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Experimental Article

Phytochemical, minerals and free radical scavenging profiles of *Phoenix dactilyfera L.* seed extract

Abiola M. Adeosun, M.Sc [a,*], Sarah O. Oni, M.Sc [b], Osasenaga M. Ighodaro, PhD [a], Okikiola H. Durosinlorun, B.Sc [a] and Omotayo M. Oyedele, B.Sc [a]

[a] *Department of Biochemistry, Lead City University, Ibadan, Nigeria*
[b] *Department of Chemistry, Lead City University, Ibadan, Nigeria*

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الملخص

أهداف البحث: يستخدم نوى تمر النخيل في الطب الشعبي لعلاج العديد من الأمراض. وقد تبين أن هذا النوى يساعد على خفض نسبة خطر الإصابة بالأمراض السرطانية، وبعض أمراض القلب والأوعية الدموية. وكذلك قد يساعد على تحسين مستوى الأداء الوظيفي وسلامة الجهاز المناعي للجسم. وعلى أساس مالدينا من معلومات تختص بتكوين النباتات وآثارها العلاجية أو الحيوية، سعت هذه الدراسة للبحث في الخصائص النباتية، ومكونات المعادن ومضادات الأكسدة لنوى تمر النخيل.

طرق البحث: تم تقييم الخصائص النباتية والمكونات المعدنية فضلا عن قدرة نوى التمر على التأثير المضاد على الجذور الحرة باستخدام البروتوكولات القياسية.

النتائج: وكانت محتويات القلويات، وفلافونيدات انثراكوينونيس، والصابونين، وتيربينويدس، والعفص (ملغ / ١٠٠جم من الوزن الجاف) ١٠٢.٢٧ موازي للأتروبين ٢٤٧، و ١.٢٣ موازي للكويرسيرتين، و٣٣٤.٩٣ موازي الإيزارين ١٩.٤٧، ١٢٤.٤١ و١٣٣.٢٠ على التوالي. أما محتوى الصوديوم، والبوتاسيوم، والكالسيوم، والمغنيسيوم، والحديد، والفوسفور، والزنك (ملغ / ١٠٠ جم من الوزن الجاف) فقد كان ٠.٦٧، ٧٨.١٢، ١٨.٢٠، ٠.٤٨، ٠.٨٢، ١٩.٣٢، و ٠.٢٥ على التوالي. أما التأثير المضاد للأكسدة للنوى ضد بعض الجذور الحرة فكان ١٠.٢١ ملغ / مل و ١.٦٧ ملغ / مل على التوالي.

الاستنتاجات: خلصت هذه الدراسة إلى أن نوى تمر النخيل غني بالفلافونويد، وهي مجموعة من المواد المضادة للأكسدة مسؤولة على ما يبدو بشكل ملموس عن إلغاء الأضرار الجانبية للجذور الحرة على الجسم، وهذا يفسر التأثير العلاجي لنوى تمر النخيل في البحث.

الكلمات المفتاحية: مضادات الأكسدة؛ نوى التمر؛ الجذور الحرة؛ المعادن؛ المواد الكيميائية النباتية

Abstract

Objectives: *Phoenix dactilyfera L* (date palm) seeds are used in traditional medicine for the treatment of several ailments. These seeds have been shown to lower the risk of cancer and some cardiovascular conditions as well as to improve the functionality and integrity of the immune system. On the basis of established associations between the composition of plants and their therapeutic or biological effects, this study sought to investigate the phytochemical, mineral, and antioxidant profiles of the date palm seed.

Methods: The phytochemical and mineral compositions as well as the free radical scavenging capacity of the date palm seed were evaluated using standard protocols.

Results: The alkaloids, flavonoids, anthraquinones, saponins, terpenoids, and tannins contents (mg/100 g dry weight) were 102.27 Atropine equivalents (ATE), 2471.23 Quercetin equivalents (QE), 334.93 Alizarin equivalents (ALE), 124.41, 19.47, and 133.20, respectively. The sodium, potassium, calcium, magnesium, iron, phosphorus, and zinc contents (mg/100 g dry weight) were 0.67, 78.12, 18.20, 0.48, 0.82, 19.32, and 0.25, respectively. The scavenging activities (EC_{50}) of the seed against 1,1-diphenyl-2-picryl hydrazyl (DPPH) and superoxide dismutase (SOD)-generated free radicals were 10.21 mg/mL and 1.67 mg/mL respectively.

Conclusions: This study concluded that the date palm seed is rich in flavonoids, a group of polyphenolic antioxidants that are apparently responsible for the appreciable free radical scavenging effects. The polyphenolic

* Corresponding address: Department of Biochemistry, P. O. Box, 30678, Lead City University, Ibadan, Nigeria.
E-mail: albaraat2020@gmail.com (A.M. Adeosun)

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compounds present in the investigated date seed may account for its therapeutic relevance in traditional medicines.

Keywords: Antioxidants; Date seeds; Free radicals; Minerals; Phytochemicals

Introduction

Phoenix dactilyfera L (date palms) are important plants of nutritional, medicinal and economic value. They are cultivated in arid regions of the world.[1] Virtually all of the parts of date palms are used in traditional medicine.[2] Date fruit is a berry consisting of a fleshy mesocarp covered by thin epicarp and endocarp surrounding the seed.[2] The date seed is a hard coated seed that is elongated, ventrally grooved and containing a small embryo. The seeds are typically discarded as a by-product by the date fruit industry or after eating, and they can be found near date industry sites where dates are processed or packed.[3] Additionally, they can be found on farms where date pastes are being produced.

Date seeds constitute 5.6−15.0% of the total fruit weight depending on maturity, variety and grade.[3,4] Date seeds contain high and variable quantities of fibre, low protein, oil content ranging from 4 to 14% and appreciable amounts of mineral elements.[1]

Date seeds are commonly used as animal feed. They are soaked in water until soft, crushed and then fed to horses, cattle, camels, sheep, goats and chickens.[5] They can also be used as a source of oil, coffee substitute and raw material for activated carbon as well as an adsorbent for dye-containing waters.[5,6] The oil content of the seeds may be suitable for use in the production of cosmetics.

Date seeds have been studied as potential sources of edible oils and pharmaceuticals.[4] The seed powder has been reported to be used as ingredient in traditional medicine to relieve ague and toothaches.[7] It has been suggested that the date seed may lower the risk of cancer and cardiovascular conditions. It is also reported to improve the functionality and integrity of the immune system.[4] Coffee grains and barley are well known to have bulk mineral contents in considerable amounts. Date palm seeds, which are employed as a coffee substitute or as coffee drinks, may also contain various essential minerals in appreciable amounts.

There is a well-known association between the therapeutic activities of plant materials and their chemical constituents; subsequent to some medicinal utilizations associated with date seeds, it is important to examine the chemical constituents of Niger variety date seeds. The plant material may contain some therapeutically promising compounds, which is what informed the current study.

Materials and Methods

Chemicals and reagents

Chemicals and reagent used in this study were purchased from Sigma Company, USA and BDH, India.

Sample preparation

A dried Khaokhara variety of date fruit (Tamr stage) was procured from date farm in the Niger republic. The fruit sample was authenticated at the Federal College of Forestry by Mr. Ogele E.I with Voucher Number FCF/OYO/2014/21976. The fruits were transported to the laboratory of the department of Biochemistry, Lead City University, Ibadan. The seeds were separated from the fruits, washed with distilled water, air dried, and ground into powder.

Phytochemical analysis

Qualitative Phytochemical screening of the date palm seeds were performed using a standard procedure by Sofowora, Harborne, and Trease and Evans.[8−10]

Determination of total alkaloid in sample

The plant extract (5 mg/mL) was dissolved in dimethyl sulphoxide (DMSO); 1 mL of 2N HCl was added and filtered. The resultant mixture was transferred to a separating funnel; 5 mL each of bromocresol green solution and phosphate buffer was added. The mixture was shaken with 1, 2, 3 and 4 mL chloroform by vigorous shaking, collected in a 10-mL volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100 g of extract.[11]

Determination of anthraquinone in sample

One gram (1.0 g) of the sample was soaked in 50 mL of distilled water for 16 h. The suspensions of the samples were heated in a water bath at 70 °C for one hour. After the suspension was cooled, 50 mL of 50% methanol was added to the sample, followed by filtration. The spectrophotometric value of the filtrate was read at a wavelength of 450 nm and compared with standard solutions containing 1 mg/100 mL of alizarin.[12]

Determination of saponin in sample

The direct spectrophotometric method described by Brunner was used for saponin analysis with slight modification. One gram (1 g) of finely ground sample was weighed into a 250-mL beaker and 100 mL Isobetyl alcohol was added. The mixture was shaken on a mechanical shaker for 3 h to ensure uniform mixing. Thereafter, the mixture was filtered with Whatman filter paper (No. 1) into a 100-mL beaker and 20 mL of 40% saturated solution of Magnesium carbonate was added. The resultant mixture was again filtered through a Whatman filter paper (No 1) to obtain a

clear colourless solution. To 1 mL of the colourless solution pipetted in 50-ml volumetric flask, 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. One mL of 1−10 ppm standard saponin solutions were treated similarly with 2 mL of 5% $FeCl_3$ solution as for the sample above. The absorbance of the standard saponin solutions and sample were read after colour development on Spectrophotometer at a wavelength of 380 nm. The percentage saponin content was calculated in mg/100 g sample.[13]

Determination of total flavonoid in sample

The total flavonoid content was determined by the aluminium chloride method. The reaction mixture (3.0 mL) comprised of 1.0 mL of extract, 1.0 mL of aluminium chloride (1.2%) and 1.0 mL of potassium acetate (120 mM) was incubated at room temperature for 30 min; absorbance measured at 415 nm. Quercetin was used as a positive control. The flavonoid content was expressed in terms of Quercetin equivalent (mg/100 g of fresh sample).[14]

Determination of tannins in sample

Tannin quantification was carried out according to the procedure described by Phan et al.[15] Five hundred milligrams of finely dried plant extract was added into a glass beaker containing 5 mL of 70% aqueous acetone. The solution was uniformly mixed and gently boiled in a water bath for 30 min. The solution was centrifuged at 3000 rpm for 10 min at 4 °C and the supernatants were collected and stored in freezing conditions. The pellet was dissolved in 5 mL of 70% aqueous acetone and re-centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were collected and mixed with freezing stored supernatants. To these supernatants, 1 mL of Folin−Denis reagent and 3 mL of sodium carbonate solution was added, and the solution was diluted to 20 mL with distilled water. The solution was mixed well and incubated at room temperature for 30 min. The absorbance was measured with a spectrophotometer at 700 nm.

Determination of terpenoids in sample

One gram (1 g) of the sample was added to 10 mL of petroleum ether and allowed to extract for 15 min. The solution was filtered and read at an absorbance of 420 nm.[12]

Mineral analysis

100 ppm stock solution of K, Mg, Ca, Na, Fe, P, Zn, Pb, were prepared by dissolving required amount of their salts in distilled water for elemental analysis of the sample. The sample was digested according to the perchloric acid digestion method.[16] 0.25 g of the sample was taken into a 50-mL flask; 6.5 mL of mixed acids solution (nitric acid, sulfuric acid, perchloric acid in ratio 5:1:0.1) was added and boiled in a fume hood on a hot plate until the digestion was completed, which completion was indicated by white fumes rising from the flasks. The digested sample was allowed to cool and then transferred into a 50-mL volumetric flask by raising the volume with distilled water. The digested sample was filtered through Whatman filter paper (No. 42). The elemental concentrations in the sample (filtrate) were determined using Shimadzu AA-670 Atomic Absorption Spectrophotometer. The mineral contents of the sample were calculated as follows:

$$\text{Cation (in sample)} = (\text{ppm in extract} - \text{blank}) \times A/W \times \text{dilution factor.}$$

A = Total volume of extract (mL), W = Weight of dry plant.

Antioxidant activities

DPPH scavenging activity

Antioxidant activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. A portion of 1.950 ml of DPPH solution (6×10^{-5} M) diluted in methanol was incubated with 50 µL of the extract, of varying concentrations. The absorbance of the mixture was measured at 515 nm, and radical scavenging activity was expressed as EC_{50}, the effective concentration, which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.[17]

Superoxide anion radical scavenging assay

The superoxide anion radical scavenging activity of the plant extract was determined by the method of Salah et al.[18] Various concentrations (0.05−5.00 mg/mL) of seed extracts (0.1 mL) were mixed with 1 mL of nitro blue tetrazolium (NBT) solution in Tris-HCl buffer (16 mM, pH 8) and 1 mL of NADH solution in Tris-HCL buffer. The reaction was started by adding 0.5 mL of phenazine methosulfate (PMS) solution to the mixture, incubated at 25 °C for 5 min; absorbance was measured against control samples at 560 nm by UV spectrophotometer. The control contained 1 mL of dimethyl sulphoxide (DMSO) instead of the test sample. Superoxide anion radical scavenging activity was compared to ascorbic acid.

$$\text{SOD scavenging effect (\%)} = [(A_1 - A_0)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of sample. The EC_{50} was used to report the radical scavenging activity.

Statistical analysis

All determinations were conducted in triplicate and statistical analysis was performed using SPSS software 16.0 (SPSS Inc., Chicago, IL) and Prism Graphpad 6.0. The results were recorded as the mean ± SD.

Results and discussion

Phytochemical composition of date seed

Phytochemicals have been associated with the pharmacological or therapeutic effects elicited by plant materials.[19,20] In this investigation, date seeds were observed to contain appreciable amounts of alkaloids, flavonoids, anthraquinone, saponin, terpenoids and tannin (Table 1).

Alkaloids are one of the most important bioactive components in natural herbs. They constitute potent therapeutic agents. The Total Alkaloid content of the date palm seed was

Table 1: Phytochemical contents (mg/100 g dry weight) of date seeds.

Phytochemicals	Amount
Alkaloids	102.27 ± 9.28 ATE
Flavonoids	2471.23 ± 3.35 QE
Anthraquinone	334.93 ± 2.82 ALE
Saponin	124.41 ± 1.93
Terpenoids	19.47 ± 0.62
Tannin	133.20 ± 0.00

ATE = Atropine Equivalent, QE = Quercetin Equivalent, ALE = Alizarin Equivalent. Results were expressed as the mean of triplicate determinations ± standard deviation.

Table 2: Mineral contents (mg/100 g dry weight) of date seeds.

Minerals	Amount
Sodium	0.67 ± 0.12
Potassium	78.12 ± 0.13
Calcium	18.20 ± 0.20
Magnesium	0.48 ± 0.10
Iron	0.82 ± 0.00
Phosphorus	19.32 ± 0.02
Lead	0.00 ± 0.00
Zinc	0.25 ± 0.00

Results were expressed as the mean of triplicate determinations ± standard deviation.

estimated as an Atropine equivalent (Table 1). Atropine and its semi-synthetic derivatives cholinomimetics are widely used in the treatment of Glaucoma, myasthenia gravis and some rare cardiac arrhythmia. Other alkaloids include ephedrine for asthma, analgesic morphine, a famous topoisomerase I (TopI) inhibitor, camptothecin and vinblastine, an anticancer alkaloid that interacts with tubulin.[21–23]

The identification of flavonoids in this study corroborates the report of Sadiq et al.[24] Flavonoids are nature's biological response modifiers because of their ability to modify the body's reaction to allergy, viruses and carcinogens. They are renowned for their free radical scavenging potency, which underlines their antibacterial, anti-inflammatory, anti-thrombotic and vasodilatory activities.[25]

Tannins have diverse effects on biological systems because they are potential metal ion chelators, protein precipitating agents, and biological antioxidants. These properties may be responsible for their appreciable anti-tumour effects and astringent activity. Tannins have been reported to improve wound healing through protein precipitation.[26]

Saponins are plant-derived anti-inflammatory compounds that may lower blood cholesterol and prevent heart disease as well as cancers. Previous studies have reported the presence of saponins in date fruits and leaves.[27,28]

Plants terpenoids have medicinal importance, quinine derived from cinchona bark have been used as anti-malarial drugs. Carotenoids, one of the major subclass of terpenes, act as biological antioxidants and protect cells and tissues from the damaging effects of free radicals.[29] The presence of terpenoids in date seeds, similar to other previously mentioned phytochemicals, underscores their medicinal relevance.

Mineral contents of date seeds

Table 2 presents the information on mineral contents found in Niger grown date seeds. In our study, essential bulk ions like potassium, calcium, magnesium, iron, and phosphorus were found to be dominant in date palm seeds, and this observation agrees with the claims of Ali-Mohamed & Khamis.[30] However, the potassium, sodium and calcium contents of the date seed are not as high as the amounts found in date fruits.[27,31] Date seeds, like their fruits, have relatively high potassium content (78.12 mg/100 g) compared to sodium content. The high potassium to sodium ratio may be of health significance in patients with cardiovascular disease or disorder who consume date palm seed extract as coffee. Phosphorous was found to be 19.32 mg/100 g and calcium content was 18.20 mg/100 g. The phosphorus and calcium content was similar to those reported in KSA-grown date fruits.[31] The relatively high calcium content is essential for healthy bone formation and development. Moreover, both minerals play essential roles in the processes of metabolism.

In-vitro antioxidant activity of date seeds

Methanol is a solvent which yield significant amounts of polyphenol compounds when used for plant extraction.[32,33] The methanol extract of date seed that is effective on 50% concentration of DPPH (EC_{50}) was estimated to be 10.21 mg/mL (Figure 1). The antioxidant activity of date seeds is significantly lower than those reported in Algerian date palm leaf extracts.[28] It is however in range with EC_{50} of Khalas, a date fruit grown in KSA.[34]

The superoxide radical scavenging capacity of the seed was estimated to be 1.67 mg/mL (Figure 2). Vayalil in 2002 following an *in vitro* study reported that date fruit scavenged hydroxyl and superoxide free radicals via protection against protein oxidation and iron induced lipid peroxidation in brain homogenate of rats in a dose

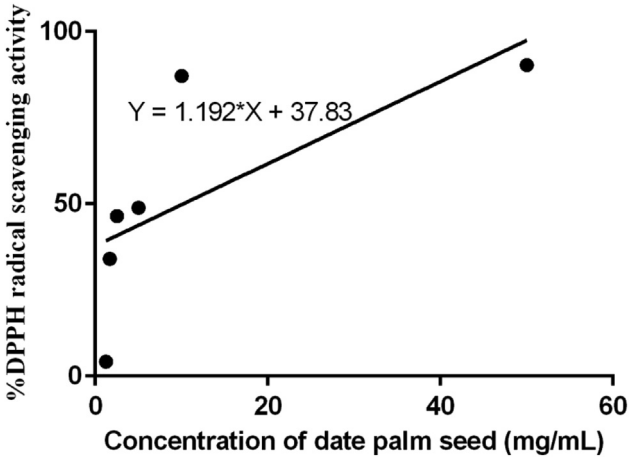

Figure 1: DPPH scavenging activity of date palm seed.

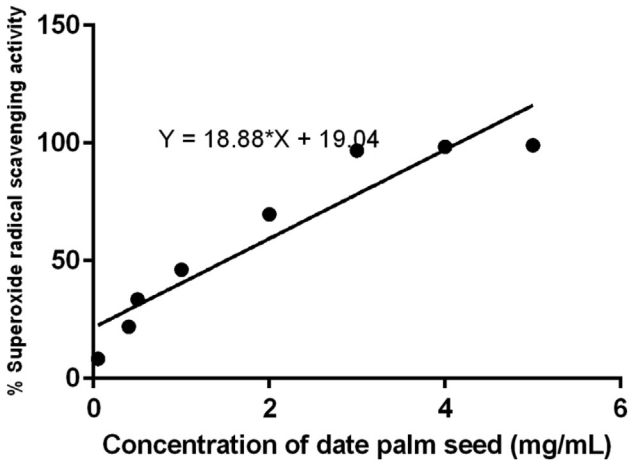

Figure 2: Superoxide radical scavenging activity of date palm seed.

dependent pattern.[35] The data gathered in this study vis-a-vis EC_{50} values suggest that date seeds may share a similar mechanism for free radical scavenging.

Conclusion

Our findings show that date seeds contain a wide range of phytochemicals and essential minerals such as potassium and calcium and are capable of eliciting notable free radical buffering effects. The seeds may therefore be of appreciable therapeutic relevance, as indicated by its local usage.

Conflict of interest

The authors declare no conflict of interest with regards to this study.

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This study was mainly funded by authors involved in the study.

Authors' contributions

AAM established design, interpreted data, performed statistical analysis, performed the experiments, make the initial draft and addressed comments to reviewers. OSO was involved in the experiment, co-designer of the project. IOM performed internal review of the paper, including some data interpretations. DOH purchased chemicals and prepared important solutions. OOM was the student that participated in all of the experiments.

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