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The Phytochemical, Elemental and Hematologic Evaluation of Crude Mesocarp Extract of *Hyphaene thebaica* (doumpalm) in Wistar Albino Rats

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ABSTRACT

The phytochemical, elemental and hematologic effect of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm) in wistar albino rats to ascertain the claims by herbalists and traditionalists in the management of anemia was evaluated. The Phytochemistry revealed the presence of tannins, saponins, steroids, carbohydrates, cardiac glycosides, flavonoids, terpenes and terpinoids in low and moderate concentrations. Alkaloids, phlobatannins and anthraquinones were absent. The elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and sodium in moderate concentration when compared to WHO concentration in ppm. The amount of manganese, zinc and silicon is low, whereas the amount of nickel, cobalt, molybdenum, arsenic and lead is negligible. There is significant ($p<0.05$) increase in red blood and white blood cells production at 1-4 weeks of extract administration, while parked cell volume and hemoglobin concentration increased at the second and third week of oral administration of 400, 600 and 800 mg kg^{-1} for four weeks of the extract respectively. This research have therefore supported the folkloric claims by traditionalists and herbalists in application of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm) in the management of anemia in Askira/Uba, Maiduguri and other Local Government areas in Borno State, Nigeria.

Key words: Phytochemical screening, elemental analysis, hematologic evaluation, crude extract, *Hyphaene thebaica*

INTRODUCTION

Drugs from plants and their derivatives have been used to treat many diseases. Phytochemical components in medicinal plants are of great importance in the manufacture of such drugs (Houghton and Raman, 1998). *Hyphaene thebaica* is reported to be found in many African countries and Indian subcontinents and have been used for many purposes (Brunken *et al*., 2008).

Roots, fruits and seeds of doum palm are used for the management of bilharzias, hypertension and sore eyes respectively in livestock (Owolarafe *et al*., 2007) and also been found to possess antioxidant property (Hsu *et al*., 2006). The ability of Medicinal plants to play vital role in chemotherapy is mainly due to the presence of secondary metabolites which the plants needs to protect themselves from insects and other disease and used by man to combat certain diseases such as diabetes, hypertension, viral, helminthes, bacterial infections and anemia (Schippmann *et al*., 2006). Active-ingredient levels can be much lower in fast-growing cultivated stocks, whereas wild populations can be older due to slow growth rates and can have higher levels of active ingredients (Schippmann *et al*., 2006). Anemia is a condition resulting from loss of blood resulting from partuirition, parasitic infection or automobile accident. Lots of medicinal plants such as *Acacia nilotica*, *Carica papaya*, *Psidium guajava*, *Cymbopogen citrates* and *Musa sapientum* have been reported to be used in the management of the above conditions (Ene and Atawodi, 2012).

The aim and objectives of this study is to evaluate the phytochemical, elemental and hematologic effect of crude mesocarp extract of *Hyphaene thebaica* in wistar albino rats.

MATERIALS AND METHODS

Plant collection and identification: Fresh mesocarp of *Hyphaene thebaica* was bought in September 2012 from Gamboru market, Borno state, north eastern, Nigeria. The seeds were authenticated by a taxonomist at the Department of Biological Science, University of Maiduguri. Voucher specimen of this plant was kept in the toxicology laboratory, University of Maiduguri for reference.

Preparation of crude *Hyphaene thebaica* mesocarp extract: Fresh mesocarp of *Hyphaene thebaica* collected were ground into fine powder and stored in a glass container. One hundred and fifty grammes of aqueous product are prepared by reflux method from three hundred and fifty grammes of initial powdered sample. The aqueous seed extract obtained was then concentrated, labelled and stored in a refrigerator at 4°C.

Phytochemical analysis of aqueous mesocarp extract: Phytochemical screening for tannins, anthraquinones, flavonoids and carbohydrates was carried out using the method described by Trease and Evans (1989, 1997) while glycosides, alkaloids, reducing sugars, monosaccharides, ketones, pentoses and terpenes by Sofowora (1982), Odebiyi and Sofowora (1978) and saponins by Harborne (1973).

Test for tannins (ferric chloride test): Two millilitre of the crude solution of the extract was added to few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour shows the presence of gallic tannins and a green-blackish colour indicates presence of catechol tannins.

Test for saponins (frothing test): Three millilitres of the crude solution of the extract was mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 minutes; it was allowed to stand for 30 min and observed for honeycomb froth which is indicative of the presence of saponins.

Test for alkaloids: One gram of the extract was dissolved in 5 mL of 10% ammonia solution and extracted with fifteen millilitre of chloroform. The chloroform portion was evaporated to dryness

and the resultant residue dissolved in 15 mL of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

Mayer's reagent (or bertrand's reagent): Drops of Mayer's reagent was 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: Two millilitres 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 presence of alkaloids.

Tests for carbohydrate (molisch's test): Few drops of Molischs solution was added to 2 mL of aqueous solution of the extract, thereafter 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 for a purple colour which is indicative of positive for carbohydrates.

Tests for carbohydrate (barfoed's test): One millilitre of aqueous solution of the extract and 1 mL of Barfoed's reagent were added into a test-tube, heated in a water bath for about 2 min. Red precipitate shows the presence of monosacchrides.

Standard test for combined reducing sugars: One millilitre of the crude solution of the extract was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid. This was neutralized with sodium hydroxide solution. The Fehling's test was repeated as indicated above and the tube was observed for brick-red precipitate that indicates the presence of combine reducing sugars.

Standard test for free reducing sugar (fehling's test): Two millilitre of the crude aqueous solution of the extract in a test tube was added 5 mL mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 min. The brick-red precipitate indicates the presence of reducing sugar.

Test for ketone: Two millilitre of crude aqueous solution of the extract was added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid and then heated over a spirit lamp flame and observed for a rose colouration, that shows presence of ketone.

Test for pentoses: Two millilitre of the aqueous solution of the extract was added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. This is heated over a spirit lamp flame and observed for red colouration, indicative of presence of pentoses.

Test for phlobatannins (hydrochloric acid test): Two millilitre of the crude aqueous solution of the extract was added dilute hydrochloric acid and observed for red precipitate that indicates presence of Phlobatannins.

Test for cardiac glycosides: Two millilitre of the crude aqueous solution of the extract was added 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5 mL 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 2 mL concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration respectively which indicates the presence of cardiac glycosides.

Test for steroids (liebermann-burchard's test): The amount of 0.5 g of the crude aqueous extract was dissolve in 10 mL anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration indicative of steroids.

Test for steroids (salkowski's test): The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

Test for flavonoids (shibita's reaction test): One gram of the crude aqueous extract was dissolved in methanol (50%, 1-2 mL) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

Test for flavonoids (pew's test): To five millilitre (5 mL) of the crude solution of the water extract was added 0.1 g of metallic zinc and 8 mL of concentrated sulphuric acid. The reaction mixture was observed for red color indicative of flavonols.

Test for anthraquinones (borntrager's reaction for free anthraquinones): One gram of the powdered seed was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for five minutes. The extract was filtered while hot and allowed to cool. To the filtrate was added equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration which is an indication of the presence of Anthraquinones. Control test were done by adding 10 mL of 10% ammonia solution in 5 mL chloroform in a test tube.

Blood sample collection: Blood samples were collected from the tail vein of the rats by snipping part of the tail for the determination of red blood cells, parked cell volume, hemoglobin concentration and white blood cells counts.

Determination of Red Blood Cells (RBC) count: The method described by researchers was used for the red blood cells count determination. This is an improved Neubauer method. The erythrocyte diluting pipette was used to draw blood from the tail vein to exactly 0.5 marks. The tip of the pipette was wiped free of blood before inserting into the erythrocyte diluting fluid and the fluid drawn into the pipette up to the 101 mark above the bulb. The pipette was gently rotated and allowed to stand for 2 min. The first few drops from the pipette were discarded before being used to charge the counting chamber. The ruled areas of the hemacytometer were thoroughly and carefully cleaned to remove grease. The cover slip was then placed on the counting chamber which

was thereafter charged with the fluid from the pipette. The chamber was left for 2 min. And cells in 5 of 25 small squares were counted under 40x objective of light microscope. The number of the red cells counted were multiplied by ten thousand (10, 000) to give number of the red blood cells in million per cubic millimeter (or $\times 10^6$ mm^3) (Coles, 1986).

Determination of Packed Cell Volume (PCV): Blood from the tail vein of the rat was allowed to run into the microhematocrit tube by capillary action until the tube is about three-quarter full. The end of the tube in contact with the blood was sealed with Plasticine and placed in a micro-haematocrit centrifuged operated at the rate of 3, 000 revolutions per minute (rpm) for 5 min, thereafter, the capillary tube was placed in a micro-haematocrit reader and the PCV read and expressed as percentage (Coles, 1986).

Determination of Hemoglobin Concentration (Hb): Colorimetric method for the determination of hemoglobin concentration was used (Dacie and Lewis, 1994; Coles, 1986). To 5 mL of Drasbsken's solution in a series of test tubes was added 0.2 cm^3 of blood and allowed to stand for 3 min to allow the blood to react with the cyanide solution properly. The colorimeter was warmed up to 10 min before use, then the content of each test tube was transferred into a cuvette and the optical density of the solution in each test tube determined using a filter of 520 nm wave length.

Determination of White Blood Cell (WBC) count: The method of Coles (1986) was used for the white blood cells count. The white blood cell pipette was used to draw blood to 0.5 marks. The tip of the pipette was thereafter wiped and used to draw WBC diluting fluid to 11 marks above the bulb; the pipette was shaken thoroughly to mix the contents and then allowed to stand for 3 min. The counting chamber was charged with the diluting fluid after discarding the first few drops. One min after charging the chamber the cells were counted with the help of light microscope at 40x objective. The cells in the four corners square were counted and multiplied by 1000 to give the total number of the cells counted in thousand per cubic millimeter ($\times 10^3 mm^3$).

Statistical analysis: The results are presented as Mean±Standard deviation. Differences between means were assessed using Analysis of variance (ANOVA) and post test using Dunnett multiple comparison test (Mead and Curnow, 1982).

RESULTS

Quantitative phytochemical analysis of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm): The phytochemical analysis of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm) revealed the presence of tannins, saponins, steroids, carbohydrates, cardiac glycosides, flavonoids, terpenes and terpinoids in low and moderate concentrations. Alkaloids, phlobatannins and anthraquinones are absent (Table 1).

Elemental analysis of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm): The elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and sodium, in moderate concentration when compared to WHO concentration in ppm. The amount of manganese, zinc and silicon is low, whereas the amount of nickel, cobalt and lead is negligible. Molybdenum and arsenic are absent in the crude extract (Table 2).

Table 1: Quantitative phytochemical analysis of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm)

Phytochemical constituents	Test	Inference
Tannins	Ferric chloride	+
	Formaldehyde	+
	Chlorogenic acid	+
Saponins	Frothing	++
	Alkaloids	-
	Dragendorff's	-
	Mayer's	-
	Wagner's	-
Carbohydrates	Molisch's	+
	Barfoed's	-
	Combine reducing sugar	++
	Free reducing sugar	++
	Ketone's	+
	Pentoses	++
Phlobatannins	Hydrochloric acid	-
Cardiac glycosides	General test	++
Steroids	Lieberman's	+
	Salkowski's	+
Flavonoids	Shinoda's	++
	Ferric Chloride	+
Terpenes/Terpinoids	Lieberman- Buchard·s	++
Anthraquinones	Salkowski's	
	Free Anthraquinones	-

-: Absent; +: Present; ++: Moderate

Table 2: Elemental analysis of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm)

Elements	Concentration (mg dL^{-1} or ppm)	WHO standard conc. (mg dL^{-1} or ppm)
Calcium (Ca^{2+})	263.9	360-800
Magnesium (Mg^{2+})	11.66	-
Manganese (Mn^{2+})	2.62	10-20
Copper (Cu^{2+})	0.661	1-3
Zinc (Zn^{2+})	8.44	15-20
Iron (Fe^{3+})	18.62	0.5-50
Sodium (Na^{+1})	76.52	-
Potassium (K^{+1})	427.6	0.1-1
Nickle (Ni)	0.07	-
Silicon (Si)	2.72	-
Cobalt (Co)	0.04	-
Lead (Pb)	0.08	1-2
Molybdenum	0.104	-
Arsenic (As)	0.031	0.02-7

The elements in the crude extract that occurred in low concentrations are copper, nickel, cobalt, lead, molybdenum and arsenic (0.661, 0.07, 0.04, 0.08, 0.104 and 0.031 ppm). Magnesium, manganese, zinc, iron and silicon occurred in moderate concentrations (11.66, 2.62, 8.44, 18.62 and 2.72 ppm) while Calcium, sodium and potassium occurred in high concentrations of 263.9, 76.52 and 427.6 ppm.

Table 3: The effect of crude mesocarp extract of *Hyphaene thebaica* (doumpalm) on hematological parameters

Parameters	Dosage (mg kg^{-1})	Period of Administration (weeks) 1	2	3	4
RBC	Control	5.2±0.13	5.3±0.15	5.3±0.14	5.4±0.09
	400	5.4±0.15	6.8±0.11^{b}	7.1±0.18^{b}	7.6±0.15^{b}
	600	13.6±0.14^{b}	7.0±0.11^{b}	7.4±0.08^{b}	8.6±0.26^{b}
	800	13.7±0.11^{b}	7.2±0.15^{b}	7.8±0.15^{b}	8.6±0.07^{b}
PCV	Control	5.2±0.13	5.3±0.15	5.3±0.14	5.4±0.09
	400	45.0±0.70	47.8±0.45^{b}	49.6±0.55^{b}	49.0±0.45^{b}
	600	44.6±0.55	48.0±0.56^{b}	49.2±0.83^{b}	50.4±0.89^{b}
	800	45.2±0.45	48.6±0.55^{b}	50.0±0.00^{b}	50.6±0.55^{b}
Hb	Control	13.5±0.18	48.6±0.55	13.6±0.14	13.6±0.09
	400	13.6±0.17	14.1±0.08	13.8±0.00^{b}	14.2±0.20^{b}
	600	13.6±0.14	14.0±0.09	14.3±0.18^{b}	14.6±0.14^{b}
	800	13.7±0.11	14.1±0.11	14.4±0.00^{b}	14.6±0.09^{b}
WBC	Control	8.3±0.18	8.4±0.16	8.4±0.11	8.5±0.08
	400	8.5±0.11^{b}	13.1±0.41^{b}	14.1±0.47^{b}	14.7±0.19^{b}
	600	8.7±0.13^{b}	13.5±0.37^{b}	14.4±0.16^{b}	15.0±0.18^{b}
	800	8.6±0.15^{b}	13.7±0.13^{b}	14.6±0.17^{b}	16.0±0.08^{b}

Values are as Mean±SD; N = 5; b = significant ($p<0.05$) increase as compared to control; RBC = Red blood cells; PCV = Packed cell volume; Hb = Hemoglobin concentration; WBC = White blood cells

The effect of crude mesocarp extract of *Hyphaene thebaica* (doumpalm) on hematological parameters: The crude mesocarp extract of *Hyphaene thebaica* (doumpalm) significantly ($p<0.05$) increased Red Blood Cells (RBC) and White Blood Cells (WBC) count throughout the four weeks, Parked Cell Volume (PCV) at second and hemoglobin concentration (Hb) concentration at third week of extract administration (Table 3). The 400 mg kg^{-1} of crude extract did not increase red blood cells count, while 600 and 800 mg kg^{-1} significantly ($p<0.05$) increased red blood cells count by 13.6±0.14 and 13.7±0.11 when compared to the mean red blood cells counts in the rats in control group 5.2±0.13 at 1st week of extract administration. At the second, third and fourth week of the extract administration, there was significant ($p<0.05$) increase in red blood cells count by 6.8±0.11, 7.0±0.11, 7.2±0.15; 7.1±0.18, 7.4±0.08, 7.8±0.15; 7.6±0.15, 8.6±0.26 and 8.6±0.07 when compared to the red blood cell levels (5.3±0.15, 5.3±0.14, 5.4±0.09) of the rats in the control group. The dosages of 400, 600 and 800 mg kg^{-1} of the extract significantly ($P<0.05$) increased packed cell volume of the rats by 47.8±0.45, 48.0±0.56, 48.6±0.55; 49.6±0.05, 49.2±0.83, 50.0±0.00; 49.0±0.45, 50.4±0.89, 50.6±0.55 when compared to the rats (5.3±0.15, 5.3±0.14, 5.4±0.09) in the control group. Hemoglobin concentration of the rats significantly ($p<0.05$) increased at third and fourth week of administration by 13.8±0.00, 14.3±0.18, 14.4±0.00 and 14.2±0.20, 14.6±0.14, 14.6±0.09) when compared to hemoglobin concentration of the rats (13.6±0.14, 13.6±0.09) in the control group respectively. The white blood cells count of the rats significantly ($p<0.05$) in throughout the four weeks of extract administration by 8.5±0.11, 8.7±0.13, 8.6±0.15; 13.1±0.41, 13.5±0.37, 13.7±0.13, 14.1±0.47, 14.4±0.16, 14.6±0.17 and 14.7±0.19, 15.0±0.18, 16.0±0.08 when compared with white blood cells counts (8.3±0.18, 8.4±0.16, 8.4±0.11, 8.5±0.08) of the rats in the control group.

DISCUSSION

The administration of the crude mesocarp extract of *Hyphaene thebaica* (doumpalm) at the limit dose of 5000 mg kg^{-1} body weight orally to wistar albino rats, did not cause death in the treated animals, hence the LD_{50} of the extract was not calculated. Researchers reported that substances with LD_{50} of 50-500 mg kg^{-1} body weight are regarded as highly toxic, those with LD_{50} of 500-1000 mg kg^{-1} are moderately toxic and those with LD_{50} above 1000 mg kg^{-1} are regarded as being of low toxicity and therefore relatively safe to be applied for treatment (Clarke and Clarke, 1977).

Medicinal plants such as *Aegel marmelos, Carissa congesta, Eugenia jambolana, Ficus carica, Phoenix sylvestris, Phyllanthus emblica, Vitis vinifera, Moringa oleifera* and *Telfaria occidentalis* experimentally tried on rats have been found to significantly increase hematological parameters (Alada, 2000; Dina *et al*., 2000; Sarswathi *et al*., 2007).

The crude mesocarp extract of *Hyphaene thebaica* orally administered at various dosages for the period of four (4) weeks significantly ($p<0.05$) increased various hematological parameters in the rats exposed. The dosage of 400, 600 and 800 mg kg^{-1} of the crude mesocarp extract significantly ($p<0.05$) increased Red Blood Cells (RBC), Packed Cell Volume (PCV), Hemoglobin concentration (Hb) and White Blood Cells (WBC) production at various times of extract administration. Some parameters started to increase significantly ($p<0.05$) at the first week and some at second week and kept on increasing throughout the period of oral administration indicating bone marrow activity.

The elemental constituent of the mesocarp extract of *Hyphaene thebaica* has significant quantity of Iron, Potassium, Zinc, Calcium, Magnesium and sodium. Zinc play vital role in cells as an essential component of enzymes such as carbonic anhydrase, alcohol dehydrogenase, lactate dehydrogenase, carboxypeptidase's and alanine peptidases (Robert *et al*., 2000).

Macro elements such as Na, K and Ca regulate the fluid balance and a number of important physiological and biochemical processes such as neuromuscular excitability, blood coagulation, secretory processes, membrane integrity/membrane transport, enzyme reaction and the release of hormones, neurotransmitters and bone mobilization respectively (Robert *et al*., 2000; Ganong, 1999).

The increase in the level of these hematological parameters observed could be as a result of presence of flavonoids as was seen exhibited by *Moringa oleifera* seeds on wistar albino rats and some mineral elements such as Iron, copper and cobalt have been reported to stimulate bone marrow activity and enhances red blood cells production and maturation (Sumati and Kapoor, 1986).

Cu have been reported as is one of the most critical trace elements in animals and is necessary for hemoglobin formation, iron absorption from GI-tract and iron mobilization from tissue stores and hence stimulating increase in the rate of hemopoisis. Copper intake results in hematological and hemorheological changes affecting both the protein content of the erythrocyte membrane and heme synthesis (Mpofu *et al*., 1999; Ozcelik *et al*., 2002).

Iron is important in the production of hemoglobin and plays an important role in flavoprotein-cytochrome system activities hence it is used to treat iron deficiency anemia (Schroeder and Kreamer, 1974; Ganong, 1999).

In this study increase in hematological parameters especially of red blood cells, packed cell volume and hemoglobin concentration is an indication suggestive of polycythemia explaining the ability of animals exposed to this extract to have increased oxygen carrying capacity to the tissues

and carbon dioxide transport capacity from the tissues to the lungs. Increase in white blood cells count indicates stimulatory effect of the extract on leucocytosis which explains the ability of *Hyphaene thebaica* crude mesocarp extract to improve immune related disease conditions. The presence of some important plant chemicals that improves hematological parameters have been reported in plants such as *Viscum album* (mistletoe) and other commonly prescribed medicinal plants (Bendich, 1993; Al-Mamary, 2002).

CONCLUSION

The effect of *Hyphaene thebaica* crude mesocarp extract on hematological parameters may be due to the presence of Flavonoids, Iron, copper and cobalt and can therefore be used in management of Anemia and immune disease conditions in humans and livestock, though due the significant presence of other macro and microelements in the mesocarp extract it can be used to improve cellular enzyme activities, neuromuscular transmission and membrane integrity/membrane transport. This fact affirms the claim and use of *Hyphaene thebaica* crude mesocarp extract in the management anemia and other forms of diseases in Maiduguri and Askira/Uba Local Government areas, Borno State, North eastern Nigeria.

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