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Gas Chromatography-mass Spectrometry (GC-MS) Comparative Chemistry of Two Indian Propolis Samples.

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ABSTRACT: *Propolis is a complex honeybee product varies in colour texture and chemical composition depending on the location of the hives and local flora. The objective of this work is to analyze and identify the different compounds in Srinagar (Jammu and Kashmir) and Coimbatore (TamilNadu) propolis samples from two different regions of India by GC-MS analysis. The chemical composition of propolis samples obtained from Srinagar (Jammu and Kashmir) and Coimbatore (TamilNadu) have been investigated by GC-MS analysis and sixteen diferent compounds have been tentatively identified. Most of these compounds have not been reported previously in Indian except one compound that is Oelic Acid from Srinagar (Jammu and Kashmir) .While comparing the two samples they have shown total different chemical compositions. The chromatographic and mass-spectral characteristics of the different compounds identified could be very useful for rapid GC-MS profiling of these propolis types for revealing their plant sources.*

Key words: *Propolis, Comparative, chemical composition, GC-MS profiling.*

INTRODUCTION

Propolis or »bee glue« is a sticky, gummy, resinous product of honeybees *(Apis mellifica* L.) that is accumulated in hives. Bees collect propolis from various leaf buds and cracks in the bark of trees[1]**.** In the northern hemisphere (Europe, North and South America and western Asia), the tree sources are: *Populus* spp., *Betula* spp., *Ulmus* spp., *Quercus* spp., *Salix* spp., *Aesculus hippocastanum* L., *Picea* spp., *Fraxinus* spp, *etc*[2]. These origins may account for the smell, color, constitution, and chemical composition of propolis. Propolis is a very complex mixture and, in general, it is composed of 50% balsams and resins, 30% wax, 10% essential oils, 5% pollen and 5% of various other substances like sugars, vitamins, *etc* [3] .Chemically, flavonoid aglycones from propolis are flavones, flavonols, flavanones, dihydroflavonols and chalcones. Other phenolic compounds are phenolic aldehydes and polyphenolic derivates of cinnamic and benzoic acid, including caffeic acid esters, terpenes, -steroids, sesquiterpenes, naphthalene and stilbene derivatives[4].

Chemical studies were also performed, starting in the late 1960s, and demonstrated the variability of propolis' composition depending on plant source [5, 6, 7 and 8]. Because of the very complex chemical composition,GC-MS became the most often used method in the 1980s for rapid chemical characterization of propolis samples of different geographic and plant origins[9].However,most of the constituents of propolis are relatively polar (flavonoids, phenolic acids and their esters, etc.) [10, 11 and 12].

With the advent of modern chromatographic techniques frequently associated with mass spectrometry (MS), many compounds have been isolated and identified in propolis [6]**.** But the complex chemical composition of propolis is frequently updated due to many regional variations. More than 300 propolis constituents have been identified using different chromatographic and spectroscopic techniques including chromatography-mass spectrometry (GC-MS). Among them, the volatile compounds are great important due to their potent biological activities.

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The great variability in chemical composition of the propolis from different regions is because honeybees extract raw materials from different plants in different ecosystems for their production of propolis[13].

The present study investigated the Comparison of composition of 70% ethanolic extracts of two propolis samples collected from two different regions of India, Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) using gas chromatography-mass spectrometry (GC-MS). The variation in composition is related to the constituents of the plant material making up the native vegetation.

MATERIALS AND METHODS

Collection and Preparation of Propolis Extracts

The crude Propolis Samples were collected from Srinagar (Jammu and Kashmir) and Coimbatore (TamilNadu) India during April 2012 and 2013 respectively by Simple Scrapping method.Scrapped propolis samples were stored at -10 °C till processing.

The samples 50mg each were grounded with mortar and pestle to a fine powder. Propolis, samples after grounded were shaked over Shaker (Hewlett-Packard SHK 1172) for 48 hrs with 70%ethanol (1:10, w/v) at room temperature in airtight Volumetric Titration flasks 250ml capacity. The 48 hrs shaked ethanolic extracts of propolis were filtered through Whatman No. 01 paper After filtration; the extracts were evaporated to dryness under vacuum at 50 °C. The ethanolic extracts of propolis were directly used for GC-MS Analysis. (Kumar *et al*., 2009).

GC-MS Analysis

The GC-MS analysis was performed with a Hewlett-Packard gas chromatograph 6890 series II Plus linked to a Hewlett-Packard 5975 mass spectrometer detector equipped with a HP5MS (30 m x 0.25 mm x 0.25 μm) capillary column operating with EI mode at 70eV . The temperature was programmed from 100 to 300 _C at a rate of 5 _C/ min. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. The injector temperature 280 _C, the interface temperature 300 _C, and the ionization voltage 70 eV.MS Scan range was 40-800m/z. (Kumar *et al*., 2009)

Identification of Compounds

Chromatographic peaks were identified by computer searches in commercial reference libraries. The available reference compounds were co-chromatographed to confirm GC retention times Good spectral matches for most of compounds could be found in the Wiley and National Bureau of Standards (NBS) mass spectral library (Kumar *et al*., 2009).

RESULTS

The comparative Chemical composition of propolis samples which were collected from the two different regions Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) India were investigated by GC-MS analysis and shows 08 individual compounds each (Table 1 and 2, and Fig. 1) and of total 16. Most of the compounds detected in Jammu and Kashmir and Tamil Nadu samples have not been previously reported in the Indian propolis except One compound that is Oleic Acid [14] and there is complete variation in the components from one region Srinagar to the other Coimbatore due to the change in topography.

The details of various compounds reported including are,

(1) Pentetic Acid. (RT16.28)

(2) Pentadecanoicacid,13-methyl, methylester. (RT17.18)

(3) 1,2 Benzenedicarboxylic acid, butyloctylester. (RT17.67)

(4) Dasycarpidan-1-methanol, acetate (ester). (RT18.07)

(5) 16-Octadecenoic acid, methyl ester. (RT18.9)

(6) Heptadecanoic acid,16-methyl ester. (RT19.12)

(7) 9-Octadecenoic acid (z);2-hydroxy-1 (hydroxymethyl)ethyl ester.(RT25.93) from Srinagar (Jammu and Kashmir) region; and

(8) 1-Heptatriacotanol. (RT 13.23)

(9) Diethyl Phthalate. (RT13.87)

(10) 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal. (RT14.4)

(11) Podocarpa-1,12-diene14-aceticacid,7-hydroxy-8,13-dimethyl-3-oxo-, e-lactone. (RT16.05)

(12) Cyclopenta(g)-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8 hexamethyl.(RT16.48)

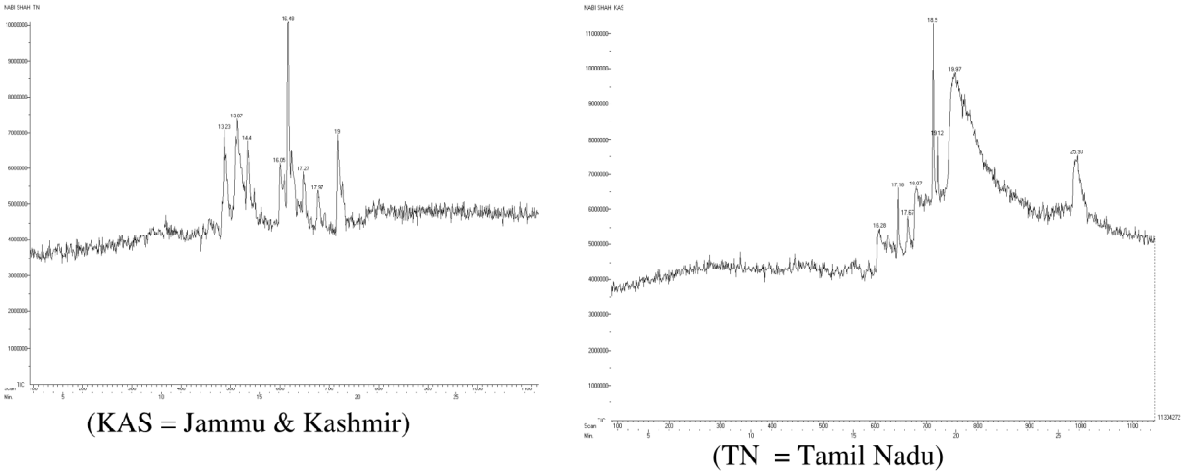
(13) Cyclopropanebutanoicacid,2-[(2-[(2-[(2 pentylcyclopropyl)methyl]cyclopropyl] methyl] cyclopropyl]methyl]-methyl ester. (RT 17.27)

(14) Acetic acid,17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17

dodecahydro-1H-cyclopenta(a)phenanthren-11-yl(ester). (RT17.97) and

(15) 16-Octadecenoic acid, methyl ester(RT 19.00) from Coimbatore (Tamil Nadu) Indian region.

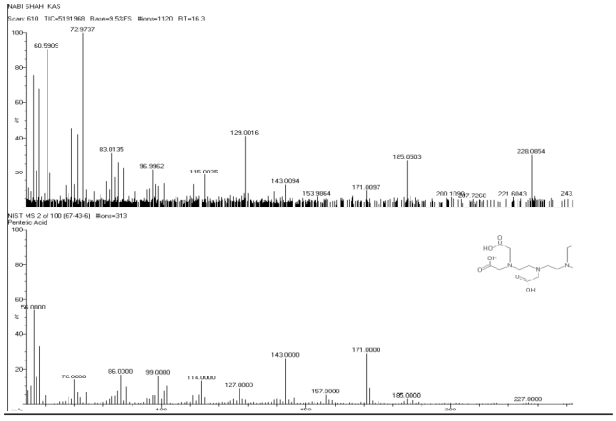
The RT values Vs TIC values for the two samples are shown in Table 02 and corresponding Chromatographs are shown in Fig 01 respectively the corresponding Chromatographs shown very good mass spectral matches.



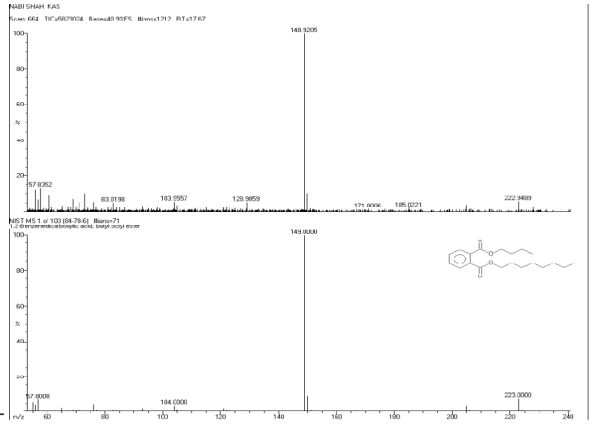
(KAS = Jammu & Kashmir)

(TN = Tamil Nadu)

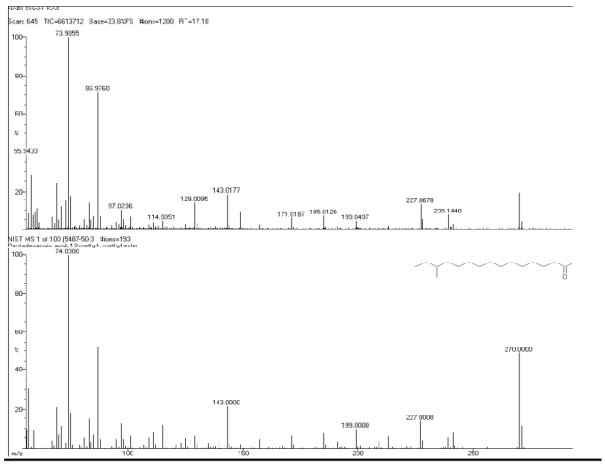
Figure 1: GC Chromatograms of ethanolic propolis extracts from (KAS) Srinagar (Jammu and Kashmir) and (TN) Coimbatore (TamilNadu) India. The No. of compounds identified correspond to those in Table 1.



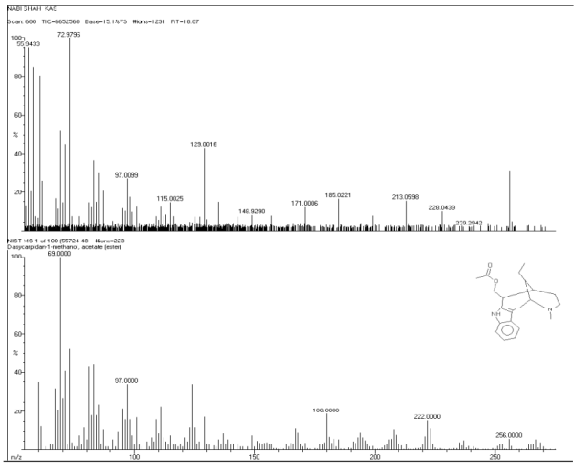
KAS Peak No. 01 ,RT = 16.3



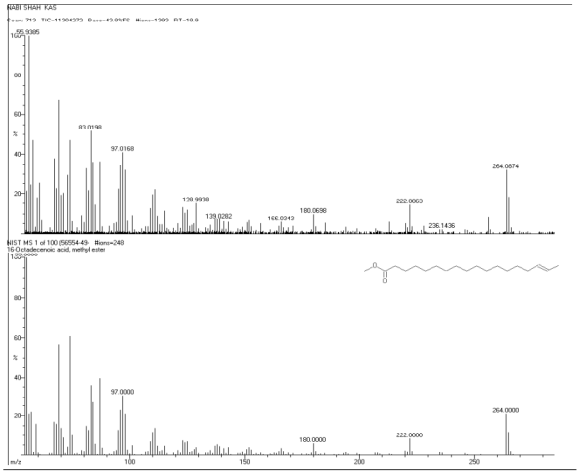
KAS Peak No.03 ,RT = 17.67



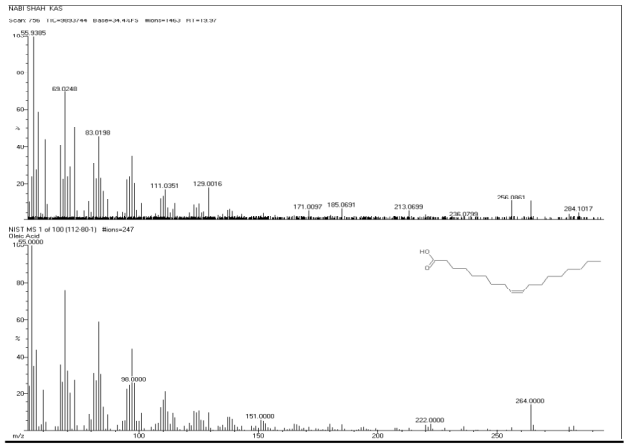
KAS Peak No. 02 ,RT = 17.18



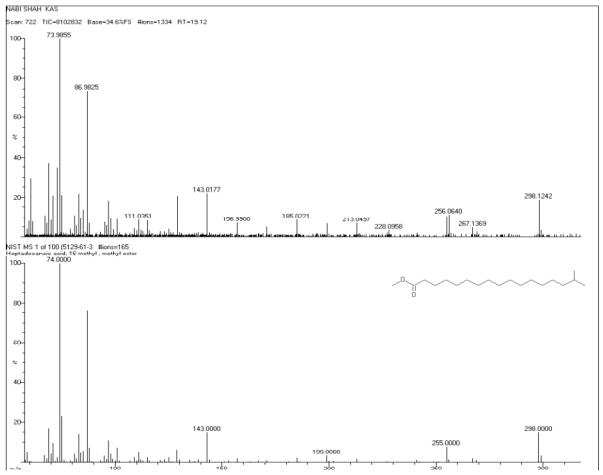
KAS Peak No.04 ,RT = 18.07



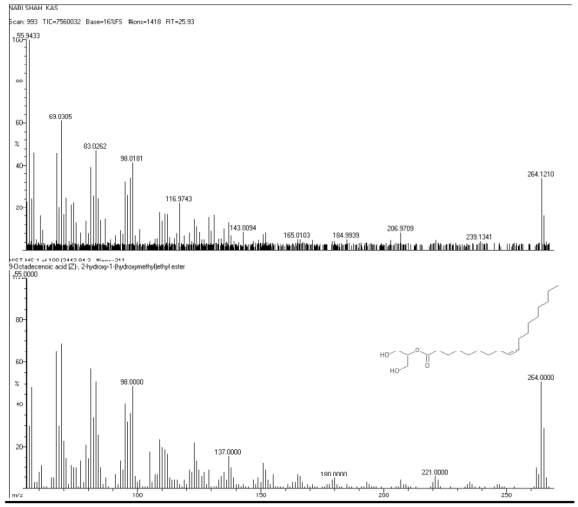
KAS Peak No.05 ,RT = 18.9



KAS Peak No.07 ,RT = 19.97

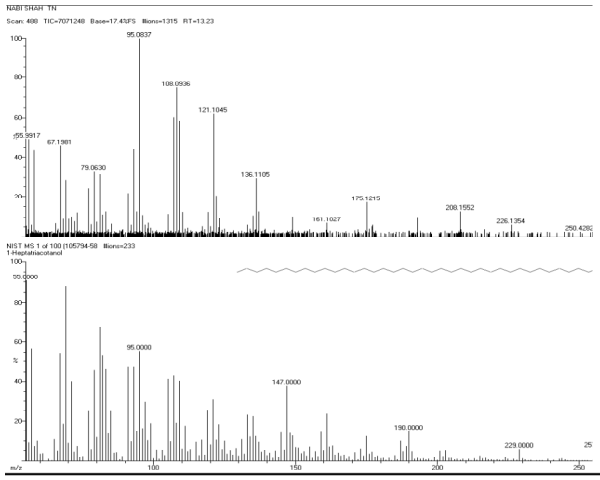


KAS Peak No.06 ,RT = 19.12

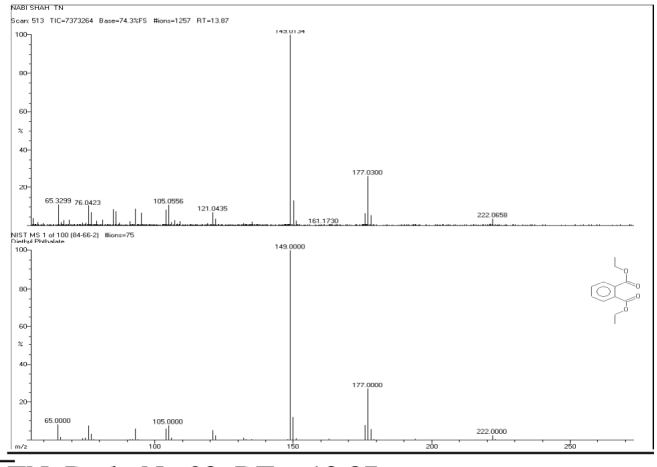


KAS Peak No.08 ,RT = 25.93

Figure 2: KAS Peak No.01 to KAS Peak No. 08 Shows Mass Spectra of Different isolated compounds from Srinagar (Jammu and Kashmir). [The Numbering of the compounds corresponds to that in Table 01 and Figure 01].



TN Peak No.01 ,RT = 13.23



TN Peak No.02 ,RT = 13.87

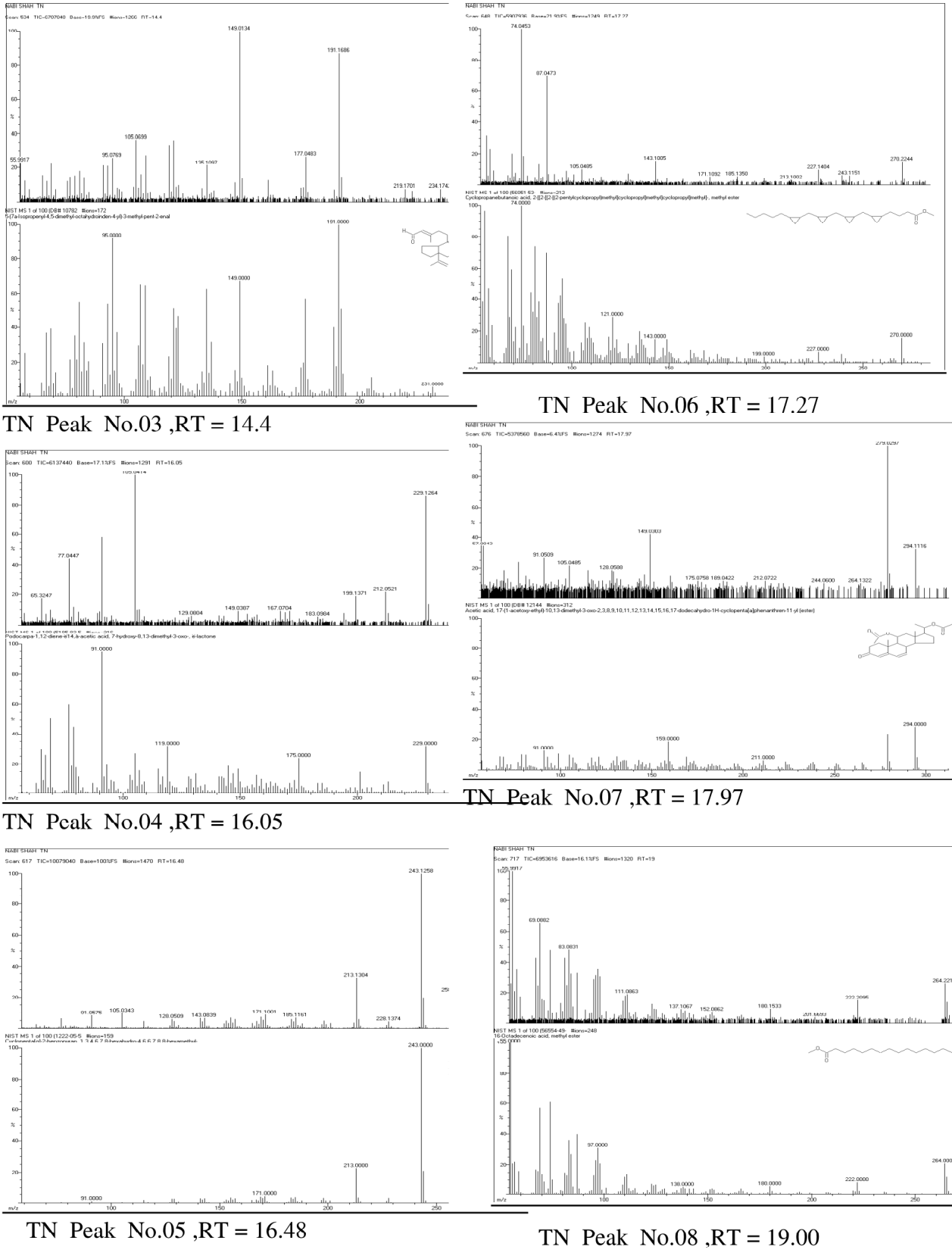


Figure 3: TN Peak No.01 to TN Peak No. 08 Shows Mass Spectra of Different isolated compounds from Coimbatore (Tamil Nadu). [The Numbering of the compounds corresponds to that in Table 01 and Figure 01]

Table 1
GC-MS Comparison of Compounds Identified in Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) Indian Propolis

Peak No.	*Srinagar (Jammu and Kashmir) Propolis*			*Coimbatore (Tamil Nadu) propolis*		
	RT	*Compound Identified*	*M⁺*	*RT*	*Compound Identified*	*M⁺*
1	16.28	*Pentetic Acid.	228	13.23	*1-Heptatriacotanol.	250
2	17.18	*Pentadecanoic acid,13-methyl,methyl ester.	270	13.87	*Diethyl Phthalate.	222
3	17.67	*1,2-Benzenedicarboxylic acid, butyl octyl ester.	222	14.4	*5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal.	231
4	18.07	*Dasycarpidan-1-methanol, acetate(ester).	256	16.05	*Podocarpa-1,12-diene14-acetic acid,7-hydroxy-8,13-dimethyl-3-oxo-,e-lactone.	257
5	18.9	*16-Octadecenoic acid, methyl ester.	264	16.48	*Cyclopenta(g)-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl.	258
6	19.12	*Heptadecanoic acid,16-methyl ester.	298	17.27	*Cyclopropanebutanoic acid,2-[(2-[(2-[(2 pentylcyclopropyl) methyl]cyclopropyl]methyl] cyclopropyl] methyl]-methyl ester.	270
7	19.97	**Oleic Acid.	284	17.97	*Acetic acid,17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-	
8	25.93	*9-Octadecenoic acid (z);2-hydroxy-1 (hydroxymethyl)ethyl ester	264	19.00	*cyclopenta(a) phenanthren-11-yl (ester). 16-Octadecenoic acid, methyl ester.	264

RT = Retention Time , M⁺ = Molecular Spectral mass, *Compounds identified first time , **Coumpounds already identified

Table 2
Comparison of Compounds Identified of their RT Vs TIC Values of Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) Indian Propolis Samples

Peak No.	*Srinagar (Jammu and Kashmir) Propolis*		*Coimbatore (Tamil Nadu) propolis*	
	RT	*TIC*	*RT*	*TIC*
1	16.28	5191968	13.23	7071248
2	17.18	6613712	13.87	7373264
3	17.67	5823024	14.4	6787040
4	18.07	6652560	16.05	6137440
5	18.9	1.1E+07	16.48	1E+07
6	19.12	8102932	17.27	5907936
7	19.97	9893744	17.97	5378560
8	25.93	7560032	19	6953616

For corresponding Chromatograph values RT = Retention Time, TIC =Total ionic current [refer Fig 01]

DISCUSSION

Variations in the chemical nature of propolis of different origin have been observed by many workers [3,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30 and 31]. It may be pointed out that even the two Indian propolis samples have been found to possess different chemical composition. It is worth noting that the majority of the compounds we have detected in the samples of Coimbatore (TamilNadu) propolis are different from those present in the Indian propolis of other origin (Gujrat zone) that has previously been investigated[14]. This may be due to some difference in the source of plants from which the propolis is collected. Similar results were found in great deal of variation in chemical composition of Srinagar (Jammu and Kashmir) and Coimbatore (Tamil Nadu) Ethanol extracts of

propolis. The total composition difference of two samples is evident in comparison to the investigations conducted by Kumar *et al* on propolis samples collected from Gujrat zone of India.We have reported the identification of 16 major com-pounds.The major compounds identified in this work could be used as chemical markers in order to classify and identify botanical origins of propolis. Further research in this area could focus on the evaluation of the biological activity of the compounds.

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