the aqueous extract. [39] This disparity could be attributed to the greater solubility of flavonoids in organic solvents, such as ethanol, than in water owing to their nonpolar or mildly polar characteristics. [40] Furthermore, this variation in flavonoid content underscores ethanol's capacity to extract a wider array of bioactive components, rendering it a superior option for optimising the medicinal and functional attributes of propolis. The increased flavonoid concentration in the ethanol extract signifies its enhanced potential for medicinal, nutraceutical and antioxidant applications. [2, 41]

Table 1: GC-MS chromatography analysis of the aqueous extract of Semarang propolis

Retention time (minutes)	Area (%)	Height	Name
3.0	2.1	1926463	2,3-Butanediol
3.1	30.2	4006012	2,3-Butanediol
26.9	11.8	1120646	Benzene, (1-Ethyl-2-Propenyl)
27.0	1.5	190001	Anthracene, 1,2,3,4,5,6,7,8-Octahydro-1-Methyl
27.3	9.7	970083	Propenoic Acid, 3-(Cycloheptatrien-7-Yl)-, Methyl Ester
28.3	5.6	452346	1-Methyl-2-Phenylcyclopropane 2
28.7	2.4	332107	Hexadecanoic Acid, Ethyl Ester
29.2	4.9	366964	(5-Nitrohex-1-Enyl)Benzene
30.7	13.4	1322574	Benzeneethanol, Beta.-Ethenyl
31.4	2.7	239412	1-(3-Butenyl)Cyclobutabenzene
32.1	2.7	494038	Ethyl Oleate
34.4	2.7	523652	Heneicosane
37.8	7.1	1342526	Heneicosane
38.1	1.3	180824	Phenol, 3-Pentadecyl
39.7	1.8	238696	Tetratriacontane

Table 2: GC-MS chromatography analysis of the ethanol extract of Semarang propolis

Retention time (minutes)	Area (%)	Height	Name
26.8	6.51	294593	Benzene, (3-Chloro-1-Propenyl)
27.3	6.88	280827	Benzene, (3-Chloro-1-Propenyl)
28.6	2.4	136281	N-Hexadecanoic Acid
28.7	16.9	1238725	Hexadecanoic Acid, Ethyl Ester
30.6	8.4	316941	Benzeneethanol, Beta.-Ethenyl
31.4	5.9	210321	1-(3-Butenyl)Cyclobutabenzene
32.0	3.0	225588	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester
32.1	28.0	1711852	Ethyl Oleate
32.6	2.2	171171	Heptadecanoic Acid, Ethyl Ester
32.8	2.8	144413	Tetradecanoic Acid, Ethyl Ester
34.4	3.3	252271	Heneicosane
37.8	5.5	442455	Heneicosane
38.2	2.0	116852	Phenol, 3-Pentadecyl
39.3	3.8	271083	Ethyl Docosanoate
39.7	2.4	132595	Tetratriacontane

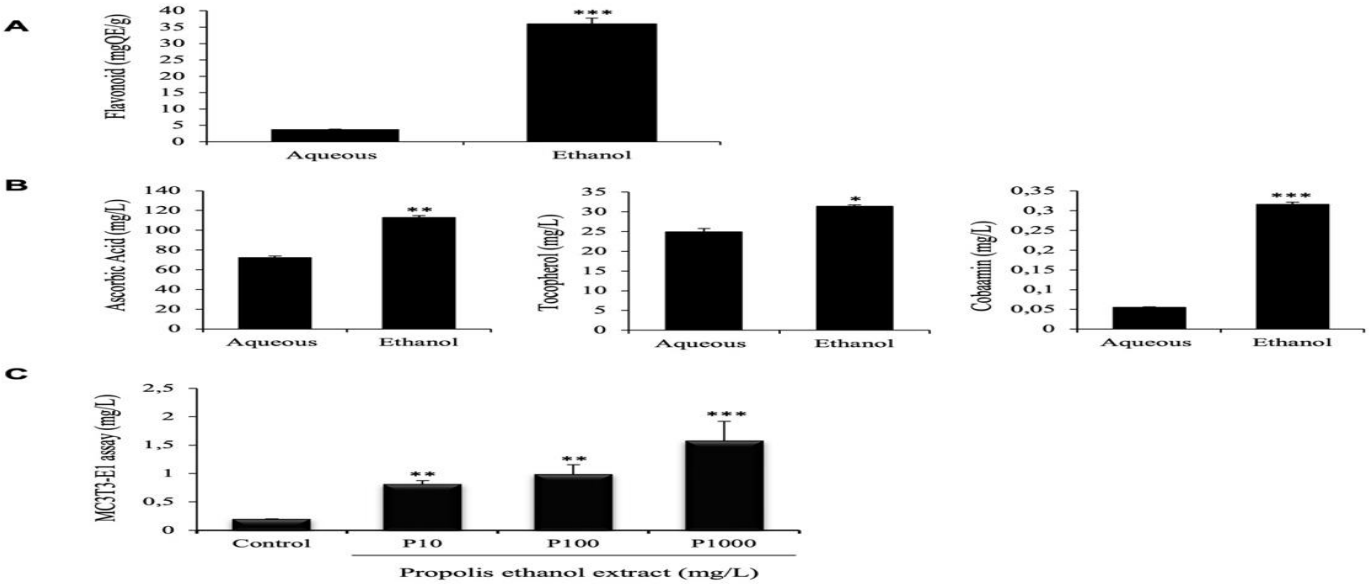

Figure 1: Comparison of Flavonoid Content, Vitamin Levels (C, E and B12) and Cell Viability of Semarang Propolis. (A) The total flavonoid content, determined at 420 nm via UV-Vis spectrophotometry and expressed as mg quercetin equivalent per gram of extract (mg QE/g), was significantly higher in the ethanol extract compared to the aqueous extract. (B) Similarly, the ethanol extract exhibited higher concentrations of vitamins C, E and B12 than the aqueous extract. (C) The ethanol extract of Semarang propolis showed a dose-dependent increase in cell viability (MTT) across concentrations (P10: 10 µg/mL, P100: 100 µg/mL and P1000: 1000 µg/mL) when compared with the control. Absorbance at 540 nm, indicating cell viability, increased significantly at higher concentrations, with P1000 demonstrating the highest cell viability enhancement. Data are represented as means ± SD from 3–5 replications. Scale bar: 100 µm. **p < 0.01, ***p < 0.000.

Table 3: The comparation of mineral elements from aqueous and ethanol extract of Semarang propolis by XRF analysis

Element	Aqueous Extract (%)	Ethanol Extract (%)	Observation
Magnesium (Mg)	0.641	Not Detected	Present in the aqueous extract but absent in the ethanol extract.
Aluminum (Al)	0.643	0.489	Higher in the aqueous extract than in the ethanol extract.
Silicon (Si)	1.89	1.07	Detected in both, but more prominent in the aqueous extract.
Phosphorus (P)	14.2	20.4	Significantly higher in the ethanol extract, suggesting higher solubility in ethanol.
Sulfur (S)	1.92	2.30	Slightly higher in the ethanol extract.
Chlorine (Cl)	7.30	26.3	Much higher in the ethanol extract, likely due to the better extraction of volatile or chlorinated compounds.
Potassium (K)	18.0	7.29	Predominantly found in the aqueous extract, which is typical for water-soluble minerals.
Calcium (Ca)	45.0	34.0	Higher in the aqueous extract, suggesting water solubility of calcium-based compounds.
Iron (Fe)	8.56	Not Detected	Found exclusively in the aqueous extract.
Zinc (Zn)	1.74	Not Detected	Found exclusively in the aqueous extract.
Bromine (Br)	Not Detected	8.12	Found exclusively in the ethanol extract, potentially linked to organic compounds extracted in ethanol.

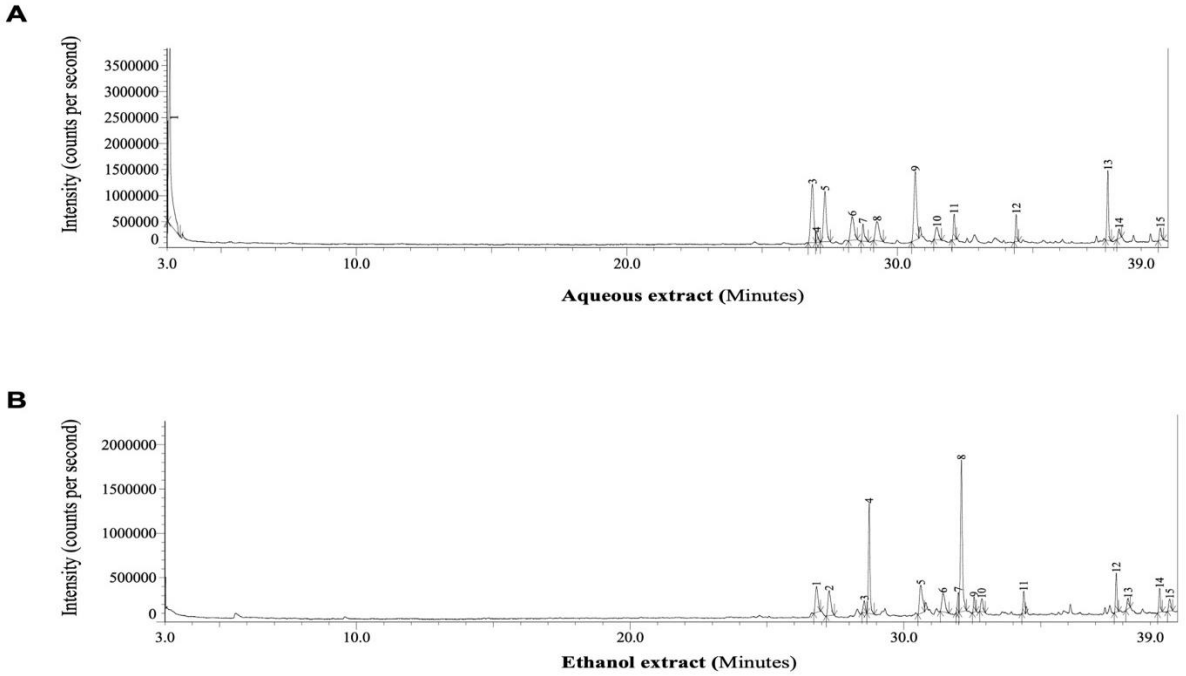

Figure 2: GC-MS chromatograms of propolis extracts**.** (A) Aqueous extract of propolis, displaying fewer and lower-intensity peaks, suggesting a different compound profile with potentially more polar constituents. The retention times and peak intensities reflect the chemical composition differences between the two extraction methods. (B) Ethanol extract of propolis, showing a higher number of detected compounds and peak intensities, indicating the efficient extraction of diverse bioactive components.

Vitamin Profile Analysis of Propolis Extracts

In addition, the results of this comparison revealed that ethanol extraction was more effective than aqueous extraction in extracting vitamins, including water-soluble (vitamin C) and fat-soluble (vitamin E) ones, from Semarang propolis. The difference was particularly evident for vitamin C, where ethanol extraction resulted in an approximately 56% higher yield, and for vitamin B12, where a five-fold increase in concentration was observed compared with aqueous extraction (Figure 1B). These findings imply that ethanol is a more efficient solvent for extracting bioactive compounds from Semarang propolis, supporting its potential for applications in pharmacological and nutraceutical formulations. [2]

MTT Assay-Based Bioactivity Assessment of Propolis Ethanol Extract

The overall findings from this investigation indicate that ethanol extraction yielded a higher concentration of primary active components and flavonoids than aqueous extraction. Therefore, an MTT experiment was performed on the MC3T3-E1 osteoblast cell line using the ethanol extract of Semarang propolis (Figure 1C). The observations suggested that Semarang propolis positively influenced MC3T3-E1 cell viability in a dose-dependent manner. When the concentration of the extract was increased from 10 µg/mL to 1000 µg/mL, cell viability improved significantly, indicating potential cytoprotective or proliferative effects in a dose-dependent manner. These observations highlight the potential therapeutic value of Semarang propolis in promoting cell health and recovery, which may have implications for its use in pharmacological and biomedical applications. [12] Further studies are warranted to examine its underlying mechanisms and dose–response associations.

Conclusion

Ethanol proved to be a more effective solvent than water for extracting bioactive compounds, flavonoids, vitamins and some chemical components from Semarang propolis. While the aqueous extract was

richer in certain minerals, the ethanol extract exhibited a broader range of bioactive properties, higher flavonoid and vitamin contents and cytoprotective effects in the MTT assay. These findings suggest that ethanol extraction maximises the pharmacological and nutraceutical potential of Semarang propolis, making it a promising candidate for applications in health supplements, cosmetics and pharmaceuticals. Further studies should aim at exploring its mechanisms of action and broader applications.

Conflict of Interest
Authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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