JYP

GC-MS Analysis of Propolis of Indian Origin

Kumar N*, Mueen Ahmed KK[1], Dang R[2], Shivananda TN[3], Das K[4]

**Lovely Professional University, Phagwara, Jalandhar; [1,2]Dept. of Pharmacognosy, Al-Ameen College of Pharmacy, Hosur road, Bangalore; [3]IIHR (Institute of Horticulture Research), Hessaraghatta, Bangalore; [4]St. John's Pharmacy College, Bangalore, India*

Address for correspondence: *Dr. Nilesh Kumar; E-mail: mpharmnilesh@gmail.com*

ABSTRACT

More than 300 constituents have been identified in different propolis samples. This study investigated the composition of ethanolic extracts of propolis samples collected from the Gujarat zone. A gas chromatography-mass spectrometry was carried out on a Agilent GC-MS 5975 under electron impact ionization (70 eV). The chromatographic column for the analysis was done by the HP5MS capillary column (30 m x 0.25 mm internal diameter). The carrier gas used was helium at a flow rate of 1 ml/min. The oven temperature was 100°C to 280°C with a constant increase of 10°C. Propolis samples were analyzed with the column held initially at 60°C for 2 min and then increased to 230°C with a 2°C/ min heating ramp and then kept at 230°C for 3 min. Finally, the temperature was increased to 280°C with a 3°C/min heating ramp. The injection was performed in split mode at 220°C. Sample volumes of 1 µl were injected and analyzed by GC-MS. The following compounds were identified for the first time in the propolis sample: p-coumeric acid, Benzyl cinnamate, 4-pentanoic acid, and Ferulic acid. The main type of the compound identified was fatty acids derivatives. Similar results were found by Silici, *et al*.[5] for the turkey propolis.

Key words: *GC-MS analysis, propolis, Honeycomb*

DOI: 10.4103/0975-1483.51876

INTRODUCTION

The term propolis derives from the Greek pro (for "in front of", "at the entrance to") and polis ("community or city") and means a substance in defense of the hive.[1] Propolis is a resinous, sticky gum, with a color that varies from yellow-green to dark brown depending on its source and age. A propolis is used to make the protective shield at the entrance of beehive.[2] It is also used to fill the cracks in the hive, to attach the corners of frames to the grooves in the hive, and to polish the cells of the honeycomb. Propolis was used especially in antiquity in Egypt. The propolis was very well known to the priests who had monopolized medicine, chemistry, and the art of mummifying corpses. A literature survey revealed that flavonoids, aromatic acid, diterpenic acid, and phenolic compounds appear to be the principle components responsible for the biological activities of propolis. This study investigated the composition of ethanolic extracts of propolis (GEEP) samples collected from the Gujarat zone. This extract was selected for GC-MS analysis as it was showing maximum activity in case of the antibacterial,[3] anti-fungal,[3] and anthelmintic[3] activity.

MATERIALS AND METHODS

Chemicals

Bis-(trimethyl-silyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (Merck-2333) were used as silylation reagents with spectrophotometric grade pyridine (Merck-7460).

Instrument

A gas chromatography-mass spectrometry was carried out on an Agilent GC-MS 5975 under electron impact ionization (70 eV). The interface temperature was 230°C, and the MS scan range was 35-450 atomic mass units (AMU). The chromatographic column for the analysis was done by HP5MS capillary column (30 m x 0.25 mm internal diameter). The carrier gas used was helium at a flow rate of 1 ml/min. The oven temperature was 100°C to 280°C with a constant increase of 10°C. Propolis samples were analyzed with the column held initially at 60°C for 2 min and then increased to 230°C with a 2°C/min heating ramp and then kept at 230°C for 3 min. Finally, the temperature was increased to 280°C with a 3°C/min heating ramp. The injection was performed in split mode at 220°C.

Sample preparation

A collection of 10 mg propolis samples were extracted with ethanol at room temperature to obtain the extract. After filtration, the extracts were combined and evaporated to dryness under a vacuum at 50°C. A total of 1 mg of

Table 1: GC-MS Analysed Compounds of Propolis

Retention time (RT)	Compounds
10.33	4-vinylphenol
11.21	Lauric acid
15.439	Myristic acid
19.088	4-pentanoic acid
19.527	Palmitic acid
20.820	p-coumeric acid
22.834	Oelic acid
22.688	Linoleic acid
23.281	Stearic acid
23.996	Caffeic acid
26.744	Benzylic acid
23.996	Benzyl cinnamate
26.253	Cinnamyl cinnamate
23.883	Ferulic acid

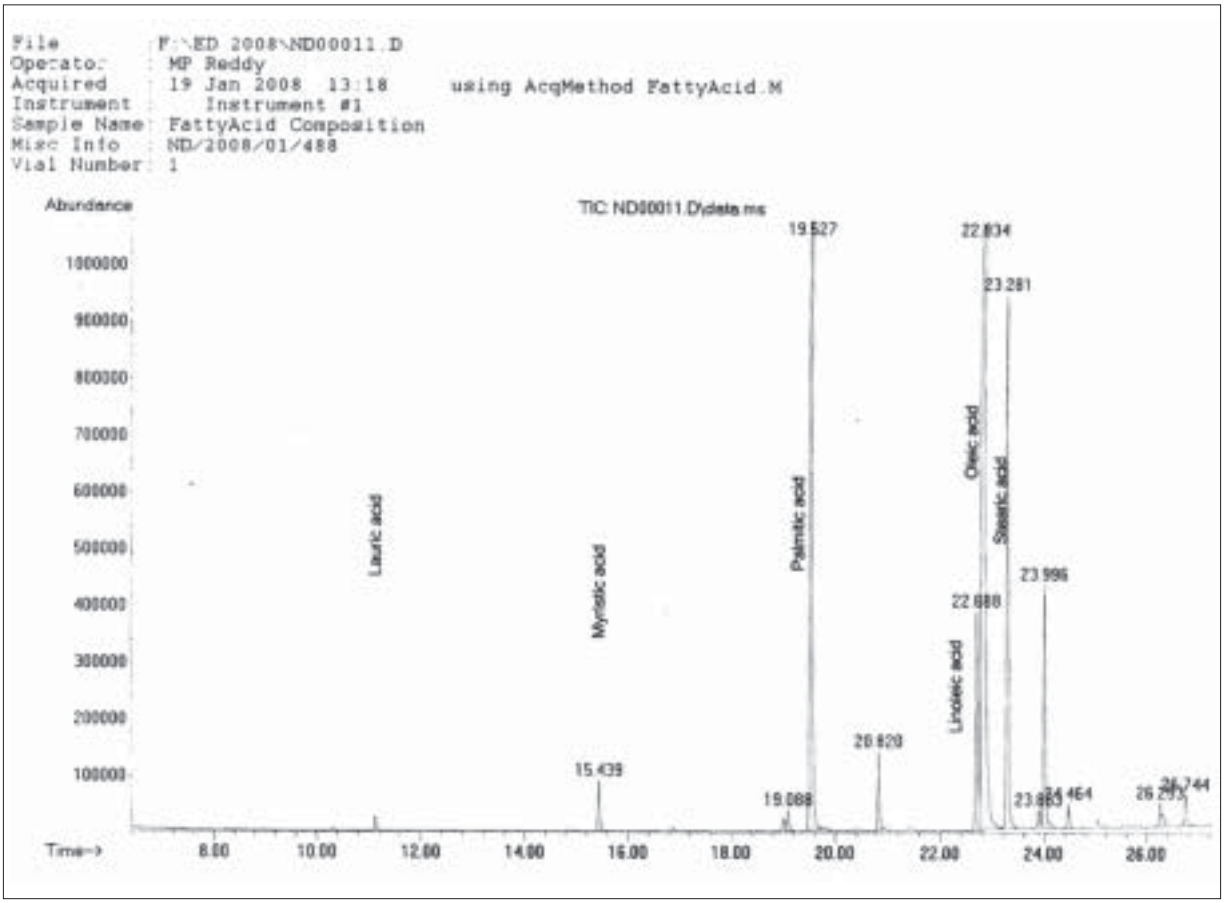

Figure 1: GC-MS Report of the Propolis of Gujarat Zone

dry extract was reacted with 50 µl pyridine + 100 µl bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100°C to prepare samples for gas chromatography. Sample volumes of 1 µl were injected and analyzed by GC-MS.

Identification of compounds

Peaks were identified by Mr. M. Prabhakara Reddy at the Bangalore Test House in Bangalore. Good spectral matches for some compounds could be found in the Wiley and National Bureau of Standards (NBS) mass spectral library.

Chromatographic Condition

Column	:	HP5MS (30 m x 0.25 i.d.)
Carrier gas	:	Helium
Oven temperature	:	100 - 280°C
Inlet temperature	:	280°C
Detector temperature (MSD)	:	230°C
Carrier gas flow rate	:	1 ml/min
Injection quantity	:	1 µl
Scan mode	:	50 – 550 mod range

RESULTS

The chemical composition of propolis, which was collected from the Gujarat zone, was investigated by GC-MS after silylation. More than 13 individual compounds were identified Table 1 and Figure 1. For this reason, the chemical composition is presented by means of the main type of compounds identified not as a percentage of individual substances. As expected, the samples displayed the typical pattern of a "poplar" propolis: they contained pinocembrin, chrysin, galangin, caffeic acid, 3- hydroxy-4-methoxycinnamic acid, 3,4-dimethoxycinnamic acid, etc. and the ratio between the main compound classes corresponded to that in *P. nigra* bud exudates.[4,5] However, there may be a variation in the components from one region to the other due to the change in topography. The following compounds were identified for the first time in the porpolis sample: p-coumeric acid, Benzyl cinnamate, 4-pentanoic acid, and Ferulic acid. The main type of the compound identified was fatty acids derivatives.

DISCUSSION

Similar results were found by Silici, *et al*. for the turkey propolis.[6,8] He reported the presence of 9- octadecanoic acid, hekzadecanoic acid, benzoic acid, 3- hydroxy-4-methoxycinnamic acid, 3,4-dimethoxycinnamic acid, benzyl benzoate, benzene ethanol, β-eudesmol, β-bisabolol, glycerine, 2-nonadecanone, 2-propen-1-one, 4*H*- 1-benzopyrane-4-on, 2-propenoic acid, heneicosane, and eicosane in all of the samples of the propolis of Turkey.[6,7] This sample of the Gujarat zone is obviously not of popular origin, its plant source is yet unknown. It will be the aim of future investigations to uncover this plant and study its biological action. This could be of interest because it has been shown that bees have the ability to find and use a propolis source in their environment, which is the best agent to protect their hives against bacterial and fungal infections. Further studies also can be conducted to determine the "type" of propolis, according to its plant source, which will be the first step for quality control of the bees' glue. This will allow us to define the type of compounds that should be quantified as main active propolis constituents.

REFERENCES

1. Burdock GA. Review of the biological properties and toxicity of bee propolis. Food Chem Toxicol 1998;36:347-63.
2. Hegazi AG. Propolis an over view. Internation Symposium on Apitherapy. Cairo, 8th- 9th March, 1997.
3. Ghisalberti EL. Propolis: A review. Bee World 1979;60:59-84.
4. Heard TA. The role of stingless bees in crop pollination. Annu Rev Entomol 1999;44:183-206.
5. Bankova V, Popova M, Bogdanov S, Sabatini AG. Chemical composition of European propolis: Expected and unexpected results. Z Naturforsch [C] 2002;57:530-3.
6. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honey bees in the same region. J Ethnopharmacol 2005;99:69-73.
7. Yaghobi SMJ, Ghorbani GR, Soleimanian ZS, Satari R. Antimicrobial activity of Iranian propolis and its chemical composition. Apidologie 2007;15:45-8.
8. Oksuz H, Duran N, Tamer C, Cetin M, Silici S. Effect of propolis in the treatment of experimental Straphylococcus aureus Keratitis in rabbits. Ophthal Res 2005;37:328-34.

Source of Support: Nil, **Conflict of Interest:** None declared.