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

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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;

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

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% Na_2CO_3 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 DDPH (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:

$$\% \; DPPH \; scarvenging \; activity = \frac{Absorbance\;(DPPH) - Absorbance\;(Extract)}{Absorbance\;(DPPH)} \; \text{X } 100$$

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 Fe^{3}+ TPTZ complex (colorless complex) to Fe^{2}+-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

buffer, 2.5 mL TPTZ in 10 mM HCl and 2.5 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. Freshly prepared working FRAP reagent was collected using syringe (1.5 mL) and mixed with 5 mL of the appropriately extract and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (1.5 mL FRAP reagent+5 mL distilled water) after 30 min incubation at 37°C. All the determinations were performed in triplicates. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mg of Trolox equivalent per gram.

Vitamin analysis

Vitamin A assay

The sample (0.5 g) was homogenized and saponified with 2.5 mL of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 mms. The saponified extract was transferred to a separating tunnel containing 10-15 mL of petroleum ether and mixed well. The tower aqueous layer was then transferred, to another separating funnel and the upper petroleum ether layer containing the carotenoids were collected. The extraction was repeated until the aqueous layer became colorless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow color was read in a spectrophotometer at 460 nm using petroleum ether as blank the amount of Vitamin A (beta-carotene equivalent) was calculated using the formulae;

$$x = \frac{\mathit{Abs\ of\ sample}}{\mathit{Abs\ of\ standard}} * \text{Conc of standard} * \frac{\mathit{volume\ of\ extracting\ solvent}}{\text{weight of sample}}$$

Vitamin E assay

Into three stoppers centrifuge was measured 1.5 mL homogenized sample, 1.5 standard and 1.5mL water (blank) respectively. Then in test and blank 1.5 mL xylene was added, stopper mixed well and centrifuged. The xylene (1 mL) layer was transferred into other stopper tubes taking care not to include any ethanol or protein. A quantity of 1 mL α, α-Dipyridyl reagents was added to each tube and mixed thoroughly, 1.5 mL of the mixture was pipette into colorimeter cuvettes and extinction of test and standard was read against the blank at 460 nm. Then, in turn beginning with the blank, 0.33 mL ferric chloride was added and the absorbance was read at 520 nm.

$$x = \frac{\text{Abs of ssmple at 520 nm} - \text{Abs of sampleat 460 nm}}{\text{Abs of standard at 460 nm} * \text{conc of standard}} * \text{Volume of sample}$$

Metal analysis

Elemental analysis

Five elements namely; Ma, Fe, Zn, Cu and K were assessed quantitatively using the method of Horwitz [25] with the aid of Atomic Absorption Spectrometer (AAS) GBC Avanta Model. Standards and digested sample were aspirated and the mean signal responses were recorded at each of the element respective wavelengths

Treatment Groups

Thirty (30) Wistar albino rats were used for the experiment. Study animals were divided into six groups each of five rats; group I was administered with 0.2 mL of normal saline and served as the study negative control, groups II -V were administered with 100 mg/kg MFE, 200 mg/kg MFE, 100 mg/kg MSE and 200 mg/kg MSE respectively, group VI represented positive control and was administered Ranferon 12R syrup. All treatments were administered daily for 28 days using the oral route. The body weights of the animals were measured daily throughout the 28 day period of study.

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 ± 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 (MFE) 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_2SO_4	-	-
Phenols and Tannins	$AlCl_3$ Test	+	+
Terpenoids	$CHCl_3$ + H_2SO_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 ($P>0.05$).

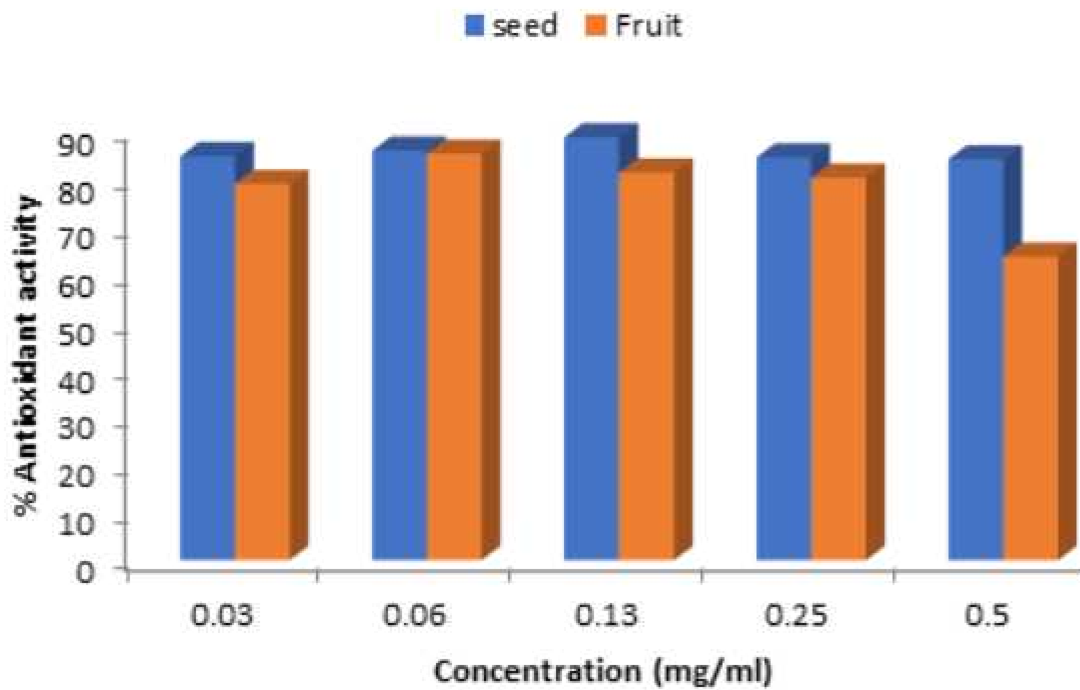

Figure 1: DPPH antioxidant activity of the methanol extract of seed and fruit of *Phoenix dactylifera*.

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.

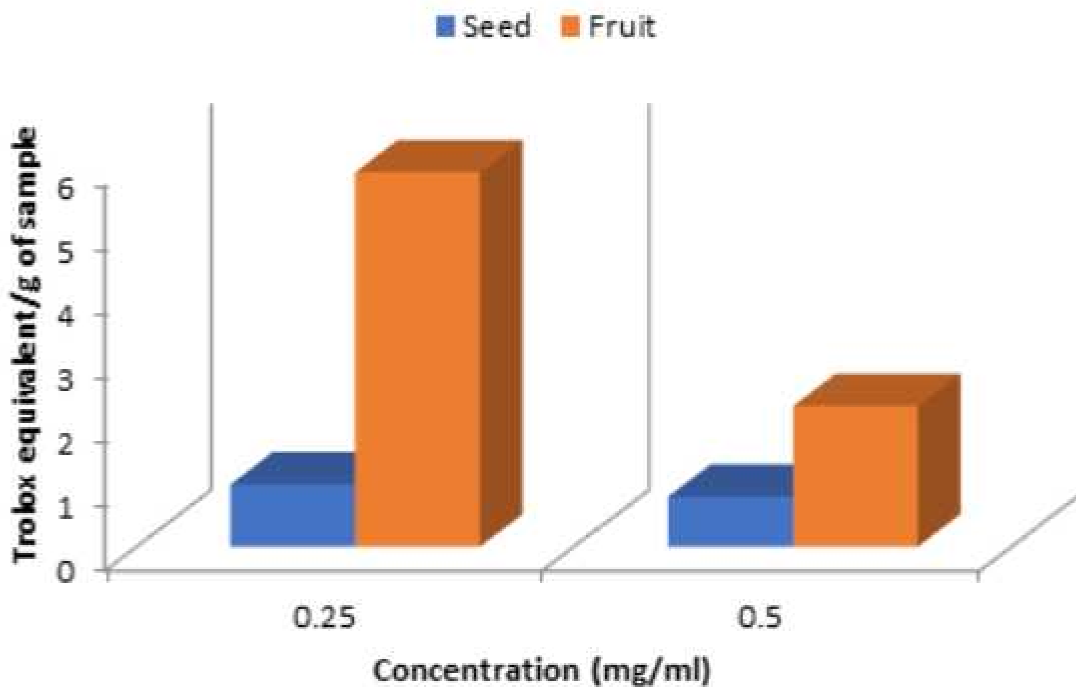

Figure 2: FRAP Antioxidant Analysis of the methanol seed and fruits Extract of *Phoenix dactylifera*

Vitamin Analysis

Table 3: Vitamin analysis of methanol seed and fruit extract of *Phoenix dactylifera*, the concentration of water-soluble vitamin A, C and E are as presented below.

SAMPLE	VIT A (IU/100g)	VIT C(mg/100g)	VIT E (mg/100g)
FRUIT	0.52	16.29	45.63
SEED	0.45	0.787	49.87

Elemental analysis

Table 4: Elemental analysis of the methanol seed and fruit extract of *Phoenix dactylifera*

Parameters	Fruit extract (mg/kg)	Seed extract (mg/kg)
Mg	0.09	0.34
Iron	0.05	0.04
Zinc	0.01	0.05
Cupper	1.42	2.42
Potassium	0.09	0.34

Hematological assay

Effect of methanolic fruit and seed extract of *Phoenix dactylifera* on Hematological parameters.

There was no significant difference ($p>0.05$) in the haematological parameters of the group administered with MFE (100 mg/kg and 200 mg/kg) and MSE (200 mg/kg) compared with the control group. Group treated with 100 mg/kg of the methanol seed extract however showed a significant ($p<0.05$) difference in the level of MPV and MCV.

Table 5: Effect of Methanol fruit and seed extract of *P.dactylifera* on hematological parameters

n=5, Data are presented as mean ± SEM, (p<0.05)

	Treatment mg/kg					
Parameters	**Control**	***MFE*-100**	***MFE*-200**	***MSE*-100**	***MSE*-200**	**Ranferon**
LYM(X10³/µL)	9.40±2.03	6.96±1.64	8.26±0.99	5.92±0.64	10.30±1.55	9.80±3.11
Mono(X10³/µL)	0.38±0.07	0.92±0.19	0.86±0.14	0.68±0.12	1.04±0.22	0.73±0.29
PLT(X104/µL)	65.46±12.54	64.74±11.49	36.94±3.02	48.80±9.80	47.44±6.37	56.28±13.94
Neutr(X10³/µL)	2.66±0.24	2.04±0.33	2.80±0.60	2.52±0.56	2.98±0.19	2.47±1.17
RBC(X106/µL)	6.99±0.27	6.88±0.32	6.09±0.47	6.46±0.31	6.51±0.39	7.05±0.72
WBC(X10³/µL)	10.63±0.24	8.48±0.65	9.93±0.63	9.22±0.64	12.07±0.27	12.97±4.21
HCT %	37.14±1.81	37.04±0.98	36.32±1.70	34.14±1.16	35.44±1.36	37.10±3.96
MCV (fl)	53.08±0.74	52.06±0.38	53.02±0.27	49.86±0.75*	51.74±0.58	52.53±0.35
MPV (fl)	7.80±0.07	7.50±0.10	7.80±0.14	7.28±0.08*	7.62±0.10	7.57±0.08
Hb (g/dl)	10.22±0.22	10.46±0.45	9.62±0.71	9.66±0.49	10.04±0.47	11.35±1.20

***RBC - Red blood cell count, WBC - White blood cell count, HGB - Hemoglobin levels, HCT - Total hematocrit, MCV - Mean corpuscular volume, MCH - Mean corpuscular hemoglobin, MCHC - Mean corpuscular hemoglobin concentration, PLT - Platelet count, LYM - Lymphocyte count, RDW - Red cell distribution width, PDW - Platelet distribution width, MPV - Mean platelet volume, PLCR - Platelet large cell ratio.

Biochemical analysis

Effect of methanolic fruit and seed extract of *Phoenix dactylifera* on Biochemical parameters.

There was no significant difference (p>0.05) in the creatinine, urea, total protein, total bilirubin, and direct bilirubin concentration. The levels of biochemical parameters remained unaltered in the group administered with MFE (100 mg/kg and 200 mg/kg) and MSE (100 mg/kg 200 mg/kg) compared with the control group.

Table 6: Effect of methanol fruit and seed extract of *P. dactylifera* on biochemical parameters

Parameters	Treatment mg/kg					
	Control	*MFE*-100	*MFE*-200	*MSE*-100	*MSE*-200	Ranferon
Creatinine	1.71±0.37	2.40±0.49	2.05±0.17	2.52±0.17	1.93±0.09	2.34±0.52
Urea	103.1±13.21	90.67±1.23	95.23±2.06	89.19±3.37	99.36±3.99	85.47±2.16
T. Protein	7.99±0.45	9.11±0.25	8.96±0.39	8.67±0.53	8.87±0.55	8.61±0.34
T. Bilirubin	3.69±0.24	4.54±0.36	4.29±0.29	3.84±0.42	3.80±0.28	4.29±0.22
D. Bilirubin	1.99±0.20	2.17±0.09	1.92±0.20	1.73±0.13	1.69±0.14	1.57±0.10
ALT	138±28.67	180.6±35.32	180±34.25	110.8±19.38	126.4±24.5	84.6±19.8
AST	1048±275.3	1136±196.5	1054±215	816.8±192.4	924±249.4	871.5±189
ALP	23.53±2.46	4.78±0.45	6.07±0.37	6.07±0.23	7.73±1.81	7.36±0.38

n=5, Data are presented as mean ± SEM, ($p<0.05$)

Relative organ weight

Effect of methanol fruit and seed extracts of *P. dactylifera* on relative organ weights of the liver and kidney

For the period of study, no deaths among experimental animals treated with both methanolic fruit and seed extracts were recorded. There were no significant ($p>0.05$) changes in the relative organ weights of the kidney and liver between various treatment groups when compared to the control group (Table 4). Change in body weight in group treated with MSE-100 was similar to the control group, however there was a significant difference ($p<0.05$) in the group treated with 100 and 200 mg/kg methanol fruits extract relative to control.

Table 7: Effect of methanol fruit and seed extracts of *P. dactylifera* on percentage weight gain and relative organ weight

	Treatment mg/kg					
Parameters	Control	*MFE*-100	*MFE*-200	*MSE*-100	*MSE*-200	Ranferon
Weight gain (%)	29.11±5.59	40.60±5.25*	50.81±4.12*	28.56±3.79	17.55±6.98	32.61±3.61
ROW-K ($X10^{-3}$)	3.48±0.12	3.11±0.16	3.16±0.10	3.46±0.11	3.50±0.08	2.92±0.16
ROW-L ($X10^{-2}$)	3.42±0.11	3.54±0.11	3.86±0.12	3.57±0.16	3.50±0.06	3.29±0.05

n=5, Data are presented as mean ± SEM, ($p<0.05$)

ROW-K- Relative organ weight of kidney, ROW -L- Relative organ weight of liver

Histopathological findings on kidney tissue

Sections showed tissue of numerous glomeruli which demonstrate no abnormalities. The histological features are consistent with normal histology under light microscope (Plates 1A-1F).

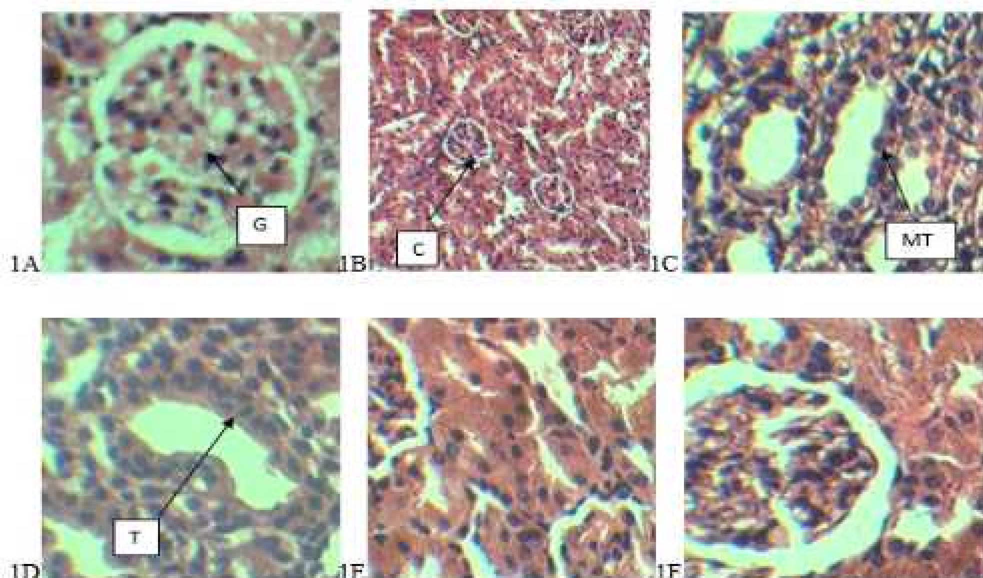

Figure 3: Sections of the kidney of rats treated with different doses of methanol seed and fruit extract of *P. dactylifera* (40x magnification). 1A) Control, 1B), 100 mg/kg MFE, 1C) 200 mg/kg MFE, 1D) 100 mg/kg MSE, 1E) 200 mg/kg MSE, 1F) ranferon.

***Glomerulus (G), Cortex comprising of glomerulus (C), medullary region composed of tubules (MT), Tubules (T).

Histopathological findings on liver tissue

Sections showed irregular shaped hexagonal plates of hepatocytes admixed with central vein. The histological features of livers of rats treated with methanol fruit and seed extracts of *P. dactylifera* are consistent with normal histology.

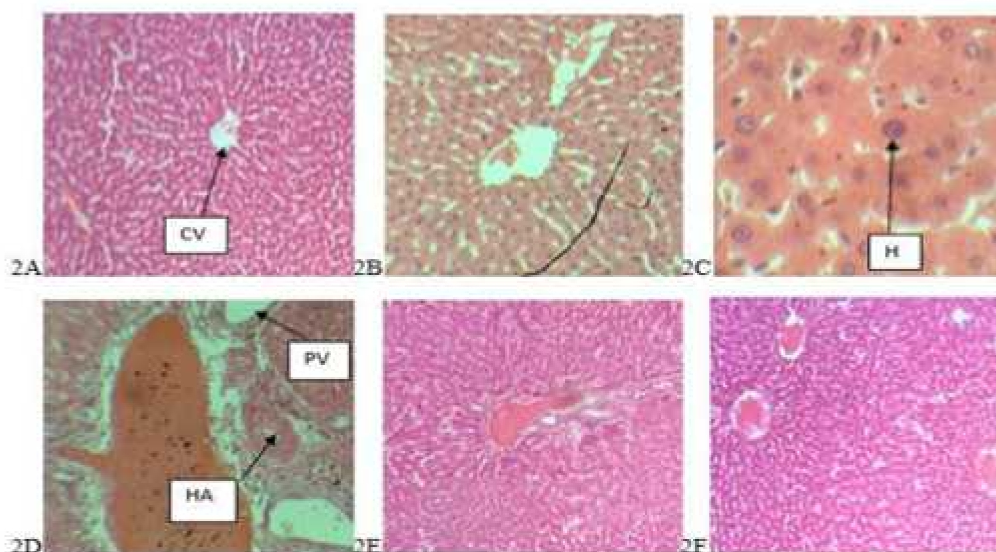

Figure 4: Section of the liver of rats treated with different doses of methanol seed and fruit extract of *P. dactylifera* (40x magnification). 1A) Control, 1B), 100 mg/kg MFE, 1C) 200 mg/kg MFE, 1D) 100 mg/kg methanolic seed extract, 1E) 200 mg/kg MSE, 1F) ranferon.

*** Central vein (CV), Hepatocytes (H), Portal vein (PV), Hepatic artery (HA).

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] antimicrobial[32] and antioxidant[33] 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 2012[28] 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 Aldhafiri[39] 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.dactilyfera*.[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,

phosphorus, potassium, zinc, selenium and manganese that are required for proper body functions. The results of the present study are in line with previous studies[47,37] that have reported varying values of micro and macro elements found in different varieties of date palms. The level of elemental minerals detected by these researchers also differs from the present study, which might be due to changes in variety, climate and soil type.[37]

There was no significant difference in the hematological parameters analyzed in this study relative to the control group; this implies that the methanol seed and fruit extract of *Phoenix datylifera* has no detrimental effect on hematolgical variables. However, a non-significant decrease was observed in the mean platelet volume (MPV) and mean corpuscular volume (MCV) of rats treated with 100 mg/kg of the seed extract. The study of MPV can provide important information on the course and prognosis in many inflammatory conditions,[48] a decrease may indicate that the average platelet size is small implying that perhaps a condition is inhibiting the production of platelets in the body.[49] MCV on the other hand measures the average size of red blood cells produced,[50] lower level of MCV indicate that RBC produced are smaller than normal which may be an indication of microcytic aneamia.[50] A similar study conducted by Orabi and Shawky (2014)[51] in Egypt reported a significant increase in the hemoglobin concentration, MCH and MCHC. The differences in the level of hematological parameters in both studies may be attributed to the difference in the geographical origin and the fresh date plant used.

The *P. dactylifera* fruits and seeds extract had no significant effect on the biochemical parameters examined except the alkaline phosphatase (ALP) levels. High level of liver and kidney biochemicals like ALT, ALP and AST are signs for hepatic damage,[26] they are measured as a part of a diagnostic evaluation of liver function test. This shows that the extracts have no deleterious effects on the liver. The methanol fruits and seeds of *Phoenix dactylifera* improved the levels of ALP, this agrees with the study of Fakhri *et al*., 2018[52] where the toxicity of aqueous extract of the seeds were evaluated. However, in the study, unlike ours the levels of AST, ALT, ALP and *albumin* were improved, this might be attributed the high dose of extract administered in the study (500, 1000, 1500 mg/kg) as compared to the present study that utilized 100 and 200 mg/kg.

Creatinine Kinase is an enzyme found primarily in the heart and skeletal muscles and to a lesser extent in the brain.[53] Significant injury to any of these structures will lead to a measurable increase in Creatinine Kinase levels,[53] but no such increase in Creatinine Kinase levels was observed in the present study. The levels of urea and creatinine in the treated rats did not show any significant difference relative to the control values, an indication that fruit and seed extracts of *Phoenix dactylifera* are neither nephrotoxic nor hepatotoxic.

An overall increase in weight was observed in all animals treated with methanol fruit extracts for the 28 days period of the study, in a dose-dependent manner, this supports the nutritional claims of the plant.[54,55] Increase in body weight is an indication that an extract promotes growth possibly by increasing the synthesis of protein (Abel *et.al*, 2013).[56] Interestingly, the changes in bodyweight in the groups of animals treated with methanol seed extract of *Phoenix dactylifera* was inversely related to the dose of the extracts, with the lowest dose giving the higher weight gain. It might have been that the seed of the plant affects rate of protein synthesis in high dose, importantly, the seed is usually not consumed with the fruit.

The weight of the kidney and liver of the treated group was not significantly altered, there was no significant difference observed in the relative organ weights relative to the control group, this may indicate that the drugs did not affect the organs adversely as increase in organ-body weight ratio may be due to inflamed organs while a decrease in the ratio typifies cellular constriction within the organs.[57,58]

The histopathology study showed no abnormalities with the tubules and glomeruli and no mononuclear inflammatory cells infiltrates in the kidney. Histopathology study of the liver displayed normal architecture in the liver of the treated groups. The data from this study shows that the methanol fruit and seed extract have no toxic effects on biochemical biomarkers of the liver as well as the histology of the liver and kidney.

CONCLUSION

In conclusion, *Phoenix dactylifera* fruit and seed extracts produced no significant increase in the hematological parameters like RBC, PCV and hemoglobin. In addition, the seed and fruit extracts of *Phoenix dactylifera* did not produce any toxic effect in the rats. They are found to be rich in phytochemicals and vitamins essential for healthy

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

ACKNOWLEDGEMENT

The authors wish to acknowledge the laboratory staff of the Department of Pharmacology and Toxicology, University of Ilorin for their support during the course of this study.

The authors declare that there is no conflict of interest.

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