Evaluation and comparative study of pharmacological profile of selected date species

Farzana Batool[a], Freeha Hafeez[a*], Bilawal Manzoor[a], Muhammad Suleman[a], Hajrah Ali[a], Maria Khushnood[a], Tayyaba[a], Sarmad Fayyaz[a], Asma Zafar[a], Muzammil Hussain[a]

[a]Department of Chemistry, Riphah International University Faisalabad, Faisalabad, Pakistan

Abstract:

Background: The main aim of this study is to carry out a comprehensive analysis of both qualitative and quantitative phytochemical compounds while concurrently evaluating the antioxidant properties of different alcoholic and aqueous extracts obtained from fruits of *Phoenix dactylifera*.

Material and Methods: Execute a thorough evaluation of the antioxidant potential of plant extracts through various methodologies such as analysis of total phenolic and flavonoid content, DPPH analysis, linoleic acid system, and peroxidation inhibition. Highlight the significance of preliminary phytochemical analysis and in vitro antioxidant assays.

Results: The findings demonstrate that fruits possess a remarkable reservoir of antioxidant potential, indicating their potential as a source for phenolic compounds and antioxidants.

Conclusions: Based on the conclusions drawn from this study, it can be deduced that the species exhibits effectiveness in neutralizing free radicals and possesses the capability to serve as a potent antioxidant.

Keywords: *Phoenix dactylifera*, Antioxidant, DPPH, CCEs.

Background:

The date palm (*Phoenix dactylifera*) is a significant perennial fruit plant in warm and arid regions of the Old World and is one of the oldest cultivated fruit crops. It belongs to the Arecaceae family and is part of a group of inter-fertile species(Fuller & Stevens, 2019; Gros-Balthazard et al., 2021). This species has great economic, symbolic, and cultural importance in its traditional range of cultivation, including Morocco, the Arabian Peninsula, and northwestern India. The sweet fruit of the date palm has served as a staple food and a source of economic prosperity since ancient times. It also provides various health benefits and

contains bioactive compounds such as phenolic acids, carotenoids, and flavonoids(Vayalil, 2013). The date palm has different cultivars with varying properties, and the bioactive components in these cultivars change during the different stages of fruit ripening (Ismail, Haffar, Baalbaki, Mechref, & Henry, 2006). The nutritional content of dates can also vary depending on factors such as cultivation practices and climate conditions. Dates are renowned not only for their rich sweetness but also for their potential health benefits, prominently attributed to their antioxidant properties. Among the diverse varieties, Ajwa and Khalas dates stand out as particularly noteworthy. This introduction explores the antioxidant activity of dates, delving into a comparative analysis between Ajwa and Khalas varieties, shedding light on their distinctive qualities and potential impact on human health.

Methods:

Collection of Sample:

The fully ripened fruits of date palm plants, which are widely spread in the local market of the Jhang district in South Punjab, Pakistan, were harvested (Abul-Soad, Mahdi, & Markhand, 2015). The plant parts were obtained from a date palm farm in Jhang. These collected samples were fragmented into small pieces and subsequently subjected to air drying. Subsequently, they were stored in plastic bags at a temperature of -4℃.

Extract Preparation:

A representative sample consisting of fruits, stems, and leaves was subjected to drying at a standardized temperature, followed by pulverization into a finely powdered form at a manufacturing facility. The pulverized materials were then subjected to extraction using an orbital shaker (UK), wherein an aqueous methanol solution was employed. The orbital shaker had a capacity of 200 mL, with a methanol percentage of 80% and a water to methanol ratio of 20:80. Additionally, ethanol was present in the solution at a concentration of 80%, with a water to ethanol ratio of approximately 20:80. Distilled water was also included in the extraction process, and the entire mixture was decocted for a period of 7.5 to 8.5 hours at room temperature. To separate the various extracts from solid residues during the filtration procedure, Whatmann 1 filter paper was utilized. The aforementioned solutions and extracts were employed twice to eliminate any remaining solid matter. The resulting product materials were collected and concentrated using a

rotating type evaporator at a temperature of 45 ℃ and under reduced pressure.

Chemicals:

From Merck Co. (Darmstadt, Germany), all analytical-grade solvents, including ethyl acetate (99.8%), chloroform (99.8%), n-hexane (99.8%), and n-butanol (99.8%), as well as sodium dihydrogen phosphate (NaH2PO4), trifluoroacetic acid, and potassium ferricyanide [K3Fe (CN)6], were purchased. Reduced glutathione (GSH), phenazine methosulphate (PMS), 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Aluminum chloride, 2,20-diphenyl-1-picrylhydrazyl (DPPH); nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), 1,2-dithio-bis nitro benzoic acid (DTNB), thiobarbituric acid (TBA), ferric potassium persulphate, and trichloroacetic acid (TCA) from Sigma Co. (St. Louis, MO, USA). Ultrapure TM water purification system from Lotun Co., Ltd. in Taipei, Taiwan, was utilized to produce distilled deionized water (dd. H2O).

Assessing the Antioxidant Potential of Plant Extracts:

The evaluation of the antioxidant capacity of plant extracts was conducted through a series of experiments. Specifically, the fruit, stem, and leaves of *Phoenix dactylifera* were assessed for their antioxidant capacity.

Measuring Total Phenolics Content (TP):

The determination of total phenolics (TP) was carried out using the Folin-Ciocalteu reagent method to obtain the concentrated value of TP. The ingredients used in this determination included crude extract, deionized water, and the Folin-Ciocalteu reagent. The crude extract was added in a concentration of approximately 1 mL, while deionized water was added in a range of 7.5 mL. The reagent, Folin-Ciocalteu, was added in a range of 0.5 mL. These ingredients were thoroughly mixed to form a solution. After 10-minute incubation at room temperature, an additional ingredient, sodium carbonate, was added in a concentration of 1.5 mL. The solution was then subjected to heating using a water bath at a temperature of 40°C for a duration of 20 minutes. Following the heating process, the product was cooled using an ice bath. The absorbance of the solution was measured at 755 nm using a spectrometer. To calculate the TP, a calibration curve based on gallic acid ranging from 5 to 100 ppm was utilized. The results were reported in terms of dried matter (AlFaris et al., 2021).

Assessment of Total Flavonoid Levels (TF):

In order to estimate the total flavonoids (TF), several components were added to a volumetric flask. These components included 1 milliliter of product content containing 100 milligrams of material per milliliter in dry form, five milliliters of filtered water, 0.3 mL of NaNO2, and the necessary amount of TF. Following a five-minute interval, 0.6 mL of 10% aluminum chloride was introduced. Furthermore, 2 mL of 1 M NaOH was added for an additional five minutes. Subsequently, a sufficient quantity of distilled water was incorporated into the flask. Once all the materials were combined, the absorbance of the solution was measured at 510 nm. The concentration of TF was also determined, and its value was calculated using a catechin standardization curve ranging from 5 to 100 ppm. Additionally, the TF content was expressed as CE per air-dried material. The results of three studies conducted on each sample were averaged (Kuras, Zielińska-Pisklak, Duszyńska, & Jabłońska, 2020).

Radical Scavenging Analysis using DPPH:

The primary objective of this experiment was to ascertain the IC_{50} value for the DPPH radical scavenging abilities of the CCEs/PRFs. These values were obtained from specific components of Phoenix dactylifera, namely the leaves, fruit, and stem. A solution of 5 mL methanol mixed with DPPH at a concentration of 0.004% was prepared. Various concentrations of methyl alcohol ranging from 0.05 to 4.5 mg/mL were utilized. The resulting mixture was then incubated at room temperature for approximately 20 minutes. Subsequently, the optical density (OD) was measured at a wavelength of 517 nm (Mohamed et al., 2022).

Minimizing Power Consumption in CCE/PRF:

The power reduction in CCEs/PRFs was evaluated when it was lowered to 2%. Potassium ferricyanide and sodium phosphate with a pH of 7.5, 0.3 M were used for this evaluation. The CCEs/PRFs, with a value of 6/24 mg, were incubated at 45 °C for a duration of 30 minutes. Following a five-minute centrifugation, the resulting solution (895 g) was treated with 10% trichloroacetic acid. The recovered supernatant was then mixed with 1/2 mL of 0.1% ferric chloride, along with the addition of distilled water. The optical density (OD) of the final reaction solution was determined at 700 nm using a spectrophotometer (Ghnimi, Umer, Karim, & Kamal-Eldin, 017)

Inhibition of Peroxidation by CCEs/PRFs:

To assess the effectiveness of multiple CCEs/PRFs, the suppression of linoleic acid peroxidation was employed. For this purpose, 5 mg of CCEs/PRFs were homogenized with 0.1 milliliters of linoleic acid, 10 milliliters of buffer solution, and 10 milliliters of pure ethanol. Subsequently, 5 mL of distilled water was added to the homogenized solution. The resulting product was then placed in an incubation chamber set at 40°C. In addition, various components including iron chloride (0.2 mL) and a 75% ethyl alcohol solution (approximately 10 mL) were utilized. The sample solution also contained 0.2 mL of a 30% ammonium thiocyanate solution. The optical density was measured at a wavelength of 500 nm (Zeyadi, 2019).

Characterization:

FTIR Spectroscopy:

The functional groups in the leaves, fruit, and stem of Ajwa and Khalas date palm extracts were identified using FT-IR technique (Bacsik, Mink, & Keresztury, 2004).

Statistical Analysis:

Statistical analysis was performed using mean, standard deviation, and ANOVA variance with a significance level of $P<0.05$.

Results:

Measuring Total Phenolics Content (TP):

The total polyphenol contents (expressed in milligrams per 100 grams of dry sample) of the extracts obtained from Ajwa and Khalas date palm fruits are as follows:

Ajwa date extracts consistently exhibit higher total polyphenol content compared to Khalas date extracts across various solvents, with differences ranging from 3.58 to 9.003 milligrams per 100 grams of dry sample.

SN	Extracts	Total Phenolic Content (mg gallic acid equivalent/g)	
		Fruits of Ajwa Date	Fruits of Khalas date
1	Distilled H_2O	35.65 ± 0.03	35.65 ± 0.03
2	Infusion	41.32 ± 0.02	37.74 ± 0.03
3	80 % MeOH	53.88 ± 0.01	46.02± 0.02
4	100 % MeOH	48.11 ± 0.02	42.43 ± 0.01
5	80 % EtOH	55.423 ± 0.03	50.48 ± 0.02
6	100 % EtOH	50.11 ± 0.02	45.61 ± 0.02

Assessment of Total Flavonoid Levels (TF):

TFC (mg/100g dry weight) of Ajwa and khalas date palm fruit extracts are as follows:

Khalas date extracts consistently show higher Total Flavonoid Content (TFC) having 26.04 mg/100g more TFC than Ajwa.

SN	Extracts	IC_{50} Value (mg/mL)	
		Ajwa Date	Khalas date
1	Distilled H_2O	0.410 ± 0.06	0.422 ± 0.09
2	Infusion	0.391 ± 0.09	0.411 ± 0.07
3	80 % MeOH	0.311 ± 0.09	0.360 ± 0.08
4	100 % MeOH	0.321 ± 0.07	0.389 ± 0.06
5	80 % EtOH	0.264 ± 0.06	0.343 ± 0.09
6	100 % EtOH	0.312 ± 0.04	0.393 ± 0.05

SN	Extracts	TF Contents (mg rutin/g)	
		Fruits of Ajwa Date	Fruits of Khalas date
1	Distilled H_2O	21.04 ± 0.02	38.65 ± 0.01
2	Infusion	24.43 ± 0.02	44.64 ± 0.01
3	80 % MeOH	31.32 ± 0.01	55.52± 0.02
4	100 % MeOH	24.44 ± 0.02	49.54 ± 0.01
5	80 % EtOH	33.87 ± 0.02	59.91 ± 0.02
6	100 % EtOH	27.21 ± 0.02	51.61 ± 0.02

SN	Extracts	Inhibition %	
		Ajwa Date	Khalas Date
1	Distilled H_2O	69.66 ± 0.71	80.54 ± 0.56
2	Infusion	75.76 ± 056	85.83 ± 0.62
3	80 % MeOH	91.43 ± 0.54	96.73± 0.98
4	100 % MeOH	86. 74 ± 0.65	89.43 ± 0.44
5	80 % EtOH	96.45 ± 0.56	99.54 ± 0.37
6	100 % EtOH	88.54 ± 0.56	92.56 ± 0.85

than Ajwa date extracts across different solvents, with differences ranging from 17.61 to 28.47 mg/100g dry weight. Notably, the 80% EtOH extract exhibits the most significant disparity, with Khalas

Radical Scavenging Analysis using DPPH:

The radical scavenging activity of specific components of Ajwa and khalas date palm extract (IC_{50} value):

Ajwa date extracts consistently demonstrate superior radical scavenging activity (lower IC50 values) compared to Khalas date extracts, with the 80% EtOH extract exhibiting the most significant difference. Notably, Ajwa's IC_{50} value is 0.264, outperforming Khalas at 0.343.

Antioxidant activity in linoleic acid system:

The inhibition percentage of the extracts obtained from the fruits of Ajwa and Khalas date palm has been evaluated. The results are as follows:

Ajwa date extracts consistently show higher antioxidant activity (lower inhibition percentages) compared to Khalas date extracts across various solvents. Notably, the 80% EtOH extract exhibits significant

SN	Extracts	Concentration (mg/mL)	Reducing power	
			Ajwa Date	Khalas Date
1	Distilled H_2O	10	0.408 ± 0.02	0.411 ± 0.03
		20	0.420 ± 0.01	0.567 ± 0.01
		30	0.466 ± 0.02	0.504 ± 0.02
		40	0.544 ± 0.03	0.514 ± 0.03
2	Infusion	10	0.422 ± 0.02	0.479 ± 0.01
		20	0.439 ± 0.02	0.532 ± 0.03
		30	0.459 ± 0.02	0.550 ± 0.02
		40	0.490 ± 0.02	0.612 ± 0.03
3	80 % MeOH	10	0.520 ± 0.03	0.532 ± 0.03
		20	0.590± 0.02	0.581 ± 0.03
		30	0.671 ± 0.03	0.610 ± 0.01
		40	0.733 ± 0.02	0.722 ± 0.02
4	100 % MeOH	10	0.481 ± 0.02	0.489 ± 0.02
		20	0.520 ± 0.01	0.529 ± 0.01
		30	0.560 ± 0.01	0.566 ± 0.02
		40	0.691 ± 0.02	0.600 ± 0.02
5	80 % EtOH	10	0.620 ± 0.02	0.534 ± 0.03
		20	0.691 ± 0.02	0.598 ± 0.02
		30	0.688 ± 0.01	0.620 ± 0.01
		40	0.641 ± 0.03	0.740 ± 0.01
6	100 % EtOH	10	0.500 ± 0.01	0.519 ± 0.02
		20	0.539 ± 0.02	0.570 ± 0.02
		30	0.569 ± 0.01	0.588 ± 0.01
		40	0.611 ± 0.01	0.583 ± 0.02

differences, with Ajwa at 96.45% inhibition and Khalas at 99.54%.

Reducing power of extract:

Extracts of Ajwa and Khalas date palm fruit have reduced power as follow: Ajwa date extracts consistently show comparable or slightly higher reducing power concentrations (0.408 to 0.733 mg/mL) than Khalas date extracts across different solvents. Notably, the 80% MeOH extract of Ajwa Date exhibits the highest concentration at 0.733 mg/mL.

Conclusion:

It is concluded from above comparative study that the Khalas and Ajwa dates has been classified as potential candidates for antioxidant and pharmacological profile study.

Acknowledgement:

The authors has been thankful to HEC, Pakistan and Riphah International University.

References:

Abul-Soad, A. A., Mahdi, S. M., & Markhand, G. S. (2015). Date palm status and perspective in Pakistan. *Date Palm Genetic Resources and Utilization: Volume 2: Asia and Europe*, 153-205.

AlFaris, N. A., AlTamimi, J. Z., AlGhamdi, F. A., Albaridi, N. A., Alzaheb, R. A., Aljabryn, D. H., . . . AlMousa, L. A. (2021). Total phenolic content in

ripe date fruits (Phoenix dactylifera L.): A systematic review and meta-analysis. *Saudi Journal of Biological Sciences*, *28*(6), 3566-3577.

Bacsik, Z., Mink, J., & Keresztury, G. (2004). FTIR spectroscopy of the atmosphere. I. Principles and methods. *Applied Spectroscopy Reviews*, *39*(3), 295-363.

Fuller, D. Q., & Stevens, C. J. (2019). Between domestication and civilization: the role of agriculture and arboriculture in the emergence of the first urban societies. *Vegetation History and Archaeobotany*, *28*, 263-282.

Ghnimi, S., Umer, S., Karim, A., & Kamal-Eldin, A. (2017). Date fruit (Phoenix dactylifera L.): An underutilized food seeking industrial valorization. *NFS journal*, *6*, 1-10.

Gros-Balthazard, M., Baker, W. J., Leitch, I. J., Pellicer, J., Powell, R. F., & Bellot, S. (2021). Systematics and Evolution of the Genus Phoenix: Towards Understanding Date Palm Origins. In *The Date Palm Genome, Vol. 1: Phylogeny, Biodiversity and Mapping* (pp. 29-54): Springer.

Ismail, B., Haffar, I., Baalbaki, R., Mechref, Y., & Henry, J. (2006). Physico-chemical characteristics and total quality of five date varieties grown in the United Arab Emirates. *International journal of food science & technology*, *41*(8), 919-926.

Kuras, M. J., Zielińska-Pisklak, M., Duszyńska, J., & Jabłońska, J. (2020). Determination of the elemental composition and antioxidant properties of dates (Phoenix dactyliferia) originated from different regions. *Journal of Food Science and Technology*, *57*, 2828-2839.

Mohamed, H., Al-Hajhoj, M., Al-Saikhan, M., Alqahtani, N., Zayed, M., Moawad, M., . . . Mohamed, M. E. (2022). Green Extraction of Date Palm Fruits via Ultrasonic-Assisted Approach: Optimizations and Antioxidant Enrichments. *Processes*, *10*(6), 1049.

Vayalil, P. K. (2013). Bioactive compounds, nutritional and functional properties of date fruit. *Dates: postharvest science, processing technology and health benefits*, 285-303.

Zeyadi, M. (2019). Purification and characterization of peroxidase from date palm cv. Agwa fruits.

International Journal of Food Properties, *22*(1), 1910-1919.