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Identification of Flavonoids in Iraqi Date Palm Pollen by HPLC

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ABSTRACT

Date palm pollen-DPP (Phoenix dactylifera L) plays an important role in traditional treatment in Iraq, especially for the treatment of fertility or as supplements, flavonoids were important components for date palm pollen, no information is available in the literature about the types of flavonoids in an Iraqi DPP variety El-Ghannmi Ahmar. The HPLC analysis revealed that in an Iraqi DPP variety El-Ghannmi Ahmar contains many types of flavonoids (13.590 µg/g lincoceric acid, 122.251 µg/g *Isorhamnetin*, 71.146 µg/g chlorogenic acid, 99.188 µg/g ferulic acid, 64.574 µg/g naringin, 109.117 µg/g apigenin, 48.391 µg/g apigenin-7-O-beta glycopyranoside, 28.883 µg/g letulin and 18.291 µg/g letulin-7-O-beta glycosides).

Key words: El-GhannmiAhmar, Date palm pollen, Flavonoids

INTRODUCTION

Date Palm Pollen (*Phoenix dactylifera*, L.)-DPP is a fine, powder-like material produced by flowering plants and gathered by bees[1], it is very important causing of human respiratory allergic disorders, involving the production of immunoglobulins and hence the release of histamine and other chemicals[2,3]. The early Egyptians and ancient Chinese used pollen as a rejuvenating medicinal agent. It has been called a “fountain of youth”[4].Regular consumption of DPP is beneficial in nephropathy, rheumatism,gastropathy, sexual debilityand DPP extract have potential protective effects on testicular dysfunction induced by altered thyroid hormones [5,6]. In addition DPP grains have antioxidant and hypolipidemic effect in which exhibited significant reduction for serum cholesterol and low density lipoptoein-cholesterol(LDL-C), triglycerides and improve the level of high density lipoprotein-cholesterol(HDL-C), also the isolated flavonoids from DPP have anti atherosclerotic effects in high dose[7].

Phytochemical screening showed that dried DPP contain sterols, flavonoids, triterpenoidal, saponins, tannins, and crude gonadotrophic substance [Egyptian cultivar][2,8,9], while in El-

Ghannmi Ahmar DPP[Iraqi cultivar] phytosterols, alkaloids, protein, carbohydrates, glycosides, phenolic compounds, tannins, terpenoids, saponins, coumarins, lignin and flavonoids[10].

Many types of flavonoids has been identified in DPP, Abbas & Ateya[11] indicated five flavonoids compounds [rutin, luteolin -7-O- β -D - glucoside, apigenin, isorhamnetin-3-O- glucoside and naringin were isolated for the first time from the pollen.While Daoud *et al* found that the Tunisian cultivar contain higher concentrations of flavonoids than Kerkennah cultivar, which was about twice as high, especially in the acetone extract,and four types of flavonoids were identified in Tunisian cultivars by HPLC,which include Quercitin, Rutin, Catechin and Epicatechin[12]. So that the aim of this study is to identify the flavonoids inEl-Ghannmi Ahmar DPP[Iraqi cultivar].

MATERIAL AND METHODS

Plant material

DPP (Phoenix dactylifera L.) variety El-Ghannmi Ahmar was collected from Samarra city,Salah Al-Din,Iraq separated from the kernels by fine gauze sieve and left in an incubator at 35°C for 3 hours.

Methods

Extraction and isolation of flavonoids (Ex-F) was done according to[13] method with some modification, while the chromatographic analyses for Ex-F was performed by HPLC (Shimadzu,10AV-LC,Japan) according to the method of the[14]with some modification by using C-18 column (150-4.6mm, 5mm). The mobile phase consisted solvent of 0.1% acetic acid in deionized water and acetonitrile at a ratio (20:80). The UV detection wavelength and the flow rate were 264nm and 0.9 ml/min, respectively. In which isolated flavonoids was dissolved in methanol(HPLC grade) at a final concentration of 100 μg /ml and then filtered through a membrane filter (0.45 μm pore size) prior to injection.Twenty microliter of Ex-F sample was injected on C18-HPLC column.

Ten stander solutions (25μg/ml) were used (lignoceric acid, isorehamanetin, chlorogenic acid, ferulic acid, naringin, apigenin, apigenin 7-O-beta-glycopyranoside, rutin, leteolin,leteolin-7-O-beta glycosides).The concentration of identified flavonoids was done according to the following equation:

$$\text{Conc. of flavonoids (μg/ml)} = \frac{\text{Area of sample}}{\text{Area of stand.}} \times C \times D$$

C=Conc. of standard solution
D=Dilution factor

RESULTS

The HPLC analysis of flavonoids in the Iraqi date palm pollen was carried out which showed 14 peaks fig.(1) with different R_t (1.257, 1.647, 2.167, 3.052, 3.84, 4.795, 5.37, 5.923, 6.757, 8.017, 8.532, 9.177, 9.945 and 10.443) min and the area for each peak were 23998, 51700, 142188, 74231, 130242,

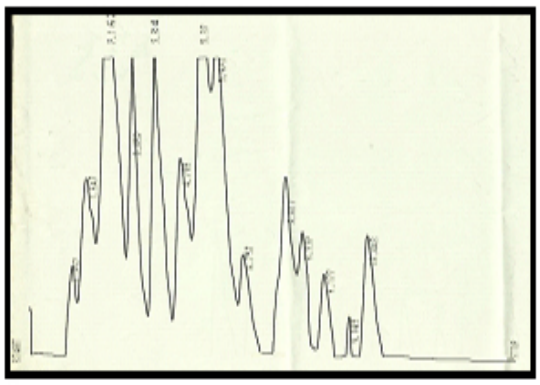

Fig. 1: HPLC Analysis of Isolated Flavonoids from Iraqi DPP

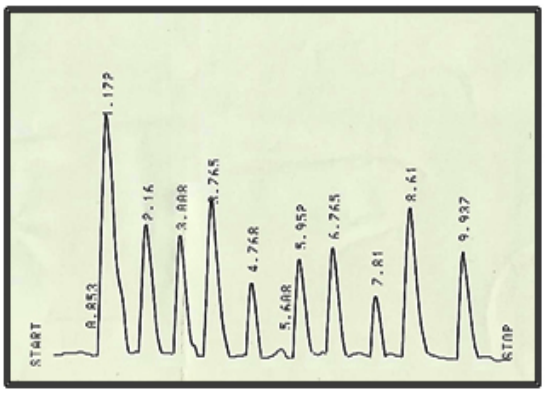

Fig. 2: HPLC Analysis of 10 Standard Flavonoids

Table 1: Retention Times and Area Under Curves for Iraqi DPP Flavonoids

Rt. (min)	Area	Identified compounds	Conc. (µg/g)
1.257	23998	Lignoceric acid	13.590
1.647	51700	Unknown	-
2.167	142188	*Isorhamnetin*	122.251
3.052	74231	Chlorogenic acid	71.146
3.84	130242	Ferulic acid	99.188
4.795	57145	Naringin	64.574
5.37	105805	Unknown	-
5.923	100658	Apigenin	109.117
6.757	48629	Apigenin-7-O-beta glycopyranoside	48.391
8.017	62767	Unknown	-
8.532	38544	Letulin	28.883
9.177	35117	Unknown	-
9.945	17723	Letulin-7-O-beta glycosides	18.291
10.443	74470	Unknown	-

57145, 105805, 100658, 48629, 62767, 38544, 35117, 17723 and 74470 which summarized in table (1).

The chromatogram of the ten standard flavonoids and phenolic compounds (lignoceric acid , *isorhamnetin*, chlorogenic acid, ferulic acid, naringin, apigenin, apigenin-7-O-beta glycopyranoside, rutin, letulin and letulin-7-O-beta glycosides) were shown in the Fig(2).

The R_t of the ten standard peaks were (1.172, 2.16, 3.008, 3.765, 4.768, 5.952, 6.765, 7.81, 8.61 and 9.937) min and the areas were 44148, 29077, 26084, 32827, 22124, 23062, 25123, 16414, 33362 and 24223 respectively, table(2).

Results obtained from chromatograms of DPP and compared with chromatogram of ten standard flavonoids and phenolic compounds, as shown in Fig.(2) and its R_t value in table(2), indicate that DPP contained 13.590µg/g lignoceric acid, 122.251 µg/g *Isorhamnetin*, 71.146 µg/g chlorogenic acid, 99.188 µg/g ferulic acid, 64.574 µg/g naringin, 109.117 µg/g apigenin, 48.391 µg/g apigenin-7-O-beta glycopyranoside, 28.883 µg/g letulin and 18.291 µg/g letulin-7-O-beta glycosides with absence of rutin, table (1).The other unknown peaks may indicate other type of flavonoids, so that we need to use other type of flavonoids as standard and more modern techniques such as GC, GC-MS, HPLC-MS or NMR for analysis the flavonoids type in DPP.

Table 2: Retention Times and Area Under Curves for Standard Flavonoids

Standard	Rt. (min)	Area
Lignoceric acid	1.172	44148
Isorhamnetin	2.16	29077
Chlorogenic acid	3.008	26084
Ferulic acid	3.765	32827
Naringin	4.768	22124
Apigenin	5.952	23062
Apigenin-7-O-beta glycopyranoside	6.765	25123
Rutin	7.81	16414
Letulin	8.61	33362
Letulin-7-O-beta glycosides	9.937	24223

Results are consistent with the previous study of [11], which indicates the presence of many types of flavonoids in Egyptian DPP naringen, luteolin -7- O- β -D - glucoside, apigenin, isorhamnetin - 3 -O- glucoside and rutin which isolated them by silica gel column chromatography and eluted by ethyl acetate, while the identification of these compounds were carried out by GC-MS and HPLC. Similarly, other study identified quercetin and rutin in DPP[8].

No information were available in the literature about the DPP content of Isorhamnetin, luteolin, chlorogenic acid, ferulic acid and apigenin-7-O-beta glycopyranoside.The content of flavonoids in pollen may be affected by many enviermintal factors such as, air pollution, in which Rezanejad study the effect of air pollution on flavonoids in pollen grains of some ornamental plants, and found that HPLC analysis demonstrated that air pollution induces flavonoids accumulation to significantly higher levels in the polluted pollen of ornamental plants than in the control[15].

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