Study of Biological Effectiveness of the Aqueous Extract of Date Pits(Phoenixdactylifera.L) Grows in the Algerian Oases

Abdelhakim Medjdoub[1*], Mohammed Said Nedjimi[1], Hayat Zerrouki[1],Teqwa Ragdi[1], Hicham Meskher[2], Salah Tlili[3] , Mohamed Lakhdar Belfar[1]

[1] Faculty of Mathematics & Matter Sciences , (VPRS) Laboratory KasdiMerbahOuargla University, 30000, Ouargla, Algeria.
[2]Division of Process Engineering, College of Science and Technology, ChadliBendjedid University, 36000, Algeria

[3]Kasdi MerbahOuargla University, LARENZA Laboratory, 30000, Ouargla, Algeria

*Corresponding author: medjdoub.hakim@yahoo.fr

Received: Aug 02, 3023; **Revised:** Sept 25 ,2023; **Accepted:** Sept 30,2023; **Published:** Oct 08 ,2023

Research Highlights

- A novel approach to prepare date palm pit has been proposed.
- Various biological activities of date palm pit have been investigated.
- The prepared extract also showed antimicrobial activity against five different bacterial strains and the yeast strain tested.
- A strong correlation existed between antioxidant activity and phenolic compound has been determined.

Abstract

The date palm pit (Phoenix dactylifera L.) is a byproduct of the date business that contains a number of bioactive compounds with potential health benefits. This study investigated the preparation of aqueous extract of date palm pit (DPP) and its biological activities, including antioxidant, anti-amylase, and antimicrobial activities.The total phenolic content of the DPP aqueous extract was 128.02 ± 0.35 µg GAE/mg gallic acid equivalent (GAE)/g extract. The total flavonoid content was 82.12 ± 0.89 µg QE/ mg /g extract.The DPP aqueous extract showed significant antioxidant activity in the DPPH free radical scavenging assay (IC50 = 492 ± 0.4 µg/mL), and the FRAP assay (RC50 = 408 ± 1.87 µg/mL).The DPP aqueous extract showed moderate anti-amylase activity (IC50 = 4.089 ± 0.78 µg/mL). The DPP aqueous extract also showed antimicrobial activity against all five bacterial strains and the yeast strain tested. The highest inhibitory zone diameter was observed against Klebsiellapneumoniae (20 mm).

Keywords: Date palm pit, aqueous extract, antioxidant activity, anti-diabetic test, antimicrobial activity.

Tob Regul Sci. ™ 2023 ;9(2):115 - 130
DOI: doi.org/10.18001/TRS.9.2.8

Introduction

The date palm (Phoenix dactylifera L.) is a dioecious tree from the Middle East and North Africa. It is one of

the world's oldest cultivated crops, and its fruit is a staple diet for many people in these areas.Date palm pits are a byproduct of the date business and were either utilized as animal feed or dumped as rubbish. Recent study, however, has revealed that date palm pits contain a number of bioactive chemicals with potential health advantages.

Date palm pits are a good source of dietary fiber, protein, and fatty acids. They also contain a variety of vitamins and minerals, including calcium, magnesium, iron, and potassium[1,2]. In addition, date palm pits contain a variety of bioactive compounds, including flavonoids, phenolic acids, tannins and sterols are abundant in date palm pits[3].

These compounds have been shown to have a variety of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. In addition, the presence of these secondary metabolites in date palm pits makes them a valuable source of bioactive compounds with potential health benefits. More research is needed to fully characterize the biological activities of date palm pits and to develop effective ways to incorporate them into food and pharmaceutical products.

The potential for date palm pits to be employed in the treatment of different illnesses and disorders has been looked at in a number of research. For instance, studies have demonstrated the effectiveness of date palm pit extract against a range of bacteria and fungi, such as Staphylococcus aureus, Escherichia coli, and Fusariumoxysporum[4]. Additionally demonstrated to have anti-inflammatory and anticancer properties is date palm pit extract [5,6].

Date palm pits can be utilized as a functional food component in addition to their potential application in the treatment of illnesses and ailments. Date palm pit flour, for example, may be used to create bread, spaghetti, and other baked products. Date palm pit oil may be used as a cooking oil as well as a salad dressing[7].

The objective of this work is to investigate the potential of date palm pit (DPP) extract as a natural source of bioactive compounds with various biological activities.The total polyphenols and flavonoids content of the extract is then determined using the Folin-Ciocalteu and

aluminum trichloride methods, respectively. LC-MS analysis is used to identify the specific chemical compounds present in the extract.

The antioxidant, anti-amylase, and antimicrobial activities of the DPP extract are then evaluated using a variety of methods. For example, the DPPH assay is used to measure the antioxidant activity of the extract, while the carrageenan-induced paw edema model is used to measure its anti-inflammatory activity. The anti-amylase activity of the extract is measured using a starch hydrolysis assay, and its antimicrobial activity is measured using an agar diffusion assay, the steps of the experiment are showing in thegraphical abstract. The Steps of Preparing the Aqueous Extracts Metabolites and Biological activities of DPP (Figure1).

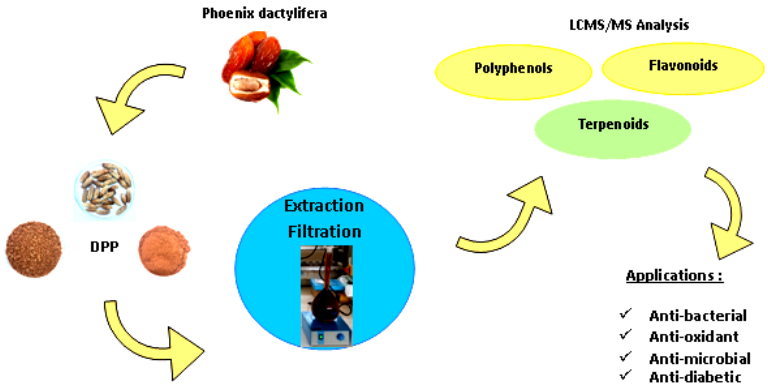


Figure 1. The Steps of Preparing the Aqueous Extracts Metabolites and Biological activities of DPP

Ripe date palm (Phœnix dactylifera L.) fruits of the Deghla Baidha variety were harvested in the Touggourt oases, Algeria, during 2022.The pits samples were removed and washed with tap water, then rinsed with deionized water to remove dust and impurities.The dry pits were crushed and ground into fine powder using a grinder (Figure.1).

2.2. Preparation the aqueous extract of date palm pit

The aqueous extract of date palm pit (DPP) was prepared by adding 25 g of DPP powder to 100 ml of distilled water. The mixture was stirred for 30 minutes at 150 rpm, then left to cool at room temperature overnight.Then filtered through Whatman No.1 paper to obtain the aqueous PLR extract. The filtered aqueous extract was collected and stored in a refrigerator at 4°C for future use(Figure.1).

2.3. Determination of total Polyphenols and Flavonoids Content

Following Singleton's technique[8], the total phenolic contents were measured using the Folin-Ciocalteu reagent and gallic acid as a reference. First, 20 µL of DPP aqueous extract or various

quantities of gallic acid (standard) were combined with 900 µL of Folin-Ciocalteu reagent (1:10). Next, 600 µL of sodium carbonate (7.5%) was added to the mixture. The sample was kept in the dark for two hours at room temperature. Later, using a spectrophotometer, the absorbance was measured at 765 nm, and the findings were represented as mg of gallic acid equivalent (GAE) per 100 g of material.

According to Kim et al. [9], a technique based on the creation of a complex flavonoid-aluminum, with the greatest absorbance at 430 nm, was used to estimate the total flavonoid levels in the DPP extract.

One milliliter of the diluted sample was combined with one milliliter of a 2% solution of aluminum trichloride (AlCl3) in methanol. Following a 15-minute incubation period at room temperature, the reaction mixture's absorbance was measured at 430 nm with a spectrophotometer, and the amount of total flavonoids was expressed as mg of quercetin equivalent (QE) per g of extract (mg QE/ g). Quercetin was used to create a calibration curve, and the findings were represented as mg quercetin equivalents (CEQ)/100 g sample.

2.4.Liquid Chromatography-Mass Spectrometry Analysis

Shimadzu 8040 Ultra-High sensitivity with UFMS technology was used, along with a binary bump Nexera XR LC-20AD. We employed direct injection without a column to optimize polyphone standards.

CID gas, 230 KPs; conversion dynode, 6.00 Kv; interface temperature, 350 C; DL temperature, 250 C; nebulizinggas flow, 3.00 L/min; heat block, 400 C; drying gas flow, 15.00 L/min were the ESI conditions.

All standards were produced in methanol at a concentration of 500 g/L. In both negative and positive ions, the ion trap mass spectrometer was employed in MRM mode (multiple reaction monitoring). Water, 0.1% formic acid, and 70% methanol made up the mobile phase. The injection volume was 6 L and the flow ratewas 0.3 mL/min.

The samples were separated using an Ultra-force C18 column (I.D. 2.5 mm 100 mm, particle size 1.8 m; Restek), and the oven temperature was set at 25 degrees Celsius. 0.1% formic acid and methanol (30:70, v/v) were used for isocratic elution. The injection volume was 10 mL, and the flow rate was 0.30 mL/min[10].

2.5.Biological activities

2.5.1.Antioxidant activities

2.5.1.1.DPPH test

The efficacy of aqueous DPP extracts in suppressing the DPPH free radical using the 2,2-diphenyl-1-picrylhydrazyl free radical was examined using Gali and Bedjou [11] technique with some modifications.

Five milliliters of a methanol solution of 1.1-diphenyl-2-picrylhydrazyl (DPPH) (0.024 mg/mL) were added to fifty microliters of varying concentrations of DPP extract in distilled water. After 30 minutes of room temperature incubation, the absorbance was measured at 517 nm in comparison to a blank. The synthetic antioxidants BHA and ascorbic acid were used as controls. The following formula was used to calculate the percentage (%) of DPPH free radical scavenging activity.

Percentage of Inhibition % = 100 × [(blank absorbance – extract absorbance) / blank absorbance]. All experiments were done in triplicate, and the findings were represented as IC50 (g/mL).

2.5.1.2.Antioxidant assay using the βcarotene bleaching.

The technique described by Assagaf et al. [12]was used to test the ability of DPP aqueous extract to inhibit βcarotene via the model system (βcarotene/linoleic acid).

In 25 µl of linoleic acid and 200 µl of Tween-80 in 1 ml of chloroform, 0.5 mg of βcarotene was dissolved. In a rotary evaporator set to 40 °C, the mixed solution was entirely evaporated under vacuum.

100 ml of distilled water should be added after vigorously shaking. After adding 0.2 ml of the test substance (1 mg/ml) to 5 ml of the aforementioned solution, absorbance was measured at 470 nm right away. The absorbance of the solutions was once more measured at the same wavelength after 120 minutes of incubation at 50 °C. The identical process was carried out once more using BHA and ascorbic acid as standards and a blank. The following equation was used to compute the antioxidant activity in the b -carotene bleaching model reported as a percentage:

Antioxidant activity (%) = (Absorbance of sample at T 0– Absorbance of sample at T120 min)/ Absorbance of control at T 0– Absorbance of control at T 120 min) *100

2.5.1.3.FRAP test.

According to Said et all's approach[13], the produced nanocomposite films' capacity to decrease iron (III) was assessed with minor modifications.

1 mL of aqueous DPP extract at various concentrations was combined with the same volume of phosphate buffer and 1% potassium ferrocyanide (K3Fe(CN)6). The resulting mixture was incubated for 20 minutes at 50 °C. After the mixture had been incubated, 1 mL of 10% trichloroacetic acid (TCA) was added. The mixture was then centrifuged for 10 minutes at 3000

rpm. A total of 1.25 ml of distilled water, 0.25 ml of ferric chloride (0.1%: w/v), and 1.25 ml of the supernatant solution from each combination were added in the end. After 10 minutes, the solution's absorbance at 700 nm was measured. The reductive capacity (RC0.5) is defined as the substrate concentration that yields an absorbance of 0.5 at 700 nm. To obtain it, a linear regression curve is employed

2.5.2.Antidiabetic assay Determination of αamylase inhibition.

The α-amylase inhibition activity was conducted using the technique of Armando et al.[14] with minor modification.

First, 25 µL of the aqueous extract was combined with 50µL of buffer solution containing 1 U/mL of -amylase and 0.02 mol/L sodium phosphate buffer (pH 6.9). This mixture was then pre-incubated at 37 °C for 10 minutes. Aqueous starch solution (0.1%) and 50µL of the mixture were then added, and the mixture was incubated at 37 °C for 60 min.A 50 µL solution of the dinitrosalicylic acid reagent was used to halt the reaction. Samples were quickly chilled in an ice bath after five minutes of incubation in a 90°C water bath. Acarabose was used as a reference sample and underwent the same testing procedures, with the result presented as a percentage of inhibition.

2.5.3.Antimicrobial activity.

The inhibitory effect of the date palm pit was tested on five human pathogenic bacterial strains: two are a Gram-negative strain (Escherichia coli ATCC 8737 andKlebsiella pneumoniae CIP8291), and three strains are Gram-positive (Bacillus subtilisATCC 6633, Staphylococcus aureus ATCCN 6538, Listeria innocuaCLIP 7491); and one yeast Candida albicans ATCC10237. Microbial strains were kept at 80 °C in brain–heart agar broths containing glycerol until they were used. The strains were grown afterward in agar broths for 24 h at 37 °C in an incubator before being tested for antibacterial activity.In brain-heart agar broths with glycerol, bacterial strains were stored at 80 °C until they were needed. After that, the strains were cultured in agar broths for 24 hours at 37 degrees Celsius in an incubator before being evaluated for their ability to combat germs.

The agar diffusion method was used to examine the efficacy of aqueous extract of date palm pit against the strains tested[15] . This test was carried out using the disk diffusion method, which involves dipping disks into each sample. To obtain bacterial suspensions with an optical density of 0.5 Mc Ferland (EQ105UFC/mL), a pure, young culture was obtained after incubation at 37°C (18 to 24 hours). Inoculations of 1 mL of each bacterial strain were made into Petri plates, which were then 4 mm thick with Muller-Hinton agar medium and allowed to dry for 3 to 5 minutes at room temperature. Next, disks (6 mm in diameter) impregnated with different concentrations of DPP were placed on the surface of the culture medium to obtain complete

contact with the agar. sterile distilled water was used as a negative control. After that, Petri plates were incubated for 24 hours at 37°C. Using a caliper, the inhibitory zone's diameter was measured in millimeters. The experiment was carried out three times for every bacterial strain[10].

3.Results and discussions

3.1.Polyphenols and flavonoids contents

Polyphenols are secondary metabolites found in practically all aromatic and therapeutic plant species. Because of their antioxidant capabilities, these metabolites are vital for human nutrition and helpful to health[16].

A quantitative analysis reveals that aqueous extract of DPP has a high phenolic and flavonoid content;with 128.02 ± 0.35 µg GAE/mg extract and 82.12 ± 0.89 µg QE/ mg extract, respectively.These values are higher to those reported for date pit extracts from different cultivars grown in the Qassim area which ranged from 29.5 to 30.2 µg GAE/mg for phenolic contents and from 23.5 to 29.2 µg QE/ mg [17].

The values obtained for TPC and TFC are significantly higher than those obtained for other date palm pits varieties; Saudi Ajwa pits[18] , different Emirati date [19] and four different varieties of Oman date [20].

Date pits contain high levels of total polyphenols compared to other organs such as fruits [21]and pulp [18]. It is generally recognized that phenolic molecules, particularly flavonoids, have a variety of biological functions, including antioxidant and antibacterial effects [22].

Due to their ability to delay the oxidative breakdown of lipids and consequently enhance food quality and nutritional content, phenolics are becoming more and more popular in the food business [23].Genotype and environmental factors have an impact on the polyphenol content of plant organs.Additionally, factors like as soil nutrient availability, temperature, light, altitude, and light intensity may affect phenylpropanoid metabolism [24].

3.2.Antioxidant activities

DPPH, FRAP, and B.carotene tests were used to assess the antioxidant capabilities of our extract, and the findings are shown in Table 1.

The aqueous extract of DPP had the highest capability for DPPH radical scavenging, with an IC50 of 320± 0.65µg/mL, whereas ascorbic acid had an IC50 of 29± 0.34µg/mL. Nevertheless, the range of our results was lesser than those found byAbdul-Hamid et al. [18].

The spectrophotometric b-carotene bleaching test was also used to assess the antioxidant properties of DPP. The findings of the current investigation demonstrated that the DPP extract

had greater levels of antioxidant activity and that this antioxidant activity increased with extract quantity.The IC50 of the aqueous extract (492 ± 0.4 µg/mL) was lower than the standard BHA (32.911 ± 0.8 µg/mL) and ascorbic acid (82.92 ± 2.98 µg/mL).

As the content of DPP extract grew, the reducing power also did so. Surprisingly, the DPP extract shown far more reducing power than the typical (BHT).

It has been claimed that a number of components are responsible for the antioxidant action of DPP. Other ingredients, such as phenolic compounds, may have contributed to this action in some measure in addition to fatty acids. Antioxidant chemicals found in the date palm, such ascorbic acid, vitamin E, carotenoids, and selenium, are responsible for the plant's antioxidant action , additionally some flavonoids and tannin's constituents [21], [25].

Table 1.Antioxidant activities of DPP extract.

	DPP extract	BHA	Ascorbic Acid
DPPH (IC50µg/mL)	320 ± 0.65	/	29 ± 0.34
βcarotene bleaching (IC50µg/mL)	492 ± 0.4	32.911 ± 0.8	82.92 ± 2.98
Reducing power assay (A0.5 µg/mL)	408 ± 1.87	/	18.05 ± 0.47

3.3. Antidiabetic assay

To assess the anti-diabetic efficacy of DPP extracts, the capacity of the various extracts to inhibit theαamylase enzyme was estimated.According to the results, the reaction of DPP extract provided the best response, with a significantly lower IC50 value than acarbose(IC50 of DPP = 4.089±0.78µg/mL, IC50 of acarbose = 18.09 ±1.78 µg/mL). This result fits well with the work of Zahnit et al. [26].

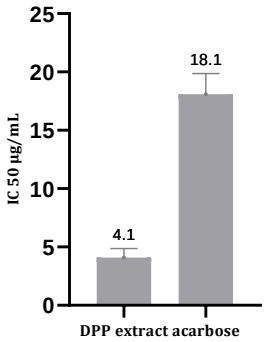


Figure 4.The anti-diabetic activity of DPP extract

3.4. Antimicrobial activity

Due to pathogenic bacteria's rising antibacterial resistance and a lack of therapeutic options, new treatment strategies are necessary[27]. According to the findings of this investigation, DPP extract has antimicrobial effects on both Gram-negative and Gram-positive bacteria and yeast tested, however meuilleur inhibition was seen in Gram-negative bacteria and yeast (Figure.5).

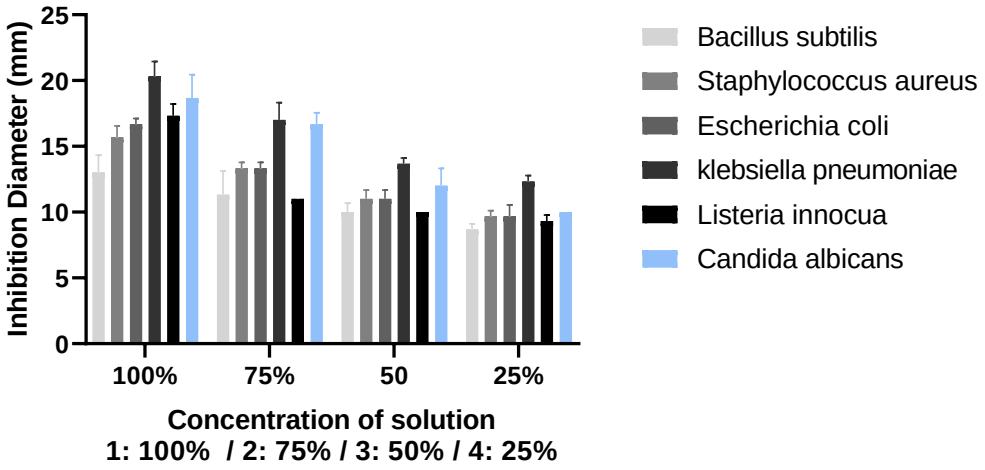
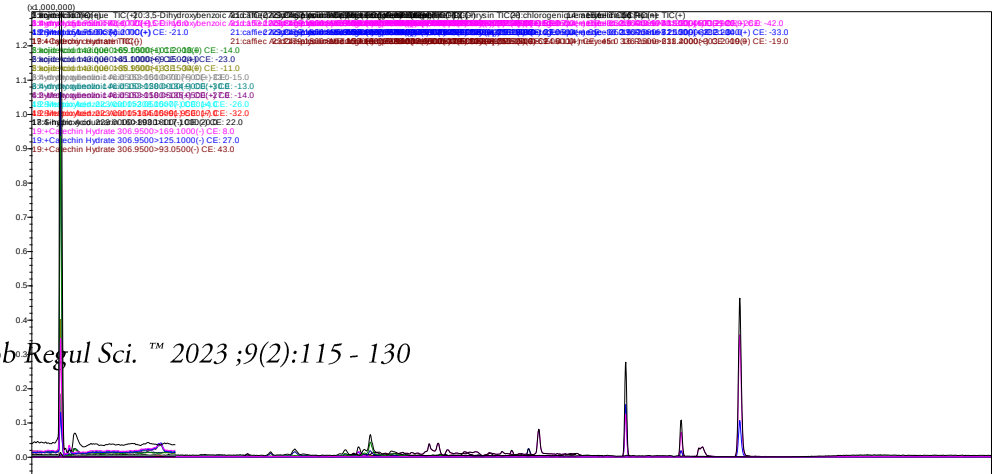


Figure5. Antibacterial activity of DPP samples against the microbe strains

3.5. LCMS/MS analysis

The LCMS/MS in liquid chromatography tandem can be used to rapidly determine the molecular weight of a of DPP extractWe employed direct injection without a column to optimize polyphone standards of DPP extract. The upper part of the display in Figure 6 a show the mass spectrum of the chromate graphic it's the Quantitative Analysis of DPP extract by LC-MS/MS with a Fast from 0 min to 240-minute Cycle. There is a regular pattern to the spectral peaks, each one of which represents the molecule with a different number of charges. The lower part of the display in Figure 6 bgraph it's aanother enlarged image spectrummore clarifying of the Quantitative Analysis of DPP extract by LC-MS/MS with a Fast from it is anotherbetween 6.5 min to 18.7-minutecycle,thefollowing (table2) explains and shown the Polyphenols and flavonoids content of DPP extractfrom the LC-MS/MSspectroscopy from Figure 6 (a)and(b).



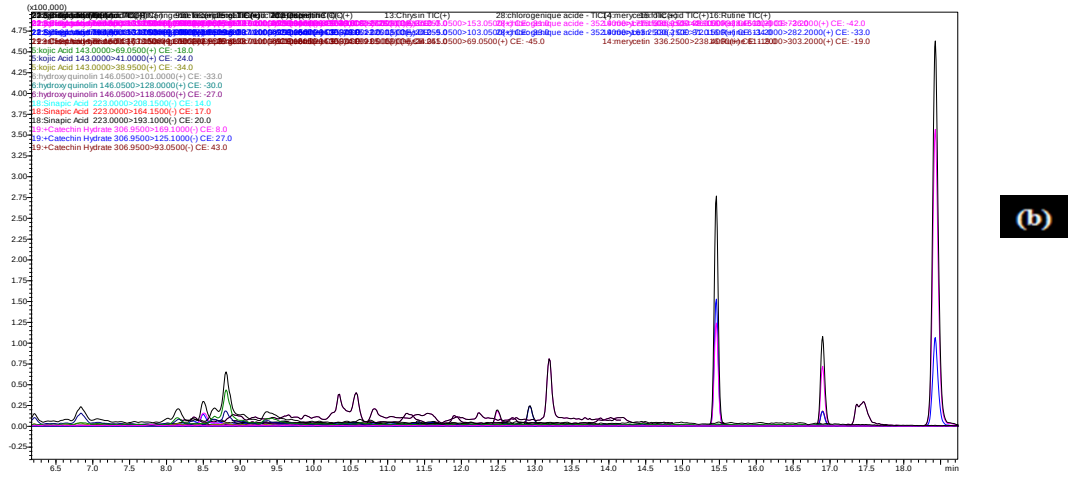


Figure 6:.(a and b) &bMolecular weight determination of DPP extract by Quantitative Analysis of DPP extract by LC-MS/MS with a Fast from 0 min to 240-minute Cycle.

ID #	Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Ret. Time	Height	Area	Area %
1	hydroxyquinolin	C9H7NO	145.16	+	146.0500 >101.0000	7.803	0	0	0
2	Thymol	C10H14O	150.22	+	151.7500 >88.1000	0.779	8782	32536	0.3807
3	2-Methoxybenzoic Acid	C8H8O3	152.15	+	153.0500 >135.0500	0.781	123021	497235	5.8182
4	4-mythoxybenzoic Acid	C8H8O3	152.15	+	153.0500 >70.7500	0.779	181817	657690	7.6958
5	acidecoumarique	C9H8O3	164.16	(+)	165.1000 >101.2000	0.769	930678	3354516	39.2518
6	kojic Acid	C6H6O4	142.11	(+)	143.0000 >69.0500	1.55	2075	25718	0.3009
7	Ferulic Acid	C10H10O4	194.18	(+)	194.9000 >177.1500	9.863	12791	158166	1.8507
8	Naringenin	C15H12O5	272.25	(+)	272.9500 >177.0000	0	5390	32082	0.3754
9	betacarotene	C40H56	536.87	(+)	537.2000 >23.1000	11.578	693	4908	0.0574
10	keampferol	C15H10O6	286.24	(+)	287.1000 >255.2500	12.258	75290	442037	5.1724
1	Quercitine	C15H10	302.23	(+)	303.1000 >262.200	12.5	178	105	1.2

1		O7			0	03	12	524	348
12	Valinin	C8H8O3	152.15	(+)	153.1000 >65.1500	8.508	0	0	0
13	Chrysin	C15H10O4	254.24	(+)	255.0500 >153.0500	12.764	0	0	0
14	merycetin	C15H10O8	318.23	(+)	336.2500 >46.1500	16.894	71815	290124	3.3948
15	folicacid	C19H19N7O6	441.14	(+)	442.9000 >323.4500	17.394	29315	336196	3.9339
16	Rutine	C27H30O16	610.5	(+)	611.2000 >73.2000	18.418	356516	1936882	22.6638
17	Catechin Hydrate	C15H16O7	308.28	(-)	306.9500 >169.1000	0	0	0	0
18	Sinapic Acid	C11H12O5	224.21	(-)	223.0000 >208.1500	0	100	729	0.0085
19	4-hydroxy coumarin	C27H30O16	162.14	(-)	160.8000 >117.1000	0.759	1943	9776	0.1144
20	3,5-Dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.1000 >109.1000	4.633	9496	71583	0.8376
21	caffiec Acid	C9H8O4	180.16	(-)	179.1500 >135.0500	8.108	0	0	0
22	Cis-p.coumaric	C9H8O3	164.16	(-)	163.1500 >119.150	9.227	1163	7079	0.0828

	Acid				0				
23	Syringic Acid	C9H10O5	198.17	(-)	196.9500 >182.0000	9.006	870	3573	0.0418
24	salysilic Acid	C7H6O3	138.12	(-)	137.1000 >93.1500	9.527	3165	89119	1.0428
25	gallic Acid	C4H4O4	170.12	(-)	169.1000 >125.0500	11.777	900	7362	0.0861
26	Luteonil	C15H10O6	286.24	(-)	284.9500 >133.0000	12.395	190	922	0.0108
27	Hespertin	C16H14O6	302.28	(-)	300.9000 >255.2500	11.677	0	0	0
28	chlorogenique acide -	C16H18O9	354.31	(-)	352.9000 >177.1500	15.456	123876	482381	5.6444

Table 2. Polyphenols and flavonoids content of DPP extract

4. Conclusion

The aqueous extract of date palm pit (DPP) was found to be a rich source of bioactive compounds, including total polyphenols and flavonoids. The LC-MS analysis of the extract revealed the presence of a variety of polyphenols. The DPP extract also exhibited a number of biological activities, including antioxidant , anti-amylase, and antimicrobial activities. In the DPPH assay, the IC50 value of the extract was found to be 320µg/ml, indicating a moderate antioxidant activity. The extract also showed a higher anti-amylase and antimicrobial activities. Overall, the findings of this study suggest that DPP extract has the potential to be used as a natural source of bioactive compounds with various biological activities. However, more research is needed to investigate the long-term safety and efficacy of DPP extract for human consumption.

References

[1] M. A. Al-Farsi et C. Y. Lee, « Nutritional and functional properties of dates: a review », Crit. Rev. Food Sci. Nutr., vol. 48, no 10, p. 877‑887, 2008.

[2] M. N. K. Khattak et al., « Anticancer activities of selected Emirati Date (Phoenix dactylifera L.) varieties pits in human triple negative breast cancer MDA-MB-231 cells », Saudi J. Biol. Sci., vol. 27, no 12, p. 3390‑3396, 2020.

[3] D. Varijakzhan, C.-M. Chong, A. Abushelaibi, K.-S. Lai, et S.-H. E. Lim, « Middle Eastern plant extracts: An alternative to modern medicine problems », Molecules, vol. 25, no 5, p. 1126, 2020.

[4] N. Bentrad, R. Gaceb-Terrak, Y. Benmalek, et F. Rahmania, « Studies on chemical composition and antimicrobial activities of bioactive molecules from date palm (Phoenix dactylifera L.) pollens and seeds », Afr. J. Tradit. Complement. Altern. Med., vol. 14, no 3, p. 242‑256, 2017.

[5] J. A. John et F. Shahidi, « Phenolic content, antioxidant and anti-inflammatory activities of seeds and leaves of date palm (Phoenix dactylifera L.) », J. Food Bioact., vol. 5, p. 120‑130, 2019.

[6] M. I. Hussain, M. Farooq, et Q. A. Syed, « Nutritional and biological characteristics of the date palm fruit (Phoenix dactylifera L.)–A review », Food Biosci., vol. 34, p. 100509, 2020.

[7] A. T. Idowu, O. O. Igiehon, A. E. Adekoya, et S. Idowu, « Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications », AIMS Agric. Food, vol. 5, no 4, p. 734‑755, 2020.

[8] V. L. Singleton et J. A. Rossi, « Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents », Am. J. Enol. Vitic., vol. 16, no 3, p. 144‑158, 1965.

[9] D.-O. Kim, S. W. Jeong, et C. Y. Lee, « Antioxidant capacity of phenolic phytochemicals from various cultivars of plums », Food Chem., vol. 81, no 3, p. 321‑326, 2003.

[10] S. Ben Amor et al., « Phytochemical Characterization and Bioactivity of Different Honey Samples Collected in the Pre-Saharan Region in Algeria », Life, vol. 12, no 7, p. 927, juin 2022, doi: 10.3390/life12070927.

[11] L. Gali et F. Bedjou, « Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated Ruta chalepensis », South Afr. J. Bot., vol. 120, p. 163‑169, 2019.

[12] H. M. Assaggaf et al., « Singular and Combined Effects of Essential Oil and Honey of Eucalyptus Globulus on Anti-Inflammatory, Antioxidant, Dermatoprotective, and

Antimicrobial Properties: In Vitro and In Vivo Findings », Molecules, vol. 27, no 16, p. 5121, 2022.

[13] Z. B.-O. S. Said et al., « Essential oils composition, antibacterial and antioxidant activities of hydrodistillated extract of Eucalyptus globulus fruits », Ind. Crops Prod., vol. 89, p. 167-175, 2016.

[14] A. Peláez-Acero et al., « The application of ultrasound in honey: Antioxidant activity, inhibitory effect on αamylase and αglucosidase, and in vitro digestibility assessment », Molecules, vol. 27, no 18, p. 5825, 2022.

[15] N. M. Atef, S. M. Shanab, S. I. Negm, et Y. A. Abbas, « Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection », Bull. Natl. Res. Cent., vol. 43, no 1, p. 1-11, 2019.

[16] F. Remok et al., « Phenolic Content, Antioxidant, Antibacterial, Antihyperglycemic, and αAmylase Inhibitory Activities of Aqueous Extract of Salvia Lavandulifolia Vahl », Pharmaceuticals, vol. 16, no 3, p. 395, 2023.

[17] E. Amin et al., « Pattern Recognition of Phytoconstituents and Bioactivities of Date Pit Extracts from Different Cultivars Grown in the Qassim Area », Separations, vol. 10, no 2, p. 102, 2023.

[18] N. A. Abdul-Hamid et al., « Metabolites and biological activities of Phoenix dactylifera L. pulp and seeds: A comparative MS and NMR based metabolomics approach », Phytochem. Lett., vol. 31, p. 20-32, 2019.

[19] M. I. Hussain et al., « Phenolic composition and antimicrobial activity of different Emirati Date (Phoenix dactylifera L.) pits: A comparative study », Plants, vol. 8, no 11, p. 497, 2019.

[20] S. S. Al Harthi, A. Mavazhe, H. Al Mahroqi, et S. A. Khan, « Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (Phoenix dactylifera L.) varieties of Oman », J. Taibah Univ. Med. Sci., vol. 10, no 3, p. 346-352, 2015.

[21] F. Masmoudi-Allouche, S. Touati, K. Mnafgui, N. Gharsallah, A. El Feki, et N. Allouche, « Phytochemical profile, antioxidant, antibacterial, antidiabetic and anti-obesity activities of fruits and pits from date palm (Phoenix dactylifera L.) grown in south of Tunisia », J. Pharmacogn. Phytochem., vol. 5, no 3, p. 15-22, 2016.

[22] I. Khlif, K. Jellali, T. Michel, M. Halabalaki, A. L. Skaltsounis, et N. Allouche, « Characteristics, phytochemical analysis and biological activities of extracts from Tunisian Chetoui Olea europaea Variety », J. Chem., vol. 2015, 2015.

[23] S. Shukla, A. Mehta, P. Mehta, et V. K. Bajpai, « Antioxidant ability and total phenolic content of aqueous leaf extract of Stevia rebaudiana Bert », Exp. Toxicol. Pathol., vol. 64, no 7-8, p. 807-811, 2012.

[24] A. Alirezalu et al., « Physicochemical characterization, antioxidant activity, and phenolic compounds of hawthorn (Crataegus spp.) fruits species for potential use in food applications », Foods, vol. 9, no 4, p. 436, 2020.

[25] L. R. Ferguson, M. Philpott, et N. Karunasinghe, « Dietary cancer and prevention using antimutagens », Toxicology, vol. 198, no 1-3, p. 147-159, 2004.

[26] W. Zahnit et al., « Phytochemical profiling, mineral elements, and biological activities of Artemisia campestris L. grown in Algeria », Horticulturae, vol. 8, no 10, p. 914, 2022.

[27] M. Sharaf et al., « Design of lipid-based nanocarrier for drug delivery has a double therapy for six common pathogens eradication », Colloids Surf. Physicochem. Eng. Asp., vol. 625, p. 126662, sept. 2021, doi: 10.1016/j.colsurfa.2021.126662.