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Physicochemical, biological, and therapeutic uses of stingless bee honey

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Stingless bee honey, also known as pot honey, is a unique product that differs from other honey (*Apis*) in terms of flavor, chemical composition, biological characteristics, and sourness. Raw and by-products made from this honey have substantial use for its diverse health benefits and human dietary requirements. The physicochemical properties of honey from stingless bee mainly rely on nectar sources, geographic locations, climate, bee species, and handling and storage conditions. The honey contains reducing sugars, water, minerals, and ash content, and its characteristic features include color, acidity, pH, electrical conductivity, and viscosity. Further, it contains several biological and therapeutic constituents such as flavonoids, antioxidants, antibacterial, wound-healing, antidiabetic, and anticancer properties, and the Maillard reaction product hydroxymethylfurfural. This review summarizes the literature on the physical and biological properties and therapeutic constituents' use of stingless bee honey. It also highlights the quality standards available worldwide and required modifications in establishing universal standards for promoting this honey.

KEYWORDS

antioxidant, honey, physicochemical, stingless bee honey, therapeutic

1 Introduction

Stingless bees are small to medium-sized bees found across Asia, Africa, America and Australia (Grüter, 2020) (Figure 1A). They visit many tropical flowering plants and have the potential to be commercial pollinators (Souza et al., 2021; Karuppaiah et al., 2023). Colonies of stingless bees are perennial and usually consist of hundreds or thousands of workers who exhibit highly eusocial behavior (Wille, 1983). About 500 stingless bee species are known around the world, belonging to the Meliponinae subfamily of the Apidae family in the Hymenoptera order (Shamsudin et al., 2019; Karuppaiah et al., 2022).

Meliponiculture is the scientific method of stingless beekeeping, which has long been practiced in tropical nations. Stingless bee honey (SBH) is highly valued and is regarded to be more effective as an all-natural remedy for treating common ailments (Zulkhairi Amin et al., 2018). SBH is also referred to as “pot-honey” as they store honey in honey pots (Figures 1B, C) rather than honeycombs (Vit et al., 2004) and is widely regarded as having, nutritive, therapeutic, and medicinal properties conferring various health benefits (Nordin et al., 2018; Souza et al., 2021).

Stingless bees dehydrate honey to a specific level and after that, the bacteria and yeasts, primarily *Bacillus* species, consume some of the sugar and ferment it anaerobically to produce alcohol, which is then fermented aerobically to produce acetic acid (Souza et al., 2006; Menezes et al., 2013). Other non-alcoholic fermentation processes also convert sugar into different kinds of acids and other byproducts that introduce bioactive components like antibiotics and antioxidant molecules to the honey (Almasaudi, 2021). The species-specific microbiomes and processing dynamics, that modify the properties of the honey are quite distinctive. Besides, it also adds enzymes and other compounds to honey, which may aid in preserving and digesting nutrients (Menezes et al., 2013).

Furthermore, the term “stingless bee honey” extracted from the Scopus database up to 2023 was used for understanding the connections between scientific output and stingless bee honey (Figure 2). According to Figure 2A, the largest circles represent the most common author-selected terms: “honey”, “stingless bee”, “physicochemical properties”, “antioxidant” and “flavonoid”. Based on the bibliometric mapping performed using widely referred VOSviewer version 1.6.19 (Kwon, 2023; Liaqat et al., 2023), we could categorize the terms into four distinct sets. The red grouping emphasizes terms that are specific to honey and its physicochemical properties. The keywords grouped in green cluster are linked to the stingless bee honey’s biological and therapeutic constituents, and the keywords assembled in blue cluster are connected to the physicochemical analysis of SBH. The terms in pink cluster focus on the microbial sensitivity tests of SBH. Figures 2A, B exemplifies that the physicochemical parameters of stingless bee honey hold great promise as a source of nutrition and therapy demonstrating a value in the human diet (Esa et al., 2022).

This review is aimed to shed light on the physicochemical properties, constituents, therapeutic benefits and quality standard requirements of SBH. The informational summary in this review will be useful in better understanding SBH, its potential use and its scope in various economic benefits (Potts et al., 2016; Souza et al., 2021) graphically depicted in Figure 3.

2 Biochemical features and constituents of SBH and *Apis* honey

The physicochemical parameters of pot honey have been extensively examined and compared to *Apis mellifera* produced floral honey (Lage et al., 2012; da Silva et al., 2013; Biluca et al., 2016; Chuttong et al., 2016; Nordin et al., 2018; Popova et al., 2021; Souza et al., 2021; Villacrés-Granda et al., 2021). Pot honey has distinct organoleptic and physicochemical properties when compared to *A. mellifera* produced floral honey (Biluca et al., 2016; Rosli et al., 2020; Popova et al., 2021). Water content, reducing sugar, acidity and pH, electrical conductivity, ash, color, viscosity, hydroxymethylfurfural (HMF), as well as various phenolic and flavonoid concentrations, are all included in the physicochemical profile of pot honey (Figure 4) (Bafo, 2019; Santisteban et al., 2019; Lavinás et al., 2023). These attributes are liable to change based on variables such as bee species, botanical sources, geographic origins, climate, collection period, processing, and

storage procedures. Supplementary Table S1 summarizes the physicochemical characteristics of stingless bees and their regional variations.

2.1 Water content

SBH has a greater water content than *Apis* honey (Avila et al., 2019a), which exceeds 20% and can approach 40% (Biluca et al., 2016). In some situations, the water content of SBH can exceed 42% (v/v), especially when collected from humid areas (Marinus, 2006; Ramli et al., 2017). Chuttong et al. (2016) reported that the average moisture content of stingless honey was 31%. *Melipona favosa* and *Melipona trinitatis* had an average moisture content of 23.5% (Bijlsma et al., 2006; Nweze et al., 2017). The moisture content of *Heterotrigona itama* and *Geniotrigona thoracica* honey is 16.1–32.1% (Julika et al., 2020; Zawawi et al., 2022). Honey’s water content is critical in inhibiting or limiting microbial growth. It is an essential factor in affecting honey’s stability and influencing microbial response and the type of microorganisms found in honey (Ikhsan et al., 2022). As a result, pot honey deteriorates more quickly than *Apis* honey (Nordin et al., 2018). The increase in water content provides an ideal environment for the growth of numerous bacteria associated with Meliponini bees. Bacilli of the genera *Bacillus* and *Streptomyces* were discovered in pot-honey samples (Ngalimat et al., 2019). These bacteria are thought to help prevent honey spoilage by suppressing the growth of pathogenic germs. The intricate interplay between honey’s water content and microbial activity underscores the significance of maintaining optimal moisture levels for honey preservation, as deviations can either enhance or compromise its shelf life and quality.

2.2 Reducing sugars

Despite having a sweet taste, stingless bee honey often has less reducing sugar than *Apis* honey (Avila et al., 2018). Each 100 g of SBH contains fructose ranging from 15.0–48.4 g, glucose ranging 12.2–40.0 g, sucrose <0.01–7.3 g, fructose + glucose ranging 15.0–80.2 g, fructose/glucose 0.78–1.6 g, and glucose/water (G/W) (0.47–1.89) (Julika et al., 2020). The fructose concentration is mainly responsible for the variation in sweetness of SBH (González-Miret et al., 2005). The variation in sugar concentration in honey is influenced by a number of variables, such as the honey’s botanical origin, the climate, and its geographic location (Pascual-Mate et al., 2018). In comparison to honey obtained in the dry season, honey extracted during the rainy months usually has a lesser amount of reducing sugars. Sugar levels decrease as a result of increased water content brought on by high humidity during the rainy season (Singh and Singh, 2018). The time of storage is yet another important component that may have an impact on the honey’s diminishing sugar content. Higher quantities of reducing sugars and a reduction in acidity have been linked to longer storage times (Karnia et al., 2020). It’s interesting to observe how various factors, from environmental conditions to storage duration, play a crucial role in shaping up the composition of stingless bee honey, affecting both its taste and nutritional characteristics.

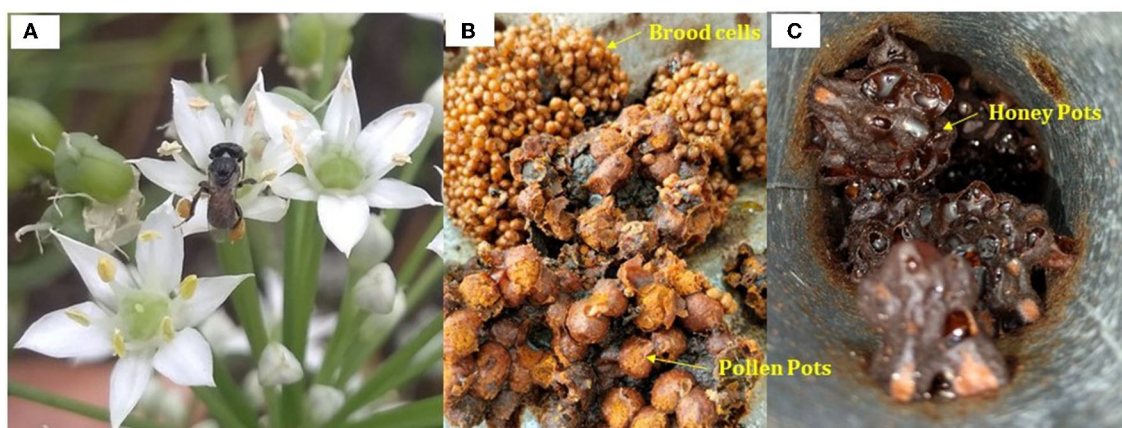


FIGURE 1

(A) Stingless bee, *Tetragonula* sp. foraging on wild onion; (B) Brood cells and pollen pots in the nest; (C) Honey pots.

2.3 Acidity and pH

Natural SBH has an acidic pH ranging from 3.2–4.5, giving it a distinct sour flavor and aroma (Sant'ana et al., 2020; Schvezov et al., 2020; Umaña et al., 2021; Andrade-Velásquez et al., 2023; Grandoa et al., 2023; López-Garay et al., 2023; Shaik et al., 2023). SBH is more acidic than *Apis* honey (Almeida-Muradian et al., 2014), which is due to the larger amount of organic acids, esters, and inorganic ions, including phosphate, chloride, sulfate, and nitrate. SBH acidity is also regulated by the source of nectar, bee species, and their enzymes or activity by bacterial communities (Finola et al., 2007; Lee et al., 2008; de Sousa et al., 2016; Machado De-Melo et al., 2018). Sugar fermentation into organic acids can also increase acidity (Evahelda et al., 2021). Fermentation takes place naturally in storage due to honey's inherently increased moisture content. Honey contains organic components that significantly lower sugar concentrations, such as fructose, glucose, maltose, and sucrose, making it ideal for the growth of osmophilic yeasts. Honey's higher moisture content (>17.1%) and storage temperature ranging from 23 to 27°C could encourage yeast development and fermentation (de Almeida-Muradian et al., 2014). These prevailing conditions favor the capacity of yeast to convert the sugars glucose and fructose into ethyl alcohol and carbon dioxide. As a result of the alcohol gets converted into acetic acid and water in the presence of oxygen, offering a sour flavor (Ismail et al., 2021). The acidic pH of honey contributes to its antibacterial qualities by averting the existence and proliferation of harmful microbes (Lee et al., 2008; da Silva et al., 2013). It is also the secret to honey's shelf-life due to its stability against microbial spoilage (Ismail et al., 2021), and it allows honey to be compatible with many food products in terms of pH and acidity.

2.4 Electrical conductivity

The mineral concentration, salts, free organic acids, ash content, moisture content, proteins, and source of nectar all have a direct impact on the electrical conductivity (EC) of SBH (Prakash

et al., 2015; Solayman et al., 2016; González-Montemayor et al., 2019). Specifically, the higher concentration of these constituents in the honey leads to high conductivity (da Silva et al., 2016; Baloš et al., 2018; Ismail et al., 2021; Mulugeta and Belay, 2022; Shaik et al., 2023). Although there are no significant differences in the electrical conductivity between SBH and *Apis* species honey, it was previously revealed to have a greater EC ranging from 0.72–0.16 mS cm⁻¹ compared to *Apis* honey (Alvarez-Suarez et al., 2018), which falls below the limit of the EC value defined by the International Honey Commission (IHC) (should not exceed 0.8 ms/cm) (Shaik et al., 2023). This variance could possibly be explained by a greater amount of organic acids (as shown by higher acidity values), resulting in higher conductivity. The role of organic acids in this context is crucial, as they contribute to the ionization of compounds in honey, leading to increased electrical conductivity. Additionally, the presence of certain minerals and salts, which are known conductive agents, may further contribute to the observed differences in EC values between SBH and *Apis* honey varieties. Further research is needed to elucidate the specific compounds responsible for the conductivity variations and their potential implications for the quality and authenticity of honey products.

2.5 Minerals and ash

The mineral composition of honey is determined by its ash content. Honey contains essential minerals such as potassium, sodium, calcium, and magnesium. In addition, honey may contain other minerals such as nickel, chlorine, phosphorus, sulfur, silicates, iron, copper, manganese, and more (Ameliya et al., 2023). SBH typically contains an appreciable amount of potassium, ranging from 10.55 mg to 448 mg 100g⁻¹, accounting for one half of the total mineral constituents (Biluca et al., 2016; Sabir et al., 2021). Potassium is an essential mineral that is vital for several physiological tasks, such as heart and muscle function. SBH contains small amounts of calcium varying from 1.12–35.2 mg 100 g⁻¹ (Biluca et al., 2016; Sabir et al., 2021), which is important for bone health and various physiological processes. Magnesium

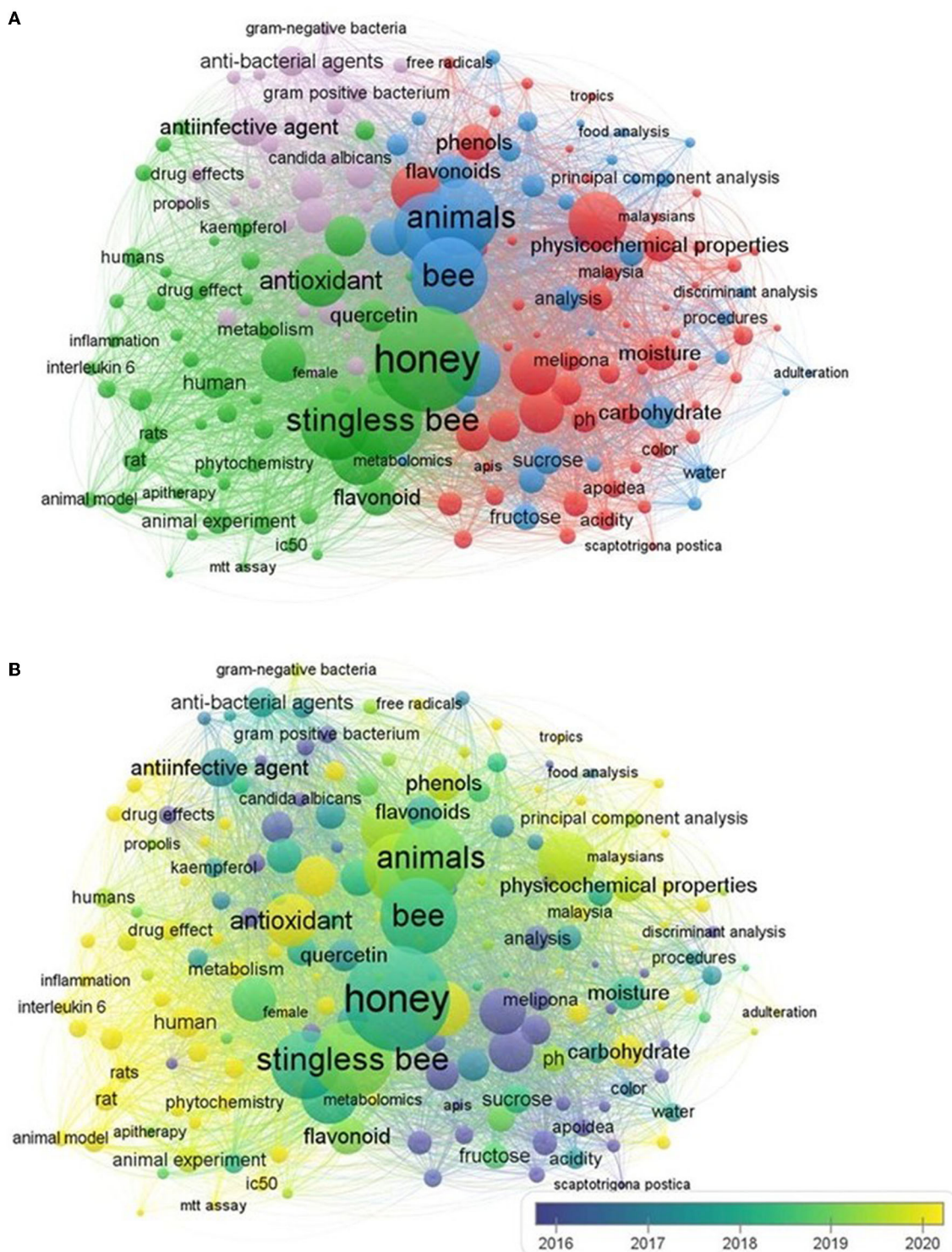
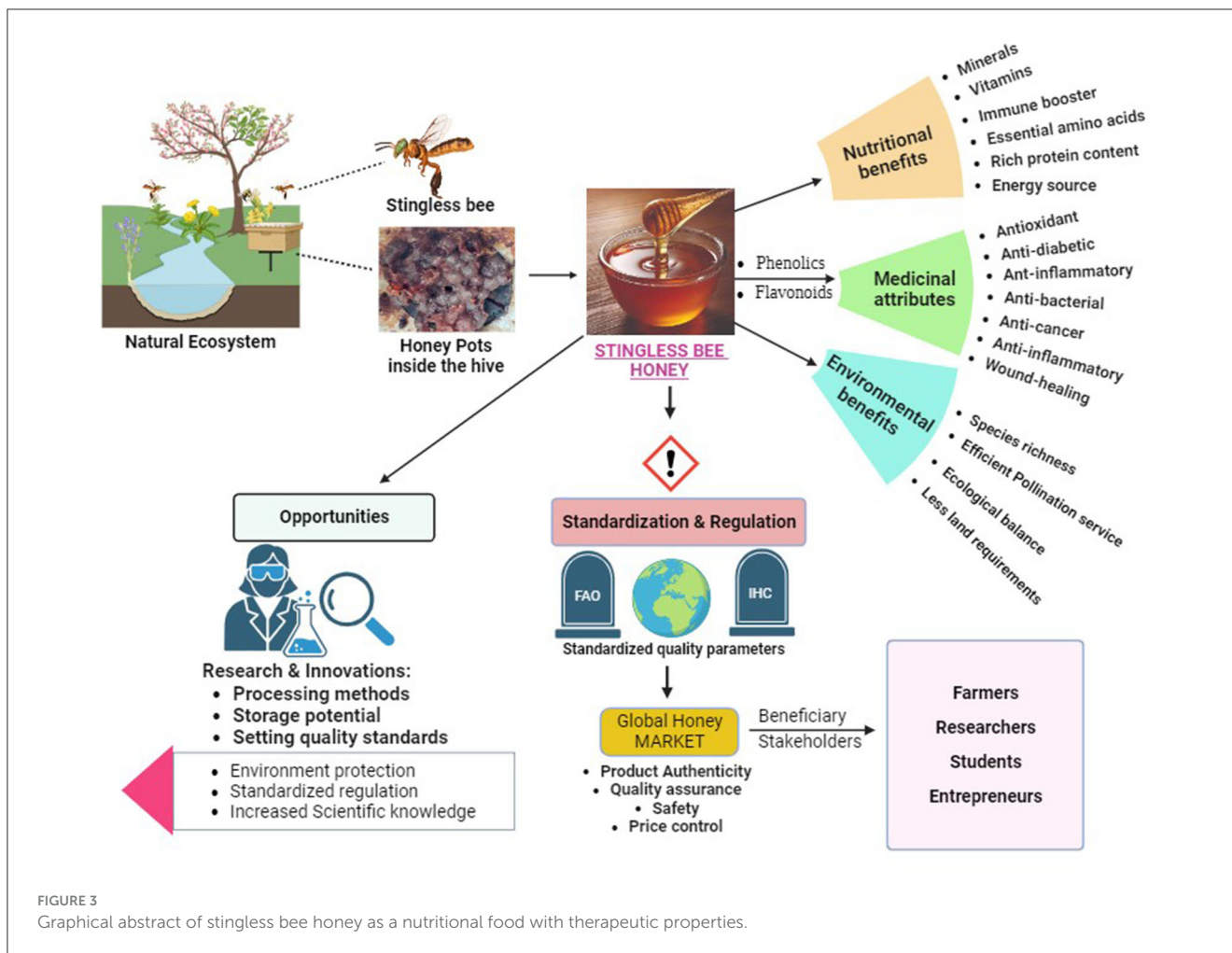


FIGURE 2

A bibliometric network map of scientific research on stingless bee honey. The network visualization based on four colored clusters (A) indicates the relatedness of the scientific journals and an overlay visualization (B) specifies a period of the occurrence of the keyword from 2016 (blue) to 2020 (yellow). This figure is created from VOSviewer version 1.6.19, and collected data were obtained from Scopus database utilizing keyword "stingless bee honey".

is essential for muscle and nerve function, as well as bone health (Ramli et al., 2020). Sodium is essential for maintaining proper fluid balance in the body. The average content of both magnesium and sodium was found to vary in SBH, ranging from 0.410 to

17.3 mg 100 g⁻¹ for Mg and from 0.730–30.4 mg 100 g⁻¹ for Na (Biluca et al., 2016; Sabir et al., 2021). Phosphorus is present in trace amounts in honey (Biluca et al., 2016), which is important for bone and tooth health and various cellular functions. Honey contains

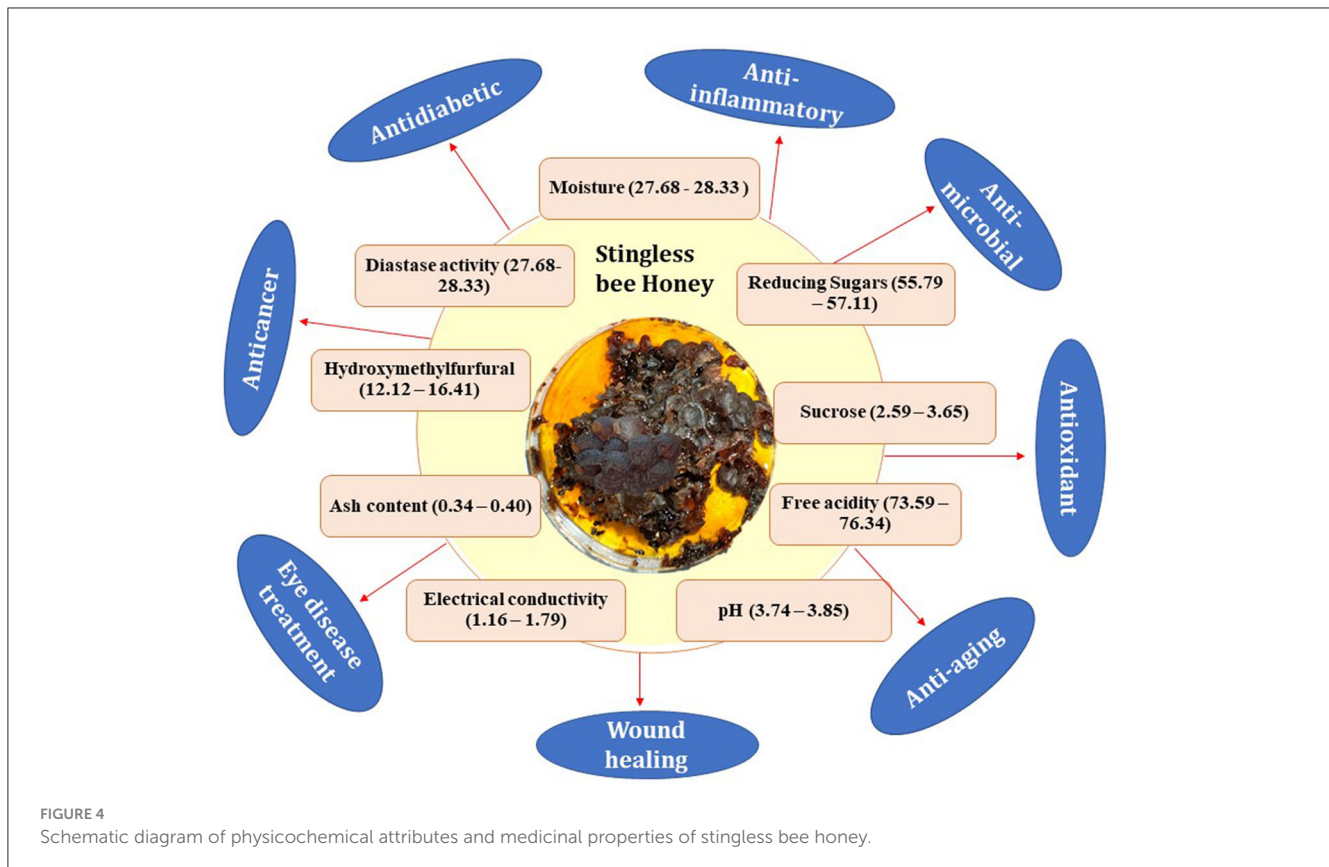


trace amounts of sulfur, which contributes to its characteristic flavor and aroma (Tafere, 2021). SBH also contain trace amounts of chlorine, an electrolyte that the body requires (Ajibola et al., 2012).

The mineral content of SBH is influenced by various factors, including the kind of flowers visited by bees for nectar, the botanical origin of the nectar, geographical location, bee species, sugar content, intensity of honey color, soil type of the plants where grown, climatic conditions, and honey processing as well as handling (Nanda et al., 2003; Lacerda et al., 2010; de Almeida-Muradian et al., 2013; Pereira et al., 2020; Gela et al., 2021). However, honey's ash level, which is correlated with its reducing sugar content and black color, suggests the presence of minerals (Venturini et al., 2007; Lacerda et al., 2010). The amount of ash content in a properly dehydrated SBH can be between 0.02 to 0.19% and 0.85% (Esa et al., 2022). The variation in mineral content underscores the intricate interplay between environmental factors and honey composition, making honey a dynamic and diverse natural product with potential health benefits derived from its rich mineral profile.

2.6 Color and viscosity

Honey's physical characteristics is initially recognized by consumers through its color (Figure 5). Scientifically, color is determined by the Pfund scale, which reveals that SBH can range from a light white hue, as shown in *Scaptotrigona pectoralis* honey (14.5 mm Pfund units), to different shades of light and dark amber, as seen in *Plebeia molesta* honey (Nordin et al., 2018; Avila et al., 2019a). *Trigona* honey is assessed to have a yellow-red hue value of 53.50 and a brightness level of 6.61 ± 0.48 (Moniruzzaman et al., 2014). Pollen sources, sugar constituents, antioxidants like carotenoids, xanthophylls, and cyan pigments, minerals, amino acids, and phenolic substances (primarily flavonoids), and 5-HMF which is produced by honey heating or prolonged storage via the Maillard reaction, all contribute to substantial changes in honey color (Shamsudin et al., 2019; Tkáč et al., 2022). The color of the honey, however, does not vary with the bee species (Nordin et al., 2018; Avila et al., 2019a). Darker honey has more minerals, polyphenolic chemicals, dextrins, and acidity than lighter honey (Machado De-Melo et al., 2018).



Viscosity is important in honey's handling, storage, processing, and parameters of sensory quality, which ultimately influence consumer preference. SBH's viscosity is lower than that of *Apis* honey (do Vale et al., 2018). According to Ameliya et al. (2023), *Trigona* honey has a viscosity of 596.33 Cp, while *Apis cerana* honey has a viscosity of 956.33 Cp. Many factors influence it, including plant species sources, water content, temperature, quantity of fructose or glucose, granulation, and the chemical composition of honey. SBH has a decreased viscosity due to its higher moisture content, low polysaccharide content, and lower sugar

content (Machado De-Melo et al., 2018). Understanding these physical characteristics, including color and viscosity, provides valuable insights into the diverse nature of honey and its appeal to consumers.

2.7 Hydroxymethylfurfural (HMF)

HMF, a heterocyclic organic molecule with six carbon atoms and functional groups for aldehyde and alcohol, is used as a measure to assess the freshness and aging of honey (Crane, 1983). It is an important intermediate product that results from two reactions; the Maillard reaction and the breakdown of 3-deoxyglucosone (Salis et al., 2021). HMF is normally absent in fresh honey, but throughout processing and aging, its content rises (Ismail et al., 2021). Low levels of HMF are anticipated in SBH due to its characteristics like high moisture and acidity content, as well as the dominance of fructose. These features limit the synthesis of HMF in honey. The concentration of HMF in SHB can range from 12.64 to 15.0 mg kg⁻¹ (Guerrini et al., 2009; Biluca et al., 2014). Unfavorable storage circumstances, higher temperatures, lengthier heating processes, pH levels, and floral sources can all alter the concentration of HMF in honey (Ismail et al., 2021). According to the Codex Alimentarius Commission (Codex Alimentarius, 2019), honey from nations or regions experiencing tropical ambient temperatures, as well as blends that include such honey, shall not exceed the HMF value of 80 mg/kg honey. Monitoring and controlling HMF levels in honey are crucial

not only for quality assurance but also for ensuring compliance with international standards and regulations. The specified limit serves as a benchmark to prevent excessive HMF formation, which could indicate improper processing, storage, or unfavorable environmental conditions. Adhering to these standards contributes to the overall quality and safety of honey products in the global market, providing consumers with confidence in the freshness and authenticity of the honey they consume.

2.8 Phenolics

Phenolic compounds are secondary metabolites found in plants and some animal products, and they are known for their potential health benefits due to their antioxidant properties, antimicrobial activity, and anti-inflammatory effects (Lin et al., 2016). SBH has a wide variety of phenolic compounds (more than 80), similar to traditional honey, such as flavonoids, phenolic acids (caffeic acid and gallic acid), and tannins (Dos Santos et al., 2021). These compounds are primarily derived from the nectar and pollen collected by the bees from various plant sources (Biluca et al., 2020). Certainly, fermentation and hydrolysis are methods known to efficiently transform glycosides into aglycones, enhancing the bioavailability of various compounds. Consequently, it is plausible that SBH may contain a higher concentration of phenolic compound aglycones as opposed to glycosides, potentially augmenting its health-promoting properties (Biluca et al., 2017; Dos Santos et al., 2021; Mokaya et al., 2022). Flavonoids have been studied for their possible health advantages, which include anti-inflammatory and antioxidant properties (Han et al., 2012). The composition of phenolic compounds in SBH can vary based on geographical location, botanical origin of the floral source, and bee species involved in honey production. The range of total phenolic content in SBH is 0.0543 ± 0.003 to 0.1760 ± 0.002 mg GAE/g (Mahani et al., 2022) to 7 to 66 mg GAE/g (da Silva et al., 2013). This biochemical variability highlights the importance of considering these factors when evaluating the potential health benefits of SBH. Overall, the rich and diverse phenolic profile of SBH underscores its significance as a natural product with promising health-promoting properties. Further research into the specific health impacts of individual phenolic compounds in SBH is warranted to fully understand its therapeutic potential.

3 Biological and therapeutic aspects of SBH

SBH contains a variety of biological, pharmacological, and physiological properties that can potentially be therapeutic for people and animals (Figure 4). The therapeutic value of these properties depends on the various categories of flavonoids and polyphenols present in stingless bee honey that have been correlated to these anti-inflammatory (Biluca et al., 2020), antidiabetic (Ali et al., 2020), antifungal (Hau-Yama et al., 2020), antimicrobial (Boorn et al., 2010; Nishio et al., 2016), antioxidant (Krishnasree and Ukkuru, 2015; Tuksitha et al., 2018), anticancer properties (Borsato et al., 2014), and ameliorating (Mohammad et al., 2020) constituents. Apinae honey has also been found to have significant medicinal uses, particularly in the treatment of

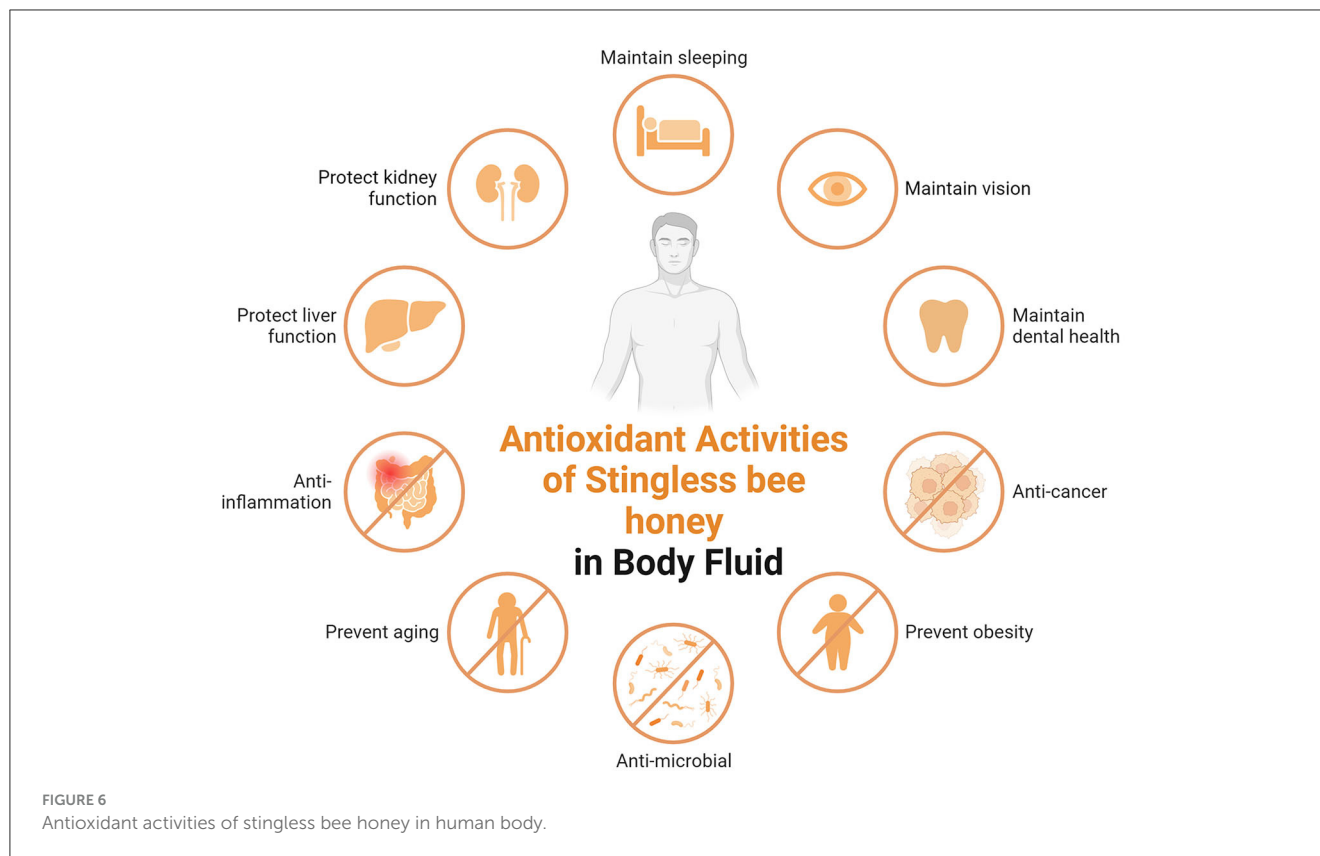
neurological disorders (Al-Himyari, 2009; Al-Rahbi et al., 2014; Saxena et al., 2014), gastrointestinal tract diseases (Haffejee and Moosa, 1985; Ali and Al-Swayeh, 1997), neurological illnesses (Al-Himyari, 2009; Al-Rahbi et al., 2014; Saxena et al., 2014), as well as improving the hormones associated with fertility (Mohamed et al., 2013; Haron et al., 2014; Mosavat et al., 2014; Rajabzadeh et al., 2015a,b). New antimicrobial agents from natural honey are of great interest, and honey from many different species and botanical origins worldwide has drawn much research (Supplementary Table S2). Honey has been used for millennia to treat various illnesses, making it a hot topic for research (Costa-Neto and Oliveira, 2000).

3.1 Anti-cancer activity, cardiovascular therapy, antioxidant and anti-inflammatory activity

Flavonoids, particularly flavanones, flavones, and flavonols, which are present in honey, have extraordinary medicinal uses, including immunosuppressive and anticancer properties (Kustiawan et al., 2014; Karabagias et al., 2016). In particular, the caffeic acid ester from *Trigona* spp. demonstrates specific chemopreventive effects. It significantly lowers the number of abnormal crypts and crypt foci, making it a viable colon cancer therapeutic alternative (Yazan et al., 2016).

Caffeic acid extracted from *Melipona subnitida* honey has been shown to be effective in the treatment of dyslipidemia, which is linked to cardiovascular disease (Bezerra et al., 2018). 4-hydroxyphenyl acid from *Heterotrigona itama* honey (Ramli et al., 2019) and gamma-Mangostin from Thailand stingless bee honey (Ishizu et al., 2019) have also shown promise in cardiovascular therapy.

Total phenolics and flavonoid content of SBH have been linked to its potential antioxidant properties (Praptiwi et al., 2023). SBH was used to formulate honey with antioxidant properties that help lessen oxidative reactions or free radicals in food systems and human health (Figure 6) (Tuksitha et al., 2018). *In vitro*, assays using Brazilian SBH have revealed 45% higher antioxidant and biological activity than those using *A. mellifera* honey (Avila et al., 2019b). According to Praptiwi et al. (2023), *T. laeviceps* honey contains 5,7-dihydroxy chromone, cnicidin, puerarin, and irisflorethin, while *Tetragonula carbonaria* honey has been illustrated to contain gallic acid, pimaric acid, and pimaric acid isomers (Massaro et al., 2014). To the anti-inflammatory properties of SBH of various species, naringenin (Copmans et al., 2018; Badrulhisham et al., 2020; Mokarrami et al., 2022), myricetin (Sun et al., 2019), and phenylalanine (Mustafa et al., 2019) add equal benefits. Kaempferol from *Trigona* spp. (Ranneh et al., 2018); Apigenin (Zhang et al., 2019b; Singh et al., 2021; Yadav et al., 2022); Chrysin (Shooshtari et al., 2020; Al-Haleem et al., 2021; Zhang et al., 2021); Catechin (Ahmed et al., 2021); Caffeic acid (Arshad et al., 2020; Kadar et al., 2022); ferulic acid (Zhang et al., 2019a,b; Park et al., 2022); and 4-hydroxybenzoic acid (Ranneh et al., 2019) from various SHB have been documented for antioxidant and anti-inflammatory activities (Vattuone et al., 2007; Suntiparapop et al., 2012; Duarte et al., 2018; Seng and Tang, 2020; Wongsa et al., 2023).



3.2 Antimicrobial and anti-diabetic activity

The antimicrobial properties of SBH are well documented by several researchers based on its botanical origin (Hbib et al., 2020; Zapata-Vahos et al., 2023). SBH is shown to have antibacterial action against Gram-positive bacteria including *Bacillus subtilis*, *Micrococcus luteus*, *B. megaterium*, and *B. brevis*, and Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas syringae* (Chanchao, 2009; Al-Hatamleh et al., 2020a,b; Cantero et al., 2021). Nishio et al. (2016) illustrated the *in-vitro* antibacterial activity of honey from *Scaptotrigona bipunctata* and *Scaptotrigona postica* against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. Miorin et al. (2003), Sgariglia et al. (2010) found that honey from *Trigona angustula* had antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative (*Pseudomonas aeruginosa* and *E. coli*). Brown et al. (2020) and Khongkwanmueang et al. (2020) reported that the honey of *M. favosa* (Tobago) and *T. laeviceps* (Thailand) contained a high amount of phenolics and flavonoids, which led to potent antimicrobial activity against yeast and pathogenic microbes (*S. aureus*, *E. coli*, *S. pyogenes*, and *H. influenza*). *Lepidotrigona arcifer* honey can also treat colds and coughs (Biswa et al., 2017). Unsaturated pyrrolizidine alkaloids (PA) of SBH have antifungal and antiviral qualities (Moreira et al., 2018; Tasca et al., 2018; Schramm et al., 2019; Mädge et al., 2020). The antibacterial properties of SBH are further correlated with the amount of hydrogen peroxide and other non-peroxide components, including lysozyme, phenolic acid, and flavonoid

(Kwakman and Zaat, 2012). These chemical compositions show that non-phenolic chemical substances were responsible for the antibacterial effect (Brodkiewicz et al., 2018). Furthermore, it may be associated with the acidic property (low pH) (Avila et al., 2018), polyphenol concentration (Daglia, 2012), and protein content (Ramon-Sierra et al., 2020).

Aziz et al. (2017) demonstrated the antihyperglycemic effects of *Apis* honey on chemically induced diabetes. Stingless bee honey showed promise as an anti-diabetic drug by lowering histopathological alterations, oxidative stress expression levels, inflammation, and apoptotic indicators in pancreatic islets. In addition, stingless bee honey raised the insulin expression level (Aziz et al., 2017; Sahlan et al., 2020). According to Krishnasree and Ukkuru (2017), *Trigona iridipennis* honey has more enzyme inhibition against amylase and glucosidase. Caffeic acid (Jung et al., 2006; Estevinho et al., 2008; Khalil and Sulaiman, 2010; Rocha et al., 2012; Spilioti et al., 2014) and catechin (Koh et al., 2004; Afroz et al., 2016) are some bioactive compounds responsible for the antihyperglycemic effects of SBH.

3.3 Wound healing, anti-allergic activity and anti-aging property

SBH has shown promising results in clinical studies on wound healing (Al-Achi, 2008; Reni-Yusli et al., 2016; Ng et al., 2017), which is useful in the treatment of pathogens, wound debridement and inflammation suppression, scarring reduction, stimulation of

angiogenesis, tissue granulation, and epithelium growth (Molan and Betts, 2004). Infections in the body, such as ulcers, skin infections, and rashes, are also treated (Kwapong et al., 2010). Protocatechuic acid, an important free phenolic acid found in SBH, is a powerful antioxidant that boosts cell proliferation and aids in healing wounds (Abd Jalil et al., 2017). Based on molecular docking modeling, polyphenol-rich SBHs having 3,5-di-O-caffeoylquinic acid, quercetin glucoside molecules, and caffeoyl-D-glucose have strong anti-allergic actions (Yong et al., 2023).

Oxygen serves as an antioxidant, safeguarding the human body from internal damage and inhibiting oxidative reactions in various types of food (Han et al., 2012). Natural honey has been shown to have anti-aging effects. SBH curtails reactive oxygen species (ROS) and free radicals produced during various metabolic activities (Habryka et al., 2021). Honey from *H. itama* increases the expression of collagen type I and metalloproteinase (MMP)-1 in human dermal fibroblast cells, acting as an anti-aging agent (Malik et al., 2020).

3.4 Other therapeutic values

In addition to its many therapeutic applications, SBH has been used to treat neurological disorders, infertility, and ocular diseases. In the course of cataract treatment, honey from *Trigona* spp. slows down the progression of the disease (Patricia, 2002), while honey from *Meliponula* spp. has been shown to shorten the duration of infection for eye disorders brought on by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ilechie et al., 2012). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels can all be boosted by eating honey. Furthermore, it has been demonstrated that giving children continual honey supplementation will shorten their bouts of diarrhea (Haffejee and Moosa, 1985) and be used to treat gastrointestinal problems brought on by ethanol as well as increased vascular permeability (Yazan et al., 2018). It prevents dementia and other cognitive diseases (Al-Himyari, 2009) and enhances memory by increasing neuron proliferation in hippocampal regions (Al-Rahbi et al., 2014).

4 Quality standards requirement for stingless bee honey

Honey, being a natural product, possesses a varied composition that is influenced by numerous factors. These factors include the botanical and geographical source of honey, the intensity of nectar availability, prevailing climatic conditions, manipulations by beekeepers, the methods of handling and packaging, the duration, and the conditions of storage (Thrasylvoulou et al., 2018). Establishing specifications for the quality criteria of SBH is crucial when it comes to its direct consumption by humans (Lemos et al., 2018). While honey standards have been adjusted based on the botanical source, the species' origin has not been considered in these modifications (Owen, 2023). It is of utmost importance that these factors are given due consideration and that quality standards are established for other varieties of SBH as well (Vit et al., 2004).

The current definition of honey, according to Codex Alimentarius (2019), pertains exclusively to honey produced

by honeybees and stored in a honeycomb made up of wax, thereby excluding honey stored by stingless bees in honey pots made of cerumen. Similarly, the directives of the International Honey Commission of the European Union (EU, 2014) limited the definition of honey to that obtained solely from the *Apis* species. Consequently, the commonly followed standards of these authorities do not encompass the definition of honey produced by stingless bees. To address this gap, the scientific community (Vit et al., 2004; Villas-Bôas and Malaspina, 2005; Carvalho et al., 2013; De Camargo et al., 2017; Malaysian Standards, 2017) has proposed regulatory standards (Table 1) for stingless bee honey, aiming to establish standardized criteria for its market acceptance and direct consumption by humans.

Stingless bees produce unique honey with physicochemical characteristics that differ from the honey produced by *A. mellifera* bees (the only once regulated). Stingless bee honey has more moisture and acidity, as well as less sugar, diastase activity, and 5-hydroxymethylfurfural (5-HMF) (Avila et al., 2018; Nordin et al., 2018). These parameters do not correspond to current regulatory restrictions (Codex Alimentarius, 2019). Moreover, the product is mostly sold informally and directly by producers without any certification of quality or authenticity, making it vulnerable to fraud. Hence, it is necessary to set general quality standards for SBH. It is crucial to revise these standards in order to accurately check the authenticity of honey products and prevent adulterated, fraudulent marketing. Failing to address this issue poses significant challenges in the market for the product.

A quality standard for SBH proposed by the Malaysian standard indicates a permissible moisture content of 35 g, sucrose of 7.5 g, and ash content of 1.0 g for each 100 g of honey. Furthermore, unlike the IHC norm, it considers a maximum reducing sugar content of 85 g per 100 g rather than a minimum content. Free acidity and diastase activity (DA) are not considered quality parameters, but the quantity of HMF content was kept close to the IHC standard. Phenolic compounds and pH values should be 2.5–3.8 in SBH (Malaysian Standards, 2017). At the same time, Vit et al. (2004) suggested increased moisture content (30 g/100 g), and sucrose (6 g/100 g) and a minimum reducing sugar content (50 mg/100 g). The ash contents were kept similar to IHC standards, while HMF should be < 30 mg/kg. In recent times, certain Brazilian states, like Bahia (Brazil, 2014) and São Paulo (Brazil, 2017), have introduced defined criteria for honey produced by stingless bees. The objective behind this is to ensure quality control and establish formal guidelines for the sale of this particular product. Consequently, establishments engaged in honey processing, accredited by the Federal Inspection Service, have managed to navigate past existing bureaucratic obstacles. They are now able to officially register and market stingless bee honey in the formal marketplace (Vit et al., 2004; Villas-Bôas and Malaspina, 2005; Carvalho et al., 2013; De Camargo et al., 2017).

Despite the growing number of studies into the physicochemical properties of these honeys, there is a great difficulty in uncovering a single standard that helps in determining their authenticity and quality (Braghini et al., 2021). Moreover, effective regulation is difficult owing to the world's large diversity of stingless bees (Braghini et al., 2021). Furthermore, when compared to *A. mellifera* bees (20 kg per hive/year) stingless bees produce less honey (1–5 kg per hive/year), (Chuttong et al., 2016). This makes it a rare product with a higher market value (US 100 USD/kg for

TABLE 1 Quality standard limits of the current legislation for Meliponini bee floral honey and *Apis mellifera* floral honey.

Reference Parameter	Codex Alimentarius (2019) and EU (2014)	Malaysian Standards (2017)		Vit et al. (2004)			Villas-Bôas and Malaspina (2005)	Carvalho et al. (2013)	De Camargo et al. (2017)
	<i>Apis mellifera</i>	Stingless bee honey (<i>Kelulut</i> honey)		<i>Melipona</i>	<i>Scaptotrigona</i>	<i>Trigona</i>	Meliponini	<i>Melipona</i>	Meliponini
		Raw honey	Processed honey						
Moisture (%)	Max 20.0	Max 35.0	Max 22.0	Max 30.0	Max 30.0	Max 30.0	Max 35.0	Max 30.0/max 19*	Max 40.0/max 20*
Sucrose (g/100 g)	Max 5.0	Max 7.5	Max 8.0	Max 6.0	Max 2.0	Max 2.0	Max 6.0	Max 6.0	Max 6.0
Fructose and glucose (sum), (g/100 g)	Min 60.0	Max 85.0	Max 90.0	Min 50.0	Min 50.0	Min 50.0	Min 50.0	Min 60.0	Min 60.0
Maltose (g/100 g)	-	Max 9.5	Max 10.0	-	-	-	-	-	-
Ash (g/100 g)	Max 0.5	Max 1.0	Max 1.0	Max 0.5	Max 0.5	Max 0.5	Max 0.6	Max 0.6	Max 0.6
Hydroxymethylfurfural (mg/kg)	Max 40.0	Max 30.0	Max 30.0	Max 40.0	Max 40.0	Max 40.0	Max 40.0	Max 10.0	Max 20.0
Free acidity (meq/1,000g)	Max 50.0	-	-	Max 70.0	Max 85.0	Max 75.0	Max 85.0	Max 50.0	Max 50.0
Diastase activity (Schade units)	Min 8.0	-	-	Min 3.0	Min 3.0	Min 7.0	Min 3.0	Min 3.0	-
pH	-	2.5–3.8	2.5–3.8	-	-	-	-	-	2.9–4.5
Electrical conductivity (mS/cm)	max 0.8	-	-	-	-	-	-	-	-

(-), not applicable.

stingless bee honey and US 20–40 USD/kg for *A. mellifera* honey) (Se et al., 2018; Shadan et al., 2018). In addition to honey, these bees manufacture other products in the hive, such as propolis and wax, which have been widely studied and deserve attention.

The physicochemical properties of Malaysian honey were influenced by many factors, including bee species, floral sources, seasonal factors, processing, geographical distribution, etc., (Ismail et al., 2021). The Malaysian *Apis* and *Trigona* honey differed remarkably in physicochemical parameters (Ismail et al., 2021). Given that stingless bee honey has very varied physicochemical characteristics, relying solely on physicochemical parameters to assess the quality and determine authenticity is insufficient (Braghini et al., 2021). To address this issue, it is of utmost importance to conduct further research focused on identifying potential chemical markers of stingless bee honey. Such markers will play a crucial role in distinguishing the entomological, geographical, and floral origin of this product (Braghini et al., 2021).

5 Conclusion

This literature review has provided an overview of the physicochemical characteristics of SBH and its prospective applications for an array of health benefits. The excellent health benefits have been outlined, including the potential to reduce inflammation, fight infections, heal wounds, treat diabetes, and fight cancer (as a chemopreventive agent). The therapeutic benefits of SBH are due to its physicochemical components, particularly their phenolic and flavonoid content. The data elucidated in this review highlights the significant variations in stingless bee honey. This further emphasizes the need for criteria and guidelines to be developed to evaluate the honey quality of stingless bees. A further difficulty for quality control is identifying species. Since there are many different types of stingless bee honey, it is difficult for the regulator to create standards. Considering the fact that many species that still need to be investigated, the increasing demand for products from stingless bees and their usage in various medicinal applications requires additional validation studies and more comprehensive methods. In-depth studies on chemical characterization still need to be included and are imperative. In particular, if research is done to identify and enhance geographical elements, The scientific understanding and awareness of the distinctive qualities of each stingless bee species' honey will be essential to increasing the value of its products. Regulations are needed to safeguard this priceless and underutilized natural product's quality, safety, and authenticity. It is vital to address the uncertainty in global legislation by establishing specific minimum standards that must be met by all nations that produce, import, or export honey.

Author contributions

AG: Conceptualization, Software, Writing—original draft, Writing—review & editing. DS: Conceptualization, Writing—original draft, Writing—review & editing. PS: Supervision,

Writing—review & editing. CP: Writing—review & editing. MP: Conceptualization, Writing—original draft, Writing—review & editing. TP: Software, Writing—review & editing. RD: Funding acquisition, Resources, Supervision, Writing—review & editing. SK: Supervision, Writing—review & editing. SR: Writing—review & editing, Formal analysis. VM: Funding acquisition, Resources, Writing—review & editing. VK: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Visualization, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2023.1324385/full#supplementary-material>

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