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The Quantitative Phytochemistry and Hypoglycaemic Properties of Crude Mesocarp Extract of *Hyphaene thebaica* (doumpalm) on Normoglycemic Wistar Albino Rats

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The quantitative Phytochemistry and hypoglycaemic properties of crude mesocarp extract of *Hyphaene thebaica* (doumpalm) on normoglycemic wistar albino rats were investigated. Fresh mesocarp of *Hyphaene thebaica* was bought in September 2012 from Gamboru market, Borno State, North eastern, Nigeria. One hundred and 50 g of aqueous product were prepared by reflux method from three hundred and 50 g of initial powdered sample. Phytochemical screening for biochemical and elemental contents were conducted. The quantitative phytochemical screening revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, terpenes and terpinoids. Elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and sodium in moderate concentration, manganese, zinc and silicon is low, whereas the amount of nickel, cobalt, molybdenum, arsenic and lead are negligible. Administration of crude mesocarp extract of *Hyphaene thebaica* in normoglycemic rats at the dosage of 400, 600 and 800 mg kg^{-1} for four weeks significantly ($p<0.05$) reduced blood glucose level of the rats at 3-4 weeks post administration. This finding validates the traditional application of *Hyphaene thebaica* crude mesocarp extract in the management of Diabetes mellitus in Borno State, Nigeria.

Key words: Phytochemistry, elemental analysis, hypoglycemia, *Hyphaene thebaica*, Albino rats

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INTRODUCTION

Hypoglycemia is experienced when the blood glucose level in the body is used up, when glucose is released into the blood stream slower than its needed or when an excessive amount of insulin is released into the blood stream. Hypoglycemia may also occur due to insulin secreting tumor of the pancreas, liver or as a response to indigestion of alcohol (Acampora *et al*., 2002; Kar *et al*., 2003; Laakso *et al*., 1990). Blood sugar level also known as serum glucose level is expressed in mmol L^{-1} or mg dL^{-1} (Ian and Soon, 2006). A patient is considered to be hypoglycemic when his/her blood sugar level is below 90 mg dL^{-1}, normoglycemic with blood sugar level of 120 mg dL^{-1} and hyperglycemic when his/her blood sugar level ranges between 140-160 mg dL^{-1} (Albertini, 1997; Ian and Soon, 2006).

Medicinal plants and other alternative medicines have been used in traditional healing around the world for a long time to treat glycaemic conditions with minimal side effects when compared to synthetic therapeutic agents (Kavishankar *et al*., 2011). Many medicinal plants have been proven to exhibit hypoglycemic activity and have been therapeutically used to manage hyperglycemic conditions; such plants include *Glycyrrhiza uralensis* fish, *Morus indica* L., *Morus inignis*, *Phyllanthus sellomiamus*, *Psacalium decompositum*, *Ocimum gratissimum*, *Sclerocarya birea*, *Tinospora cordifolia Miers*, *Tinospora crispa* and *Zizyphus sativa Gaertn* (Kavishankar *et al*., 2011).

These plants confers their hypoglycemic activity through various mechanisms such as stimulation of pancreatic islets beta cells to release insulin, inhibits hormones that increase blood glucose, increase the number, affinity, or sensitivity of the insulin receptors to insulin, decrease release of glycogen from the liver, resist lipid peroxidation, correct lipid and protein metabolic disorder and improves microcirculation in the body (Cho *et al*., 2002). The aim and objectives of this study is to determine the hypoglycemic activity of the crude mesocarp extract of *Hyphaene thebaica* in wistar albino rats and the possible application of this product in the management of hyperglycemic conditions in humans.

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 grams 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) and 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 color shows the presence of gallic tannins and a green-blackish color indicates presence of catechol tannins.

Test for saponins (frothing test): Three millilitre 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 min; 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 15 mL 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 millilitre 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 monosaccharides.

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 1 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 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 5 min. 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.

Effect of *Hyphaene thebaica* crude extract on normoglycemic wistar albino rats: Twenty rats weighing 110-200 g were used. They were divided into four groups of 5 rats each. The rats in group A (control) were administered distilled water only. Those in groups B, C and D were administered 400, 600 and 800 mg kg^{-1} of *Hyphaene thebaica* extract respectively. Blood glucose level was determined using one touch glucose meter (Asatoor and King, 1954) at 7, 14, 21 and 28 days post extract administration.

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

RESULTS AND DISCUSSION

The quantitative phytochemical screening revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, terpenes and terpinoids (Table 1). The elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and sodium in moderate concentration, manganese, zinc and silicon is low, whereas the amount of nickel, cobalt, molybdenum, arsenic and lead are negligible (Table 2).

Administration of crude mesocarp extract of *Hyphaene thebaica* in normoglycemic rats at the dosage of 400, 600 and 800 mg kg^{-1} for four weeks significantly ($p<0.05$) reduced blood glucose level of the rats at 3-4 weeks post administration (Table 3).

The crude mesocarp of *Hyphaene thebaica* is found to possess some degree of hypoglycemic activity. The hypoglycemic activity may be due to the plant possessing some significant quantity of biochemical principles such as triterpenes, flavonoids and saponins in the crude mesocarp extract (Table 1). Triterpenes isolated from the root and Bark of *Astragalus membranaceus* and *Bumelia sartorum* is found to decrease blood glucose concentration by increasing plasma insulin level (Almeida *et al*., 1985; Yu *et al*., 2006). Flavonoids in n-butanol fraction of *Bauhinia forficate* and *Garcinia kola* seed extract have been shown to exhibit significant hypoglycemic activity (Iwu *et al*., 1990; De Sousa *et al*., 2004). Saponins extract from the seed of fenugreek significantly decreased hyperglycemia in rabbits (Frati *et al*., 1990). Flavones, glycosides and sterols found in *C. javanica* are considered to be antidiabetic compounds, hence its hypoglycemic effect (Diatewa *et al*., 2004; Dhanabal *et al*., 2007; Rastogi and Mehrotra, 1993). The presence of some elements such as calcium, zinc, magnesium, manganese and copper in medicinal have been reported to stimulate beta cells regeneration, activity and insulin production (Akhtar and Iqbal, 1991). *Hyphaene thebaica* crude mesocarp extract possesses most of these elements such as calcium, zinc, magnesium, manganese and copper (Table 2) in

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	+
Triterpenes/terpinoids	Lieberman-Buchard,s	++
	Salkowski's	++
Anthraquinones	Free anthraquinones	-

-: Absent, +: Low, ++: Moderate

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

Elements	Concentration (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+})	-	-
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

Table 3: Effect of the crude mesocarp extract of *Hyphaene thebaica* on blood sugar level in wistar albino rats

Dosage (mg kg^{-1})	Time (weeks) 1	2	3	4
Control	117.00±10.36	119.80±9.070	119.00±9.570	115.60±8.560
400	123.80±11.82	118.00±8.090	102.60±25.37	83.60±14.94[b]
600	120.60±3.180	109.80±14.85	96.00±17.62[b]	84.60±11.76[b]
800	123.00±7.380	110.80±11.17	86.00±17.62[b]	76.00±12.96[b]

Values are as Mean±SD, N = 5, [b]Significant ($p<0.05$) decrease as compared to control

significant amount which could be the reason for its hypoglycemic effect. *Hyphaene thebaica* have been reported to posse's high fiber content which could also be the reason for its hypoglycemic activity as seen in mulberry leaves with high fiber content of 13.85% causing hypoglycemia (Reid, 1990). The graded oral doses of 400, 600 and 800 mg kg^{-1} of the extract administered for the period of four weeks significantly ($p<0.05$) reduced blood glucose level in wistar albino rats by 83.60±14.94, 96.00±17.62, 84.86±11.76, 86.00±17.62 and 76.00±12.96, respectively (Table 3).

CONCLUSION AND RECOMMENDATIONS

The crude mesocarp of *Hyphaene thebaica* have been found to posses some vital biochemical substances such as triterpenes, flavonoids, glycosides, sterols and micro and macroelements such as calcium, zinc, magnesium, manganese and copper that have been reported to stimulate beta cells regeneration, activity and insulin production reported in various research. This might be the reason for the hypoglycemic effect of the crude mesocarp of *Hyphaene thebaica* in wistar albino rats. The mesocarp of this plant can be recommended in the management of hyperglycemic conditions e.g., Diabetes mellitus.

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REFERENCES

Acampora, B., R.A. Jackson, J.B. Hamburg, P.M. Blix and M.I. Hawa, 2002. The Influence of hyperglycemia on glucose uptake and other metabolic responses. J. Clin. Endocrinol. Metab., 63: 594-604.

Akhtar, M.S. and J. Iqbal, 1991. Evaluation of the hypoglycemic effect of *Achyranthes aspera* in normal and alloxan diabetic rabbits. J. Ethnopharmacol., 31: 49-57.

Albertini, K.G.M.M., 1997. The clinical implications of impaired glucose tolerance. Diabetes Med., 13: 927-937.

Almeida, R.N., J.M.B. Filho and S.R. Naik, 1985. Chemistry and Pharmacology of an ethanol extract of *Bumelia sartorum*. J. Ethnopharmacol., 14: 173-185.

Asatoor, A.M. and E.J. King, 1954. Simplified Calorimetric Blood Sugar Method. 29th Edn., Harold Varley, Arnold Heineman Publishers, India, pp: 56-58.

Cho, S.Y., J.Y. Park, E.M. Park, M.S. Choi and M.Y. Lee *et al*., 2002. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. Clin. Chim. Acta, 317: 109-117.

De Sousa, E., L. Zanatta, I. Seifriz, T.B. Creczynski-Pasa, M.G. Pizzolatti, B. Szpoganicz and F.R.M.B. Silva, 2004. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(α)-dirhamnoside from *Bauhinia forcata* leaves. J. Nat. Prod., 67: 829-832.

Dhanabal, S.P., M.K. Mohan-Maruga Raja, M. Ramanathan and B. Suresh, 2007. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats. Fitoterapia, 78: 288-291.

Diatewa, M., C.B. Sambaa, T.C.H. Assaha and A.A. Abenaa, 2004. Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* Baillon in normal and alloxan-induced diabetic rats. J. Ethnopharmacol., 92: 229-232.

Frati, A.C., E. Jiminez and C. Raoul Ariza, 1990. Hypoglycemic effect of *Opuntia ficus indica* in non-insulin dependent Diabetes mellitus patients. Phytother. Res., 4: 195-197.

Harborne, B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, Pages: 278.

Ian, W.C. and S. Soon, 2006. Effect of *Arachis hypogea* in alloxan induced diabetic rats. J. Ethnopharmacol., 9: 553-555.

Iwu, M.M., O.A. Igboko, C.O. Okunji and M.S. Tempesta, 1990. Antidiabetic and aldose reductase activities of biflavanones of Garcinia kola. J. Pharm. Pharmacol., 42: 290-292.

Kar, A., B.K. Choudhary and N.G. Bandyopadhyay, 2003. Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. J. Ethnopharmacol., 84: 105-108.

Kavishankar, G.B., N. Lakshmimidevi, S. Mahadeva Murthy, H.S. Prakash and S.R. Niranjana, 2011. Diabetes and medicinal plants. Int. J. Pharm. Biomed. Sci., 2: 65-80.

Laakso, M., S.V. Edelman, G. Bretchel and A.D. Baron, 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J. Clin. Investigation, 85: 1844-1852.

Mead, R. and R.N. Curnow, 1982. A Simple Experiment in Statistical Method in Agriculture Biology. Chapman Hall, London, pp: 33-46.

Odebiyi, O.O. and E.A. Sofowora, 1978. Phytochemical screening of Nigerian medicinal plants II. Lloydia, 41: 234-246.

Rastogi, R.P. and B.N. Mehrotra, 1993. Compendium of Indian Medicinal Plants. Vol. 5, Central Drug Research Institute, Lucknow, New Delhi, pp: 175-176.

Reid, N.W., 1990. Dietary Fibre: Chemical and Biological Aspects. Royal Society of Chemistry, Cambridge, Pages: 340.

Sofowora, A., 1982. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons, Chichester, UK., ISBN-13: 9780471103677, pp: 179.

Trease, G.E. and W.C. Evans, 1989. Trease and Evans Pharmacognosy: A Physicians's Guide to Herbal Medicine. 13th Edn., Bailliere Tindall, London.

Trease, G.E. and W.C. Evans, 1997. A Text Book of Pharmacognosy. 14th Edn., W.B. Saunders, London, pp: 13-53.

Yu, J., Y. Zhang, S. Sun, J. Shen and J. Qiu *et al*., 2006. Inhibitory effects of astragaloside IV on diabetic peripheral neuropathy in rats. Can J. Physiol. Pharmacol., 84: 579-587.