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Sub-acute Administration of Aqueous Pericarp Extract of *Hyphaene thebaica* (Doum palm) on Haematological Parameters in Wistar Albino Rats

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ABSTRACT

This study is to ascertain the effect of sub-acute oral administration of aqueous pericarp extract of Hyphaene thebaica *(Doum palm) on some haematological parameters in Wistar albino rats using graded doses (250, 500 & 750 mg/kg) of the aqueous pericarp extract and Vitamin C (500mg/kg) as a standard drug. The fresh pericarp of the plant collected was ground into a fine powder, and the aqueous product was prepared using the reflux method. The study used twenty-nine (29) Wistar albino rats, 4 for the acute toxicity using the up and down method and 25 for the haematological parameters. The blood sample was taken from the experimental rats after 28 days of extract administration. The sample was used for parked cell volume (PCV, %), red blood cell count (×106mm3), haemoglobin concentration (Hb,%), mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), white blood cells count and differential leucocytic counts determination using a standard method. Phytochemical analysis of the aqueous pericarp extract of Hyphaene thebaica (Doum palm) revealed the presence of secondary metabolites such as saponins, carbohydrates, cardiac glycosides, cardenolides and flavonoids, respectively. There was a significant ($P<0.05$) increase in parked cell volume, haemoglobin concentration (HB), red blood cell count, white blood cell count (WBC) and differential leukocytic count (DLC) in the Wistar rats during the 28 days of treatment with aqueous* Hyphaene thebaica *pericarp extract and Vitamin C (standard drug). The comparative effect of graded doses of the plant extract and vitamin C showed that the aqueous plant extract had influenced the haematological parameters more than the standard drug (Vit. C). The information obtained from this study proved the claims by traditionalists and herbalists in Northeastern Nigeria on the use of pericarp extract of* Hyphaene thebaica *as a haematinic and blood extender. This study also indicated the plant effect on a differential leucocytic count, where neutrophils, eosinophils and basophils were increased. Hence, the plant product is in clinical trials for the immune system in biological subjects.*

Keywords: *Doum palm, oral administration, Hyphaene thebaica, hematological parameters, Wistar rats*

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INTRODUCTION

Hyphaene thebaica(L) Mart belongs to the family Palmae, the subfamily Borassoideae and belongs to the family Arecaceae. This plant is commonly known as doum palm, which means permanence in allusion to the persistence of the tree under abnormal conditions. Hyphaene thebaica is a desert palm native to Egypt, Sub-Saharan Africa and West India. It is commonly called the African doum palm (Amin and Paleologu, 1973; Dosumu *et al*., 2006). It belongs to the family of Arecaceae. The tree is in Egypt, Senegal, Sudan, Central Africa, Nigeria, Tanzania and Mauritania (Walter, 1971). Various parts of *Hyphaene thebaica* (Doum palm) are used in the management of conditions such as bilharzia, hypertension, cough, cancer, diabetes and other forms of bacterial infections, while the seed is used in the management of sore eyes in livestock (Adaya *et al*., 1977; Di Carlo *et al*., 1993; Wolarafe *et al*., 2007).

The application of synthetic antibiotics in third-world countries is not always possible due to their high cost and adulteration of products. Therefore, opportunistic infections resist in various forms to the synthetic antibiotics used. The preparation obtained from plants in many African communities is used for different medications to overcome the problems of antibiotic resistance (Fabiola, 1998). Approximately one-quarter of prescribed drugs contain plant extract or active ingredients obtained from or moulded from plant substances. Plant-derived products have a diversity of phytochemicals such as steroids, phenolic acids, flavonoids, tannins, lignin and other compounds (Cowan, 1999).

Ethno-medicine was introduced into mainstream healthcare delivery to complement orthodox medicine in achieving the World Health Organization's Healthcare for All goal (Abdulrahman, 1992). Medicinal Plants are a source of large amounts of drugs comprising different groups such as antispasmodics, emetics, anti-cancer, antimicrobials, etc. *Hyphaene thebaica* extracts are used in treating bilharzia, haematuria, and bleeding, after childbirth and as a haematinic agent (Adaya et al., 1977; Von Maydell, 1986). In a similar study using ethanolic pulp extract of the plant, Kamis *et al*. (2003) reported that at high concentrations, the plant is hypolipidemic, hepatotoxic and nephrotoxic. However, Modu et al. (2001) used an aqueous pulp extract of *Hyphaene thebaica* (L) Mart found the extract to be hypolipidemic but nontoxic (Bidlack *et al*., 2000; Prashant *et al*., 2011; Padayatty et al., 2003).

Hyphaene thebaica is a desert palm native to Egypt, Sub-Saharan Africa and West India. It is African doum palm (Dosumu *et al*., 2006). It belongs to the family of Arecaceae. Various extracts of *Hyphaene thebaica* treat hypertension, bilharzias and a haematinic agent (Adaya *et al*., 1977). The aqueous extract of the doum fruit showed antioxidant activity due to its substantial water-soluble phenolic contents (Hsu, 2006). The aqueous fruit pulp extract treats *Diabetes mellitus* (Shehu *et al*., 2015).

It is estimated that approximately one-quarter of prescribed drugs contain plant extracts or active ingredients obtained from or moulded from plant substances (Tripathi L.

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and Tripathi J., 2003). Plant-derived products comprise a great diversity of phytochemicals such as steroids, phenolic acids, flavonoids, glycosides, terpenes and terpenoids, anthraquinones, tannins, lignin and other compounds (Cowan, 1999; Bidlack*et al*., 2000).

The significance is to provide scientific information backing or refuting the claims of using aqueous pericarp extract of *Hyphaene thebaica* (Doum palm) by traditionalists and herbalists in Northeastern Nigeria as a haematinic or blood extender.

MATERIALS AND METHODS

Plant collection and Identification

The fresh pericarp of *Hyphaene thebaica* (Doum palm) was bought in September 2023 at Tashan Bama market, Maiduguri, Borno State, North-Eastern Nigeria. The seeds were authenticated by a taxonomist at the Department of Biological Science, University of Maiduguri. Voucher specimen No. 95 of the authenticated plant was deposited at the Department of Veterinary Pharmacology and Toxicology, University of Maiduguri for reference.

Experimental Animals and Treatment

A total of twenty-nine (29) Wistar albino rats were used; 4 rats for acute toxicity study using the up and down method and 25 rats for haematological parameters determination. The twenty-five (25) rats were divided into five (5) groups (A -E) of five (5) rats each. Group A was used as control (naive), Groups (B - D) were administered graded doses (250, 500 & 750 mg/kg) of aqueous pericarp extract of Hyphaene thebaica, Group E (Positive control) was administered vitamin C. Blood samples were taken from these rats after 21 days of extract administration. The samples obtained were used for parked cell volume (PCV,%), red blood cell count (x10°mm3), haemoglobin concentration (Hb,%), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), White blood cells count and Differential leucocytic counts determination using the method of Coles (1986).

Preparation of aqueous *Hyphaene thebaica* (Doum palm) Pericarp extract

The fresh pericarp of *H. thebaica* (Doum palm) collected was ground into powder and stored in a glass bottle. One hundred and twenty-six grams (126 g) of the aqueous product was prepared by reflux from 300g of the initial powdered sample. The aqueous seed extract obtained was then concentrated, labelled and stored in a refrigerator at 4°C until use.

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Phytochemical analysis of aqueous pericarp extract of *Hyphaene thebaica* (Doum palm)

The aqueous extract obtained from the pericarp of the plant was subjected to a phytochemical test using standard methods (Trease and Evans, 1989; Odebiyi and Sofowora, 1978).

Test for tannins (Ferric chloride test

A 2ml of the aqueous solution of the extract was added to a few drops of 10% ferric chloride solution (light yellow). The blackish-blue color showed the presence of garlic tannins and a green-blackish color indicated the presence of catechol tannins. Test for saponins (Frothing test) 3ml of the aqueous solution of the extract was mixed with 10ml of distilled water in a test tube. The test tube was stopped and shaken vigorously for about 5 minutes; it was allowed to stand for 30 minutes and observed for honeycomb, which indicated the presence of saponins.

Test for alkaloids

A 1g extract was dissolved in 5 ml of 10% ammonia solution and extracted with 15 ml chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15ml of dilute sulphuric acid. One-quarter of the solution was used for the general alkaloid test while the remaining was used for specific tests Mayer's reagent (or Bertrand's reagent). Drops of Mayer's reagent were added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids. Dragendorff's reagent 2ml of acidic solution in the second test tube was neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed which was indicative of the presence of alkaloids.

Tests for carbohydrates (Molisch's test)

A few drops of Molisch's solution were added to 2 ml of aqueous solution of the extract, and a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed as a purple color which indicated the presence of carbohydrates.

Tests for carbohydrate (Barfoed's test)

A 1ml of aqueous extract and 1ml of Barfoed's reagent was added into a test tube, and heated in a water bath at 60°C for about 2 minutes. The formation of a red precipitate indicated the presence of Monosaccharides.

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The standard test for combined reducing sugars

A 1ml of the aqueous solution of the extract was hydrolyzed by heating with 5ml of dilute hydrochloric acid. This was neutralized with a sodium hydroxide solution. The Fehling's test was repeated as mentioned previously and the tube was observed for brick-red precipitates that indicated the presence of combined reducing sugars.

Standard test for free reducing sugar (Fehling's test)

A 2ml of the aqueous solution of the extract in a test tube was added to a 5 ml mixture of equal volumes of Fehling's solutions I and II and heated in a water bath for about 2min. A brick-red precipitate was formed as the reaction between the aqueous pericarp extract of *Hyphaene thebaica* and Fehling solution I and II which indicated the presence of reducing sugars.

Test for ketenes'

A 2ml of aqueous solution of the extract was added to a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid; and then heated over a spirit lamp flame. This was observed for a rose colouration that showed the presence of ketones.

Test for pentoses

A 2ml of the aqueous solution of the extract was added to an equal volume of concentrated hydrochloric acid containing little phloroglucinol, and then this was heated over a spirit lamp flame; and was observed for red coloration, indicative of the presence of pentoses.

Test for phlobatannins (Hydrochloric acid test)

A 2ml of the aqueous solution of the extract was added to dilute hydrochloric acid and observed for red a precipitate formation that indicated the presence of phlobatannins.

Test for Cardiac glycosides

A 2ml of the aqueous solution of the extract was added to 3 drops of a strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5ml of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2ml concentrated sulphuric acid in a

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test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration, respectively. This indicated the presence of cardiac glycosides. Test for steroids (Liebermann-Burchard's test) 0.5g of the extract was dissolved in 10ml anhydrous chloroforms and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution was mixed with 1ml of acetic anhydride followed by 1ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green coloration which indicated the presence of steroids.

Test for steroids (Salkowski's test)

The second portion of the solution was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colouration, indicative of steroid ring Test for flavonoids (Shibita's reaction test) 1g of the water extract was dissolved in methanol (50%, 1-2 ml) by heating. Metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution which became red and orange indicated the presence of flavonols and flavones, respectively.

Test for Flavonoids (Pew's test)

To 5ml of the aqueous solution of the water extract was added, 0.1g of metallic zinc and 8ml of concentrated sulphuric acid. The reaction mixture was observed for red colouration; indicative of the presence of flavonoids.

Test for Anthraquinones (Borntrager's reaction for free Anthraquinones)

A 1g of the powdered seed was placed in a dry test tube and 20ml of chloroform was added. This was heated in a steam bath for 5min. The extract was filtered while hot and allowed to cool. To the filtrate was added an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink coloration, which is an indication of the presence of anthraquinones. Control tests were done by adding 10ml of 10% ammonia solution in 5 ml chloroform in a test tube.

Determination of Red Blood Cell Count (RBC)

In determining the red blood cell count according to Cole (1986), the following materials were used. Diluting fluid (Hayem's fluid), red cell pipette, and counting chamber (hemocytometer of improved Neubauer type). The red cell pipette has a graduation mark (0.5 and 1) on the capillary stem below and 101 above the bulb with a red bean inside the pipette. The pipette was cleared and dried. The blood was sucked up to 0.5 marks and immediately Hayem's diluting fluid was drawn up to 101 marks. The blood was mixed

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thoroughly with the diluting fluid for one minute. The counting chamber and the coverslip were dried and cleaned with cotton wool. The coverslip was placed on the counting chamber to fit in. The first few drops of the fluid from the pipette were discarded and the tip of the pipette was brought in contact with the exposed part of the chamber to allow fluid to flow under the coverslip. The cells were allowed to settle for 1-3 minutes and the cells in the 5 central squares of the chamber were counted under the x40 objective of the light microscope. The number of red cells counted was multiplied by ten thousand (10,000) to give the number of red cells in millions per cubic millimeter (x106mm3).

Determination of White Blood Cell Count (WBC)

The white blood cells were counted using a hemocytometer as described by (Schalm *et al.*, 1975). A leukocyte diluting fluid pipette was used to draw blood exactly to the 0.5 mark, the tip of the pipette was wiped free of excess blood and then used to draw in the leukocyte diluting fluid (Turk's solution) to mark 11. The pipette was shaken thoroughly to mix and then allowed for 3 minutes. About 2-3 drops of the fluid were discarded from the pipette and the tip of the pipette was brought close to the chamber, and the next drop fell near the coverslip and the chamber was filled due to capillary action. The counting chamber was allowed for one minute before counting using the x40 objective of the light microscope. The cells in the four corners were counted considering those cells inside the squares and from two sides. The number of cells counted was multiplied by 50 to give the total number of cells per cubic millimeters (×109/L).

Determination of Packed Cell Volume (PCV)

The blood samples were collected from the tail vein of the rats by allowing them to run into a heparinized capillary tube by capillary action until the tube was three-quarters filled. The end of the tube in contact with the blood was sealed with plasticine and centrifuged at 15,000 revolutions per minute (rpm) for 5 minutes. The PCV was then read as a percentage (%) using a microhematocrit reader.

Determination of Hemoglobin Concentration (Hb)

The haemoglobin concentration was determined by the colourimetric method. About 0.2ml of blood from the tail vein of the rats was pipetted into a test tube containing 5ml of Drabkin's solution and was vigorously shaken. The mixture was allowed to stand for about 3 minutes for the blood to react with the cyanide solution properly. The colourimeter was switched on for ten minutes to stabilize before use. The mixture was transferred into a clean cuvette and then placed in a colourimeter using a filter of 520 wavelengths to determine the

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optical density of a sample. The haemoglobin concentration (%) corresponding to the optical density was read using a standard chat.

Differential Leukocyte Count

A drop of blood was placed at one end of a clean, grease-free glass slide. Using a cover slip, the blood was allowed to spread along its edge at an angle of approximately 45°. The cover slip was pushed along the slide drawing the blood until it was smeared. The film was allowed to air dry then fixed with 100% methanol and allowed for 1 minute, then rinsed. It was stained with Giemsa stain. The film was covered with one volume of Giemsa stain and allowed for 2 minutes. Two volumes of buffered distilled water with a pH of 6.8 were added and mixed gently. It was allowed to stay for 10 minutes and then washed to remove the excess stain. The slide was then viewed using a microscope under an oil immersion objective. The film was examined by moving three fields along the edge, two folds up, two fields at the middle and two fields down starting at the end of the smear. The sequence was continued until a maximum of 100 cells were counted. The main types of leukocytes identified were eosinophils, neutrophils and basophils. The value of each leukocyte type was expressed as a percentage (×109/L) (Cole, 1986).

Statistical Analysis

Data obtained were analyzed and expressed as Mean ± Standard deviation (SD). Statistical analysis of data was performed using Graph Pad Prism 5.03 and Microsoft Excel. Data were analyzed by ANOVA along with Bonferroni multiple comparison post hoc test. A value of ($p \leq 0.05$) was considered statistically significant (Mead and Curnow, 1982).

RESULTS

The extract of 75.14% $^{w}/_{w}$ had a brown colour and readily dissolved in water. The Phytochemical analysis of the aqueous pericarp extract of *Hyphaene thebaica* (Doum palm) revealed that saponins, carbohydrates, cardiac glycosides, Cardenolides and flavonoids were the most abundant. Tannins, alkaloids, terpenoids and anthraquinones were absent in the aqueous pericarp extract (Table 1) below.

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Table 1: Qualitative Phytochemistry of the crude aqueous Pericarp extract of *Hyphaene thebaica* (Doum palm)

Phytochemical constituents		Tests	Inference
Tannin	1.	Ferric chloride	-
	2.	Lead acetate	-
Saponins	1.	Frothing	+
Alkaloid	1.	Dragendorff's	-
	2.	Wagner's	-
Carbohydrate	1.	Molisch's	+
	2.	Barfoed's	+
	3.	Combine reducing sugar	+
	4.	Free reducing sugar	+
	5.	Ketone's	+
Terpinoids	1.	Hydrochloric acid	-
Cardiac glycosides	1.	Salkowski's	+
	2.	Liebermann – Burchard	+
Cardenolides	1.	Keller-Killians	+
Flavonoid	1.	Shinoda's	+
	2.	Ferric chloride	-
	3.	Lead acetate	-
	4.	Sodium hydroxide	+
Anthraquinones	1.	Free Anthroquinones	-
	2.	Combined Anthroquinones	-

Key: + Present - Absent

The result of the effect of aqueous pericarp extract of *Hyphaene thebaica* on parked cell volume (PCV), haemoglobin concentration (HB), and red blood cell count (RBC), white blood cell count (WBC) and differential leukocytic count (DLC) is shown in Table 2 below. There was a significant ($P<0.05$) increase in parked cells volume of (66.42 ± 4.46b) in rats treated with 750 mg/kg of the plant, and the insignificant increase was observed in rats administered the other doses and Vitamin C (standard drug) during 28 days treatment. There was significant ($P<0.05$) dose-dependent ($P<0.05$) increase in haemoglobin concentration (HB) of (41.86 ± 4.41**b,** 47.72 ± 1.86**c,**48.68 ± 1.36**d)** in rats treated with (250, 500 and 750 mg/kg) of aqueous *Hyphaene thebaica* pericarp extract, whereas insignificant ($P>0.05$) increase was observed in the group of rats treated with Vit C (standard drug). There was a significant ($P<0.05$) increase in red blood cell count (8.70 ± 0.21**b***)* in rats treated with 750 mg/kg of the plant extract whereas an insignificant in the same parameter was observed in rats administered other doses and Vitamin C (standard drug) during the 28 days treatment. There was a significant ($P<0.05$) dose-dependent increase in white blood cell count (WBC), (17.12 ± 1.96b**,** 28.78 ± 1.45c**,** 32.40 ± 2.43d**)** in rats treated with (250, 500 and 750 mg/kg)

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of aqueous pericarp extract of *Hyphaene thebaica*, significant (P<0.05) increase of (29.30 ± 3.84e) was also observed in the group of rats treated with the standard drug (Vit C). There was a significant dose-dependent (P<0.05) increase in the differential leukocytic count (DLC), i.e. neutrophils, eosinophils and basophils during 28days treatment with aqueous pericarp extract of *Hyphaene thebaica*and Vitamin C (standard drug).

Table 2: Effect of acute exposure of Aqueous Pericarp extract of *Hyphaene thebaica* (Doum palm) on some haematological parameters in Wistar Albino rats

Groups/Treatments	Mean ± SD (Haematological parameters)						
	PCV (%)	HB (%)	RBC ($x10^6mm^3$)	WBC ($\times10^9$/L)	Neutrophil ($\times10^9$/L)	Eosinophils ($\times10^9$/L)	Basophil ($\times10^9$/L)
Control	45.90 ± 4.92[a]	26.38 ± 1.78[a]	7.26 ± 1.29[a]	8.18 ± 0.87[a]	7.30 ± 0.15[a]	0.76 ± 0.11[a]	0.70 ± 0.070[a]
250 mg/kg	51.88 ± 6.25[a]	41.86 ± 4.41[b]	7.54 ± 0.74[a]	17.12 ± 1.96[b]	8.30 ± 0.20[b]	1.42 ± 0.16[b]	1.30 ± 0.12[b]
500 mg/kg	53.80 ± 9.68[a]	47.72 ± 1.86[c]	6.94 ± 0.56[a]	28.78 ± 1.45[c]	8.28 ± 0.26[c]	2.34 ± 0.11[c]	2.46 ± 0.32[c]
750 mg/kg	66.42 ± 4.46[b]	48.68 ± 1.36[d]	8.70 ± 0.21[b]	32.40 ± 2.43[d]	13.12 ± 0.51[d]	2.78 ± 0.13[d]	3.44 ± 0.09[d]
250 mg/kg Vit C	45.16 ± 1.92[a]	27.78 ± 4.35[a]	6.86 ± 0.86[a]	29.30 ± 3.84[e]	5.70 ± 0.43[e]	3.42 ± 0.15[e]	4.46 ± 0.26[e]

Key: *Mean ± SD*
a – Significantly (P < 0.05) lower than the normal value
b, c, d, e – Significantly (P < 0.05) higher than the normal value

DISCUSSION

Phytochemistry studies chemicals produced by medicinal and toxic plants, particularly the secondary metabolites. Phytochemistry takes plants' structural compositions, biosynthetic pathways, functions and mechanisms of actions of these metabolites in the living system. The study of phytochemicals has been instrumental in discovering new plant and natural products of commercial value in various industries such as the traditional and complementary medicine systems, pharmaceutical, nutriceuticals, and dietary supplement industries. Secondary metabolites are important plant constituents for effective therapeutic activities. It was reported that the presence of this specific group of compounds showed specific medicinal actions and was sometimes traditionally reported, but there are few ways of scientific validation or clinical trials of these secondary metabolites (Agusta, 2003; Veena *et al*., 2015). Consumption of medicinal herbs protects and heals some ailments and has been the principal therapy in prehistoric times until the discovery of synthetic drugs in the nineteenth century (Rao *et al*., 2014).

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The *Hyphaene thebaica* (Doum palm) pericarp extract of 75.14% w/w of the dry pericarp extracted *in vitro*, the product had a brown colour and readily dissolved in water. The result of the Phytochemical analysis of the aqueous pericarp extract of *Hyphaene thebaica* (Doum palm) presented in Table 1 showed the presence of saponins, carbohydrates, cardiac glycosides, cardenolides and flavonoids as the most abundant compounds, tannins, alkaloids, terpenoids and anthraquinones were absent in the aqueous pericarp extract of the plant.

There were reports that saponins possess anti-anemic potential. Saponins are also known to inhibit platelet aggregation, thrombosis and various forms of intravascular coagulation. Plants containing saponins have been successfully used in the management of hepatitis, as tonics, and sedatives, and to promote revitalized blood circulation (Falcone et al., 1997; Wang *et al*., 2005). This corresponded with the findings in this research, where the aqueous extract of *Hyphaene thebaica* (Doum palm) contained saponins and exhibited anti-anemic potentials.

The result of the effect of the aqueous *Hyphaene thebaica* pericarp extract on parked cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), white blood cells count (WBC) and differential leukocytic count (DLC) is shown in Table 2. There was a significant ($P<0.05$) increase in parked cell volume (66.42 ± 4.46**b***)* in rats treated with 750 mg/kg of the plant, whereas insignificant in the same parameter was observed in the oral administration of the other doses and Vitamin C that was used as a standard drug during the 28 days treatment. There was a significant ($P<0.05$) dose-dependent ($P<0.05$) in haemoglobin concentration (HB) (41.86 ± 4.41b, 47.72 ± 1.86c, 48.68 ± 1.36d**)** in rats treated with (250, 500 and 750 mg/kg) of aqueous *Hyphaene thebaica* pericarp extract, whereas insignificant ($P>0.05$) in the group of rats treated with Vitamin C (Standard drug). There was a significant ($P<0.05$) increase in red blood cell count of (8.70 ± 0.21b*)* in rats treated with 750 mg/kg of the plant, with no increase in the same parameter observed in the oral administration of the other doses including Vitamin C that was used as a standard drug during 28 days treatment. There was a significant ($P<0.05$) dose-dependent increase in white blood cell count (WBC) of (17.12 ± 1.96b**,** 28.78 ± 1.45c**,** 32.40 ± 2.43d**)** in rats treated with (250, 500 and 750 mg/kg) of aqueous *Hyphaene thebaica* pericarp extract, significant ($P>0.05$) increase of (29.30 ± 3.84e) was also observed in the group of rats treated with the standard drug (Vit C). There was a significant ($P<0.05$) dose-dependent ($P<0.05$) increase in the differential leukocytic count (DLC), i.e. neutrophils, eosinophils and basophils during 28 days of treatment with aqueous *Hyphaene thebaica* pericarp extract and Vitamin C as a standard drug.

Anaemia is a medical condition in which the number and size of red blood cells, or haemoglobin concentration, drops below the physiological level, affecting the tendency of the haemoglobin to carry oxygen to the body tissue (WHO, 2017). Anaemia is the most frequently encountered public health condition worldwide, particularly in developing and underdeveloped countries (Sanogo, 1992). The causes of anaemia are multiple among which

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are iron deficiency, deficiency of vitamins such as vitamin B12, A and C as well as foliqueriboflavin acid that influences the formation of haemoglobin in the body (GOI, 2013; WHO, 2017). Those most at risk of anaemia are infants, children at their intensive growth stage, the elderly and pregnant women (Zinebi *et al*., 2017).

A report by some researchers indicated that oral administration of a plant *Tectona grandis* (Lamiaceae) extract to rats, previously treated with phenylhydrazine, increased haemoglobin concentration, red blood cell count, hematocrit and reticulocyte count (Diallo *et al*., 2016). Veena *et al*. (2015) also reported that the combination of aqueous extract of *Azadirachta indica* (Meliaceae) and *Emblica officinalis* (Euphorbiaceaea) produced significant anti-anemic activity. The aqueous extract was administered for 15 days at 200 and 400 mg/kg body weight in rats previously treated with 60 mg/kg b.wt. of Phenyl hydrazine (PHZ). The results indicated a significant increase in haematological parameters. Study on the anti-anaemic potential of three plant extracts namely *Mangifera indica* (Anacardiaceae), *Telfairia occidentalis* (Cucurbitaceae) and *Amaranthus hybridus* (Amaranthaceae) on phenyl hydrazine induced anaemia in rabbits showed an anti-anaemic effect. Phytochemical analysis of these plants is found to possess saponins, cardiac glycosides and flavonoids that are presumed to have anti-anaemic activities in the laboratory animals subjected to the extract of these plants (Ogbe *et al*., 2010).

The anti-anemic potential of medicinal plant extracts observed *in vitro* experiments in laboratory animals could be due to the presence of different plant phytochemicals namely saponins, tannins, alkaloids and flavonoids present in these medicinal plants which act as a source of powerful antioxidants that prevent or repair damaged blood cells by free radicals neutralization or absorption of highly reactive oxygen species at the cellular surfaces of blood cells (Ogbe *et al*., 2010).

CONCLUSION

The qualitative phytochemical analysis of the aqueous pericarp extract of *Hyphaene thebaica* (Doum palm) revealed the presence of secondary metabolites such as saponins, carbohydrates, cardiac glycosides, cardenolides and flavonoids. The pericarp of the plant has anti-anaemic properties which influence the increase in parked cell volume, haemoglobin concentration (HB), red blood cell count, white blood cell count (WBC) and differential leukocytic count (DLC) in the Wistar rats during 28 days treatment. This study proved the claims by traditionalists and herbalists in Northeastern Nigeria on the use of pericarp extract of *Hyphaene thebaica* as a haematinic and blood extender. The plant also significantly affects the differential leucocytic count, where neutrophils, eosinophils, and basophils have significantly ($P<0.05$) increased, technically indicating that the pericarp of the plant is an immune booster.

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RECOMMENDATION

The *In-vitro* clinical trial of the aqueous extract of *Hyphaene thebaica* in biological subjects should be tried so that the plant product can be used in massive anti-anaemic drug production like Vitamin C in bulk. In further experiments using aqueous pericarp extract of *Hyphaene thebaica*, the standard drug (Vitamin C) can be increased to see whether better anti-anaemic properties can be obtained at a higher dose.

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