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### GC-MS ANALYSIS OF PHYTOCOMPONENTS IN THE METHANOLIC EXTRACT OF PROPOLIS OF HONEY BEE

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#### ABSTRACT

Propolis is a resinous Bee hive product and has been long used in folk medicine of different nations as early as 3000 BC. It consists of exudates from plants mixed with beeswax. The present study was carried out to identify the phytochemicals present in the methanolic extract of the propolis of honey bee *Apis mellifera* by GC-MS analysis. From the GC-MS results thirteen compounds were identified as major constituents, they are Ethylhexanol, 3 ethyl 3 methylheptane, Dodecane, 1,1 dimethylethyl, Tetradecane, 4,6 dimethyl, Tetracosane, Diethyl phthalate, Dibutylphthalate, Dibutylphthalate, Hexadecanoic acid, Octadecenoic acid, 1,2 benzenedicarboxylic acid, Hexatriacontane. These different active phytochemicals have been found to possess a wide range of biological activities, which may help in the protection against incurable diseases.

#### KEYWORDS

GC-MS, Propolis, *Apis mellifera*, Folk medicine and Phytochemicals.

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#### INTRODUCTION

Propolis is a honeybee product with a broad spectrum of biological properties<sup>1</sup>. As a resinous substance, Propolis is prepared by the honeybees to seal the cracks, smooth walls, and to keep moisture and temperature stable in the hive all year around. Propolis is widely used in traditional medicine and is reported to have pharmacological activities. Besides its traditional uses, it has recently gained popularity as a food supplement in numerous countries, claimed to improve health and prevent diseases<sup>2,3</sup>. Various biological activities, such as

anticancer, antioxidant, antibiotic effects have been reported for propolis and its component<sup>4</sup>.

Propolis is a complex resinous mixture which contains approximately 50% of resin and balsam, 30% of wax, 10% of essential and aromatic oils, 5% of pollen, and 5% of impurities<sup>5</sup>. The chemical composition of propolis is highly variable mainly due to the variability of plant species growing around the hive, from which the bees collect the exudates<sup>6,7</sup>.

The chemical composition of propolis appeared to be extremely complex and more than 180 compounds have been identified so far<sup>8</sup>, the most important ones being polyphenols. Now it is known that bees collect propolis from different plant buds<sup>9</sup>. The aim of the present work is to study the composition of propolis by gas chromatography-mass spectrometry (GC-MS) an effective combination for chemical analysis, and biomedical applications of propolis.

## MATERIAL AND METHODS

### Collection of the Sample

Samples of propolis produced by *Apis mellifera* were obtained from Apiary centers in Bangalore. The collected propolis samples were kept desiccated in the dark until analysis.

### Preparation of Extract

50 g of powdered propolis was extracted with 250mL methanol at the temperature between 60 and 65°C for 24 h by using soxhlet extractor. The solvent was evaporated by rotary vacuum evaporator to obtain viscous semisolid masses. This semi dry methanolic crude extract was subjected to GC-MS analysis.

### Gas Chromatography-Mass Spectrometry analysis

The methanolic extract of the propolis of was subjected to GC-MS analysis on a GC- MS Clar us 500 Perkin Elmer system comprising a AOC- 20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Restek Rtx R- 5, (30meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at

70 eV; helium (99.999%) was used as carrier as at a constant flow of 1ml/min and an injection volume of 1.0 µl was employed (split ratio of 10:1); injector temperature 280°C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 60°C/ min to 280°C, then ending with an isothermal for 15min at 280°C. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes.

### Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

## RESULTS

GC-MS chromatogram of the methanolic extract of Propolis showed thirteen major peaks (Figure No.1) and have been identified after comparison of the mass spectra with NIST library indicating the presence of thirteen phytocomponents. From the results, it was observed that presence of Ethylhexanol, 3 ethyl, 3 methylheptane, Dodecane, 1, 1 dimethylethyl, Tetradecane, 4, 6 dimethyl, Tetracosane, Diethyl phthalate, Dibutylphthalate, Dibutylphthalate, Hexadecanoic acid, Octadecenoic acid, 1, 2 benzenedicarboxylic acid, Hexatricontane were the major components in the extract. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) and biological activities of prevailing compounds are summarized in Table No.1.

## DISCUSSION

In the present study, the GCMS analysis of the methanolic extracts of propolis Sample-showed the presence of 13 compounds. 75% of compounds present in Propolis Sample showed the property of biological activity.

The compounds namely Octatrienal, Tetracosane, Hexadecanoic acid are reported to possess

antioxidant property<sup>12</sup>. The two compounds namely 1, 2 benzene dicarboxylic acid, Diethyl phthalate are antimicrobial<sup>13</sup>. Octadecenoic acid is reported to be cancer preventive. Ethyl hexanol has the property of antifungal<sup>10</sup> activity; Heptane is antiallergic<sup>11</sup>, Octadecenoic acid and Hexatriacontane is well-known for its anti-inflammatory activity<sup>14</sup>. 1, 1dimethylethyl has free radical scavenging capacity<sup>12</sup>.

These results showed that GCMS is a technique capable to provide an overall view of Propolis composition. This technique is a useful and alternative tool for pharmaceutical application. Since literature survey accounts for qualitative and quantitative variations of propolis in different location, standardized method will allow connecting a particular propolis variety to specific biological activity and will be helpful for further drug development.

**Table No.1: Phytocomponents identified in the of propolis sample of *Apis Mellifera* by GC-MS**

S.No	Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Peak area	Structure	Activity
1	13.466	Ethylhexanol	C <sub>8</sub> H <sub>18</sub> O	130.23	7.01		Antifungal <sup>10</sup>
2	14.180	3 ethyl 3 methylheptane	C <sub>7</sub> H <sub>16</sub>	100.201	3.28		Antiallergic <sup>11</sup>
3	17.953	Dodecane	C <sub>12</sub> H <sub>26</sub>	170.334	2.52		No activity
4	19.328	1,1 dimethylethyl	C <sub>10</sub> H <sub>14</sub> O	168.66	4.28		free radical scavenging <sup>12</sup>
5	19.877	4,6 dimethyl	C <sub>14</sub> H <sub>30</sub>	198.388	3.42		No activity
6	22.562	Tetradecane	C <sub>14</sub> H <sub>30</sub>	198.388	1.75		No activity
7	24.575	Tetracosane	C <sub>24</sub> H <sub>50</sub>	338.65	1.93		Antioxidant <sup>12</sup>
8	26.75	Diethyl phthalate	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	166.1308	44.35		Antimicrobial <sup>13</sup>
9	31.131	Dibutylpbhthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.34			No activity
10	33.527	Hexadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.771	2.35		Antioxidant <sup>12</sup>
11	36.192	Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.461	1.04		cancer preventive, antioxidant <sup>12</sup> , anti-inflammatory <sup>14</sup>
12	41.433	1,2 benzenedicarboxylic acid	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub>	330.42	6.35		Cytotoxicity, antimicrobial <sup>13</sup>
13	43.163	Hexatriacontane	C <sub>36</sub> H <sub>74</sub>	506.5790	1.43		Antiinflammatory <sup>14</sup> and analgesic activity <sup>14</sup>

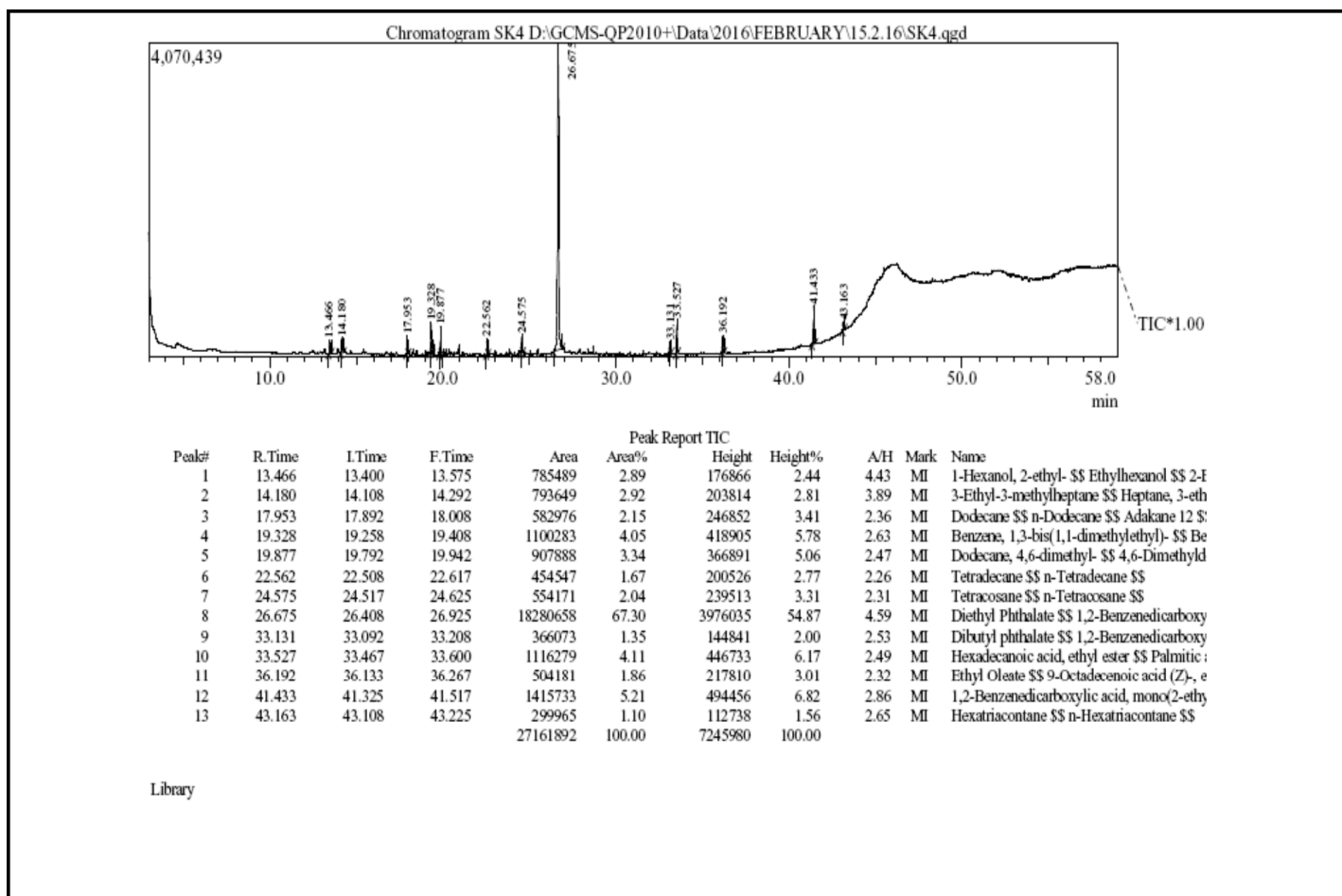


Figure No.1: GC- MS chromatogram of the methanolic extract of propolis of honeybee *Apismellifera*

## CONCLUSION

The presence of various bioactive compounds justifies the uses of propolis for various ailments by local population. However, if individual phytochemical constituents are isolated from propolis and subjecting it to pharmacological activity will definitely give fruitful results. These findings showed that bioactive compounds present in propolis will alleviate many diseases and can be used for better human health.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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