Study the active compounds of Barhi date seed extracts

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Abstract

Recently, there has been increased interest in the use of date seeds, mainly in various nutritional and health fields. Their is significant impact on human health, including antioxidant, antimicrobial, anti-inflammatory , anti-mutagenic, and anti-cancer effects. The potential use of date seeds in many nutritional and health systems is due to their possession of several properties that may lead to improving sensory and functional qualities and extending the shelf life of foods, given that a large amount of date seeds is produced as waste.

This study included the diagnosis of bioactive compounds in the aqueous and alcoholic extracts of date seeds powder using gas chromatography-mass spectrometry (GC-MS). The antioxidant activity and total phenol content were studied. The results showed the appearance of a number of active compounds, volatile oils, phenolic compounds, and flavor compounds. The active compounds were observed in the alcoholic extract of date kernels, where several peaks of different active compounds appeared with a retention time (RT) ranging from 4.386 to 29.044 minutes, including compounds that gave the highest area%, reaching 24.1099%. Several peaks also appeared for different bioactive compounds of the aqueous extract of date kernel powder with a retention time (RT) ranging from 4.241 to 35.764 minutes, including compounds that gave the highest area%, reaching 21.845%. The results of estimating the antioxidant activity showed that the alcoholic extract was higher, reaching the highest activity at 1.25 mg/ml was 89.53%, while the aqueous extract was 45.76%.

The highest total phenol content was found in the alcoholic extract at a concentration of 2.5 mg/ml (70.53%), while the highest total phenol content was found in the aqueous extract at the same concentration (36.18%).

Keywords: Date seed extract, active compounds, GC-MS, phenols.

1. Introduction

The date palm (Phoenix dactylifera L.) is an important crop in most Middle Eastern countries. Date kernels, or seeds or pits, are a by-product of date processing plants. Despite the high nutritional value of date kernels as a source of carbohydrate, dietary fiber, protein, oil, natural antioxidants, and bioactive polyphenols, they remain underutilized and usually used as a by-product. Nonetheless, recent research has been focusing on the potential of date kernels to serve as an alternative source of bioactive agents in various products, such as medicine supplements, cosmetics, and foods [5,4] Food functionality is a continuously increasing need for the production of food, and this should be met sustainably. Therefore, the highest priority should be given to natural compounds and their derivatives possessing bioactive activity. But in some cases, natural resources might be limited, and other by-products can be utilized to meet the growing demand for food additives[27]

Intake of date seed powder enriched functional foods can help humans reach FDA and other daily guidelines for fiber intake. Fibers is a vital ingredient because it plays numerous functions in food systems, from fat substitutes, fat absorbers (e.g., during cooking), stabilizers, to binders[34,5]. Date seed powder is of great concern in biomedical applications due to the fact that it has high concentrations of bioactive compounds[10]. Date seeds are highly active antioxidants due to the fact that

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they have high concentrations of phenolics and tocopherols. This by-product can therefore be used as a natural preservative with particular strong potential, bearing in mind that market demand is skewed towards natural preservatives with no adverse effects on the health value or sensory characteristics of foods [20].World date production annually was estimated at around 7.5 million tons in 2008 (FAOSTAT).

Given that 10% of this total production is date pit weight (as per the estimated average percentage [16,15], the total accumulated annual global date pit amount is approximately 750,000 tons. Furthermore, there has been a massive rise in the land area used in date palm cultivation; therefore, total production will continue to rise in the future. Unfortunately, date pits are usually discarded and used as animal feed [5], or at best, used in Arabic coffee [29]. Date pits have been used to produce beverages that are comparable in character to Arabic coffee for centuries since they are in excess and inexpensive in the Arab world. Researchers have more recently looked into whether date pits, which are full of fibers, can be added to bakery foods [9,11,6], for improved shelf life[25], as a fat replacement [7,14], and as a meat tenderizer of natural origin in an attempt to increase juiciness, texture, and flavor [26] . In some research, the consumption of date pit powder has proved to be useful in enhancing nutritional and oxidative stress, anti-inflammatory status, mental state, exercise performance, and fatigue resulting from exercise during high-intensity training among runners [24]. Apart from that, previous studies in animals and humans have investigated the effects of date pits, dates have positive effects on antioxidant defense systems, promoting markers, oxidative stress, inflammation, hyperglycemia, memory, and learning disabilities as a low-cost food supplement [12,19].

2. Materials and Method

followed the method [8] with some necessary changes, the Samples were collected from local markets in Basra Governorate, Iraq, and were in good condition, intact, and fully ripe. The pulp was manually separated from the kernel, then the date kernels were collected, washed with distilled water, and dried in an electric oven at 40-45°C for 6 hours. The kernels were ground in an electric grinder to obtain date kernel powder. The kernels were stored in dark glass containers in the refrigerator until use.

2.1. Extract Preparation

Alcoholic Extract

Alcoholic extracts were prepared by dissolving 100 g of date seed powder in 500 ml of ethanol (70%), mixing them well, and leaving them to stand for 24 hours at a laboratory temperature of 25-30°C. The mixtures were filtered using a Whatman No. 1 evaporator. They were concentrated using a rotary evaporator under vacuum at 40°C until dry at laboratory temperature. The extracts were placed in glass bottles and stored in a refrigerator at 4°C [13] .

Aqueous Extract

The method of [30] was followed in preparing the aqueous extract, Then mix 20 g of date seed powder with distilled water (500 ml).. The extract was then placed in a rotating magnetic stirrer to extract the maximum possible amount of the extract for one day at laboratory temperature, after that centrifuged at 2,500 rpm for 10 minutes. The extract was filtered using Whatman No. 1 filter paper and the resulting mixture was concentrated using a rotary evaporator until it reached a thick liquid. Then it was placed in an incubator. at 37°C for 48 hours. To obtain a dry powder ultimate, the extract was stored at 4°C in glass bottles.

2.2 Antioxidants Activity

Determination of (1, 1-diphenyl-2-picrylhydrazyl) (DPPH) free radica

The free radical scavenging activity of the extract was estimated based on the scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, according to the procedure described in [31]. A sample of the extract (1 ml) was mixed with 4 ml of methanol and 1 ml of a

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reagent solution (10 mM DPPH in ethanol). This solution contained only the DPPH solution instead of the sample. The mixture was shaken vigorously and left at room temperature. After 30 minutes, a decrease in the absorbance of the test mixture (The removal efficiency of DPPH free radicals was measured at a wavelength of 517 nm.. The removal effect was calculated using the following equation.

Inhibition percentage = {1 - [Antibody sample/Antibody control group]} × 100

Estimation of total phenol content

The phenolic content of the alcoholic and aqueous extracts of date seed powder was amounted using the Folin-Ciocalteau method mentioned in[3] by dissolving 1 g of the extracts in 46 ml of distilled water, adding 1 ml of Folin-Ciocalteau reagent whith mixing well. After 3 minutes, 3 ml of 2% sodium carbonate (Na2CO3) was added and the mixture was left for 2 hours with intermittent shaking. Different concentrations of gallic acid were prepared ranging from (0-100 mg/ml), after which the absorbance was measured at a wavelength of 760 nm.

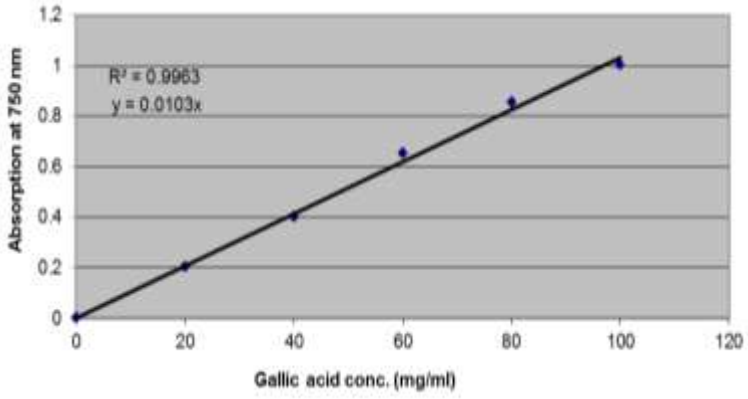

Figure No. (1): Standard curve for gallic acid

Characterization of Bioactive Compounds in Date Kernels by (GC-MS)

Compunds activity in date kernel extracts were identified by gas chromatography-mass spectrometry (GC-MS) at the Nahran Omar Laboratories in Basra using an HP-5ms capillary column with an injection volume of 1 µL, helium gas at a flow rate of 0.5 mL/s, and an injector and connector temperature of 250°C. The oven program was set at 80°C for 4 minutes and increased to 280°C for 20 minutes at a rate of 10°C/min. The spectra of the discrete peaks of the components were compared to the spectra database of the National Institute of Standards and Technology (NIST) Program Library (2014) [17] .

3. Results and Discussion

3.1.Bioactive Compounds in Date Kernels Using Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a method that allows for the qualitative identification of bioactive compounds resulting from biosynthetic processes and plant raw material extraction techniques. Active compounds and essential oils contribute to the desired flavor, antibacterial and antifungal activity, and antioxidant efficacy of food products [21]. Table (1) shows the most important active compounds in the alcoholic extract of date kernels, as detected by GC-MS. Figure (2) shows several peaks of various active compounds, such as volatile oils and flavor compounds, with retention times (RT) ranging from 4.386 to 29.044 minutes. The compounds with the highest Area% were identified, reaching

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24.1099%, such as 5-hydroxymethylfurfural with a retention time (RT) of 14.77 min, which appeared to be a phenolic compound with antioxidant and antifungal properties [28] and for pharmaceutical, polymer, and many food and industrial applications [18] . In addition, compounds such as 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, Hexadecanoic acid, methyl ester, and Methanamine, N-methoxy, appeared with a retention time of 13.498, 22.539, and 4.386 min, respectively, and Area% was 16.167, 8.418, and 6.754, respectively. These compounds can be used as emulsifiers in Food products, food additives, and anti-corrosion agents in bread [32].

Table No. (1) Active compounds in the alcoholic extract of date seed powder

Peak	Compounds name	R.T.	Area %
1	Methanamine, N-methoxy-	4.386	6.7549
2	Butanal, 3-methyl-	4.716	3.9642
3	Chloromethylmethyl sulfide	6.302	0.9378
4	Chloromethylmethyl sulfide	6.562	2.4166
5	2-Furanmethanol	7.269	4.596
6	4-Cyclopentene-1,3-dione	7.881	1.3025
7	2-Thiazolamine, 4,5-dihydro-	8.117	2.4133
8	1,2-Cyclopentanedione	8.926	1.0728
9	2-Furanmethanol, 5-methyl-	9.5	0.3352
10	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.947	2.5918
11	2H-Pyran-2,6(3H)-dione	10.364	1.0726
12	Propanal, 3-methoxy-	10.984	1.614
13	2-Furancarboxylic acid, hydrazide	12.013	1.1579
14	Ethyl acetoacetate	12.783	0.7293
15	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	13.498	16.1672
16	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	13.985	0.7663
17	Heptane, 4-ethyl-	14.393	2.8807
18	5-Hydroxymethylfurfural	14.77	24.1099
19	3-Methyl-4-methylamino-1,2,4-triazole-5-thiol	16.035	3.5518
20	3,5-Dichloro-2,4-dimethylphenol	16.679	0.6915
21	1-Tridecene	17.049	0.367
22	Thiazolidine, 3-acetyl-2-methylene-	17.96	0.8948
23	Scyllo-Inositol, 1-C-methyl-	18.117	0.5201
24	Dodecanoic acid	18.596	0.3563
25	1H-1,2,3-triazolo[4,5-d]pyrimidin-7-ol, 5-mercapto-	20.214	1.3619
26	Tridecanoic acid, 12-methyl-, methyl ester	20.426	1.5472
27	Pentadecanoic acid	21.848	0.2831
28	Hexadecanoic acid, methyl ester	22.539	8.4182
29	n-Hexadecanoic acid	22.869	1.1528
30	Methyl stearate	24.409	1.1056
31	Oleic Acid	24.535	0.7483
32	Octadecanoic acid	24.715	0.2721
33	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	25.909	1.0438
34	Ethyl stearate, 9,12-diepoxy	27.119	0.3284

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35	cis-Vaccenic acid	27.355	0.3805
36	Benzamide, 2,6-difluoro-3-methyl-N-methyl-N-butyl-	28.297	0.6744
37	[1-(3,4-Difluorobenzyl)-1,2,3-triazol-4-yl]methyl morpholine-4-carboxylate	29.044	1.4192

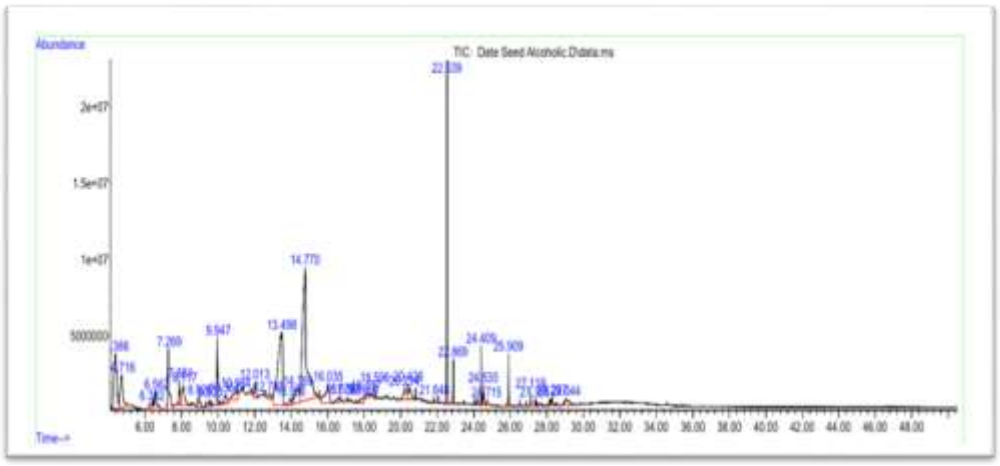

Figure No. (2): GC-MS profile of the alcoholic extract of date seed powder

Table (2) shows the of the aqueous extract of date seed powder by GC-MS analysis, which showed several peaks for different bioactive compounds with a retention time (RT) ranging from 4.241 to 35.764 minutes, where the compound Trimethoxybenzylamine, PFP-2,4,5 had the highest retention time (RT) and the compound Carbonyl sulfide had the lowest retention time (RT). The compounds with the highest Area% were identified, reaching 21.845%, Hydroxymethylfurfural-5, which is a phenolic compound that acts as an antioxidant[36], protects the heart from oxidative stress [35], and also protects against oxidative alcoholic liver damage and has an anti-inflammatory effect [23] .

Table No. (2) Active compounds in the aqueous extract of date seed powder

Peak	Name	RT	Formula
1	Carbonyl sulfide	4.241	COS
2	2-Propenoic acid, ethenyl ester	5.811	C5H6O2
3	Propanoic acid, butyl ester	6.563	C7H14O2
4	3-Furaldehyde	6.872	C5H4O2
5	Methylenecyclopropanecarboxylic acid	7.779	C5H6O2
6	1H-Pyrazole, 1,3-dimethyl-	8.078	C5H8N2
7	l-Alanine, N-methoxycarbonyl-, methyl ester	8.341	C6H11NO4
8	6-pentylpiperidin-2-one	9.153	C10H19NO
9	2-Furancarboxaldehyde, 5-methyl-	9.712	C6H6O2
10	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	10.143	C6H8O4
11	Hexa-1,3,5-triyne	10.358	C6H2
12	2H-Pyran-2,6(3H)-dione	10.463	C5H4O3
13	2H-Pyran, 3,4-dihydro-2-methoxy-	10.535	C6H10O2
14	Methane, isocyanato-	10.827	C2H3NO
15	3-Heptene, 3-ethyl-	11.37	C9H18

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16	Valproic Acid	11.408	C8H16O2
17	2,4-Dioxohexahydro-1,3,5-triazine	11.462	C3H5N3O2
18	2,4,5-Trihydroxypyrimidine	11.743	C4H4N2O3
19	2-Furancarboxylic acid, hydrazide	12.01	C5H6N2O2
20	2,4,5-Trihydroxypyrimidine	12.054	C4H4N2O3
21	3-Pentanone	12.254	C5H10O
22	Silicon tetrafluoride	12.36	F4Si
23	2(3H)-Furanone, 5-acetyldihydro-	12.745	C6H8O3
24	Methyl 2-[methoxy(methyl)amino]-2-methylpropanoate	13.169	C7H15NO3
25	1-Methyl-5-fluorouracil	13.307	C5H5FN2O2
26	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	13.813	C6H6O4
27	2(5H)-Furanone	13.955	C4H4O2
28	Catechol	13.979	C6H6O2
29	5-(Hydroxymethyl)dihydrofuran-2(3H)-one	14.132	C5H8O3
30	5-Acetoxymethyl-2-furaldehyde	14.257	C8H8O4
31	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester	14.471	C7H10O4
32	3,4-Furandimethanol	14.51	C6H8O3
33	3-Butene-1,2-diol, 1-(2-furanyl)-	14.525	C8H10O3
34	5-Hydroxymethylfurfural	14.583	C6H6O3
35	2,4,5-Trihydroxypyrimidine	14.781	C4H4N2O3
36	Disulfide, propyl 1-(propylthio)ethyl	14.809	C8H18S3
37	Butanedioic acid	14.976	C4H6O4
38	Cyclotetrasiloxane, octamethyl-	15.342	C8H24O4Si4
39	1-(Methylthio)-3-pentanone	15.408	C6H12OS
40	3-Methyl-5-fluorouracil	15.612	C5H5FN2O2
41	Glutaric acid, 3-methylbut-2-en-1-yl 1-naphthyl ester	15.769	C20H22O4
42	Methyl (2R,3R,4S)-2,4-dimethyl-3-hydroxyhexanoate	15.933	C9H18O3
43	1,3-Dioxepane, 5-methyl-2-pentadecyl-	16.141	C21H42O2
44	2H-Pyrazole-3-carboxylic acid, 2-methyl-	16.67	C5H6N2O2
45	Butanedioic acid, bis(2-methylpropyl) ester	17.151	C12H22O4
46	3-Ethoxy-4-methoxyphenol	17.218	C9H12O3
47	Ethanone, 1-(2,4,6-trihydroxyphenyl)-	17.587	C8H8O4
48	2-Thiophenecarboxylic acid, 5-methyl-, methyl ester	17.88	C7H8O2S
49	Carbonic acid, isobutyl 2-nitro-5-fluorophenyl ester	19.4	C11H12FNO5
50	1-Naphthalenecarboxaldehyde, 4-methoxy-	20.01	C12H10O2
51	1,2-Ethanediamine, N-1-naphthalenyl-	20.035	C12H14N2
52	n-Hexadecanoic acid	22.844	C16H32O2
53	Oleic Acid	24.526	C18H34O2
54	5,7-Dihydroxy-3-[2,3-dihydro-4-hydroxy-2-(2-hydroxyisopropyl) benzofuran-7-yl] chromone	28.945	C20H18O7

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55	5-(3-Chloro-phenylcarbamoyl)-3H-imidazole-4-carboxylic acid, ethyl ester	29.105	C13H12ClN3O3
56	4-Methyl-2,4-bis (p-hydroxyphenyl) pent-1-ene, 2TMS derivative	31.116	C24H36O2Si2
57	4-Methyl-2,4-bis (p-hydroxyphenyl) pent-1-ene, 2 TMS derivative	31.117	C24H36O2Si2
58	Silane, dimethyl(4-(2-phenylprop-2-yl)phenoxy)pentyloxy-	33.518	C22H32O2Si
59	2,4,5-Trimethoxybenzylamine, PFP	35.674	C13H14F5NO4

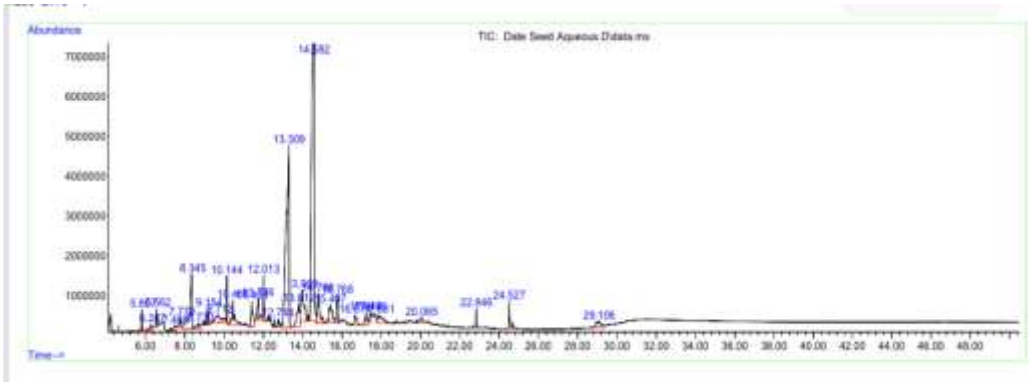

Figure No. (3): GC-MS profile of the aqueous extract of date seed powder.

3.2. Antioxidant activity

Determination of the free radical scavenging activity of DPPH. (1, 1-diphenyl-2-picrylhydrazyl):

DPPH's antioxidant activity depends on scavenging free radicals. Table (3) shows the DPPH activity of aqueous and alcoholic extracts of date pits at different concentrations. The activity increased with increasing extract concentrations. The aqueous and alcoholic extracts showed antioxidant activity, reaching 89.53% for the alcoholic extract at 1.25 mg/ml and 45.76% for the aqueous extract at the same concentration. The antioxidant activity of the alcoholic extracts reached (87.05, 84.15, 80.11, and 74.9) % for concentrations (1, 0.75, 0.5 and 0.25) mg/ml respectively, while the aqueous extracts gave an efficiency of (43.08, 40, 36.77 and 30.15) % for the same concentrations above, where the efficiency was attributed to the phenol content in the extracts and that the ability to capture the root increased with increasing concentrations for the two extracts. These results were consistent with the results mentioned by [33] where it was found that the alcoholic extracts have the highest ability to remove DPPH radicals, and in general, phenolic compounds that are able to lose a hydrogen atom are more effective in removing in removing DPPH radicals [22]. The results showed that there were significant differences at the significance level ($P<0.05$) between the DPPH values of the aqueous and alcoholic extracts for all concentrations, and differences were found between the different concentrations of the extracts between the rows.

Table No. (3): Removal of DPPH radical from alcoholic and aqueous extracts of date seed powder

DPPH%		
Concentrations mg/ml	Alcoholic extract	Aqueous extract
0.25	74.9 Aa	30.15Ab
0.5	80.11Ba	36.77Bb
0.75	84.15Ba	40 Bb
1	87.05 Ba	43.08 Bb
1.25	89.53 Ba	45.76 Ba

A: There are significant differences in the same column at a significance level for (P<0.05) some concentrations of the alcoholic extract

B: There are significant differences in the same column at a significance level for (P<0.05) some concentrations of the aqueous extract.

2. Total Phenolic content

In Table (4) The results showed significant differences at a significance level for (P<0.05) between the aqueous and alcoholic extracts of date pits, as the alcoholic extracts had a higher phenolic content than the aqueous extracts, as the phenolic content of the alcoholic extracts was higher than the aqueous extracts of date pits, which reached 70.53 mg/ml for the alcoholic extract at a concentration of 2.5 mg/ml and 38.7 mg/ml for the aqueous extract at the same concentration. These results were in agreement with [2], who mentioned that water has a weak ability to extract phenols and flavonoids in date seeds due to the low solubility of these components in water.

Table No. (4): Phenolic content of aqueous and alcoholic extracts of date seed extract

Concentrations mg/ml	Alcoholic extract	Aqueous extract
0.5	25Aa	Bb60.11
1	29.86 Aa	62.73 Bb
1.5	32.48 Aa	65.22 Bb
2	Aa36.43	Bb67.64
2.5	Aa38.7	Bb70.53

A: No notable differenceswere found at the level of (P<0.05(For all concentrations in one column of the alcoholic extract

B: No notable differences at the level of (P<0.05(For all concentrations in one column of the aqueous extract

The results indicated that date seed powder possesses strong antioxidant activity due to its high content of polyphenolic compounds. These results are consistent with a study conducted by [1].

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Conclusion

The importance of the active compounds in date seed powder was revealed using GC-MS technology. The study confirmed that date seed powder is rich in bioactive compounds and is a natural source of them. It also contains antioxidants and phenolic compounds, which effectively remove DPPH free radicals. These qualities give date seed powder numerous properties that enable it to be used in many medical, health, and nutritional fields.

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