ISSN 0974 – 5211

Journal of Natural Products
Volume 4 (2011)
www.JournalofNaturalProducts.com

Research Paper

Chemical analysis of the essential oils from *Punica granatum*, *Vitis vinifera* and *Cucurbita maxima* seeds growing in Lebanon by GC/MS

Youssef Bakkour[1,2], Salim Makhoul[1], Hanna El-Nakat[1*], Fawaz El Omar[2]
[1] Department of Chemistry, Faculty of Science, University of Balamand,
P.O. Box 100, Tripoli, Lebanon
[2] Laboratory of Applied Chemistry (LAC), Faculty of Science III, Lebanese University,
P.O. Box 826, Tripoli, Lebanon
* Corresponding Author

(Received 08 January 2011; Revised 09-14 January2011; Accepted 15 January2011)

ABSTRACT

Eight major chemicals are identified after analyzing the essential oil of the seeds of three medicinal plants *Punica granatum*, *Vitis vinifera* and *Cucurbita maxima*, using Gas Chromatography/Mass Spectrometry (GC-MS). Only three of the identified chemicals (Farnesene, Docosane and Tetracosane) were found in all three samples, but in varying proportions. Trans-Squalene was found in *Cucurbita maxima* and *Punica granatum* only. The remaining four compounds (Octanoate ethyl ester, Doconoate ethyl ester, Palmiate ethyl ester, and Linoleic acid ethyl ester) were found exclusively in the *Vitis vinifera* seed oil, being probably the reason behind the precise application of this oil in traditional herbal medicine. A deep investigation of the medicinal uses of the identified constituents justifies many traditional beliefs concerning these seed oils.

Keywords: *Punica granatum*; *Vitis vinifera*; *Cucurbita maxima*; Essential oil; GC-MS.

INTRODUCTION

Nowadays more light is being shed on the importance of medicinal plants, many of which have always been used as mere traditional or folk remedies, nonetheless today, they are being studied and analyzed for potential biological activities that will thus explain why the locals have always used them for treating various diseases and illnesses (Tiwari, 2008). Many of these remedies are based on the essential oil of the plants, for most of the chemical constituents of these oils are terpenoids, characterized by a low molecular weight which allows easy transport across cell membranes to induce different biological activities including anti-microbial, anti-inflammatory, and anti-oxidant effects (Kaplan, et al., 2001).

In Lebanon, we can find many plants which are grown not only to be used in the food and drink industry, but also their oils are used as nutritional supplements and

as medical treatments. This study focuses on the seed oil of three medicinal plants grown in the Bekaa Valley in Lebanon: *Punica granatum, Vitis vinifera* and *Cucurbita maxima.* The seeds of *Punica granatum* are considered a tonic for the heart and throat, and the plant is proven effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation, all of which are steps in atherosclerosis and cardiovascular disease (Kaplan, et al., 2001). The seed oil of *Vitis vinifera*, or grape seed oil, in its turn, has been appreciated for its antioxidant properties as well as radioprotective effects (Thorsten, et al., 2008; Passos, et al., 2010). *Cucurbita maxima*, or pepita pumpkin, has also been recognized for many health benefits, concentrated in the seed oil, including the prevention of the growth and reduction of the size of prostate, retardation of the progression of hypertension, mitigation of hypercholesterolemia and arthritis, reduction of bladder and urethral pressure, alleviation of diabetes by promoting hypoglycemic activity, and lowering levels of gastric, breast, lung, and colorectal cancer (Pranabendu, et al., 2009).

The aim of this study is to identify the seeds essential oil's composition of these three plants using GC/MS, based on their retention time and retention index in order to try to find a plausible explanation for the numerous medical applications of these plants.

MATERIALS AND METHODS

Plant material: Mature Fruits were gathered from the west region of the Bekaa Valley in Lebanon and identified by Mr. Kamal Akl, botanist, and their seeds were extracted, dried out away from direct sunlight and stored in a dry area till the time of the experiment. Table 1 summarizes the source and month of cultivation of each fruit.

Table- 1: Origin and month of cultivation of the three studied plants

Plant	Source	Month of Cultivation
Punica granatum	West Bekaa	August
Vitis vinifera	Central Bekaa	September
Cucurbita maxima	West and Central Bekaa	October

Essential oil extraction: The samples treated above were submitted to cold pressure oil extraction, during which the seeds are finely ground into a paste. The paste is spread on fiber disks, which are stacked on top of each other, then placed into the press under ambient to cold temperature, without heating. The obtained liquids are then separated by a standard process of decantation. For extraction of 10 ml of seed oil 25g of *Cucurbita maxima*, 50 to 80g of *Vitis vinifera* or 2000g of *Punica granatum* were used. The essential oil of the three seed classes is collected and stored at -4^0C until used. After each extraction the disks are properly cleaned from the remains of paste.

Gas chromatography/mass spectrometry: A Schimadzu QP 2010 plus gas chromatography system interfaced to a 2010 mass spectrometer was used for analysis of the samples. The separation was performed on a 30 m x 0.25 mm internal diameter fused silica capillary column coated with 0.25 µm film Rtx-5MS. The injector and the detector temperatures were respectively 250 and 280°C. The oven temperature was held at 40°C for 5 min, and programmed from 40 to 100°C at 4°C/min then to 280°C at 19°C/min and finally maintained at 280°C for 5 min. Split injection was conducted with a split ratio of 5:10. Helium was used as carrier gas, and flow-rate was 1.62 ml/min. The mass spectra were recorded over a range of 30-1000 atomic mass unit at 0.5s/scan. Solvent cut time was 3 min. Ionization energy was 70 eV. The inlet and ionization source temperature were 280°C. The chemical composition of the oil was

identified by comparing their spectra with those of a NIST library and confirmed by comparing their retention indices with data published in various literatures.

RESULTS AND DISCUSSION

The main constituents of the essential oil of the seeds of *Punica granatum*, *Vitis vinifera* and *Cucurbita maxima* cultivated in the west region of the Bekaa Valley and studied in this experiment are presented in table 2. Table 2 summarizes our findings showing the constituents which are common to more than one plant, as well as the ones which are specific to one of them, in addition the percent area of each of these constituents and their retention time.

Table-2: Identified chemical constituents.

Name	RT	RI	% Area		
			Vitis vinifera	***Cucurbita maxima***	***Punica granatum***
Octanoate ethyl ester	21,767	1202	1.84	0	0
Doconoate ethyl ester	28,317	1399	2.25	0	0
Farnesene	31,250	1452	33.59	17.25	27.75
Palmiate ethyl ester	32,758	1993	7.33	0	0
Linoleic acid ethyl ester	33,633	2159	36.25	0	0
Docosane	34,225	2200	10.02	16.86	15.07
Tetracosane	35,142	2400	3.08	5.04	5.12
Trans-Squalene	37,392	2790	0	38.03	20.68
Total	-	-	94.36	77.18	68.62

- RT: Retention time; RI: Retention Index.

The amazing ability of essential oils to penetrate tissue has been proven repeatedly in scientific experiments. Essential oils penetrate tissue roughly 100 times faster than water and 10 000 times faster than salts (Pawar, et al., 2006). In the present study, Farnesene was found in all three essential oils, in varying proportions, giving the *Vitis vinifera* oil the strongest odor, especially when combined with the Linoleic acid ethyl ester also found in abundance in grapes (Mookherjee, et al., 1984). Trans-Squalene is believed to be an antiviral compound for treating hepatitis C virus carriers (Elsherbini, et al., 1999). The ethyl esters identified only in the *Vitis vinifera* sample are secondary metabolites produced by many fungi. Their natural physiological role is not known but in fermentations of alcoholic beverages and other food products they play a key role as flavor compounds (Saerens, et al., 2008). The use of essential oils in medicinal purposes needs to address the issues of safety and toxicity (Hammer, et al., 1999) and a further biological study is believed to be useful in identifying the different biological effects of these seed oil components and their mode of action and effect of non-target organisms (Tiwari, 2008).

Acknowledgments: The authors are grateful to Mr. Kamal Akl for providing useful information and offering the plants as a gift to be studied and investigated and to the management committee of scientific research at the Lebanese University for the financial support.

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