GC/MS characterization of Egyptian propolis different extracts

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Abstract

Propolis is a natural substance known to be beneficial for human health and used as a folk medicine in many parts of the world. In this study, propolis powder was extracted by different solutions; water, hydroalcohol, ethanol and hexane and the resulted extracts were analyzed by GC/MS. All results together give evidence that the solvent used for extractions significantly affect the content of the individual compounds and each extract was associated with different number of fractions and has its own m/z range. These characteristics variation may have an impact on the biological activity of propolis.

Key words: GC/MS, Egyptian propolis, Water, Ethanol, Hexane.

1. INTRODUCTION

Propolis is a resinous substance collected by *Apis mellifera* L. from buds and exudates of different plant sources. It is also mixed with bees wax, pollen, and some certain enzymes from bees' saliva **(Pietta P. G, 2002)** The chemical composition of propolis is diverse and complex. Approximately, 300 compounds have been identified from propolis, including flavonoids, phenolic acids, terpenoids, steroids, and amino acids **(Bankova V. S, 2000)**. Propolis has been

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used as a traditional medicine for thousands of years; thus, it has been extensively investigated in many application fields **(Bankova V. S, 2000 and Tang T.-X., 2014)**. Propolis covers a broad spectrum of biological effects from anticancer (**Sun L.-P, 2012)** and antioxidant **(Hatano A, 2012)** to antiviral **(Vaijwade D. N. S, 2014)** and anti-inflammatory **(Wang K, 2014)** properties. These biological properties can mainly be ascribed to phenolic compounds, in particular phenolic acids and flavonoids.

The main functions of propolis are attributed to key chemical components such as flavonoids, phenolic acids, and their esters. Since these lipophilic compounds are readily extracted by alcohol, recent studies and applications on propolis have mainly focused on ethanol extracts of propolis (EEP). There has also been much work on water extracts of propolis (WEP) and its volatile oils. The methods used for analysis and discrimination of propolis include HPLC **(Markham K. R, 1996, Guo X. L, 2011 and Barrientos L, 2013)**, HPLC-ESI-MS **(Volpi N, 2006)**, GC-MS **(Kartal M., 2002, Isidorov V. A, 2014)**, LC-MS **(Gardana C, 2007)**, and DHS-GC-O-MS **(Yang C, 2010)**

In the present study, we used both polar and non-polar solvents to extract the propolis powder and apply the GC/MS technique to characterize and discriminate them.

2. Materials and Methods

2.1. Propolis

Propolis powder has been obtained from the Agricultural Research Center (Giza, Egypt).

2.2. Extraction and sample preparation

Four different extracts of propolis (water, hydroalcohol, ethanol, and hexane) were prepared by soaking 10 g of the propolis powder in the desired solvent for 24 h. Then, the resulted suspension was filtered through the filter paper followed

by the micro-filter (0.45 µm). The resulted filtrate was then evaporated by nitrogen gas under reduced pressure until dryness. The dried extract was then taken in a dark glass bottle, weighted and kept in 4^{o}C in a suitable organic solvent.

2.3. GC/MS analyses

A finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-1 column, 30 m x 0.32 mm (internal diameter) , was employed with helium as carrier gas (He pressure , 20 Mpa/cm^{2}; injector temperature, 310 $^{\circ}$C; GC temperature program, 85-310 ^{o}C at 3 ^{o}C/min (10 min. intial hold).The mass spectra were recorded in electron ionization (EI) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39-650 atomic mass units (amu).

3. Results and Discussion

In the chromatograms of the propolis extracts it was possible to identify several compounds which belong to several classes. These compounds were presented in each sample with different intensities and area percentage. Figure 1 shows the typical chromatograms of all propolis extracts. The profile of water extract is different from the other three extracts with m/z range 355.1 - 642. The major compound is one of the plant's essential oils, phenylpropene; 1, 2, 3 Trimethyoxy-5-prop-2-enylbenzene with an area percentage of 50.78 % and methylbenzoate (3.14 %).

The other three extracts display wider m/z range; 169.3 - 610, 253.1 - 637.1 and 183.3 - 539.4 that corresponds to hydroalcohol, ethanol and hexane extracts. The major constituent presented in the hydroalcohol extrtact was found to be benzlalcohol (13.28 %) associated to multiple organic constituents as given in table 1. On the other hand, the major constituent in the ethanol extract was methylpentanoic acid (12.88 %). This extract was uniquely characterized by the presence of another major compound with area percentage of 12.16 % that was identified as methylbenzoate.

The chromatogram of propolis hexane extract shows similar constituents to ethanol and hydroalcohol extracts but, with higher concentration of acetophenone compound (8.8 %). Table 1 summerizes all the GC-MS results obtained from the different propolis extracts.

Each propolis extract has its own characteristic mixture of compounds. The variability of constituents of propolis extracts and accordingly the main compound(s) may explain the potential variation in the biomedical effectiveness of propolis extracts.

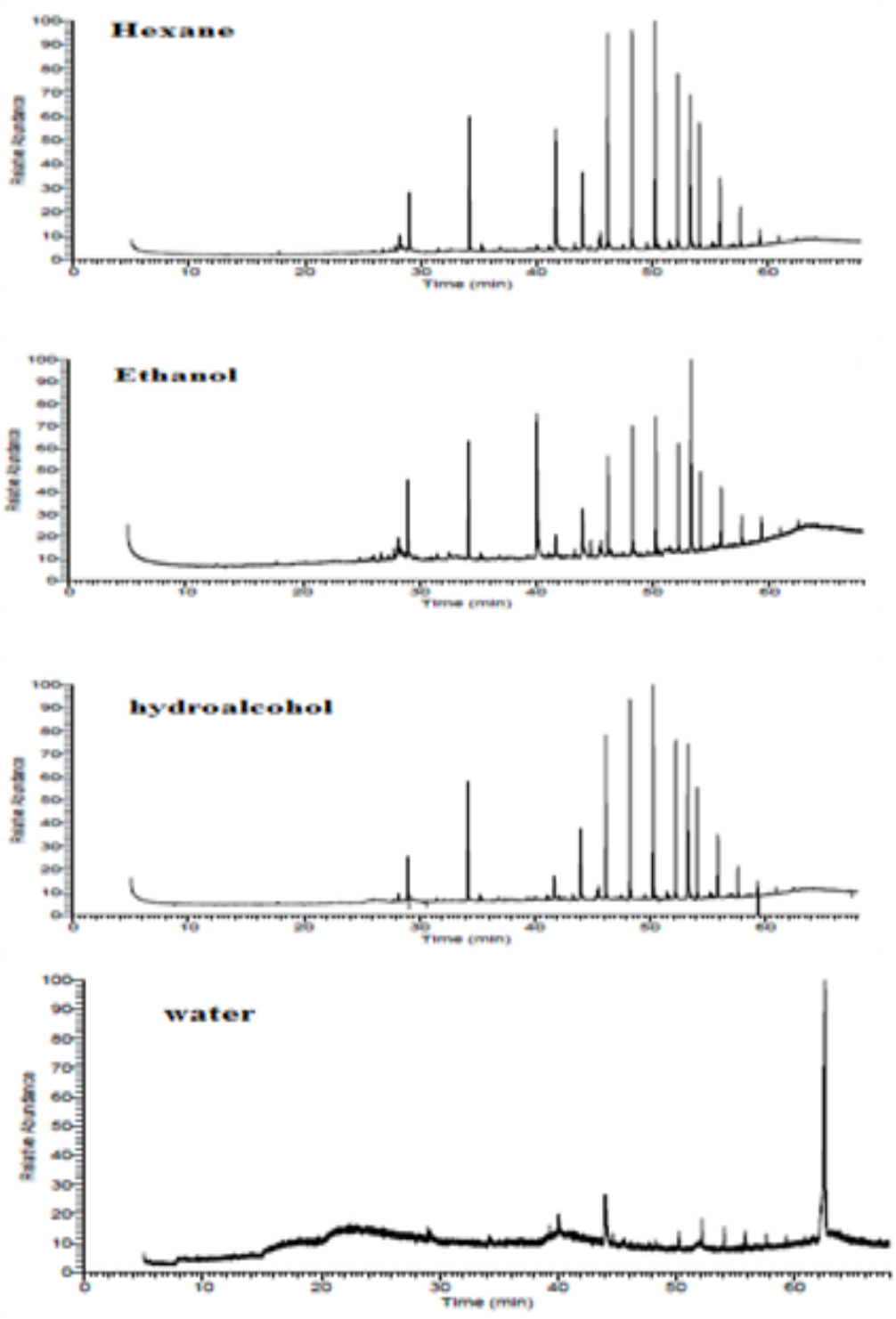

Figure 1. Typical mass spectra obtained for all propolis extracts

Table I: Chemical composition assessed by GC/MS of different Egyptian Propolis extracts.

R_t (min)	Compound name	Area%			
		Water extract	Hydroal-cohol extract	Ethanol extract	Hexane extract
28.99	Acetic acid		3.53	5.21	3.43
34.22	Benzaldhyde		7.4	6.39	6.82
40.07	Methylbenzoate	3.18		12.16	
43.99	2-methyl butanoic acid		4.96	3.34	4.15
46.18	Benzyl acetate		10.52	6.68	13.53
48.3	Ester of 2- methyl propanoic acid		12.83	7.65	11.21
50.3	Benzyl alcohol	1.82	13.28	8.17	12.01
54.1	Geranyl or nerolidyl ester	2.43	10.79	12.88	8.56
55.9	Octanoic acid	1.84	3.99	3.72	3.5
57.64	unindentified acid	1.36	2.12	1.78	2.01
59.32	unindentified acid	1.24	1.46	1.84	0.9
62.4	Phenylpropene	50.78			

4. Conclusion:

The chromatogram of propolis hexane extract shows similar constituents to ethanol and hydroalcohol extracts but, with higher concentration of acetophenone compound (8.8 %). Each propolis extract has its own characteristic mixture of compounds. The variability of constituents of propolis extracts and accordingly the main compound(s) may explain the potential variation in the biomedical effectiveness of propolis extracts.

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الملخص باللغة العربية

خصائص المميزة للمستخلصات المختلفة للدنج المصري GC/MS

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الدنج من المواد الطبيعة الذي لديه فوائد عديد ة في صحة الانسان وفي الطب الشعبي فـي معظم أنحاء العالم. تتضمن هذه الدراسة ، إستخلاص مسحوق الدنج بعدد من المحاليل المختلفة (المياه ، الكحول المخفف، الايثانول، الهكسان) والمستخلصات الناتجة تم تحليلها عن طريق GC/ MS . كل النتائج اعطت دليل بأن المذيب المستخدم للمستخلصات يؤثر بشكل كبير علي محتوي المركبات منفردة، وكل مستخلص مرتبط بعدد من المركبات المختلفة ولديه مدي مختلف من ال M/Z .هذه الخصائص المختلفة لديها تأئير علي الفاعلية البيولوجية للدنج.