PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITIES OF AQUEOUS *HYPHAENE THEBAICA* FRUIT PULP

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ABSTRACT

Natural products from plants including fruits, seeds, barks, leaves and other plant parts are endowed with various phytochemicals and nutritional elements with diverse nutraceutical and pharmacological potentials for the management and treatment of various human health disorders including oxidative stress related diseases such as diabetes, cancer, cardiovascular diseases, inflammatory disorders, amongst others. These diseases are fueled by the actions of free radicals whose effect on the body tissues and organs can be neutralized by antioxidants. Natural and synthetic antioxidants have the capacity to offset the excessive outcome of free radicals. Our study investigated the preliminary phytochemical screening and in-vitro antioxidant activities of a Nigerian edible wild fruit; Hyphaene thebaica, otherwise known as doum palm or gingerbread palm which is a member of the mint family (Arecaceae). It is popularly called 'Goruba' or 'Goriba' by the Hausa tribe who are the major consumer of the fruit in Nigeria. The fruit and other parts of the plant is used traditionally in the treatment of diabetes, hematuria and in elimination of worm. The outcome of our preliminary phytochemical screening using spectrophotometric methods showed that the aqueous fruit pulp extract of H. thebaica is packed with appreciable amounts of vital phytochemicals such as phenols, flavonoids, reducing sugars, tannins, saponins, steroids and others. The in-vitro free radical scavenging assays also indicated that the extract has significant ability to scavenge free radicals in comparison with standard antioxidant drug ascorbic acid. These result outcomes suggest that doum fruits will be valuable in the treatment and management of oxidative stress related disorder.

Key Words; wild fruits, *Hyphaene thebaica*, doum fruit, oxidative stress, antioxidants, phytochemicals, *in-vitro*, nutraceuticals.

INTRODUCTION

It is sarcastic that oxygen, a key element for continuity of life could also pose danger under certain conditions and cause damages to the human body. Majority of the possibly destructive effects of oxygen are as a result of the formation and subsequent action of some chemical compounds referred to as Reactive Oxygen Species (ROS), they have a propensity to donate oxygen to other substances (Lobo et al., 2010). Prolonged uninhibited oxidative stress has been associated with several human inflammation diseases like hepatitis, atherosclerosis, asthma, cancer, stroke, retinal damage, arthritis amongst others. Free radicals, specifically reactive oxygen species have been recognized to play a critical part in the pathogenic processes in several of these diseases, from responses to growth factor stimulation to generation of inflammatory responses (Oyinloye et al., 2015). Both natural and synthetic antioxidants are known to have the ability to counteract the excessive effect of reactive oxygen species and free radicals. Numerous natural products like fruits, vegetables, herbal plants, edible flowers, grains amongst other food sources have been shown to have antioxidant activities (Augustyniak et al., 2006). Several researches have established the potential of wild fruits as profound sources of antioxidants (Prakash et al., 2012; Egea et al., 2010; Lamien-Meda et al., 2008). This study seeks to investigate the *in vitro* antioxidant activity of an edible but rarely consumed wild

fruit; *Hyphaene thebaica*, popularly called *doum* palm or gingerbread palm. It is a member of the mint family (Arecaceae). It is usually found in close proximity to the River Nile in Sudan and Egypt and can also be found in sub-Saharan Africa, especially in the northern part of Africa and West India. It is likewise native to the Saudi Arabia, Yemen, Israel and Sinai (Abdel-Farid et al., 2019; El-Beltagi et al., 2018). It grows in valleys, at oases as well as rocky hillsides and it is regarded as a drought-tolerant and fire-resistant plant. It is a dioecious palm that grows up to a height of 17 m (56 ft). The stem, which can have a circumference of up to 90 cm is alienated into two branches, with each branch further separated into two branches. The split ends of the branches comprise of tufts of large leaves. The bark is smooth, dark gray in colour and has scars of fallen leaves. Woody fruits germinate from the female palm which grows continuously on the tree for an extended period. It is usually 6–10 × 6–8 cm, smooth, rectangular to cubical with rounded edges, it is usually shiny brown when ripe and has a single ivory coloured seed within it (El-Beltagi et al., 2018; Orwa et al., 2009).

Doum palm is regarded as life-sustaining by desert dwellers; hence, it is characterized as a famine food. The outer cover of the fruit is edible; it can be pounded to form powder which is often dried and added to food as seasoning agent. Additionally, the fruit pulp is used in cooking, the unripe kernel is also edible but the ripe kernel is hard and is mainly used as vegetable ivory (Siddeeg et al., 2019). The sweet drink prepared from hot water infusion of the dried fruit pulp is usually taken as a health boost and is greatly cherished (Farag and Paré, 2013). Traditionally, *doum* extract is used in the management of hematuria, particularly after delivery. It is generally taken as a hematinic suspension. In Egypt, the tea prepared from *doum* is popularly believed to cure diabetes. Additionally, the resin of the tree has been established to have diaphoretic as well as diuretic properties, it is popularly recommended for the treatment of tapeworm and as remedy to alleviate animal bites (El-Gendy et al, 2008).

MATERIALS AND METHODS

Collection of Sample, Preparation and Extraction

Ripe wholesome fruits of *H. thebaica* were obtained from Sabongari market in Kano state, Nigeria in the month of September 2020. The fruit was authenticated in the Department of Botany, University of Lagos. The fruits were washed under running tap water, drained and split open to remove the seed while the pulp was dried for 12 days. It was pulverized into fine powder which was macerated in distilled water for 24 hours, after which it was filtered through a muslin cloth to remove the shaft. The filtrate was left to stand in the refrigerator for 24 hours and the supernatant was removed. The sediment was dried in the oven at 40 $^{\circ}$C and subsequently, the dried crude extract obtained was stored in the refrigerator for further analysis.

Preliminary Phytochemicals Analysis

Qualitative

Test for the presence of phenols, alkaloids, saponin, tannin, terpenoids, phlobatannins, reducing sugar, flavonoids and steroids was carried out as previously described (Gul et al., 2017; Trease and Evans 1989) with some modifications.

Test for Phenols: 0.5g of crude extracts was dissolved in distilled water, thereafter, 2 ml of $FeCl_3$ solution was mixed with it. The formation of a deep bluish green solution shows presence of phenols.

Test for phlobatannins: 0.5 g of powder crude extract was properly dissolved in distill water, 1 % aqueous hydrochloric acid was added and was boiled in hot water bath with continuous stirring. The formation of red colored precipitate is an indication of the presence of phlobatannins.

Test for reducing Sugar: 0.50 g of crude powdered sample was dissolved 5 ml of distilled water, and was put into a test tube in which 1 ml of Fehling solution A and 1 ml of Fehling solution B have been

put, the tube was inserted in a water-bath at 60° C. A positive test is shown by a green suspension and a red precipitate.

Test for terpenoids: 0.5 g of sample dissolved in 5 ml of distilled water was mixed with 2 ml of chloroform, 3 ml concentrated H_2S0_4 was carefully added to form a layer. The formation of a reddish-brown colored interface is an indication of positive results for the presence of terpenoids

Test for flavonoids: 0.5 g of sample was dissolved in 10 ml of distill water, 5 ml of dilute ammonia solution was added followed by addition of 1 ml concentrated H_2S0_4. Occurrence of yellow color which disappeared on standing shows the presence of flavonoids in the sample.

Test for alkaloids: Alkaloids were detected using the Dragendroff's test. 0.2 g of sample was dissolved in 5ml of distilled water, 5 ml of 2 % HCl was poured in a test tube having the sample. 1 ml of Dragendroff's reagent was added and appearance of orange brown precipitate showed the presence of alkaloids.

Test for tannins: 0.2 g of the samples was dissolved in 5 ml distilled water, to this was added a few drops of 1 % lead acetate. Formation of a yellowish precipitate indicated the presence of tannins.

Test for saponins: 5 ml of distilled water was added to a solution of the plant extract in a test tube and was vigorously agitated. The frothing solution formed was mixed with few drops of olive oil and then mixed vigorously, the appearance of foam showed the presence of saponins.

Test for steroids: 2 ml of chloroform and concentrated H_2SO_4 were mixed with the 5 ml of previously dissolved aqueous plant extract. The occurrence of red colouration indicated the presence of steroids.

Quantitative Analysis

Determination of alkaloids: 0.5 g of extract was dissolved in 96 % ethanol and 20 % H_2S0_4 (1:1) mixture. 1 ml of the filtrate was put into 5 ml of 60 % H_2S0_4, this was left undisturbed for 5 min. Thereafter, 5 ml of 0.5 % formaldehyde was added and left for additional 3 hr. The reading was taken at absorbance of 565 nm (Harborne, 1976).

Estimation of flavonoids: The amount of flavonoid in *H. thebaica* was ascertained spectrophotometrically. 0.5 g of sample was added to 5 ml of dilute hydrochloric acid and boiled for 30 min, it was filtered after allowing it to cool. 1 ml of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1 % ammonia solution and the absorbance was measured at 420 nm (Harborne, 1976).

Estimation of Saponins: *H. thebaica* extract were dissolved in 80 % methanol thereafter, 2 ml of Vanilin in ethanol was introduced, mixed properly and then 2 ml of 72 % hydrogen tetraoxosuphate phosphate acid solution was added. The mixture was thoroughly mixed and heated on a water bath at 600 °C for 10 min, the absorbance was measured at 544 nm against reagent blank (Madhu, 2016).

Estimation of phenol: The total phenolics content in *H. thebaica* extract was determined with the *Folin-Ciocalteu*'s reagent. A solution of the extract was mixed with 0.4 ml *Folin- Ciocalteu*'s reagent (diluted 1:10 v/v). 5 min later, 4 ml of sodium carbonate solution was added and the final volume was made up to 10 ml with distilled water and left to stand for 90 min at 25 °C. Thereafter, absorbance of the sample was taken against the blank at 725 nm with a spectrophotometer. These data were used to estimate the total phenolics content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Khatri and Chhetri, 2020).

Estimation of Steroids: 1 g of the extract was macerated with 20 ml of ethanol and filtered. To 2 ml of the filtrate, 2 ml of chromogen solution was added and the solution left undisturbed for 30 min. Thereafter, the absorbance was read at 550 nm (Ekwueme et al., 2015).

Estimation of Tannins: 1 g of the test sample was macerated with 50 ml of methanol and filtered. To 5 ml of the filtrate, 0.3 ml of 0.1 N ferric chloride in 0.1 N HCl and 0.3 ml of 0.0008 M of potassium ferric cyanide was added. Then, the absorbance of the solution was measured at 720 nm (Ekwueme et al., 2015).

Estimation of reducing sugar: The reducing sugar content was estimated with the 3,5-dinitrosalicylic acid (DNSA) method based on the methodology of Krivorotova and Sereikaite, (2014) with some modification. DNSA reagent was made by dissolving 1 g of DNSA and 30 g of sodium-potassium tartaric acid in 80 ml of 0.5 N NaOH at 45°C, the solution was allowed to cool and diluted to 100 ml with distilled water. 2 ml of DNSA reagent was mixed with 1 ml of plant extract and kept at 95 °C for 5 min. Thereafter, 7 ml of distilled water was added to the solution and the absorbance of the resulting solution was measured at 540 nm. The reducing sugar content was derived from the calibration curve of standard D-glucose, and the results were expressed as mg D-glucose equivalent per gram dry extract weight.

In-vitro Antioxidant Activity Determination

DPPH radical scavenging assay: 0.1 mM solution of DPPH in ethanol was prepared; 1ml the solution was added to 1 ml of extract dissolved in water at different concentrations. The mixture was mixed thoroughly and kept at room temperature for 30 min. Thereafter, the absorbance was measured at 517 nm with a UV-Spectrophotometer. Decrease in absorbance of the reaction mixture is an indication of free radical scavenging activity (Aksoy et al., 2013).

The percent DPPH scavenging effect was calculated using the equation;

DPPH Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract.

Nitric oxide Scavenging activity: 10 mM of sodium nitroprusside in phosphate buffer saline was mixed with varied concentrations of *H. thebaica* extract, and incubated for 30min, 0.5ml of Griess reagent (1 % sulfanilamide in 5 % phosphoric acid and 0.1 % naphthylethylenediamine dihydrochloride) was added. Thereafter, the absorbance was measured at 550nm using ascorbic acid as standard. The inhibitory concentration of the extract needed to reduce 50 % of nitric oxide formation was determined (Patel and Patel, 2011).

$$\text{Nitric oxide (inhibition \%)} = \frac{\text{A (control)} - \text{A (sample)}}{\text{A (control)}} \times 100$$

Reducing power assay: The reducing power assay was done based on the method of Oyaizu (1986) with slight modification. Reaction was carried out in a mixture containing 1 ml of sample (25-100 µg/ml), 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 %, w/v potassium ferrocyanate [K3Fe(CN)6] by incubating at 50 °C for 20 min. After the addition of 2.5 ml trichloroacetic acid (10 %, w/v), the mixture was centrifuged at 5000 rpm for 10 min. The upper layer (5 ml) was mixed with 0.5 ml of fresh $FeCl_3$ (0.1 %, w/v), and the absorbance at 700 nm was measured against a blank. Gallic acid was used as the control.

Statistical analysis

Analysis was done using the GraphPad Prism Version 9.1.2 (GraphPad Software, San Diego California, USA) using Student's T-test and $P < 0.05$ was regarded as significant. Data are expressed as mean ± SEM of three determinations.

Results

Qualitative and Quantitative Phytochemicals Analysis

The outcome of the qualitative analysis for the presence of phytochemicals is as shown in Table 1. All the important phytochemicals tested were confirmed to be present except phlobatannin. Result of the quantitative analysis is presented in Table 2, it shows significant amounts of the tested phytochemicals expressed in mg/100g. It shows the presence of good amount of total phenol content (114.01 ± 2.44 mg/100g). The reducing sugar in the extract is shown to be 69.04 ± 0.42 mg/100g. The flavonoids content is 43.63±1.64 mg/100g, while 27.46 ± 0.65 g/100mg of steroids and

52.51 ± 0.61 mg/100g of alkaloids were found in the aqueous extract. 82.31 ± 1.00 mg/100g of tannins and 55.21 ± 0.95 mg/100g of saponins was also discovered present in the aqueous fruit extract.

Table 1: Occurrence of selected phytochemicals in aqueous extracts of *H. thebaica*

Phytochemical s	Occurrence
Phenols	+
Reducing sugar	+
Flavonoids	+
Alkaloids	+
Terpenoids	+
Steroids	+
Saponins	+
Tannins	+
Phlobatannin	-

Note: represents present, - represents absent

Table 2: Quantity of selected phytochemicals in *H. thebaica*, values in mg/100g of sample

Phytochemical s	Amount (mg/100g)
Phenols	114.01 ± 2.44
Reducing sugar	69.04 ± 0.42
Flavonoids	43.63 ± 1.64
Alkaloids	52.51 ± 0.61
Steroids	27.46 ± 0.65
Tannins	82.31 ± 1.00
Saponins	55.21 ± 0.95

Note: Individual value is a mean of three determination ±SEM

Antioxidant and free radical scavenging activities

The scavenging activity of the DPPH radical was tested by reduction of the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. The experimental observations of scavenging effect of *H. thebaica* extract with the DPPH radical are presented in Table 3. Also presented in Table 3 is the outcome of the ability of our extract to inhibit the accumulation of nitrite, a stable oxidation product of nitric oxide in comparison with ascorbic acid. It is observed from the result that the DPPH scavenging activity and nitric oxide scavenging activity increases with increase in the concentration of the doses administered for both our extract and ascorbic acid. The best percentage inhibition for both our extract and the control in both assays is at a concentration of 100 µg/ml. Statistical analysis indicates that there is no significant difference ($P < 0.05$) between the activity of the *H. thebaica* aqueous fruit extract and the standard drug used as control. The scavenging activity of our extract is dosage concentration dependent.

For the evaluation of the reductive capacity, the transformation of Fe^{3+} to Fe^{2+} in the presence of the aqueous extract of *H. thebaica* samples and the standard drug ascorbic acid was determined. Table 4 illustrates the reducing power capability of our extract in comparison with ascorbic acid. Statistical analysis indicates that there is no significant difference ($P < 0.05$) between the reductive powers of our extract in comparison with the standard drug ascorbic acid.

Table 3: *In-vitro* free radical scavenging effect of aqueous *H. thebaica*

Sample	Concentration (μg/ml)	DPPH scavenging activity (% inhibition)	Nitric oxide scavenging activity (% inhibition)
H. thebaica	**25**	31.25 ± 0.99	36.53 ± 0.99
	50	50.90 ± 0.44	53.19 ± 1.00
	75	65.98 ± 0.83	69.45 ± 0.71
	100	77.55 ± 1.04	79.63 ± 0.78
Ascorbic acid	**25**	45.00 ± 0.55	48.25 ± 0.75
	50	56.44 ± 1.11	63.46 ± 0.87
	75	77.35 ± 1.04	77.35 ± 2.67
	100	90.25 ± 0.93	85.31 ± 0.98

Note: Individual value is a mean of three determination ±SEM

Table 4: Reducing power ability of *H. thebaica*

Concentration (μg/ml)	*H. thebaica*	Ascorbic acid
25	0.19 ± 0.01	0.24 ± 0.01
50	0.29 ± 0.01	0.38 ± 0.01
75	0.39 ± 0.02	0.49 ± 0.01
100	0.51 ± 0.02	0.63 ± 0.01

Note: Individual value is a mean of three determination ±SEM

Discussion

Plants are endowed with various phytochemicals which have been shown to be responsible for various biological activities exhibited by different plant parts including the fruits. Naturally, these phytochemicals are important machineries for proper physiological functions of plants like protection against pathogens, protection against ultra violet light and herbivores and aids pollination (Mohd Dom et al., 2020). Phytochemicals also known as secondary plant metabolites offer profound health benefits to humans. They act as synergistic agents, enabling nutrients to be absorbed more effectively by the body. The beneficial importance of phytochemicals includes; low toxicity, low cost, easy to obtain and most importantly their biological properties (Nyamai et al., 2016).

The present study investigated the occurrence and quantity of selected phytochemicals in the *aqueous doum* palm fruit extract and the antioxidant potential of the extract in comparison with ascorbic acid a standard antioxidant. Result of the qualitative analysis is consistent with previous studies which recorded the presence of saponins, phenols, flavonoids, tannins, reducing sugars, alkaloids, steroids and terpenoids but the absence of phlobatannins in extracts of *doum* fruit (Abubakar 2017; Auwal et al., 2013).

Phenolic compounds are known to enhance the quality and nutritional value of plant parts by impacting on the flavor, aroma, colour and taste, they are also vital in plant defense mechanisms to offset the effect of Reactive Oxygen Species (ROS) so as to prevent molecular damage (Tamilselvi et al., 2012). It is noteworthy that the result obtained in this study collaborates previous research which reported that the highest amount of total phenols was obtained from the fruits of *H. thebaica* in comparison with other plant parts investigated (Taha et al., 2020).

Recent studies equally reported the presence of ample amount of carbohydrate in the fruit, leaves and male parts of the extract of *doum* plant with the fruit having the highest value (Datti et al., 2020; Taha et al., 2020). Sugar plays a crucial part in plants as nutrient and central signaling molecules which control gene expression linked to growth and development, metabolism, response to stress and disease resistance. Both reducing and non-reducing sugar perform vital role in the central metabolic

pathways and aid in the production of secondary metabolites which boost the medicinal activities of plants (Khatri et al., 2020).

Like in previous studies (Taha et al., 2020; Farag and Paré, 2013), our quantitative analysis of phytoconstituents of our extract shows the presence of appreciable quantity of flavonoids in the fruit extract. Flavonoids which occur in all photosynthesizing plant are a main colouring constituent of flowering plants. They are usually responsible for preventing fatty acids oxidation, protection of vitamins and enhancing enzyme activities hence, ensuring protection against diseases. The extensive biological properties of flavonoids help in promoting good health and reducing risk of diseases in human (Tiwari and Husain, 2017).

Several studies have established that wild edible fruits are packed with significant quantity of alkaloids which are phytochemicals that contain nitrogen derived from several amino acids (Anhwange et al., 2015; Barcelo, 2015; Ruiz et al., 2014). Alkaloids with therapeutic properties chiefly act by influencing chemical transmitters of the nervous system such as, serotonin, dopamine, acetylcholine and γ-aminobutyric acid. Alkaloids are reported to have antidiabetic, antiarrhythmics, antihypertensive, anticancer and antimalarial effects (Nyamai et al., 2016).

Plant steroids are secondary metabolites which can be classified into numerous groups on the basis of taxonomic considerations, their functions as well as structures. They perform vital physiological functions within plants. They are of great pharmacological importance to mankind, thus, they are used as herbal supplements in many countries (Gunaherath et al., 2006).

Saponins have the ability to lower blood cholesterol by averting its reabsorption, thus, making it useful in cardiovascular disease. Additionally, research has shown that saponins possess antitumor and anti-mutagenic actions. They can reduce the risk of human cancers by preventing the growth of cancer cells. Saponins are thought to react with the cholesterol rich membranes of cancer cells, thus, restraining cell growth and viability. The non-sugar part of saponins is said to have a direct antioxidant activity and may help in reducing risk of cancer and cardiac diseases (Ajiboye, et al., 2013).

Tannins are present in the fruit extract in appreciable amount. Tannins are polyphenols which are obtainable from many parts of different plant species including the fruit, fruit pod, wood, tree bark, wood, leaves as well as the roots. Tannins have been reported to be capable of stimulating the receptor cells to enhance carbohydrate utilization (Nyamai et al., 2016). Tea, wine, fruits and vegetables which are rich in tannins can be taken in definite quantity to maintain good health by preventing oxidative damages due to generation of free radicals. Tannic acid isolated from herbs and fruits can be of great use in the development of nutraceuticals and pharmaceuticals (Ghosh, 2015).

Four concentrations of *H. thebaica* was investigated for antioxidant activities with DPPH free radical scavenging assays, nitric oxide scavenging activity assay and reducing power activity assay using ascorbic acid as standard in all assays. Results of the DPPH free radical scavenging assay shows that the percentage inhibition increases with increase in extract concentration. There is no significant difference between the free radical scavenging ability of our extract and that of the standard drug ascorbic acid.

The DPPH radicals were utilized as a substrate to appraise the free radical scavenging ability of *aqueous* fruit extract of *H. thebaica*. This assay involves the reaction of antioxidants with a stable free radical 2, 2-diphenyl-1-picry hydrazyl. This leads to a reduction of DPPH concentration by the antioxidant thus, decreasing the optical absorbance of DPPH (Kumar et al., 2010). The inhibitory effect of *H. thebaica* extract on nitric acid production showed inhibition in a concentration-dependent manner. This is an indication that the extract may be useful in preventing the effect of excessive production of nitric oxide in human body. Nitric oxide is implicated in inflammation, cancer and other pathological human disorders (Ebrahimzadeh, and Bahramian, 2009).

A dose-dependent reductive power was established for both the extract and the standard drug, with the extract exhibiting a greatly remarkable reducing power in comparison with ascorbic acid. The

reducing power of the extracts increased with increase in concentration suggesting that the electron donating capability of our extract is concentration dependent. Reducing power assay involves the measurement of the reductive ability of an antioxidant which is evaluated by the transformation of iron (III) to iron (II) in the presence of the plant extracts through the donation of electrons. Electron donation is a vital mechanism by which antioxidants encourage the formation of less reactive specie (Lue et al., 2010).

The reported free radical scavenging activity of the fruit extract might be attributed to the presence of high quantity of phenols and flavonoids as reported in the phytochemicals screening. A good correlation between these two phytochemicals and their antioxidant activity has been previously established (Hu et al., 2016; Abuashwashi 2016).

Conclusion

The outcome of the various antioxidant assays is an indication that *aqueous* extracts of *H. thebaica* fruit might be very valuable in the management of oxidative stress related disorders. Further research is required to evaluate the antioxidant activity of the extract *in vivo*. There is also need for the identification, isolation and characterization of the bioactive component of the fruit extract responsible for the reported antioxidant activity, this will hopefully lead to the use of the fruit in supplementation, production of nutraceuticals and drug formulation.

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