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Research Article

# Phytochemical Composition and Antioxidant Potential of *Hyphaene* thebaica Fruit

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# INTRODUCTION

Medicinal plants are plants that have the inherent capacity or potential to treat or prevent a variety of illnesses. Plants have been used for therapeutic purposes since antiquity. There is a wealth of archaeological evidence that people used medicinal herbs during the Paleolithic period (approximately 60,000 years ago)<sup>1</sup>. Other nonhuman primates have also been observed to ingest medicinal herbs to alleviate disease. In underdeveloped nations, where infectious illnesses are widespread and contemporary healthcare facilities are woefully inadequate, medicinal plant usage is more visible and tactile<sup>2</sup>.

Different phytochemicals have been reported to have other ameliorative effects. These include polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthone, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulfides, inorganic ions, guanidines, which are extracted from different parts of the various plants (root, stem, leaf, flower, fruit, and other)<sup>3</sup>. The decrease of the antioxidant activity defense mechanism inherent to the body, which affects the generation of hydroxyl radicals, results in lipid peroxidation as the pancreatic beta cells are more susceptible to oxidative damage<sup>4</sup>.

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### Abstract

The nature and application of medicinal plants in managing complications of various ailments are attributed partly to the presence of different phytochemicals. Oxidative stress due to increased levels of reactive oxygen species is a challenge in the management of such ailments, which might lead to death. This study aimed to determine the phytochemical composition and antioxidant potential of Hyphaene thebaica fruit. The phytochemicals in methanol, aqueous, and ethyl acetate extract were qualitatively determined, followed by quantitation of the ethyl acetate extract as it contains phytochemicals absent in the other extracts. The in vitro antioxidant potential of the ethyl acetate extract of *H. thebaica* fruit was also determined. The result revealed the presence of alkaloids, saponins, terpenoids, and flavonoids in all the extracts. However, steroids were not detected in the aqueous extract, and glycoside was detected only in the ethyl acetate extract. Flavonoids (5.80±0.20%) were higher than all the other phytochemicals in the ethyl acetate extract, followed by saponins which were present up to 2.50±0.11%, then terpenoids. Glycosides were present in higher amounts compared to steroids. However, alkaloids (0.08±0.05%) were in smaller amounts compared to the other phytochemicals quantified. The antioxidant activity showed a concentration-dependent increase in absorbance of the extract as displayed by the standard (ascorbic acid). The extract had an IC<sub>50</sub> of 52.21  $\mu$ g/mL, significantly (p <0.05) higher than that of the standard (14.10  $\mu$ g/mL). Due to its phytochemical composition and antioxidant activity, H. thebaica can be used as a medicinal plant.

Received: June 14<sup>th</sup>, 2022 1<sup>st</sup> Revised: September 20<sup>th</sup>, 2022 Accepted: October 9<sup>th</sup>, 2022 Published: November 30<sup>th</sup>, 2022 *Hyphaene thebaica* (Doum palm fruit; **Figure 1**) is a desert palm tree with edible oval fruit native to the Nile valley, a member of the palm family Arecaceae, and a source of potent antioxidants<sup>5</sup>. The fruit contains flavonoids (quercetin, hesperetin, and naringin), steroids, terpenes and tannins, carbohydrates, cardiac glycosides, terpenes, and terpenoids, besides various metals<sup>6</sup>. *Hyphaene thebaica* fruit has significant antimicrobial activities attributed to flavonoids' presence. Also, the aqueous extract of *H. thebaica* fruits showed antioxidant activity due to the substantial amount of their water-soluble phenolic contents<sup>5</sup>. *Hyphaene thebaica* fruit also has hypolipidemic activity, as the administration of the fruit decoction significantly lowers blood cholesterol, glucose, triglycerides, and total lipids<sup>7</sup>.

Medicinal plants usually act gently and support deficient systems and processes, making symptom relief a section of medicinal plants' therapeutic strategies. Therefore the need for investigations into the potential and efficacy of these medicinal sources to further develop the sources for maximum efficiency<sup>8</sup>. Several works of literature revealed different phytochemical compositions and antioxidant activity of *H. thebaica* fruit. Thus, this study aimed to determine the phytochemical composition and antioxidant potential of *H. thebaica* fruit.

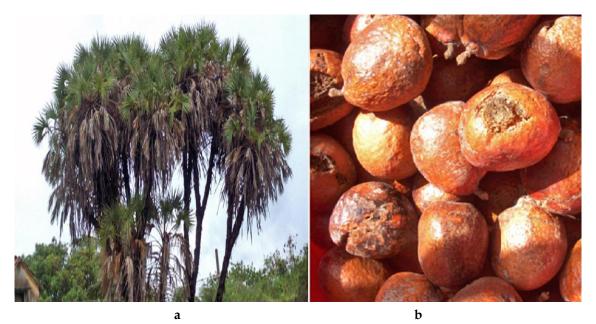


Figure 1. Hyphaene thebaica (a) tree and (b) fruit<sup>9</sup>.

# MATERIALS AND METHODS

### Materials

*Hyphaene thebaica* fruit was collected from Mayo-belwa Local Government Area of Adamawa state, Nigeria. Mayo-Belwa has a latitude of 9°3'10.38"N and a longitude of 12°3'27.17"E. The plant was authenticated by a Botanist with the Department of Plant Sciences, Modibbo Adama University, Yola. The voucher specimen (MAUH101) was deposited in the herbarium. The fruit pulp was dried, and the mesocarp was removed and grounded into powder using mortar and pestle. The chemicals and instruments used include DPPH, methanol, chloroform, ethyl acetate, and incubator/oven UNISCOPE SM9053. All other chemicals and reagents were of AnarlaR.

### Methods

### Extract preparation

*Hyphaene thebaica* fruit pulp powder (500 g) was macerated with 1.5 L of distilled water, methanol, and ethyl acetate in a glass jar for two days at room temperature. The extract was filtered and concentrated to dryness in the oven<sup>10</sup>.

#### Qualitative phytochemical analysis<sup>10,11</sup>

- 1. Alkaloid: To 2 mL of the extract, 2 mL of 10% HCl was added, followed by 2 mL of Mayer's reagent. The formation of an orange precipitate indicated a positive result.
- 2. Saponin: To 2 mL of the extract, 2 mL of distilled water was added. The mixture was agitated in a test tube for 5 minutes. The appearance of a layer of foam indicated a positive result.
- 3. Tannin: To 2 mL of the extract, five drops of 0.1% ferric chloride were added. The formation of a brownish-green or blueblack coloration indicated a positive result.
- 4. Steroid: To 2 mL of the extract, 10 mL of chloroform was added, and then 10 mL of concentrated sulphuric acid was added by the side of the test tube. The formation of a reddish upper layer and yellow sulphuric acid layer with green fluorescence indicated a positive result.
- 5. Glycoside: To 2 mL of acetic acid, 2 mL of the extract was added. The mixture was cooled in a cold-water bath, and 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Color development from blue to bluish-green indicated the presence of glycosides.
- 6. Terpenoid: To 2 mL of the extract, 2 mL of chloroform, and 1 mL of concentrated sulphuric acid were carefully added to form a layer. A transparent upper and lower layer with reddish-brown interphase indicated a positive result.
- 7. Flavonoid: To 2 mL of the extract, 10% sodium hydroxide was added. A yellow color was formed, which turned colorless upon the addition of 2 mL of dilute hydrochloric acid, indicating a positive result.

#### Determination of total alkaloids content

Total alkaloids were determined by the gravimetric method as previously described<sup>12</sup>. Briefly, 0.5 g of the extract was weighed into a conical flask containing 10 mL of 10% ammonium hydroxide to convert alkaloidal salts into the free base; the mixture was stirred and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one-quarter of its original volume on a water bath, and concentrated ammonium hydroxide solution was added dropwise to the mixture to precipitate the alkaloids. The precipitate was filtered using a weighed filter paper and washed with 10% ammonium hydroxide solution. The precipitate was dried with the filter paper in an oven at 60°C for 30 minutes and then reweighed and calculated thus **Equation 1**.

% Total alkaloids = 
$$\frac{weight of residue}{weight of sample} \times 100$$
 [1]

#### Determination of total saponins content

Total saponins were determined according to the previous method<sup>13</sup>. Briefly, 0.5 g extract was introduced into a conical flask, and 10 mL of 20% aqueous ethanol was added. The sample was heated over a water bath for one hour with continuous stirring at about 55°C. The concentrate was transferred into a 250 mL separator funnel, and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, and the ether layer was discarded. About 10 mL of n-butanol was added, followed by 2 mL of 5% aqueous NaCl. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight and calculated thus **Equation 2**.

$$\% Total saponins = \frac{weight of residue}{weight of sample} \times 100$$
[2]

#### Determination of total steroids content

The steroid content was determined using the method described previously<sup>12</sup>. Briefly, 0.5 g of the sample was hydrolyzed by boiling 50 mL of the hydrochloric acid solution for about 30 minutes. It was filtered, and the filtrate was transferred to a separating funnel. An equal volume of ethyl acetate was added to it, mixed well, and allowed to separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100°C for 5 minutes in a water bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture became turbid, and a pre-weighed filter paper was used to filter the mixture properly. The dry extract was then cooled in a desiccator and reweighed. The process was repeated two mere times, and an average was obtained. The concentration of steroids was determined and expressed as a percentage thus **Equation 3**.

% Total steroids = 
$$\frac{weight of residue}{weight of sample} \times 100$$
 [3]

#### Determination of total glycosides content

Total glycosides were determined as described previously<sup>14</sup>. Briefly, 0.5 g of the extract was weighed into a 100 mL volumetric flask with 10 mL of 70% of ethanol in it. It was boiled for 2 minutes in the water bath, filtered, and the filtrate was diluted with 20 mL of distilled water. Afterward, 2 mL of 10% lead acetate was added to this volumetric flask to precipitate the chlorophyll, tannins, and alkaloids. It was then filtered with the filtrate transferred to a separating funnel with 10 mL of chloroform. The funnel was rotated repeatedly. Two layers were formed, and the lower organic layer was collected (chloroform), dried, and weighed. The percentage of total glycosides contents was determined thus **Equation 4**.

% Total glycosides = 
$$\frac{weight of residue}{weight of sample} \times 100$$
 [4]

#### Determination of total terpenoids

Total terpenoids were determined by the gravimetric method described previously<sup>11</sup>. Briefly, 0.5 g of the sample was taken and soaked in 10 mL of ethanol for 24 hours. The extract, after filtration, was extracted with 10 mL of petroleum ether using a separating funnel. The ether extract was separated in pre-weighed crucibles and waited for its complete drying. Ether was evaporated, and the yield (%) of total terpenoids contents was measured thus **Equation 5**.

% Total terpenoids = 
$$\frac{weight of residue}{weight of sample} \times 100$$
 [5]

### Determination of total flavonoids content

Total flavonoids were determined according to the method described previously<sup>12</sup>. About 0.5 g of the extract was mixed with 10 mL of 80% aqueous methanol. The whole solution was filtered through the Whatman filter paper. The filtrate was transferred to a pre-weighed crucible and evaporated into dryness over a water bath, and weighed thus **Equation 6**.

% Total flavonoids = 
$$\frac{weight of residue}{weight of sample} \times 100$$
 [6]

#### Determination of antioxidant activity

Evaluation of the DPPH radical scavenging method was adopted, as reported previously<sup>15</sup>. The free radical scavenging activity of the extract was measured by DPPH. Here 0.1 mM solution of DPPH in methanol was prepared and added to different concentrations of the extract (20, 40, 60, 80, and 100  $\mu$ g/mL) prepared in methanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer, with ascorbic acid as standard. The procedure was done in triplicate. The lower absorbance of the reaction mixture indicated higher free radical activity. The half-maximal inhibition concentration (IC<sub>50</sub>) value was determined. The percentage DPPH scavenging effect was calculated by using the following Equation 7.

% DPPH scavenged = 
$$\frac{Absorbance \ control - Absorbance \ sample}{Absorbance \ control} \times 100$$
 [7]

#### Statistical analysis

Data were expressed as mean  $\pm$  standard error of the mean ( $\pm$  SEM). Differences among group means were assessed by One-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. Group means were considered to be significantly different at p <0.05. Data were statistically evaluated using Statistical Package for the Social Sciences (SPSS) version 22 Software.

### **RESULTS AND DISCUSSION**

The phytochemical composition of methanol, aqueous, and ethyl acetate extracts of *H. thebaica* fruit are shown in **Table I**. The result revealed the presence of alkaloids, saponins, steroids, terpenoids, and flavonoids in the methanol extracts, with the absence of tannins, phenols, and glycosides. Alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids were

detected in the ethyl acetate extract without tannins and phenols. The result also revealed the presence of alkaloids, saponins, terpenoids, and flavonoids in the aqueous extract, with the absence of tannins, phenols, steroids, and glycosides.

The ethyl acetate extract contains glycosides that were not detected in methanol and aqueous extract. The steroid was also detected in ethyl acetate extract, which was not detected in the aqueous extract though present in the methanol extract. The glycosides of luteolin and chrysoeriol flavones in *H. thebaica* fruit were reported previously<sup>10</sup>. The present study agrees with a study previously reported<sup>16</sup> for detecting the presence of flavonoids and tannins in the aqueous extract of *H. thebaica* fruit. The previous study<sup>17</sup> on the phytochemical composition of crude mesocarp extract of *H. thebaica* revealed the presence of tannins, saponins, steroids, glycosides, flavonoids, and terpenoids in low and moderate concentrations, though alkaloids were not detected. However, in the present study, alkaloids were detected. Another study detected saponins, flavonoids, glycosides, terpenoids, and steroids in *H. thebaica* fruit without alkaloids<sup>16</sup>. The present study agrees with this, as the phytochemicals were all detected without alkaloids. In a similar study<sup>18</sup>, alkaloids, flavonoids, terpenoids, and saponin in *H. thebaica* were reported previously. However, glycosides were not detected, which was detected in the present study. Different phytochemicals have been reported to have various anti-diabetic activities. These include polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthone, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulfides, inorganic ions, and guanidines, which are extracted from different parts of the various plants (root, stem, leaf, flower, fruit, and other)<sup>319</sup>.

Table I.	fruit.	ne phytochemical composition of	methanol, aqueous, and ethyl aceta	ite extracts of <i>H. thebaica</i>
	Component	Methanol extract	Ethyl acetate extract	Aqueous extract

Component	Methanol extract	Ethyl acetate extract	Aqueous extract
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	-	-	-
Phenols	-	-	-
Steroids	+	+	-
Glycosides	-	+	-
Terpenoids	+	+	+
Flavonoids	+	+	+

Note: + = Phytochemical detected (present); - = Phytochemical was not detected (absent).

The quantitative phytochemical composition of the ethyl acetate extract of H. thebaica fruit is presented in **Table II**. The result revealed the presence of flavonoids ( $5.80\pm0.20\%$ ) higher than all the other phytochemicals. This was followed by saponins which were present up to  $2.50\pm0.11\%$ , then terpenoids ( $2.30\pm0.09\%$ ). Glycosides ( $2.10\pm0.10\%$ ) were present in a higher amount compared to steroids ( $1.11\pm0.08\%$ ). The alkaloids ( $0.08\pm0.05\%$ ) were in smaller amounts than the other phytochemicals quantified.

 Table II.
 Quantitative determination of the phytochemical composition of ethyl acetate extract of *H. thebaica* fruit.

Components	Concentration (%)	
Alkaloids	0.80 ±0.05	
Saponins	2.50 ±0.11	
Steroids	1.11 ±0.08	
Glycosides	2.10 ±0.10	
Terpenoids	2.30 ±0.09	
Flavonoids	5.80 ±0.20	

Note: Values were in triplicates determinations (± SEM).

The flavonoids value reported in the present study was higher than the value (2.1%) previously reported<sup>10</sup> for the methanol mesocarp extract of *H. thebaica*, which might be due to the lower polarity of ethyl acetate compared to methanol as flavonoids are medium or semi-polar compounds. A similar study<sup>20</sup> reported the flavonoid compounds of methanol and ethanol extract of *H. thebaica* as 4.1% and 2.1%, respectively. These values were lower than the value obtained in this study (5.80±0.20%). In a similar study<sup>16</sup> previously reported on the methanolic extract of *H. thebaica* fruit, saponins were in lower amounts than the value (2.50±0.11%) reported in the present study. In another study<sup>16</sup>, flavonoids (21.21±0.03%) in the

methanol extract of *H. thebaica* were reported in higher amounts than the value ( $5.80\pm0.20\%$ ) reported in the present study. Saponins ( $2.50\pm0.11\%$ ) reported in the present study were lower than values reported previously<sup>16</sup> for methanol ( $8.32\pm0.02\%$ ) and aqueous ( $6.27\pm0.05\%$ ) extract of *H. thebaica* fruit. The flavonoid value ( $5.80\pm0.20\%$ ) reported in the present study was higher than the value ( $20.4\pm2.0\%$ ) of the methanolic extract of *H. thebaica* reported previously<sup>5</sup>. The difference in the values might be due to the difference in the solvent used for extraction. Flavonoid compounds are speculated as one of the most widespread groups of natural constituents in plants, with the ability to adsorb and neutralize free radicals through the scavenging or chelating process<sup>21,22</sup>.

Alkaloids have been reported to have different pharmacological applications, which include antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine)<sup>23</sup>, antibacterial (e.g., chelerythrine)<sup>24</sup>, and anti-hyperglycemic activities (e.g., piperine), psychotropic (e.g., psilocin) and stimulant activities (e.g., cocaine, caffeine, and nicotine) used as recreational drugs<sup>25</sup>. Plant terpenoids were reported to have antimicrobial, antioxidant, anticancer, neuroprotective, and chemoprotective properties<sup>26-28</sup>. Terpenoids are regarded as the most abundant group of secondary metabolites, with over 30,000 known compounds, including steroids<sup>29</sup>.

The result of the *in vitro* antioxidant potential of ethyl acetate extract of *H. thebaica* fruit using DPPH is presented in **Table III**. The result showed a concentration-dependent increase in absorbance of the extract as displayed by the standard (ascorbic acid), with the absorbance ranging from  $38.48 - 66.45\pm0.01\%$  and  $68.23 - 74.40\pm0.01\%$  for the extract and standard (ascorbic acid) respectively, though the absorbance of the extract was significantly (p <0.05) lower than that of the standard. Ethyl acetate extract of *H. thebaica* fruit had a half-maximal inhibitory concentration (IC<sub>50</sub>) of  $52.21 \,\mu\text{g/mL}$ , which was significantly (p <0.05) higher than that of the standard (14.10  $\mu\text{g/mL}$ ). In a similar study<sup>30</sup>, the antioxidant activity of ethyl acetate extract of *H. thebaica* fruit showed strong DPPH scavenging activity at concentrations (400, 600, and 800  $\mu\text{g/mL}$ ) more than ethanolic extract and standard, which were 70.20\%, 88.70\%, and 95.80\%, respectively with a lower IC<sub>50</sub> value. The low IC<sub>50</sub> indicated a stronger free radical inhibition of the ethyl acetate extract.

DPPH has been an effective method to determine the ability to scavenge free radicals by converting DPPH into the more stable DPPH-H after acquiring an electron or hydrogen radical. Recent studies revealed the antioxidant activity of leaves and fruit of *H. thebaica* using different solvents for extraction<sup>5,19,30</sup>. Free radicals generated in disease conditions which include diabetes, cancer, neurodegenerative diseases, and others, bring about oxidative stress to the subject. The ability to scavenge these free radicals is essential in managing these diseases<sup>31</sup>.

Concentration (%)	Absorbance	e (%)
Concentration (76)	Ethyl acetate extract	Ascorbic acid
20	38.48 ±0.01 <sup>a</sup>	68.23 ±0.01
40	45.04 ±0.02 <sup>a</sup>	74.05 ±0.02
60	53.63 ±0.01 <sup>a</sup>	74.09 ±0.01
80	58.10±0.01 <sup>a</sup>	75.94 ±0.00
100	66.45 ±0.01 <sup>a</sup>	76.14 ±0.00

Table III.	In vitro antioxidant activity of ethyl acetate extract of H. thebaica fruit using DPPH.	
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Note:

 $IC_{50}$  for ethyl acetate extract = 52.21  $\mu$ g/mL.

IC<sub>50</sub> for ascorbic acid =  $14.10 \,\mu\text{g/mL}$ .

 $Values were in triplicates determinations (\pm SEM). Values in the same row with {}^{a} superscript were significantly (p < 0.05) lower than the standard (ascorbic acid).$ 

The presence of secondary metabolites such as flavonoids, polyphenols, glycosides, alkaloids, and glycosaponins with different pharmacological capabilities, such as antioxidant and hepato-protective, reduce oxidative stress<sup>32</sup>. Their ability to inhibit lipid peroxidation, capillary fragility, permeability, platelets aggregation, and various lipoxygenase enzyme activities was also reported<sup>33</sup>. The presence of malic and citric acid in 61% and 34% of the total organic acid content in *H. thebaica* fruit were previously reported<sup>10</sup>. Malic acid is commonly found in unripe fruit and contributes to its sour taste, whereas citric acid is an antioxidant with the ability to chelate metals that could account for *H. thebaica* antioxidant activity<sup>34</sup>. The antioxidant activity of the ethyl acetate extract of *H. thebaica* fruit might be attributed to malic and citric acid. The DPPH IC<sub>50</sub> value (52.21  $\mu$ g/mL) reported in this study for the ethyl acetate extract was within the range of values (>50  $\mu$ g/mL) reported

previously for the methanol extract of *H. thebaica* fruit<sup>20</sup>. The difference in the IC<sub>50</sub> values reported might be due to factors such as the type of solvent, composition of the solvent, and apparatuses used in the extraction<sup>35</sup>.

# CONCLUSION

In conclusion, the presence of phytochemicals and promising radical scavenging potential of *H. thebaica* fruit indicates that the fruit can be applied in the management of various ailments such as free radical-linked diseases.

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# **AUTHORS' CONTRIBUTION**

Muhammad Mubarak Dahiru carried out the research and wrote the article. Margret Samuel Nadro conceptualised the central research idea, provided the theoretical framework and supervised the whole research.

# DATA AVAILABILITY

None.

# **CONFLICT OF INTEREST**

The authors declares that there is no conflict of interest.

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