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Comprehensive Insights into Malaysian Stingless Bee Honey: Biodiversity, Therapeutic Benefits, and Analytical Approaches

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Stingless bee honey (SBH), produced by diverse species of stingless bees (*Meliponini*), has gained significant attention for its unique chemical composition and therapeutic properties. Unlike honey produced by traditional honeybees, SBH contains higher concentrations of nutrients, minerals, and bioactive compounds, offering potential health benefits including anti- inflammatory, antimicrobial, antioxidant, antidiabetic, and wound-healing properties. This review presents comprehensive insights into the biodiversity, therapeutic benefits, and chemical constituents of Malaysian SBH, with a focus on its ecological and medicinal value. The most commonly farmed species in Malaysia, such as *Heterotrigona itama* and *Geniotrigona thoracica*, are highlighted for their roles in honey production and their distinct bioactive profiles. Additionally, this paper explores various analytical methods employed for SBH authentication, including physicochemical analysis, chromatographic techniques, spectroscopic methods, and DNA-based approaches. Emerging technologies, such as electronic sensors and artificial intelligence, are also discussed for their potential to improve the efficiency and accuracy of SBH quality control and discrimination. This review serves as a valuable resource for researchers and industry stakeholders, encouraging further studies into SBH's potential applications in healthcare and the food industry.

Keywords: Stingless bee honey (SBH); therapeutic properties; biodiversity; chemical constituents; authentication methods

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Honey, a widely cherished natural sweetener, is defined by the Codex Alimentarius (2001) as a "natural sweet substance produced by honeybees". These bees process nectar from plants or other botanical secretions by combining it with enzymes, depositing it in honeycombs, and dehydrating it to allow ripening [1]. Among the diverse groups of bees, stingless bees (*Meliponini*) are particularly significant, comprising approximately 605 species worldwide, making them the most diverse group of corbiculate bees. This clade also includes honeybees (*Apini*), bumble bees (*Bombini*), and orchid bees (*Euglossini*) [2, 3].

Stingless bees have been culturally and ecologically significant, particularly in tropical regions, where they play an integral role in pollination and honey production. These bees range in size from 2 to 14 mm and exhibit varied adaptations to their habitats. In Malaysia, commonly observed stingless bee species include *Heterotrigona itama*, *Geniotrigona thoracica*, *Tetragonula apicalis*, and *Lepidotrigona terminata*, among others [4]. Their honey, often referred to as stingless bee honey (SBH), is highly valued for its unique nutritional and medicinal properties.

Meliponiculture, the farming of stingless bees, is an emerging industry in Malaysia, celebrated for its simplicity and manageable practices compared to traditional apiculture. The industry gained momentum following research by the Malaysian Agricultural Research and Development Institute (MARDI) in 2007 and subsequent workshops, such as those conducted by Universiti Sains Malaysia in 2010 [5, 6]. Initially practiced on a small scale, meliponid-culture expanded significantly due to the growing recognition of SBH's benefits and its byproducts, such as bee bread and propolis, which exhibit high economic potential.

Stingless bee honey differs significantly from honeybee honey, particularly in its chemical composition. The smaller size of stingless bees allows them to access a diverse range of floral sources, enriching the honey with polyphenols and other bioactive compounds. SBH contains higher concentrations of nutrients and minerals but also has higher moisture content, distinct sugar profiles, and susceptibility to adulteration, necessitating advanced analytical methods for its authentication [7].

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Numerous studies highlight the therapeutic benefits of SBH, including its anti-inflammatory, antimicrobial, antibacterial, antidiabetic, and wound-healing properties, largely attributed to its flavonoids and phenolic content [8]. This review aims to provide a comprehensive exploration of Malaysian stingless bee honey, including its biodiversity, therapeutic benefits, and chemical constituents. Additionally, it evaluates various analytical methods for SBH discrimination, such as physicochemical analyses, chromatographic techniques, spectroscopic methods, DNA-based approaches, and emerging technologies, offering a valuable reference for researchers and supporting its industrial and healthcare applications.

METHODOLOGY

A literature search covering 26 years of publications (1998 to 2024) was conducted across three key databases which were ScienceDirect, Scopus, and Google Scholar as well as Google Search. Only English-language articles were selected. The scientific names of the stingless bee species were verified using the Malaysia Biodiversity Information System (MyBIS). For the chemical structures of naturally occurring metabolites previously identified in *Geniotrigona thoracica* and *Heterotrigona itama*, ChemDraw Professional v.17.1 software was used.

The search combined the keywords "*Geniotrigona thoracica*" and "*Heterotrigona itama*" with terms such as "medicinal", "traditional", "ethnomedicinal", "pharmacological" and "phytochemical". To create a thorough profile of the biological and pharmacological properties of these two stingless bee species, additional terms, including "antioxidant", "antimicrobial", "antibacterial", "anti-diabetic", "anti-cancer", "anti-inflammatory", "anti-proliferative" and "stingless bee honey method discrimination" were also used. This search yielded around 469 articles from Google Scholar, Science-Direct, and Scopus up to October 2024. A total of 51 articles were used in this review paper.

DISCUSSION

Malaysian Common Stingless Honeybee Description and Taxonomy

The most common stingless bee species in Malaysia is *Heterotrigona itama*, which is highly valued for its adaptability to the tropical Malaysian climate and widespread cultivation for honey production and crop pollination [9]. This species is popular among Malaysian beekeepers due to its large population, ease of management, and high honey yield. *H. itama* honey is renowned for its unique medicinal and nutritional properties, with studies highlighting its potential health benefits, such as antioxidant and antimicrobial activity [10].

In contrast, *Geniotrigona thoracica* is particularly prized for its medicinal qualities, including notable antioxidant effects [11]. Its honey, which is rich and slightly acidic, contains high levels of polyphenols, making it especially suitable for therapeutic applications [12]. Other important stingless bee species in Malaysia include *Tetragonula laeviceps*, *Tetragonula fuscobalteata*, and *Lepidotrigona terminata*, which also contribute to honey production and pollination. However, *Heterotrigona itama* remains the most widely farmed and recognized species in the region, solidifying its status as a cornerstone of Malaysia's stingless bee industry [13]. Plate 1 is the morphological and Table 1 is a comparison of the key morphological traits of five notable Malaysian stingless bee species. Each species' unique morphology not only aids in taxonomic classification but also has implications for their adaptability, behavior, and interactions with their environment [14].

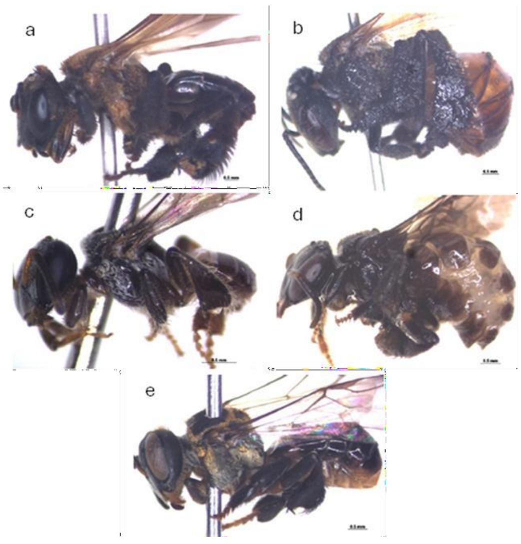


Figure 1. Stingless bee species in Malaysia; a) *Geniotrigona thoracica*, b) *Heterotrigona itama*, c) *Tetragonula fuscobalteata*, d) *Tetragonula laeviceps*, and e) *Lepidotrigona terminata* [14].

Table 1. Malaysian common stingless bee honey description.

Species	Body Length (mm)	Head Width (mm)	Head Length (mm)	Unique Features
Geniotrigona thoracica	8.12 - 8.65 (avg. 8.44)	3.20 - 3.39 (avg. 3.30)	2.58 - 2.64 (avg. 2.61)	Brown mesoscutum with two black vertical stripes; forewings 7.93 - 8.59 mm; black elongated hind tibiae (3.20 - 3.30 mm)
Heterotrigona itama	4.78 - 5.87 (avg. 5.58)	2.70 - 2.85 (avg. 2.78)	1.96 - 2.06 (avg. 2.02)	Commonly managed by beekeepers; pterostigma 0.85 - 0.90 mm; tibia length 2.00 - 2.20 mm, width 0.78 - 0.91 mm
Tetragonula fuscobalteata	3.16 - 3.33 (avg. 3.26)	1.46 – 1.52 (avg. 1.48)	0.73 – 0.76 (avg. 0.74)	Smallest stingless bee; black mesoscutum with alternating smooth and hairy bands; brown gastral tergite with pale yellow setae
Tetragonula laeviceps	4.24 - 4.37 (avg. 4.31)	1.40 – 1.58 (avg. 1.49)	0.70 - 0.79 (avg. 0.74)	Small head with sparse long setae; mandibles with two teeth; rounded wings with weak venation; dark brown abdomen
Lepidotrigona terminata	3.16 - 3.33 (avg. 3.26)	1.44 – 1.46 (avg. 1.45)	0.72 – 0.73 (avg. 0.72)	Black frons with white hair, distinguishable by frons color and patterning on mesoscutum

Medicinal Properties of Stingless Bee Honey in Malaysia

Stingless bee honey (SBH), commonly referred to as "kelulut honey" in Malaysia, has gained recognition for its unique physicochemical properties and exceptional medicinal potential. This honey is produced by various species of stingless bees, such as *Heterotrigona itama* and *Geniotrigona thoracica*, and is distinct from *Apis* honey due to its higher moisture content, tangy taste, and diverse bioactive compounds. The growing body of research underscores the therapeutic applications of SBH in Malaysia, making it a promising natural product in traditional and modern medicine.

Antioxidant Properties

SBH is a potent source of natural antioxidants, attributed to its high content of phenolic acids, flavonoids, and other phytochemicals. These compounds play a critical role in neutralizing free radicals, thereby reducing oxidative stress and preventing chronic diseases such as cancer, cardiovascular ailments, and neurodegenerative disorders [15]. Studies have shown that Malaysian SBH exhibits higher antioxidant activity compared to other types of honey, primarily due to its unique floral sources in tropical environments [16]. Several studies have demonstrated that stingless honeybees from various geographical regions exhibit significant but variable antioxidant activity. The biological properties of honey are largely attributed to the presence of polyphenols, particularly flavonoids. However, the combined presence of various compounds, including phenolic acids, flavonoids, ascorbic acid, catalase, and peroxidase, seems to substantially impact honey's antioxidant activity [17].

Khongkwanmueang (2020) reported that the antioxidant activity of honey is influenced by the quantity and types of phenolic and flavonoid compounds, as well as other components like proteins and minerals. Honey from the *T. laeviceps* species complex showed antioxidant activity, with EC50 values ranging from 15.70 to 52.07 mg/mL, and an average value of 20.10 ± 0.60 mg/mL. These results suggest that the antioxidant activity of the honey samples is closely related to the levels of phenolic and flavonoid compounds, indicating that these bioactive compounds play a crucial role in their antioxidant properties [18].

Antibacterial and Antimicrobial Effects

The antimicrobial properties of SBH are well-documented and are largely attributed to its low pH, high osmolarity, and the presence of bioactive compounds like hydrogen peroxide and flavonoids. Malaysian SBH has demonstrated effectiveness against a wide range of pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [19]. Its efficacy against antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), highlights its potential as an alternative therapeutic agent [20]. The anti-microbial properties of stingless bee honey (SBH) are well-documented, with researchers linking these effects to its botanical origins. SBH has demonstrated antibacterial activity against Gram-positive bacteria, including *Bacillus subtilis*, *Micrococcus luteus*, *B. megaterium*, and *B. brevis*, as well as Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas syringae* [21].

Additionally, a study by Boorn (2010) found that eleven varieties of stingless bee honey, including those from *Trigona carbonaria*, possess antimicrobial effects against various microorganisms collected from thirteen clinical samples, as well as standard reference strains [22]. Furthermore, *Trigona laeviceps*, a species native to Thailand, produces honey with antimicrobial properties effective against bacterial strains such as *E. coli* and *S. aureus*, the fungal strain *Aspergillus niger*, and two yeast types, *Auriobasidium pullulans* and *Candida albicans* [23]. A study by Zakaria (2020) shows that stingless bee honey demonstrated the highest antibacterial activity, showing significant inhibition against both *Escherichia coli* and *Staphylococcus aureus*, with greater effectiveness observed against *S. aureus*. The antimicrobial properties of honey are largely attributed to the production of hydrogen peroxide and the presence of phenolic acids, such as gallic, caffeic, benzoic, ferulic, and cinnamic acids, which play a crucial role in its antibacterial efficacy [17].

Wound Healing and Anti-inflammatory Properties

SBH has gained attention for its ability to promote wound healing and reduce inflammation. Its high moisture content, coupled with bioactive compounds like flavonoids, contributes to tissue repair and accelerates epithelialization [24]. A study on diabetic wound healing revealed that SBH significantly enhanced wound closure rates and reduced inflammatory markers, making it a promising treatment for chronic wounds [23]. It is thought to possess antioxidant, antibacterial, anti-inflammatory, and natural moisturizing properties that support the wound healing process. Key components such as its moisture content, water activity, pH, peroxide and non- peroxide activity, phenolic acids, flavonoids, vitamins, and enzymes likely contribute to its effectiveness in accelerating wound healing [5].

A previous in vitro study demonstrated that stingless bee honey effectively modulates inflammatory responses in lipopolysaccharide (LPS)-stimulated macrophages [25]. Treatment with stingless bee honey resulted in a 23.0% reduction in TNF-α levels and a 43.9% decrease in IL-6 secretion, alongside significant inhibition of interferon secretion, reaching up to 88.8%. These findings are consistent with prior in vivo research, where stingless bee honey reduced circulating levels of key inflammatory markers, including C-reactive protein (CRP), TNF-α, IL- 1β, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1). Additionally, it attenuated NF-κB and p38 MAPK signaling pathways in various tissues of LPS-induced rats. Beyond its anti-inflammatory effects, stingless bee honey was shown to enhance antioxidant defenses, further reinforcing its potential as a natural anti- inflammatory [26].

Antidiabetic Potential

The antidiabetic properties of SBH have been explored in several studies, with promising results. SBH has been shown to regulate blood glucose levels and improve insulin sensitivity, attributed to its low glycemic index and the presence of phenolic compounds that modulate glucose metabolism [27]. Research also indicates that SBH can reduce oxidative stress and inflammation associated with diabetes, further supporting its use as a complementary therapy. Researchers have explored the potential of stingless bee honey from the *Geniotrigona thoracica* species, which is native to the tropical regions of Southeast Asia, to protect the pancreas and maintain metabolic profiles in diabetic conditions. Aziz et al. (2017) [28] discovered that this honey holds significant promise as a protective agent against pancreatic damage and dysfunction. When administered to diabetic rats, *G. thoracica* honey prevented increases in fasting blood glucose, total cholesterol, triglycerides, and low-density lipoprotein levels. At the same time, it led to increases in high-density lipoprotein and serum insulin levels, while reducing histopathological changes and the expression of markers related to oxidative stress, inflammation, and apoptosis in pancreatic islets [29].

Gastroprotective Effects

SBH has demonstrated significant antiulcer effects in an ethanol-induced gastric ulcer model, as evidenced by macroscopic and microscopic evaluations. Severe diffuse hemorrhage was prominent in the positive control group due to vascular injury caused by absolute ethanol, whereas treatment groups showed only mild and localized hemorrhage. SBH treatment notably reduced the total ulcer area and ulcer index compared to the positive control group, highlighting its potential gastroprotective properties [30].

Immunomodulatory Effects

G. thoracica honey was shown to enhance the levels of the anti-inflammatory cytokine IL-13 by 163.1 ± 14.8% ($p < 0.05$), prompting further investigation into its immunomodulatory properties. This honey significantly reduced TLR4 expression by 59.3 ± 3.5% ($p < 0.001$) and increased mannose receptor levels by 67.3 ± 2.4% ($p < 0.001$). Additionally, it enhanced phagocytic activity by 25.0 ± 7.7% ($p < 0.01$) and decreased macrophage cell death by 32.1 ± 1.7% ($p < 0.001$). Collectively, these findings underscore the potential of stingless bee honey from *Melipona quadriafasciata* as an immunomodulatory agent, capable of attenuating pro-inflammatory markers and promoting anti-inflammatory responses in J774 cells [31].

Neuroprotective Benefits

Emerging research suggests that SBH may have neuroprotective properties, offering potential benefits in preventing or managing neurodegenerative diseases such as Alzheimer's and Parkinson's. The honey's antioxidant compounds, particularly phenolics, have been shown to protect neurons from oxidative damage and modulate signaling pathways involved in brain health [5].

A previous study investigating the impact of stingless bee honey (SBH) supplementation on the histological structure of rat hippocampi revealed its neuroprotective potential. SBH supplementation preserved the number of hippocampal pyramidal cells in the CA1 region, even following a high-carbohydrate, high-fat (HCHF) diet. These findings suggest that SBH may mitigate metabolic syndrome (MetS)-related alterations in the brain [32].

Research indicates that long-term dietary intake of honey, sucrose, or a sugar-free diet can influence anxiety and spatial memory in rats. Notably, honey-fed rats exhibited less reduction in spatial memory and showed decreased anxiety levels compared to those on other diets by the end of the study. Interestingly, rats on a sugar-free diet generally performed worse than honey-fed rats, suggesting that the cognitive and behavioral benefits observed in honey-fed rats were not solely attributable to the lower glycemic index (GI) of honey. These findings point to additional bioactive components in honey, such as antioxidants, that may contribute to its positive effects [33].

Anti-cancer Effects

Martinotti (2004) highlighted that cancer patients have increasingly embraced and become more informed about complementary and alternative medicine (CAM) practices. Among the various CAM modalities, honey has emerged as a significant natural product of interest. Produced by honeybees from nectar, honey holds a prominent position in alternative therapies due to its therapeutic properties and multifaceted health benefits [34].

Yaacob (2017) reviewed the cytotoxic effects of stingless bee honey on various cancer cell lines, revealing that it exhibits sensitivity to liver hepatocellular carcinoma (HepG2) and lung bronchus carcinoma (ChaGo-I) cell lines. However, the honey was found to be ineffective against colon carcinoma (SW620), human gastric carcinoma (KATO-III), and ductal carcinoma (BT474) cell lines. This suggests that stingless bee honey has differential effects depending on the type of cancer cell line. Further research into the cytotoxic activity of specific pure compounds derived from phenolic acids in honey, such as kaempferol, apigenin, caffeic acid phenethyl ester (CAPE), and naringenin, showed that these compounds exert cytotoxic effects on ChaGo-I, KATO-III, and BT474 cell lines, respectively [30]. This finding indicates that individual phenolic acid compounds in honey can be cytotoxic to certain cancer cells [35].

The effects of *H. itama* sp. honey on the morphology of U-87 MG cells were evaluated at concentrations of 0.625%, 1.25%, and 10% over 24, 48, and 72 hours of treatment. In untreated control cells, increased proliferation was observed up to 72 hours, accompanied by minimal cell detachment indicative of natural cell turnover. In contrast, cells treated with honey exhibited distinct morphological changes, including shrinkage and a rod-like appearance. After 72 hours, most cells rounded up and lost adhesion, consistent with apoptosis-induced cell death. The highest level of cell death was recorded at a honey concentration of 10%, highlighting its potential cytotoxic effect on U-87 MG cells [36].

The cytotoxicity of *Tetragonula itama* honey against HSC-2 cells was assessed using the MTT assay following treatments with various honey concentrations for 24, 48, and 72 hours. Untreated cells served as controls, demonstrating 100% cell proliferation. The results indicated a significant, dose-dependent inhibition of HSC-2 cell growth upon exposure to *T. itama* honey, highlighting its potential as an antiproliferative agent [37].

Chemical Constituents of Stingless Bee Honey

Stingless bee honey (SBH) is distinguished by its unique physicochemical properties and a rich profile of bioactive compounds, making it a valuable subject in the study of functional foods and natural therapeutics. Its composition, including reducing sugars, carotenoids, amino acids, and polyphenolic compounds, underpins its notable therapeutic effects, with detailed metabolites summarized in Table 2.

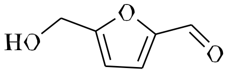
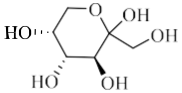
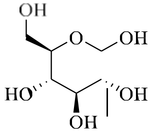
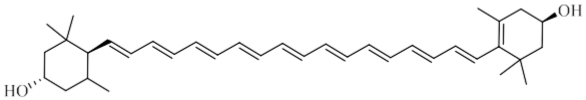
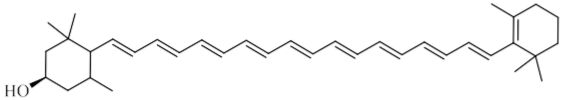
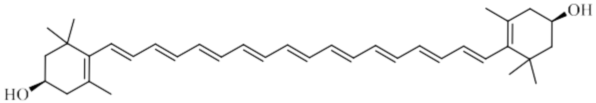
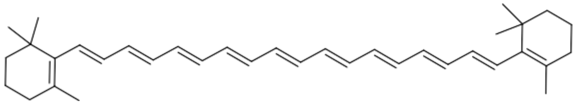
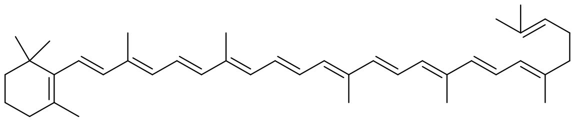
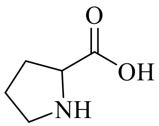
Julika (2020) reported that SBH contains lower levels of reducing sugars than Apis honey, despite its sweet flavor [38]. Hydroxymethylfurfural (HMF), an organic compound crucial for assessing honey freshness and aging, is present in varying levels depending on environmental and storage conditions [39]. SBH composition per 100 grams includes fructose (15.0–48.4 g), glucose (12.2–40.0 g), and minimal sucrose (<0.01–7.3 g). The combined fructose-glucose content ranges from 15.0–80.2 g, with a fructose/glucose ratio of 0.78–1.6 and a glucose/water (G/W) ratio of 0.47–1.89, contributing to its distinct taste and nutritional properties [38, 39].

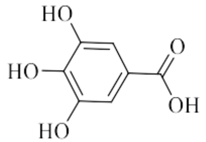
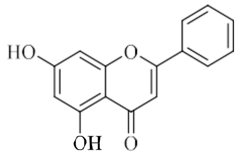
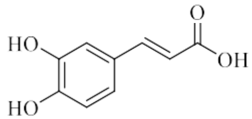
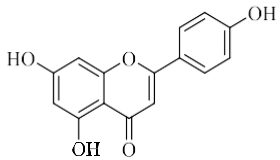
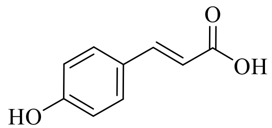
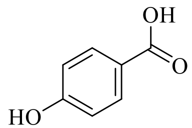
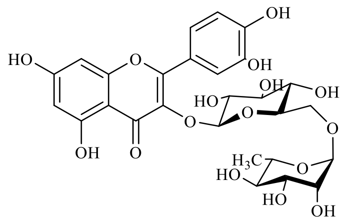
Carotenoids, including lutein, cryptoxanthin, zeaxanthin, β-carotene, and γ-carotene, were detected in SBH at varying concentrations. Habib et al. (2014) noted significant variability, with zeaxanthin showing the highest levels (up to 38.66 µg/100 g), while other carotenoids, such as lutein and β-carotene, were often below detectable limits. Honey sourced from arid regions generally exhibited higher carotenoid content, highlighting environmental influences on its composition [40].

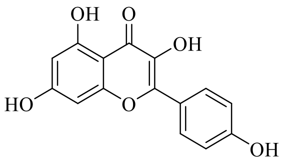
In a study by Hassan et al. (2023), three samples of *H. itama* honey harvested in 2018 exhibited proline levels below 180 mg/kg, potentially due to proline degradation during extended storage. In contrast, *T. apicalis* and *G. thoracica* honey from the same bee farm, also harvested in 2018, showed significantly higher proline levels exceeding 700 mg/kg. These results suggest that proline accumulation in stingless bee honey may be influenced by factors such as floral composition and geographical origin [41].

Additionally, the high phenolic and flavonoid content in stingless bee honey is attributed to polyphenolic compounds such as gallic acid, chrysin, caffeic acid, apigenin, *p*-coumaric acid, 4-hydroxybenzoic acid, rutin (quercetin-3-O-rutinoside), and kaempferol, which are known for their potent antioxidant properties [42].

Table 2. Metabolites from stingless bee honey.

Class Compound	Compound	Structure	Molecular Formula	Molecular Weight	References
Reducing Sugar	Hydroxymethylfurfural (HMF)		$C_6H_6O_3$	126.11	(Julika et. al., 2020)
	Fructose		$C_6H_{12}O$	180.16	(Julika et. al., 2020)
	Glucose		$C_6H_{12}O$	180.16	(Julika et. al., 2020)
Carotenoids	Lutein		$C_{40}H_{56}O_2$	568.87	(Habib et. al., 2024)
	Cryptoxanthin		$C_{40}H_{56}O_2$	552.85	(Habib et. al., 2024)
	Zeaxanthin		$C_{40}H_{56}O_2$	568.88	(Habib et. al., 2024)
	β-carotene		$C_{40}H_{56}$	536.87	(Habib et. al., 2024)
	γ-carotene		$C_{40}H_{56}$	536.88	(Habib et. al., 2024)
Amino Acids	Proline		$C_5H_9NO_2$	115.13	(Hassan et. al., 2023)

Polyphenol	Gallic acid		$C_7H_6O_5$	170.12	(Esa et. al., 2022)
	Chrysin		$C_{15}H_{10}O_4$	254.24	(Esa et. al., 2022)
	Caffeic acid		$C_9H_8O_4$	180.16	(Esa et. al., 2022)
	Apigenin		$C_{15}H_{10}O_5$	270.05	(Esa et. al., 2022)
	p-coumaric acid		$C_9H_8O_3$	164.05	(Esa et. al., 2022)
	4-hydroxybenzoic acid		$C_7H_6O_3$	138.12	(Esa et. al., 2022)
	Rutin		$C_{27}H_{30}O_{16}$	610.52	(Esa et. al., 2022)

Kaempferol		$C_{15}H_{10}O_6$	286.23	(Esa et. al., 2022)

Methods for Stingless Bee Honey Discrimination

Various methods were used for stingless bee honey discrimination, including physicochemical analyses, chromatographic techniques, spectroscopic methods, DNA-based approaches, and emerging technologies.

Physicochemical Analysis

Physicochemical analysis is a foundational approach for evaluating stingless bee honey's quality and authenticity. This method examines parameters such as moisture content, pH, electrical conductivity, free acidity, and sugar profile. Stingless bee honey is characterized by its high moisture content, often exceeding 30%, and notable acidity, both of which set it apart from Apis honey [16]. Additionally, the sugar composition dominated by fructose and glucose can be analyzed using High-Performance Liquid Chromatography (HPLC), which is particularly useful for distinguishing its botanical or geographical origin [43]. Despite being straightforward and effective in assessing general quality, physicochemical analysis may lack the sensitivity to detect adulteration or subtle compositional variations linked to specific floral sources.

Chromatographic Techniques

Chromatographic methods are widely utilized to provide detailed profiles of stingless bee honey's components, offering high sensitivity and specificity. Gas Chromatography-Mass Spectrometry (GC-MS) is particularly effective in identifying volatile organic compounds (VOCs), which serve as biomarkers for the honey's floral origin. This technique highlights the unique aromatic profile of stingless bee honey, reflecting its tropical plant sources [44]. Similarly, Liquid Chromatography-Mass Spectrometry (LC-MS) is employed to analyze bioactive compounds such as phenolic acids and flavonoids, which contribute to its antioxidant and therapeutic properties [12]. While these techniques provide valuable insights, they are resource-intensive, requiring specialized equipment and technical expertise, limiting their application for routine analysis.

Spectroscopic Methods

Spectroscopy has emerged as a rapid, non-destructive tool for honey authentication and quality control. Among these methods, Fourier Transform Infrared (FTIR) spectroscopy is widely applied to identify functional group vibrations associated with sugars, organic acids, and adulterants, making it an efficient tool for detecting sugar syrups [20]. Raman spectroscopy, though relatively new, shows promise as a non-invasive method for characterizing chemical profiles unique to stingless bee honey. However, fluorescence interference remains a challenge in its application. Ultraviolet-Visible (UV-Vis) spectroscopy is another approach that can be used for adulterated honey [46]. While spectroscopic methods are versatile, their full potential often relies on integration with advanced data analysis techniques.

DNA-Based Methods

DNA-based approaches offer precise tools for verifying the floral and entomological origins of stingless bee honey. Pollen metabarcoding involves extracting DNA from pollen grains present in the honey, enabling detailed botanical profiling. This technique has been successfully applied to identify floral species, including rare or endemic plants contributing to the honey's unique characteristics [47,48]. Additionally, honeybee DNA analysis can identify the specific stingless bee species responsible for honey production, providing insights into geographical and species-specific variations [49]. While these methods are highly accurate and provide detailed information, they require advanced laboratory facilities and expertise, making them less practical for routine applications.

Isotopic Analysis

Isotopic analysis, particularly Stable Carbon Isotope Ratio Analysis (SCIRA), is a powerful method for detecting honey adulteration. This technique differentiates between natural sugars from C3 plants (e.g., nectar sources) and added sugars from C4 plants, such as cane sugar or corn syrup. Stingless bee honey possesses a distinct isotopic signature that not only distinguishes it from Apis honey but also aids in identifying adulteration [50]. Although isotopic analysis offers exceptional accuracy and reliability, its high cost and specialized equipment requirements limit its widespread adoption, especially in resource-constrained settings.

Emerging Techniques

Emerging technologies are transforming the landscape of stingless bee honey authentication by offering innovative solutions that are rapid, accurate, and scalable. The electronic tongue and nose systems mimic human sensory perception to analyze taste and aroma profiles, enabling the discrimination of flavor compounds and volatile organic compounds (VOCs) unique to stingless bee honey. Chemometric techniques combine spectroscopic or chromatographic data with multivariate statistical analysis, improving classification accuracy for botanical and geographical origins [51]. Additionally, Artificial Intelligence (AI) is being integrated into honey authentication workflows to analyze large datasets, detect subtle patterns, and improve precision in quality control processes [12]. These advancements, though promising, require further development to make them cost-effective and user-friendly for commercial and artisanal markets.

CONCLUSION

In conclusion, Malaysian stingless bee honey (SBH) is a valuable natural product with unique therapeutic and nutritional benefits, attributed to its diverse chemical constituents, such as flavonoids and phenolic compounds. The growing meliponiculture industry highlights the potential of SBH, especially from species like *Heterotrigona itama* and *Geniotrigona thoracica*, which are prized for their high honey yield and medicinal properties. However, its authentication requires advanced analytical methods, including physicochemical, chromatographic, and spectroscopic techniques, with emerging technologies like AI offering promising solutions. Future research should focus on optimizing SBH production and refining authentication methods to further enhance its application in health and industry.

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