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STINGLESS BEE-DERIVED NATURAL COMPOUNDS AS ANTI-INFECTIVE AGENTS: COMPARATIVE GC-MS PROFILING OF Itama sp. AND Tetragonula laeviceps

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Abstract

Background: Stingless bee honey is recognized for its therapeutic value, which is attributed to a diverse array of bioactive compounds.
Objective: This study aimed to compare the chemical profiles of honey from Tetragonula laeviceps and Itama sp. using gas chromatography-mass spectrometry (GC-MS), focusing on antimicrobial constituents.
Methods: Honey samples were collected from Bali, Indonesia, and analyzed using GC-MS. Compounds were identified through spectral matching with established libraries, and primary constituents were compared based on relative abundance.
Results: T. laeviceps honey showed a high concentration of the rare sugar allose (7.6%), azulene (1.02%), and octadecanoic acid (2.07%), whereas Itama honey exhibited elevated levels of levoglucosan (5.35%), aromatic esters, and 3-methyl-2-furylacetone (2.58%). Notably, 5-hydroxymethylfurfural (HMF), known for its antimicrobial properties, was present in both samples at comparable levels (1–2%).
Conclusions: The metabolite variations between the two species reflect differences in foraging and resin collection behavior, influencing honey bioactivity. The unique profiles suggest that both types of honey possess valuable antimicrobial potential and could serve as sources for developing novel anti-infective agents.

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INTRODUCTION

Honey, as well as other substances with medicinal properties, is produced by stingless bees (Meliponini). Tetragonula laeviceps (here shortened as T. laeviceps) and Heterotrigona Itama (often referred to simply as Itama sp.) are two prevalent stingless bee species in Southeast Asia. Honey, the resinous substance that bees collect and modify to safeguard their colonies, is abundant in secondary metabolites, including flavonoids, phenolic acids, terpenes, and waxes. The compounds exhibit substantial variation in the local flora and the species of bees (1,2). Traditional medicine has employed honey and other bee products from stingless bees due to their significant antibacterial, antifungal, and anti-inflammatory properties. Recent research has verified that stingless bee honey possesses substantial antimicrobial properties, particularly in the context of Gram-positive bacteria (3). This bioactivity is ascribed to honey's diverse chemistry, as more than 200 compounds have been identified in honey samples worldwide. The chemical composition of stingless bee products can vary significantly between species despite the increasing interest in this topic. The honey of one species may contain unique compounds that are not present in the honey of another due to differences in plant resin sources and insect foraging preferences. It is crucial to compare these profiles, as it can indicate which metabolites may be responsible for bioactivities (5–7). Moreover, recent studies have highlighted the potential of specific stingless bee-derived compounds, such as flavonoids and fatty acid esters, as promising anti-infective agents capable of disrupting microbial biofilms and inhibiting pathogen growth (8,9). This reinforces the value of chemical profiling as a tool to uncover natural alternatives in the fight against infectious diseases.

Despite the increasing number of studies reporting the antimicrobial properties of stingless bee products, most investigations have been limited to generalized bioactivity assays or bulk chemical assessments, such as total phenolic or flavonoid content. Comprehensive, species-specific chemical profiling at the molecular level remains underexplored, particularly using advanced techniques like gas chromatography-mass spectrometry (GC-MS). Moreover, comparative metabolomic analyses between stingless bee species, such as T. laeviceps and Itama sp., are scarce, leaving the relationship between species-specific chemical composition and anti-infective potential poorly understood. This gap hampers the rational selection of bee species or their derived products for targeted therapeutic applications. Therefore, a detailed comparative analysis of their secondary metabolite profiles is essential to identify key bioactive constituents and elucidate their anti-infective activity's chemical basis, thereby supporting their potential development as natural antimicrobial agents.

This study used GC-MS to analyze the secondary metabolites found in the honey (or honey-derived extracts) of T. devices and Itama sp. We focus on compounds that demonstrate a notable relative abundance, especially those recognized for their antimicrobial or anti-infective characteristics. The objective is to emphasize the critical similarities and differences in the chemical arsenals of these two species and to explore the potential of these natural compounds as cures or preventives for infectious diseases by comparing their profiles. Particular emphasis is placed on identifying furans, organic acids, and terpenes, which have been previously associated with notable antimicrobial activity. The findings of this comparative study will enhance our comprehension of the chemistry of stingless bees and may serve as a guide for the creation of novel natural antimicrobial

agents. By identifying specific metabolites that are abundant and bioactive in each species, this research enables the targeted exploration of compounds with promising mechanisms of action against pathogenic microorganisms.

MATERIALS AND METHODS

Sample Collection: Honey samples were obtained from Itama sp. and T. laeviceps bee colonies in the same geographic region (Gianyar, Bali). Each honey sample was cleaned of debris and stored at 4 °C in the dark until extraction. To ensure comparability, both species' samples were collected during the same season and from hives with similar environmental exposure.

GC-MS Analysis: An Agilent Technologies 7890B GC system coupled with a 5977A mass selective detector was used. The GC had a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature program was: 50 °C hold for 2 min, then ramp at 3 °C/min to 300 °C, hold for 10 min. Helium was the carrier gas at a constant 1.0 mL/min flow. The injection volume was one µL with a split ratio 1:20. The injector and transfer line temperatures were set to 250 °C and 280 °C, respectively. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. Mass spectra were acquired in the range of 50–550 m/z.

Compound Identification: Peaks in the total ion chromatogram were integrated, and their mass spectra were compared against the NIST and Wiley mass spectral libraries for identification. A match quality above 70% was considered acceptable for tentative identification, and wherever possible, identifications were confirmed by comparing retention indices with literature values. Identified compounds were recorded with their retention time (RT) and relative abundance, expressed as a percentage of the total ion chromatogram area (% area). Only compounds comprising at least 0.5% of the total area in at least one species were considered "notable" and included in comparisons to focus on major constituents.

Data Analysis: The list of identified metabolites for each species was compiled, and compounds present in both species were noted. The absence of unique compounds in the other species was confirmed by checking that no matching retention time and mass spectral signal were found. A bar graph was prepared to compare the relative concentrations of selected primary metabolites between Itama sp. and T. laeviceps (Figure 1). A table (Table 1) listed key metabolites, their retention times, and relative abundances in each species. "Not detected (ND)" indicates compounds not present above trace levels in a given species. This comparative analysis emphasizes compounds with higher area and potential antimicrobial relevance.

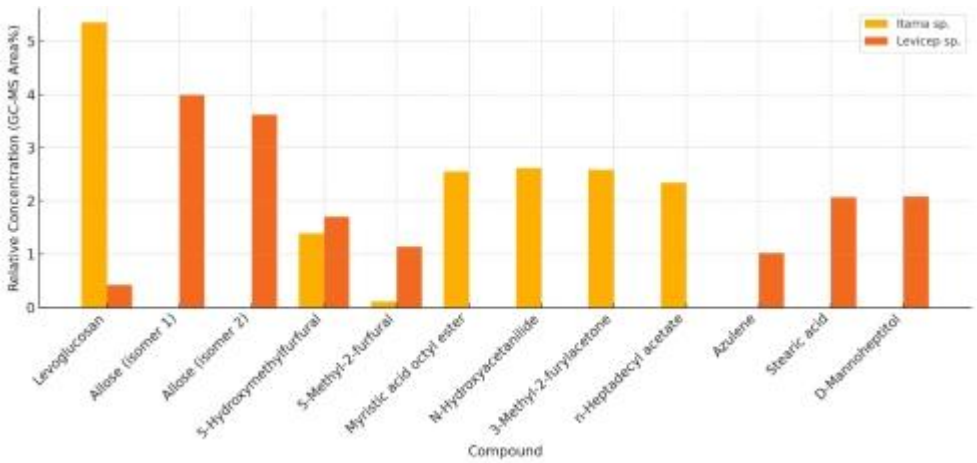
RESULTS AND DISCUSSIONS

GC-MS Profiles

The GC-MS analysis revealed the complex chemical compositions of both Itama sp. and T. laeviceps extracts. Itama sp. revealed approximately 80 peaks, including sugars, furan derivatives, and various aliphatic and aromatic constituents. T. devices showed a similar level of complexity in their chromatogram, with about 90 peaks, but the main

compounds were distributed differently. The amounts of the essential compounds (more than 1% of the total area) found in each species are shown in picture 1. It was readily apparent that the two species share some common metabolites but exhibit distinct dominant compounds. Figure 1 displays the relative concentrations of six representative primary metabolites in both species for direct comparison. It is essential to mention that levoglucosan (1,6-anhydro-β-D-glucose), a type of sugar alcohol (8), was the main ingredient in the Itama sp. extract, making up 5.35% of the total area measured by GC-MS. In contrast, T. laeviceps showed very little levoglucosan, at only 0.42%. Conversely, T. laeviceps exhibited minimal levoglucosan detection (0.42%).

Conversely, a high concentration of allose, a rare hexose sugar (9), distinguished the T. laeviceps chromatogram. T. laeviceps showed two peaks of allose (likely different forms) at around 15.6–15.8 minutes, making up about 7.6% of the total area. These sugar compounds (levoglucosan in Itama and allose in T. laeviceps) were some of the most common ones found, indicating a fundamental difference in the resin or nectar makeup used by each bee species. In addition to carbohydrates, both profiles contained various furan derivatives, albeit in varying quantities. Itama sp. had 1.39%, and T. laeviceps had 1.70% of 5-hydroxymethylfurfural (HMF), a substance made from carbohydrates, and both had similar amounts. Itama sp. contained only 0.11% of 5-methyl-2-furaldehyde (5-methylfurfural), whereas T. laeviceps contained approximately 1.14%. A related compound, 5-methyl-2-furaldehyde (5-methylfurfural), was also a minimal component. These compounds are frequently produced because of the heating or maturation of honey or plant resins, which implies that both extracts contain some heat-derived products (3). Such products could result from natural thermal processing in the hive or during sample processing.



Picture 1. Comparative abundance of selected primary, secondary metabolites in Itama sp. vs. T. laeviceps honey extracts. Bars represent each compound's relative concentration (GC-MS area) in the two species. Itama sp. (yellow) shows higher levels of levoglucosan and furanyl compounds, whereas T. laeviceps (orange) is richer in allose (hexose sugar), stearic acid, and azulene. 5-Hydroxymethylfurfural (5-HMF) is

present in both species at similar levels. Octyl myristate (myristic acid octyl ester) was abundant in Itama sp. but not detected in T. laeviceps, while azulene and stearic acid were unique to T. laeviceps.

Numerous intriguing aromatic and terpenoid compounds distinguished the two species. T. laeviceps extract contained a unique concentration of azulene, an aromatic bicyclic sesquiterpene (3). In its pure form, this compound stands out for its intense blue color. The volatiles of T. laeviceps contained approximately 1.02% azulene (RT ~7.834 min), which was not detected in Itama sp. On the other hand, Itama sp. showed a strong signal for an aromatic N-phenolic compound called N-hydroxyacetanilide (related to acetaminophen) at RT 16.97 min (2.61%). At the same time, T. laeviceps did not have this compound. T. laeviceps did not contain this compound. The presence of N-hydroxyacetanilide in Itama honey is intriguing, as it is not a typical honey constituent. It could be a byproduct of other biochemical transformations or plant sources. Although it is not recognized as an antimicrobial, the presence of this substance emphasizes the chemical diversity of Itama honey (2,10).

Aliphatic acids and esters were also in varying quantities in the two bee species. A long-chain saturated fatty acid, octadecanoic acid (stearic acid, RT 20.87 min, 2.07%), was the source of a distinct peak in T. laeviceps. In contrast, Itama sp. exhibited only trace quantities of free stearic or palmitic acid. Itama honey, on the other hand, had a considerable amount of esters. Myristic acid, octyl ester (octyl tetradecanoate), was one of the main constituents in Itama honey. It eluted at RT 7.55 min and accounted for 2.55% of the extract. Additionally, Itama sp. had a notable long-chain ester, n-heptadecyl acetate (heptadecanol acetate, RT 18.45 min), making up 2.34%. These esters were either absent or present at significantly reduced levels in T. laeviceps. For instance, a trace of heptadecyl acetate was tentatively detected in T. laeviceps, but it was below the 0.5% threshold for reporting.

Lastly, the honey extracts of both species contained moderate levels of other cyclic or aromatic compounds. For example, Itama sp. had about 2.58% of a sweet-smelling furanone called 3-methyl-2-furylacetone (also known as 1-(3-methyl-2-furyl)-2-propanone, RT 15.53 min). T. laeviceps did not have this compound. T. laeviceps sp. did not exhibit this compound. On the other hand, T. laeviceps showed about 2.09% of D-mannoheptitol (which is also called perseitol or styracitol, RT 17.93 min), a seven-carbon sugar alcohol found in some stingless bee honeys and certain plant saps. Mannoheptitol was not detectable in Itama sp. Itama sp. honey seems to have more substances from resin and products formed at high temperatures, while T. laeviceps honey might have more plant nectar or sap because it has a lot of sugar, alcohol, and sugar.

Table 1. Key secondary metabolites were identified in *Itama sp*. and *T. laeviceps* honey extracts (GC-MS data). Retention times (RT) and relative concentrations (as % of total ion chromatogram area) are given for each compound in both species. "ND" denotes not detected above trace level.

Compound	RT (*Itama*)	Area% (*Itama*)	RT (*T. laeviceps*)	Area% (*T. laeviceps*)
Levoglucosan (1,6-Anhydro-β-D-glucose)	15.899	5.35	16.106	0.42
Allose (hexose sugar, isomer 1)	–	–	15.617	3.99
Allose (hexose sugar, isomer 2)	–	–	15.771	3.62
5-Hydroxymethylfurfural (HMF)	11.636	1.39	11.230	1.70
5-Methyl-2-furfural (5-methylfurfural)	4.630	0.11	4.240	1.14
Myristic acid, octyl ester (octyl tetradecanoate)	7.548	2.55	–	–
N-Hydroxyacetanilide (p-hydroxyacetanilide)	16.974	2.61	–	–
3-Methyl-2-furyl acetone (furanyl acetone)	15.525	2.58	–	–
n-Heptadecyl acetate (heptadecanol acetate)	18.451	2.34	–	–
Azulene (bicyclic sesquiterpene)	–	–	7.834	1.02
Octadecanoic acid (stearic acid)	–	–	20.870	2.07
D-Mannoheptitol (perseitol, 7-C sugar alcohol)	–	–	17.932	2.09

Note: RT = retention time in minutes. "ND" (not detected) is indicated by an absence (–) of a peak corresponding to that compound in the given species. All compounds listed were identified with library match scores ≥80% except *N*-hydroxyacetanilide (match ~70%, tentative). Allose appeared as two separate GC peaks (likely α- and β-anomeric forms); both are listed.

Discussion

This comparative study offers a unique perspective on how two stingless bee species utilize their honey constituents to implement distinct chemical defenses. The data shows that T. laeviceps and Itama sp. honey have a variety of secondary metabolites, with some similarities but many different compounds. These distinctions may significantly affect their prospective application in the fight against infections. The prevalence of sugar-derived compounds in both species, albeit in distinct forms, is a noteworthy discovery. The honey of Itama sp. was abundant in levoglucosan, a dehydration product of glucose (11,12). Levoglucosan is not known to fight germs; it probably comes from heating plant materials, like when they burn a little or get very hot in resin sources. Its presence implies that Itama beekeepers may accumulate resin or sap subjected to heat from sun-exposed tree resins or as a byproduct of beeswax heating during honey production (13).

Conversely, T. laeviceps honey contained substantial quantities of the intact sugar allose. Despite not typically being antimicrobial, researchers have identified allose, a rare sugar, as having anti-inflammatory and antioxidant properties (11). The high allose content may suggest that T. laeviceps honey contains more untransformed plant fluid or nectar (possibly because of variations in how this species processes honey or the plants it visits). Even though sugars like allose and sugar alcohols (like mannoheptitol in T. laeviceps) don't kill germs directly, they might change the environment around them or help transport other beneficial compounds. Also, uncommon carbohydrates might shift the balance of microbial communities or act as prebiotics, which could help improve anti-infective effects in a complicated way (14,15).

Due to their recognized bioactivities, the presence of furan compounds in both species is of particular interest. Recent studies have shown that 5-hydroxymethylfurfural (HMF) in Itama and T. laeviceps extracts can prevent bacteria like Pseudomonas aeruginosa from communicating and producing harmful substances (16). This effectively suppresses bacterial communication and virulence factor production. This indicates that the presence of HMF in stingless bee honey may help prevent biofilm formation, a critical factor in chronic infections, thereby inhibiting the establishment of bacterial infections (14).

Even though HMF is only present in moderate amounts in honey (about 1–2%), it may work better with other helpful compounds to boost its ability to fight germs. Similarly, 5-methylfurfural, which is commonly found in T. laeviceps, is another type of sugar that could boost antimicrobial activity. Furfural and its alkylated derivatives are recognized for their bacteriostatic effects on specific microorganisms (17). They have been employed as industrial antiseptics in the past and have the potential to disrupt microbial metabolism. The higher amount of 5-methylfurfural in T. laeviceps honey might boost its ability to stop the growth of microbes, especially in bacteria that are sensitive to furan aldehydes (18).

The content of terpenoid and aromatic compounds is a significant distinction between the species. Azulene, an azulenoid sesquiterpene, was present in T. laeviceps honey. The anti-inflammatory and moderate antimicrobial properties of azulene (and its derivatives, such as chamazulene and guaiazulene) are well-known for chamomile and other medicinal plants. Azulene has been reported to possess antibacterial properties, particularly when activated by light or in specific formulations (it is even being investigated in antimicrobial photodynamic therapy) (19). The presence of azulene in T. laeviceps honey is significant because it suggests that the bees may be accumulating resin or sap from plants that contain azulene, such as composites or other plants that impart this compound.

The anti-inflammatory properties of azulene and the antimicrobial properties of honey can be combined to reduce tissue inflammation, while other components are used to combat pathogens. In contrast, Itama sp. honey did not contain azulene but an uncommon aromatic amide, N-hydroxyacetanilide (20,21). This molecule, structurally like the painkiller acetaminophen and not classified as an antibiotic, highlights the biochemical uniqueness of Itama honey. The exact role of N-hydroxyacetanilide in honey remains ambiguous; it may serve as an antioxidant or interact with other compounds, although it does not indicate that it fights germs (20).

The identification of several fatty acids and esters offers insight into the potential antibacterial effects that these pollinators may exert through non-polar molecules. Medium- to long-chain fatty acids are commonly found in honey and can break microbial cell

membranes, demonstrating antibacterial and antifungal capabilities (22). In our investigation, T. laeviceps comprised merely a small proportion of free stearic acid (about 2%), whereas Itama sp. Free saturated fatty acids, such as stearic acid, exhibit moderate antibacterial properties; generally, shorter-chain (C8–C12) or unsaturated fatty acids demonstrate more potency.

A significant distinction between the species lies in the concentration of terpenoid and aromatic chemicals. Itama sp. had a distinctive aromatic amide, N-hydroxyacetanilide. This molecule, chemically analogous to the painkiller acetaminophen, is not a recognized antibiotic, yet its existence underscores the biochemical distinctiveness of Itama honey(21). It may derive from plant metabolites (some plants synthesize acetanilide-like molecules as defensive chemicals) or interactions with bee metabolism. The biological function of N-hydroxyacetanilide in honey remains ambiguous; it may serve as an antioxidant or have a synergistic impact; however, it does not explicitly indicate antibacterial activity (22).

Nonetheless, stearic acid can still impede the growth of specific Gram-positive bacteria by incorporating into and disrupting their membranes. Stearic acid in T. laeviceps honey may provide an inhospitable environment for microbial invaders (23). Itama honey contains octyl myristate, heptadecyl acetate, and long-chain wax esters. These chemicals may lack significant antibacterial properties alone, but they fulfill alternative functions: they can render honey hydrophobic, aiding in sealing gaps and repelling moisture. Establishing a water-resistant barrier indirectly inhibits microbial colonization, as microorganisms struggle to proliferate on dry, resinous surfaces. Furthermore, upon hydrolysis (by enzymes or over time), these esters may liberate fatty acids (specifically myristic acid in the instance of octyl myristate), which has antibacterial properties (myristic acid can compromise cell membranes, albeit with less efficacy than lauric acid) (4,24).

Prior studies on Brazilian Apis honey with unusually elevated fatty acid levels indicated that fatty acids such as oleic, palmitic, linoleic, and stearic acids may contribute to its antibacterial properties. Despite later fractionation revealing that other chemicals, such as benzophenones, are the principal active agents, the hypothesis that fatty components contribute to honey's antibacterial properties remains probable. In our instance, T. laeviceps honey contains free stearic acid. At the same time, Itama sp. possesses long-chain esters, indicating divergent strategies: T. laeviceps may depend more on the direct action of a fatty acid, while Itama honey may focus on forming a protective waxy barrier and the gradual release of active lipids (5,6).

It is crucial to highlight that these chemicals function synergistically in natural honey. Although individual constituents such as HMF or fatty acids exert quantifiable effects on microorganisms, the honey extract frequently demonstrates superior activity to any standalone component. This happens because different components work better together; for example, when HMF and fatty acid are combined, they can break down bacterial cells and stop them from communicating, which boosts the overall impact (4,11,18). Similarly, the sugars in T. laeviceps honey might help keep moisture or create a protective layer, allowing other medicinal compounds like azulene or stearic acid to work against bacteria. The intricate formulation probably affects several microbial pathways simultaneously, complicating the development of resistance in pathogens (19,23,25). This combination of processes is one reason honey has been effective against germs for ages and is a promising source for drug discovery amid increasing antibiotic resistance. The Itama sp. and T.

laeviceps results indicate that honey from various stingless bee species may be customized for medical use.

Itama sp. honey, which contains furans and aromatic compounds, might be particularly good for treating skin infections or acting as a disinfectant because it is expected to be strong against Gram-positive bacteria, common in wounds and skin infections (26). The ingredients in Itama sp. honey, like furfural derivatives and phenolics, make it a good option for oral health products that help stop bacteria that cause plaque (27,28). T. laeviceps honey, which has azulene and a lot of sugars, could be a good option for a dressing that reduces inflammation and fights germs; azulene helps reduce swelling, while HMF and fatty acids quietly stop bacteria from growing, and sugars help keep the area moist for healing. Indeed, additional bioassay-guided investigations are necessary to validate these ideas; nonetheless, our chemical analysis offers a framework for anticipated activity (2,15).

GC-MS methodology predominantly analyzed the lower polarity component of honey. Further research, like using LC-MS, would probably find more polar antioxidant compounds (flavonoids, phenolic acids), especially in Itama honey, which could improve its ability to fight infections since flavonoids are known for their strong antibacterial effects (14). Consequently, the observed discrepancies may be much more pronounced when accounting for the entire range of chemicals. Additional polar compounds, such as phenolic glycosides or peptides, may also influence T. laeviceps honey, but this GC-MS analysis did not detect them (15). The limitation of this study is the absence of quantitative calibration; the area percentage indicates relative abundance but not absolute concentrations. However, we justified our emphasis on contrasting relative profiles by conducting both samples under identical settings. Another consideration is that certain chemicals, such as N-hydroxyacetanilide, were tentatively identified; validating them with standards would reinforce the conclusions. Notwithstanding these reservations, the principal chemicals are unequivocally identified, and the observed patterns are resilient.

CLINICAL IMPLICATION

Identifying bioactive secondary metabolites in stingless bee products, particularly those with demonstrated anti-infective potential, such as hydroxymethylfurfural and azulene, provides a scientific basis for their integration into developing alternative therapies for managing infectious diseases. From a clinical perspective, these findings suggest that stingless bee-derived products hold potential as adjunct or alternative treatments for antibiotic-resistant bacteria or chronic biofilm-associated infections. Identifying species-specific chemical profiles could guide the targeted development of natural antimicrobial formulations for topical use or incorporation into biomaterials. Additionally, these extracts' natural origin and multi-compound synergy may reduce the risk of resistance development, offering a sustainable alternative to conventional antimicrobials.

LIMITATIONS

This study was limited by using GC-MS alone, which primarily detects volatile and semi-volatile compounds. As a result, non-volatile bioactive constituents such as flavonoids and phenolic acids may not have been fully captured. Additionally, the absence of quantitative standards restricts the ability to report absolute concentrations, and no

biological assays were conducted to confirm antimicrobial activity. Future work should include LC-MS analysis, bioactivity-guided fractionation, and in vitro antimicrobial testing to validate the therapeutic potential of identified compounds.

CONCLUSIONS

The study found that while both types of honey contain complex mixtures of active chemicals, the types and amounts of these secondary metabolites differ. Itama sp. honey has higher levels of levoglucosan, furfural derivatives, and unique aromatic compounds. In contrast, T. laeviceps honey is known for having more intact sugars (like allose), sugar alcohols, and specific terpenoids such as azulene and long-chain fatty acids.

Numerous discovered chemicals in these profiles significantly possess recognized antibacterial or therapeutic effects. 5-Hydroxymethylfurfural, found in both species, can block bacteria from communicating and forming biofilms, suggesting it might help prevent infections. Azulene, present in T. laeviceps, has anti-inflammatory and antibacterial advantages that may augment the medicinal applications of honey.

The fatty acids and esters function as natural antibacterial agents and enhance the physical barrier qualities of honey. The interaction of these varied chemicals in each honey extract likely supports their effectiveness in traditional uses, including wound healing and infection prevention. Our findings confirm that stingless bee honey is a substantial source of natural compounds with pharmacological potential. The differences between Itama and T. laeviceps honey highlight the importance of choosing the right bee species when making honey-based medicines, as different species might create honey better suited for specific diseases or health issues. Both types of honey have room for improvement: Itama sp. honey, known for its strong furanics and phenolics, and T. laeviceps honey, recognized for its gentle anti-inflammatory azulene and sugar-based components.

Future studies should focus on breaking down these honey extracts to test their effects, looking at how different chemicals work together, and checking their effectiveness and safety in living organisms. Utilizing the chemical variety developed in stingless bee hives may yield novel, multi-targeted antimicrobial medicines when innovative solutions are urgently required to combat resistant diseases.

CONFLICT OF INTEREST

The author declares no conflict of interest related to this study.

AUTOR CONTRIBUTIONS

The sole author was responsible for the conception and design of the study, acquisition, and analysis of data, interpretation of results, manuscript drafting, and final approval of the version to be submitted for publication.

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