1st International Electronic Conference on Metabolomics
1-30 November 2016
chaired by Dr. Peter Meikle
sponsored by metabolites

Qualitative analysis of phenolic metabolites from date palm (Phoenix dactylifera L.) tree by using high-performance liquid chromatography hyphenated with mass-spectrometry detection system

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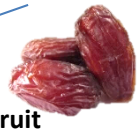
Phenolic metabolites from date palm

Skin



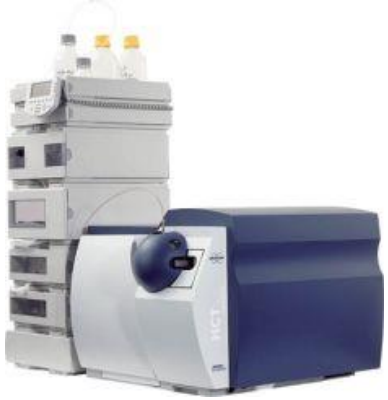
Fruit

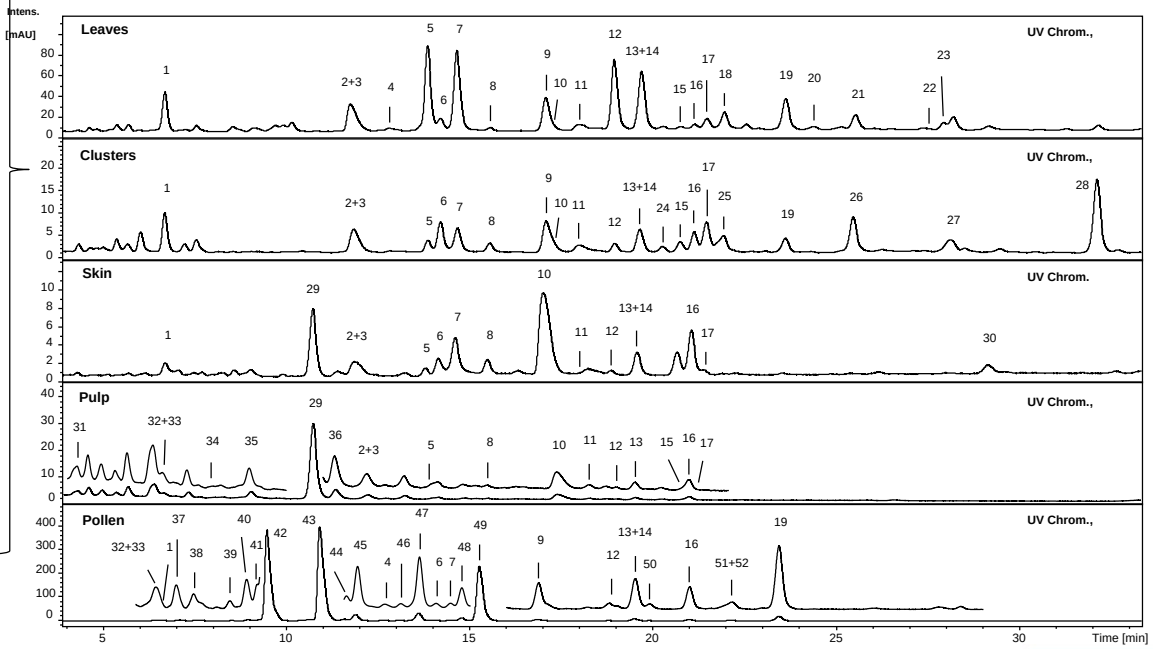
Pulp

Fronds (Leaves)

Clusters

Pollen





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Abstract:
Date palm (*Phoenix dactylifera* L.) is an important fruit tree mainly grown in the Middle East and North Africa. This plant has been widely used as a food and also in the folk medicine, due to its health-promoting properties. In spite of many previous works concerning the phenolic composition of dates fruits, studies on the phenolics in the other different parts of the tree are still scarce. Therefore, analysis of dates composition was carried out by HPLC–DAD-ESI/MS^n as a powerful screening tool for exploring its phenolic metabolites. Over 50 phenolics have been characterized in the date palm samples analyzed. Of which, about 30 compounds are described herein in the dates' material for the first time. Remarkably, kaempferol glycosides and malonyl derivatives detected in this work haven't been reported previously in *P. dactylifera*. The method used provides more information on dates' chemical composition. Also, the information obtained should help nutritionists and food technologists to become aware of the benefits of using this traditional plant in contemporary diets as potential sources of antioxidants. Additionally, data in this work may support the ancient and current use of this plant parts in as a source for functional ingredients in the medicinal, pharmaceutical, and dietary uses.

Keywords: *Phoenix dactylifera L.*; Dates' by-products; Secondary metabolites; HPLC-DAD-ESI/MSn

Introduction

- The date palm (Phoenix dactylifera L., Arecaceae), one of the oldest plants cultivated by mankind, is broadly distributed in many regions of the world, including Asia, Africa, Arabian countries, and the Middle East.

- At present, 2000 or more different cultivars of the date palm exist around the world.

- The worldwide production of P. dactylifera fruits is about 7.5 million mt (FAOSTAT, 2016).

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Introduction

- The date palm is a multi-purpose, important tree with abundant nutritional, therapeutic, socio-economic, and environmental attributes, besides its ability to grow in various climatic conditions. Fruit has been utilized since ancient times as an important staple food and in ethnomedicine in different parts of the world.
- The medicinal value of date fruits is related to the therapeutic implications in the control of diseases, through antioxidant, anti-inflammatory, anti-tumor, and anti-diabetic effects.
- They have antifungal, antiviral, antibacterial, immunomodulatory, antiparasitic, hepatoprotective, antiinflammatory and anticoccidial activities.

Introduction & objective

- Other parts of P. dactylifera have been used commonly in traditional medicine for treatment of hypertension, memory disturbances, diabetes, fever, paralysis, atherosclerosis, and nervous disorders. The pollen has been acclaimed as an aphrodisiac.
- Date extracts possess antioxidant and free radical scavenging capacities and exhibit potent anti-oxidative properties which is ascribed mostly to the phenolic composition and act to curb the oxidative damage to lipids, nucleic acids, and proteins. Several studies on the phenolic composition of dates have been conducted in the last decade. These focus on the fruits of different and little information exists concerning other parts of the trees and by-products (skin, leaves, clusters, and pollen), with regard to their phenolic compounds.

Introduction & objective

- The present work investigates different anatomical parts (pulp, skin, leaves, clusters, and pollen) of date trees of the variety Medjool growing in Palestine, with regard to their bioactive phenolic compounds (flavonoids and phenolic acids) extracted with methanol/water and analyzed using HPLC-DAD-ESI/MSn as a powerful analytical technique.

- There is a considerable economic importance for the disposal of by-products of the date industry, it is important to identify the potential applications of the new sources of phytochemicals.

- Distinct anatomical parts of dates, including clusters and leaves, have been compared from this perspective for the first time.

Materials and methods

- Samples of Medjool dates (fruits, leaves, clusters, and pollen), were collected from the crops of 2015/2016.
- Upon receiving the samples, first they were washed with water. Then, they were cut and kept in the freezer (-20°C).
- The fruits' skins (peels) were separated manually from the flesh.
- All samples were ground using a household grinder and thereafter lyophilized.The lyophilized samples were kept at -20°C until extraction.
- For each lyophilized sample (skin, leaves, clusters, and pollen), 0.6 g was extracted with methanol-water in an ultrasound bath for 1 h, left to stand overnight in the freezer at -18°C.
- After that, the samples were sonicated again for 1 h, centrifuged for 5 min at 12000 rpm, and the supernatants collected and filtered through 0.45-µm PVC filters. Finally, the filtrates were injected into the HPLC system.

Materials and methods

- Chromatographic analyses were carried out on a Kinetex C18 column (150 mm × 4.6 mm, 5 µm particle size; Phenomenex, Macclesfield, UK).
- The mobile phase was a mixture of (A) 1% formic acid and (B) acetonitrile. The flow rate was 0.8 mL min^{-1} in a linear gradient, starting with 12% B and reaching 25% B at 30 min, 50% B at 35 min, and 60% B at 37 min.
- The injection volume was 8 µL. Chromatograms were recorded at 340 nm. The LC-UV-ESI/MS^n analyses were carried out in an Agilent HPLC 1200 series.

Results and discussion

- The overall analysis of the phenolic compounds revealed that there was a qualitative similarity among the different dates parts analyzed. The method used provided tentative identification of more than 50 compounds: chiefly flavonoid glycosides of quercetin, luteolin, apigenin, chrysoeriol, kaempferol, isorhamnetin, 3-methyl-isorhamnetin, sulfates, and malonyl derivatives.
- In the present work, more than 30 phenolic derivatives are described for the first time in dates. To the best of our knowledge, kaempferol glycosides and malonyl derivatives have not been described previously in *P. dactylifera*.
- The MS data, MS^n fragmentation pattern, and UV information obtained have been of great help in the interpretation of the compounds detected and in their structural identification, s no standards commercially available.

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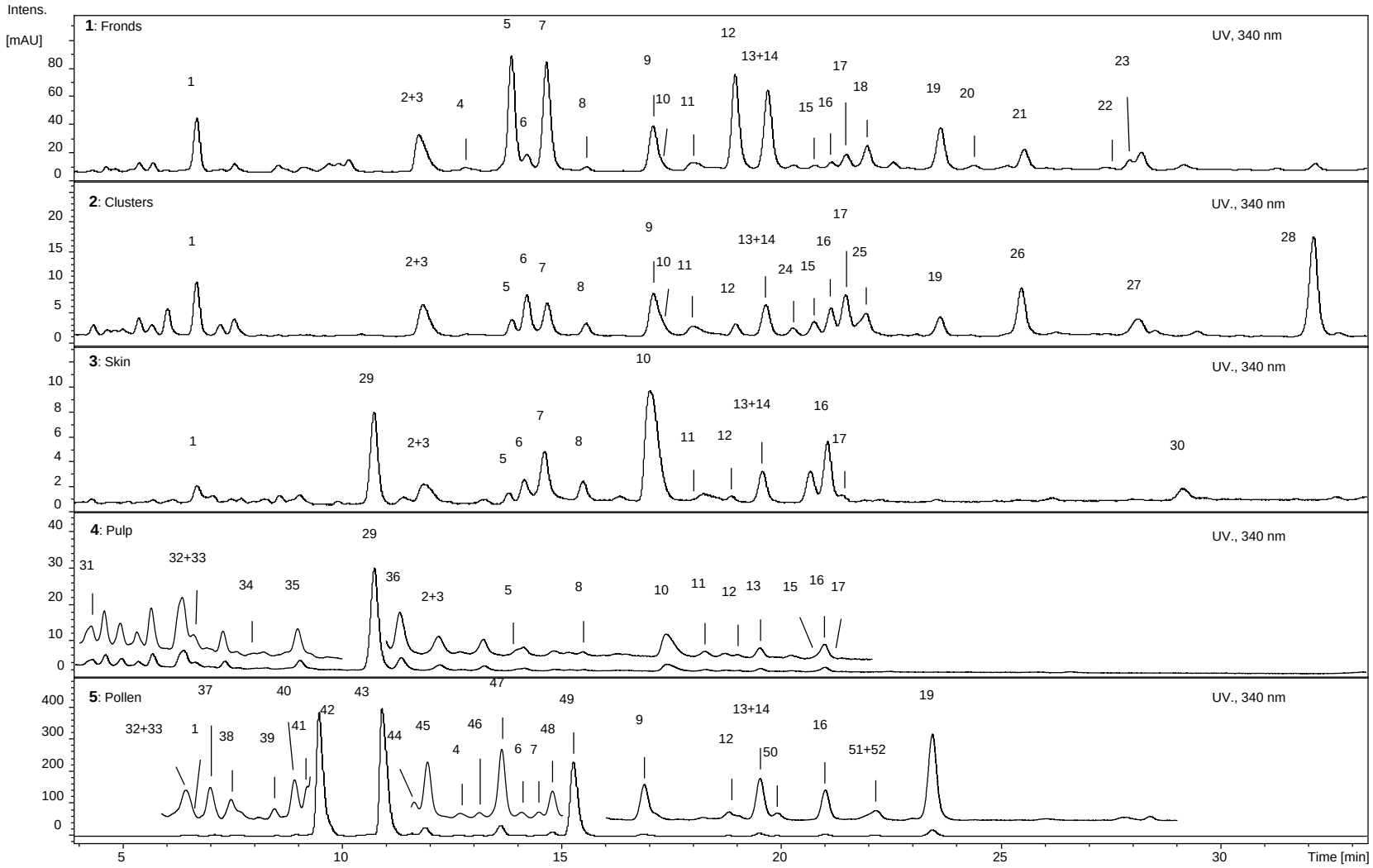
Peak #	Phenolic compound	[M-H]$^-$	Pp	Sn	Fd	Cr	Pn
1	Apigenin-6,8-di-*C*-Hx	593	-	+	+	+	+
2	Q-3-Hx(6Sulft)	543	+	+	+	+	-
3	L-7-Hx(6Sulft)	527	+	+	+	+	-
4	I-3-Hx(6Sulft)	557	-	-	+	-	+
5	Q-3-(6Rh)Hx	609	+	+	+	+	-
6	L-7-(6Rh)Hx	593	+	+	+	+	+
7	Q-3-Hx	463	-	+	+	+	+
8	L-7-(2Rh)Hx	593	+	+	+	+	-
9	Q-3-(2Mln)Hx	549	-	-	+	+	+
10	Chr-7-Hx(6Sulft)	541	+	+	+	+	-
11	MeI-7-Hx(6Sulft)	571	+	+	+	+	-
12	I-3-(6Rh)Hx	623	+	+	+	+	+
13	Chr-7-(6Rh)Hx	607	+	+	+	+	+
14	I-3-Hx	477	-	+	+	+	+
15	Chr-7-Hx	461	+	-	+	+	-
16	Chr-7-(6Rh)Hx isom.	607	+	+	+	+	+
17	MeI-7-Hx	491	+	+	+	+	-
18	MeI-7-Hx(6sulfate) deriv.	737	-	-	+	-	-
19	I-3-(6Mln)Hx	563	-	-	+	+	+
20	MeI-7-Hx(6sulfate) deriv.	737	-	-	+	-	-
21	MeI-7(2Rh)Hx deriv.	833	-	-	+	-	-
22	MeI-7(2Rh)Hx deriv.	833	-	-	+	-	-
23	MeI-7(2Rh)Hx deriv.	833	-	-	+	-	-
24	MeI-7-(6Rh)Hx	637	-	-	-	+	-
25	MeI-7-(2Rh)Hx	637	-	-	-	+	-
26	Ferulic acid deriv.	693	-	-	-	+	-
27	Ferulic acid deriv.	693	-	-	-	+	-
29	Ferulic acid	193	+	+	-	-	-
30	Luteolin	285	-	+	-	-	-
31	Q-3-(6Rh)Hx-7-Hx	771	+	-	-	-	-
32	Q-3-(2Hx[6Sulft])Hx	705	+	-	-	-	+
33	L-7-(2Hx[6Sulft])Hx	689	+	-	-	-	-
34	Chr-7-(2Hx[6Sulft])Hx	703	+	-	-	-	-
35	L-7-(2Hx[6Sulft])Hx isom.	689	-	-	-	-	+
36	Q-3-(2Sulft, 6Rh)Hx	689	-	-	-	-	+
37	I-3-(2Hx[6Sulft])Hx	719	-	-	-	-	+
38	Q-3-(6Hx)Hx-7-Hx	787	-	-	-	-	+
39	I-3-(6Hx)Hx-7-Hx	801	-	-	-	-	+
40	Q-3-(2Hx)Hx-7-Hx	787	-	-	-	-	+
41	Q-3-(6Rh)Hx-7-Hx isom.	771	-	-	-	-	+
42	Q-3,7-diHx	625	-	-	-	-	+
43	I-3,4´-diHx	639	-	-	-	-	+
44	Q-3(2Mln, 3/4Hx)Hx	711	-	-	-	-	+
45	Q-3-(2Rh)Hx	609	-	-	-	-	+
46	I-3-Hx-4´-Pent	609	-	-	-	-	+
47	I-3-(2Hx, 3/4Mln)Hx	725	-	-	-	-	+
48	K-3-(2Rh)Hx	593	-	-	-	-	+
49	I-3-(2Rh)Hx	623	-	-	-	-	+
50	I-3-(2R, 6Mln)Hx	709	-	-	-	-	+
51	I-3-Hx	477	-	-	-	-	+
52	Chr-7-Hx	461	-	-	-	-	+

Phenolic compounds identified together with their distribution and presence in the different parts (Pulp, Skin, Frond, Cluster, and Pollen) of the date palm tree



HPLC-UV profiles (340 nm) of phenolic compounds detected in different parts of *Phoenix dactilifera*. **1**: Fronds, **2**: Clusters, **3**: Skin, **4**: Pulp, **5**: Pollen

Conclusions

- Although, there are many previous studies dealing with the phenolic composition of dates, the present work characterizes novel phenolic compounds.

- Analysis by HPLC–DAD-ESI/MS^n has proved to be a powerful tool for screening different anatomical parts of date palms for the occurrence of phenolics. Thus, over 50 phenolic compounds were characterized in the date palm samples tested.
- Of Them, 33 have never been reported before in date palm and are described herein for the first time.

- The method used could provide more information on the chemical composition of date palms, which may be useful for further research into the effects of this plant on human health.
- Moreover, the information presented should help consumers and food technologists to become aware of the benefits of using this traditional plant in contemporary diets as a potential source of antioxidants. In addition, the qualitative data in this study may explain the ancient and current uses - pharmaceutical, medicinal, cosmetic, and dietary - of this plant.

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