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Volatile compounds in Trigona honey and their potential as antimicrobials

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Abstract

Indonesia is rich in natural resources, one of which is honey, which needs to be developed to improve quality and have competitiveness. This study aimed to determine the volatile compounds in Trigona honey and their potential as antimicrobials. This research was carried out in four stages, namely the sample preparation stage, the extraction stage, the volatile compound identification stage, and the determination of volatile antimicrobial compounds stage. Trigona honey samples were obtained from Masamba, South Sulawesi. The extraction was carried out in the chemical laboratory of the Faculty of Chemistry, Hasanuddin University. Honey was extracted with two non-polar (n-hexane) and semi-polar (ethyl acetate) organic solvents. Adjustment of volatile compounds extracted using Gas chromatography-mass spectrometry (GC-MS) was carried out at the The State Police of the Republic of Indonesia (POLRI) Forensic Laboratory. A total of twenty-seven volatile organic compounds including carbonates, acids, aldehydes, ketones, alcohols, phenolics, amines, imines and oximes were detected in the Trigona honey. Of the twenty-seven volatile compounds found in Trigona honey, there were six compounds that have potential as antimicrobials namely heptadecane, benzoic acids 1,3 dioxolane, eicosane, 3-hydroxy-4-methoxybenzaldehyde and phenolic compounds.

1. Introduction

Honey is a sweet liquid from plant nectar, which the bees process into honey and store in the cells of the hive. Since thousands of years, honey has been a natural food or drink that plays an important role in life (Cahyaningrum, 2019).

Based on data from the Central Statistics Agency (BPS), Indonesia produced 189,780 litres of honey in 2021. This number has increased compared to the previous year's of 51,338.26 litres. By region, the largest honey production is in Java. In 2020 honey production on Java Island was 41614.26 litres, followed by Sumatra Island at 4010.00 litres, Kalimantan Island at 3007.00 litres and Sulawesi Island at 500.00 litres (BPS-Statistik Indonesia, 2021).

Therefore, developing local honey with good quality and competitiveness is necessary. One of the local honeys in South Sulawesi is Trigona honey, which comes from Trigona spp. bees. There are three types of trigona bees in South Sulawesi, each colony consisting of 300–80,000 thousand heads (Siregar *et al*., 2011). Trigona bees are a type of bee that are small and do not sting. The amount of honey produced by the Trigona type is less than that of the Apis honey-producing bees, and it is more difficult to harvest from the hive (Suhendra and Feby Nopriandy, 2021). The trigona bee is a small black insect with a body length of 3-4 mm and a wingspan of approximately 8 mm. Worker bees have large heads and long jaws. Although the queen is 3-4 times the size of the worker, she has a large belly like a moth, is brown, and has short wings (Surata, 2017).

One of the important components of honey is the volatile compound. Volatile compounds are volatile in honey due to their low molecular weight. Volatile compounds are the main factor responsible for the aroma. The aroma profile is one of a food product's most distinctive features for evaluating its organoleptic quality and authenticity. The aroma of honey is very complex, involving dozens of volatile compounds (Tian *et al*., 2018; Shiddiq *et al*., 2021). These compounds are different for each honey depending on the origin of the flowers, the composition of the flower nectar sucked by

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the honey, and their geographical origin so that they give different aroma profiles to honey and are characteristics that can be used to distinguish honey based on their botanical origin (Tian *et al*., 2018). Several volatile compounds in honey have antimicrobial potential (Burgut, 2020). This study aimed to identify the volatile compounds of Trigona honey and their potential as antimicrobials.

2. Materials and methods

The research design was exploratory to identify volatile compounds and proximate analysis of South Sulawesi trigona honey. The research was conducted at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University, to extract Trigona honey, and at the Forensic Laboratory of South Sulawesi Province to identify the volatile compounds of Trigona honey.

2.1 Sample preparation

Sampling was carried out in Masamba District, South Sulawesi Province. Honey samples are stored in glass bottles that are cool and dry. Initially, the honey was weighed (1 g) and diluted with 1 mL of aquabidest. The mixture was vortexed for 2 -3 mins to ensure the mixing of honey and aquabidest before liquid-liquid extraction and continued with compound identification using GC-MS.

2.2 Liquids extraction

Two organic solvents (n-hexane and ethyl acetate) with increasing polarity were selected. First, n-hexane (4 mL) was added to a sealed vial containing diluted honey. Then, the mixture was vortexed at 1500 rpm for 2 mins before the sonicate for 20 mins to separate the organic layer from the aqueous layer. The top layer containing the organic solvent was transferred to a closed vial while the bottom layer was added with the same solvent then the above method was repeated three times. The first, second, and third extracted organic layers were combined and centrifuged at 2500 rpm for 10 mins, and the top layer was pipetted. Then, Na_2SO_4 was added to remove the remaining water, and ready to be analysed using Gas chromatography-mass spectrometry (GC-MS).

2.3 Analysis using gas chromatography-mass spectrometry

GC-MS analysis was performed on an Agilent 5975c. The column was HP-5MS fused silica capillary column, and helium running at constant pressure was used as the carrier gas. The following conditions were used: initial temperature 40°C, equilibration time 5 mins, run 25°C/min, final temperature 310°C. The NIST and Wiley libraries identified all peaks based on mass spectral matching (≥90%). Only compounds with 90% or greater spectral matching accuracy are reported.

2.4 Determination of volatile antimicrobial compounds

The volatile compounds obtained from the GC-MS results are then identified which compounds belong to antimicrobials using literature studies, matching them with the literature in the form of research results both from within and outside the country.

3. Results and discussion

Overall, twenty-seven volatile compounds from Trigona honey have been identified. Eighteen volatile compounds were obtained from the extraction of trigona honey using a non-polar solvent (n-hexane), and nine volatile compounds were obtained from the extraction of trigona honey using a semi-polar solvent (ethyl acetate).

Based on chemical structure analysis, there are chemical compounds from different groups in trigona honey, including hydrocarbons, acids, aldehydes, ketones, alcohols, phenolics, amines, imines, and oximes.

Table 1 shows the volatile compounds of Trigona South Sulawesi honey after being extracted using two organic solvents and injected into the GC-MS tool, retention time and concentration.

Table 1. Trigona honey volatile compounds from the results of extraction using organic solvents (N-hexane).

No	Name Compound	RT	Percentage (%)
1	2,2,7,7-tetramethyloctane dodecane	12.69	0.70
2	Heptadecane	12.82	0.83
3	1-Hexadecanol	13	0.83
4	Decan	13.1	0.89
5	Benzoic Acid	13.13	0.80
6	Dodecane	13.67	0.69
7	1,3-Dioxolane	14.53	0.64
8	Oxalic Acid	14.57	0.65
9	Tetradecane	14.94	0.72
10	2,5-Cyclohexadiene-1,4 Dione	15.47	0.97
11	Eicosane	15.68	1.27
12	3-Chlorooxanilic acid	15.75	0.48
13	3-Hydroxy-4-Metoxybenzaldehyde	15.94	0.38
14	Phenethylamine	16.22	1.91
15	Silicic Acid	16.43	0.92
16	Phenol	16.74	1.53
17	Propiophenone	16.83	0.61
18	1H-Indole-2-carboxylic acid	17.01	0.94

The volatile compounds in trigona honey belonging to the hydrocarbon group were 2,2,7,7-tetramethyloctane dodecane, Heptadecane, decan, Dodecane, 1,3–

Dioxolane, Tetradecane, 2,5-Cyclohexadiene-1,4 Dione, Eicosane, Ethane and Toluene.

The volatile compound belonging to the alcohol group is 1-Hexadecanol. Volatile compounds belonging to the acid group were benzoic acid, oxalic acid, 3-chlorooxanilic acid, silicic acid, 1H-Indole-2-carboxylic acid, benzene acetic and 1,2-benzenedicarboxylic acid.

Compounds belonging to the aldehyde group are 3-hydroxy-4 methoxy benzaldehyde or called isovanillin. Compounds belonging to the ketone group were propiophenone, methyl isobutyl ketone, 3,5,5-trimethyl-2-cyclohexane-1-one or called is phorone.

The compounds of the phenolic group are phenols and the compounds of the imine group are oxime, methoxy-phenyl. Compounds belonging to the oxime group are 3-ethoxyphenylacetone hydroxy oxime. Compounds belonging to the amine group were phenethylamine and (2) 1H-indol-2-amine.

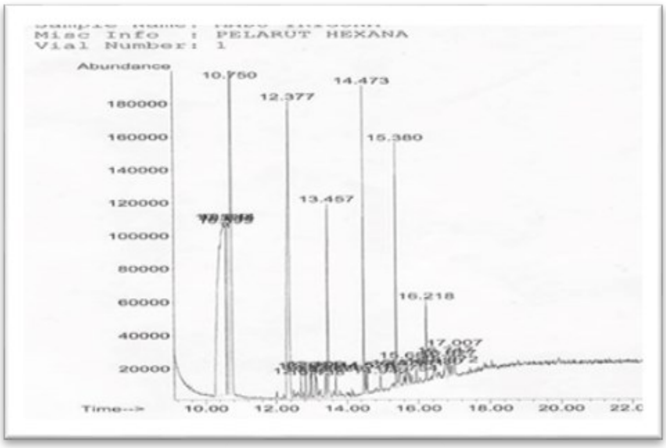

Figure 1. Chromatogram obtained after running Trigona honey n-hexane extract samples in GC-MS.

Honey has antibacterial activity. One reason is the presence of compounds that are antibacterial in honey. Antimicrobial substances are compounds that can kill or inhibit the growth of microorganisms. Antimicrobial substances can kill microorganisms (microbicidal) or inhibit the growth of microorganisms (micro biostatic).

Several compounds have antibacterial properties in the volatile compounds of honey Trigona South Sulawesi. Of the 27 volatile compounds found in Trigona honey, six compounds have potential as antimicrobials, namely heptadecane, benzoic acids 1,3 dioxolane, eicosane, 3-hydroxy-4-methoxy benzaldehyde, and phenolic compounds.

Heptadecane compounds can inhibit the de novo synthesis of fatty acids and improve the conditions of several oxidative stress-related diseases. They are also known as anti-inflammatories in their ability to suppress the expression of COX-2 and iNOS (second NF-kB-related genes) in mice (Kim *et al*., 2013).

The second volatile compound as an antimicrobial is benzoic acids. This compound is used in the synthesis of perfumes and dyes. Benzoic acid (C_6H_5COOH) is a widely used preservative and is often used in food ingredients. Benzoic acid is commonly used in acidic foodstuffs to prevent the growth of yeast (yeast) and mold (downy mildew). Benzoic acid is more effective against yeast than mold. Benzoic acid is used as an antimicrobial in preserved sour fruits; at a pH of 2.5-4.0, the use of benzoic acid becomes more effective in these conditions. The activity of benzoic acid as an antimicrobial can inhibit fungi and bacteria (fungistatic and bacteriostatic) and kill fungi and bacteria (fungicide and bactericidal) by penetrating or damaging the microbial cell membrane tissue, resulting in cell death (Rorong, 2014).

The third compound classified as antimicrobial among the volatile compounds in trigona honey is 1,3-Dioxolane. This compound has solid antibiotic potential against *Escherichia coli* bacteria (Syarifuddin *et al*., 2019).

The fourth volatile compound that is classified as antimicrobial is eicosane. Eicosane is an antimicrobial compound in the marine microalgae Tetrahemes Chui, which is effective against *E. coli*, *Staphylocccus aureus*, *Candida albicans* and *Aspergillus flavus* (Syarifuddin *et al*., 2019).

The fifth volatile compound that is classified as an antimicrobial is 3-hydroxy-4-methoxy benzaldehyde or also called isovanillin. Isovanillin is a member of the benzaldehyde class, which has a role as a plant metabolite, antidiarrheal drug, antifungal agent, HIV protease inhibitor, and animal metabolite (Rose *et al*., 2023).

Table 2. Trigona honey volatile compounds from extraction using organic solvents (Ethyl acetate).

No	Name Compound	RT	Percentage (%)
1	Ethane	9.62	3.66
2	Methyl Isobutyl Ketone	9.76	3.20
3	Oxime, Methoxy-phenyl	11.57	21.83
4	Toluene	10.17	35.69
5	3-Ethoxyphenylacetone Hydroxy oxime	12.05	0.72
6	Benzene acetic Acid	13.46	1.72
7	3,5,5-Trimethyl-2-cyclohexen-1-one	13.51	15.41
8	1H-Indol-2-Amine	14.48	0.46
9	1,2-Benzenedicarboxylic Acid	16.04	1.55

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The sixth trigona honey volatile compound, which acts as an antimicrobial, is a phenolic compound. Phenolic compounds are the best source of antioxidants. Phenolic compounds also function as antimicrobials. Several studies have proven that this compound exhibits high antibacterial activity against Staphylococcus epidermidis and Pseudomonas aeruginosa. Tannic acid and epigallocatechin gallate present the highest microbial inhibition potential (Mandal *et al*., 2017). Likewise, for the bacteria *Listeria monocytogenes*, *E. coli*, *Salmonella enterica* serovar Enteritidis, *S. aureus* and *Bacillus subtilis* (Mandal *et al*., 2017). The phenolic compounds in honey and propolis have an extraordinary affinity for two enzymes in the novel coronavirus (2019-nCoV), the main protease enzymes (Mpro and RNA-dependent RNA polymerase) (Shaldam *et al*., 2021).

The mechanism of action of phenolic compounds as antibacterial is to poison protoplasm, damage and penetrate cell walls, and precipitate microbial cell proteins. Phenolic components can also denature the enzymes responsible for spore germination or affect the amino acids involved in the germination process (Natural Food Antimicrobial Systems, 2000).

These compounds play a role in fighting bacteria, so honey is used as a treatment. One of them is the treatment of respiratory tract infections. Honey has been shown to relieve symptoms of tracheal and lung diseases (Lauer *et al*., 2020; Abuelgasim *et al*., 2021). The use of honey in aerosol form has been shown to reduce the number of airway inflammatory cells in bronchoalveolar lavage fluid and inhibit goblet cell hyperplasia (Kamaruzaman *et al*., 2014). Honey (aerosol) and propolis in the steam inhalation procedure can help reduce the symptoms of COVID-19 (Al Naggar *et al*., 2021; Shaldam *et al*., 2021).

Honey has been used in many cultures since ancient times as an effective remedy for its therapeutic properties including antioxidant, anti-inflammatory, antibacterial and immunomodulatory properties. Its antibacterial activity is attributed to four prominent factors: hydrogen peroxide levels, hyperosmolarity (low water activity), acidity (pH) (Kwakman *et al*., 2010).

Another factor that makes honey have potential as an antimicrobial is the composition of the honey. Trigona honey is a solution of saturated or supersaturated carbohydrates containing the most glucose and fructose (87.41%) Apparently, the osmotic effect of honey is due to the fact that the strong interaction between sugar and water molecules leaves very little or no water to support growth of microorganisms (bacteria and fungi as they thrive in moist environments); As a result, they become dehydrated and eventually die (Mandal and Mandal, 2011).

The water content of Trigona honey is relatively low, namely 10.90% (Astuti *et al*., 2019), which meets the 2013 Indonesian National Standard (SNI 01-3545-2013) stating that good quality honey has a maximum water content value of 22% (Badan Standarisasi Nasional Indonesia, 2013).

In addition, honey is acidic. The pH value of Trigona honey is 4.0 due to the presence of organic acids such as benzoic acid, oxalic acid, salicylic acid and is low enough to inhibit the growth of most pathogenic organisms for which the optimal growth pH is usually between 7.2 and 7.4 (Adityarini *et al*., 2020).

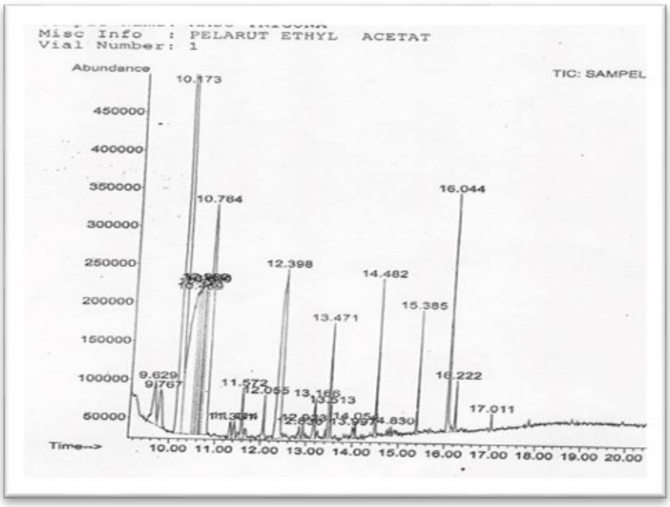

Figure 2. The chromatogram obtained after running Trigona honey ethyl acetate extract samples in GC-MS.

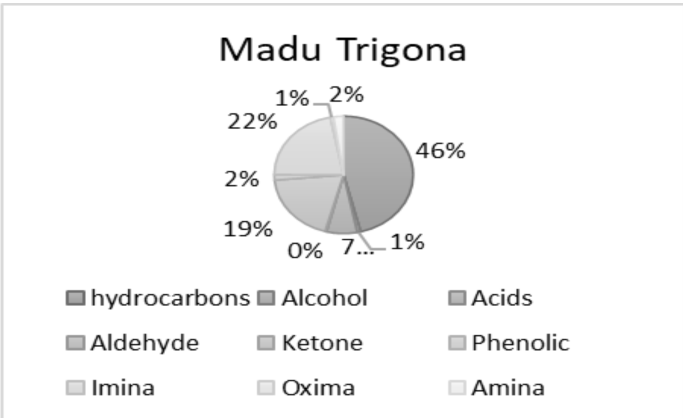

Figure 3. Percentage of Trigona honey by group (chemical structure).

4. Conclusion

Overall, 27 volatile compounds from Trigona honey have been identified, which are divided into several groups, namely hydrocarbons (46.06%), imines (21.83%), ketones (19.22%), acids (7.06%), amines (2.37%), phenolics (1.53%). %), alcohol (0.83%), oxime (0.72%) and aldehyde (0.38%). Based on the literature,

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of the 27 volatile compounds found in Trigona honey, six compounds have potential as antimicrobials, namely heptadecane, benzoic acids, 1,3 dioxolane, eicosane, 3-hydroxy-4-methoxy benzaldehyde, and phenolic compounds.

Conflict of interest

All authors declare that they have no conflicts of interest.

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