GC-MS analysis of phytocomponents in the ethanolic extract of Propolis of stingless Bee

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ABSTRACT

Propolis, a resinous product derived from beehives, has been utilized in folk medicine across various cultures for over 3,000 years. Stingless bees, native pollinators of valuable medicinal plants, produce propolis as a highly adhesive, natural mixture consisting of bee secretions and plant resins. The pharmacological properties of stingless bee propolis are broad and varied, influenced by factors such as geographical location, climate, bee species, and the availability of botanical resources for foraging. The objectives of this study were twofold: first, to determine the chemical compounds present in the ethanolic extract of stingless bee propolis collected from the Community Enterprise for Stingless Bee Rearing and Propagation in Ban Khlong Don, Thailand, using gas chromatography-mass spectrometry (GC-MS); and second, to investigate the antioxidative and anti-inflammatory potential of stingless bee propolis. For GC-MS analysis, the injector temperature was set to 280 °C in splitless mode. The oven temperature was initially maintained at 60 °C for 4 minutes, increased to 150 °C at a rate of 10 °C/min for 15 minutes, and finally raised to 310 °C. Mass spectrometry parameters included a source temperature of 280 °C, transfer temperature of 150 °C, a solvent delay of 2 minutes, and a scan range of 35–500 Da, with a total GC run time of 40.5 minutes. Phytochemical compounds were identified by comparing their mass spectra with entries in the National Institute of Standards and Technology (NIST) library. Five major compounds—Ethyl oleate, Fumaric acid, (R)-(-)-14-Methyl-8-hexane-1-ol, Phenol, and Squalene—were identified, each showing significant pharmacological activities, particularly in antioxidative and anti-inflammatory responses.

Keyword: Gas chromatography-mass spectrometry (GC-MS), Stingless bee extract., Suan Sunandha Rajabhat University. , Tetragonula pegdeni,Anti-oxidant,Anti-inflammatory.

1. Introduction

Stingless bees are insects that play a crucial role in ecosystems, particularly in pollinating various plants such as durian, mango, and other tropical fruit and flowering plants, which positively impacts agricultural quality and yields (Slaa et al., 2006; Heard, 1999). Stingless bees differ from other bee species in that they do not have a stinger, are smaller in size, and cannot produce venom. Instead, stingless bees use biting as a defense mechanism against threats, making them safe to farm and widely applicable in agriculture in Thailand (Michener, 2000; Inoue et al., 1985).

Propolis is an important product produced by stingless bees, made from flower pollen, nectar, and secretions. Its main component is resin, which is used for nest repair and has attracted significant research attention for its biological and medicinal properties (Bankova et al., 2000). Propolis contains a variety of chemical compounds, including flavonoids, phenolic acids, and terpenes, which have antioxidant, antibacterial, antifungal, and antiviral properties (Banskota et al., 2001; Marcucci, 1995). Research has found that flavonoids in propolis, such as galangin, pinocembrin, and pinostrobin, exhibit potent antimicrobial effects and stimulate the immune system (Dimov et al., 1992). Additionally, ferulic acid and caffeic acid are key compounds that demonstrate antimicrobial effects, playing a significant role in preventing skin diseases and inflammatory wounds (Debuyser, 1983; Ghisalberti, 1979).

The composition of stingless bee propolis varies based on environmental factors and the types of plants present in each area, resulting in propolis with distinct characteristics in terms of color, scent, taste, and biological properties (Barth et al., 2011; Souza et al., 2007). Thailand, with its rich plant diversity and favorable climate for stingless bee farming, is a significant source of propolis with high potential for commercial use, especially in international markets where there is demand for natural, environmentally friendly products (Boorn et al., 2010).

However, the production of propolis in Thailand still lacks comprehensive research and clear standards, leading to variations in product quality. This research, therefore, focuses on studying the chemical composition of propolis from stingless bees in Thailand using Gas Chromatography-Mass Spectrometry (GC-MS) to analyze key compounds and evaluate beneficial biological properties. The findings of this study will help improve the quality and standards of stingless bee products, enabling them to compete in the global market (Greenaway et al., 1990; Burdock, 1998).

The objective was to

1. determine the chemical compounds present in the ethanolic extract of stingless bee propolis collected from the Community Enterprise for Stingless Bee Rearing and Propagation in Ban Khlong Don, Thailand, using gas chromatography-mass spectrometry (GC-MS)
2. investigate the antioxidative and anti-inflammatory potential of stingless bee propolis.

2. Methodology

1. Propolis Extraction Using Crude Ethanol Extract

In this study, propolis was extracted using a 95% ethanol solution at a ratio of 1:10 (300 grams of propolis to 3,000 µL/g of ethanol). The propolis was immersed in ethanol for three days, after which the resulting solution was filtered through filter paper. The filtered extract was then centrifuged at 7,000 rpm at 4°C for 15 minutes to precipitate any solids. The supernatant was concentrated to dryness using a rotary evaporator to yield a crude ethanol extract of propolis. The final extract was weighed and stored in a dark environment at 4°C. The extraction yield (% yield) was subsequently calculated. The antioxidant activity of the extract was assessed using the DPPH assay, which measures radical scavenging activity using the reagent 2,2-Diphenyl-1-picrylhydrazyl. Additionally, the total phenolic content of the extract was determined using the Folin-Ciocalteu reagent. The chemical composition of the propolis sample was analyzed using gas chromatography-mass spectrometry (GC-MS).

2.Analysis of Propolis Sample Using Gas Chromatography-Mass Spectrometry (GC-MS)

For GC-MS analysis, the injector temperature was set to 280 °C in splitless mode. The oven temperature was initially maintained at 60 °C for 4 minutes, increased to 150 °C at a rate of 10 °C/min for 15 minutes, and finally raised to 310 °C. Mass spectrometry parameters included a source temperature of 280 °C, transfer temperature of 150 °C, a solvent delay of 2 minutes, and a scan range of 35–500 Da, with a total GC run time of 40.5 minutes. Phytochemical compounds were identified by comparing their mass spectra with entries in the National Institute of Standards and Technology (NIST) library.

3. Results

The GC-MS analysis of the stingless bee propolis extract revealed the presence of several bioactive compounds with potential health benefits. Five major components were identified in the ethanol extract based on their retention times and mass spectra. Ethyl oleate was detected at a retention time (RT) of 37.251 minutes, accounting for 31.36% of the total area, and is known for its anti-inflammatory and antimicrobial properties. (R)-(-)-14-Methyl-8-hexadecyn-1-ol was observed at RT 39.441 minutes, comprising 15.96% of the area, which may contribute to the biological activities of the extract Fumaric acid, dodecyl 3-methylphenyl ester appeared at RT 40.435 minutes with 19.83% of the area and is recognized for its antioxidant potential. Phenol, 3-heptadecyl- was identified at RT 42.535 minutes, making up 14.29% of the area, known for its antioxidant properties essential in neutralizing free radicals. Finally, Squalene was detected at RT 43.495 minutes, constituting 19.09% of the area, a compound widely acknowledged for its role as a natural antioxidant and precursor in sterol biosynthesis.

Table 1. GC-MS spectral analysis of ethanolic extract of *Tetragonula pagdeni*

Compound	Retention Time (min)	Area %	Potential Activity
Ethyl Oleate	37.251	18.68	Antimicrobial, anti-inflammatory
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	39.441	15.96	Bioactive properties
Fumaric Acid, Dodecyl 3-methylphenyl Ester	40.435	29.30	Antioxidant, anti-inflammatory
Phenol, 3-heptadecyl-	42.535	16.97	Antioxidant
Squalene	43.495	19.08	Antioxidant, sterol precursor

The GC-MS analysis of the ethanolic extract of *Tetragonula pagdeni* propolis identifies five primary compounds, each with significant bioactive potential. These compounds, as highlighted in the table, include Ethyl Oleate, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Fumaric Acid Dodecyl 3-methylphenyl Ester, Phenol 3-heptadecyl, and Squalene. Their retention times, area percentages, and potential activities suggest diverse therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, and sterol precursor functions.

Antimicrobial and Anti-inflammatory. Properties Ethyl Oleate, which constitutes 18.68% of the extract, is known for its antimicrobial and anti-inflammatory properties. Studies show that Ethyl Oleate has demonstrated effectiveness against various microbial strains and can reduce inflammation by modulating immune responses (Chen et al., 2017). Its presence in *Tetragonula pagdeni* propolis aligns with previous findings on the antimicrobial properties of bee propolis (Silva-Carvalho et al., 2015).

Bioactive Properties of Alkanols. The compound (R)-(-)-14-Methyl-8-hexadecyn-1-ol, with 15.96% area, exhibits bioactive properties. Alkanols such as this compound are known to possess lipid-soluble properties that aid in cellular interactions and may contribute to the overall bioactivity of the extract (Medeiros et al., 2018).

Antioxidant Activity. Fumaric Acid Dodecyl 3-methylphenyl Ester and Phenol 3-heptadecyl, constituting 29.30% and 16.97% of the extract respectively, show significant antioxidant potential. Antioxidants in propolis have been shown to neutralize free radicals, which can protect cells from oxidative stress (Ahn et al., 2007). This aligns with the function of Fumaric Acid derivatives as potent antioxidants in natural products (Yang et al., 2013).

Squalene as a Sterol Precursor Squalene, representing 19.08% of the extract, serves as a precursor for sterol synthesis, which is crucial for cell membrane integrity and function. Additionally, squalene's antioxidant capacity supports the health benefits of Tetragonula pagdeni propolis, as it has been found effective in skin barrier improvement and cellular protection (Kim et al., 2015).

The compounds identified in Tetragonula pagdeni propolis ethanolic extract exhibit notable bioactivities, especially antioxidant and anti-inflammatory properties, which are consistent with the therapeutic effects traditionally associated with propolis. These findings contribute to understanding the biochemical basis of the health benefits of stingless bee propolis and align with previous studies emphasizing its role in natural medicine and potential applications in pharmaceutical products.

4. CONCLUSION AND FUTURE WORK

The GC-MS analysis of the ethanol extract from stingless bee propolis revealed several key bioactive compounds, notably Ethyl oleate, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Fumaric acid (dodecyl 3-methylphenyl ester), Phenol (3-heptadecyl-), and Squalene. These compounds support the traditional medicinal uses of propolis due to their known bioactivities.

Ethyl oleate is widely used for its anti-inflammatory properties and as a non-toxic solvent, indicating potential in drug formulations. (R)-(-)-14-Methyl-8-hexadecyn-1-ol has demonstrated antimicrobial activity, which may contribute to propolis's traditional infection-fighting applications. Fumaric acid (dodecyl 3-methylphenyl ester) shows antioxidant properties, supporting its use in managing oxidative stress. Phenol (3-heptadecyl-) acts as an antimicrobial agent, useful in natural preservative applications. Lastly, Squalene is an antioxidant and skin-protective compound, indicating propolis's suitability for skincare and anti-aging products.

In summary, the identified compounds highlight the therapeutic potential of stingless bee propolis in pharmaceuticals and cosmetics. Further isolation and testing of these compounds could optimize their use in health products.

In future product development, we aim to create a range of innovative skincare solutions, including a nourishing sleeping mask, a convenient cleansing stick, and a protective sunscreen stick. Each product will be crafted with high-quality, bioactive ingredients to ensure effectiveness and ease of use. These formulations will be designed to meet the needs of diverse skin types, delivering targeted benefits through thoughtful, user-friendly packaging. As we expand our product line, we will continue to prioritize natural, skin-beneficial ingredients and sustainable practices, enhancing our commitment to effective, everyday skincare.

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