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Volatile Constituents and Antioxidant Activity of Spathes from Five Uncommon Varieties of *Phoenix dactylifera* L.

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Abstract

This study deals with the characterization of chemical composition of spathe essential oil of five male varieties of *Phoenix dactylifera* L. including Ghir (GHI), Lar (LAR), Khosooyeh (KHO), Boyer (BOY) and Shahani (SHA). The essential oil samples were prepared through hydro-distillation using a Clevenger-type apparatus. The analysis of oil samples was carried out following dehydration process. Hexane diluted samples were injected into a gas chromatograph attached to a mass spectrometer (GC/MS). Kovats indices (KI) was calculated for each compound and their mass fragmentation pattern were studied. The results compared with the relevant information procured from Wiley nl7 library and the Pherobase and NIST databases. The results of quantitative analysis of oil specimens indicated that GHI (0.03%) and LAR (0.07%) cultivars contain the lowest and the highest extraction yields of essential oil, respectively. The oil samples were further screened for their DPPH free radical scavenging effects by means of a thin layer chromatographic method, which showed a positive indication of anti-DPPH activity through the visualization of spots and a yellow color change on spraying the chromatoplates with a 0.2 % methanolic solution of DPPH. The results also indicated that 3,4-dimethoxytoluene is the main component in all *Phoenix dactylifera* L. spathe oil samples. This methoxylated aromatic compound showed the highest concentration in BOY (62.45%) and the lowest proportion in KHO cultivar (41.57%). BOY spathe oil was superior in the manifestation of DPPH free radical scavenging activity as compared with other cultivars.

Keywords: Essential oil, *Phoenix dactylifera* Spathe, GC/MS.

1. Introduction

Phoenix dactylifera L., commonly known as date palm, is a flowering plant species in the family Arecaceae. It is widely grown in the southern regions of Iran for its nutritive fruits and economic values. Over 400 date palm varieties are being cultivated in Iran out of which at least 100 varieties are usually grown in Fars. Date fruit has been considered as a folk remedy for the treatment of atherosclerosis, hypertension, diabetes, and cancer (1-3). The fruit pulp is rich in phytochemicals such as phenolics, flavonoids, carotenoids, anthocyanins, sterols, and procyanidins (4). The ratio and concentration of these constituents depend on the variety, proper pollination, stage of fruit picking, and climatic conditions such as temperature, humidity, rain, and soil conditions. These ecological and environmental factors also affect the number and types of volatile constituents of the plant. 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 (5). The spathes are removed during pollination of date tree. It has a specific fragrance particularly when it is fresh and is used in large scale production of Tarooneh

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hydrodistilled water in Fars province. This water distillate 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 (6,7).

2. Material and methods

2.1. Chemicals

Methanol, aluminum chloride, sodium carbonate, acetate buffer, iron chloride, hydrochloric acid, ammonium persulphate, Folin-Ciocalteu reagent and TLC plates were purchased from Merck (Darmstadt, Germany). Ethanol % 96 was from Zakaria Co., Jahrom, Iran. Quercetin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, USA.

2.2. Plant material and samples preparation

The spathe of five male cultivar varieties of *P. dactylifera* L. including Ghir (GHI), Lar (LAR), Khosooyeh (KHO), Boyer (BOY) and Shahani (SHA) were collected at flowering stage from Jahrom in April 2017. All spathe specimens were identified by the plant taxonomist of Jahrom Agricultural Research Center under herbarium Nos. MRCH-92-37 to MRCH-92-41 that were deposited at Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Samples were dried in shade, separately crushed (100 g) in a grinder, and subjected to hydrodistillation for 4 h using a Clevenger type apparatus. The essential oil samples were separated and further dried over anhydrous sodium sulphate and stored at 4 ºC prior to GC/Mass analysis and antioxidant assay.

2.3. GC/MASS Analysis

This analysis was performed on a gas chromatograph 7890A system coupled with a mass detector, 5975 C, Agilent technologies. Fused-silica capillary column (HP-5MS, 5% phenyl methyl siloxane, 30 m ×0.25 mm ×0.25 µm, Agilent technologies) was used. Oven temperature was programmed to rise from 60 to 280 ºC at a rate of 10 ºC/min and held at 280 ºC for 10 min. Helium was used as the carrier gas with a flow rate of 1 mL/min.The interface temperature was 280 ºC. A volume of 1 µL of the essential oil was injected in split mode (1:50) and mass spectra were acquired in EI mode (70 eV) in a mass range of 30-600 m/z. Identification and quantification of essential oil components were based on calculation of KI (Kovats Index) for each constituents and comparison of data obtained with the information given in Wiley nl7 and Adams, NIST, and Pherobase mass spectral libraries as well as the values reported in the related literature (18). In order to confirm the structure of each oil component inspection of mass spectral fragmentation pattern of the compound was also performed and the results were compared with the reported values.

2.4. DPPH Screening Method

2.4.1. Analysis of antioxidant constituents by thin layer chromatography

The antioxidant constituents of various essential oil samples were analyzed using thin layer chromatography (TLC) followed by DPPH (2,2- Diphenyl-1-picrylhydrazyl). A 100 µg/ml solution of essential oil in n-hexane was loaded on TLC plates (Merck, 10 x 10 cm). The plates were developed in toluene-ethyl acetate (97:3) to separate various chemical constituents of the oil samples. The developed chromatogram was dried and further sprayed with a 0.2% methanolic solution of DPPH. The plates were air dried and incubated for 10 min at room temperature and further observed under visible and UV light (254 and 366 nm). Various separated spots were noted with distinct Rf values. The corresponding active antioxidant constituents of essential oil samples were detected as yellowish cream spots on reaction with DPPH by resolved bands on thin-layer chromatoplate. After visual comparison with the intensity of bleached color of the TLC band of positive standard, the antioxidant strengths of seagrass constituents were tentatively categorized as strong and weak activities. All detected active antioxidant constituents were noted on the plate based on distinctive Rf values (8-10).

3. Results and discussion

As evidenced by the results, the spathes essential oil content were found to be different among the tested *P. dactylifera* varieties (Figure

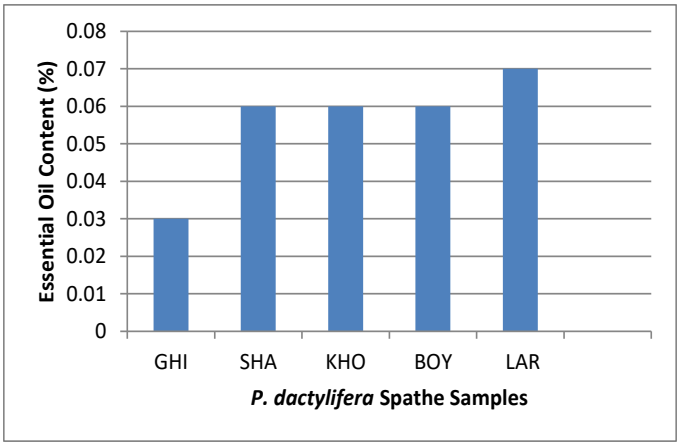

Figure 1. Comparison of Spathe essential oil content among *P. dactylifera* varieties.

1). Following the gas chromatography /mass spectrometric analysis of the spathe essential oil from five uncommon varieties of *Phoenix dactylifera*, their chemical components were identified. Table 1-6 represents the chemical composition of essential oil from the spathes of five *P. dactylifera* varieties. 3,4-Dimethoxytoluene was identified as the main aromatic component in all the studied essential oil samples. The comparison of essential oil components in KHO and GHI samples indicated that 3,4-dimethoxytoluene the most abundant compound of GHI volatile oil (44.31%) comprises only 6.82% of KHO oil components (Table 1 & 2) . β-Caryophyllene was found to be the second most abundant composition detected in GHI variety (27.38%) while this component was absent in KHO oil. 2,6-Dimethoxytoluene, the third major component of GHI variety (13.54%), showed a very low contribution in KHO oil components (1.75%). Moreover, caryophyllene oxide forms an abundance of 22.21% in KHO oil, but its contribution to GHI variety was only 2.5% (Table 1 & 2). β-Caryophyllene is the second most abundant component of KHO oil was not observed among the oil chemical components of GHI variety. 9,12,15-octadecatrienoic acid and hexadecanoic acid comprising 17.04% and 66.06%, respectively of KHO volatile oil composition were not detected in GHI spathe oil (Table 1 & 2). In case of LAR essential oil specimen, the major component was found to be 3,4-dimethoxytoluene (48.8%), followed by p-methylanisole (19.41%), 2,6-dimethoxytoluene (16.9%) and β-caryophyllene (6.31%) (Table 3). Comparison of essential oil components of LAR with GHI and KHO indicated that both LAR and GHI essential oil contain 3,4-dimethoxytoluene as

Table 1. Volatile Spathe constituents of *P. dactylifera*, GHI variety.

No.	Component	Retention time	Area%	KI	Method of Identification
1	P-Methyl anisole	6.508	2.69	1021	MS-KI
2	n-Nonanal	8.519	0.47	1104	MS-KI
3	3,4-Dimethoxytoluene	12.115	44.31	1242	MS-KI
4	2,6-Dimethoxytoluene	12.483	13.54	1256	MS-KI
5	Phytol	31.437	0.52	2113	MS-KI
6	β -Caryophyllene	16.861	27.38	1427	MS-KI
7	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	17.542	0.6	1454	MS-KI
8	α-caryophyllene	17.67	2.15	1459	MS-KI
9	Caryophyllene oxide	20.777	2.05	1590	MS-KI
10	Farnesyl acetone B	27.812	3.62	1921	MS-KI
11	Unknown	14.249	2.68	1324	MS

Table 2. Volatile Spathe Constituents of *P. dactylifera*, KHO variety.

No.	Component	Retention time	Area%	KI	Method of Identification
1	3,4-Dimethoxytoluene	12.051	6.82	1240	MS-KI
2	2,4-Dimethoxytoluene	12.459	1.75	1255	MS-KI
3	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	31.939	17.04	2141	MS-KI
4	β-caryophyllene	16.831	22	1425	MS-KI
5	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	17.554	0.59	1455	MS-KI
6	α-caryophyllene	17.67	2.43	1459	MS-KI
7	Caryophyllene oxide	20.783	22.21	1590	MS-KI
8	Unkhnown	21.383	1.21	1616	MS
9	Unkhnown	21.977	0.34	1643	MS
10	Pentadecanoic acid	26.57	0.42	1859	MS-KI
11	Unknown	26.827	0.74	1872	MS
12	Farnesyl acetone c	27.812	4.78	1921	MS-KI
13	Hexadecanoic acid	28.634	14.66	1963	MS-KI
14	Unknown	31.443	1.92	2114	MS
15	Linoleic acid	31.805	2.53	2133	MS-KI
16	Unknown	14.255	0.56	1324	MS

the most abundant constituent, which is still higher in LAR variety (48.8 vs. 44.31%). p-Methylanisole accounts for a small amount of GHI volatile oil composition (2.69%). The other component of the spathe essential oil of LAR variety was found to be 2,6-dimethoxytoluene, which is actually of slightly higher percentage compared with GHI variety (16.09% vs. 13.54%). β-Caryophyllene, as the third major component of GHI volatile oil, comprised far higher contribution, 27.38% compared to 6.31% in LAR variety. Caryophyllene oxide (22.21%) was characterized as the most abundant constituent in KHO oil. Other constituents of this specimen including β-Caryophyllene, 9,12,15-octadecatrienoic acid methyl ester, hexadecanoic acid, and 3,4-dimethoxytoluene were detected with 22.0, 17.04, 14.66, and 6.82% contributions, respectively. While the content of 3,4-dimethoxytoluene is far higher in LAR sample (48.8%), compounds such as 9,12,15-octadecatrienoic acid methyl ester and hexadecanoic acid were not detected in LAR variety (Table 3). The difference in

Table 3. Volatile Spathe constituents of *P. dactylifera*, LAR variety

No.	Component	Retention time	Area%	KI	Method of Identification
1	p-Methyl anisole	6.526	19.43	1021	MS-KI
2	n-Nonanal	8.519	1.07	1104	MS-KI
3	3,4-Dimethoxytoluene	12.092	48.8	1241	MS-KI
4	2,6-Dimethoxytoluene	12.471	16.09	1255	MS-KI
5	Farnesyl acetone B	27.806	3.69	1921	MS
6	trans-Caryophyllene	16.819	6.31	1425	MS-KI
7	α-caryophyllene	17.664	0.92	1459	MS-KI
8	Caryophyllene oxide	20.771	1.93	1590	MS-KI
9	Unknown	14.243	1.23	1323	MS-KI

Table 4. Volatile Spathe constituents of *P. dactylifera*, Shahani variety

No.	Component	Retention time	Area%	KI	Method of Identification
1	P-Methyl anisole	6.52	4.04	1021	MS-KI
2	n-Nonanal	8.531	2.78	1105	MS-KI
3	3,4-Dimethoxytoluene	12.139	60.84	1243	MS-KI
4	2,6-Dimethoxytoluene	12.5	14.58	1257	MS-KI
5	Unknown	14.266	1.82	1324	MS
6	β-Caryophyllene	16.842	2.55	1426	MS-KI
7	α-Ionone	16.988	0.96	1432	MS-KI
8	(E)-Geranylacetone	17.559	1.87	1455	MS-KI
9	β-Ionone	18.428	1.31	1490	MS-KI
10	Caryophyllene oxide	20.8	4.69	1591	MS-KI
11	Farnesyl acetone C	27.829	3.38	1922	MS-KI

the profile of constituents in these two varieties also revealed that para-methylanisole (19.43%) in LAR variety was not detected in KHO, and 2,6-dimethoxytoluene of LAR oil (16.09%) showed only 1.75% contribution in KHO oil. The investigation of volatile oil composition obtained from SHA variety confirms that 3,4-dimethoxytoluene together with its isomer 2,6-dimethoxytoluene and caryophyllene oxide are the most abundant components with 60.84, 14.58, 4.69%,.respectively (Table 4).

Comparison of the oil constituents in SHA and GHI clearly revealed that in both varieties, 3,4-dimethoxytoluene is the most abundant composition. However, the percentage of this compound is significantly higher in SHA (60.84% vs. 44.31%) than GHI variety. The contribution of 2,6-dimethoxytoluene is somewhat similar in these two specimens (14.58% in SHA and 13.54% in GHI) (Table 1 & 4). The third abundant compound, caryophyllene oxide of Shahani (4.69%), accounts for only 2.05% of GHI specimen, while the GHI oil second abundant compound, β-caryophyllene (27.38%), was comparatively detected at very low contribution in SHA spathe oil. By comparing the volatile oil obtained from the above two varieties, it can be concluded that the most abundant compound of SHA oil is 3,4-dimethoxytoluene with 60.84%, which comprises only 5.82% of KHO spathe oil. The isomer of this compound, 2,6-dimethoxytoluene, the second most abundant compound observed in SHA (14.58%) comprises 1.75% of KHO oil. Caryophyllene ox-

Table 5. Volatile Spathe constituents of *P. dactylifera*, Boyer variety

No.	Component	Retention time	Area%	KI	Method of Identification
1	L-Linalool	8.414	1.12	1100	MS-KI
2	3,4-Dimethoxytoluene	12.057	62.45	1240	MS-KI
3	2,6-Dimethoxytoluene	12.453	21.28	1255	MS-KI
4	Unknown	14.249	4.53	1324	MS
5	5,9-Undecadien-2-one, 6,10-dimethyl (E)-	17.542	0.75	1454	MS-KI
6	α-Amorphene	18.329	0.7	1486	MS-KI
7	Caryophyllene oxide	20.771	0.83	1590	MS-KI
8	Pentadecanal	23.569	1.64	1715	MS-KI
9	Unknown	23.767	0.93	1724	MS
10	Farnesyl acetone B	27.806	4.76	1921	MS-KI
11	Phytol	31.431	1.0	2113	MS-KI

ide, another ingredient in SHA oil, was found to be the most abundant compound (22.21%) detected in KHO oil. β-Caryophyllene, 9,12,15-octadecatrienoic acid methyl ester, and hexadecanoic acid with 22.2%, 17.04%, and 14.66%, respectively, were the second to fourth major components of KHO oil that were not detected in SHA (Table 2 and 4). Comparison of the components in volatile oil samples of LAR and SHA shows that 3,4-dimethoxytoluene is the most abundant compound of both varieties (60.84 in SHA and 48.8% in LAR). 2,6-dimethoxytoluene is the Shahani second major component (Table 3 and 4). Nevertheless, the amount of this substance in these two varieties is largely similar (14.58% in SHA and 16.09% in LAR). The third marker compound in Shahani oil specimen, caryophyllene oxide, was detected at the level of 4.69%, comprising only 1.93% of LAR oil variety. However, p-methylanisole, the second abundant component of LAR (19.43%), was only 4.04% of SHA oil. β-Caryophyllene was the fourth abundant material in LAR specimen (6.6%), while this substance showed 2.55% contribution in shahani oil.

The most abundant compound of Boyer oil was found to be 3,4-dimethoxytoluene followed by 2,6-dimethoxytoluene and farnesylacetone-B with 62.45, 21.28, and 4.76%, respectively (Table 5). Comparison of the major components of the oil of BOY and GHI revealed, 3,4-dimethoxytoluene as the major component in both varieties, but with a far lower content in GHI oil (62.45% in BOY and 44.31% in GHI). 2,6-Dimethoxytoluene was found as the third major substance in GHI spathe essential oil (13.54%). On the other hand, the third abundant component of Boyer oil was indicated to be farnesylacetone-B (4.76%), while this compound accounted for only 3.62% of GHI spathe oil composition (Table 1 and 5). This compound was not found among the SHA and LAR oil

Table 6. Comparison of Spathe essential oil components among *P. dactylifera* varieties.

No	Chemical Component	Ghir (%)	Khosooyeh (%)	Lar (%)	Shahani (%)	Boyer (%)
1	P-Methyl Anisole	2.69	---	19.43	4.04	---
2	L-Linalool	---	---	---	---	1.12
3	n-Nonanal	0.47	---	1.07	2.78	---
4	3,4-Dimethoxytoluene	44.31	6.82	48.8	60.84	62.45
5	2,6-Dimethoxytoluene	13.54	1.75	16.09	14.58	21.28
6	β-Caryophyllene	27.38	22.0	6.31	2.55	---
7	α-Ionone	---	---	---	0.96	---
8	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	0.6	0.59	---	---	0.75
9	(E)-Geranylacetone	---	---	---	1.87	---
10	α-caryophyllene	2.15	2.43	0.92	---	---
11	α-Amorphene	---	---	---	---	0.7
12	β-Ionone	---	---	---	1.31	---
13	Caryophyllene oxide	2.05	22.21	1.93	4.69	0.83
14	Pentadecanal	---	---	---	---	1.64
15	Pentadecanoic acid	---	0.42	---	---	---
16	Farnesyl acetone B	---	---	3.69	---	4.76
17	Farnesyl acetone C	3.62	4.78	---	3.38	---
18	Hexadecanoic acid	---	14.66	---	---	---
19	Phytol	0.52	---	---	---	1.0
20	Linoleic acid	---	2.53	---	---	---
21	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	---	17.04	---	---	---

Table 7. Combination of terpenic and non- terpenic components of various *P. dactylifera* Spathe oils.

Cultivar	% Terpenic compounds	% Non-terpenic compounds
LAR	12.58	85.39
GHI	36.32	61.01
SHA	14.76	82.24
BOY	7.46	86.07
KHO	52.01	43.22

constituents. β-caryophyllene, the second abundant component in GHI (27.37%), was not detected among the chemical content of BOY oil. The second and fourth major components of the LAR spathe oil were characterized as p-methylanisole and β-caryophyllene, indicating 19.4 and 31.6% of the volatile oil composition, respectively. Comparing SHA and BOY spathe volatile oil compositions (Table 4 and 5), it could be seen that 3,4-dimethoxytoluene content was very close in both varieties (62.45% in Boyer and 60.84% in Shahani), but 2,6-dimethoxytoluene content showed remarkable difference in these varieties (21.28% in BOY and 14.58% in SHA (Table 6). Based on the results, the tested spathe essential oil specimens were rich in methoxylated aromatic compounds such as 3,4- and 2,6-dimethoxytoluene and p-methylanisole. Sesquiterpenes such as β-Caryophyllene, oxygenated sesquiterpenes including caryophyllene oxide and farnesyl acetone, monoterpene hydrocarbons, and fatty acid esters showed to be other major classes of volatile components in *P. dactylifera* spathe oil (Table 6). Results indicated that the content of non-terpenic compounds was far higher than the terpenic components in the tested essential oil samples (Table 7). As can be observed in thin-layer chromatographic plate prepared with essential oil samples (Figure 2), the presence of various antioxidant compounds with different polarities among the chemical composition of the essential oil content might be the reason for the emergence of DPPH inhibitory effect of the studied spathe essential oil samples of *P. dactylifera*. As declared by the results, the antioxidant properties of the spathe essential oil of the studied date cultivars thus were revealed through manifestation of DPPH inhibitory properties. Results of the present study indicate the importance of various spathe

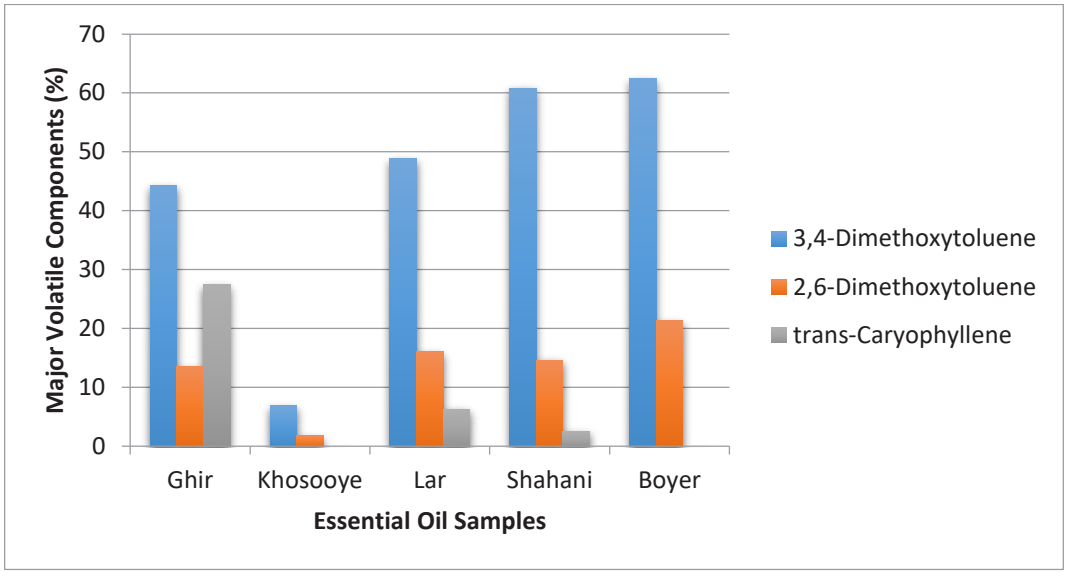

Figure 2. Comparison of the most abundant chemical components of Spathe essential oil of *P. dactylifera* varieties.

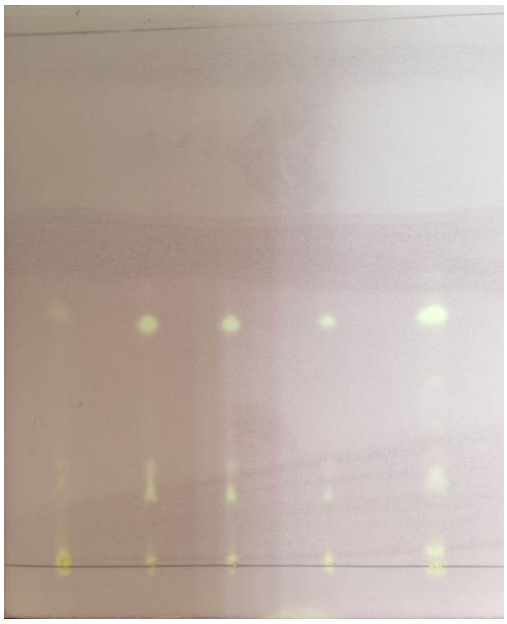

Figure 3. Thin layer chromatogram of various spathe essential oil samples.

essential oil composition of *P. dactylifera*. On the other hand, these data may be exploited in order to identify and standardize the volatile oil constituents for comprehensive use in the food industry.

Results of the present study indicated the highest content of 3,4-dimethoxytoluene (60.84%), which has not been reported earlier in Shahani spathe oil (Figure 3). Farnesylacetone with an abundance of 20.48%, 2,6-Dimethoxytoluene (15.95%), and caryophyllene oxide (4.69%), respectively, are other major compounds of Shahani oil. Methyl geranate, 1,2-benzenedicarboxylic acid-2-methylpropyl ester, 2,13,14-trimethylheptadecane, hexadecanoic acid, and henigosan were reported in Shahani spathe oil previously, which were not found in Shahani oil specimen in the present study (36). On the other hand, the volatile constituents such as β-caryophyllene and caryophyllene oxide were not detected in Shahani spathe oil in the previous studies.

4. Conclusion

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 spathe water distillate of *P. dactylifera* containing volatile constituents is being produced in large scale and is widely used as a beverage particularly in Fars, the southern state 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* spathe 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 many botanical similarities between date varieties, remarkable variations could be discerned among the types and concentration of the major constituents as well as the minor chemical components of various spathes essential oil samples. Another important finding obtained from this study is the presence of a decreased number of constituents in the essential oil of some varieties as compared with others. These variations in the volatile components depended 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 concluded that the spathe oil of some date varieties were 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 including farnesyl acetone, caryophyllene in addition to the major methoxylated aromatic compounds such as 3,4- and 2,6-dimethoxytoluene. Consequently, an 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 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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Conflict of Interest

None declared.

5. References

1. Vayalil P.K. Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Arecaceae). *J Agric Food Chem.* 2002;50:610-7.
2. Duke J.A. 1992. Handbook of Phytochemical Constituents of GRAS Herbs and other Economic Plants.CRC Press, Boca Raton, 251.
3. Tahraoui A, El-Hilaly J, Z.H. Israili, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *J Ethnopharmacol.* 2007;110:105-17.
4. Baliga M S, Baliga B R V, Kandathil S M, Bhat H P, Vayalil P K. A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). *Food Res Int.* 2011;44:1812-22.
5. Zaid A. 2002. (ed.): Date palm Cultivation. FAO Plant production and protection paper 156, Rev.1.
6. Mikki M.S, Al -Taisan S M, Abdul Aziz A. 1989. Isolation of the chemical constituents of the spathe of date palm, Al-Hassa Regional Agricultural Research Centre. Saudi Arabia, 244-98.
7. Baraem I, Jeya H, Imad H, Riad B. Date consumption and dietary significance in the U nited Arab Emirates. *J Sci Food Agric*. 2006;86:1196-201.
8. Sharif S, Kitaz A, Al-Kayali R. TLC Screening and Evaluation of Antioxidant, Antibacterial Activity of Onopordon Macrocephalum by Bioautography Method. *Iranian J Pharm Sci.* 2016;12:1-8.
9. Dewanjee S, Gangopadhyay M, Bhattacharya N, Khanra R, Dua TK. Bioautography and Its Scope in the Field of Natural Product Chemistry. *J Pharm Analysis.* 2015;5:75-84.
10. R. Ragupathi Raja Kannan, R. Arumugam, S. Meenakshi. Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India. *Inter J Chem Technol Res*. 2010;2:1526-30.
11. Adams R.P.2007.Identification of Essential Oil Components by Gas Chromatography/ mass Spectrometry,4th;Allured Publishing:Carol Stream,IL,USA.
12. Mass spectra library.2001.NIST/EPA/ NIH:USA, http://www.nist.gov/srd/nlstla.htm.
13. The pharobase [updated 2012; cited 5/2013]. Available from: www.thepharobase. com
14. Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK. A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). *Food Res Int*. 2011;44:1812-22.
15. Mard SA, Jalalvand K, Jafarinejad M, Balochi H, Naseri MKG. Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of Phoenix dactylifera palm leaves and its fractions in alloxan-induced diabetic rats. *Malays J Med Sci.* 2010;17:4-13.
16. Abedi A, Parviz M, Karimian SM, Rodsari HRS. Aphrodisiac activity of aqueous extract of Phoenix dactylifera pollen in male rats. *Advances in Sexual Medicine*. 2013;3:28-34.
17. Ardekani MRS, Khanavi M, Hajimahmoodi M, Jahangiri M, Hadjiakhoondi A. Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. *Iran J Pharm Re*s. 2010;9:141.
18. Al-Shahib W, Marshall RJ. The fruit of the date

palm: its possible use as the best food for the future? *Int J Food Sci Nutr.* 2003;54:247-59.
19. Saafi EB, El Arem A, Issaoui M, Hammami M, Achour L. Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. *J Food Sci Technol.* 2009;44:2314-9.
20. Maxwell SR. Prospects for the use of antioxidant therapies. *Drugs.* 1995;49:345-61.
21. Witt EH, Reznick AZ, Viguie CA, Starke-Reed P, Packer L. Exercise, oxidative damage and effects of antioxidant manipulation. *J Nutr.* 1992;122:766-73.
22. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. *Int J Biochem Cell Biol.* 2007;39:44-84.
23. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med.* 2010;49:1603-16.
24. Jadhav HR, Bhutani K. Antioxidant properties of Indian medicinal plants. *Phytother Res*. 2002;16:771-3.
25. Larson RA. The antioxidants of higher plants. *Phytochemistry.* 1988;27:969-78.
26. Lanigan R, Yamarik T. Final report on the safety assessment of BHT (1). *Int J Toxicol.* 2002;21:19-94.
27. Devi R, Narayan S, Mohan KV, Sabitha K, Devi C. Effect of a polyherbal formulation, Ambrex, on butylated hydroxy toluene (BHT) induced toxicity in rats. *Indian J Exp Biol.* 2003;41:1294-9.
28. Faine L, Rodrigues H, Galhardi C, Ebaid G, Diniz Y, Fernandes A, et al. Butyl hydroxytoluene (BHT)-induced oxidative stress: Effects on serum lipids and cardiac energy metabolism in rats. *Exp Toxicol Pathol.* 2006;57:221-6.
29. Morales FJ, Jiménez-Pérez S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chem.* 2001;72:119-25.
30. Singh G, Kapoor I, Pandey S, Singh U, Singh R. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytother Res.* 2002;16:680-2.
31. Lawless J. The illustrated encyclopedia of essential oils: the complete guide to the use of oils in aromatherapy and herbalism. Shaftesbury, Dorset: Element Books Ltd. p. 256.
32. Sur S, Tuljupa F, Sur L. Gas chromatographic determination of monoterpenes in essential oil medicinal plants. *J Chromatogr A.* 1991;542:451-8.
33. Lucchesi ME, Chemat F, Smadja J. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *J Chromatogr A.* 2004;1043:323-7.
34. Moein S, Moein MR. Relationship between antioxidant properties and phenolics in Zhumeria majdae. *J Med Plant Res.* 2010;4(7):517-21.
35. Hildebert Wagner, Sabine Bladt. Plant drug analysis, A thin layer chromatography. 2nd edition, 1996, p. 385.
36. Demirci B, Tsikolia M, Bernier UR, Agramonte NM, Alqasoumi SI, Al-Yahya MA, et al. Phoenix dactylifera L. spathe essential oil: chemical composition and repellent activity against the yellow fever mosquito. *Acta Trop.* 2013;128:557-60.