

---

## PHYTOCHEMICAL SCREENING AND SOME NUTRITIONAL PROFILES OF *HYPHAENE THEBAICA*

Mustapha Garba Muhammad<sup>1\*</sup>, Musbahu Muhammad Sani<sup>2</sup>, Nafiu Ibrahim Ismail<sup>1</sup>, Adam Isah Adam<sup>1</sup>, Rabia Muhammad Abdullahi<sup>3</sup>

<sup>1</sup>Department of Life Sciences, School of Technology, Kano State Polytechnic, P.M.B. 3348, Kano State, Nigeria, <sup>2</sup>Medical Biochemistry Unit, Faculty of Basic Medical Sciences, Federal University Dutse, Jigawa State, Nigeria. \*Corresponding author E-mail: [mgm4real@yahoo.com](mailto:mgm4real@yahoo.com)

### ABSTRACT

*Hyphaene thebaica* (Doum Palm) is a desert palm tree which is widely used in medicine for the treatment/management of several diseases including cancer and cardiovascular diseases. This research is aimed at evaluating the secondary metabolites and nutritional compositions of different extracts of *H. thebaica* fruit pulp. Qualitative phytochemical analyses of the aqueous, ethanol and *n*-hexane extracts of *H. thebaica* fruit pulp revealed the presence of phytoconstituents such as Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone and Essential oils. Quantitative nutritional compositions (proximate, vitamins and mineral analysis) of each extract were also determined and the results were statistically analysed and compared within and between the aqueous, ethanol and *n*-hexane extract groups. Statistical analyses indicated that there is significant difference ( $p < 0.05$ ) when the vitamins (A, C and E) content were compared amongst all the extracts groups. Significant difference ( $p < 0.05$ ) was observed between Sodium, Calcium, Magnesium, Potassium and Iron content of the different extract groups when compared. However no significance was observed ( $p > 0.05$ ) when copper content of all the extracts are compared. Further research towards identifying active principles that can be used in the treatment or management of pathologic conditions like cancer and cardiovascular diseases is therefore recommended.

**Keywords:** *Hyphaene thebaica*, Secondary metabolites, Phytochemicals, Vitamins, Minerals, Pathologic conditions.

## 1.0 INTRODUCTION

Doum palm (*Hyphaene thebaica*) is a desert palm tree with edible oval fruit, originally native to Egypt, Sub-Saharan Africa, and Western India (Hsu *et al.*, 2006; Abdel-Rahman 2019). It also grows very well in the Northern part of Nigeria. It is a member of the palm family, *Arecaceae*, that extends to a length of 6–9 feet and has branched stems with 65–75 centimeter lengthy fan-shaped leaves (Fakhar *et al.*, 2022). It is dichotomous and arborescent in nature. It is listed as one of the useful plants of the world (Fletcher, 1997). It is represented by the genus *Hyphaene*, the fruit of interest in the current study. Its fibre and leaflets are used by people along the Nile to weave mats, baskets and other household items (Siddeeg *et al.*, 2019). Doum palm fruit is also a source of potent antioxidants (Hsu *et al.*, 2006). The fruit has a brown outer fibrous flesh which is normally chewed and spewed out. Doum palm kernel is edible when it is unripe but hard when it is ripe. Moreover, doum palm is also used for local craft, for construction and the root is also medicinal. The foliage is used to make mats, ropes, baskets, and hats while the stem with the leaves are used for construction purpose (Moussa *et al.*, 1998). Roots of doum palm are used for treatment of bilharzias while the fruit is often chewed to control hypertension (Orwa *et al.*, 2009).

The demand for herbal medicine has been increasing by the day as consumers perceive these forms of healing as safe as and more effective than the synthetic drugs. This trend of using alternative and complimentary healthcare has prompted scientists to investigate the various biological activities of medicinal plants (Wendakoon *et al.*, 2011).

Phytochemicals are the chemicals that present naturally in plants. They play a significant role against number of ailments such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Phytochemicals manage/treat some diseases without causing negative effect to human beings these can also be considered as man-friendly medicines (Smith-Warner *et al.*, 2003). As a consequence, these evidences accelerated the search for antioxidants principles, which led to the identification of natural resources and isolation of active antioxidant molecules. Many plants have been identified as having potential antioxidant activities and their consumption recommended (Kitts *et al.*, 2000; Lee *et al.*, 2003; Piao *et al.*, 2008; Kilani *et al.*, 2008; Wang *et al.*, 2009).

Beyond these targeted phytochemical reports, analysis of secondary metabolites classes in doum is yet to be reported to provide insight into the breadth of chemical constituents and proximate activity. Secondary metabolites or phytochemicals from plants have eminent pharmacological activities such as anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anticarcinogenic (Krishnamachari, 2017).

The medicinal value of this plant lies in its phytochemicals, antioxidant vitamins contents and mineral contents which produces definite physiological actions on the human body. The most important of these phytochemical components are alkaloids, tannins, flavonoid and phenolic compounds (Shariff, 2001). Natural antioxidant compounds which are widely distributed in plants are capable of terminating a free radical-mediated by oxidative reaction and would have beneficial activities in protecting the human body from such diseases (Havsteen, 2002). Therefore, the objective of the present study was to qualitatively and quantitatively evaluate the phytochemical, proximate, mineral as well as some non-B vitamin content of the aqueous, ethanol and n-hexane, extracts of *H. thebaica* fruit pulp.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of Plant Samples

Fresh fully ripe fruits of *H. thebaica* were simultaneously collected from Kano Zoological Garden, Kano State, Nigeria. The samples were immediately taken to the Laboratory of Life Sciences Department, Kano State Polytechnic, Nigeria for storage. The plant samples were identified and authenticated at the Herbarium unit of Plant Biology Department, Bayero University Kano. The identification voucher number of sample specimen assigned BUKHAN 0380 and deposited at the unit for future reference.

### 2.2 Processing of the samples

The plant samples were gently washed with water to remove dirt and dust. The outer skin of the fruit was scrapped off using a sharp knife. The outer skin portion was retained and separately homogenized using mortar and pestle. The ground samples were stored in a labelled air tight container and kept at room temperature which was immediately used for subsequent analysis.

### 2.3 Extract preparation

30 g of fine powder of plant materials were extracted with 300 mL of an appropriate solvent (distilled water, ethanol and N-hexane) in a round bottom flask with magnetic stirrer for 24 h at room temperature respectively; this gave a ratio of 1:10. The pulp extracts were then centrifuged at 5000 rpm for 15 min. An external magnetic field was applied to the magnetic stirrer to mix the solutions which facilitates the rotating of the small magnetic bar placed in the mixture of interest (Yashashri *et al.*, 2017).

### 2.4 Phytochemical Screening of the extract samples

Phytochemicals screening was carried out for all the extracts by the standard methods (Tiwari *et al.*, 2011).

**2.4.1 Test for Saponins:** 0.5 g of all the extracts would be mixed and shaken with 2 mL of water. The tubes were allowed to stand in a vertical position and were observed for 30 min. a honey comb froth that persists for 10 min indicates the presence of saponins.

**2.4.2 Test for Tannins (Gelatin Test):** To all the extract, 1 % gelatin solution containing sodium chloride were added. Formation of white precipitate indicates the presence of tannins

**2.4.3 Test for Phenols (Ferric Chloride Test):** The extracts fruit pulp of *H. thebaica* were treated with 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

**2.4.4 Test for cardiac glycosides (Cardenolides Test):** Extracts were treated with sodium nitropruside in pyridine and 20% sodium hydroxide. Formation of red colour, fades to brownish yellow indicates the presence of cardiac glycosides.

**2.4.5 Test for Anthranol Glycosides (Modified Borntrager's Test):** The extracts were treated with ferric chloride solution and immersed in boiling water for 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and

treated with ammonia solution. Formation of rose-pink to blood red colour indicates the presence of anthranol glycosides.

**2.4.6 Test for Anthraquinone (Ammonium hydroxide Test):** 10 mg of extracts were dissolved in isopropyl alcohol followed by addition of concentrated ammonium hydroxide solution. Formation of red colour shows the presence of anthraquinone.

**2.4.7 Test for Flavonoids (Alkaline Reagent Test):** Extracts were treated with 2 mL of sodium hydroxide solution followed by addition of 3 drops of dilute hydrochloric acid. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

**2.4.8 Test for Alkaloids (Dragendorff's/ Kraut's Test):** Extracts were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of reddish-brown precipitate indicates the presence of alkaloids.

**2.4.9 Test for Terpenoid (Noller's Test):** Each extract solution was warmed with a piece of tin and a few drops of thionyl chloride. Violet or purple colouration indicates the presence of terpenoid.

**2.4.10 Test for Diterpenes (Copper acetate Test):** Extracts were dissolved in distilled water and treated with 3 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

**2.4.11 Test for Triterpenes (Salkowski's Test):** Extracts were treated with few drops of concentrated Sulphuric acid, shaken well and allowed to stand. Appearance of golden yellow layer indicates the presence of triterpenes.

**3.4.12 Test for Reducing Sugars (Benedict's Test):** Extracts were treated with 0.5 mL Benedict's reagent and heated gently for 2 min. Green, yellow or red precipitate indicates the presence of reducing sugars.

**2.4.13 Test for Phytosterols (Liebermann-Burchard's Test):** Extracts were dissolved in 2 mL acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

**2.4.14 Test for Coumarin:** 0.5 mg moistened extracts were taken in test tube, mouth of test tube was covered with 1N NaOH treated filter paper, heated for few min. in water bath. To 2 mL of the each extract solution, a few drops of alcoholic sodium hydroxide would be added. Appearance of yellow fluorescence colour indicates the presence of coumarin.

**2.4.15 Test for Steroid (Liebermann-Burchard's Test):** Extracts were dissolved in 2 mL acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Appearance of purple colour, which changes to blue or green colour, shows the presence of steroid.

**2.4.16 Test for Quinone (Sulphuric acid Test):** Each extract solution was in isopropyl alcohol treated with a few drops of concentrated sulphuric acid. or aqueous sodium hydroxide solution. A red colour formation indicates the presence of quinoid compound.

**2.4.17 Test for Essential oil (Spot/Stain Test):** A small quantity of dried powdered sample of each extract were pressed between two filter papers. Formation of grease spot indicates the presence of essential oil.

**2.4.18 Test for Phlobatannins (hydrochloric acid test):** 2 mL of the crude aqueous solution of all extracts were added 1% hydrochloric acid and observed for red precipitate that indicates presence of Phlobatannins.

**2.4.19 Test for Ketone:** 2 mL of crude aqueous solution of each extracts were added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid and then heated over a spirit lamp flame and observe for a rose colouration, that shows presence of ketone.

**3.4.20 Test for Pentoses:** 2 mL of the aqueous solution of each extracts were added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. It was heated over a spirit lamp flame and observed for red colouration, indicative of presence of pentoses.

## 2.5 Determination of Proximate Compositions

The dried powdered samples of each extract was brought to uniform size by sieving. They were then be determined for moisture, protein, ash, and fiber by the methods of Association of Official Analytical Chemists (AOAC) 2005 (Shumaila & Mahpara, 2009). The proximate compositions of the fruit pulp of doum extracts would be assessed by the methods of Oluduro (2012).

### 2.5.1 Determination of moisture

#### Procedure

1.5 g of well-mixed dried powdered sample of each extract was accurately weighed in clean, dried crucible ( $W_1$ ). The crucible was heated in an oven at 100-105°C for 6-12 h until a constant weight was obtained. Then the crucible was placed in the desicator for 30 min to cool. After cooling it would be weighed again ( $W_2$ ). The percent moisture was calculated by the formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2 \times 100}{\text{Wt. of Sample}}$$

Where

$W_1$  = Initial weight of crucible Sample

$W_2$  = Final weight of crucible + Sample

### 2.5.2 Determination of ash content

#### Procedure

Clean empty crucible was placed in a muffle furnace at 60 °C for 1 h, cooled in desicator and then weight of empty crucible was noted ( $W_1$ ). 1 g of each dried extract sample was taken in crucible ( $W_2$ ). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 50 °C for 2-4 h. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. After ashing furnace is switch off. The crucible was cooled and weighed ( $W_3$ ). Percent ash was calculated by the formula:

$$\% \text{ Ash} = \frac{\text{Difference in Wt. of Ash} \times 100}{\text{Wt. of Sample}}$$

Difference in wt. of Ash =  $W_3 - W_1$

### 2.5.3 Determination of crude protein

#### Procedure

Protein in the sample was determined by Kjeldahl method. 1 g of dried sample was taken in digestion flask. 8 mL of concentrated  $H_2SO_4$  and 8 g of digestion mixture i.e.  $K_2SO_4$ :  $CuSO_4$  (8:1) was added. The flask was swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture become clear (blue green in colour). It needs 2 h to complete. The digest was cooled and transferred to 100 mL volumetric flask and volume was made up to mark by the addition of distilled water. 10 mL of digest was introduced into the distillation tube then 10 mL of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 min and  $NH_3$  produced was collected as  $NH_4OH$  in a conical flask containing 20 mL of 4 % boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appeared due to  $NH_4OH$ . The distillate was titrated against standard 0.1 N HCl solutions till the appearance of pink color. A blank would also be run through all the steps as above. Percent crude protein content of the sample was calculated from the percent nitrogen by using the formula:

$$\% \text{ Crude Protein} = 6.25 \times \% \text{ N}$$

$$\% \text{ Nitrogen} = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Wt. of the Sample} \times V}$$

Where

S=Sample titration reading

B=Blank titration reading

N=Normality of HCl

D=Dilution of sample after digestion

V=Volume taken for distillation

0.014=Milli equivalent weight of Nitrogen

### 2.5.4 Determination of crude lipid

#### Procedure

Crude lipid would be determined by ether extraction method using Soxhlet apparatus. Approximately 1 g of moisture free extract samples would be wrapped in filter paper, placed in lipid free thimble and then introduced into the extraction tube. Weighed, cleaned and dried receiving beaker would be filled with petroleum ether and fitted into the apparatus. The water and heater would be turned on to start extraction. After 4 - 6 siphoning, ether would be allowed to evaporate and beaker disconnected before last siphoning. The extracts would be transferred into clean glass dish with ether washing. Ether would be evaporated on water bath. The dish



would then be placed in an oven at 105 °C for 2 h and cooled in a desiccator. The percent crude fat is determined by using the following formula:

$$\% \text{ Crude Lipid} = \frac{\text{Wt. of Ether Extract} \times 100}{\text{Wt. of the Sample}}$$

### 2.5.5 Determination of crude fiber

#### Procedure

2 g sample ( $W_0$ ) was weighed and transferred to a porous crucible. The crucible was placed into Dosi-fiber unit and kept the valve in “Off” position. 150 mL of preheated  $H_2SO_4$  solution and some drops of foam-suppressor was added to each column. Then the cooling circuit would be opened and the heating elements turned on (power at 90 %). When boiling starts, the power was reduced to 30 % and allowed for 30 min. Valves was opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from sample. The same procedure was used for alkali digestion by using KOH instead of  $H_2SO_4$ . The samples was dried in an oven at 150 °C for 1 h and allowed to cool in a desiccator and weigh ( $W_1$ ). The samples crucibles was kept in muffle furnace at 55 °C for 3 - 4 h. The samples was cooled in desiccator and weigh again ( $W_2$ ). Calculation was done by using the formula:

$$\% \text{ Crude Fibre} = \frac{W_1 - W_2 \times 100}{W_0}$$

### 2.5.6 Determination of Carbohydrate

#### Procedure

The carbohydrate content was determined by subtracting the sum up percentage of compositions of moisture, protein, fiber, and ash contents from 100.

$$\% \text{Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude lipid} + \% \text{ crude fibre} + \% \text{ ash})$$

## 2.6 Determination of Vitamins Content

### 2.6.1 Determination of vitamin A (Rutkowski *et al.*, 2006)

#### Procedure

Into a conical flask containing 25 mL of 95% ethanol, 5g of samples were macerated, placed and maintained at a temperature of about 70 – 80°C in a water bath for 20 min with periodic shaking. Both extracts were decanted, allowed to cool and the volume measured by means of measuring cylinder and recorded as volume (V). 1 mL of the analysed liquid was measured into a test-tube I (centrifugal) with a tight stopper and 1 mL of KOH solution was added, the tube was stoppered and shaken vigorously for 1 min followed by heating in a water bath (60°C, 20 min). The tube was cooled in cold water and 1 mL of xylene was added. The tube was stoppered, vigorously shaken again for 1 min and then centrifuged (1500rpm, 10 min). The whole of the separated extracts (upper layer) were collect and transferred to test tube II made of “soft” (sodium) glass. The absorbance  $A_1$  of the obtained extracts were measured at 335 nm against xylene. The extract in the test tube II was irradiated with UV light for 30 minutes, then the absorbance  $A_2$  was measured. The concentration  $C_x$  of vitamin A in the analysed liquid was calculated using the formula:

$$C_x = (A_1 - A_2) \times 22.23$$

where:

22.23 = multiplier received on basis of the absorption coefficient of 1% solution of vitamin A in xylene at 335 nm in a measuring cuvette of thickness = 1 cm.

### **2.6.2 Determination of Vitamin C by Titrimetric Method (Abdullahi, 2015)**

#### **Procedure**

5 mL of samples containing about 0.1 mg of vitamin C were pipetted into boiling tube. 1 mL of glacial acetic acid was added and titrated with dye solution to a faint permanent pink colour. The titre value was recorded. The titration repeated with 5 mL of water for the blank (B) and 5 mL of ascorbic acid standard solution(s) and the vitamin C content of the test sample was calculated.

### **2.6.3 Determination of Vitamin E**

#### **Procedure**

1 g of each sample was macerated with 20 mL of ethanol and then filtered. 0.2 % ferric chloride in ethanol and 1 mL of 0.5 %  $\alpha$ - $\alpha$ -dipyridine to 1 mL of the filtrate. This was diluted to 5 mL with distilled water. Absorbance was taken at 520 nm (Anaduaka *et al.*, 2013)

## **2.7 Mineral contents determination**

Mineral contents were determined by Atomic Absorption Spectrometry (AAS) and Flame Photometry (FP) according to the official method of Association of Official Analytical Chemists (AOAC) 2005.

### **2.7.1 Determination of Calcium (Ca), Iron (Fe), Magnesium (Mg), Copper (Cu), Manganese (Mn) and Zinc (Zn).**

#### **Principle**

Atoms of an element were vaporized and atomized in the flame. The atoms absorb the light at a characteristic wavelength. The source of the light was hollow cathode lamp, which is made up of the same element, as that which was determined. The lamp produces radiation of an appropriate wavelength, which while passing through the flame absorbed by the free atoms of the sample. The amount of the energy absorbed is proportional to the concentration of the element present in the sample (AOAC, 2005).

### **2.7.2 Determination of sodium (Na) and potassium (K) by flame photometry**

#### **Principle**

To determine Na and K, analysis of the sample were done by the method of flame photometry. The same wet digested sample solutions were used for the determination of Na and K (AOAC, 2005). In this technique, the digested sample was aspirated through a nebulizer (or aspirator) into the flame. After the sample matrix evaporates, the sample was atomized. Atoms then reach an excited state by absorbing heat from the flame. The amount of energy emitted from the flame is equivalent to the quantity/concentration of an element present in the sample.



## 2.8 Statistical Analyses

The data were collected from various parameters of *H. thebaica* and presented as mean  $\pm$  standard deviation. Sample means were subjected to One – way Analysis of Variance (ANOVA) statistical analysis using Statistical Package for Social Sciences (SPSS) Version 20.0 (IBM Inc.).

## 4.0 RESULTS

The results of qualitative phytochemical compositions screened in aqueous, ethanol and n-hexane extracts of *H. thebaica* are presented in table 1; All the phytochemicals screened (Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone, Essential oil, Phlobatanin, Ketone, Pentose and Anthraquinone) are present in ethanol and aqueous extracts with exception phlobatanin, essential oil, ketone, pentose and anthraquinone which were absent in ethanol and aqueous extracts respectively. Cardiac glycoside, anthranol glycoside, terpenoid, triterpenoid, reducing suger, steroid and essensial oil are present in n-hexane extract while saponin, tannin, phenol,, flavonoid, alkaloid, diterpenes, phytosterol, coumarin, quinone, phbobatanin, ketone, pentose and anthraquinone are absent in n-hexane extract.

**Table 1:** Show the qualitative results of secondary metabolites of *H. thebaica*.

Parameters	Aqueous Extract	Ethanol Extract	n-Hexane Extract
Saponin	+	+	-
Tannin	+	+	-
Phenol	+	+	-
Cardiac glycoside	+	+	+
Anthranol glycoside	+	+	+
Flavonoid	+	+	-
Alkaloid	+	+	-
Terpenoid	+	+	+
Diterpenes	+	+	-
Triterpenoids	+	+	+
Reducing sugar	+	+	+
Phytosterol	+	+	-
Coumarin	+	+	-
Steroids	+	+	+
Quinone	+	+	-
Essential oil	-	+	+
Phlobatanin	-	-	-
Ketone	-	-	-
Pentose	-	-	-
Anthraquinone	-	-	-

Key: + = present, - = absent

The results of quantitative compositions of proximate parameters (Carbohydrate, protein, Ash, moisture, fat and fibre) of *H. thebaica* fruit pulp are shown in table 2; Carbohydrate

(62.33±0.76 %), moisture (22.40±0.40 %) and fiber content (7.26±0.25 %) have the highest concentration while lipid (0.92±0.02 %) and protein (2.82±0.10 %) lowest.

**Table 2:** Determination of proximate compositions of *H. thebaica*.

Parameters	(%)
Protein	2.82±0.10
Lipid	0.92±0.02
Carbohydrate	62.33±0.76
Ash	4.04±0.04
Fiber	7.26±0.25
Moisture	22.40±0.40

Values are expressed as mean ± standard deviation of 3 replicates.

Table 3 presents the results of quantitative analysis of vitamins present in aqueous, ethanol and n-hexane extracts. Aqueous extract exhibited higher contents of vitamins A (13.08±1.83 µmol/L), n-hexane extract had the highest content of vitamin E (39.77±1.08mg/L) and ethanol extract had the highest content of vitamin C (14.36±0.01mg/L). However, it was observed that, there is statistically significant difference ( $p < 0.05$ ) when the vitamins (A, C and E) content were compared with all the extracts.

**Table 3:** Determination of antioxidant vitamins present in aqueous,ethanolic and n-hexane extracts of *H. thebaica*.

Extracts	Vitamin C (mg/L)	Vitamin E (mg/L)	Vitamin A (µmol/L)
Aqueous	8.08±0.26	17.17±0.57 <sup>b</sup>	13.08±1.83 <sup>c</sup>
Ethanol	14.36±0.01 <sup>a</sup>	20.05±0.12 <sup>b</sup>	11.56±0.38 <sup>c</sup>
n-hexane	7.42±0.41	39.77±1.08 <sup>b</sup>	5.78±0.01 <sup>c</sup>

Values are expressed as mean ± standard deviation of 3 replicates; a, b and c= significant difference at  $p < 0.05$  when the extracts are compared.

Table 4 presents the quantitative compositions of some minerals present in aqueous, ethanol and n-hexane extracts. Ethanol extract had higher of Sodium (76.78±0.17 mg/L), aqueous had higher content of Calcium (18.73±0.82 mg/L) and Iron (0.87±0.01mg/L), n-hexane had higher content of Magnesium (22.62±0.02 mg/L), aqueous and n-hexane had the same content of Zinc (0.25±0.04 mg/L), n-hexane had higher content of Potassium (569.33±0.47 mg/L), Copper (0.06±0.06 mg/L), and Manganese (0.13±0.02 mg/L) respectively. Statistical analysis showed significant difference ( $p < 0.05$ ) between Sodium, Calcium, Magnesium, Potassium and Iron content when compared with all the extracts (n-hexane, ethanol and aqueous). However no significance observed ( $p > 0.05$ ) when copper content of all the extracts are compared.

**Table 4:** Determination of mineral contents in n-hexane, ethanolic and aqueous extracts of *H. thebaica*.

Parameters (mg/L)	n-Hexane	Ethanol	Aqueous
Sodium	74.78±0.37 <sup>a</sup>	76.78±0.17 <sup>a</sup>	60.13±0.02 <sup>a</sup>
Calcium	17.11±0.02 <sup>b</sup>	16.67±0.13 <sup>b</sup>	18.73±0.82 <sup>b</sup>
Magnesium	22.62±0.02 <sup>c</sup>	14.03±0.17 <sup>c</sup>	15.22±0.14 <sup>c</sup>
Zinc	0.25±0.04	0.15±0.00 <sup>d</sup>	0.25±0.00
Potassium	569.33±0.47 <sup>e</sup>	413.20±1.87 <sup>e</sup>	298.73±1.14 <sup>e</sup>
Copper	0.06±0.06	0.05±0.06	0.05±0.09
Iron	0.56±0.02 <sup>f</sup>	0.66±0.12 <sup>f</sup>	0.87±0.01 <sup>f</sup>
Manganese	0.13±0.02	0.02±0.01 <sup>g</sup>	0.05±0.02

Values are expressed as mean ± standard deviation of 3 replicates; a, b, c, d, e, f and g = significant difference at  $p < 0.05$  when the extracts are compared.

## Discussion

The importance of plant secondary metabolites and their potential effect on human health are the interest of growing number of researches. Consumers are becoming a more concerned about the kind of diet they consume, showing greater interests in natural substances that seems safer and health promoting (Fakhar *et al.*, 2022). In the present study, the phytochemical, proximate, non-B vitamins and some minerals were qualitatively and quantitatively evaluated. The qualitative phytochemical screening of the aqueous, ethanol and n-hexane extracts of *Hyphaene thebaica* revealed the presence of Saponin, Tannin, Phenol, Cardiac glycoside, Anthranol glycoside, Flavonoid, Alkaloid, Terpenoid, Diterpenes, Triterpenes, Reducing sugar, Phytosterol, Coumarin, Steroids, Quinone, Essential oil, Phlobatanin, Ketone, Pentose and Anthraquinone compounds which have a vital in fighting against diseases producing pathogens. The results of the present study are in agreement with the findings of Ibrahim and Birniwa, (2024).

It was observed that the ethanol extract contains more phytochemical principles, followed by the aqueous and the least in terms of phytochemical constituents is the n-hexane extract. This observation might be due to the non-polar nature of the n-hexane extract while most of phyto-constituents of the *H. thebaica* fruit pulp are mostly polar in nature. This makes polar solvents (aqueous and ethanol) the best interms of extracting this phytochemicals than the non polar solvent (n-hexane). Therefore this chemicals can be used pharmacologically to develop new compounds for health benefits as shown in table 1.

The edible part of the fruit of Doum contains 74.0% soluble sugars, 22.0% of its starchy substances and 37.0% sucrose, also *H thebaica* contain a high percentage of potassium (Abdel-Muti, 2002) which is in agreement with the findings of this research and potassium content in then-hexane (569.33±0.47), ethanol (413.20±1.87) and aqueous extracts (298.73±1.14) respectively. FAO (2006) recorded the chemical compositions (per 100 g) of African Doum of 4.00, 3.80, 0.80, 84.10 and 7.30% for moisture, protein, fats, carbohydrates and ash, respectively. Tonga *et al.*, reported the carbohydrate (60.49±3.48), fiber (11.70±2.4), protein

( $3.12 \pm 0.77$ ), ash ( $6.85 \pm 0.81$ ) and moisture content ( $6.92 \pm 0.01$ ) respectively which are nearly close to the findings of this present study.

The mesocarp of *H. thebaica* fruits has some minerals such as magnesium, cobalt, copper, zinc, calcium and iron (Nwosu *et al.*, 2008). Abdel-Rahman *et al.*, (2014) stated that Doum fruit has adequate amount of calcium (Ca), iron (Fe), phosphorus (P), sodium (Na), magnesium (Mg), manganese (Mn) and potassium (K). This study found that the fruits of *Borassus aethiopum*, *Carissa edulis*, *Chrysophyllum albidum*, *Detarium microcarpum*, and *Hyphaene thebaica* have potential and significant amounts of proximate components and mineral elements that could be harnessed industrially to meet our daily nutritional requirements in times of scarcity (Bello *et al.*, 2021).

According to different researches, Doum flour contains significant quantity of important minerals such as potassium, sodium, calcium, magnesium, as well as phosphorus. The flour also contains vitamin B complex, carbohydrates, and fibers, all of which are beneficial to one's health. Several investigations have found that doum flour extracts are rich in phenolic and flavonoid compounds (Seleem 2015).

Our findings are similar to that of Khider *et al.*, 2022 who reported that Doum exhibit high content of minerals which increases the nutritional value.

In their findings, Abdullahi *et al.*, 2022, reported that the presence of phytochemicals including steroids, saponin, tannins, phlobatannins, terpenoids, alkaloid, glycoside, and flavonoids, in the aqueous extract of Doum (*Hyphaene thebaica*) fruit. Datti *et al.* (2021) reported that the Doum fruit was found to contain minerals including Potassium (3366.21 mg/100 g), Calcium (292.04 mg/100 g), Sodium (212.27 mg/100 g), Magnesium (177.14 mg/100 g), Iron (4.86 mg/100 g), Manganese (0.83 mg/100 g), Zinc (0.68 mg/100 g), Copper (0.40 mg/100 g), Nickel (0.32 mg/100 g) as well as Cobalt (0.12 mg/100 g).

Vitamin A content is highest in n-hexane extract than the other extracts (water and ethanol) probably because vitamin A is hydrophobic/lipophilic in nature hence dissolves in non polar solvent. The best solvents for extracting vitamin A in this research is n-hexane followed by ethanol and then water. The primary site of storing vitamin A in humans is kidneys, liver and adipose tissues and it is in the form of long chain fatty esters and provitamins (carotenoids). Its antioxidant action is exhibited through its strong radical quenching ability, thus making it powerful in hypoxic condition (Harabawy and Mosleh, 2014; Palace *et al.*, 1999). The extracts of *H. thebaica* should be used as supplement for this antioxidant vitamins because its supplementation has been shown to lower the risk of coronary heart disease and ischemic heart disease. Dalirani *et al.*, (2011) reports that, vitamin A was observed to reduce injury and scarring following pyelonephritis.

There is significant difference between aqueous and ethanol extracts of vitamin C content at  $p < 0.05$  but no significant difference is observed when aqueous and n-hexane extracts compared at  $p > 0.05$ . Vitamin C is hydrophilic/lipophobic in nature hence dissolves in polar solvents. The best solvents for extracting vitamin C is polar compounds like ethanol and water, non polar compounds like n-hexane can also be used for extraction purposes. Vitamin C plays a significant role in regulating hydrogen peroxide levels in oxidative stress, it was reported by Zaidi *et al.*, (2005) that vitamin C regulate the levels of SOD, catalase, GST, GSH and MDA in immobilized stressed rats. The antioxidant properties of vitamin C have been shown to prevent/alleviate some pathologic conditions including viral diseases, hypoxia-reperfusion induced apoptosis via the release of cytochrome C and the activation of caspases 9 and 3 in human endothelial cells (Montecinos *et al.*, 2007). Table 3 shows the results of vitamin E

content of aqueous, ethanol and N-hexane extracts *H thebaica*. The content of vitamin E of aqueous and ethanol extracts does not differ significantly at  $p > 0.05$ , however, when aqueous and ethanol extracts are compared with N-hexane extract significant difference is observed at  $p < 0.05$ . probably because the solvents (water and ethanol) of extraction is polar solvent. Vitamin E could scavenge free radicals through a mechanism of electron transfer to yield a Vitamin E cation radical, which subsequently undergoes fast de-protonation to produce a vitamin E radical. In other cases, vitamin E decomposes lipid peroxy radicals and lipid hydroperoxide resulting in the formation of vitamin E radicals (Niki, 2014).

## CONCLUSION

It was found that fruit palm extracts of *H thebaica* have higher contents of some nutritional profile that is proximate, antioxidant vitamins and mineral contents and the extracts of doum palm show the presence of some secondary metabolites screened in this study. The results of this study revealed that *H. thebaica* fruit pulp could be a significant source of antioxidant vitamins, minerals, some secondary metabolites and might be suggested for certain pathologic conditions like oxidative stress diseases, cancer, diabetes mellitus and cardiovascular diseases. Intensive research towards isolating, identifying active compounds and their mechanism of action is needed for better understanding of the manner and ability to treat/ manage diseases for the well being and welfare of humans.

## Declarations

### Author contribution statement

Mustapha Garba Muhammad, Musbahu Muhammad Sani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote and edit the paper.

Nafiu Ibrahim Ismail, Adam Isah Adam: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Rabia Muhammad Abdullahi: Performed the experiments; proofread and edited the manuscript,

## Funding statement

This research project was supported by the Tertiary Education Trust Fund (TETFund) [grant number TETF/DR&D/CE/POLY/KANO/RG/2021/VOL.I].

## Competing interest statement

The authors declare no conflict of interest.

## REFERENCES

- Abdel-Muti, O. M. S. (2002). Nutritive value of wild plants of the Sudan. *Arab Journal of Food and Nutrition* 3(3):6–67
- Abdel-Rahman, N. A., Ismail, I. A. & Elshafe'a, E. B. B. (2014) Characterization of some Sudanese edible forest fruits. *Journal of Agri-Food and Applied Sciences.*,2(2): 39–44
- Abdel-Raman, N.A.G (2019) "*Hyphaene thebaica* (Doum): distribution, composition and utilization. In *Wild Fruits: Composition, Nutritional Value and Products*, Springer, 2019, p. 427-434.
- Abdullahi F.H. (2015). Effect of locally prepared virgin coconut oil on oxidative stress markers in experimental rats, Department of Biochemistry, Bayero University, Kano, Nigeria. pp 54
- Abdullahi, A. N., Abdulmumin, T. M., Sheshe, S. M., Ismail, S. Y., Murtala, M., Ibrahim, A. M., Hassan, M. K., Bichi, S. A., Sarki, S. I. & Abubakar, S. (2022). Hypolipidemic Effect of Aqueous Fruit Extract of Doum Palm ( *Hyphaene Chemical & Pharmaceutical Research* Hypolipidemic Effect of Aqueous Fruit Extract of Doum Palm ( *Hyphaene Thebaica* ) in Wistar Rat. *Chemical & Pharmaceutical Research*, 4(3): 1–4.
- Anaduaka, Emeka G., Ogugua, Victor N, Egba, Simeon and Apeh, Victor O.I. (2013). Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leaf and stem. *American Journal of Ethnomedicine*, Vol. 4, No. 2, 175-201
- AOAC (2005) Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.
- Bello, M.I., Joshua, T., Paul, Y.J., Salifu, O.S., Nicholas, Z.M. and Magaji, B.S. (2021). Proximate and mineral elements compositions of some selected wild plants fruits (*Borassus aethiopum*, *Carissa edulis*, *Chrysophyllum albidum*, *Detarium microcarpum*, and *Hyphaene thebaica*. Vol. 6, Pp. 62-66, 2021. Vol. 6, Pp. 62-66, 2021 ISSN: 2734-2182. <https://doi.org/10.26765/DRJPHET01378932156>
- Dalirani, R., Yousefi Zoshk, M., Sharifian, M., Mohkam, M., Karimi, A., Fahimzad, A., & Varzandefar, M. (2011). Role of vitamin A in preventing renal scarring after acute pyelonephritis. *Iranian Journal of Kidney Disease*, 5(5), 320-323.
- Datti, Y. Ibrahim, M. Salihu, I. Abdulhadi, M. Muhammad, S. M. Abubakar, S. A. & Halima, S. (2021). Mineral Content , Proximate Composition and the Antioxidant Properties of the Mineral Content , Proximate Composition and the Antioxidant Properties of the Ethanol Extract of *Hyphaene thebaica* L . from Gezawa Town ,. *Asian Journal of Applied Chemistry Research.*, 6(2): 33–40.
- Fakhar Islam, Farhan Saeed, Muhammad Afzaal, Muzzamal Hussain, Entessar Al Jbawi, Muhammad Armghan Khalid & Muhammad Asif Khan (2022). Nutritional and functional properties of *Hyphaene thebaica* L. flour: a critical treatise and review, *International Journal of Food Properties*, 25:1, 1234-1245, DOI: 10.1080/10942912.2022.2078836
- FAO (2006). Composition and characteristics of selected palm products. FAO Corporate Document Repository. Forestry Department. Tropical Palm. FAO. Rome
- Fletcher R. (1997). Listing of useful plants of the world. Australian New Crops <http://www.newcrops.uq.edu.au/listing/hyphaenethebaica>.



- Harabawy, A.S.A., & Mosleh, Y.Y.I (2014). The role of vitamins A, C, E and selenium as antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia, *Oreochromis niloticus*. *Ecotoxicology and Environmental Safety*, 104(0), 28-35. doi:<http://dx.doi.org/10.1016/j.ecoenv.2014.02.015>
- Havsteen BH. (2002). The biochemistry and medical significance of the flavonoids. *Journal of Pharmacology and Therapeutics* 96,67-202
- Hsu B, Coupar IM, Ng K. (2006). Antioxidant Activity of Hot Water Extract from the Fruit of the Doum Palm, *Hyphaene thebaica*. *Food Chem., Elsevier Science Direct*. 98: 317–328.
- Ibrahim, I.S and Birniwa, A.H (2024). Phytochemical Screening, Ftir Characterization, And Antimicrobial Activity Of Doum Palm (*Hyphaene Thebaica*). *FUDMA Journal of Sciences (FJS)*. Vol. 8 No. 2, April, 2024, pp 163 – 169. DOI: <https://doi.org/10.33003/fjs2024-08022274>
- Khider, M., Seliem, K. A. E., Mustafa, W., & Ebid, A. (2022). Development of Functional Synbiotic Flavored Fermented Skim Milk Drinks Supplemented with Doum (*Hyphaene thebaica* L.) and Carob (*Ceratonia siliqua*) Fruits Powder for Nutritional, Antimicrobial and High Antioxidant Activities. *Food and Nutrition Sciences*, 13: 878–905.
- Kilani S, Sghaier M, Limem L, Bouhlel I, Boubaker J Bhourri W, Skandrani L, Neffatti A, Ammarb R, Dijoux- Franca M, Ghedira K, Chekir-Ghedira L (2008). In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. *Bioresource Technol*. 99: 9004–9008.
- Kitts D, Yuan Y, Wijewickreme AN, Hu C (2000). Antioxidant properties of a North American ginseng extract. *Molecular Cellular Biochem*, 203: 1–10.
- Krishnamachari Harini, Nithyalakshmi,. (2017) Phytochemical Analysis and Antioxidant Potential of Cucumis Melo Seeds. *Int. J. Life. Sci. Scienti. Res.*, 3(1); 863-867.
- Lee SE, Hyun JH, Ha J, Jeong H, Kim JH (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sci. African Journal of Pure and Applied Chemistry Vol. 3 (10)*, pp. 197-201.
- Montecinos, V., Guzmán, P., Barra, V., Villagrán, M., Muñoz-Montesino, C., Sotomayor, K., Sotomayor, P., & Vera, J.C. (2007). Vitamin C is an essential antioxidant that enhances survival of oxidatively stressed human vascular endothelial cells in the presence of a vast molar excess of glutathione. *Journal of Biological Chemistry*, 282(21), 15506-15515.
- Moussa H, Hank A, Margolis HA, Dube P, Odongo J. (1998). Factors Affecting the Germination of Doum Palm (*Hyphaene thebaica* Mart.) Seeds from the Semi-Arid Zone of Niger, West Africa. For. Ecol. Elsevier Science Direct, *Int. J. Life. Sci. Scienti. Res.*, 104: 27-41.
- Niki, E. (2014). Role of vitamin E as a lipid-soluble peroxy radical scavenger: in vitro and in vivo evidence. *Free Radical Biology and Medicine*, 66, 3-12.
- Nwosu, F. O., Dosumu, O. O., & Okocha, J. O. C. (2008). The potential of *Terminalia catappa* (Almond) and *Hyphaene thebaica* (Dum palm) fruits as raw materials for livestock feed. *Afr. J. Biotechnol.*, 7: 4576- 4580.
- Oluduro A.O. (2012). Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South Western Nigeria, *Malaysian Journal of Microbiology*, 8(2), 59-67.

- Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. (2009). AgroForest Tree Data Base: A Tree Reference and Selection version 4.0 (<http://www.worldagroforestry.org/sites/treedbs/treedatabases-asp>)
- Palace, V. P., Khaper, N., Qin, Q., & Singal, P. K. (1999). Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radic Biol Med*, 26(5-6), 746-761.
- Piao M, Kang k, Dong R, OkKo D, Wang Z, You H, Kim H, Kim J, Kang S, Hyun J. (2008). Hyperoside prevents oxidative damage induced By hydrogen peroxide in lung fibroblast cells via an antioxidant effect. *J Biochimica Biophysica Acta*. 1780 : 1448–1457.
- Review. *Internationale Pharmaceutica Scientia.*; 1(1):98-106.
- Rutkowski M., Grzegorzcyk K., Gendek E., Kedziora J.,(2006). Laboratory convenient modification of Bessey method for vitamin A determination in blood plasma *J.Physiol. Pharm.* 57(Suppl. 2), 221.
- Seleem, H. A. (2015). Effect of Blending Doum (Hyphaene Thebaica) Powder with Wheat Flour on the Nutritional Value and Quality of Cake. *Food Nutr. Sci.*, 6(7), 622–632. DOI: 10.4236/fns.2015.67066
- Shariff ZU. (2001) Modern Herbal Therapy for Common Ailments. *Nature Pharmacy Series Vol.1*, Spectrum Books Ltd., Ibadan, Nigeria in Association with Safari Books (Export) Ltd. UK, pp. 9-84.
- Shumaila Gul and Mahpara Safdar, (2009). Proximate Composition and Mineral Analysis of Cinnamon. *Pakistan Journal of Nutrition*, 8:1456-1460.
- Siddeeg, A.; Salih, Z. A.; Al-Farga, A.; Ata-Elfadeel, E. M. A.; Ali, A. O. (2019) Physiochemical, Nutritional and Functional Properties of Doum (Hyphene Thebaica) Powder and Its Application in Some Processed Food Products. *J. Nutri. Food Sci. Forecast.* 2(1), 1009.
- Smith-Warner, S. A.; Spiegelman, D; Yaun, S. S.; Albanes, D; Beeson, W. L.; Van Den Brandt, P. A.; Feskanich, D; Folsom, A. R.; Fraser, G. E.; Freudenheim, J. L.; Giovannucci, E; Goldbohm, R. A.; Graham, S; Kushi, L. H.; Miller, A. B.; Pietinen, P; Rohan, T. E.; Speizer, F. E.; Willett, W. C.; Hunter, D. J. (2003). "Fruits, vegetables and lung cancer: A pooled analysis of cohort studies". *International Journal of Cancer*. 107(6), 1001–11.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011). Phytochemical screening and Extraction: A
- Tonga, M.A. Mohammed, M.I. Baba Buro, S.I. Muhammad S. and Umar M.I (2021). Proximate and Essential Elements Analyses of Some Fruits used as Animal Feed in Arid Environment, Nigeria. *Nigerian Research Journal of Chemical Sciences*. Volume 9, Issue 1.
- Wang B, Zhang W, Duan X, Li X. (2009). In vitro antioxidative activities of the extract and semipurified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). *Food Chem.* 113: 1101-1105.
- Wendakoon, C., Calderon, P., & Gagnon, D. (2011). Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. *Journal of Medicinally Active Plants*, 1(2), 60-68.

- Yashashri H, Javalgikar A, Mane L, Kale S & Chikodi P (2017). Application of Magnetic Stirrer for Influencing Extraction Method on *Tectona grandis* as Analgesic. Activity. *International Journal of Pharmaceutical and Clinical Research*; 9(9): 634-637.
- Zaidi, S. M., Al-Qirim, T. M., & Banu, N. (2005). Effects of antioxidant vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver. *Drugs in R & D*, 6(3), 157-165. doi:634 [pii]