

VARIATION OF SPATHE ESSENTIAL OIL COMPOSITION OF TEN IRANIAN DATE VARIETIES (*PHOENIX DACTYLIFERA* L.)

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ABSTRACT: The variations in the volatile constituents of hydrodistilled essential oil of spathes from ten varieties of *Phoenix dactylifera* L. including Kabkab (KAB), Shahani (SHA), Piarom (PIA), Hanaei (HAN), Tovarz (TOV), Shakri (SHK), Magti (MAG), Ghasb (GHS), Siah (SIA) and Gioni (GIO) were studied by GC/FID and GC/MS techniques. The highest Yield of spathes oil were found to be 0.36%. for Tovarz while Piarom and Siah varieties each with 0.03% declared the lowest content of essential oil. Twenty four compounds were detected in the spathes oil. As evidenced by the results obtained, in the spathes oils of *Phoenix dactylifera* L., Shakri cultivar afforded the highest content of methoxylated aromatics (89.95%) from the total identified compounds. 3,4-dimethoxytoluene was found to be the major component in Gioni essential oil with 82.17%. This compound showed the lowest percentage in Shahani oil with 36.74%. The other major chemical components among all the varieties investigated were 2,6-dimethoxytoluene (6.89-15.95%), farnesyl acetone (E,E) (1.74-20.85%), methyl geranate (1.1-5.60%), geranyl acetone(1.31-4.81%) and p-methylanisole (0.53-4.43%) respectively. Tovarz variety showed the maximum content of aliphatic aldehydes, oxygenated mono- and sesquiterpenes and fatty acids with 6.11, 5.86, 31.58 and 1.05% respectively. As indicated by the results, methoxylated aromatics showed the highest contribution in the chemical composition of spathes essential oil of *P. dactylifera* varieties while oxygenated sesquiterpenes and monoterpenes were among the quantitatively important components.

Key words: *Phoenix dactylifera* L., essential oil, GC/FID, GC/MS

INTRODUCTION

Date palm tree with the scientific name *Phoenix dactylifera* L. is a member of the family Arecaceae which is extensively cultivated in the southern part of Iran due to its nutritive and economical impacts. It is estimated that out of above 400 date palm varieties cultivated in Iran at least 100 varieties are usually grown in Fars, the southern state of Iran. Date fruit is considered as a folk remedy for the treatment of atherosclerosis, hypertension, diabetes and cancer [1-3]. The fruit pulp is rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins, and flavonoids [4]. The ratio and concentration of these constituents depend on the variety, proper pollination, stage of fruit picking, location and soil conditions. These factors may also affect the overall content of volatile compounds in other organs of the plant. Both volatile and non volatile constituents contribute to the various nutritional and pharmacological properties of the fruit and other parts of the plant [5, 6]. The inflorescence of date palm tree, in its early stages of growth is enclosed in a hard covering / envelope known as spathe which splits open as the flowers reach maturation [7]. The spathes are removed during pollination and insemination of date. Spathe is commonly called “Tarooneh” in Persian and has a specific fragrance particularly when it is fresh and is utilized in large scale production of Tarooneh hydrodistilled water in Fars province. This water contains volatile components and is widely consumed as a beverage to improve heart functioning in local and traditional health practice. It also possesses analgesic and anti inflammatory effects [8, 9].

Although various groups of active phytochemicals reported from *Phoenix dactylifera* L. fruit and pits [10, 11, 12] but literature search revealed only few reports on the chemical composition of date spathes essential oil declaring the investigation on a single variety of *Phoenix dactylifera* L. This work was therefore undertaken to study variations in the chemical constituents of spathes essential oils isolated from ten varieties of *Phoenix dactylifera* L. cultivars cultivated in Fars state. This task has been performed by the use of GC/FID and GC/MS which are the most rapid and reliable instrumental techniques for characterisation of volatile constituents of essential oils [13,14].

MATERIALS AND METHODS

Plant material

The spathes of ten varieties of *Phoenix dactylifera* L. were collected from Jahrom in the southern part of Fars state in March 2012 at the initial stage of fruit development. The taxonomic identification of plant materials was carried out by the Jahrom Department of Agricultural Research, and Faculty of Pharmacy, Shiraz University of Medical Sciences. The varieties selected for the present study included Kabkab, Shahani, Piarom, Hanaei, Tovarz, Shakri, Magti, Ghasb, Siah and Gioni. The voucher specimens (MRCH/92/1–MRCH/92/10 respectively) have been deposited in the herbarium of Medicinal Plants Processing Research Centre at Shiraz University of Medical Sciences, Shiraz- Iran.

Essential oil Extraction

The freshly collected plant materials from each cultivar (200 g) was crushed in a grinder and subjected to hydro distillation for 4 hours using a Clevenger type apparatus. The oil was separated, dried over anhydrous sodium sulphate and stored at 4° C in the dark. Each oil sample was dried over anhydrous sodium sulfate, filtered and diluted with analytical grade petroleum ether 1:5 v/v prior to the moment of analysis.

Identification of Essential Oil Components

Initially the diluted solution of essential oil samples were injected to GC/FID and the analysis was carried out in order to obtain a suitable analytical condition. These analyses were performed on a gas chromatograph, model 7890A, Agilent technologies, equipped with HP-5MS column (30 m, 0.32 mm, 0.52 µm film thicknesses) and a flame ionization detector (FID). Nitrogen gas was used as carrier gas with a flow rate of 1.0 ml/min and the split ratio was 1:50. The injector and detector temperatures were adjusted at 250 and 300°C respectively while the column temperature was linearly programmed from 60 to 220°C (at a rate of 5°C/min) and maintained at 220°C for 10 min. For GC/MS method, helium with 99.999% purity was used as carrier gas with the same flow rate as for GC/FID. The transfer line temperature was kept at 280°C and the MS spectrometer (Agilent technologies model 5975C) operating in the EI mode (70 ev) in a mass range of 30-600 m/z. For each analysis, 1.0 µL of diluted essential oil sample (1:5 v/v in petroleum ether) was consecutively injected manually.

The components of essential oil samples were identified via GC/MS by comparison of their Kovats retention indices (KI), relative to C9- C30 n -alkanes under the same operating conditions with those of the reported values [15, 16]. Further identification was made by comparison of Kovats indices (KI) and mass spectra respectively with Adams and Wiley (7nl) libraries and the structural information procured from interpretation of mass spectra of each chemical component [17,18]. Comparison of results were also made with those of authentic samples previously recorded in our laboratory [19]. MSD Chem Station software (Agilent Technologies) was used for data analysis.

RESULTS AND DISCUSSION

The essential oil isolated from the spathes of ten Iranian date varieties were analyzed by GC/FID and GC/MS techniques. The highest yield of essential oil were found to be 0.36% for Tovarz variety while Piarom and Siah showed the lowest content of essential oil with 0.03%. Table 1 shows the chemical components of spathes essential oils of various *P. dactylifera* L. varieties. An inspection of the compounds listed in table 1 shows that Siah oil with 98.83% has the highest number of identified compounds. As shown in table 1 the major chemical components among all varieties were found to be 3,4-dimethoxy toluene and Gioni variety with 82.17 % declared the highest percentage of this compound. Table 1 also shows 2,6-dimethoxy toluene as the second abundant phenolic constituent of essential oil samples with highest percentage in Shahani variety (15.95 %). The spathe oil of this variety also contains highest percentage of *p*-methylanisole (4.43 %) as another methoxylated aromatic compound.

Farnesyl acetone(E,E), a sesquiterpene ketone was detected in all varieties(1.74- 20.84%) and the oil of Shahani bears the highest proportion of this compound while the Z,Z isomer was found solely in Kabkab oil (1.71%) . Geranyl acetone, a monoterpene ketone constituting 1.31- 4.81% were also quantitatively important component in various date spathes essential oils except Hanaei and Shakri oils that were lacking this compound(Table 1, Figure 1).

Piarom oil declared a concentration of 4.81% for this compound being the highest among all varieties. This variety of *P.dactylifera* also contains highest concentration of methyl geranate while Siah was the single variety devoid of this compound (Table 1).The oxygenated sesquiterpene, caryophyllene oxide (0.83%) was only found in Shakri oil. The diterpene, neocembren A (0.76%) and the oxygenated diterpene, phytol (0.83%) were found respectively in Piarom and Shahani cultivars. Overall inspection of the results in table 2 indicated that methoxylated aromatics have the highest concentration in the chemical components of all *P.dactylifera* spathe essential oil samples and Hanaei was found to contain 94.28% of this class of compounds. Terpenic compounds showed lower contributions in the total chemical composition of the oil samples. Oxygenated sesquiterpenes were found to be the second major class of compounds and showed the highest content in Tovarz variety (27.26%).

Table 1. Percentage chemical composition of the spathe essential oil of ten date varieties (*Phoenix dactylifera* L.)

Compound	SHA	PIA	KAB	HAN	TOV	SHK	MAG	SIA	GIO	GHS	R ^{Ical} .	R ^{lit} .	Identification
Heptanal	-	-	-	-	1.10	-	-	-	-	-	902	902	MS-KI
P-Methyl anisole	4.43	2.24	0.53	1.27	0.71	2.71	0.87	4.08	-	2.25	1021	1020	MS-KI
Linalool	-	-	-	-	-	-	0.96	-	-	-	1100	1098	MS-KI
Nonanal	1.78	3.21	1.82	-	4.29	1.04	2.71	1.60	1.24	7.25	1105	1104	MS-KI
3,4-Dimethoxy-toluene	36.74	60	50.82	82.16	41.57	79.3	64.68	73.99	82.17	60.57	1236	1230	MS-KI
2,6-Dimethoxy-toluene	15.95	6.89	15.62	11.05	7.27	7.94	13.66	8.38	6.91	10.70	1259	1264	MS-KI
Thymol	-	-	-	-	-	-	1.55	-	-	-	1291	1290	MS-KI
Carvacrol	-	-	-	-	-	-	4.39	-	-	-	1301	1299	MS-KI
Methyl geranate	1.48	1.1	3.99	2.53	2.49	2.59	2.72	-	5.60	2.49	1323	1322	MS-KI
Caryophyllene	-	-	-	-	-	2.08	-	-	-	-	1424	1428	MS-KI
α-Ionone	1.47	2.23	1.43	-	2.03	-	-	-	-	1.75	1430	1426	MS-KI
Naphthalene, 1-methoxy	-	0.63	1.90	-	-	-	-	-	-	0.75	1449	1450	MS-KI
Geranyl acetone	4.60	4.81	3.50	-	4.32	-	1.67	1.34	1.31	3.86	1454	1453	MS-KI
β-Ionone	2.56	3.01	2.75	-	3.83	-	-	1.04	1.03	2.74	1489	1485	MS-KI
Caryophyllene oxide	-	-	-	-	-	0.83	-	-	-	-	1586	1581	MS-KI
Unknown	1.97	-	-	-	-	-	-	-	-	-	1631	-	-
Pentadecanal	1.53	-	-	-	0.72	-	-	-	-	-	1714	1711	MS-KI
Unknown	-	-	2.45	-	-	-	-	-	-	-	1724	-	-
Farnesyl acetone(z,z)	-	-	1.74	-	-	-	-	-	-	-	1861	1861	MS-KI
Nonadecane	0.7	-	1.07	-	-	-	-	-	-	-	1899	2000	MS-KI
Farnesyl acetone(E,E)	20.84	9.23	11.79	2.98	27.26	3.53	6.79	8.40	1.74	7.64	1920	1921	MS-KI
Neocembren-A	-	0.76	-	-	-	-	-	-	-	-	1965	1959	MS-KI
Palmitic acid	0.88	-	-	-	1.05	-	-	-	-	-	1980	1984	MS-KI
Z-phytol	0.85	-	-	-	-	-	-	-	-	-	2112	2114	MS-KI
Total Identification	92.33	93.01	92.97	97.46	94.15	97.43	97.28	98.83	94.4	97.51	-	-	-

Table 2 also presents other classes of chemical compounds found in the composition of essential oil samples such as aliphatic aldehydes, monoterpene hydrocarbons, oxygenated mono- and sesquiterpenes and fatty acids. According to these findings, Tovarz variety enriched with diverse classes of chemical components as aliphatic aldehydes (6.11%), oxygenated monoterpenes (5.86%), oxygenated sesquiterpenes (31.58%) and fatty acids (1.05%) in addition to methylated aromatics when compared with essential oil samples obtained from other varieties of *P. dactylifera* L. As presented in table 2 monoterpene hydrocarbons were detected only in Magti variety with 6.90%. Looking into the percentage contribution of four major chemical components in the analysed spathe essential oil samples reveals that 3,4-dimethoxytoluene was the dominant component in *P. dactylifera* spathe essential oils while other major chemical components were found to be 2,6-dimethoxytoluene, farnesylacetone and geranylacetone (Figure 1). Among the four main chemical components, 3,4-dimethoxytoluene, 2,6-dimethoxytoluene and farnesyl acetone were found in all essential oil samples but geranyl acetone was solely detected in the essential oils of six varieties of *P. dactylifera*. p-Methyl anisole were obtained from all essential oil samples except the one extracted from Gioni (Table 1, Figure 1).

Table 2. Chemical classes of compounds (%) in the spathe essential oil of date varieties (*Phoenix dactylifera* L.)

Classes of compounds	SHA	PIA	KAB	HAN	TOV	SHK	MAG	SIA	GIO	GHS
Aliphatic aldehydes	3.31	3.21	1.82	-	6.11	1.04	2.71	1.60	1.24	7.25
Methoxylated aromatics	57.12	69.76	68.87	94.48	49.55	89.95	79.21	86.45	89.08	74.27
Monoterpenes	-	-	-	-	-	-	6.9	-	-	-
Oxygenated monoterpenes	4.03	5.24	4.18	-	5.86	-	-	1.04	1.03	4.49
Sesquiterpenes	-	-	-	-	-	2.08	-	-	-	-
Oxygenated sesquiterpenes	25.44	14.04	17.03	2.98	31.58	4.36	8.46	9.74	3.05	11.5
Hydrocarbons	0.7	-	1.07	-	-	-	-	-	-	-
Fatty acid hydrocarbons	0.88	-	-	-	1.05	-	-	-	-	-
Diterpenes	-	0.76	-	-	-	-	-	-	-	-
Oxygenated diterpenes	0.85	-	-	-	-	-	-	-	-	-

Kabkab (KAB), Shahani (SHA), Piarom (PIA), Hanaei (HAN), Tovarz (TOV), Shakri (SHK), Magti (MAG), Ghasb (GHS), Siah (SIA) and Gioni (GIO)

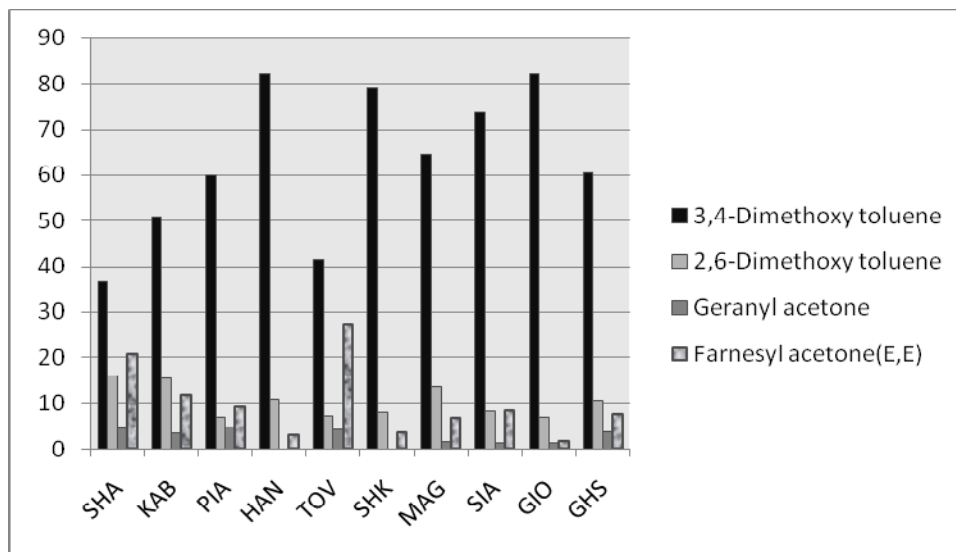


Figure 1. Variation of four major compounds in spathe essential oil of ten varieties of *Phoenix dactylifera* L.

The convincing evidence for the above findings is the results of Demirci et al. that represented 3,4-dimethoxy toluene as the major constituent in the spathes essential oil of a single variety of *P. dactylifera* L. [20]. They introduced 2,4-dimethoxytoluene, β -caryophyllene, p-cresyl methyl ether and caryophyllene oxide as other major components while in the present study, the major compounds were found to be 2,6-dimethoxytoluene, farnesyl acetone and geranylacetone in addition to 3,4-dimethoxy toluene. However the conclusion made by some other authors that oxygenated monoterpenes, thymol and carvacrol constitute the major components of the spathes essential oil require more evidence [21]. It is interesting to note that neither our previous results of GC/FID and GC-MS studies on twenty varieties of date spathes from the same climatic region nor the results procured from other climatic zones support this claim [20]. As can be seen in Table 1 thymol and carvacrol were detected in the lowest proportions only in Magti variety and the remaining nine varieties were found to be totally devoid of these two components. Other characterized chemical components of the oil samples such as α - and β -ionones are presented in table 1. The results given in table I and II thus reflect variations in the type and proportion of chemical composition of the essential oil isolated from various cultivars of *P. dactylifera*. These variations may be attributed mainly to the variety, location, seasonal and climatic changes and the time and process of pollination [22]. It is to be noted that some of the minor compounds are generated during the process of hydrodistillation at elevated temperature.

CONCLUSION AND RECOMMENDATION

The increasing importance of essential oils in various pharmaceutical, food and fragrance industries has prompted extensive need for the careful analysis of their volatile chemical composition. The spa the water ditillate containing volatile constituents is being produced in large scale and widely used as a beverage particularly in southern states of Iran. The results of present study may offer precise information on the volatile chemical composition of spathes essential oil of *P. dactylifera*. These results also provide the required analytical information for standardization and quality control of spathes water distillate.

The use of *P. dactylifera* spa the water distillate in Iranian folk medicine, may be partly explained by the presence of compounds identified here as the chemical components of date spathes oil. Despite the many botanical similarities between date varieties, remarkable variations could be discerned among the types and concentration of major as well as minor chemical components of various spathes essential oil samples. Another important findings obtained from this study is the presence of a decreased number of constituents in the essential oil of some varieties as compared to others. These variations in the volatile components depend strongly on the genetic background of the individual cultivars and obviously affect the overall quality and the specific aroma of the essential oils. The present study also conclude that the spathes oil of some date varieties are good sources of diverse volatile components showing less remarkable fluctuations in the types and concentrations of chemical components while in some other varieties the content of oil and the concentration of individual compounds were highly variable. Moreover the present study declared that spathes oil can be considered as a source of bioactive compounds like farnesyl acetone, geranylacetone and methyl geranate in addition to other compounds of biological interest. Consequently, individual approach should be applied to each cultivar in order to verify the purity of volatile oils and to detect adulterations in spathe distillate products. Separation of the bioactive chemical constituents of the oil for various functional food and therapeutic applications may also remain a subject of interest for future studies.

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