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Chemical Profiling of Bee Venom and Beeswax: A GC-MS Analysis

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Abstract
Problem: The aim of the study was to find out chemical profiling of bee venom and beeswax of Apis mellifera through GCMS analysis**. Approach:** The sample of the bee venom and beeswax were purchased from private apiary located in Dera Bassi, Punjab, India. The chemical profile of thebee venom water extract and beeswax acetonitrile extract were analysed with the help of gas chromatography-massspectrometry**. Findings:** Result showed presence of hydrocarbons, terpene, ketones, carboxylic acid, alcohols, esters, phenols, steroids, retinoid, opioid and amines in bee venom and a variety of hydrocarbons in beeswax as observed in the GC-MS analysis. **Conclusion:** Products of bees showcase an amazing variety of compounds. Their distinct qualities and possible advantages for people are a result of their compositions. Even though a lot of bee products have showed potential in a variety of settings, more research is often needed to properly comprehend their usefulness and safety.

Keywords: Gas chromatography- mass spectrometry, Bee Venom, Bee Wax, Chemical Profiling.

Introduction

Historically, natural products and their structural equivalents have made substantial contributions to pharmacology, notably in the treatment of cancer and infectious diseases. (Atanasov et al., 2015;Rana and Bajwa, 2023). Natural products have unique qualities that set them apart from conventional synthetic molecules, which confer a great deal of advantages in the field ofalternative medicines (Cragg andNewman,2013). Their significant importance for infectious diseases and cancer can be attributed to the fact that they have undergone structural optimization via progression to fulfill certain biological functions, including the regulation of innate defense mechanisms and interactions (often competition)with other organisms (Atanasov et al.,2021).Their usage in traditional medicine may also shed light ontheirefficacyand safety.

The beesproducts are one such natural product whose therapeuticabilities have long been acknowledged and employed in traditional medicine. (Kolayli and Keskin,2020).Bees are a large family of social insects that are members of the

Apidae family. They include honey bees, stingless bees, and many more distinct species. The two most well-known species which are domesticated andutilized nowadays in beekeeping are, namely western-Apis mellifera Linnaeus (mostly found in Europe, Africa, America, and Asia) and eastern- Apis cerana Fabricius which are native to Southeast Asia (Zhang et a-6920l., 2019). These honeybees produce a number of substances and keep them in their hives, that may be advantageous to human health. Bee products providenumerous health benefits and are becoming increasingly popular in the era of medicalresearch (Dumitru et al., 2022;Smritiet al., 2024). Apitherapy, which is a type of complementary medicine uses the bioactive characteristics of bees products to prevent and /or treat various ailments (LiverTox,2022).
Bee venom, also known as apitoxin,serves as a significant defense mechanism for honey bees and is produced within poison glands located in their abdominal cavity. This colorless and transparent liquid contains proteins that can result in localized inflammation or, in severe instances, trigger serious allergic responses. Furthermore, it contains various enzymes and peptides that have therapeutic potential against a broad spectrum of health conditions. Several research studies mentioned the idea of employing bee venom (direct bee sting orinjectableform) tocureavariety of complicationseither in vivoor in vitro.

Materials and Methods

Collection of Samples

Three samples of Bee venom and beeswax of Apis mellifera were purchased from a private apiary located in Dera Bassi, Punjab.

Sample Preparation

The process of Rybak and Szezesna,(2004) was followed with minor changes in the amount, 100mg of the bee venom sample was dissolved in 2ml of distilled water. The mixture wascontinuouslystirred until the sample got dissolved.
For beeswax, Issa et al. (2020) extraction process was carried out with minorvariations in the temperature, time and by taking acetonitrile as solvent.10g of the solidified beeswax was first melted using a water bath at a temperature between60°C -70°C for about 15-20 mins. The melted beeswax was then transferred to a beakercontaining 50ml of acetonitrile. The solution was stirred continuously till a clear solution wasobtained.The samples were then sent for GC-MS analysis.

GC-MS Analysis

The chemical profiling of the bee venom and beeswax samples were analyzed by the process of GC-M Susing Thermo Trace 1300 GC couple dwithtriple Quadrupole MSTSQ8000. A solution of 1 μlwas introduced into the system with an injector temperature of 290°C. The temperature within the gas chromatography (GC) oven was programmed to start at 50°C, held for 1min, then increased to 225°C at a rate of 20°C/min, followed by a further increase to 270°C at a rate of 40°C/min. Helium was employed as the carrier gas, maintaining a steady flow rate of 1mm/min. The analytical scanning was performed in mass spectrometry mode across a range of 50 to 600amu, with the ion source temperature set at 250°C. The total runtime for the analysis was 24mins, and all peaks were identified through mass spectral comparison using the NIST 2.0 library.

Statistical analyses: Peaks were identified by analyzing individual samples and cross-referencing the obtained results with commercial reference libraries, such as the NIST library.

Results and Discussion

The study resulted in the identification of 41 individual compounds which belong to variouschemicalclasseslikehydrocarbons,alcohols,ketones,carboxylicacids,steroids,esters, retinoids,phenols, terpenes, amines, etc. The compounds with RT value of 5.14, which are α-Phellandrene, Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- and α-Pinene, have acquired the highest peak with an area of24.29%of thetotal peakarea. Among the identified compounds, terpenes like phellandrene, pinene, terpinenyl acetate and and rographolide were present which are known to possess promising pharma cologicalactivities (Thangaleela et al., 2022; Salehi et al., 2019; Yanet al., 2018; Chowdhury and Kumar, 2020). Terpenes are a class of chemical molecules mostly found in plants and trees,including citrus fruits like lemon and mandarin, tea, cannabis, thyme, and trees and possessattractivebiologicalcharacteristicsincludinganalgesicandanticonvulsant effects(DelPrado-Audelo et al., 2021). α-Phellandrene has the potential to be employed in the treatmentof inflammatory illnesses such as osteoarthritis, rheumatoid arthritis, allergies, and so on(Bizzoetal.,2009).Pinene, which has been detected in trace amounts in bee venom,(Isidorov et al.,2023), is a component of hepatic and renal medicines (Sybilskaetal.,1994). It is also utilized as an antibacterial substance due to its toxic effects on membranes (Alma et al., 2004). Furthermore, pinene has been shown to suppress the growth of breast cancer and leukaemia (Zhou et al., 2004). Terpinyl acetate is a terpene ester commonly found in various essential oils (Vaičiulytėet al., 2021). Though it is primarily used in perfumery and fragrance industry, due to its aroma, they are also known to exhibits multi target-directed ligand potential(MTDL) in Alzheimer's disease (Chowdhury and Kumar, 2020). Andrographolide is anotherwell-known terpene known for its potential health benefits. It is commonly used in traditionalherbal medicine in various parts of the world (Yanet al., 2018). It is known for its anti-inflammatory properties (Liet al., 2022).

A number of alcohols were also seen in the sample like 2-Nonadecanol, 1,6-Octadien-3-ol, 1-Hexadecanol, 3-Dodecen-1-ol, etc. Alcohols are present in small amounts and contribute tothe overall composition and effects of bee venom. Decanol is a long-chain alcohol which haspreviously been identified in bee venom (Isidorov et al., 2023). It is used in the production of perfumes and other fragrances. It is also being studied as a penetrationenhancer for transdermal delivery of drug because of its propensity to permeate the skin (Kanikkannan and Singh, 2002). 1,6-Octadien-3-ol, 3,7-dimethyl, also known as linalool, is a terpenoid alcoholand has demonstrated effect on the central nervous system, primarily as an anti-depressant drug. Alcohols in bee venom are often found in essential oils and natural extracts, contributing to their aromas and properties.

Retinal, a retinoid, is an aldehyde of vitamin A (Hruszkewycz et al., 2011) which plays acrucialrolein vision as acomponent ofthe visualpigment rhodopsin. Dimenoxadolisanopioid is an anal gesicthat cause susualopioi deffects like numbness and drowsiness. It is a benzilic acid derivative and was used for the relief of moderate to severepain. However, it was withdrawn from the market in many places due to concerns about itspotentialfor abuse, addiction, andadverse effects(NCBI).9, 10-Secocholesta-5,7,10(19)-triene is a derivative of vitamin D3 (Yakhimovich et al.,1976).Vitamin-D derivatives are important in various physiological processes, including calcium metabolism, bone health, and immune system regulation (Nagubandi et al.,1981). Phenolic compounds are known to improve one's health by lowering the likelihood of developing metabolic diseases (Rahman et al., 2021). In this sample, Phosphonic acid, (p-hydroxyphenyl)-, which is a phenol compound that has a phosphonic acid group attached to the phenyl ring was identified.

Aromatic hydrocarbons like benzene(4-bromobutyl), o-cymene, p-cymene, benzene,1-methyl-3-(1-methylethyl), benzene, 2-ethyl-1,3-dimethyl and benzene, 1-ethyl-2,4-dimethylwere identified in the sample. 1-pentadecene, 8-heptadecene, 1,3-cyclohexadiene, cetene and cyclohexene were the identified alkenes. α-Farnesene, a sesquiterpene hydrocarbon commonly found in essential oils, were also seen in the results.

Apart from these, carboxylic acids like acetic acid, ketones and a number of esters were also seen. Though these compounds are present in a small amount, but they make the overall profile of bee venom. Many of these components were seen for the very first time in a bee venom sample which might be due to the variations in nearby flora, geographical location, climatic conditions or even the storage conditions might have made a difference.

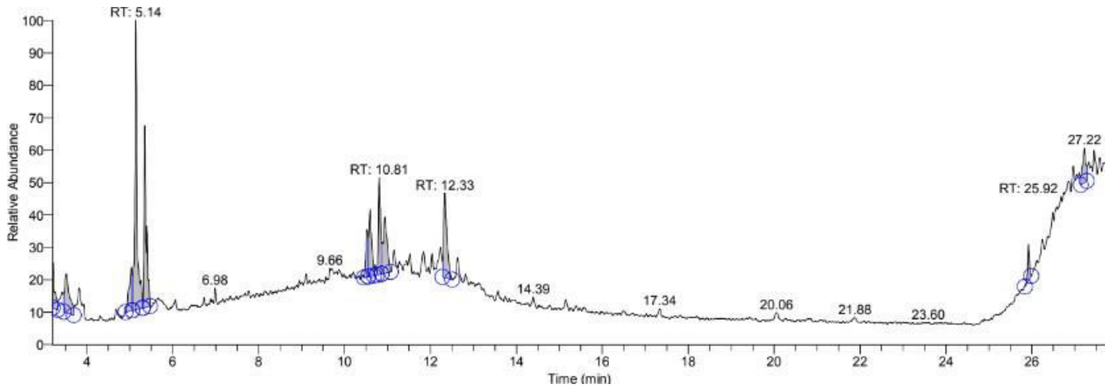

Fig 1- GC-MS chromatogram graph of bee venom sample

Table 1: The list of compounds identified in the bee venom sample

Cpd	Name	Classification	Formula	RT
1.	Cyclopentadecanone,4-methyl-	Ketone	$C_{16}H_{30}O$	3.22
2.	3-Dodecen-1-ol,(Z)	Alcohol	$C_{12}H_{24}O$	3.22
3.	Oxirane, [(tetradecyloxy)methyl] -	Epoxide	$C_{17}H_{34}O_2$	3.22
4.	Dimenoxadol	Benzilic acid derivative/ Opioid	$C_{20}H_{25}NO_3$	3.22
5.	1-Pentadecene, 2-methyl-	Hydrocarbon	$C_{16}H_{32}$	3.22
6.	Benzene, (4-bromobutyl)-	Aromatic Hydrocarbon	$C_{10}H_{13}Br$	3.52
7.	Benzenesulfonamide, 5-amino-2-methyl-N-phenyl-	Amine	$C_{13}H_{14}N_2O_2S$	3.52
8.	Benzene,1,1'-[(1,2-dimethyl-1,2-ethanediyl)bis(oxymethylene)] bis	Organic Compound	$C_{18}H_{22}O_2$	3.52
9.	Urea, (phenylmethoxy)-	Urea group and phenyl methoxy group	$C_8H_{10}N_2O_2$	3.52
10.	Phosphonic acid, (p-hydroxyphenyl)-	Phenol	$C_6H_7O_4P$	5.03

11.	Phenylphosphoricacid	Phosphoric acid derivative	$C_6H_7O_4P$	5.03
12.	Acetic acid, bromo-phenyl ester	Carboxylic Acid Derivative	$C_8H_7BrO_2$	5.03
13.	Carbamic acid, phenyl-, phenyl ester	Carbamic acid ester	$C_{13}H_{11}NO_2$	5.03
14.	Phenyl α-chloropropionate	Ester	$C_9H_9ClO_2$	5.03
15.	α-Phellandrene	Terpene	$C_{10}H_{16}$	5.14
16.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Hydrocarbon	$C_{10}H_{16}$	5.14
17.	α-Pinene	Terpene	$C_{10}H_{16}$	5.14
18.	o-Cymene	Aromatic Hydrocarbon	$C_{10}H_{14}$	5.35
19.	Benzene,1-methyl-3-(1methylethyl)-	Hydrocarbon	$C_{10}H_{14}$	5.35
20.	Benzene,2-ethyl-1,3-dimethyl-	Hydrocarbon	$C_{10}H_{14}$	5.35
21.	Benzene,1-ethyl-2,4-dimethyl-	Hydrocarbon	$C_{10}H_{14}$	5.35
22.	p-Cymene	Aromatic hydrocarbon	$C_{10}H_{14}$	5.35
23.	n-Dodecyl methacrylate	Esters	$C_{16}H_{30}O_2$	10.52
24.	8-Heptadecene	Hydrocarbon	$C_{17}H_{34}$	10.52
25.	1-Hexadecanol	Alcohol	$C_{16}H_{34}O$	10.52
26.	1-Dodecanol,3,7,11-trimethyl-	Alcohol	$C_{15}H_{32}O$	10.52
27.	Cetene	Hydrocarbon	$C_{16}H_{32}$	10.52
28.	Cyclotridecane	Hydrocarbon	$C_{13}H_{26}$	10.60
29.	4-Terpinenylacetate	Terpene alcohol	$C_{12}H_{20}O_2$	10.81
30.	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*,S*)]-	Hydrocarbon	$C_{15}H_{24}$	10.81
31.	Hydrocinnamanilide	Amide	$C_{15}H_{15}NO$	10.81
32.	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-	Hydrocarbon	$C_{15}H_{24}$	10.81
33.	Linalyl phenylacetate	Ester	$C_{18}H_{24}O_2$	10.81

34.	Andrographolide	Diterpenoid lactone	$C_{20}H_{30}O_5$	12.33
35.	1,6-Octadien-3-ol, 3,7-dimethyl-, propanoate	Alcohol, Carboxylic acid derivative	$C_{13}H_{22}O_2$	12.33
36.	α-Farnesene	Hydrocarbon	$C_{15}H_{24}$	12.33
37.	2-Methyl-1-undecanol	Alcohol	$C_{12}H_{26}O$	25.92
38.	2-Nonadecanol	Alcohol	$C_{19}H_{40}O$	25.92
39.	Bis(tridecyl)phthalate	Phthalate Ester	$C_{34}H_{58}O_4$	25.92
40.	Retinal	Retinoids	$C_{20}H_{28}O$	27.22
41.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	Secosteroid	$C_{27}H_{44}O_3$	27.22

Table 2: Categorization of the compounds identified in the bee venom sample

Hydrocarbons	1-Pentadecene, 2-methyl-Benzene,(4-bromobutyl)- Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- o-Cymene Benzene,1-methyl-3-(1-methylethyl)-Benzene, 2-ethyl-1,3-dimethyl-Benzene,1-ethyl-2,4-dimethyl- p-Cymene 8-HeptadeceneCeteneCyclotridecane 1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*,S*)]- Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl- α-Farnesene
Terpene	αPhellandreneα-Pinene 4-Terpinenylacetate Andrographolide
Ketones	Cyclopentadecanone,4-methyl-

Carboxylic Acid	Aceticacid,bromo-, phenylester 3,7-dimethyl-,propanoate
Alcohols	3-Dodecen-1-ol, (Z)1-Hexadecanol -Dodecanol,3,7,11-trimethyl-1,6-Octadien-3-ol 2-Methyl-1-undecanol 2-Nonadecanol
Esters	Carbamicacid,phenyl-, Phenylester Phenyl α-chloropropionate n-Dodecylmethacrylate Linalyl phenylacetateBis(tridecyl)phthalate
Phenols	Phosphonic acid,(p-hydroxyphenyl)-
Steroids	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-
Retinoids	Retinal
Opioid	Dimenoxadol
Amines	Benzene sulfonamide, 5-amino-2-methyl-N-phenyl-

Analysis of the beeswax sample

The result showed the presence of 8 individual compounds which belong to a single chemical group, namely hydrocarbons. Hydrocarbons constitute 12-16% of the total composition of beeswax (Fratinietal.,2016). Unbranched saturated hydrocarbons (n-alkanes) make up approximately 67% of all beeswax hydrocarbons. Branched saturated hydrocarbons (iso-alkanes) and unsaturated hydrocarbons (alkenes) contribute significantly less, approximately 2%. Alkenes are typically found as cis isomers (Waśetal.,2014). All the identified hydrocarbons belong to unbranched alkane and have a chain length of C20-C44.

Eicosane, tetracosane, triacontane, tetratriacontane, etc. are the components that contribute to overall composition of beeswax (Baron et al., 2015). These components present in bees wax contribute to its waterproofing properties and help protect the honeycomb structure from moisture and environmental factors. The hydrocarbons in beeswax contribute to its texture, water resistance, and other physical characteristics. Beeswax is used in various applications, including cosmetics, skin care products, candles, and food, due to its unique properties. The presence of hydrocarbons, along with other components like fatty acids, esters, and alcohols, gives beeswax its distinctive qualities that make it valuable in these applications.

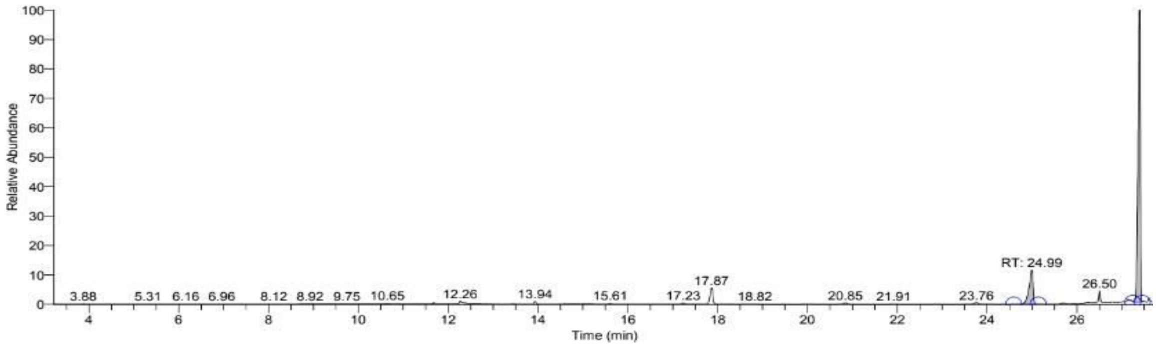

Fig 2: GC-MS chromatogram graph of beeswax sample

Table 3: The list of compounds identified in the beeswax sample

Cpd	Name	Classification	Formula	RT
1.	Eicosane	**Hydrocarbon**	$C_{20}H_{42}$	24.99
2.	Eicosane,7-hexyl-	**Hydrocarbon**	$C_{26}H_{54}$	24.99
3.	Tetracosane	**Hydrocarbon**	$C_{24}H_{50}$	24.99
4.	Heneicosane	**Hydrocarbon**	$C_{21}H_{44}$	24.99
5.	Eicosane,10-methyl-	**Hydrocarbon**	$C_{21}H_{44}$	24.99
6.	Tetratriacontane	**Hydrocarbon**	$C_{34}H_{70}$	27.39
7.	Tetratetracontane	**Hydrocarbon**	$C_{44}H_{90}$	27.39
8.	Triacontane	**Hydrocarbon**	$C_{30}H_{62}$	27.39

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Authorship and Contributorship

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Conclusion and future perspectives

Bee products in this study like bee venom and beeswax, are fascinating and complex substances that are produced by bees and have been utilized by humans for various purposes throughout history. These bee products highlight the incredible diversity of compounds.Theircompositions contribute to their unique properties and potential benefits for humans. However, it's important to note that while many bee products have shown promise in various contexts, more research is often needed to fully understand their mechanisms of action and ensure their safety and efficacy.

In conclusion, bee products are not only remarkable in their natural complexity but also offera wide range of potential benefits for human health and various industries. The study of theseproducts continues to reveal insights into their compositions, properties, and uses, opening up avenues for research, innovation, and sustainable practices in beekeeping and related fields.

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