



FLAVONOIDS AND GLYCOSIDIC FLAVONOIDS CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF IRAQI DATE PALM (*PHOENIX DACTYLIFERA* L.) SEEDS

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Abstract

The current study include evaluation the concentration of total flavonoids in Date palm (*Phoenix dactylifera*, L.) seeds-DPS, isolate the total flavonoids and glycosidic flavonoids from DPS, evaluate the Total antioxidant capacity-TAC of crude DPS and their isolated extracts and identified the flavonoids and glycosidic flavonoids by HPLC. The results indicate that DPS contain $66.844 \pm 1.854 \mu\text{g/g}$ of total flavonoids, the total antioxidant capacity were $192.609 \pm 2.881 \mu\text{mol/L}$, $205.419 \pm 2.495 \mu\text{mol/L}$, $119.422 \pm 5.695 \mu\text{mol/L}$ for crude seeds, isolated flavonoids extract and glycosidic flavonoids respectively. Otherwise the HPLC analysis identified 15 types of flavonoids with different concentration in crude seeds extract and in the isolated parts, this compounds include Rhamnosyl-di-hexosyl quercetin sulphate, Rhamnosyl dihexosyl methyl quercetin, Apigenin di-C-hexoside, Rhamnosyl dihexosyl luteolin, luteolin, Rhamnosyl dihexosyl methyl luteolin, Rhamnosyl hexosyl luteolin, Rhamnosyl hexosyl quercetin, dihexosyl quercetin, quercetin, Hxosyl leuteolin sulfate, apeginin, Hxosyl methyl leuteolin sulfate and Isorhamnetin. From all the above results we can conclude that DPS and their isolated extracts (Flavonoids and glycosidic flavonoid) could be an excellent source of antioxidants in medicine preparation and food.

Key words: Date palm seeds, Antioxidant, Flavonoids, Glycosidic flavonoids

Introduction

Date palm (*Phoenix dactylifera*, L.) is the most important and economical species grown in Iraq, North Africa and west Asia, extensively planted in the Arab countries. Date palm is a multipurpose tree providing, fiber, minerals, vitamins and carbohydrates besides having certain medicinal properties (Al-Abbad *et al.*, 2011; Al-Shahib *et al.*, 2003; Al-Abdoulhadi *et al.*, 2011), used mainly for its fruit, fiber and as a construction material (Lombard *et al.*, 2001).

The chemical composition of different parts of Date palm were study from different researchers worldwide, and also isolate the different secondary metabolites, In which Al-Samarrai *et al.*, isolated and identification of phenolic compounds, Flavonoids in Iraqi Date Palm Pollen-DPP (variety El-Ghanmi Ahmar) by HPLC, The HPLC analysis revealed many types of flavonoids such as naringin, letulin, Isorhamnetin, chlorogenic acid, apigenin, lincoceric acid, ferulic acid, apigenin-7-O-beta

glycopyranoside and letulin-7-O-beta glycosides, while the dry fruits of date palm revealed the presence of different types of phenolic acids, which include caffeic acid, ferulic acid, gallic acid, p-hydroxybenzoic acid, p-coumaric acid, protocatechuic acid, syringic acid and vanillic acid (Regnault-Roger *et al.*, 1987).

The chemical composition of date palm seeds-DPS were investigate from many researcher, Metoui *et al.*, found that the DPS contain flavonoid, Glycosidic flavonoids, anthocyanin, tannins and Polyphenol. The polyphenols and flavonoids often provide beneficial bioactive properties to the plants itself and also to animals for repair their functions and homeostasis as well as preventing diseases (Lancon *et al.*, 2013). In which the clinical studies showed that the DPS acts as antioxidant, free radical scavenging, anticancer, anti-inflammatory antimicrobial, antimutagenic with hepatoprotective (Khalid *et al.*, 2017; Chandrasekaran *et al.*, 2013). So the aim of the present study was to identify the active metabolic compounds (flavonoids and Glycosidic flavonoids) in Iraqi

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DPS and in its extract by HPLC and evaluate the total antioxidant capacity.

Materials and Methods

Plant material

DPS (*Phoenix dactylifera* L.) variety Barhy was collected from Samarra city, Salah Al-Din, The date seeds were washed to remove any adhering flesh, dried at 50°C on an oven, made as fine powder and kept in a dark container until used Fig.1.

Chemicals

Standards chemicals materials were used in this study: such as Ethanol, sodium hydroxide, sodium nitrate, Aluminum chloride, quercetin, sodium acetatetrihydrate, 2, 4, 6- tripyridyl- S-triazine, Hydrochloric acid, Ferric chloride, Iron(II) sulphate, Rhamnosyl-di-hexosyl quercetin sulphate, Rhamnosyl dihexosyl methyl quercetin, Apigenin di-C-hexoside, Rhamnosyl dihexosyl luteolin, luteolin -Rhamnosyl dihexosyl methyl luteolin, Rhamnosyl hexosyl luteolin, Rhamnosyl hexosyl quercetin, dihexosyl quercetin, Hxosyl leuteolin sulfate, apeginin, Hxosyl methyl leuteolin sulfate and Isorhamnetin were purchased from Sigma Chemical Company (USA) and the HPLC-grade solvents like methanol, formic acid and water were purchased from Merck (Germany).

Methods:

Preparation of Extract:

Extraction of total flavonoids from DPS *Phoenix dactylifera* L. :The extraction method was done according to (Chan *et al.*, 2006), The solvent was evaporated, The extract was collected and labeled the vial as DPS-TF Fig. 1. and kept at 4°C until used.

Extraction of Glycosidic flavonoids from DPS: One hundred grams of DPS were soaked in (3 X 500ml) 70% ethanol(prepared in 5% Sodium hydroxide) with continuous stirring, 48hours at room temperature. The extract was filtrate and evaporated under reduced pressure to dryness, collected and labeled the vial as DPS-GF Fig. 1 and kept

at 4°C until used.

Identification of flavonoids by High-Performance Liquid Chromatography-HPLC: Identification of flavonoids in *Date palm seeds*, *DDPS-TF* and *DPS-GF* were carried out according (Rodríguez *et al.*, 2001). method with some modification, The main compound were separated on Fast liquid chromatography-FLC column under the optimum condition:

Column: Phenomenex C-18, µm particle size(50 × 2.0 mm I.D)

-Mobile phase: linear gradient of solvent A(5% formic acid in water); solvent B was 5% formic acid in methanol, 0% B to 100% B for 20 min.

-Flow rate:1.4ml/min

-Detection: UV 370nm.

-Preparation of samples for HPLC analysis

Five gram of seeds powder or extract was homogenized in polyton (2min in ice) with 10ml of (4mM sodium fluoride in methanol to inactivate polyphenoloxidases and prevent phenolic degradation due to browning) (Chaira *et al.*, 2013). The homogenates were kept in ice until centrifuged (16000g) for 15min at (2-5)°C. The supernatant was recovered and filtered through 0.45mm filters and directly analyzed by HPLC. Twenty five (25µg/ml) of fifteen standard flavonoids were used, which include(Rhamnosyl-di-hexosyl quercetin sulphate, Rhamnosyl dihexosyl methyl quercetin, Apigenin di-C-hexoside, Rhamnosyl dihexosyl luteolin, luteolin, Rhamnosyl dihexosyl methyl luteolin, Rhamnosyl hexosyl luteolin, Rhamnosyl hexosyl quercetin, dihexosyl quercetin, quercetin, Hxosyl leuteolin sulfate, apeginin, Hxosyl methyl leuteolin sulfate and Isorhamnetin.

The concentration of identified, flavonoids was done, according to, the following equation:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times 25 \times D$$

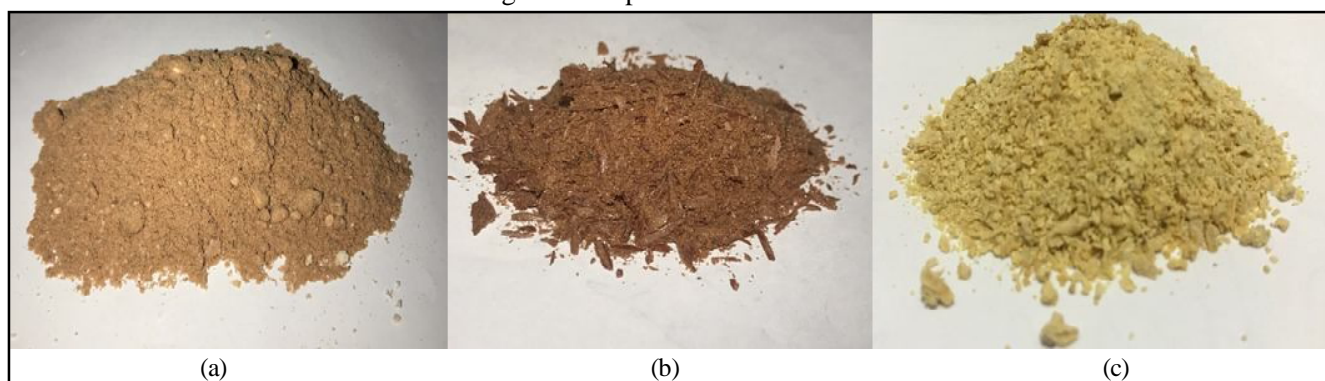


Fig.1: Date palm seed (a-seed powder

b-isolated flavonoids

c-isolated Glycosidic flavonoids).

Area of sample

D = Dilution factor

Quantitative determination of total flavonoids:

Total flavonoids concentration in Date palm seeds was determine by using Aluminum chloride spectrophotometric method (Olajire *et al.*, 2004), quercetin was using as standard. All test was done triplicate and the concentration of flavonoids for each

Table 1: Total antioxidant capacity for crude seeds, *DDPS-TF* and *DPS-GF*.

Extracts	Mean±SD (µmol/L)
Date palm seeds	192.609±2.881
Total flavonoids extract	205.419±2.495
Glycosidic flavonoids	119.422±5.695

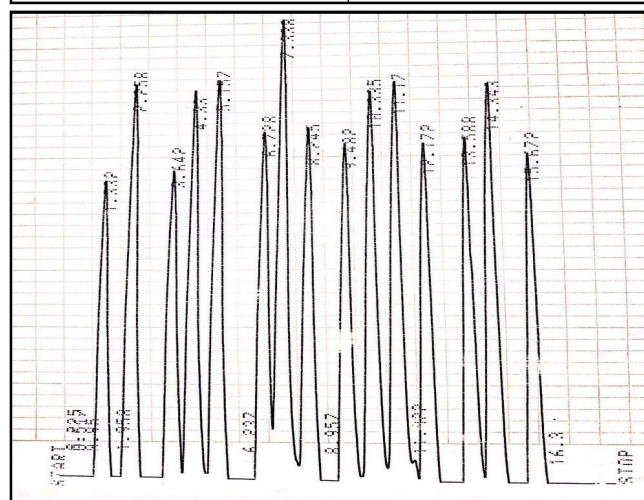


Fig. 2: HPLC Analysis of fifteen standard Flavonoids and glycosidic flavonoids.

Table 1: The Rt and area under curve for standard Flavonoids and glycosidic flavonoids.

No.	Standard compound	Retent-ion time	Area un-der peakv
		(min)	µv
1-	Rhamnosyl-di-hexosyl quercetin sulphate	1.33	185075
2-	Rhamnosyl dihexosyl methyl quercetin	2.25	281258
3-	Apigenin di-C-hexoside	3.64	191465
4-	Rhamnosyl dihexosyl luteolin	4.33	236415
5-	Luteolin	5.15	276478
6-	Rhamnosyl dihexosyl methyl luteolin	6.72	195041
7-	Rhamnosyl hexosyl luteolin	7.33	277396
8-	Rhamnosyl hexosyl quercetin	8.24	215477
9-	dihexosyl quercetin	9.48	208700
10-	Rhamnosyl hexosyl methyl quercetin	10.33	244771
11-	Quercetin	11.17	243369
12-	Hxosyl leuteolin sulfate	12.17	252994
13-	Apeginin	13.58	202765
14-	Hxosyl methyl leuteolin sulfate	14.34	276689
15-	Isorhamnetin	15.67	190049

extract were expressed as mean ± standard deviation(mean ± SD).

Total antioxidant capacity-TAC:

The determination of TAC for date palm seed, *DDPS-TF* and *DPS-GF* were done by using ferric reducing-antioxidant power -FRAP method (Benzie *et al.*, 1996). The TAC for each sample were estimated against a standard solution of ferrous sulphate, each test was done triplicate.

Results

The present study was carried out to identify the total flavonoids concentration in Date palm, the result showed that mean ± SD was 66.844 ± 1.854µg/g and the total antioxidant capacity were 192.609 ± 2.881 µmol/L, 205.419 ± 2.495 µmol/L, 119.422 ± 5.695 µmol/L for crude seeds, *DDPS-TF* and *DPS-GF* respectively, table 1.

The results indicate that the high antioxidant capacity was for total flavonoids as compared with two other extract(Date palm seeds and Glycosidic flavonoids).

The HPLC analysis of standard flavonoids were done by using 15 standard of flavonoids and glycosidic flavonoids, in which Fig. 2 show the fifteen pecks with different Retention time-Rt and table 2 showed the Rt and area under curve for each standard.

The HPLC analysis of date palm seeds extract showed twelve pecks with different Rt as shown in Fig. 3 and area under curve values for each peck in table 2. Results obtained from chromatograms of date palm seeds extract and compared with chromatogram of 15 standard flavonoids and glycosidic flavonoids, as shown in Fig. 2 and its Rt value in table 2, indicate that DPP contained results obtained from chromatograms in Fig. 3 and compared with chromatogram of Fig. 2 for the 15 standard flavonoids and glycosidic flavonoids indicate that the types of flavonoids found in date palm seeds extract, were 766.10µg/g of Rhamnosyl-di-hexosyl quercetin sulphate, 2369.8µg/g of Apigenin di-C-hexoside, 1776.87 µg/g of Rhamnosyl dihexosyl luteolin, 1168.60 µg/g Rhamnosyl hexosyl luteolin, 3546.22 µg/g Rhamnosyl hexosyl quercetin, 2044.81 µg/g dihexosyl quercetin, 2647.32 µg/g Rhamnosyl hexosyl methyl quercetin, 1997.55 µg/g quercetin, 1617.45 µg/g Hxosyl leuteolin sulfate, 2259.32 µg/g apeginin, 2477.03 µg/g Hxosyl methyl leuteolin sulfate and 2855.921 µg/g Isorhamnetin.

The Fig. 4 showed the chromatogram of the HPLC analysis for flavonoids and glycosidic flavonoids in *DPS-TF* extract, which indicate eighteen

peaks with different Rt and area under curve values, table 3.

While the types of flavonoids found in DPS-TF extract were 1442.96 $\mu\text{g/g}$ of Rhamnosyl-di-hexosyl quercetin sulphate, 1142.45 $\mu\text{g/g}$ of Rhamnosyl dihexosyl methyl quercetin, 2750.64 $\mu\text{g/g}$ Apigenin di-C-hexoside, 1568.19 $\mu\text{g/g}$ of Rhamnosyl dihexosyl luteolin, 1466.01 $\mu\text{g/g}$ luteolin, 2398.93 $\mu\text{g/g}$ Rhamnosyl dihexosyl methyl luteolin, 2140.01 $\mu\text{g/g}$ Rhamnosyl hexosyl luteolin, 3526.99 $\mu\text{g/g}$ Rhamnosyl hexosyl quercetin, 2860.72 $\mu\text{g/g}$ dihexosyl quercetin, 3545.06 $\mu\text{g/g}$ Rhamnosyl hexosyl methyl quercetin, 2501.56 $\mu\text{g/g}$ quercetin, 2674.73 $\mu\text{g/g}$ Hxosyl leuteolin sulfate, 2796.71 $\mu\text{g/g}$ apeginin, 3249.82 $\mu\text{g/g}$ Hxosyl methyl leuteolin sulfate and 3419.40 $\mu\text{g/g}$ Isorhamnetin with three unknown peaks.

The Fig. 5 showed the chromatogram of the HPLC analysis for flavonoids and glycosidic flavonoids in DPS-GF extract indicate thirteen peaks with different Rt and area under curve values, table 4.

The results indicate present different types of flavonoids in DPS-GF extract with different concentration, and all the peaks of chromatogram in Fig. 4 were identified, which include 1780.23 $\mu\text{g/g}$ of Rhamnosyl-di-hexosyl quercetin sulphate, 1489.26 $\mu\text{g/g}$ of Rhamnosyl dihexosyl methyl quercetin, 1608.32 $\mu\text{g/g}$ of Rhamnosyl dihexosyl luteolin, 2480.30 $\mu\text{g/g}$ Rhamnosyl dihexosyl methyl

luteolin, 1207.63 $\mu\text{g/g}$ Rhamnosyl hexosyl luteolin, 3066.82 $\mu\text{g/g}$ Rhamnosyl hexosyl quercetin, 1978.94 $\mu\text{g/g}$ dihexosyl quercetin, 2097.35 $\mu\text{g/g}$ Rhamnosyl hexosyl methyl quercetin, 1541.83 $\mu\text{g/g}$ quercetin, 1143.86 $\mu\text{g/g}$ Hxosyl leuteolin sulfate, 2493.94 $\mu\text{g/g}$ apeginin,

Table 2: The Rt, area under curve and concentration of flavonoids and glycosidic flavonoids in date palm seeds extract.

Identified Compounds	Rt	Area	Conc. ($\mu\text{g/g}$)
Rhamnosyl-di-hexosyl quercetin sulphate	1.323	56715	766.10
Apigenin di-C-hexoside	3.67	181495	2369.8
Rhamnosyl dihexosyl luteolin	4.327	168032	1776.87
Rhamnosyl hexosyl luteolin	7.344	129667	1168.60
Rhamnosyl hexosyl quercetin	8.265	305645	3546.22
dihexosyl quercetin	9.505	170701	2044.81
Rhamnosyl hexosyl methyl quercetin	10.353	259195	2647.32
quercetin	11.16	194457	1997.55
Hxosyl leuteolin sulfate	12.17	163683	1617.45
apeginin	13.587	183247	2259.32
Hxosyl methyl leuteolin sulfate	14.353	274147	2477.03
Isorhamnetin	15.663	217106	2855.921

Table 3: The Rt, area under curve and concentration of flavonoids and glycosidic flavonoids in DPS-TF extract from date palm seeds.

Identified Compounds	Rt	Area	Conc. ($\mu\text{g/g}$)
Rhamnosyl-di-hexosyl quercetin sulphate	1.23	106823	1442.96
Rhamnosyl dihexosyl methyl quercetin	2.237	128530	1142.45
Apigenin di-C-hexoside	3.658	210661	2750.64
Rhamnosyl dihexosyl luteolin	4.312	148298	1568.19
Luteolin	5.15	162127	1466.01
Unknown	5.807	36525	—
Unknown	6.308	28.694	—
Rhamnosyl dihexosyl methyl luteolin	6.713	187156	2398.93
Rhamnosyl hexosyl luteolin	7.325	237453	2140.01
Rhamnosyl hexosyl quercetin	8.24	303950	3526.99
Unknown	8.907	72012	—
dihexosyl quercetin	9.467	238813	2860.72
Rhamnosyl hexosyl methyl quercetin	10.325	347092	3545.06
quercetin	11.143	243521	2501.56
Hxosyl leuteolin sulfate	12.137	270677	2674.73
apeginin	13.565	226830	2796.71
Hxosyl methyl leuteolin sulfate	14.325	359676	3249.82
Isorhamnetin	15.63	259942	3419.40

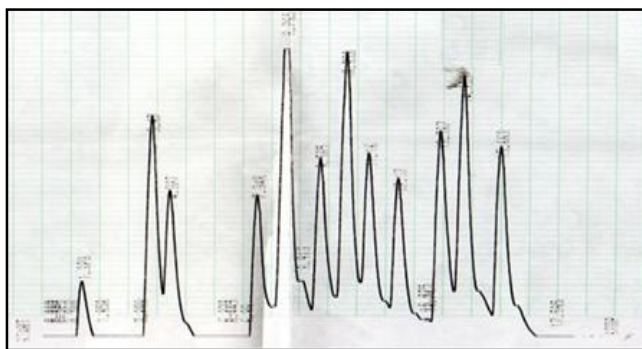


Fig. 3: HPLC Analysis of flavonoids in Date palm seeds extract.

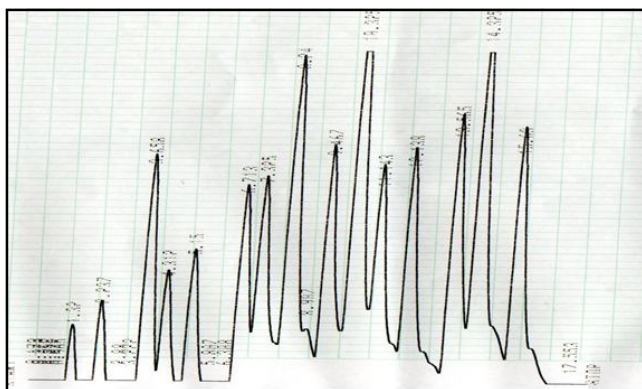


Fig. 4: HPLC Analysis for total flavonoids in DPS-TF extract.

1941.403 μ g/g Hxosyl methyl leuteolin sulfate and 2293.40 μ g/g Isorhamnetin.

Discussion

The flavonoids are a large group of polyphenolic compounds synthesized by plants by phenylpropanoid pathway. Its present in plants to provide potential and versatile health benefits via *in-vitro* chelating activity and radical scavenging. Which due to their ability to reduce the free radical formation and hence exhibit several biological activities (Tapan *et al.*, 2016). Many studies suggest that flavonoids are well known for antioxidant, anti-allergic, anti-thrombitic, anti-inflammatory, anticancer

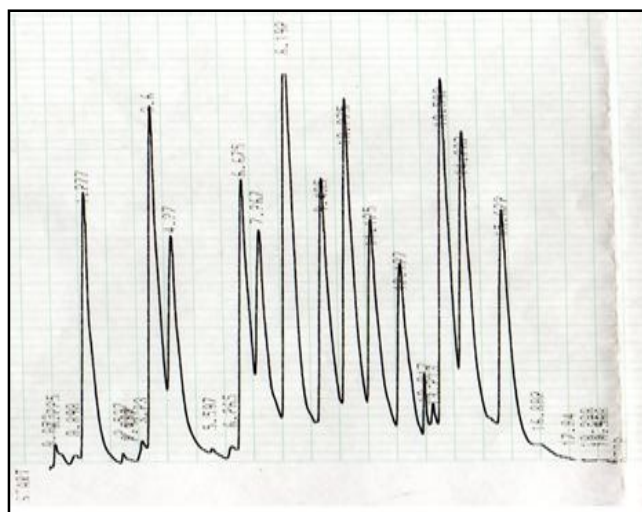


Fig. 5: HPLC Analysis for flavonoids and glycosidic flavonoids in DPS-GF extract.

Table 4: The Rt, area under curve and concentration of flavonoids and glycosidic flavonoids in DPS-GF extract.

Identified Compounds	Rt	Area	Conc. (μ g/g)
Rhamnosyl dihexosyl quercetin sulphate	1.277	131791	1780.23
Rhamnosyl dihexosyl methyl quercetin	2.6	167547	1489.26
Rhamnosyl dihexosyl luteolin	4.27	152093	1608.32
Rhamnosyl dihexosyl methyl luteolin	6.675	135808	2480.30
Rhamnosyl hexosyl luteolin	7.267	133997	1207.63
Rhamnosyl hexosyl quercetin	8.192	264326	3066.82
dihexosyl quercetin	9.435	165202	1978.94
Rhamnosyl hexosyl methyl quercetin	10.275	205349	2097.35
quercetin	11.125	150094	1541.83
Hxosyl leuteolin sulfate	12.127	115756	1143.86
apeginin	13.592	202274	2493.94
Hxosyl methyl leuteolin sulfate	14.287	214866	1941.403
Isorhamnetin	15.622	174344	2293.40

and have hepatoprotective effect (Maheshkumar *et al.*, 2012; Alsamarrai, *et al.*, 2020). Nevertheless the plant content of antioxidant in general, could be affected by the level of maturity, cultivar, growing conditions such as the soil state, location, climate, agriculture practices and agriculture practices (Wu, *et al.*, 2004).

The date palm seeds contain a wide range of impotent phytochemical compounds which have antioxidant properties such as such as phenolic compounds and flavonoids. Bouhlali et al found that the total flavonoids in date palm seeds range between 1.224 to 1.659 mg/100g of dry weight depending to the cultivar of date fruits, this concentration for flavonoids is higher the concentration of flavonoids in the present study, that's due to the different of cultivar, maturity and other environmental factors, while the antioxidant activity for the seeds extract in the present study are in agreement with the finding of this study, no information in the literature about the antioxidant activity for total flavonoids extract and glycosidic flavonoids extract which isolated from date palm seed in the present study.

22- S.I. Al-Mulhem and S.N. El-Rahman, Characteristic Analysis, Antioxidant Components and Antioxidant Activity of Date Fruits, Date Seeds and Palm Shell. Clin Med Case 2017; 1: 101-107.

Ammar *et al.*, identified seven types of glycosidic flavonoids in the methanolic extract of date palm seeds, include isoquercetin, acacetin-7-O- β -D- neohesperopyranoside, luteolin-7-o- β -D-neohesperopyranoside-3'-O-methylether, apigenin 7-O- α -D-apiofuranoside, Luteolin -7-O- β -D- neohesperopyranoside, genistein -8-C- β -D- glucopyranoside and apigenin-7-O- α -D-apiofuranosyl-(1 \rightarrow 2)-O- β -glucopyranoside, while El-Rahman and Al-Mulhem indicate that Apigenin and Quercetin were absent in date palm seeds.

The identification of fifteen compound in date palm seeds were carried out for the first time in the present study, and also no information about the isolation the total flavonoids and glycosidic flavonoids were available in the literature and also about the identification of flavonoids and glycosidic flavonoids in isolated parts from date palm seeds.

Conclusion

The present study was carried out for the first time for Iraqi date palm seed to identify their antioxidant activity and flavonoid contents. So we can conclude that DPS and their isolated extracts (Flavonoids and glycosidic flavonoid) could be an excellent source of antioxidants in medicine preparation and food.

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