GCMS Profiling of Volatile Constituents in Indian Propolis

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Abstract
Propolis is a complex resinous substance collected by bees from plants and used as a construction material and protective substance in the hive. It consists of volatile and non-volatile constituents; the latter is known to attribute to the various biological activities of propolis. The volatile chemistry of Indian propolis has not been studied extensively which prompted the present investigation on the volatile compounds of propolis samples obtained from five different phytogeographical regions of India by GCMS analysis. The findings of the study indicated the presence of 40 bioactive compounds in the propolis samples with various biological activities. Among the various chemical compounds identified in the propolis extracts, two novel compounds namely deoxy- podocarpol and p-1-indanyl phenol were reported for the first time. These results suggested that the propolis samples are rich in bioactive compounds with many biological activities. It was concluded from the study that the novel compounds identified in Indian propolis have the potential to be developed into pharmaceutical drugs.

Keywords: GCMS, Indian propolis, deoxy podocarpol, p-1-indanyl phenol

Introduction
The traditional medicine of different cultures around the world has recognized the importance of bee products, most notably propolis (Gemiarto *et al.,* 2015). Propolis is a resinous substance that honeybees collect from buds, leaves, barks, and exudates of many trees and plants (Lotti *et al*., 2010). Propolis is used by the honey bees for various functions such as blocking the entrance of intruders to the nest, maintaining the inner temperature at approximately 35°C and as a sealant for unwanted open spaces in the hive. It also aids in hardening the cell walls and achieve sterile conditions within the beehive (Salatino *et al* 2005). A broad range of pharmacological activities of propolis have been documented in several studies such as anticancer, antimicrobial, anti-inflammatory, antiviral, as antibiotic, antifungal, antineoplastic, anti-oxidative activities (Belfar *et al*.,2015)

The functional pharmacological activities of propolis and its effective therapeutic applications have generated growing curiosity about its chemistry (Bankova *et al*., 1987). Propolis composition is extremely complex and variable, showing the presence of beeswax, resin, essential oils, and pollen. (Galeotti *et al.,* 2018) Propolis generally consists of resin and balsam (50%), which is composed of flavonoids and related phenolic acids, generally called as the polyphenolic fraction, waxes (30%), essential and aromatic oils (10%), pollen (5%), and other organic compounds (5%). (Thomson, 1990).The waxes are secreted by the honey bees, while resin and oils are obtained from plants, generally derived from secretions or by cutting fragments of vegetative tissues. (Galeotti *et al*., 2018)

The bee waxes and hydrocarbons predominantly make up the non-polar fraction of propolis. The propolis waxes are secreted by honey bees, and the differences between propolis waxes and waxes present in hexagonal combs arise from the genetic factors of the bees and are independent of the botanical sources. These include various alkanes, alkenes, alkadienes, monoesters, diesters, aromatic esters, fatty acids, their esters, and glycerol, and steroids. These non-volatile components do not contribute any significant pharmacological activity to propolis. (Ristivojević *et al,* 2015)

The polar fraction consists of mainly volatile constituents which originate from the plant exudates, bee metabolism (amino acids, glycerol phosphates) and contamination with honey (various sugars) (Groot, 2013) Various classes of volatile compounds present in the chemical profile of propolis are responsible for its complex chemical composition (Silici & Kutluca, 2005). Propolis contains different monoterpenes and sesquiterpenes as its volatile components (Bankova, 1994) Other components of volatile oils consist of alcohols, especially aromatic alcohols, phenols, aldehydes, ketones, acids (from Acetic acid to Stearic acid), esters, alkanes, benzylated benzene, and naphthalene. (Ferracini, 1995)

The volatile fraction of propolis has not been studied extensively because the volatile compounds constitute only 10% of the total composition compared to 50% of the resinous substances in propolis. (Miguel and Antunes, 2011) These essential oils which are present in minute quantities confer aroma and biological activity to propolis (Bankova *et al.,* 2000) Although, the volatile constituents comprise a small portion of the propolis composition, their chemical information is helpful in the chemical characterization of propolis and can also lead to the discovery of novel bioactive compounds. (Bankova *et al.,* 2014) The volatile constituents possess different biological activities, which in turn attribute to the biological activity of propolis. Identification of volatile compounds in propolis also provides valuable information about the geographical origin of propolis (Miguel and Figueiredo, 2017)

Propolis from various geographical regions exhibit variability in their volatile composition due to the collection of resins and plant exudates by the bees from local flora varying according to their respective climatic conditions, terrain, water availability and other environmental factors (Moţ *et al.,* 2011). Significant differences are thus observed in the volatile chemistry of propolis between different countries (Bankova *et al.*, 2014)· The volatile constituents of propolis from the temperate and tropical zones display tremendous variation in their chemical composition (Torres *et al.*, 2008). Propolis from temperate zones, is known to contain mainly flavonoid aglycones (flavones and flavanones), phenolic acids, and their esters. (Bankova, 2005b; Bankova, Castro, & Marcucci, 2000; Bankova, Popova, Bogdanov, & Sabatini, 2002) On the other hand, propolis in tropical regions also shows volatility in its chemical composition (Torres *et al.*, 2008). Tropical propolis is rich in phenolics, flavonoids, prenylated derivatives of p-coumaric acids, terpenes, lignans, and benzophenones. (Bankova, Popova, & Trusheva, 2018; Marcucci, 1994; Salatino *et al.*, 2011) However, propolis from tropical countries is still not well understood, although it has demonstrated promising biological activities and unique chemical composition in these countries (Katekhaye *et al.*, 2019) In fact, the essential oils of propolis samples within the same country have also shown unpredictable composition (Bankova *et al.*, 2014).

India has a vast diversity of flora and shows regional variations in climatic conditions, therefore it is predicted that propolis collected from different parts of the country will differ in their chemical composition. (Kasote, 2017) Worldwide, there has been extensive research on propolis, but the research on Indian propolis is still in its infancy. (Kapare *et al*., 2019) Propolis has been the subject of substantial research in America, Australia, the United Kingdom and especially Eastern Europe over recent decades (Rathod *et al* 2011).But, there is scant information on the chemistry and applications of Indian propolis. (Naik *et al*., 2013) Only a handful of studies have documented the chemical composition of Indian propolis. (Thirugnanasampandan *et al.,* 2012)

Owing to its extremely complex chemical composition, GCMS became the most preferred technique in the 1980s for faster chemical characterization of propolis samples from various geographical and botanical origins (Greenaway *et al.,* 1990). The isolation and identification of several compounds of propolis has been possible because of the development of modern chromatographic techniques combined with mass spectroscopy (MS). (Shah *et al.,* 2014) GC–MS helps to prevent the loss and degradation of volatile components and is therefore very often used for the characterization of propolis volatiles. (Pavlovic *et al*., 2020) GCMS technique has long been used for the in-depth analysis of the major volatile and semi-volatile chemical compounds of propolis (Haile and Dekebo, 2013) Rapid GC-MS profiling of the various propolis types involves the identification of different compounds by their chromatographic and mass spectral characteristics to reveal their plant sources (Shah *et al.,* 2014) It is also helpful in establishing the geographical origins of the propolis samples (Melliou *et al.,* 2007).

Since the volatile chemistry of Indian propolis has not been extensively studies which prompted the present investigation on the volatile compounds of propolis using GCMS technique. The current study aims to establish the degree of variation in the volatile composition of propolis samples collected within India by GCMS analysis and identify novel phenolic compounds from Indian propolis which have further potential to be developed into novel pharmaceutical drugs.

Materials and method

Collection of propolis samples

In the present study, raw propolis samples were collected from five different geographical regions of India namely Rohtas from Bihar, Latur from Maharashtra, Sawai Madhopur from Rajasthan, Rewari from Haryana and Kota from Rajasthan. The samples were collected by scraping the frames of beehives from apiaries housing *Apis mellifera* colonies. The samples were physically cleaned and pulverized into small particles. The samples were stored in a cool place for further investigations.

Preparation of ethanolic extract of propolis (EEP)

Raw propolis samples were physically cleaned and pulverized into small particles. The crude propolis extracts were prepared by crushing 20 g of propolis into small pieces and extracted with 100 ml of 70% ethanol and placed on a magnetic stirrer for two days with intermittent shaking at room temperature. The extract was filtered by the Whatman filter paper to remove insoluble matter and waxes. After filtration, the obtained ethanolic extracts of propolis were evaporated to dryness under reduced pressure at 50°C by rotary evaporator. The dried extracts were stored in air-tight vials at – 20 °C for further GC-MS analysis.

Gas chromatography and Mass spectrum analysis

The propolis samples were investigated by GCMS analysis to analyze the possible differences in the chemical composition of the propolis samples and to suggest similarities and differences between samples. The dried propolis extracts (0.5mg) were dissolved in 1mL of HPLC grade methanol to appropriate concentrations and analyzed in a GC-MS device. A GC system Thermo Scientific-Trace 1310 coupled to a triple quadruple mass Spectrometer (TSQ 8000, Thermo Scientific) was used for the analysis of extracted propolis samples. The GC was equipped with a DB-5MS column (30 m in length × 0.25 mm in internal diameter × 0.25 μm in film thickness). Helium (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min. The temperature of the GC injection port was 250°C. An injection volume of 1 μL of the sample was employed (Split ratio 1:30). The GC oven temperature was programmed initially at 40°C for 2 minutes and then increased to 240°C at a rate of 5°C /minute. Finally, the temperature was increased to 300°C at a rate of 20°C/min for 2 minutes. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70eV and the mass analyzer operated in full scan mode from 50 to 1000 atomic mass units in a full scan mode. The MS transfer line was maintained at a temperature of 300°C and the MS ion source temperature was maintained at 230°C. The relative quantity of the chemical compounds present in each of the propolis extracts was expressed as a percentage based on the peak area produced in the chromatogram.

Identification of Components

The interpretation of the mass spectrum of GC-MS was conducted using the mass spectral database of the National Institute Standard and Technology (NIST Lib version 2.2). The spectrum of the unknown component was compared with the spectrum of the known components stored in the database. The number of hits used to identify the compound and the name, molecular weight, molecular formula, molecular structure of the compounds was recorded from the NIST library. The identification of chemical compounds in the crude extract was based on the similarity of GC retention time and mass spectra (%) with the standards.

Results and discussion

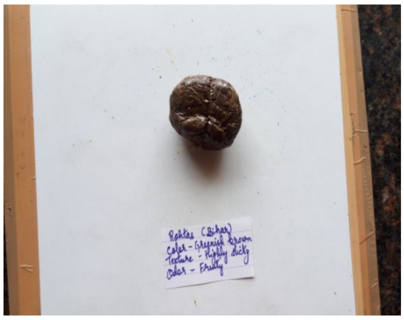
GCMS analysis of various extracts of Indian propolis

In the present study, GCMS analysis was used for the profiling of volatile components in the propolis samples from five different phytogeographical regions of India namely Rohtas from Bihar, Latur from Maharashtra, Sawai Madhopur from Rajasthan, Rewari from Haryana and Kota from Rajasthan. The samples were explored for their phytochemical composition by GC-MS analysis. Details of place of collection of propolis samples collected in India and the coordinates of the geographical regions are shown in Table 1.

Table 1: Details of place of collection of propolis samples collected in India (Census of India, 2011)

State	District	Block/ Tehsil	Village	Geographical position
Bihar	Rohtas	Sasaram	Misripur	25.0686° N, 84.0167° E
Maharashtra	Latur	Jalkot	Atnoor	18.4088° N, 76.5604° E
Rajasthan	Sawai Madhopur	Gangapur	Phulwara	26.0378° N, 76.3522° E
Haryana	Rewari	Rewari	Bhanwari	28.1928°N, 76.6239° E
Rajasthan	Kota	Sangod	Narsinghpura	25.2138° N, 75.8648° E





The results of GC-MS analysis revealed that there is variability in the chemical composition of the volatile fraction of the five propolis samples. It is evident from the present study that the samples exhibit significant differences in their chemical composition depending on the location of the collection.

Many chemicals including alcohols, aromatic compounds, flavanones, linear hydrocarbons, alkenes, aromatic acids, aromatic esters, fatty acid esters, terpenoids, and other chemical substances were found in the propolis samples. The different chemical profiles of the samples demonstrate that the botanical sources of propolis vary significantly, even in the same state and the same season. Most of the identified compounds in Indian propolis were found to possess many biological activities.

The volatile profiling of propolis extracts led to the identification of over 40 major compounds in the propolis extracts. The analysis indicated the presence of two novel bioactive compounds viz. deoxy- podocarpol and p-1-indanyl phenol, among the various chemical compounds identified in the propolis extracts. The two compounds were identified for the first time in Indian propolis and the rest of the compounds were already reported from various parts of the world as revealed by the extensive literature survey.

The phytochemical composition of the methanolic extracts of propolis sample from Rohtas, Bihar, as identified by GC-MS analysis revealed the presence of ten compounds. GC-MS analysis of propolis sample from Rohtas, Bihar with their retention time (RT), molecular formula, molecular weight and peak area (%) are presented in (Table 2). The GC-MS chromatogram of the extract from Rohtas, Bihar is also given in Fig.1.

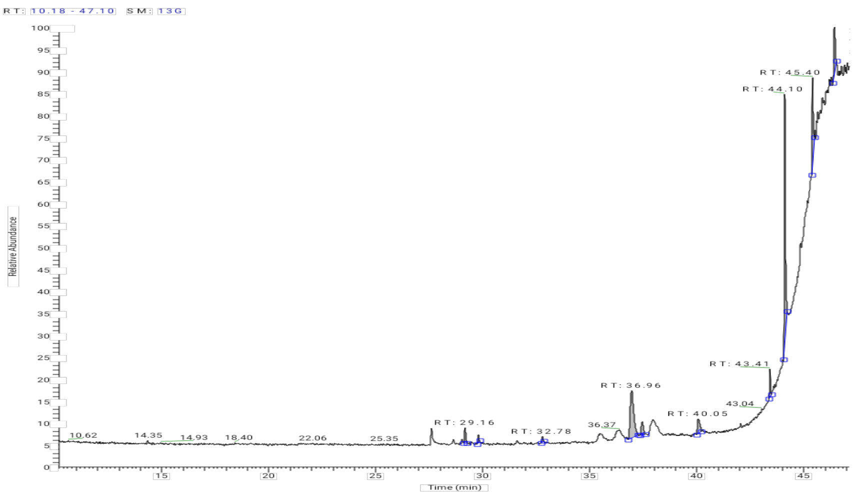
The major compounds were found to be Pinocembrin (38.42%), deoxy- podocarpol (23.87%), Naringenin(12.42%), 1,2,4,5-tetramethyl- 3- (3-phenylpropyl)-benzene (8.73%), Triphenylethene (4.95%), (E)-1-Phenyl-1-butene (3.97%), Cinnamyl cinnamate (3.01%), Viridiflorol (2.83%), cis-Eudesm-6-en-11-ol (0.93%), and α-Bisabolol (0.87 %). Out of all these compounds, deoxy-podocarpol was identified for the first time in the propolis sample from Rohtas, Bihar.

Table 2: GC-MS analysis of propolis extract from Rohtas, Bihar

Sl. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound
1	29.16	Viridiflorol	$C_{15}H_{26}O$	222.372	2.83	Sesquiterpenoid
2	29.79	α-Bisabolol	$C_{15}H_{26}O$	222.372	0.87	Sesquiterpenoid
3	32.78	cis-Eudesm-6-en-11-ol	$C_{15}H_{26}O$	222.372	0.93	Sesquiterpenoid
4	36.96	Podocarpol, deoxy-	$C_{17}H_{24}O$	244.378	23.87	Alcohol
5	37.45	(E)-1-Phenyl-1-butene	$C_{10}H_{12}$	132.206	3.97	Alkene
6	40.05	Triphenylethene	$C_{20}H_{16}$	256.348	4.95	Aromatic hydrocarbon
7	43.41	Cinnamyl cinnamate	$C_{18}H_{16}O_2$	264.324	3.01	Ester

8	44.1	Dihydrochrysin	$C_{15}H_{12}O_4$	256.257	38.42	Flavanone
9	45.4	Naringenin	$C_{15}H_{12}O_5$	272.256	12.42	Flavanone
10	46.41	Benzene, 1,2,4,5-tetramethyl-3-(3-phenylpropyl)-	$C_{19}H_{24}$	252.401	8.73	Aromatic Compound

Figure 1: GC-MS chromatogram of propolis from Rohtas, Bihar



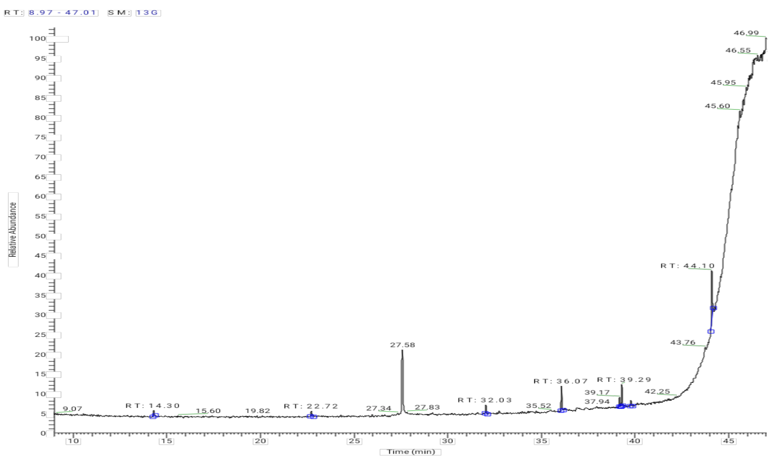
The chemical composition of the methanolic extract of propolis sample from Latur, Maharashtra was analyzed using GC - MS. The analysis by gas chromatography-mass spectrometry of propolis sample from Latur, Maharashtra allowed the identification of eight compounds. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), and peak area (%), nature of compounds in the propolis from Latur, Maharashtra are presented in Table 3. The GC -MS spectrum of the chemical compounds identified in the propolis from Latur, Maharashtra is illustrated in Figure 2.

The prevailing compounds were Pinocembrin or Dihydrochrysin, (41.67%), Ethyl ester hexadecanoic acid (17.66%) and Ethyl Oleate (14.71%) were also found to constitute a major amount. Isopropyl linoleate (7.12%) and Ethyl ester undecanoic acid (6.07%) also showed a good presence. The compounds Ethyl ester decanoic acid (4.88%), Ethyl ester octadecanoic acid (4.2%) and Undecane (3.7%) were present in lesser amounts.

Table 3: GC-MS analysis of propolis from Latur, Maharashtra

Sl. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound
1	14.3	Undecane	$C_{11}H_{24}$	156.313	3.7	Alkane
2	22.72	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200.322	4.88	Fatty acid ethyl ester
3	32.03	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214.349	6.07	Fatty acid ethyl ester
4	36.07	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.484	17.66	Fatty acid ethyl ester
5	39.17	Isopropyl linoleate	$C_{21}H_{38}O_2$	322.533	7.12	Fatty acid ethyl ester
6	39.29	Ethyl Oleate	$C_{20}H_{38}O_2$	310.522	14.71	Fatty acid ethyl ester
7	39.78	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.538	4.2	Fatty acid ethyl ester
8	44.1	Dihydrochrysin	$C_{15}H_{12}O_4$	256.257	41.67	Flavanone

Figure 2: GC-MS chromatogram of propolis of Latur, Maharashtra



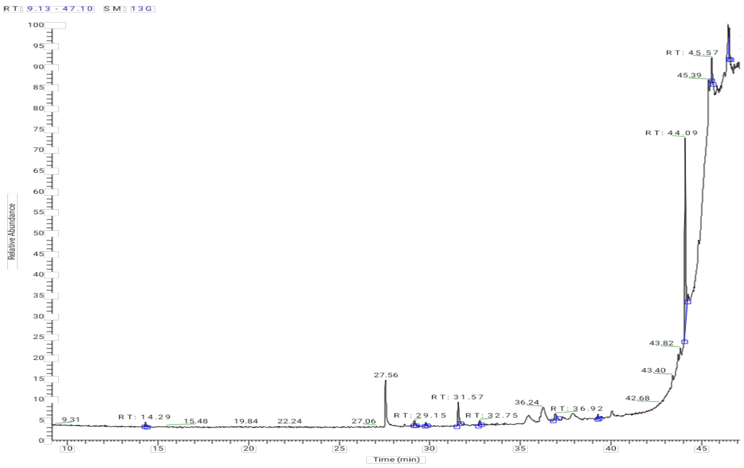
The GC-MS analysis of the propolis from Sawai Madhopur**,** Rajasthan was performed and ten major peaks were detected. The sample from Sawai Madhopur, Rajasthan was characterized by the presence of 10 individual compounds. The active principles with their retention time (RT), Molecular formula, Molecular weight (MW) and concentration (%), compound nature of the compounds in the propolis from Sawai Madhopur are presented in Table 4. The mass spectra of the chemical compounds identified in Sawai Madhopur, Rajasthan are presented in Figure 3.

The major compounds present in the sample are: Dihydrochrysin (63.09%) was the most prevalent. p -1- indanyl phenol (9.63%), Betulinaldehyde (7.98%). Spironolactone (6.64%), 1-butenyl-E-benzene(4.57%) also showed good presence. The compounds α- Eudesmol (2.33%), Cryptomeridiol (2.31%), Undecane (1.38%), Longiborneol (1.14%), Cavonyl (0.94 %) were found to be present in trace amounts. Out of these, p-1-indanyl phenol has been identified for the first time in propolis from Sawai Madhopur, Rajasthan.

Table 4: GC-MS analysis of propolis from Sawai Madhopur, Rajasthan

Sl. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound
1	14.29	Undecane	$C_{11}H_{24}$	156.313	1.38	Alkane
2	29.15	α-Eudesmol	$C_{15}H_{26}O$	222.372	2.33	Sesquiterpenoid
3	29.78	Longiborneol	$C_{15}H_{26}O$	222.372	1.14	Sesquiterpenoid
4	31.57	Phenol, p-1-indanyl-	$C_{15}H_{14}O$	210.276	9.63	Phenol
5	32.75	Cryptomeridiol	$C_{15}H_{28}O_2$	240.387	2.31	Sesquiterpene diol
6	36.92	Benzene, 1-butenyl-, (E)-	$C_{10}H_{12}$	132.206	4.57	Benzene
7	39.29	Cavonyl	$C_{12}H_{16}N_2O_3$	236.271	0.94	Barbiturate derivative
8	44.09	Dihydrochrysin	$C_{15}H_{12}O_4$	256.257	63.09	Flavanone
9	45.57	Spironolactone	$C_{24}H_{32}O_4S$	416.576	6.64	Steroidal 17α-spirolactone
10	46.54	Betulinaldehyde	$C_{30}H_{48}O_2$	440.712	7.98	Pentacyclic triterpenoids

Figure 3: GC-MS chromatogram of propolis from Sawai Madhopur, Rajasthan



Relatively complex chemical composition was exhibited by the sample from Rewari, Haryana. The GC-MS analysis of the propolis sample from Rewari showed the presence of 24 compounds that were identified using a computer search on a NIST MS data library based on mass spectral fragmentation patterns. The active principles with their retention time (RT), Molecular formula, Molecular weight (MW) and concentration (%), compound nature is presented in Table 5. The GC-MS spectrum of Rewari, Haryana confirmed the presence of various components with different retention times as illustrated in Figure 4.

The sample showed high content of Pinocembrin or Dihydrochrysin, (54.89%) was the most prevalent. Naringenin (10.93%) and Chrysin (8.05%) were also found in reasonably good amounts. The compounds 1-Pentamethyldisilyloxy-3-phenylprop-2-ene (3.67%) and Emodin (2.83%) were present in lesser quantities. All other compounds like p-1-indanyl phenol,1.5- Diphenyl-1,5-hexadiene, Undecane, α-Eudesmol, γ- Eudesmol, α-Bisabolol, 7-(2- Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol, Methyl 13-methylpentadecanoate, Cryptomeridiol, Methyl 13-methylpentadecanoate, 1,5-Diphenyl-1,5-hexadiene, Ethane, 1-(o-ethylphenyl)-1-phenyl-Hexadecanoic acid, ethyl ester, 1-n-Hexadecylindan, Cyclic octaatomic sulfur, Linoleic acid ethyl ester, 10-Octadecanoic acid, methyl ester, Ethyl Oleate, Cinnamyl cinnamate were found to be present in trace amounts.

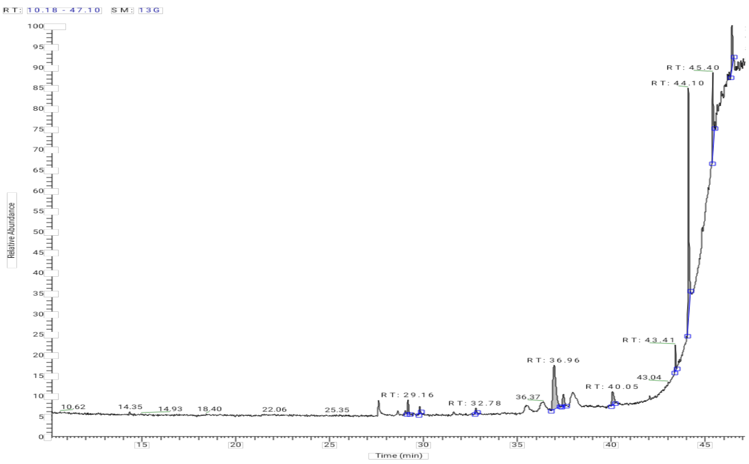
Table 5: GC-MS analysis of propolis extract from Rewari, Haryana

Sl. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound
1	14.29	Undecane	$C_{11}H_{24}$	156.313	0.07	Alkane
2	28.6	γ-Eudesmol	$C_{15}H_{26}O$	222.372	0.29	Sesquiterpenoid

3	29.14	α-Eudesmol	$C_{15}H_{26}O$	222.372	0.88	Sesquiterpenoid
4	29.77	α-Bisabolol	$C_{15}H_{26}O$	222.372	0.39	Sesquiterpene alcohol
5	31.57	Phenol, p-1-indanyl-	$C_{15}H_{14}O$	210.276	1.75	Phenol
6	31.88	Cryptomeridiol	$C_{15}H_{28}O_2$	240.387	0.13	Sesquiterpene diol
7	32.74	Cryptomeridiol	$C_{15}H_{28}O_2$	240.387	0.69	Sesquiterpene diol
8	34.74	Methyl 13-methylpentadecanoate	$C_{17}H_{34}O_2$	270.457	0.07	Fatty acid methyl ester
9	35.43	1,5-Diphenyl-1,5-hexadiene	$C_{18}H_{18}$	234.140	1.03	Alkadiene
10	36.07	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.484	0.32	Fatty acid ethyl ester
11	36.23	Ethane, 1-(o-ethylphenyl) -1-phenyl-	$C_{16}H_{18}$	210.31	0.95	Aromatic hydrocarbon
12	36.92	1-n-Hexadecylindan	$C_{25}H_{42}$	342.611	0.19	Indene
13	37.33	Cyclic octaatomic sulfur	S_8	256.48	0.24	Homomonocyclic compound
14	38.08	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.495	0.13	Fatty acid ethyl ester
15	39.18	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.506	0.05	Fatty acid ethyl ester
16	39.29	Ethyl Oleate	$C_{20}H_{38}O_2$	310.522	0.28	Fatty acid ethyl ester
17	43.40	Cinnamyl cinnamate	$C_{18}H_{16}O_2$	264.324	0.58	Ester
18	44.11	Dihydrochrysin	$C_{15}H_{12}O_4$	256.257	54.89	Flavanone
19	44.94	Naringenin	$C_{15}H_{12}O_5$	272.256	10.93	Flavanone
20	45.40	Naringenin	$C_{15}H_{12}O_5$	272.256	10.43	Flavanone
21	45.59	Chrysin	$C_{15}H_{10}O_4$	254.241	8.05	Flavone
22	45.83	2-Ethylsulfanyl-4-(4-methoxy-phenyl)-6-methyl-nicotinonitrile	$C_{16}H_{16}N_2OS$	284.4	1.17	Pyridine

23	45.96	Emodin	$C_{15}H_{10}O_5$	270.24	2.83	Anthraquinone
24	46.41	1-Pentamethyldisilyloxy-3-phenylprop-2-ene	$C_{14}H_{24}OSi_2$	264.51	3.67	Phenylpropanoids

Figure 4: GC-MS chromatogram of propolis from Rewari, Haryana



GC-MS chromatogram analysis of propolis sample from Kota, Rajasthan showed 5 peaks which were identified after comparison of the mass spectra with the NIST library indicating the presence of five chemical constituents. The major compounds have been identified based on the percentage peak area in the Chromatogram. The active principles with their retention time (RT), Molecular formula (MF), Molecular weight (MW), Peak Area (%), nature of the compound in the propolis from Kota, Rajasthan are presented in Table 6. The mass spectrum of the chemical compounds identified in Kota, Rajasthan is presented in Figure 5.

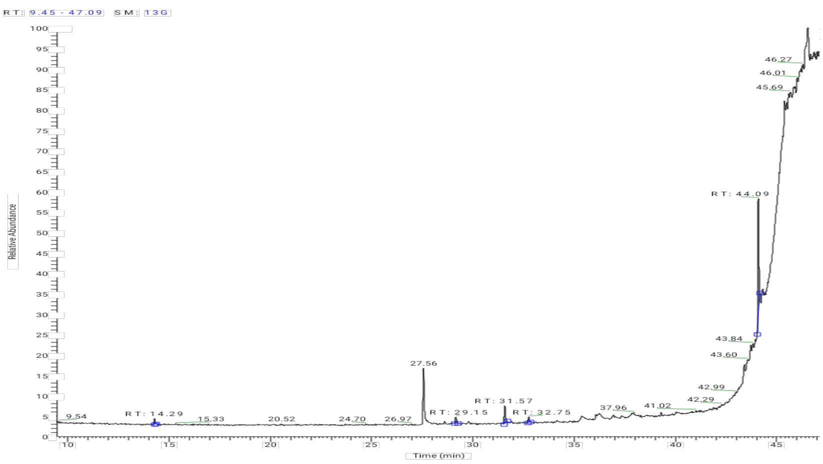
It was observed that the main types of compounds in the propolis from Kota are: Pinocembrin or Dihydrochrysin, (73.51%) was the most prevalent. A second major compound p-1-indanyl- phenol (13.86%) was also found in high amounts. The compounds epi-γ-Eudesmol,(5.67 %), cis-Eudesm-6-en-11-ol (4.15%) and 3,5-dimethyl-octane (2.81%) were found in comparatively lesser amounts.

Table 6: GC-MS spectral of propolis from Kota, Rajasthan

Sl. No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound
1	14.29	Octane, 3,5-dimethyl	$C_{10}H_{22}$	142.286	2.81	Alkane
2	29.15	epi-γ-Eudesmol	$C_{15}H_{26}O$	222.372	5.67	Sesquiterpenoid
3	31.57	Phenol, p-1-indanyl-	$C_{15}H_{14}O$	210.276	13.86	Phenol
4	32.75	cis-Eudesm-6-en-11-ol	$C_{15}H_{26}O$	222.372	4.15	Sesquiterpenoid

5	44.09	Dihydrochrysin	$C_{15}H_{12}O_4$	256.257	73.51	Flavanone

Figure 5: GC-MS chromatogram of propolis from Kota, Rajasthan



This publication is the first to report the presence of two novel phenolic acids- deoxy podocarpol and p-1-indanyl phenol in Indian propolis. (Kumar *et al.,* 2009) reported the presence of novel compounds in Indian propolis like p-coumaric acid, Benzyl cinnamate, 4-pentanoic acid, and Ferulic acid, identified for the first time in the Propolis from Gujarat zone. The presence of 24 compounds (9 known and 15 novel compounds) in Indian stingless bee propolis from Raigad District of Maharashtra State was reported by (Choudhari *et al.,* 2012)

(Kumar *et al,* 2011) reported the presence of the novel compound 1,4 Di-O-Acetyl-2,3,5-tri-O-Methylribitol (C12H22O7), identified for the first time in propolis from *Apis mellifera* and *Trigona sp.* collected from Coimbatore, Tamil Nadu, India. (Shah *et al.,* 2014) analyzed the propolis samples from Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) and identified Sixteen different compounds that have not been reported previously in Indian propolis (Ramnath *et al.,* 2015) analyzed the chemical composition of six Indian propolis samples collected from six different Indian regions like Karnal from Haryana State, Hamirpur from Himachal Pradesh, Sarangpur from Uttaranchal, Palladam from Tamil Nadu, Hubli from Karnataka and Sultanbatteri from Kerala using GCMS analysis. In the study, 93 compounds were identified and 14 being new for Indian propolis.

Compared with the findings of this research, although these constituents are somewhat different from those which have been previously reported in propolis from other geographical regions of India by several researchers, however, it is interesting to note that there are some common constituents between the samples. (Naik *et. al.* 2013) have reported the chemical composition of the essential oil obtained from Indian propolis (Mahabaleshwar, Maharashtra). The essential oil was shown to contain long-chain alkanes (tricosane, hexacosane, heptacosane, heneicosane), terpenoids (linalool, methyleugenol, geraniol) and phenols ((Z)-ethyl cinnamate) as major groups of compounds

(Shashikala *et al.*, 2016) reported the presence of thirteen compounds in the propolis sample from Bangalore, Karnataka. The major constituents were Ethylhexanol, 3 ethyl, 3 methylheptane, Dodecane, 1,1, dimethylethyl, Tetradecane, 4,6 dimethyl, Tetracosane, Diethyl phthalate, Dibutylpbhthalate, Hexadecanoic acid, Octadecanoic acid, 1,2, benzenedicarboxylic acid, Hexatricontane.

(D'Souza *et al.*, 2016) identified flavonoids, alkaloids, phytosterols, triterpenes and glycosides from the propolis from the Shahapur region of Maharashtra. (Kalia *et al.,* 2015) reported the presence of various flavonoids, esters, ketones, and acids that were identified in propolis from Chandigarh, Punjab. The major compounds in EEP were 4', 5, 7-Trihydroxy flavanone, 4H-1-benzopyran4-one, isoquinoline, propanone and cinnamic acid (Shashikala *et al.,* 2016) quantified flavonoids in six samples of propolis obtained from different locations in Karnataka and Tamil Nadu by GCMS analysis and reported high concentrations of flavonoids in propolis samples from Bangalore, Coorg, and Coimbatore. (Verma *et al*, 2017) studied the propolis obtained from Patiala (Punjab) and Delhi, propolis sample from Delhi showed the presence of flavonoids, phenolics, terpenoids, and fatty acids whereas the propolis extract from Punjab showed the presence of polycyclic hydrocarbon derivatives in addition to flavonoids, phenolics, terpenoids, steroids, and fatty acids. (Thirugnanasampandan *et al.*, 2012) analyzed propolis from Tamil Nadu, India and identified the presence of fatty acids and phenolic substances. (Shubharani *et al.,* 2014) compared the chemical constituents of Indian propolis samples collected from different locations in India like Karnal from Haryana, Dehradun from Uttarakhand, Coimbatore from Tamil Nadu and Bijapur from Karnataka by GCMS technique. The results of the four samples showed the presence of 44 compounds in the samples.

Conclusion

The results of the present study by GCMS analysis revealed variation in the volatile profiles of propolis samples collected from different geographical regions of India. The volatile profile represents a chemical fingerprint of propolis since both the nature and the amount of volatile compounds are characteristics of the botanical source. The characterization of the volatile profiles of the propolis samples helped in identifying marker compounds that differentiate propolis from various geographical regions in India. Volatile profiling of Indian propolis led to the identification of many volatile constituents with various biological activities.

The present study can also guide us in further research on the isolation and purification of the two novel phenolic compounds deoxy- podocarpol and p-1-indanyl phenol identified in Indian propolis. Besides, it is also necessary to investigate the various pharmacological activities of the two novel phenolic compounds. It is evident from the present study that the knowledge of volatile compounds in Indian propolis is far from being exhaustive. Further research is needed to reveal the chemistry of volatile compounds of propolis from other unexplored regions of India. Also, volatile constituents in Indian propolis may prove to be a promising drug in the near future and can be the subject of pharmaceutical research.

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