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ANALYSIS OF PHYTOCHEMICAL COMPOUNDS IN WATER AND ETHANOL EXTRACTS OF MALAYSIAN PROPOLIS

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ABSTRACT

Propolis is a complex resinous material with many pharmacological and biological properties attributed to the presence of many volatile and non-volatile phytochemical compounds which have not been analysed in details for Malaysian propolis. In this study, we determined and compared volatile phytochemical compounds in water and ethanol extracts of Malaysian propolis (*Trigona itama*) using gas chromatography-mass spectrometry analysis. Twelve and twenty five volatile phytochemicals were identified from water and ethanol extracts of Malaysian propolis, respectively. The three major phytochemical compounds in water extract propolis were norolean-12-ene (1.73%), 2,3-butanediol (1.69%) and 2,6,10,15,19,23 hexamethyl (1.12%) while in ethanol extract propolis were 1,3-benzenediol-5-pentadecyl (4.18%), cycloartenol (4.06%) and phenol-3-pentadecyl (1.01%). This study indicated that Malaysian propolis phytochemical compounds have different solubility in water and ethanol with more chemicals compounds presence in ethanol extract propolis. Further studies are needed to evaluate the non-volatile phytochemical compounds as well as antioxidant and biological properties of this Malaysian propolis.

KEYWORDS: **GC-MS analysis, propolis, extract, ethanol, water**

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INTRODUCTION

Bee (family *Apidae*) product called propolis has been known as an agent in local medicine and some food industries worldwide[1]. This natural substance is found collected by certain group of bees known as worker bees from different exudates of the area vegetation which they use to fill spaces and construct their hive walls for protection and shelter[2,3]. It is known to contain resins, wax, balsams, and pollen[4]. Since 300 BC, propolis is reported to have medicinal value with many biological functions including antioxidant activity[5]. Some scientists find propolis to possess versatile pharmacological actions such as antiviral, antibacterial, fungicidal, antitumor growth and antiulcer activities[1,6]. Most of the substance or compounds isolated from propolis include flavonoids, diterpenoids, caffeic acid esters, isoflavonoids, geranyl flavanones and lignans[7]. Some of these compounds are responsible for the use of propolis in complementary medicine, cosmetics and food industries[2,8,9]. GC-MS may identify and separate different chemical substances within a test based on their volatility in which they evaporate into gas. It is highly compatible method of analyzing and identifying the volatile phytochemical compounds in a natural product. For example, a study using gas-liquid chromatography and mass spectrometry (GC-MS) analysis for the chemical compositions of *Ixora coccinea* Linn has identified 17 different compounds[10,11]. To date, no study has been reported on the detailed analysis of Malaysian propolis. In this study, therefore, we investigated and compared the phytochemical compounds from both water (WEP) and ethanol (EEP) extracts of Malaysian propolis using an analytical method that combines the features of GC-MS.

MATERIALS AND METHODS

Extract preparation

Raw propolis from stingless bee *Trigona itama* purchased from a local beekeeper in Kota Bharu, Kelantan, Malaysia was washed with distilled water and dried. Then it was frozen at -80 ºC and grounded.

Water extracts of propolis (WEP)

Water extract of Malaysian propolis was prepared using the method as described previously[12,13] with some modification. Thirty gram of grounded propolis was added into 100 mL of deionized water and mixed vigorously at room temperature for 30 min. The mixture was incubated at room temperature on a horizontal shaker for 6 hours daily for a week. Then it was filtered using filter paper and the filtrate was kept in a freezer at 4 ºC overnight. The next day, it was filtered again using filter paper to remove wax and freeze dried at 5 mmHg pressure and - 50 ºC. The yield of WEP was obtained as percentage of the original mass used.

Ethanolic extract of propolis (EEP)

Ethanol extract of Malaysian propolis was carried out using the method as described previously[4] with some modification. Thirty gram of grounded propolis was added into 100 mL of deionized water and mixed vigorously at room temperature for 30 min. The mixture was incubated at room temperature on a horizontal shaker for 6 hours daily for a week. Then it was filtered using filter paper and the filtrate was kept in a freezer at 4 ºC overnight. The next day, it was filtered again using filter paper to remove wax. The filtrate was then concentrated using a rotatory evaporator at 60 ºC followed by freeze drying under 5 mmHg pressure at - 50 ºC. The EEP yield was calculated as the percentage of the original mass used.

Gas chromatography-mass spectrometry (GC-MS) analysis

Both extracts were separately dissolved in methanol (0.01 g/mL) and vortexed for 1 min. The samples were then analyzed using an Agilent-5 GC-MS spectrometer with a HP-5 capillary column (30.0 m x 250 µm) equipped with MSD 5973 detector and split/splitless injection system. Seventy Ev electrons were

used for ionization in the analysis and helium gas was applied to mediate the flow at a rate of 1.0 mL/min. The oven temperature program was as follows: injector temperature 280 ^{0}C, initial oven temperature 2 min at 150 ºC, then 10 ºC/min to 290 ºC and finally 290 ºC at 10 min. The injection was performed in splitless mode with injection port temperature of 250 $^{\circ}$C. The data acquisition was carried out and compounds were identified using the WileyRegistry8e database[10].

Component identification

The interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) and Wiley Registry of Mass Spectral data's, New York (Wiley). The phytochemical compounds analysis was identified based on mass spectral matching with (NIST) and WILEY libraries. However, some information of the component of the test materials such as retention time and percentage peak area was also obtained using this analysis. The chemical compounds with percentage peak of the area more than 0.07% are considered and recorded in this study.

RESULTS AND DISCUSSION

Propolis is a natural product containing many chemicals compounds with many benefits to mankind in which it is used as complementary medicine, cosmetics and in some industries[6]. Previous studies have shown the non-volatile phytochemical compounds in Brazilian, Chinese and Turkish propolis[3,9,12,14,15]. Some of the phytochemical compounds found in propolis include flavonoid and phenolic compounds using different methods of preparation. In this study we used GC-MS analysis to determine and compare the phytochemical compounds in WEP and EEP of Malaysian propolis. GC-MS chromatogram and phytochemical compounds for water extract of Malaysian propolis are shown in Figure 1 and Table 1, respectively. Twelve volatile phytochemical compounds were identified in WEP in which norolean-12-ene had the highest percentage peak area of 1.73 and retention time of 17.62 while ethylreoctadecanoate had the lowest percentage peak area of 0.20 and retention time of 13.16. Figure 2 and Table 2 show the GC-MS chromatogram and phytochemical compounds for ethanol extract of Malaysian propolis, respectively. Twenty five volatile phytochemical compounds are identified from EEP in which 1,3-benzenediol-5-pentadecyl had the highest percentage peak area of 4.18 and retention time of 15.83 and 2,5-furandione-3-3-methyl had the lowest percentage peak area of 0.17 and retention time of 5.78. The three major phytochemical compounds in water extract propolis were norolean-12-ene (1.73%), 2,3-butanediol (1.69%) and 2,6,10,15,19,23 hexamethyl (1.12%) while in ethanol extract propolis were 1,3-benzenediol-5-pentadecyl (4.18%), cycloartenol (4.06%) and phenol-3-pentadecyl (1.01%). However, the difference in total compound number, type and amount of the various phytochemical compounds identified in WEP and EEP of Malaysian propolis using different mediums showed their difference in terms of solubility. As compared to WEP from Turkey, 17 chemical compounds are detected[14] which are different from our findings in WEP of Malaysian propolis. On the other hand, it has been reported that there are 20 chemical compounds found in EEP of Brazilian red propolis[4] while 24 and 18 chemical compounds in two EEP samples of Turkish propolis[15] as compared to our findings. It is noted that both benzoic and hexadeconoic acids found in our EEP of Malaysian propolis are also detected in EEP of Brazilian and Turkish propolis[4,15]. However, most of the chemical compounds in our findings are different from Turkish and Brazilian red propolis as the chemical composition of propolis is dependent on its geographical location[16]. The activities of phytochemical compounds in water and ethanol extracts of Malaysian propolis based on Dr Duke's Phytochemical and ethno botanical database[17] are summarized in Table 3. Some of the compounds have medicinal and biological properties while some are used in skin care and industrial products.

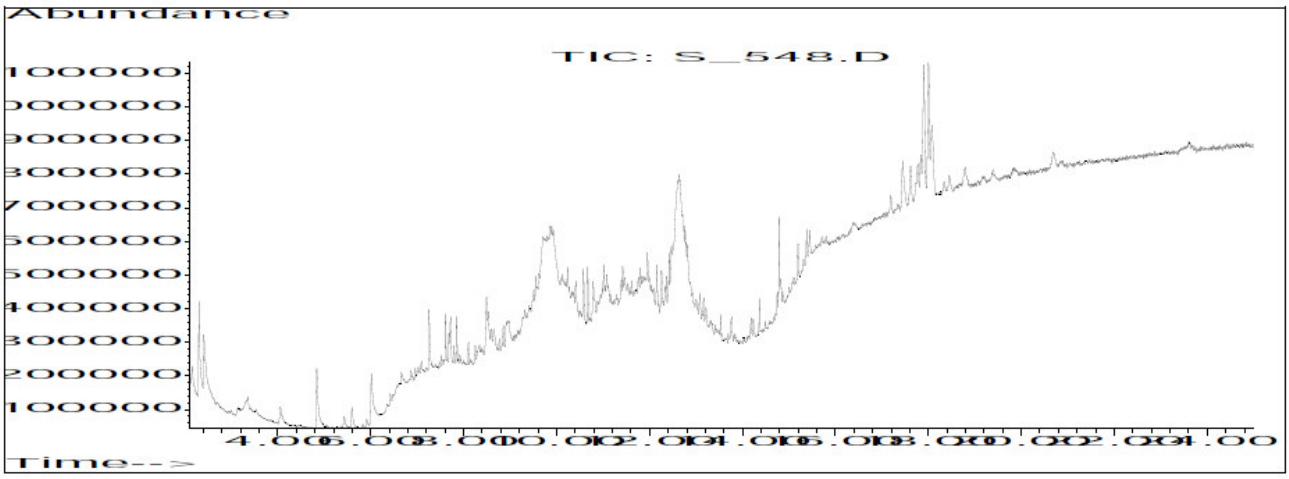

Figure 1
GC-MS chromatogram for water extract of Malaysian propolis.

Table 1
Phytochemical compounds in water extract of Malaysian propolis

No.	Compound name	Retention time	Peak Area (%)
1.	2,3-butanediol	2.41	1.69
2.	2(3h) furanone, dihydro	4.84	1.03
3.	2,3,5,6-tetramethyl pyrazine	7.61	0.40
4.	phenylethylalcohol	7.85	0.27
5.	2-ketoisophorone	8.10	0.14
6.	hexadecanoic acid	12.47	0.81
7.	9-octadeconioc acid	13.09	0.39
8.	ethyloctadecanoate	13.16	0.20
9.	1,2-benzenedicarboxylic acid	14.37	0.24
10.	eicosane	14.79	0.77
11.	2,6,10,15,19,23-hexamethyl	15.20	1.12
12.	norolean-12-ene	17.62	1.73

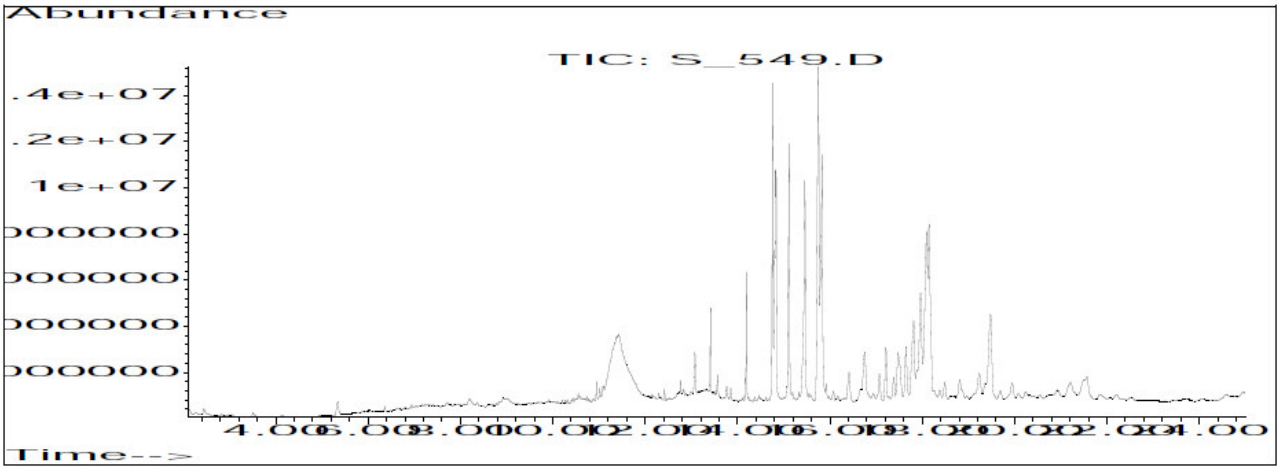

Figure 2
GC-MS chromatogram for alcohol extract of Malaysian propolis

Table 2
Phytochemical compounds in alcohol extract of Malaysian propolis

No.	Compound name	Retention time	Peak Area (%)
1.	2,5- furandione-3-methyl	5.78	0.17
2.	2,4-dihydrxyl-2,5-dimethyl-3(2h) furan-3-one	6.37	0.20
3.	2,5-furandione	6.95	0.19
4.	bezeneethanol	7.87	0.19
5.	benzoic acid	8.37	0.39
6.	benzeneacetic acid	8.93	0.52
7.	isocaryophyllene	9.92	0.20
8.	alpha copaene	10.21	0.27
9.	dodecanoic acid	10.56	0.22
10.	methylester	12.16	0.19
11.	hexadecanoic acid	12.30	0.35
12.	5-octadecene	12.77	0.28
13.	8-octadecenoic acid	12.84	0.45
14.	9-octadecenoic acid	13.09	0.87
15.	9-octadecen-1-ol	13.42	0.80
16.	phenol 3-pentadecyl	14.20	1.01
17.	2-hydroxy-1-(hydroxymethyl) ethyl ester	14.24	0.17
18.	2-ethythexyl ester	14.37	0.22
19.	5-heptyresorcinol	14.47	0.34
20.	1,3-benzenediol-5-pentadecyl	15.83	4.18
21.	Lanosta-8,24-dien-3-ol	17.22	0.92
22.	cycloartenol	18.10	4.06
23.	9,19-cyclolanostan-3-ol	18.49	0.55
24.	9,19-cyclo-9-beta-lonostane-3-beta-25-diol	18.63	0.26
25.	9,19-cyclolanostan-3-ol	20.56	0.32

Table 3
Activity of the compounds identified in the WEP and EEP of the Malaysian propolis

No.	Compound name	Activity
1.	2,3- butanediol	used as flavor
2.	2 (3h) furanone, dihydro	no activity report
3.	2,3,5,6- tetramethyl pyrazine	cosmetic, nutty flavor, fragrance agents
4.	phenylethylalcohol	bacteriostatic agent, antimicrobial, preservative
5.	2-ketoisophorone	no activity report
6.	hexadecanoic acid	antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, flavor
7.	9-ethyl ester	nematicide, antieczemic, antiacne, antiarthritic, insecticide
8.	ethyloctadecanoate	anti-inflammatory hypocholesterolemic cancer preventive, hepatoprotective
9.	1,2-benzenedicarboxylic acid	used in dyes, perfumes
10.	eicosane	antiasthma, smooth muscle relaxant
11.	2,6,10,15,19,23-hexamethyl	no activity report
12.	norolean-12-ene	anti-inflammatory, antiulcer
13.	2,5- furandione-3-methyl	oils, lubricants
14.	2,4-dihydroxyl-2,5-dimethyl-3(2h)furan -3-one	no activity report
15.	2,5-furandione	use as wet strength of paper
16.	bezeneethanol	antimicrobial, flavor, perfume, preservative
17.	benzoic acid	food preservatives
18.	benzeneacetic acid	used in perfumes, drugs (penicillin, amphetamine)
19.	isocaryophyllene	antinociceptive, neuroprotective, anxiolytic, antidepressant
20.	alpha copaene	pesticide
21.	dodecanoic acid	antimicrobial, soaps, shampoo
22.	methyl ester	nematicide, insectifuge antihistaminic, antieczemic, antiacne
23.	5-octadecene	no activity report
24.	8-octadecenoic acid	anti- inflammatory, hypocholesterolemic cancer preventive, hepatoprotective
25.	9-octadecenoic acid	anti-inflammatory, cancer preventive, hepatoprotective, antieczemic, antiacne
26.	9-octadecen-1-ol	no activity report
27.	phenol-3-pentadecyl	skin cream, lotions, cosmetic, shampoos
28.	2-ethythexyl ester	lubricants, plastic wrap
29.	5-heptyresorcinol	anti-aging, anticancer, skin whitening
30.	1,3-benzenediol-5-pentadecyl	no activity report

CONCLUSION

In the present study, twelve and twenty five volatile phytochemical compounds were identified from the water and ethanol extracts of Malaysian propolis, respectively, using GC-MS analysis which have various medicinal and biological properties. It is needed to further identify the non-volatile phytochemical compounds as well as antioxidant and biological properties of this Malaysian propolis which may be beneficial for human nutrition and health.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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