

Phytochemical and antioxidant screening of the fruit and seed extract of *Phoenix dactylifera* linn and evaluation of its toxicological profile in albino rats

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ABSTRACT

Background: *Phoenix dactylifera* (date palm) is a common plant with various known benefits. The plant's health benefits have been associated with its high antioxidant profile.

Objectives: To conduct phytochemical, antioxidant, metal and vitamin analysis, and evaluate the hematological and biochemical effects of the fruit and seed extracts of *Phoenix dactylifera*.

Methods: Evaluation of *Phoenix dactylifera* for phytochemicals was conducted using standard methods. Quantitative determination of total phenolics, total flavonoids, and various in vitro antioxidant activities (DPPH and FRAP) was carried out using colorimetric methods.

Results: Phytochemical analysis shows the presence of alkaloids, flavonoids, terpenoids, phenols and tannins in both extracts. The antioxidant activity was also found to be 83.86 to 88.43% for seeds and 63.49 to 85.06% for fruit. The fruit and seed extracts contained Vit A (0.52/0.46 IU/100g), C (16.26/0.79 mg/100g), and E (45.63/49.87 mg/100g). Metal analysis indicated the presence of Mg, Zn, Ca, Cu, I and K with the seeds having a higher concentration of the metals than the fruits.

Serum biochemical analysis indicated no deleterious effects on vital organs, no significant changes observed in the levels of ALT, AST, albumin, bilirubin and total protein. The histopathological findings in the kidney and liver of rats treated with the extract were found to be normal. Hematological parameters showed no significant difference from control but MPV and MCV concentration were decreased significantly ($p < 0.05$) in animals treated with 100 mg/kg of the methanol seed extract. The body weights increased in a dose-dependent manner in rats treated with fruits extract. Conversely, a decrease in body weight was observed in those treated with seeds extract.

Conclusion: The seed and fruit extract of *Phoenix dactylifera* did not produce any toxic effect in the rats.

Keywords: Antioxidants, *Phoenix dactylifera*, fruits, seed extract?

INTRODUCTION

In recent times, the seeds and fruits of many plants, including some of the commonly consumed herbs and spices in our daily food, have been safely and effectively used in complementary and alternative medicine (CAM).^{1,2} Due to the increased commercial exploitation of medicinal foods, all varieties of fruits and vegetables are being re-evaluated for their phytochemical composition and invariably, their health benefits.³ Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms when taken as a medicine or as a part of daily diet.⁴ Several health benefits such as chemoprevention of cancer, prevention of diabetes and cardiovascular diseases as well as cholesterol-lowering properties, among others have been attributed to phytochemicals present in plants.^{5,6}

Phoenix dactylifera, commonly known as date or date palm is a flowering plant species that belongs to the palm family *Arecaceae* and cultivated for its edible sweet fruit called dates which is consumed in fresh, dried or processed forms.⁴ Various species of *Phoenix dactylifera* are widely cultivated across the Middle East, Northern Africa and South Asia, and it is naturalized in many tropical and subtropical regions around the world.⁷ *P. dactylifera* plays a significant role in the economical and the environmental condition in those areas where it is widely cultivated.

The date palm fruit has been reported to contain phytochemical substance such as flavonoid and phenolic compounds and has been employed for the treatment of various infectious diseases, cancer and its powerful antioxidant has been well documented.^{8,9} Date fruit has nutritional values rich in carbohydrates, dietary fibers, proteins, minerals and vitamin B complex.¹⁰ They also provide essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese.^{11,10}

The edible part of the date palm tree has been recognized to possess many medicinal properties when consumed alone or in mixture with other medicinal herbs.⁵ In recent years, a huge interest in the abundant health promoting properties of date fruits had led to many pharmacological studies (*in-vitro* and *in-vivo*) as well as the identification and quantification of different classes of their phytochemicals.¹² The concentration and composition of these phytochemical constituents are widely varied depending on several parameters, including date variety,

stage of fruit picking, storage, postharvest processing, the geographical origin of the dates and soil conditions.^{13,14,15} There are reports that the chemical constituents and functional composition of date fruits are dramatically changed during date maturing period with increasing in levels of reducing sugars, while fiber, mineral, and vitamin levels decreasing steadily.^{16,17,15}

Several health promoting effects of the date palm have been reported in *in-vitro* and *in-vivo* studies using either the pure aqueous and mixed aqueous/organic solvent extracts. However, the non-mixed organic solvent extracts of date fruits are currently not sufficiently covered in the literature. Organic solvents extracts of date fruits will lower the carbohydrate content, which is highly water soluble, concentration in the extracted materials.⁴ The aim of this study is to evaluate the phytochemical constituent, analyze metal and vitamin content, evaluate the antioxidant activity and carry out the effect of methanolic extract of fruit and seed of *Phoenix dactylifera* on hematological and biochemical parameters.

MATERIALS AND METHODS

Collection and Identification of Plant

Dry *Phoenix dactylifera* fruits and seeds were purchased from Oja-Oba market in Ilorin, Kwara State, Nigeria. The collected dried fruit was identified in the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria.

Animals

Thirty female wistar albino rats, 100-120 g obtained from the Animal house of the University of Ilorin were used for this study. They were given a period of acclimatization and free access to food *ad libitum* in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin.

Ethical clearance

Ethical approval was obtained from the University of Ilorin Ethics Review Committee and a voucher number FPS-ERC/ANS/2023/2 assigned. The experiment followed the Guidelines for laboratory procedures laid down by the University of Ilorin Ethics Committee on Research, and the International Animal Care and Use Committee (IACUC) in Nigeria.

Drugs and Reagents

Ranferon 12 syrup was obtained from a registered pharmacy in Ilorin. All other reagents and consumables

were obtained from the department of Pharmacology laboratory, University of Ilorin and they were of analytical grades.

Preparation of Methanolic fruits extract (MFE) and Methanolic seed extract (MSE) of *P. dactylifera*

Three hundred grams (300 g) of freshly blended dried fruits of *P. dactylifera* was extracted exhaustively with 1.6 L of 70% methanol for 48 hours then filtered; the filtrate was evaporated to concentrate the crude extract. The fruits extract was put in universal bottles and stored in a cool at -4°C place until required. The same procedure was repeated to extract the seeds. Percentage yield was calculated using the formula below;

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered material}} \times 100$$

PHYTOCHEMICAL ANALYSIS

Phytochemical Screening

The crude methanolic extracts of bark and leaves were tested for the presence of steroids, alkaloids, cardiac glycoside, flavonoids, saponins, terpenoids, phenols and tannins using standard procedures as described in literatures.^{18,19,20}

The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals

Total phenolic content

Total phenolic contents was evaluated with Folin-Ciocalteu's phenol reagent (Akinmoladun et al., 2007)²¹, 5 mL of the extract solution was mixed with 5 mL Folin-Ciocalteu reagent previously diluted with water. After 5 minutes, 4 mL of 7% Na₂CO₃ solution was added to the mixture. The tubes were shaken for 5 seconds and

allowed to stand for 30 min at 40°C for color development. The absorbance of the sample was measured at 765 nm, Gallic acid was used as standard (1 mg/mL). The test was performed in triplicate and the results were determined from the standard curve and expressed as gallic acid equivalent (mg/g of extracted compound).

Total flavonoid content

Total flavonoid content was determined using the aluminum colorimetric method adapted from Banothu et al., (2017).²² A calibration curve of quercetin was prepared in the range of 0-200 g/ mL. Briefly, 0.5 mL solution of plant extract in methanol was mixed with 2 mL of 2% aluminum chloride, the absorbance was measured at 420 nm at room temperature after one hour. All the tests were performed in triplicates. Flavonoid contents was determined from the standard curve and expressed as quercetin equivalent (mg/g of extracted compound).

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) Assay

DPPH radical scavenging activity was determined according to the method described by Ghasemzadeh et al., in 2010.²³ A Sample stock solution (1.0 mg/mL) was diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/mL in methanol. 1.5 mL of the different concentrations was added to 1.5 mL of methanolic solution DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as positive control while the mixture without the extract was taken as blank. Two replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

$$\% \text{ DPPH scavenging activity} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$

The Ferric reducing antioxidant power (FRAP) Colourimetric Test

The antioxidant capacity of the medicinal plants was estimated spectrophotometrically following the procedure of Owojuyigbe et al., (2020).²⁴ The method is based on the reduction of Fe³⁺ TPTZ complex (colorless

complex) to Fe²⁺-tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm.

FRAP reagent was prepared by mixing 25 mL of acetate

Sample collection for analysis

Twenty-four (24) hours after the administration of the last dose of treatments, animals from all the groups were euthanized and blood was obtained through cardiac puncture into plain bottles for biochemical assays of liver and kidney parameters. Heparinized vacutainer tubes were utilized for blood samples intended for hematological analysis

Hematological parameters

The white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) were determined using a fully automated hematology analyzer (Pentra-XL 80, Horiba ABX, USA).

Biochemical analysis

The procedure described by Yakubu *et al* (2009)²⁶ was adopted in the preparation of serum used for the biochemical analysis. The blood samples were allowed to clot and then centrifuged at 3,000 x g for 10 minutes and serum was aspirated using Pasteur pipette into sample bottles. Liver and kidney biochemical analysis were carried on the processed samples to assay the changes in

the levels of the following parameters; creatinine, urea, calcium, phosphorus, albumin, bilirubin, alkaline phosphatase (ALP), g-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by spectrophotometric determination using assay kits obtained from Roche Diagnostics GmbH (Mannheim, Germany).²⁶

Histopathological examination

The euthanized animals were dissected frontally, and perfused using normal saline to rid the organs of blood. The kidney and liver were excised and stored in plain bottles containing 4% Paraformaldehyde. Tissue samples were prepared for histopathological examination according to the method described by Krause (2001).²⁷ The photomicrographs were produced in bright light at x 400 magnification.

Relative organ weight

Percentage change in body weight (WT-A), weight of kidney (WT-K), weight of liver (WT-L) were noted and used to determine the relative organ weight of kidney (RW-K) and relative liver weight (RW-L). Relative organ weight (ROW) was determined as follows;

$$ROW = \frac{\text{Absolute Organ Weight}}{\text{Body Weight of Animal}} * 100$$

Statistical Analysis

Data were expressed as mean $\pm$ standard error of mean. The results were analyzed using Graph Pad version 9.1; one Way Analysis of Variance (ANOVA) followed by Dunnett's test was used. Statistical significance was set at $p < 0.05$.

RESULTS

Percentage yield

The percentage yield of the methanol fruit (MFE) and seed (MSE) extract of *Phoenix dactylifera* were 26.87% and 6.4% respectively.

Phytochemical Analysis

Alkaloids, flavonoids, terpenoids, phenols and tannins were found to be present in both methanol seed and fruit extract of *Phoenix dactylifera* (Table 1).

Table 1: Phytochemical screening of the methanol extract of fruit and seed of *Phoenix dactylifera*.

Phytochemical Constituent	Test	Seed	Fruits
Alkaloids	Wagner's Reagent	+	+
	Mayer's Reagent	+	+
Cardiac Glycosides	Keller-Kilani Test	-	-
Flavonoids	Alkaline (NaOH) reagent Test	+	+
	Shinoda Test	+	+
Saponins	Frothing Test	-	-
Steroids	CHCl ₃ + Conc. H ₂ SO ₄	-	-
Phenols and Tannins	AlCl ₃ Test	+	+
Terpenoids	CHCl ₃ + H ₂ SO ₄	+	+

Key: + = present, - = absent

Total phenolic and flavonoid Content

The total phenolic and flavonoid contents in the extracts of the seed and fruit of *Phoenix dactylifera* determined were found to be 0.22 ± 0.02 and 0.24 ± 0.01 mg/g gallic acid equivalent and 0.13 ± 0.01 and 0.06 ± 0.01 mg/g Quercetin equivalent respectively.

Table 2: Total phenol and flavonoids contents of the methanol extract of seed and fruits extract of *P.dactylifera*.

	Total phenolic content (mg GAE/g)	Total flavonoids content (mg QE/g)
Seed extract	0.22 ± 0.02	0.13 ± 0.01
Fruit extract	0.24 ± 0.01	0.06 ± 0.01

GAE = Gallic acid equivalent, QE = Quercetin equivalent

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) Assay

DPPH antioxidant activity was assayed at different concentration for both seeds and fruit methanol crude extract. The seed was observed to have better antioxidant activity at all concentrations tested relative to the fruit extract, though not significant ($P > 0.05$).

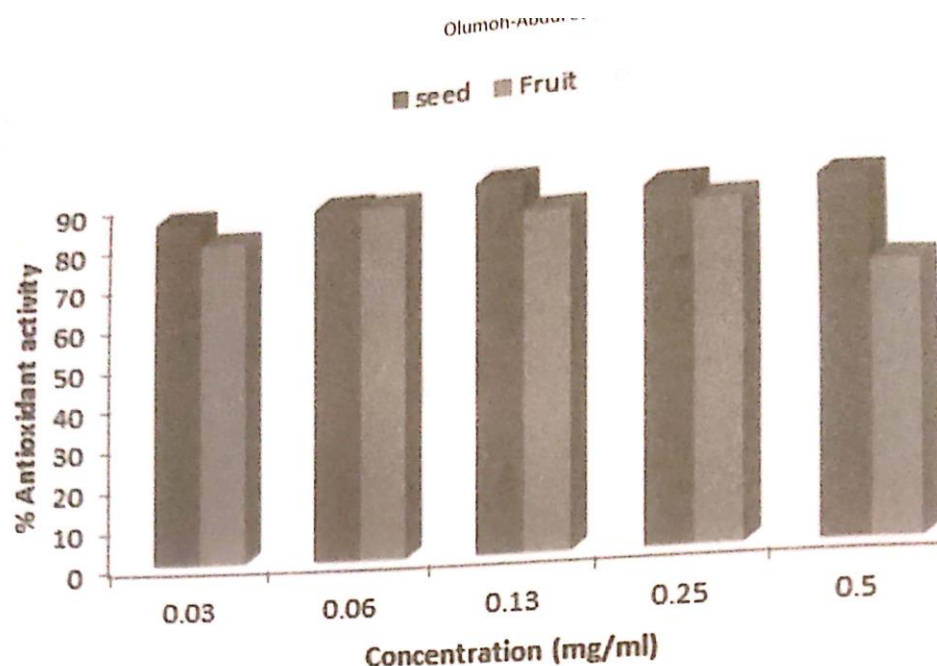


Figure 1: DPPH antioxidant activity of the methanol extract of seed and fruit of *Phoenix dactylifera*.

The Ferric reducing antioxidant power (FRAP) Colorimetric Test

Ferric reducing antioxidant power of methanol seed extract was found to be significantly ($P < 0.05$) higher compared to the methanol fruit extract at both concentrations assayed.

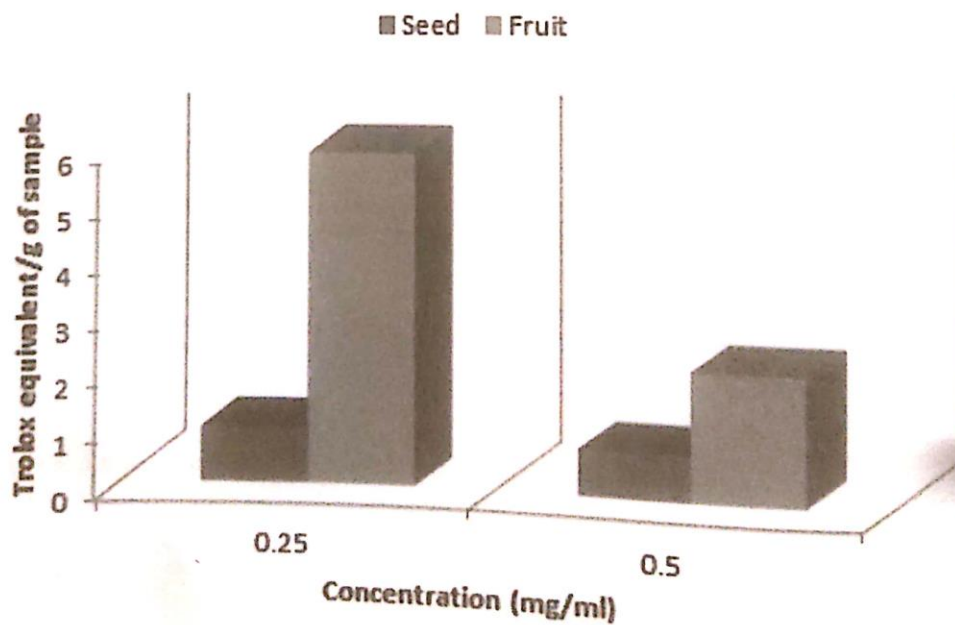


Figure 2: FRAP Antioxidant Analysis of the methanol seed and fruits Extract of *Phoenix dactylifera*

DISCUSSION

The phytochemical screening of crude methanol extracts of seed and fruit samples of *Phoenix dactylifera* revealed the presence of some secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols and tannins. The presence of these phytochemicals in date palm has been widely reported^{17,28,29} and they are believed to be responsible for the several medicinal and dietary benefits of the plant like the anti-inflammatory and antioxidants activities. The wide range of activities of alkaloids, flavonoids, terpenoids, phenols and tannins have been greatly reported in literatures, for instance alkaloid have been reported to possess anti-inflammatory,³⁰ antimalarial,³¹ antimicrobial³² and antioxidant³³ activities while tannins have antibacterial,³⁴ antitumor activities.³⁵ This has led to the general assumption that phytochemicals derived from plants have abundant health benefit,⁴ however their concentration and composition in date palm as with other plants depend on several makers such as the variety, stage of fruit picking, storage, post-harvest processing, geographical origin and the soil conditions.^{14,15}

The results revealed that the methanol extract of seed and fruit of *Phoenix dactylifera* has similar total phenolic content. Comparing the phenolic content from this study with the data available from other studies, it is found the values of phenolic content were lower (0.22 and 0.24 mg GEA/g for seed and fruit respectively). Anjum *et al.*, in 2012²⁸ reported a range of 2.288 - 5.648 mg/g gallic acid for methanol extract of varieties of date extracts in Pakistan while Nadeem *et al.*³⁶ documented a range of 142.5 to 298.02 mg gallic acid in 100 g fruit in 2019. The lower phenolic content observed in this study may be due to the separation of the seed and fruit of date palm for our study. Generally, dates are rich sources of phenolic compounds embedded with several health benefits; however, the phenolic content can be affected by parameter such as the geographical environment, soil type and the stage of harvest.³⁷

Flavonoids are of great importance as they help human body to fight against diseases and possess potent antioxidants tendency.³⁸ The total flavonoid content for both seed and fruit were also similar with the seed having slightly higher flavonoid content, although the values were lower compared to what has been recorded in most literatures. It is however in tandem with the work F.K Aldhafiri³⁹ that recorded a flavonoid content that was as low as 0.01 in a group of species of date palm examined.

Antioxidant assays showed that the seed and fruit extract evaluated in this study have excellent antioxidant capacity, however the seeds have better antioxidant activity compared to the fruit. The antioxidant activities of date palm have been widely attributed to the presence of large amount of polyphenolic compounds^{40,41} and are associated with their medicinal values.³⁸ In this study, the antioxidant activity of MSE and MFE of *P. dactylifera* was measured using two kinds of assays; FRAP and DPPH. It is imperative to employ more than one type of antioxidant capacity measurement to cover the various mechanisms of antioxidant action.⁴²

DPPH is a stable organic free radical that is absorbed maximally at 517 nm, It is based on the ability of the antioxidant to scavenge the DPPH radical that can donate electron or hydrogen atom. The free radical scavenging activity is a widely used method of evaluating the antioxidant activity in vitro.⁴³ FRAP assay on the other hand is a commonly used test to determine the antioxidant capacity of different extract, this method is based on the ability of sample to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}).²⁴ Data presented in this study demonstrated that the methanol seed and fruit extract of date palm are rich in antioxidants, this corresponds with reported works that have documented the high antioxidant capacity of *P. dactylifera*.^{44,38,45}

The seeds and fruits extract of date palm were tested for nutrient values of different vitamins content. Vitamins A, C and E were found in various concentrations which are in line with the vitamin contents provided in Food and Agricultural Organization of the United Nations)⁴⁶ i.e., 0.01-0.05 mg per 100 g vitamin B2 and 0.33-2.2 mg per 100 g B3 in fresh fruit⁴⁶. Vitamins are important for essential body functions such as tissue repair, waste removal, growth, proper and energy production.³⁷ The concentrations of Vitamin A and E in the seeds and fruit extracts were similar while the fruit contained a higher concentration of Vitamin C. The good nutritional value of dates has been widely attributed to the abundance of vitamin C in the plant.

Minerals are inorganic elements which are required by every living cell for proper functioning and structure maintenance. The methanol fruit and seed extract of *P. dactylifera* used in the study showed the presence of different minerals in varying concentrations. However, as compared to the fruit extract, the seed had higher concentrations of these minerals. Date palm is enriched with essential minerals such as calcium, iron, magnesium,

living and their administration for 28 days produced no deleterious effect on hematology parameters, liver and the kidney indices.

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The authors declare that there is no conflict of interest.

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