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Alpha amylase inhibition and antioxidant potential of *Raphia hookeri* (Palmae)

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ABSTRACT

This study was carried out to investigate α-amylase inhibitory and antioxidant activities of extract and fractions of *Raphia hookeri* stembark. The results showed that the extract prevented the digestion of starch by inhibiting α-amylase in a dose dependent manner with maximum inhibitory effect (72.86 %, 1.00 $mgml^{-1}$). The aqueous fraction demonstrated highest inhibition (67.22%, 0.50 $mgml^{-1}$) which was less than acarbose (68.90%, 0.08 $mgml^{-1}$) used as positive control. The hexane, chloroform and ethylacetate fractions showed lower inhibitory activity (12.16, 9.06, 35.97%) respectively. The total phenolic content of the *R. hookeri* extract measured using Folin Ciocalteu reagent in terms of gallic acid equivalent (GAE) was found to be 109.10 ± 3.24 mgg^{-1}. The antioxidant activity of the extract increased with concentration and the aqueous fraction produced the highest radical scavenging effect (95.04 ± 0.57%, 0.10 $mgml^{-1}$) which was significantly less ($P < 0.05$) that of ascorbic acid. The hexane, chloroform and ethylacetate fractions however demonstrated lower radical scavenging activity (18.4, 11.1 and 94.9 %) respectively. The reducing ability of the extract was comparable to that of ascorbic acid and the aqueous fraction produced the best activity. This study indicates that the extract of *Raphia hookeri* has α-amylase inhibitory and antioxidant activities which reside mainly in ethylacetate and aqueous fractions.

KEYWORDS

Raphia hookeri;
Amylase inhibition;
Antioxidant;
Radical scavenging;
Total phenol.

INTRODUCTION

Diabetes mellitus is a common and prevalent disease affecting the citizens of both developed and developing countries. It is caused by the abnormality of carbohydrates metabolism which is linked to low blood insulin level or insensitivity of target organ to insulin. It is estimated that 25% of the world population is affected by the disease[1]. The digestion of starch by α-amylase is known to contribute to sharp increase in blood glucose leading to postprandial hyperglycemia which has been implicated in type 2 diabetes. Diabetic patients are known to be under oxidative stress due to imbalance in free radical generation and scavenging ability. The inhibition of α-amylase enzyme and oxidative stress could thus be a veritable tool in the management of hyperglycemia and type 2 diabetes[2,3]. Medicinal plants are sources of secondary metabolites with potential therapeutic effect and they exhibit various pharmacological activities including antidiabetic properties[4]. Although many herbal preparations are being prescribed as antidiabetic agents, the search for new anti diabetic

agents from medicinal plants is on the increase.

Raphia hookeri Mann & Wendl (Palmae) is a tall palm, trunk up to 10 m high and 10 cm in diameter. It is the most common and widely dispersed *Raphia* of the forest area. It is a valued source of palm wine and fibre[5]. The root of *R. hookeri* was reported to have effect on blood glucose[6]. The stem bark of the plant was claimed by herb sellers to be useful in treatment of diabetes in Nigeria. Given this background, the objective of the present study was to investigate *in-vitro* α-amylase inhibition and antioxidant activities of the stem bark extract of the plant.

EXPERIMENTAL

Plant material

The stem barks of *R. hookeri* were collected at Ikorodu, Lagos State, Nigeria and identified by Mr. Daramola of the Herbarium Unit, Department of Botany, University of Lagos after comparing with voucher specimen number LUH 947. The barks were washed and dried at 40°C and milled to produce fine powder.

Extraction and fractionation

About 300 g of the powdered of the stem bark was extracted with methanol (2.5 l) using Soxhlet apparatus for 48 h. The extract was concentrated under reduced pressure using rotatory evaporator until a semi-solid sticky mass was obtained. The yield was 5.52 %w/w. Extract (20.0 g) was pre-dissolved in a mixture of methanol and water then partitioned with n-hexane, chloroform and ethylacetate in separating funnel successively and yielded hexane, chloroform, ethylacetate and residual aqueous fractions that were concentrated and subjected to *in- vitro* α-amylase inhibitory and antioxidant investigations.

Amylase assay

The amylase inhibition assay was carried out by a method previously described by Kwon *et al.*,[7]. A mixture of 500 µl of extract (0.05-2.0 $mgml^{-1}$) and 500 µL of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing porcine pancreatic α-amylase (0.5 $mgml^{-1}$; Sigma Chemical Company, St. Louis, MO) were mixed for 10 min, then 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at timed intervals. The reaction mixtures were allowed to stand for 10 min. were stopped with 1.0 ml of dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted with 10 ml distilled water and absorbance was measured at 540 nm. The α-amylase inhibitory activities of the hexane, chloroform, ethylacetate and aqueous fractions were tested at 0.2 $mgml^{-1}$ concentration using similar procedure. The inhibition of α-amylase was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract\_}}{A_{control}} \times 100$$

1, 1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity.

DPPH radical scavenging ability of the extract was determined according to the method of Adesegun *et al.*,[8]. The solution of extract (1.0 ml, 0.005-0.2 $mgml^{-1}$) was diluted to 20% of the original concentration with methanol and 1.0 ml of methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (1 mM) was added. The mixture was shaken vigorously and allowed to react in dark for 30 min. The absorbance of the resulting mixture was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. The radical scavenging ability of the hexane, chloroform, ethylacetate and aqueous fractions were tested at 0.1 $mgml^{-1}$ using similar procedure. Ascorbic acid was used as positive control and deionized water in place of extract or the control was used as blank. All analysis was performed in triplicate and the capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenging Effect (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blankl} was the absorbance of the negative control and A_{sample} was the absorbance in the presence of the sample of extract or standard.

Total phenolic content

The total phenolic content of the extract was determined using Folin Ciocalteu reagent according to the method of Wolfe *et al.*[9]. The extract (5 $mgml^{-1}$, 1.0 mL) was mixed thoroughly with 5 ml Folin-Ciocalteu

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reagent (1:9 in distilled water) and after 5 min, 4.0 ml of sodium carbonate (0.7 M) was added and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance was measured at 765 nm with a spectrophotometer. All determinations were carried out in triplicate. The total phenolic content in the extract was expressed as mg/g gallic acid equivalent using the calibration curve.

Ferric reducing power

The reducing power was determined according to the method described by Oliveira *et al*.[10]. The extract (1.0 ml, 0.05-2.0 $mgml^{-1}$) was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) was added then the mixture was incubated at 50^0C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture and centrifuged at 4000 rev/min for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm against a blank in the spectrophotometer. The reducing power of hexane, chloroform, ethylacetate and aqueous fractions were tested at 0.2 $mgml^{-1}$ concentration using similar procedure Ascorbic acid was used as positive controls. Higher absorbance of the reaction mixture indicated increased reducing power.

Statistics

All experiments results were expressed as mean ± SEM and evaluated by analysis of variance (ANOVA) and $P < 0.05$ was regarded as statistically significant.

RESULTS &DISCUSSION

Research on medicinal plants has received greater attention in recent times due to interesting discoveries arising from their biological activities. The hydrolysis of starch could be delayed by the use medicinal plants with compounds that inhibit intestinal carbohydrate hydrolyzing enzymes. Alpha amylase is a very important enzyme in carbohydrate digestion and is a therapeutic lead for regulation of postprandial hyperglycemia common in type 2 diabetes. Several medicinal plants have been reported to elicit hypoglycemic effect through inhibitory effect on α-amylase[11,12]. The inhibitory effects of R. hookeri stem bark extract and acarbose are shown in Figure 1. The result of this present study indicates that the extract of possessed concentration dependent inhibitory effect on the starch breakdown with IC_{50} 0.14 $mgml^{-1}$ compare to 0.04 $mgml^{-1}$ of acarbose. The activity was however significantly ($P < 0.05$) less than that of acarbose with maximum effect 72.86% at 1.0 $mgml^{-1}$. This suggested that the extract may possess compounds which are inhibitors of α -amylase and could be one of the mechanisms for the hypoglycemic activity of stem extract. The inhibitory activity of the fractions (0.5 $mgml^{-1}$) follows the order aqueous (67.22%) > (ethylacetate 35.97%) > hexane (12.16%) > chloroform (9.06%) indicating that the activity resides mainly in the aqueous and ethylacetate fractions.

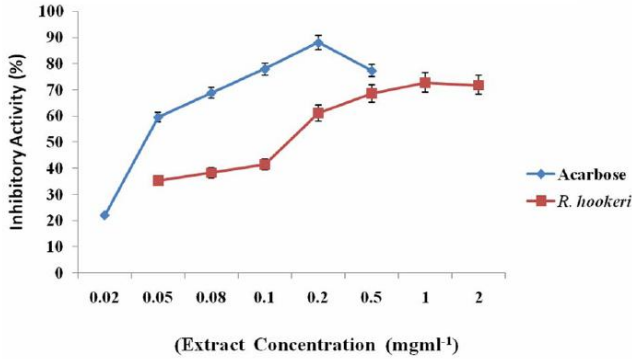

Figure 1: Alpha Amylase Inhibitory Activity of *R. hookeri* Extract and Acarbose

Diabetes mellitus is often associated with elevated level of oxidative damage, reduced level of antioxidant defenses and lipid peroxidation. Investigation of antioxidant potential of natural products could be a useful tool by which potential medical benefit could be assessed. Plants also produce antioxidants that have reported to reduce the occurrence of diabetes[13]. In this study, antioxidant activities of *R. hookeri* stem bark extract and its fractions were determined by the DPPH, ferric reducing and total phenolic content assays.

The DPPH free radical can accept electron or hydrogen radical and become stable and this is commonly used in assessment of radical scavenging power of natural products[14]. The radical scavenging ability of the extract and ascorbic used as positive control is shown Figure 2. The DPPH radical scavenging ability of the crude extract and ascorbic acid increase with concentration, IC_{50} 0.04 and 0.03 $mgml^{-1}$ extract and ascorbic acid respectively. The free radical scavenging activity of hexane, chloroform, ethylacetate and aqueous frac-

tions (0.1 mg/ml) were 18.40 ± 1.64, 11.10 ± 0.88, 94.96 ± 0.15 and 95.04 ± 0.57 % respectively. The effect of the samples on the radical may due to their hydrogen donating ability. The decrease in absorbance of the DPPH solution was caused by scavenging power of the sample by donation of hydrogen[15].

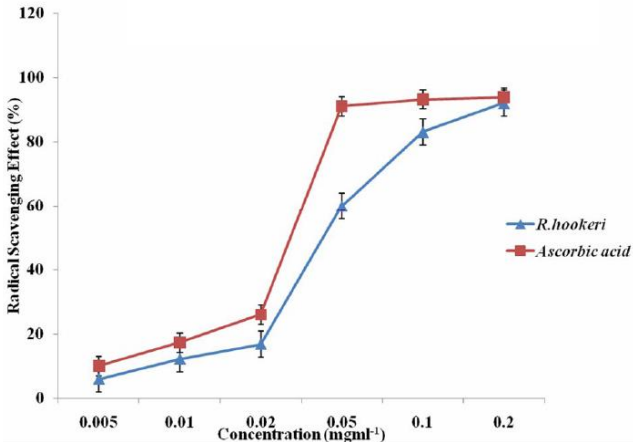

Figure 2: Radical Scavenging Effects of *R. hookeri* and Ascorbic acid

Ferric reducing assay is used in determination of antioxidant effect of natural product in terms of its ability to donate electron. Figure 3 showed the reducing power of the extract and ascorbic acid. The reducing power of the extract increased with increasing concentration and there is no significant difference ($P > 0.05$) compared to ascorbic acid. The reducing power for hexane, chloroform, ethylacetate and aqueous fraction at 0.1 mg/ml were 0.104 ± 0.014, 0.946 ± 0.048, 1.462 ± 0.086 and 1.834 ± 0.049 respectively. The aqueous fraction demonstrated the best reducing ability while the n-hexane fraction had the least. Antioxidants in the extract reduced Fe^{3+}/ferricyanide complex to the ferrous form suggesting that the extract is an electron donor and could react with free radicals and convert them to more stable forms[16].

The total phenolic content in extract as measured by the Folin-Ciocalteu method was 109.01 ± 3.24 mgg^{-1} in terms of gallic acid equivalent. Phenolics are major groups of compounds with high oxygen potential, hydrogen donors and singlet oxygen quenchers that act as antioxidants or free radical terminators. The high polyphenols in this plant suggested that it could useful in treatment of diseases related to high level of free radicals in the body such as diabetes mellitus. Polyphenols have been reported to inhibit intestinal α-amylase thus the observed inhibitory properties of the extract might be due to this class of compound[17].

CONCLUSION

The present study demonstrated that *R. hookeri* stem bark possesses α-amylase inhibitory potential, radical scavenging and reducing activities and these reside mainly in ethylacetate and aqueous fractions thus justify its folklore use of the plant in treating diabetes. Further work needs to be carried out to isolate the compounds responsible for the activity.

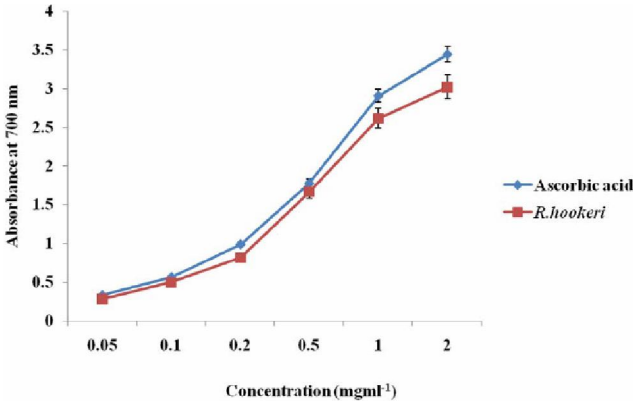

Figure 3: Reducing Power of *R. hookeri* and Ascorbic acid

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