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Characterization of Chemical Composition of Methanolic Extract of Honey from Haryana Region in India

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Abstract
Problem: The main of this study was to find out the chemical composition of methanolic extract of honey through gas chromatography- mass spectrometry. Honey is a complex blend of nutrients and bioactive substances with several biological functions. Bees absorb the floral nectar from a variety of flora to make honey, which is then converted into simple sugars and stored inside the honeycomb. **Approach:** A specific honey's Volatile Organic Compounds (VOCs) can be utilized to distinguish it from honey that comes from different botanical and geographic floral sources. In this study, the chemical profile of the methanolic extract of honey sample is analyzed with the help of gas chromatography- mass spectrometry.The sample of honey was collected from the Jhajjar region of Haryana, India, and the chemical profile of the methanol extract of the honey sample was analyzed with the help of gas chromatography-mass spectrometry. **Finding:** Results showed the presence of hydrocarbons, alcohols, aldehydes, amino acids, ketones, and esters were observed in the GC-MS result analysis. **Conclusion:** Current findings authenticate the huge diversity of chemical composition in honey, and hence, further study on honey is required due to its broad spectrum of potential medicinal, nutritional, and other applications.

Keywords: Gas chromatography- mass spectrometry, honey, chemical composition, volatile components.

Introduction

Bee products has become very important commercially due to its nutritional qualities, medicinal properties as well as healing powers (Rana, 2021; Rana et al., 2022a&b;Rana and Kumar, 2022; Rana and Parmar, 2022; Khakhlary and Rana, 2023; Rana and Kumar 2023;Rana and Bajwa, 2023). Among them, honey has been used in traditional medicine since ancient times (Robotti, 2017; Montaseret al., 2023). It is an essential product of bees made from plant nectar and has long been a great nutritional alternative for many generations owing to its medicinal properties (Eteraf-Oskouei and Najaf, 2013; Nayik et al., 2014; Dekebo et al., 2018). Honey bees consume nectar, floral secretions, or other living components of plants and then convert these substances by mixing them with specific compounds of their own secretions (Ajibola et al., 2012) and after mixing these were placed in the honeycomb, dehydrated, and allowed to develop and mature (Khan et al., 2018).

It is a complex mixture of organic and inorganic components (Khan et al., 2018). Approximately 300 varieties of honey have been recognized (Lay-flurrie, 2008). These variants are associated with many types of nectar gathered by honeybees. Its major component is carbohydrates, which account for 95-97% of its dry weight. It also contains significant amounts of proteins, vitamins, amino acids, minerals, organic acids, flavonoids, polyphenols, reducing substances, alkaloids, glycosides, cardiac glycosides, anthraquinone, and volatile compounds (Betts, 2008; Islam et al., 2012 and Samarghandianet al., 2017). The medicinal/pharmaceutical properties of honey are due to the manifestation of different antioxidant compounds such as; phenolic acids, flavonoids and polyphenols (Talha et al., 2023).

The composition and activity of honey is dependent upon environmental conditions and botanical sources such as; floral source, geographic origin, weather/climatic conditions, processing, storage and its handling (Belitz, 2009). It can be pale yellow, dark reddish, or almost black in color depending upon temperature and its storage time (Beretta et al., 2005).

In traditional medicine, honey was used for a wide range of ailments, including ulcer and wound healing, eye disorders, asthma, tuberculosis, throat infections, hiccups, thirst, exhaustion, hepatitis, dizziness, constipation, worm infestation, piles and eczema (Bansal et al., 2005; Eteraf-Oskouei and Najaf, 2013; Talha et al., 2023). People are not entirely familiar with its mechanism and scope (Blair et al., 2009), therefore more study is being done on therapeutic uses of honey in the twenty-first century. Thus it is now quickly becoming a component of modern medicine (Majtan and Martin, 2010).

In this study, the GC-MS analysis of the volatile compounds in honey was studied in order to better understand the resource's potential for future therapeutic uses.

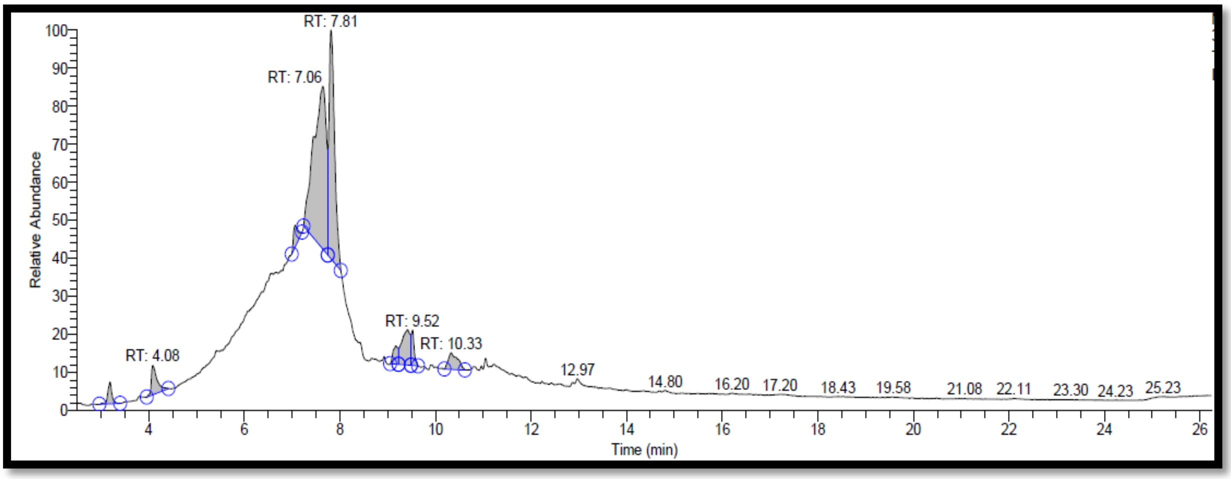

Fig 1: Chromatographic analysis of methanolic extract of honey

Materials and Methods

Collection of Honey

The honey sample from the honey bee species Apis mellifera was obtained from a private apiary located in Jhajjar, Haryana, India. This honey sample was a multiflora type.

Préparation of a methanolic extract of honey

The multiflora honey sample was prepared for the GC-MS analysis using methanol. 5g sample of honey was dissolved in 50ml of methanol. The solution was continuously stirred until there was a clear solution. The sample was then sent for GC-MS analysis.

GC-MS Analysis

The chemical profiling of the pollen sample was analyzed by the process of GC-MS using Thermo Trace 1300 GC coupled with triple quadrupole MS TSQ8000. 1μl of the solution was injected at an injector temperature of 290°C.The GC oven temperature was programmed from 50°C (held for 1 min) to 225°C at a rate of 20°C /minute which was further increased to 270 °C at a rate of 40°C/minute. The carrier gas was helium at a constant flow rate of 1ml/min. Mass spectrometry mode was used during analytical scanning from 50–600 atomic mass units (amu).The ion source temperature was set to 250 °C. The run time was 24 mins. The peaks were all detected using mass spectral matching from the NIST 2.0 library.

Statistical analysis

The identification of peaks was performed by taking a single sample and comparing the results with commercial reference libraries such as the NIST library.

Results and Discussion

The GC-MS analysis result led to the identification of 30 individual compounds that belong to the classes of various organic compounds including hydrocarbons, alcohols, aldehydes, esters, carboxylic acids, fatty acids, ketones, etc.

Cpd	Name	Classification	Formula	RT
	Trifluoroguanidine	Amidines	$CH_2 F_3 N_3$	3.19
	Ethylhydroxylamine	Alcohol	$C_2 H_7NO$	3.19
	p-Dioxane-2,3-diol	Heterocyclic compound	$C_4 H_8\ 0_4$	3.19
	Methane, (methylsulfinyl)(methylth io)	Hydrocarbon	$C_3 H_8 O S_2$	3.19
	Pyrazole, 1,4-dimethyl	Heterocyclic aromatic compound, Hydrocarbon	$C_5 H_8\ N_2$	4.08
	Furfural	Aldehyde	$C_5 H_4 O_2$	4.08
	3-Furaldehyde	Aldehyde	$C_4 H_5 O_2$	4.08
	2-Furancarboxylic acid, 1-cyclopentylethyl ester	Carboxylic Acid, ester	$C_{12} H_{16} O_3$	4.08
	L-Histidine, 1-methyl	Amino acid	$C_7 H_{11} N_3 O_2$	4.08
	á-d-Mannofuranoside, O-geranyl	Sugar	$C_{16} H_{28} O_6$	7.06
	Butanoic acid, 3-methyl-, 3,7-dimethyl-2,6-octadienyl ester	Fatty acid, ester	$C_{15} H_{26} O_2$	7.06
	4,8-Decadienal, 5,9-dimethyl	Aldehyde	$C_{12}H_{20}O$	7.06
	Geranyl vinyl ether	Hydrocarbon	$C_{12} H_{20}O$	7.06
	2-Octen-1-ol, 3,7-dimethyl-	Alcohol	$C_{10} H_{20}O$	7.64
	Citronellol	Alcohol	$C_{10} H_{20}O$	7.64
	6-Octen-1-ol,3,7-dimethyl-, formate	Alcohol, Formic acid	$C_{11}H_{20}O_2$	7.64

	Citronellyl butyrate	Ester	$C_{14} H_{26} O_2$	7.64
	Geraniol	Alcohol	$C_{10} H_{18} O$	7.81
	2,6-Octadien-1-ol,3,7 dimethyl-	Alcohol	$C_{10} H_{18} O$	7.81
	4-Hepten-3-one, 4-methyl-	Ketone	$C_8 H_{14}O$	7.81
	4-Octen-3-one, 6-ethyl-7-hydroxy	Ketone	$C_{10} H_{18} O_2$	7.81
	ç-Muurolene	Hydrocarbon	$C_{15} H_{24}$	9.17
	1H-Cyclopenta[1,3]cyclopropa [1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)	Hydrocarbon	$C_{15} H_{24}$	9.17
	.alfa.-Copaene	Hydrocarbon	$C_{15} H_{24}$	9.17
	Naphthalene, 1,2,3,4,4,5,6,8a-octahydro-7-methyl-4-methylene-1 -(1-methylethyl)	Hydrocarbon	$C_{15} H_{24}$	9.52
	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3 a-ol	Alcohol, Hydrocarbon	$C_{14} H_{26} O_2$	10.33
	4ah-cycloprop[e]azulen-4a-ol, Decahydro-1,1,4,7-tetramethyl	Alcohol, Hydrocarbon	$C_{15} H_{26}O$	10.33
	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)-	Carboxylic acid, Hydraxine	$C_8 H_{15} N_{30}$	10.33
	2-Butyl-5-methyl-3-(2-methylprop-2-enyl) cyclohexanone	Ketone	$C_{15} H_{26}O$	10.33

	10-Methyl-8-tetradecen-1-ol Acetate	Alcohol, Carboxylic acid	$C_{17}H_{32}O_2$	10.33

The main compounds found in the study can be categorized in the following groups mentioned in table 2.

Hydrocarbons	Methane 1,4-dimethyl ç-Muurolene Octahydro-7-methyl-3-methylene-4-(1-methylethyl) Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1 1-Isobutyl-7,7-dimethyl- Decahydro-1,1,4,7-tetramethyl
Alcohols	2-Octen-1-ol, 3,7-dimethyl- Citronellol 6-Octen-1-ol,3,7-dimethyl Geraniol Ethylhydroxylamine 2,6-Octadien-1-ol,3,7 dimethyl- 10-Methyl-8-tetradecen-1-ol 4ah-cycloprop[e]azulen-4a-ol octahydro-isobenzofuran-3a-ol
Ketones	4-Octen-3-one,6-ethyl-7-hydroxy 4-Hepten-3-one, 4-methyl- 2-Butyl-5-methyl-3-(2-methylprop-2-enyl) cyclohexanone

Carboxylic Acid	2-Furancarboxylic acid Hydrazinecarboxamide,2-(2-methylcyclohexylidene) Acetate
Esters	3-methyl-, 3,7-dimethyl-2,6-octadienyl ester Citronellyl butyrate
Aldehydes	4,8-Decadienal, 5,9-dimethyl 3-Furaldehyde Furfural
Fatty Acid/Carboxylic Acid	Butanoic acid
Amino Acid	Histidine

It was found that the compounds, that come under the RT values of 7.64 and 7.81, which are 2-octen-1-ol, 3,7-dimethyl,citronellol, formate, citronellyl butyrate, geraniol, 2,6-octadien-1-ol,3,7 dimethyl, 4-hepten-3-one, 4-methyl and 4-octen-3-one,6-ethyl-7-hydroxy, have acquired the highest peaks (Fig.1). The compounds with RT value 7.64 have the highest peak area of 46.90% followed by the compounds with RT value 7.81 with an area of 29.99% of the total peak area. The other compounds with RT value 3.19, 4.08, 7.06, 9.17, 9.42, 9.52 and 10.33 are present in small amounts with an area percentage of 2.04%, 3.95%, 2.09%, 2.13%, 6.92%, 2.38% and 3.60% respectively (Fig.1).

It is generally known that the volatile fraction composition, which in turn depends on the nectar composition and floral source, greatly influences the aroma of bee honey (Kaškonienė et al., 2008). The same goes for alcohol. Alcohols are a crucial component of honey (Barra etal., 2010) and may have many useful properties. Among the identified alcohols, citronellol is one such type of alcohol that has been promising pharmacological properties. Citronellol is a monoterpene alcohol found in the essential oils of a variety of plants that are used in cooking and traditional medicine. Citronellol offers numerous pharmacological activities, including anti-inflammatory and analgesic properties, as well as minimal toxicological activity, making it an effective choice in the search for novel drugs (Santos et al., 2019). Geraniol, which is also a monoterpene alcohol, is shown to be a potential antimicrobial agent in many studies (Chen and Viljoen, 2010). While these alcohols are not found in abundance in honey, it is possible that trace amounts of these alcohols or related compounds could be present in honey if bees collect nectar from flowers that contain these alcohol-rich essential oils.

Honey is a naturally occurring source of organic acids (Keke and Cinkmanis, 2019). Despite their small amount, organic acids have a crucial role in several aspects of honey, including organoleptic, physical, and chemical qualities (Chakir et al., 2016; Aljohar et al., 2018). Organic acids can also be used to determine the freshness and authenticity of honey (Teczan et al., 2011). Organic acids have also been found to contribute to honey's antibacterial and antioxidant effects (Cavia etal., 2006).Organic acids like Acetic Acid, Butanoic Acid, and Formic Acid etc. are usually found in honey (Barra et al., 2010), although their presence is generally in very low concentrations, they contribute to the overall flavor and aroma profile of honey.

Furan is an organic compound that can potentially be found in trace amounts in honey. Furan-derived compounds observed in these honey samples were furfural, 3-furaldehyde and 2-furancarboxylic acid. These are compounds resulting from the degradation of sugar and can form as a result of heat processing (Castro-Vázquezet al., 2008). They are cyclic aldehyde compounds that are lacking in fresh samples but can be formed as a result of the Maillard reaction if honey is exposed to high temperatures or prolonged heating (Apriceno et al., 2018).

The only amino acid identified in the sample was histidine. Honey contains amino acids in relatively small amounts which are derived from the floral nectar of insect pollination. The types and concentrations of amino acids found in honey are related to the amino acid content of sources used as food by bees, which in turn varies depending on the source, location, and period of year (Noor et al., 2019).

Ketones are typically present in very low concentrations in honey and are part of the wide range of volatile compounds that contribute to honey's flavor and aroma diversity. The ketones found in this sample were 4-hepten-3-one, 4-octen-3-one and cyclohexanone. These compounds were not found to be common in the composition of honey.

Pyrazole, which is a heterocyclic aromatic compound, was also identified in the sample. Pyrazole was also previously identified in Gelam honey (Ismail et al., 2021) and is reported to be associated with a broad range of biological activities such as anti-inflammatory, anti-microbial, anticancer, anti-fungal, anti-tubercular, anti-convulsant, anti-viral (Naim et al., 2016), making them important in drug discovery and development. Though hydrocarbons are one of the many volatile compounds found in honey but their occurrence is not very much common. They are not typically found naturally in honey and may have come into contact with honey during the processing, packaging, or storage stages. ç-Muurolene, a sesquiterpene hydrocarbon, which is a type of natural organic compound found in many plants (Madboly etal., 2023). Sesquiterpenes are a class of terpenes, which are aromatic compounds responsible for the scents and flavors of various plants.

Benzene and its derivatives are also known to play an important role in honey (Manyi-Loh etal., 2011). Some esters, hydrazines and other heterocyclic compounds were also identified which make the overall volatile profile of honey. The organoleptic and nutritious qualities of honey are, nevertheless due to this smaller fraction of the overall composition.

Nonetheless, the mixture of volatile organic compounds present in honey is shaped by both the composition of the nectar it originates from and the specific floral sources. This relationship between nectar composition and floral origin could also contribute to the determination of the honey's geographical source. Furthermore, changes in the concentration of volatile compounds within honey can arise over time due to factors like temperature exposure and the duration of exposure during storage (Cuevas-Glory et al., 2008).

Due to variances in volatile content, honeys produced from diverse floral sources may have significantly different aromas and tastes. As a result, research into volatile components has gained credibility and space in recent years, as it allows for the classification of products based on certain parameters and the prediction of the quantity and quality of honey. Specific volatile organic compounds in honey have favorable health qualities and may thus contribute to honey's overall biological activity

Conclusion

Honey is a natural substance that is produced by Apismellifera from the nectar of flowers containing different compounds like hydrocarbons, alcohols, ketones, aldehydes, acids, esters and amino acids. Composition of these volatile organic compounds present in honey depends on floral origin and nectar composition. This relationship between nectar composition and floral origin could also contribute to the determination of the honey's geographical source. Different aroma and flavor of multi-flora honey is result of variances in volatile compounds content. Further research based on volatile compounds allows the better understanding of how these contribute to honey's overall biological activity.

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