

Assessment of the Antioxidative Properties of *Hyphaene Thebaica* Fruit and Its Comparative Inhibitory Activities with Butylhydroxyanisole on α -Amylase and α -Glucosidase Enzymes

Research Article

Abstract

Aim: To assess the antioxidant capacity of *Hyphaene thebaica* and investigate its interaction with enzymes linked with type -2-diabetes (α -amylase and α -glucosidase) in comparison with a purified antioxidant compound, Butylhydroxyanisole (BHA).

Methods: One gram (1g) of the sample was weighed into 20mL of distilled water and was left for 24 hours, for the aqueous preparation. The phenolic contents (total phenol and total flavonoid), ABTS• (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) and 1,1-diphenyl-2-picrylhydrazyl (DPPH), scavenging activity was also studied. The ability of the aqueous *hyphaene thebaica* fruit (AHTF) extract and BHA, to inhibit diabetes enzymes in rat pancreas *in vitro* were investigated. Furthermore, the major phytochemical present in AHTF were also identified.

Results: The result showed that AHTF extract had some antioxidant capacity, it had a higher α -amylase enzyme inhibitory activity than that of BHA. But BHA exhibited the higher potentials.

Conclusion: This may signify that the antidiabetic potential of *hyphaene thebaica*, is a synergy of both the antioxidant capacity and free radical scavenging activities, linked with the added phytochemicals present, especially flavonoids and alkaloids, which where both present. Though BHA has a more favourable result than the natural, cheap, available *hyphaene thebaica* the fruit can be useful as nutraceutical or active agent in the formulation of herbal drugs.

Keywords: Medicinal plant; Oxidative stress; Diabetes mellitus; α -amylase, α -glucosidase, *Hyphaene thebaica* fruit

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Abbreviations: AHTF: Aqueous *Hyphaene Thebaica* Fruit; ROS: Reactive Oxygen Species; HT: *Hyphaene Thebaica*; BHA: Butylhydroxyanisole;

Introduction

The use of plants in the treatment and management of diseases has been an age long practice in all parts of the world, especially Africa. Through the practical knowledge gained over the years, folklore medicine has become the main option against orthodox medicine. This is because of the expensive nature of the available drugs, its scarcity and moderate to serious side effects. In the case of diabetes mellitus, in which Insulin injection is administered most persons prefer to use plant for treatments, as to avoid the pains and adverse effect of the injection [1]. The use of herbs is predetermined by the ethnicity and socio-cultural beliefs of the inhabitants of that particular area. Plant medicine is receiving more attention because it is considered safer, cheaper, and with minimal side effects; especially in developing nations and gaining credence in the developed nations also [2].

Diabetes mellitus is a group of metabolic diseases which

is characterized by high blood glucose levels; this might be as a result of, defects of insulin action, insulin secretion or both [3]. It has received much attention and it is one of the growing health concerns worldwide. In 2010, Boyle et al. [4] estimated that the annual new cases of diabetes will increase from about 8 cases per 1,000 (2008) to about 15 in 1,000, by the 2050. Thus, assuming a low incidence and modestly high mortality rate, it has also been projected (among adult population) there will be an increase from 14% in 2010 to 21% of the US population by 2050. This projected increase in the incidence of Diabetes in the US population, succinctly indicates that there is much to be done in developing nations of the world. Further scientific studies are required as to authenticate the folkloric plants used as medicine in the management and treatment of diabetes.

Free radicals are known to have a double effect; they are involved in signalling pathway in the normal body system of differentiation and movement across cellular membranes. Their negative effect is due to their very unstable and very reactive nature; so they transfer their unpaired electron to cellular components and molecules, leading to their disruption and oxidative damage [5]. The body has a natural system, whereby the

produced antioxidants will scavenge and neutralize free radicals and thereby protect the cellular membranes and molecules from oxidative damage [6]. Increase in post prandial blood glucose has been linked to the generation of reactive oxygen species (ROS), thus leading to an imbalance between natural endogenous antioxidants and the ROS. Oxidative damage has can be associated with diabetes complications such as the development of diabetes-specific microvascular pathology in the retina, renal and neuronal cells of the peripheral nerves. Also, accelerated atherosclerotic macrovascular disease which affects arteries that supply the heart, brain and lower extremities, which are the main leading cause of death in diabetics [7,8].

A major therapeutic approach in the management of hyperglycemia is through the decrease in the level of glucose absorbed. The inhibition of key enzymes involved in carbohydrate breakdown by α -amylase and the release of glucose from disaccharide by α -glucosidase in the small intestines, both actions lead to a delay in the absorption of blood glucose into the blood stream, and, consequently blocking a sharp rise in the blood glucose level, which is typical with type 2- diabetes [9]. Natural inhibitors from plant sources are the main emphasis for the intervention and management of diabetes, especially in developing nations because it is readily available, affordability is guaranteed, simpler indigenous technologies for administration can be learnt or acquired and most importantly the side effects are minimal.

Free radicals formed by normal body metabolism system are responsible for oxidative damage, consequently leading to degenerative diseases and accelerates aging process [10]. Since humans cannot produce antioxidants, the main source of antioxidants are primarily plants, and this accounts for a wide variety of biological potentials, and these includes, anti inflammatory, antimicrobial and antioxidant activities [11,12].

The phytochemicals found in plants such as, alkaloids, flavonoids, saponins, terpenes, etc., especially the polyphenolic compounds mainly flavonoids and alkaloids, in their combined activities increases the antioxidant capacity of such plants [13,14].

Hyphaene thebaica (L) mart, is an example of such medicinal plants and belongs to the subfamily Borassoideae. Also known as doum palm or ginger bread tree .It's grows better in hot, arid and dry regions, can be found in the Northern part of Nigeria, Kano, Maiduguri [15]. Emam et al. [16], investigated the effect of *Hyphaene thebaica*, and found out that it lowered the carbohydrate metabolism, in vivo, reducing the postprandial blood glucose.

Butylhydroxylanisole (BHA) is an organic compound, chemically a derivative of phenol, which is useful for its antioxidant properties. European and U.S. regulations allow small amounts to be used as a food additive. In addition to this use, BHA is widely used to prevent oxidation in fluids (e.g. fuel, oil) and other materials where free radicals must be controlled [17].

Hyphaene thebaica fruit is very cheap, readily available and has a simple means of administration, orally, it is expedient to investigate its antioxidant capacity, phytochemical constituent and inhibitory activity of α -amylase and α -glucosidase enzymatic action in comparison to Butylhydroxylanisole (BHA), a synthetic antioxidant.

Materials and Methods

Sample collection and Preparation

Fruits of the *Hyphaene thebaica* (HT) were collected from Kano, Nigeria (March, 2015). Authentication was performed by Mr. T. D. Mshelbwala, a forester and a taxonomist, in the Forestry department in the College of Forestry, Jos. The fruit were then cracked to collect its epicarp; shade dried for 10days, milled into fine powder, and was stored in an airtight plastic container for further analyses.

Chemicals and equipment

Folin-Ciocalteu's phenol reagent, gallic acid and anhydrous sodium carbonate used were products of Fluka (Buchs, Switzerland). Quercetin and DPPH (2, 2-diphenyl-1picrylhydrazyl), were products of Merck (Darmstadt, Germany), Iron chloride, porcine pancreatic α -amylase (EC 3.2.1.1) and α -glucosidase (EC 3.2.1.20) were products of Sigma-Aldrich (USA). Iron (III) chloride 6-hydrate and trichloroacetic acid Fisher products. The distilled water used was obtained from the Chemistry Department at Federal University of Technology, Akure. Optical absorbance was measured with a UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). Methanol, acetic, gallic, caffeic ellagic acid, kaempferol and chlorogenic acids were purchased from Merck (Darmstadt, Germany).

Aqueous extract preparation

1 g each of the samples was weighed into 20 mL of distilled water and was left for 24 hours [18]. The mixture after 24 hours was filtered and the filtrate centrifuged at $805 \times g$ for 10 minutes. The clear supernatant collected was used for the assay.

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. [19]. Briefly, appropriate dilutions of the pastes were oxidized with 2.5 ml 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at $45^\circ C$ and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. [20], briefly 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, $50\mu l$ 10% $AlCl_3$, $50\mu l$ 1 M potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

ABTS scavenging ability

The ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging ability of both extracts were determined according to the method described by Re et al. [21]. The ABTS•

was generated by reacting an (7 mmol/L) ABTS aqueous solution with K2S2O8 (2.45 mmol/L, final concentration) in the dark for 16 h and adjusting the Abs734nm to 0.700 with ethanol. 0.2mL of appropriate dilution of the extract was added to 2.0mL ABTS' solution and the absorbance were measured at 734nm after 15 min. Trolox was used as standard and trolox equivalent antioxidant capacity was subsequently calculated.

Phytochemical screening

The methods described by Trease and Evans [22] were used for phytochemical screening of aqueous extract of *the baica hyphaene* for the presence of bioactive compound.

1,1-diphenyl-2 picrylhrazyl radical scavenging ability (DPPH)

The free radical scavenging ability of the pastes against 1,1-diphenyl-2 picrylhrazyl (DPPH) free radical was evaluated as described by Gyamfi et al. [23]. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

α -Amylase inhibition assay

This was measured using the dinitrosalicylic acid method adapted from Bernfeld [24]. Appropriate dilution of the pastes (500 μ l) and 500 μ l of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic α -amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25° C for 10 min. Then, 500 μ l of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures was incubated at 25° C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm. The EC50 (the extract concentration inhibiting 50% of the α -amylase activity) of the pastes was calculated.

α -Glucosidase inhibition assay

Appropriate dilution of the extract (50 μ l) and 100 μ l of α -glucosidase solution (1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) were incubated at 25° C for 10 min. Then, 50 μ l of 5 mM p-nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25° C for 5 min before reading the absorbance at 405 nm in the spectrophotometer. The α -glucosidase inhibitory activity was expressed as percentage inhibition. The EC50 of the pastes was calculated [25].

Determination of IC₅₀ Values

IC₅₀ (extract concentration causing 50% enzyme inhibition) values for the enzyme inhibitory activity assays were calculated using nonlinear regression analysis.

Data analysis

The results of three replicates were pooled and expressed as mean \pm standard deviation (SD). One-way analysis of variance

(ANOVA) and least significance difference (LSD) were carried out [26]. Significance was accepted at $p \leq 0.05$.

Results

Butylhydroxylanisole (BHA) is an organic compound, chemically a derivative of phenol, which is useful for its antioxidant properties. European and U.S. regulations allow small amounts to be used as a food additive. In addition to this use, BHA is widely used to prevent oxidation in fluids (e.g. fuel, oil) and other materials where free radicals must be controlled. More recently, they cannot be classified simply as diluents but also as a means of increasing weight, consistency and volume, and may even promote fast disintegration, dissolution and particularly stability [27]. The result of the total phenol and flavonoid content of the Aqueous *Hyphaene thebaica* fruit (AHTF) is presented in Table 1. The result revealed that AHTF had some antioxidant capacity, total phenol and total flavonoid content were 23.57mg GAE/g and 64.4mg.QUE/g respectively.

Table 1: Total phenol and Total flavonoid content of *Hyphaene thebaica* (AHTF)

Total Phenol	23.574 \pm 0.40 mg. GAE/g
Total Flavonoid	64.404 \pm 0.65 mg. QUE/g

From Table 2, the result revealed that both extracts scavenged DPPH radical in a dose dependent manner (0.02 - 0.16 mg/ml) (Figure 1), however as revealed by the IC₅₀ (extract concentration causing 50% DPPH radical scavenging ability) values, BHA had the higher inhibitory effects at (0.022mg/ml) in DPPH scavenging ability. The AHTF had the least inhibitory effect for DPPH scavenging (0.034mg/ml) effect.

Table 2: The IC₅₀ values of DPPH, α -amylase inhibition and α -glucosidase inhibition.

	DPPH	α -Amylase Inhibition	α -Glucosidase Inhibition
AHTF	0.081 ^a	0.071 ^a	0.12 ^a
BHA	0.066 ^b	0.083 ^a	0.067 ^b

Values represent means \pm standard deviation (n=3). Values with the same superscript along the column are not significantly (P<0.05) different.

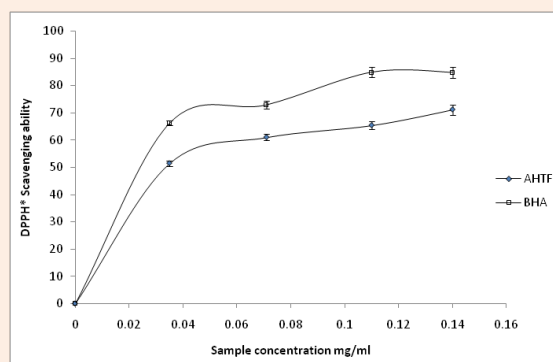


Figure 1: The DPPH scavenging ability of AHTF and BHA.

The ABTS⁺ scavenging ability of both extracts is presented in Figure 2. The result revealed that both extracts scavenged ABTS⁺ in solution. However BHA extract (0.96 mmol. TEAC/g) had the higher ABTS⁺ scavenging ability while AHTF (0.23 mmol. TEAC/g) had the lower ability.

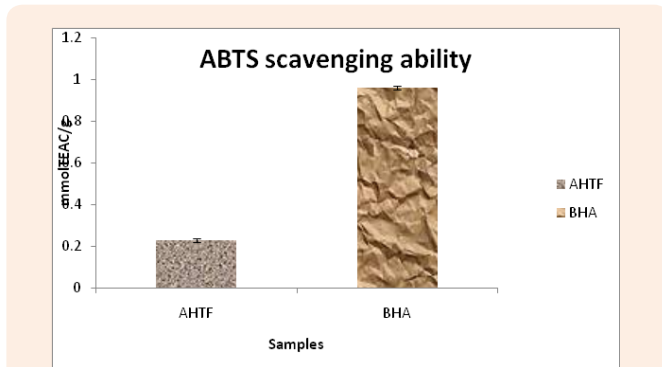


Figure 2: The ABTS⁺ scavenging ability of AHTF and BHA extracts.

The inhibitory effect of AHTF and BHA on α -amylase and α -glucosidase enzymes is shown in Figures 3 & 4. The result revealed that both extracts inhibited both α -amylase and α -glucosidase enzyme activities in a dose dependent manner (0-0.14mg/ml); however as revealed by the IC₅₀ values in Table 2, AHTF extract had the higher inhibitory effect for α -amylase-0.071mg/ml, in comparison to BHA with the least inhibitory effect for α -amylase-0.083mg/ml. But BHA had the higher inhibitory effect for α -glucosidase-0.067mg/ml, with AHTF extract with the least inhibitory effect for α -glucosidase-0.12mg/ml.

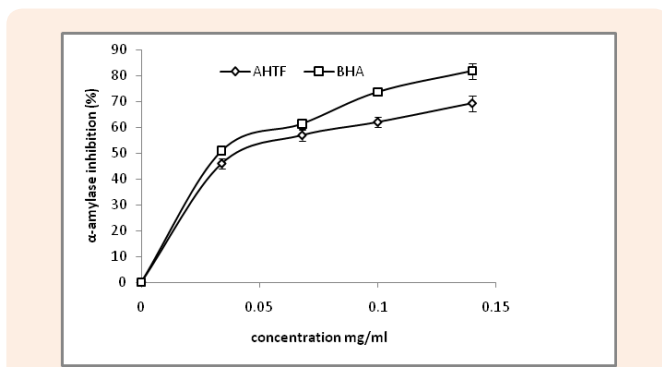


Figure 3: The α -amylase inhibitory activity of AHTF and BHA extracts.

Table 3: The result of the phytochemical screening, both qualitative and quantitative of AHTF.

	Qualitative Analysis	Quantitative Analysis
Alkaloids	++	19%
Saponins	++	17%
Tannins	+	10%
Flavonoids	+++	42%

Keywords: + Present; ++ More Present; +++Highly present

The values from Table 4, shows the presence of Flavonoids and alkaloids more than the other two phytochemicals, saponins and tannins. This also was reaffirmed by the quantitative analysis.

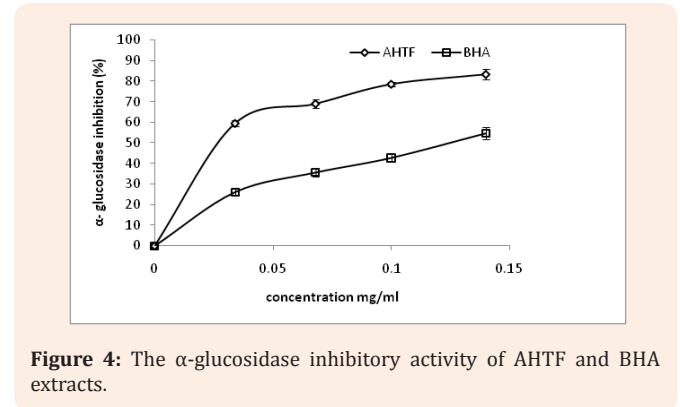


Figure 4: The α -glucosidase inhibitory activity of AHTF and BHA extracts.

Discussion

The phytochemical components in plants, such as, alkaloids, flavonoids, tannins and phenolic compounds; are the potents behind the medicinal values accrued by plants. They are useful in aiding the physiological functions of the body [28]. Table 3, showed the result of both the quantitative and qualitative phytochemical screening. It indicated a strong presence of flavonoids, and it has been found out that they exhibit other biological effects such as anti-inflammatory, vasodilatory, anticancer, antiviral and antioxidant properties [29,30]. AHTF has good quantity of flavonoids which indicates an important basis for its medicinal use.

Antioxidants are chemical substances that are effective in the physiological function of human body; this is done through inhibition or delay of the formation of free radicals and peroxidation of cellular membranes. This action leads to the prevention and management of degenerative diseases in the human body [31]. An imbalance in the body system wherein the free radicals activities are more than the activities of its antioxidant enzymes leads to oxidative damage and consequently oxidative stress. From research work, phenolic compounds have been found to protect the cellular membranes and tissues from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The results of the total phenol and flavonoid content of AHTF was higher than that of Snake tomato and two other variety as reported by Ademosun et al. [32].

One of the main importances of antioxidants is its ability to scavenge free radicals, inhibiting further degradative action by which cellular biomolecules are damaged. The DPPH⁺ and ABTS⁺ scavenging ability of both AHTF and BHA, showed a higher scavenging ability of BHA more than AHTF. But, considering the affordability of AHTF, it is economically agreed to be better than the purified antioxidant. The DPPH⁺ and ABTS⁺ scavenging ability of AHTF was found to be higher than that reported by Oboh et al. [33].

Modern research in the formulation of drugs and therapeutic agents in the management of diabetes (type-2) has laid more

emphasis on the inhibition of starch metabolizing enzymes such as α -amylase and α -glucosidase. The inhibition of these key enzymes, consequently will lead to a reduced blood glucose [34,35].

As shown in this study AHTF has a higher dose dependent inhibition of α -amylase than BHT, a purified antioxidant. This result shows that, having an increased antioxidative potential does not guarantee the required enzyme inhibition. Phytochemicals and polyphenolics are very crucial in the management of degenerative diseases, because they work in synergy with the antioxidant activities of the plant related materials. This study gives an insight to the dependability of developing nations on herbal medicine and its vast abundant supplies. Also from this study, it may be that AHTF, can be used in the management of diabetes, through its synergistic actions of the phytoconstituents and its ability to inhibit key enzymes linked to type-2 diabetes.

Conclusion

With the antioxidant activities of Butylhydroxylanisole, a "pure" antioxidant, in comparison antidiabetic enzyme inhibition with *Hyphaene thebaica*, showed that the aqueous plant extract can be used to manage and/or prevent type -2 diabetes. This may be due to the higher inhibition of α -glucosidase by aqueous extract of *Hyphaene thebaica* and its flavonoid and alkaloid rich content of phytochemicals. However, further *in vivo* experiments and clinical trials are recommended.

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