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RESEARCH ARTICLE

Bio-friendly Extraction of *Geniotrigona thoracica* Propolis Using Plant-based Oils: Phytochemical Composition and Bioactivities

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Abstract Stingless bees propolis is increasingly recognized as a rich source of bioactive compounds with potential applications in health and skincare. Conventional ethanol-based extraction raises concerns due to skin irritation and regulatory restrictions, underscoring the need for safer alternatives. This study evaluated plant-based oils as bio-friendly plant-based extraction media for *Geniotrigona thoracica* propolis, using coconut (COP), olive (OOP), and sunflower (SOP) oils, with ethanol (EEP) and water (WEP) as comparators. Recoverable extract yields were determined, while total phenolic content (TPC) and total flavonoid content (TFC) were quantified using Folin–Ciocalteu and aluminium chloride colorimetric assays, respectively. Phytochemical composition was profiled by gas chromatography–mass spectrometry (GC–MS). Antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays, while cytotoxicity towards human keratinocytes (HaCaT) was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. COP achieved the highest recoverable extract yield (10.00%) and TPC (54.40 mg GAE/g), statistically comparable to EEP (53.50 mg GAE/g) but significantly higher than other extracts. TFC was greatest in EEP (66.54 mg QE/g), followed by COP (46.55 mg QE/g). GC-MS analysis revealed distinct chemical fingerprints across extracts, encompassing fatty acids, esters, aldehydes, hydrocarbons, and phenolic derivatives. COP exhibited significantly stronger DPPH radical scavenging capacity (50.85 ± 8.70%) than all other extracts, while its ferric reducing capacity (659.00 µM Fe^{2+}) was comparable to EEP (597.37 µM Fe^{2+}). For ABTS, EEP (33.13 ± 4.48%) and COP (26.60 ± 2.88%) did not differ significantly and were both higher than the other extracts. All extracts were non-toxic to HaCaT cells at the concentrations tested. Overall, coconut oil demonstrated a favorable balance of phytochemical recovery, strong antioxidant activity, and cytocompatibility, supporting its potential as a plant-based extraction medium for bio-friendly propolis-based dermatological formulations.

Keywords: Antioxidant activity, cytotoxicity, GC-MS, plant-based extraction, stingless bee propolis.

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Introduction

Propolis is a resinous substance produced by bees from plant resins, wax, and salivary secretions. Rich in polyphenols, flavonoids, terpenoids, and esters, it exhibits diverse biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects [1]. These bioactivities have driven growing interest in propolis as a natural ingredient for functional foods, cosmetics, and pharmaceuticals [2,3].

Stingless bees, a diverse group of eusocial bees, produce a distinctive type of propolis, often referred to as "pot-honey propolis," which differs chemically from that of honeybees (*Apis mellifera)* [4,5]. In Malaysia, *Geniotrigona thoracica* has gained particular attention due to its adaptability to tropical ecosystems and its ability to produce propolis rich in bioactive compounds such as caffeic acid derivatives, flavonoids, and terpenoid [6,7,8]. Several studies have documented its strong antioxidant, antimicrobial, and wound-healing activities, making it an attractive candidate for cosmeceutical and dermatological applications [6,9,10].

Ethanol is the most widely used solvent for propolis extraction, as it effectively dissolves a broad range of phenolic compounds [1,4]. However, ethanol-based extracts face limitations, particularly for topical formulations intended for sensitive skin and formulation compatibility [11]. Moreover, solvent residues from ethanol extraction may necessitate additional processing to comply with safety and regulatory standards [12]. In contrast, water extraction is considered safer but generally inefficient for recovering lipophilic bioactives that play an important role in skin health and topical applications [11,13].

Plant-based oils such as coconut, olive, and sunflower oils have emerged as bio-friendly alternatives for propolis extraction [12,14,15]. These oils are non-toxic, naturally compatible with topical formulations, and possess intrinsic health benefits. Coconut oil, rich in medium-chain triglycerides (MCT), can enhance the solubility and stability of phenolic and terpenoid compounds [16]. Olive oil contains bioactive constituents, including hydroxytyrosol and oleuropein [17], and its monounsaturated lipid matrix may facilitate the oxidative stability of phenolic compounds during extraction and storage [18]. Sunflower oil, abundant in linoleic acid, offers emollient and skin-conditioning properties desirable in cosmetic applications [17]. Despite these advantages, there is limited systematic research comparing oil-based extraction to conventional ethanol and water extractions for *G. thoracica* propolis.

The present study aimed to evaluate the recoverable extract yield, phytochemical composition, antioxidant activity, and cytotoxicity of *G. thoracica* propolis extracted using three plant-based oils: coconut oil propolis (COP), olive oil propolis (OOP), and sunflower oil propolis (SOP). These extracts were compared with ethanolic propolis extract (EEP) and aqueous propolis extract (WEP) to systematically assess the influence of extraction medium on the recovery of bioactive constituents and their biological activities. Cytotoxicity was assessed using human keratinocyte (HaCaT) cells to establish the preliminary safety of the extracts for potential topical and dermatological applications. The findings are expected to provide scientific evidence supporting the use of plant-based oil extraction in the development of safe, effective, and bio-friendly propolis-derived products for topical and cosmetic applications.

Materials and Methods

Collection of *G. thoracica* propolis

Raw propolis was collected in May 2025 from established hives located in Kampung Luat, Lenggong, Perak, Malaysia (5.0594° N, 100.9838° E). The stingless bee species was identified by an entomologist from the Malaysian Agricultural Research and Development Institute (MARDI), and the corresponding voucher specimen has been deposited at the MARDI Museum under accession number 29338. The colonies were kept in wooden nest boxes within a managed apiary, surrounded by a diverse range of nectar and resin sources within a 3 km foraging radius. The area included multiple fruit-bearing trees such as *Cocos nucifera* (coconut), *Syzygium malaccense* (Malay apple), *Garcinia mangostana* (mangosteen), *Durio zibethinus* (durian), *Artocarpus heterophyllus* (jackfruit), *Artocarpus integer* (chempedak), *Musa* spp. (banana), *Morus alba* (mulberry), and *Syzygium aqueum* (watery rose apple). Flowering plants were also abundant, including *Antigonon leptopus* (coral vine), *Melaleuca cajuputi* (gelam putih), *Xanthostemon chrysanthus* (golden penda), *Jasminum sambac* (Arabian jasmine), and *Mimusops elengi* (Spanish cherry). Additional vegetation included *Hevea brasiliensis* (rubber tree) and mixed grasses (*Poaceae*), which contribute as supplementary pollen and resin sources. The raw propolis was manually cleaned to remove debris and stored at –20 °C until further processing. Prior to extraction, frozen samples were ground into fine particles.

Preparation of Propolis Extracts

Oil-based extraction

Oil-based extractions were conducted using three food-grade plant-based oils namely coconut oil, olive oil, and sunflower oil, designated as coconut oil propolis (COP), olive oil propolis (OOP), and sunflower oil propolis (SOP), respectively. The oil-based extraction procedure was adapted from Kubiliene et al. [19], with modifications to standardize the extraction conditions across all extraction media and the subsequent analytical sample preparation. Briefly, ground propolis (10 g) was mixed with 100 g of respective oil (1:10 w/w) and incubated in a shaking water bath (Julabo, Seelbach, Germany) at 45 °C for 48 h with agitation at 250 rpm. The mixtures were filtered through Whatman No.1 paper to remove solid residues, and the filtrates were stored in amber bottles at 4 °C until further use.

Ethanolic and water extraction

Ethanolic and water extractions were performed under comparable standardized conditions to allow direct comparison across extraction media. For the EEP, ground propolis was macerated in 70% ethanol (1:10 w/v) at 45 °C for 48 h under continuous shaking (250 rpm), following Zohdi et al. [20] with modifications. The mixture was filtered, concentrated under reduced pressure at 40 °C using rotary evaporation, freeze-dried, and stored at 4 °C until use. For the WEP, ground propolis (10 g) was suspended in 100 mL of distilled water (1:10 w/v) and incubated under identical conditions (45 °C, 250 rpm, 48 h). The mixture was filtered, concentrated under reduced pressure, freeze-dried, and stored at 4 °C in light-protected conditions.

Post-extraction analytical clean-up

Prior to phytochemical characterization and bioactivity assays, all crude extracts underwent a standardized post-extraction analytical clean-up procedure. This procedure was adapted from the liquid–liquid partitioning approach described by Kubiliene et al. [19] for oily propolis extracts, with modifications to accommodate all extraction media. Its primary purpose was to remove residual waxes, triglycerides, and other non-polar matrix components that may interfere with chromatographic, spectrophotometric, and cell-based assays. Applying the same clean-up procedure to all extracts also ensured standardized downstream sample preparation, thereby enabling direct comparison of the analytically recoverable extract yield across the different extraction media.

Briefly, aliquots of each crude extract (5 mL) were transferred to separatory funnels and partitioned with equal volumes of methanol and hexane. The mixtures were vigorously shaken and allowed to separate into two layers. The resulting methanol fraction was concentrated to dryness using a SpeedVac concentrator (Eppendorf, Hamburg, Germany). The resulting dried residue was subsequently used for determination of the analytically recoverable extract yield, phytochemical characterization, and bioactivity assays. Blank extraction media subjected to the same analytical clean-up procedure were included for background correction during colorimetric analyses.

The percentage of recoverable extract yield was calculated relative to the initial mass of raw propolis using the formula:

$$\textbf{Recoverable extract yield (\%)} = \frac{Mass\ of\ dried\ residue\ after\ clean{-}up\ (g)}{Initial\ mass\ of\ raw\ propolis\ (g)}\ x\ 100$$

Determination of Total Phenolic Content (TPC)

TPC of the propolis extracts was assessed using the Folin–Ciocalteu method, with gallic acid as the calibration standard [21]. Standard solutions were prepared in the range of 5 to 1000 µg/mL to construct the calibration curve. In a 96-well plate, 25 µL of each extract or standard was mixed with 100 µL of 25% (v/v) Folin–Ciocalteu reagent and incubated for four minutes at room temperature with gentle shaking. Subsequently, 75 µL of 7.5% (w/v) sodium carbonate was added to each well. After two hours of incubation at ambient temperature, absorbance was measured at 765 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Results were calculated from the gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

TFC was determined using the aluminum chloride colorimetric method with quercetin as the reference compound [22]. Standard quercetin solutions ranging from 5 to 1000 µg/mL were used to generate the calibration curve. In each well of a 96-well plate, 20 µL of extract (1 mg/mL) or standard solution was

combined with 20 µL of 10% (w/v) aluminum chloride, 20 µL of 1 M potassium acetate, and 140 µL of distilled water. The plate was shaken gently for one minute and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 415 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Results were calculated from the quercetin standard curve and expressed as milligrams quercetin equivalents per gram of extract (mg QE/g).

Determination of the chemical profile of propolis extracts using Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 280 °C at 5 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 40-400 amu.

For identification of phytochemical composition, co-injection with the selected standards (major components) was used, together with correspondence of retention indices and mass spectra with respect to those occurring in libraries and literature [9,23-26].

Antioxidant activity of propolis extracts

DPPH radical scavenging assay

The antioxidant capacity of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following Adli et al. [27]. Briefly, 100 µL of each extract at concentrations ranging from 7.81 to 1000 µg/mL, prepared in dimethyl sulfoxide (DMSO), was mixed with 100 µL of 0.1 mM DPPH solution in methanol. Trolox was used as the reference standard. A negative control was prepared by mixing 200 µL of DPPH solution with 25 µL of DMSO. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was recorded at 517 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). The percentage of radical scavenging activity was calculated using the formula:

$$Inhibition\ (\%) = \frac{(Absorbance\ of\ blank - Absorbance\ of\ sample)}{Absorbance\ of\ blank}\ X\ 100$$

ABTS Radical Scavenging assay

ABTS•⁺ radicals were generated by mixing 5 mL of 7 mM ABTS stock solution with 88 µL of 140 mM potassium persulfate and incubating in the dark for 16 hours. The solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The assay was conducted following Ferreira et al. [28] with modifications. In each well of a 96-well plate, 25 µL of extract (7.81–1000 µg/mL) was added to 275 µL of ABTS•⁺ solution. After 30 minutes of incubation at room temperature, absorbance was read at 734 nm. The percentage of ABTS•⁺ inhibition was calculated using the following equation:

$$Inhibition\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control}\ X\ 100$$

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of the extracts was determined by the FRAP assay following Idris et al. [6]. Extracts were prepared at a concentration of 1000 µg/mL. Briefly, 30 µL of each extract was mixed with 300 µL of freshly prepared FRAP reagent, consisting of acetate buffer, ferric chloride, and 2,4,6-tripyridyl-s-triazine (TPTZ) solution, in a 96-well plate. The reaction mixture was incubated at 37 °C for 30 minutes, after which absorbance was measured at 593 nm. A calibration curve was prepared using ferrous sulfate, and the reducing capacity was expressed as micromoles of ferrous ion equivalents (µM Fe^{2+}).

Cytotoxicity evaluation of propolis extract on human keratinocyte (HaCaT) cells

Cell culture

HaCaT cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells between passage 10 and 15 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. Cultures were maintained at 37 °C in a humidified incubator (Thermo Fisher Scientific, USA) under 5% CO_2 and sub-cultured when reaching 80–95% confluence.

MTT assay

Cytotoxicity was assessed using the MTT assay. HaCaT cells were seeded into 96-well plates at a density of 1 × 10^4 cells/well and allowed to adhere overnight. Cells were then treated with extracts at concentrations ranging from 7.81 to 1000 µg/mL for 24 hours. MTT solution (0.5 mg/mL) was added and incubated for 4 hours. The resulting formazan crystals were solubilized with DMSO, and absorbance was measured at 570 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Vehicle-treated cells (0.5% DMSO) served as negative controls. Cell viability (%) was calculated as:

$$\text{Cell viability (\%)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Dose–response curves were generated, and IC_{50} values were determined by non-linear regression.

Statistical Analysis

All analytical assays were performed in triplicate as technical replicates and data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test using GraphPad Prism version 7.0 (GraphPad Software, CA, USA), with $P < 0.05$ considered statistically significant. Recoverable extract yield was determined from a single extraction for each extraction medium and is therefore presented descriptively.

Results and Discussion

Recoverable extract yield, total phenolic and flavonoid contents

The percentage of recoverable extract yields varied according to the extraction medium (Table 1). Since all extracts underwent an identical post-extraction analytical clean-up prior to recovery determination, these values reflect the amount of analytically recoverable propolis constituents remaining after standardized sample preparation rather than the crude extraction yield alone. COP produced the highest recovery (10.00%), followed by SOP (9.50%) and EEP (8.50%). In contrast, OOP and WEP yielded lower amounts (7.10% each).

Table 1. The percentage of recoverable extract yield, total phenolic and flavonoid contents of propolis extracts.

Extracts	Recoverable extract yield (%)	Total phenolic content (mg GAE/g)	Total flavonoids content (mg QE/g)
COP	10.00	54.40 ± 0.01^{c}	46.55 ± 0.01^{c}
OOP	7.10	33.22 ± 0.03^{b}	36.98 ± 0.01^{b}
SOP	9.50	27.73 ± 0.02^{b}	29.40 ± 0.01^{b}
EEP	8.50	53.50 ± 0.03^{c}	66.54 ± 0.02^{d}
WEP	7.10	13.87 ± 0.04^{a}	10.06 ± 0.01^{a}

Values are reported as mean ± SD. GAE: Gallic acid equivalent; QE: Quercetin equivalent. Different letters indicate significant differences among the tested extracts ($p < 0.05$).

Coconut oil's strong performance is likely due to its high concentration of MCT. These compounds have been shown to improve the solubility of lipophilic phytochemicals and maintain oxidative stability during extraction [16]. SOP produced an intermediate recoverable extract yield (9.50%), suggesting that sunflower oil was less effective than coconut oil but more effective than water and olive oil under the present extraction conditions. EEP produced a comparable recoverable yield (8.50%), consistent with the well-established ability of aqueous ethanol to extract a broad spectrum of phenolic and moderately

lipophilic constituents from propolis [11]. In contrast, the lower yields for OOP and WEP (7.10% each) likely reflect poor compatibility with the resinous matrix of propolis. Olive oil's long-chain monounsaturated fatty acids do not dissolve these compounds as effectively as coconut oil's MCT [17]. Meanwhile, water's high polarity limits its ability to dissolve the largely non-polar resin and wax fractions that make up the bulk of propolis [11]. As the present work was based on single-batch extractions, no statistical variation is reported, and the values should be interpreted as indicative rather than conclusive.

Phytochemical quantification further highlighted the efficiency of COP and EEP. COP recorded the highest TPC (54.40 mg GAE/g), which was comparable to EEP (53.50 mg GAE/g) but significantly higher than OOP, SOP, and WEP ($p < 0.05$). For TFC, EEP exhibited the highest concentration (66.54 mg QE/g), significantly exceeding COP (46.55 mg QE/g). though both were significantly higher than the remaining groups ($p < 0.05$). Conversely, WEP produced the lowest TPC (13.87 mg GAE/g) and TFC (10.06 mg QE/g), indicating its limited ability to recover bioactive compounds.

Parallel to the yield trends observed earlier, the high MCT content in coconut oil accounts for COP's high TPC, as these triglycerides dissolve phenolic compounds [16]. Ethanol is typically used as the standard solvent for propolis because its intermediate polarity extracts both phenolic acids and flavonoids [11,12]. In this context, the similar TPC values between COP and EEP indicate that coconut oil, despite its lower polarity, can recover phenolic constituents as effectively as ethanol. Furthermore, COP's higher TFC compared to OOP, SOP, and WEP likely reflects the lipophilic nature of propolis flavonoids, which dissolve more readily in coconut oil than in water or the other oils [11]. While EEP retained the highest overall TFC due to ethanol's efficiency in extracting a wide range of flavonoids [11,12], these findings demonstrate that coconut oil is a viable alternative capable of recovering a substantial portion of these compounds.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC–MS profiling identified 22 chemical constituents across the five extracts, comprising fatty acids, esters, aldehydes, hydrocarbons, a ketone, a furanone, and phenolic derivatives (Table 2). Five compounds (1-hexadecene, methyl palmitate, methyl stearate, palmitic acid, and stearic acid) were consistently detected in COP, OOP, SOP, and EEP but were absent from WEP, indicating preferential recovery by the oil-based and ethanolic extraction media.

Table 2. Chemical compounds identified in propolis extracts using GC-MS.

No.	Compounds	COP	OOP	SOP	EEP	WEP
1	1-Hexadecene	✓	✓	✓	✓	
2	2,4-Di-*tert*-butylphenol	✓			✓	✓
3	2-Heptadecanone	✓			✓	
4	2-Methoxy-4-vinylphenol	✓			✓	✓
5	2-Tetradecene, *(E)*-	✓		✓	✓	
6	3-Tetradecene, *(E)*-				✓	
7	4-Oxononanal		✓		✓	✓
8	5-Pentyl-2(5H)-furanone		✓			✓
9	Caprylic acid	✓				
10	Cyclopentadecane		✓		✓	
11	Heptadecane			✓	✓	
12	Lauric acid	✓				
13	Lauric aldehyde	✓	✓	✓		
14	Methyl caprate	✓				
15	Methyl myristate	✓				
16	Methyl palmitate	✓	✓	✓	✓	
17	Methyl stearate	✓	✓	✓	✓	
18	Myristaldehyde	✓				
19	Palmitaldehyde		✓		✓	
20	Palmitic acid	✓	✓	✓	✓	
21	Stearaldehyde	✓			✓	
22	Stearic acid	✓	✓	✓	✓	

Several compounds detected in COP, including caprylic acid, lauric acid, lauric aldehyde, methyl caprate, methyl myristate, and myristaldehyde, are well-recognized constituents of coconut oil and therefore likely reflect the chemical contribution of the extraction medium. Accordingly, the GC–MS profile represents the combined composition of the oil–propolis extract rather than propolis alone. Caprylic acid, lauric acid, methyl caprate, methyl myristate, and myristaldehyde were detected exclusively in COP, whereas lauric aldehyde was also present in OOP and SOP. These medium-chain fatty acid derivatives have been associated with antimicrobial and anti-inflammatory activities [29]. COP also contained the phenolic derivatives 2,4-di-tert-butylphenol and 2-methoxy-4-vinylphenol, both reported to possess antioxidant and radical-scavenging activities [30,32,33]. Their detection in COP and EEP is consistent with the higher TPC values of these extracts, whereas their presence in WEP suggests that water recovered only limited amounts of selected low-molecular-weight phenolic constituents despite its lower extraction efficiency.

EEP exhibited the second-highest number of detected compounds (15) and the broadest chemical diversity, comprising hydrocarbons, aldehydes, esters, phenolic derivatives, and a ketone, consistent with ethanol's well-established ability to extract a broad range of propolis constituents [11,12]. In contrast, OOP and SOP yielded narrower chemical profiles dominated by hydrocarbons, esters, fatty acids, and aldehydes, whereas WEP produced the fewest detectable compounds, consistent with its lower extract yield and TPC/TFC values.

To further characterize the phytochemical composition of the COP, the retention time (RT), molecular weight (MW), and molecular formula (MF) of the compounds identified by GC–MS are summarized in Table 3. The corresponding chemical structures of the major identified compounds are presented in Figure 1.

Table 3. Detailed GC–MS identification of compounds detected in coconut oil propolis extract (COP)

No.	Compounds	RT (min)	MW (g/mol)	Molecular Formula
1	1-Hexadecene	40.672	224	$C_{16}H_{32}$
2	2,4-Di-*tert*-butylphenol	37.867	206	$C_{14}H_{22}O$
3	2-Heptadecanone	51.637	254	$C_{17}H_{34}O$
4	2-Methoxy-4-vinylphenol	29.099	150	$C_9H_{10}O_2$
5	2-Tetradecene, *(E)*-	32.501	196	$C_{14}H_{28}$
6	Caprylic acid	23.130	144	$C_8H_{16}O_2$
7	Lauric acid	40.038	200	$C_{12}H_{24}O_2$
8	Lauric aldehyde	33.268	184	$C_{12}H_{24}O$
9	Methyl caprate	29.568	186	$C_{11}H_{22}O_2$
10	Methyl myristate	45.623	242	$C_{15}H_{30}O_2$
11	Methyl palmitate	52.445	270	$C_{17}H_{34}O_2$
12	Methyl stearate	58.661	298	$C_{19}H_{38}O_2$
13	Myristaldehyde	41.471	212	$C_{14}H_{28}O$
14	Palmitic acid	52.445	256	$C_{16}H_{32}O_2$
15	Stearaldehyde	45.222	268	$C_{18}H_{36}O$
16	Stearic acid	58.661	284	$C_{18}H_{36}O_2$

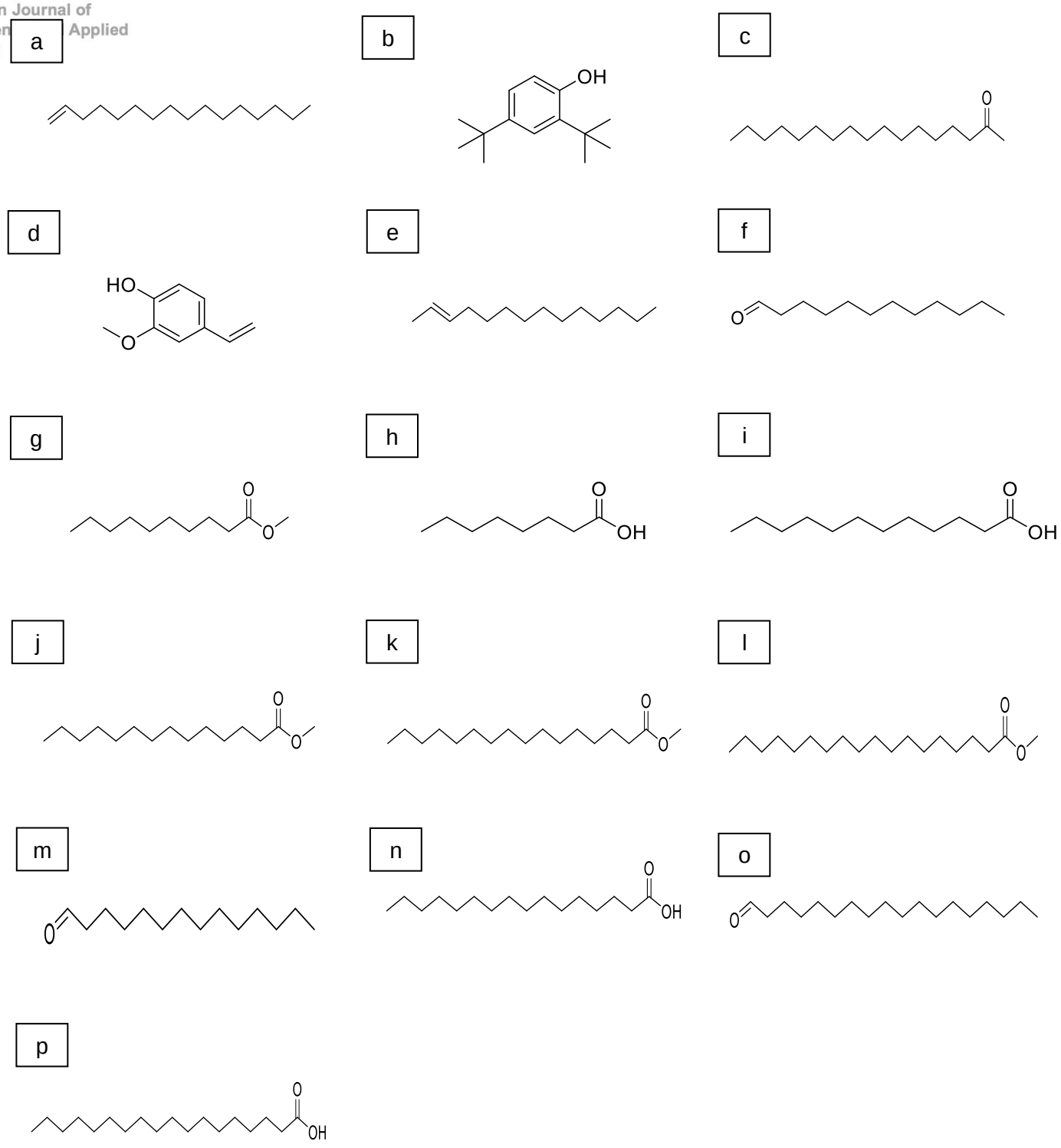

Figure 1. Chemical structure of 16 major compounds identified in coconut oil propolis extract (COP) based on GC–MS analysis. (a) 1-Hexadecene, (b) 2,4-Di-tert-butylphenol, (c) 2-Heptadecanone, (d) 2-Methoxy-4-vinylphenol, (e) 2-Tetradecene, (E)-, (f) Caprylic acid, (g) Lauric acid, (h) Lauric aldehyde, (i) Methyl caprate, (j) Methyl myristate, (k) Methyl palmitate, (l) Methyl stearate, (m) Myristaldehyde, (n) Palmitic acid, (o) Stearaldehyde, (p) Stearic acid.

Antioxidant activity

The antioxidant capacity of the extracts at 1000 µg/mL is summarized in Table 4. The DPPH assay evaluated the free radical scavenging capacity of the extracts by measuring their ability to donate hydrogen atoms or electrons to reduce the stable DPPH• radical [34]. COP exhibited the highest radical scavenging activity (50.85 ± 8.70%), significantly greater than EEP (39.25 ± 1.30%), OOP (26.64 ± 2.96%), SOP (7.12 ± 1.62%), and WEP (3.26 ± 2.29%) ($p < 0.05$). This observation is consistent with the higher TPC of COP and the presence of the phenolic derivatives 2,4-di-*tert*-butylphenol and 2-methoxy-4-vinylphenol identified by GC–MS. Coconut oil has also been reported to enhance the solubility of phenolic constituents, which may contribute to the observed antioxidant activity [17,35]. In contrast, the long-chain triglyceride matrices of olive and sunflower oils may limit the recovery of hydrogen-donating phenolics, resulting in comparatively lower activity [16].

Table 4. Antioxidant activities of propolis extracts at 1000 µg/mL.

Extracts	DPPH inhibition (%)	ABTS inhibition (%)	FRAP values (µM Fe^{2+})
COP	50.85 ± 8.70^{c}	26.60 ± 2.88^{a,b}	659.00 ± 0.06^{d}
OOP	26.64 ± 2.96^{b}	17.89 ± 4.31^{a}	372.47 ± 0.02^{c}
SOP	7.12 ± 1.62^{a}	24.14 ± 2.13^{a}	153.00 ± 0.01^{b}
EEP	39.25 ± 1.30^{b}	33.13 ± 4.48^{b}	597.37 ± 0.06^{d}
WEP	3.26 ± 2.29^{a}	12.35 ± 2.94^{a}	51.20 ± 0.01^{a}
Trolox	90.91 ± 0.05^{d}	70.50 ± 13.36^{c}	4883.20 ± 0.09^{e}

Values are reported as mean ± SD. DPPH:2,2-diphenyl-1-picrylhydrazyl; ABTS:2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric reducing antioxidant power. Different letters indicate significant differences among the tested extracts ($p<0.05$).

In the ABTS assay, EEP demonstrated the highest inhibition (33.13 ± 4.48%), although the value was not significantly different from COP (26.60 ± 2.88%) ($p > 0.05$). SOP (24.14 ± 2.13%), OOP (17.89 ± 4.31%), and WEP (12.35 ± 2.94%) were significantly lower compared with EEP ($p < 0.05$). In contrast to the DPPH assay, which is more responsive to lipophilic hydrogen-donating antioxidants [34], the ABTS assay detects both hydrophilic and lipophilic antioxidant species [36]. The comparable ABTS activities observed for COP and EEP therefore suggest that although coconut oil efficiently recovered lipophilic antioxidant constituents contributing to DPPH scavenging, ethanol extracted a broader spectrum of both hydrophilic and lipophilic phenolic compounds, resulting in similar overall ABTS radical scavenging capacity. This difference highlights that antioxidant activity depends not only on total phenolic content but also on the chemical composition and polarity of the extracted antioxidants [34,36,37].

The FRAP assay measured the electron-donating capacity of the extracts, as reflected by their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions [38]. Similarly, COP exhibited the highest ferric reducing power (659.00 ± 0.06 µM Fe^{2+}), which was comparable to EEP (597.37 ± 0.06 µM Fe^{2+}), while both extracts showed significantly greater reducing capacity than OOP, SOP, and WEP ($p < 0.05$). These findings are consistent with their higher TPC values, supporting the contribution of phenolic constituents to electron transfer-based antioxidant activity [11,12,15]. Overall, the antioxidant activity of COP was in agreement with its higher recoverable extract yield, elevated phenolic content, and the presence of antioxidant-associated phenolic derivatives identified by GC–MS.

Cytotoxicity

The cytotoxic effects of the extracts on HaCaT cells are presented in Table 5. All extracts exhibited IC_{50} values above 600 µg/mL, indicating low cytotoxicity [39,40]. COP (801.87 µg/mL) and SOP (797.09 µg/mL) recorded higher IC_{50} values than EEP (642.94 µg/mL) and OOP (656.49 µg/mL), whereas WEP exhibited the highest IC_{50} value (6983.02 µg/mL), indicating the lowest cytotoxicity. Nevertheless, all extracts remained within the non-cytotoxic range, indicating acceptable cytocompatibility toward HaCaT cells. Although WEP demonstrated the lowest cytotoxicity, its low recoverable extract yield, reduced TPC and TFC values, and limited antioxidant activity reduce its suitability as an extraction medium.

Table 5. Cytotoxicity of propolis extracts towards HaCaT cells.

Extracts	IC_{50} (µg/mL)
COP	801.87 ± 38.45[b]
OOP	656.49 ± 34.82[a]
SOP	797.09 ± 10.47[b]
EEP	642.94 ± 42.17[a]
WEP	6983.02 ± 151.23[c]

The present findings indicate that COP provides antioxidant activity comparable to EEP while maintaining acceptable cytocompatibility. While Ferreira et al. [37] reported lower antioxidant recovery in oil-based extracts compared with ethanol, our findings indicate that coconut oil can achieve comparable antioxidant activity, suggesting that extraction efficiency is influenced by both oil type and experimental conditions. Collectively, the present study supports the potential of coconut oil as a cytocompatible medium for cosmetic and dermatological applications.

Conclusions

This study demonstrated that the choice of extraction medium influenced the recoverable extract yield, phytochemical composition, antioxidant activity, and cytocompatibility of *G. thoracica* propolis. Among the extraction media evaluated, COP achieved the highest recoverable extract yield, the highest phenolic content, the strongest radical scavenging activity, and the greatest ferric reducing power, while remaining non-cytotoxic toward HaCaT cells. GC–MS profiling further identified phenolic derivatives and medium-chain fatty acid-related constituents in COP that may contribute to these effects. Collectively, these findings suggest that coconut oil is a promising plant-based extraction medium for *G. thoracica* propolis, with potential application in topical and dermatological product development. As this study was based on single-batch extractions, the findings should be regarded as preliminary. Further validation using larger, replicated sample sets, comprehensive chemical profiling, and additional skin-relevant bioassays are warranted to strengthen translational relevance and inform future product development.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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