

ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT DATE PALM (PHOENIX DACTYLIFERA L.) SEED VARIETIES: AN IN VITRO ASSESSMENT

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DOI:

10.38106/LMRJ.2024.6.1-04

Received: 14.02.2024

Accepted: 03.03.2024

Published: 31.03.2024

ABSTRACT

The date palm (*Phoenix dactylifera* L.) fruit contains variety of bioactive constituents including phenolic compounds. This research aimed to analyze the phenolic constituents and antioxidant potential of crude methanol extract samples from seeds of different date varieties, Aseel (As), Ambar (Am), Ajwa Saudi (AJS), Ajwa Khairpur (AJK), Khipro (KP) and Karblyan (KB). Date seeds are regarded as waste product. However, it contains secondary chemicals of biomedical significance. Colorimetric methods were used for the evaluation of phytochemical and antioxidant capacity. The Folin-Ciocalteu method was used for the assessment of phenolic content. The antioxidant potential was evaluated through phosphomolybdenum test, potassium ferricyanide test, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate scavenging test methods. All samples differ significantly in terms of antioxidant activities and quantity of secondary metabolites (phenolic and flavonoid contents). When all samples were compared, As was shown to have the highest amount of phenolic and flavonoids, while AJ(S) had the lowest content. The KP sample had the highest overall antioxidant and reducing potential, while the AJ(K) and Am samples showed the lowest, respectively. Significant DPPH scavenging capacity has been demonstrated in all samples. In order to extend the shelf life of food products, date seeds may consequently provide ideal material for the bio-functional food industry.

Key Words: Date palm; Phytochemicals; Phenolic content; Antioxidant; Flavonoids

INTRODUCTION

Date palm is the primary plant that characterizes, represents, and defines dry and semiarid-areas of North Africa and the Middle East. Due to its adaptation to tropical or subtropical temperatures, this crop is the world's oldest perennial fruit tree (> 4000 B.C). With around 1 million metric tons of date seeds, the world produced up to 8.5 million metric tons of dates in 2016. Among the nations that produce dates, Pakistan comes on the sixth number (1). Its taxonomical classification is given in Figure 1. Dates are rich source of nutritional and bioactive constituents. The seeds make about 10-15% of overall weight of the fruit and are enclosed in a fleshy pericarp. In addition, date seeds continue to be a concern for the date processing business. Sometimes seeds are utilized as feed for livestock in Middle Eastern and South Asian nations (2). In comparison to controls, broilers fed diets enriched with date seeds gained more weight (3). Additional research suggests that seeds contain nutrients that are beneficial for animal diets and reports a positive supplementary effect on animal diets (4). When comparing date seeds with the wheat bran and oats, they are a richer source of fiber (5). Consuming date seeds lowers the risk of dyslipidemia, diabetes, obesity, hypertension, and other metabolic disorders (6). Protein, fiber, vitamins, and bioactive substances are some examples of components could be associated with these effects (7). Phenolic compounds are among the bioactive substances that have been found to have antioxidant, anti-carcinogenic, anti-microbial, anti-mutagenic, and anti-inflammatory properties. Date seeds may contain nutritious and bioactive components with high added value that may be extracted and used to bio-functional foods. Despite the 300 different date variants growing in district Khairpur, there is little information available on the phytochemical analysis and antioxidant activity of the seeds produced from

these trees as byproducts. So, the primary objective of the current study was to evaluate and compare the existing variations in phytochemical content and antioxidant activities of seeds from six distinct varieties of date (*P. dactylifera* L.) seeds.

Kingdom:	Plantae
Sub-kingdom:	Tracheobionta
Division:	Magnoliophyta
Class	Monocotyledon
Order:	Arecales
Family:	Arecaceae
Genus:	Phoenix
Specie:	Dactylifera
Botanical name:	<i>P. dactylifera</i>



Figure 1. Taxonomy of date palm (*P. dactylifera* L.)

METHODS

COLLECTION AND PROCESSING OF THE SAMPLE

Various varieties of dates were collected from Shah Abdul Latif University or purchased from Saudi Arabia. The seeds were taken from all date varieties, washed, and shade dried. Coarse powder was prepared using Grinder. After that, extract was prepared by soaking the sample powder in methanol (1:4 w/v) for three days at room temperature and frequent shaking. The procedure was repeated, filtered through Whatman No.1 filter paper and concentrated using rotary evaporator (R-200 Buchi, Switzerland). Finally, the samples were completely dried in an oven (VacuCell, Einrichtungen GmbH). The six samples were labeled, including: Aseel (As), Ambar (Am), Ajwa Saudi (AJS), Ajwa Khairpur (AJK), Khipro (KP); Karblyan (KB) and kept in air-tight containers at 4°C until further analysis.

ASSESSMENT OF PHYTOCHEMICALS

Quantification of Total Phenolic Content

The reaction mixture was prepared by mixing the sample, Folin-Ciocalteu reagent, and sodium carbonate (1:9:9, V/V) for the assessment of the phenolic content. The mixture was incubated at 30°C for one hour. The optical density was recorded at 725 nm. The presence of phenolic compounds was evaluated from the regression curve of the Galic acid ($y = 0.0617x - 0.0525$, $R^2 = 0.9834$), used as standard phenolic compound. While, phenolic compounds were quantified as Gallic acid equivalents (GAE).

Quantification of Total Flavonoid Content

Reaction mixture was prepared by mixing sample, potassium acetate and aluminium chloride (2:1:9, V/V) for the assessment of the flavonoid content. The mixture was incubated at ambient temperature for half an hour. The optical density was recorded at 415 nm. The presence of flavonoid compounds was evaluated from the regression curve of the Quercetin ($y = 0.0429x - 0.1092$, $R^2 = 0.9909$), used as a standard flavonoid compound. While, flavonoid compounds were quantified as Quercetin equivalents (QE).

ANTIOXIDANT ACTIVITIES

Assessment of free radical scavenging potential

As a stable radical with strong absorbance at 517 nm, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was utilized to examine the capacity of samples to scavenge this free radical. Assay was performed following the previously described method with minor modification (8). The reaction mixture consisting of sample and DPPH solution was incubated at room temperature for one hour in a dark environment. Then, absorbance was measured at 517 nm. Vitamin C was used as the standard scavenging chemical. The following formula was used to determine the percentage of scavenging activity:

$$\%SA = (1 - ODS/ODNC) \times 100$$

SA: Scavenging activity; ODS: optical density of sample; ODNC: optical density of negative control.

Assessment of total antioxidant potential

The total antioxidant potential of the extracts was estimated using a phosphomolybdenum-based colorimetric assay by following previously described method (8). Total antioxidant reagent comprised of ammonium molybdate, sulfuric acid and sodium phosphate at the concentrations of 4 Mm, 0.6 M and 28 mM, respectively. The sample and total antioxidant reagent (1:9 v/v) was poured in Eppendorf tubes. Then, reaction mixture was incubated in boiling water for one and half hour. Followed by cooling the mixture at room temperature and measuring the optical density at 630 nm. Standard curve of Vitamin C was prepared and vitamin C equivalents were used to express the overall antioxidant activity of the samples. The assay was performed in triplicate and repeated three times.

Assessment of total reducing potential

Total reducing potential of the samples was investigated by following previously described method (9). Reaction mixture for assessment of total reducing potential comprised of extract sample, phosphate buffer (pH 6.6), potassium ferric cyanide at the concentrations of 400 µg, 0.2 M, 0.6 M and 1%, respectively and mixture was subjected to incubated for half an hour in water bath at 50°C. Subsequently, trichloroacetic acid was added to stop the reaction. At the end optical density was measured at 630 nm. Standard curve of Vitamin C was prepared and vitamin C equivalents were used to express the overall total reducing potential of the extract samples. The assay was performed in triplicate and repeated three times.

Statistical analysis

All experiments were carried out in triplicate, repeated thrice and presented as Standard Deviation (\pm SD). GraphPad Prism (version 5.01 for Windows, California, USA) was used to analyze the data and determine statistical significance at the level of p-value < 0.05.

RESULTS

PHYTOCHEMICAL ANALYSIS

The class of phytochemical and their quantities in extracts depends on the nature of the solvents. The biological potential of plants is generally thought to depend on phytochemical profile such as polyphenolic constituents and flavonoids. Because of this, the quantification of phenolic and flavonoid constituents in each date palm cultivar was measured through in vitro assays using the calibration curve of the corresponding standards. Total six cultivars of date palm, As, Am, AJS, AJK, KP, and KB, were examined for their phytochemical quantification.

Among all tested sample Aseel showed the highest concentration of the phenolic content. While, AJ(S) were observed to have the lowest level of the flavonoid content. The date seed samples used in our investigation revealed phenolic compounds as gallic acid equivalents in various kinds, with As > KP > AJ(K) > KB > Am > AJ(S), respectively. The results of the phenolic compounds are shown in figure 2.

Flavonoid are the important secondary metabolite phytochemical and date seed sample were explored for presence of these compounds. The experiment displayed the quantity of flavonoid compounds in order of As > AJ(K) > KP > Am > KB > AJ(S), respectively, as equivalents of quercetin in all samples (Figure 3).

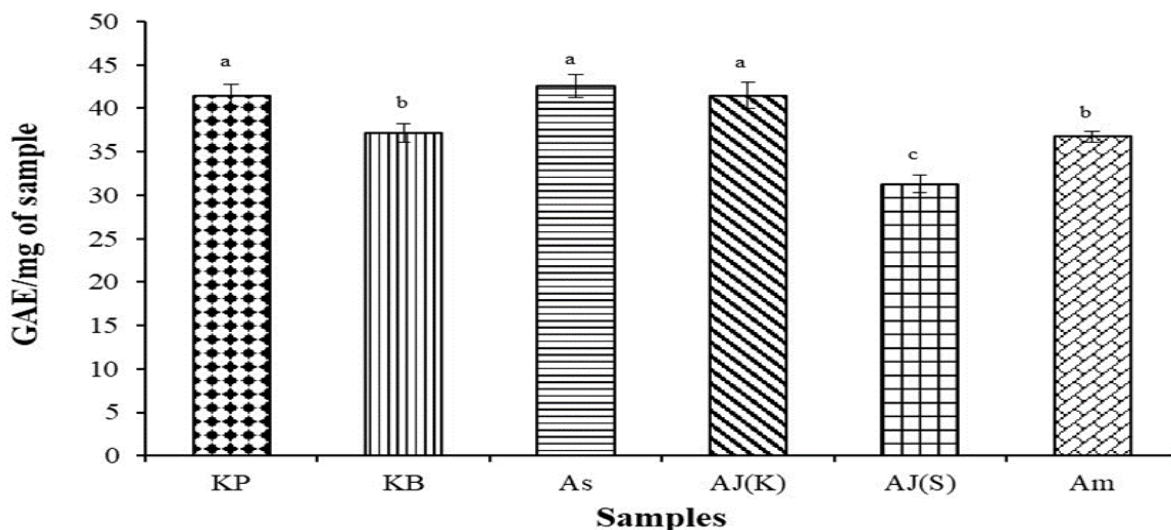


Figure 2. Quantification of total phenolic content in crude methanol extract of date seed samples. The results are shown as mean \pm SD of three experiment performed in triplicate and phenolic quantity was expressed as gallic acid equivalent (GAE)/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am, respectively.

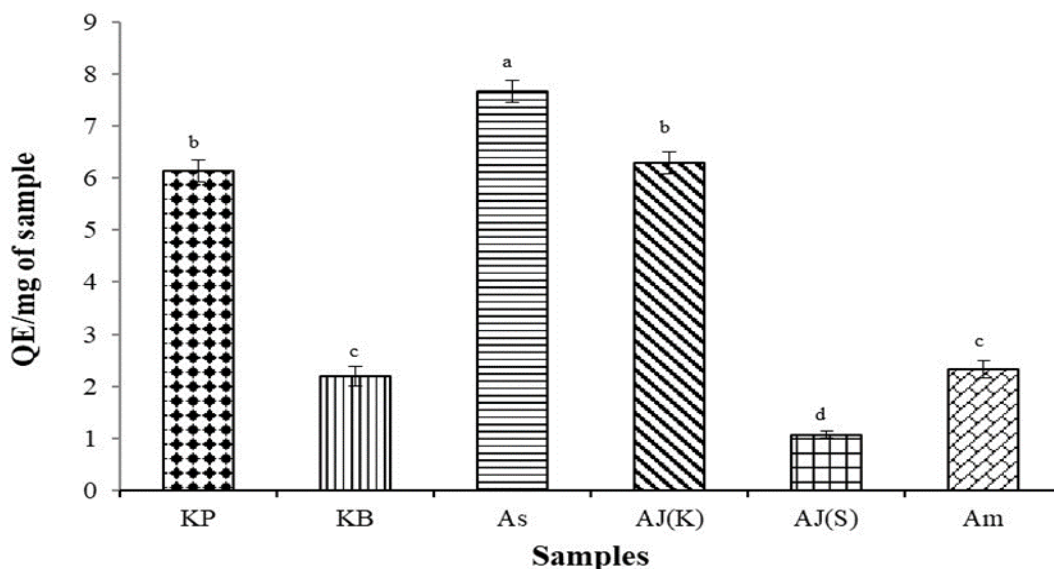


Figure 3. Quantification of total flavonoid content in crude methanol extract of date seed samples. The results are shown as mean \pm SD of three experiment performed in triplicate and phenolic quantity was expressed as quercetin equivalent (QE)/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am, respectively.

ANTIOXIDANT ACTIVITIES

In the present study multimode antioxidant activity was performed using different *in vitro* assays such as total antioxidant assay through ammonium molybdate reduction potential, total reducing potential through potassium ferricyanide colorimetric method and free radical (DPPH) scavenging assay for six different cultivars of date palm seeds including As, Am, AJS, AJK, KP, and KB.

Crude methanolic extract of all samples showed the total antioxidant capacity in order of KP > Am > As > AJ(S) > As > KB > AJ(K), respectively as quantified as ascorbic acid equivalents. The results are shown in Figure 4. Among all tested sample KP was observed to highest Total antioxidant activity and AJ(K) has lowest activity.

The total reducing power seed samples in the increasing order of KP > AJ(K) > AJ(S) > As > KB > Am, respectively. The results are presented in Figure 5. Among all tested sample KP was observed to highest total reducing potential, while Am was observed to have lowest.

In addition to these, all samples exhibited the > 90% DPPH scavenging potential at the concentration of 200 µg/ml. Therefore, again evaluated at lower concentration of 7.4, 22.2 and 66.6 µg/ml and significant activity was observed by the samples as shown in Figure 6.

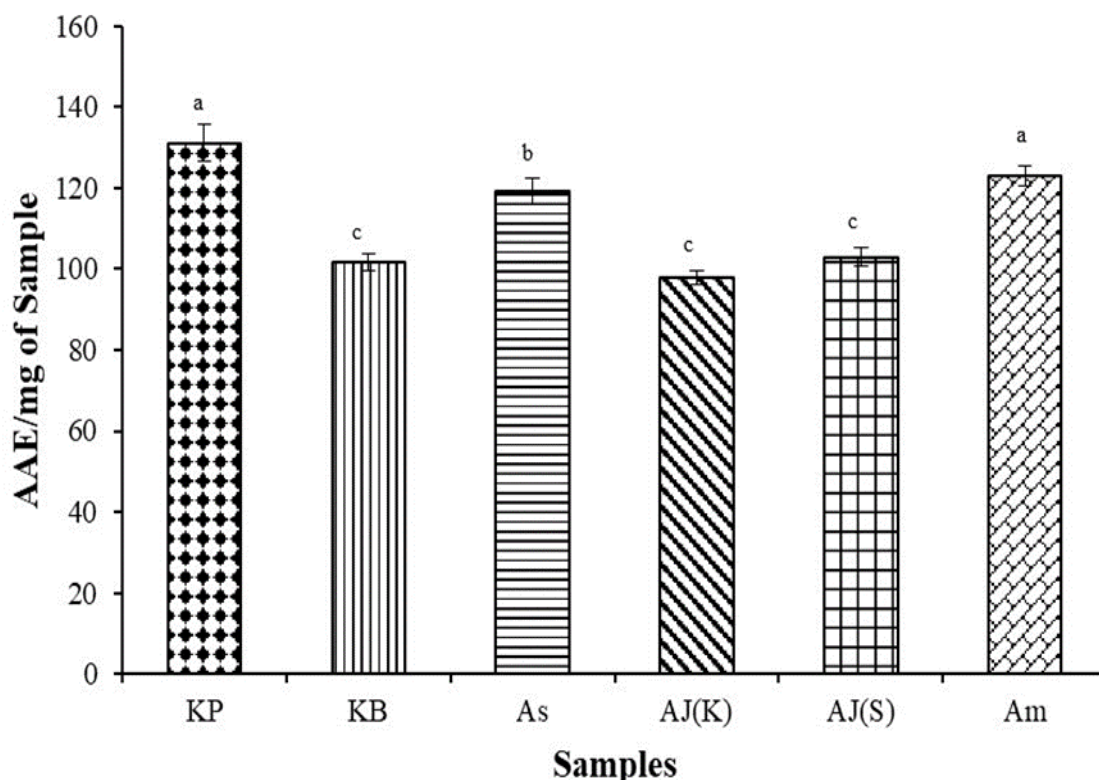


Figure 4. Assessment of Total antioxidant potential of crude methanol extract of date seed samples. The results are shown as mean ± SD of three experiment performed in triplicate and expressed as µg AAE/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

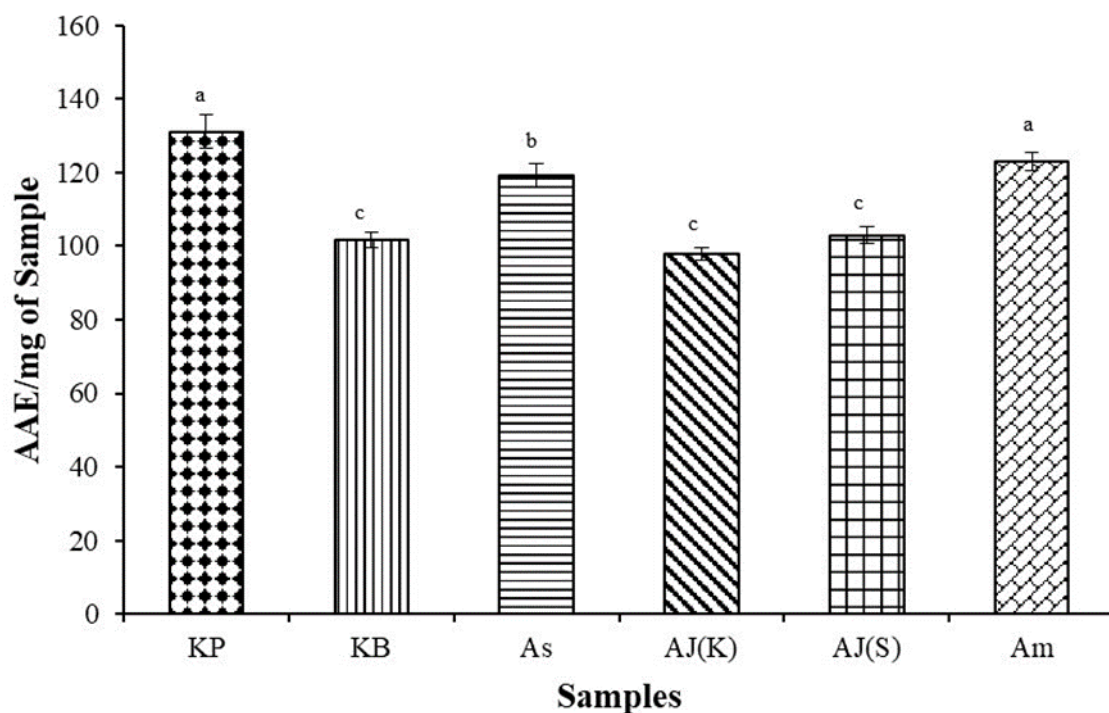


Figure 5. Assessment of total reducing potential of crude methanol extract of date seed samples. The results are shown as mean \pm SD of three experiment performed in triplicate and expressed as μg AAE/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

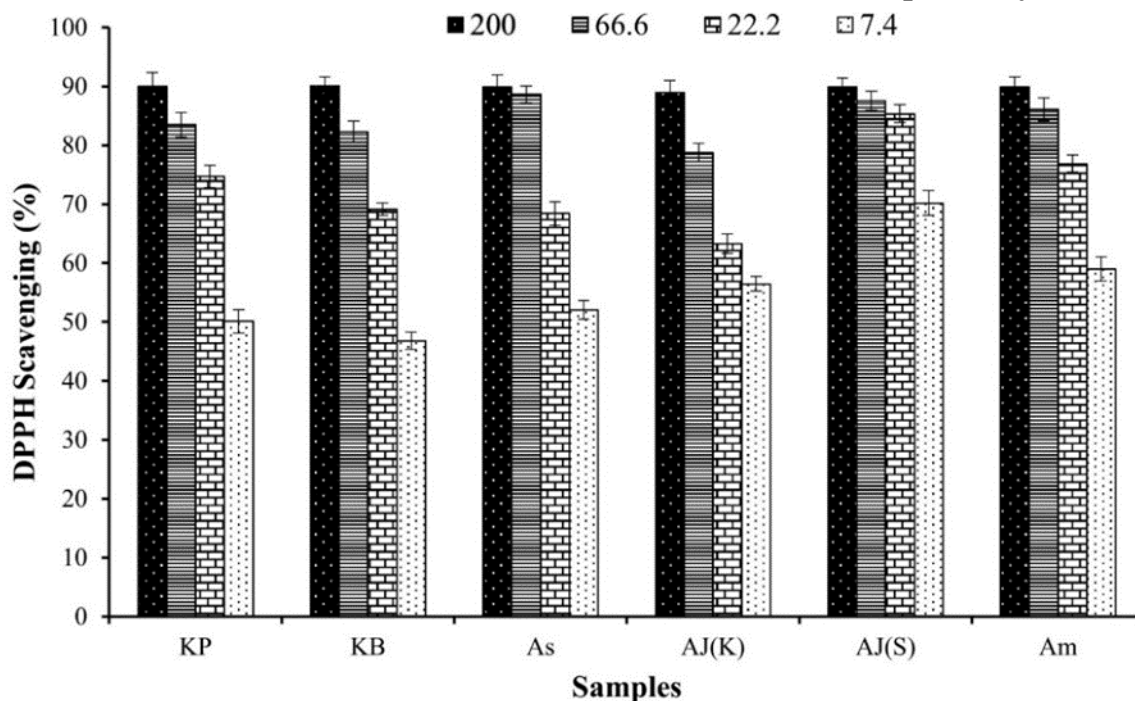


Figure 6. Free radical (DPPH) scavenging potential of the crude methanolic extract of date seeds. The results are shown as mean \pm SD at indicated concentrations ($\mu\text{g/ml}$). Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

DISCUSSION

Plants have long been used for the treatment and management a wide range of diseases. The fruit of date palm is a rich source of nutrients and high content of bioactive substances like phenolic compounds. It also possesses variety of therapeutic potential such as antioxidant, antimicrobial, anti-inflammatory and others (10). Date palm is supposed as a complete diet and medicinal plant as phytochemical studies have explained that the date palm contains anthocyanins, phenolics, sterols, carotenoids, and flavonoids. Due to the existence of distinct bioactive constituents, several parts of the tree, including the edible portion of fruits, seeds, leaves, and the spathe, have been linked to a variety of health benefits and therapeutic potential (11, 12).

Secondary plant metabolites with potential benefits for farm animals include polyphenols and flavonoids. These phytoconstituents are related to the taste, and astringency of plant-based products. They are divided into various groups, with the main bioactive components being flavonoids, lignans, phenolic acids, and stilbenes (13). Plant flavonoids have been considered as functional secondary metabolites with potential advantages, such as antioxidant and radical-scavenging properties or protection against various chronic, cardiovascular, or carcinogenic illnesses (14). Recently, a large number of *in vitro* and *in vivo* studies, as well as the identification and quantification of several classes of phytochemicals, have been conducted worldwide in response to the many health advantages of dates (15). Dates fluctuate in form, size, and weight according on the area. Furthermore, they differ in terms of their physical, chemical, and organoleptic properties (16).

In the current study, six variants of date palm, As, Am, AJS, AJK, KP, and KB, were examined for their phytochemical quantification, including their phenolic and flavonoid content and antioxidant activity, using several *in vitro* tests. Date seeds are continuously distinguished by a high concentration in phenolic compounds despite the fact that many standards have been utilized for phenolic measurement. More phenolic constituents are found in date seeds than other by-products of date (17). A previous study supports our findings, where phytochemicals of three date kinds (Shahal, Um-sellah, and Mabseeli) have been reported (7). In addition to this, the chemicals found in other date seeds and cultivars, obtained from other nations and under various conditions, are positively correlate with present study (18).

Phenolic and flavonoids are one of the important groups of secondary metabolites with significant bioactivities and therapeutic potential. The date seed samples used in our investigation revealed the presence of significant quantity of phenolic and flavonoid compounds (Figure 2). Variations of phytochemicals and biological activities in different studies can be attributed towards variety of factors such as environmental variables, cultivars, fruit maturity, and extraction conditions (19). Other variables like variety, geographic origin, growth circumstances, fertilizer, soil type, season, maturity, sunshine during ripening, or storage conditions might partially account for the observed variation among studies. Date seeds often contain more flavonoids than date fruits (20). Furthermore, the types of solvents and their polarity have an impact on the solubility of polyphenols (14, 21), and the date-seeds contain a variety of polyphenols, each of which may have a distinct polarity. In comparison to a solvent that is just aqueous, acetone in aqueous solution dissolves hydrophilic and high molecular weight molecules (10). One of study reported that phenolics and flavonoids had comparable extraction patterns, with 50% aqueous acetone producing the best results comparative to pure acetone (7). However, in our case methanol was used as solvent for extractions. Date seeds can be utilized to make extracts high in phenolics and flavonoids that could be used as useful substances for both people and animals, according to their chemical makeup.

Since antioxidants scavenge free radicals associated to a number of illnesses, including cancer, arthritis, heart diseases, diabetes, and Parkinson's disease, they have drawn a lot of interest. Catalase, superoxide dismutase, and glutathione peroxidase are examples of the antioxidants that the body naturally synthesizes to defend against free radicals. Altered production in these antioxidant results oxidative stress which is associated with diseases (16). Furthermore, antioxidants can be taken in nutrition to cope with these oxidative stress related

conditions. Due the presence of the bioactive chemicals like phenolic constituents, date seeds have the potential to be used as function foods (16).

Possible natural antioxidant action of plant extract, phenolic and flavonoid compounds is well recognized (21). In this investigation, the widely used multimode antioxidant approach was utilized to measure the antioxidant capacity of plant products. Date seeds' high antioxidant capacity may thus promote their usage as natural antioxidants for therapeutic, nutraceutical, or pharmacological applications (22). Through the phosphomolybdenum technique, total antioxidant capacity was assessed and substantial activity was observed in all samples. Date seed samples were shown to have overall significant reducing power as measured through potassium ferricyanide technique. Total antioxidant potential and total reducing power activity was expressed as Ascorbic acid equivalents. All sample exhibited significant free radical (DPPH) scavenging activity. DPPH has frequently been used to evaluate the antioxidant capability of substances, such as plant extracts, and may be used to precisely titrate the oxidisable groups of biomolecules (23). In the present study all sample were found active in scavenging the DPPH free radical. Seeds samples of all six cultivars have shown considerable antioxidant activity. For thousands of years, dates have been a staple meal throughout the Middle East, and different parts of the world. There are many different cultivars of dates around the globe. Each kind of date has demonstrated therapeutic benefit in the prevention of different diseases (24). The presence of flavonoids, phenolics, and other antioxidant substances (Vitamin-C, -E, carotenoids and other phytochemicals) in these by-products is responsible for their antioxidant action (25, 26). A prior study demonstrated that the phenolic content of date fruit was substantially linked with its ability to scavenge DPPH radicals. Compared to other fleshy fruits like figs, prunes, or raisins, dates and their pits would have more antioxidant activity (7, 27). For this reason, exploiting natural resources like plants, algae, and their products is a better, safer choice when searching for bio-functional or therapeutic compounds

CONCLUSION

Date palm (*P. dactylifera* L.) seeds are regarded as a problematic waste product, contain secondary chemicals that have biological activities, and initiated the research direction for promising applications in various fields. The present investigation highlights the antioxidant value and phytochemical assessment of different varieties of date seeds. All samples differ significantly in terms of antioxidant activities and quantity of secondary metabolites (phenolic and flavonoid contents). When comparing all samples, As was shown to have the highest amount of phenolic and flavonoids, while AJ(S) had the lowest levels. The KP samples had the highest overall antioxidant and reducing potential, whereas the AJ(K) and Am samples showed the lowest, respectively. The more research is recommended to characterize, isolate the phytochemicals and investigate other properties for possible use in food and biomedical industries.

Conflict of interest:

Authors declare no conflict of interest

Funding source:

The study did not receive any external funding

Ethical Approval:

The study was approved by local research ethics committee.

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