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Research Paper

α-amylase inhibition and antioxidant activity of *Pterocarpus osun* Craib

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ABSTRACT

In this study, methanol leaf extract of *Pterocarpus osun* and its fractions were assessed for anti-diabetic ability using *in vitro* α-amylase inhibitory potential and antioxidant activities determined by DPPH radical scavenging effect, reducing power and total phenolic content. Our results showed that the extract had a dose dependent prevention of digestion of carbohydrates by inhibiting α-amylase. The maximum effect was observed at 2.0mg/ml was (74.98±4.35%, $P<0.05$). At 0.2mg/ml, the aqueous fraction demonstrated 71.87±3.41% inhibition which is comparable to 88.10±4.59% of acarbose used as reference drug. The hexane and chloroform fractions demonstrated lower ($P<0.05$) inhibitory activity; 16.16 and 14.34% respectively. The total phenolic content of the *P. osun* extract measured using Folin Ciocalteu reagent in terms of gallic acid equivalent (GAE) was found to be 97.01±0.02mg/g. The DPPH radical scavenging activity of the extract increased with concentration but was significantly less than ($P<0.05$) ascorbic acid used as positive control. The radical scavenging activity of the aqueous fraction (94.60±3.45%) at 0.2mg/ml was however higher than that of reference drug. The hexane and chloroform fractions however showed lower radical scavenging activity; 12.35±0.07 and 46.20±1.23% respectively. The extract showed dose-dependent increase in reducing power. The aqueous fraction however showed highest reducing ability while the n-hexane fraction had lowest. This study indicates that α-amylase inhibitory and antioxidant activities of the methanol extract of *P. osun* resides in the aqueous fraction.

Keywords: *Pterocarpus osun*; α-amylase inhibiton; Antioxidant activity; Antidiabetes.

INTRODUCTION

Diabetes mellitus (DM) is fast becoming leading cause of mortality in the developed and developing world. It is often due to excessive calorie intake, hyperglycemia and oxidative stress. One of steps for managing DM is the management of postprandial hyperglycemia by reducing the digestion of carbohydrates by α-glucosidases (Fowler, 2007). Medicinal plants have been reported to contribute to the management of type 2 diabetes by inhibiting carbohydrate hydrolyzing enzymes like pancreatic amylase. This delays carbohydrate digestion and prolong carbohydrate digestion time thus reducing glucose absorption rate and postprandial plasma glucose level (Jung, et al.,

2006; Kwon, et al., 2006). Oxidative stress and free radicals have also been implicated in type 2 diabetes (Droge, 2002).

In Nigeria, the sap of the bark of *Pterocarpus osun* (Leguminosae) is used as dye and the wood is used in carpentry (Burkill, 1995). Antidiabetic properties of other species like *P. indicus* and *P. marsupium* had been established (Joshi, et al., 2004; Ragasa, et al., 2005) however there were very little reports on the activities of the leaf of *P. osun*. In the present study, we report for the first time antioxidant and anti-amylase activities of the plant.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *P. osun* were collected at Ikire, Oyo State, Nigeria in May 2010 and identified by Mr. I. K. Odewo of the Herbarium Unit, Department of Botany, University of Lagos after comparing with voucher specimen LUH 3153. The leaves were dried at 40°C in an oven and milled to produce fine powder.

Extraction and Fractionation: Previous studies on another species (Joshi, et al., 2004) and preliminary studies in our laboratory showed methanol extract to be effective. About 400g of the powdered plant material was extracted in May 2010 with methanol (2.5L) using Soxhlet apparatus for 48h. The extract was concentrated under reduced pressure using rotatory evaporator at 40°C until a semi-solid sticky mass was obtained. The yield was 7.64%w/w. Extract (20g) was dissolved in a mixture of methanol and water then partitioned between n-hexane and chloroform successively to obtain hexane, chloroform and 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 (Kwon, et al., 2006). A total of 500µl of extract (0.05-2.0mg/ml) and 500µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing porcine pancreatic α-amylase (0.5mg/ml; Sigma Chemical Company, St. Louis, MO) were mixed at 32°C for 10min, then 500µl of 1% starch solution in 0.02M sodium phosphate buffer (P^H 6.9 with 0.006M sodium chloride) was added to each tube at timed intervals. The reaction mixtures were incubated at 32°C for 10min. The reaction was stopped with 1.0ml of dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5min and then cooled to room temperature. The reaction mixture was diluted with 10ml distilled water and absorbance was measured at 540nm. The α-amylase inhibitory activities of the hexane, chloroform and aqueous fractions were tested at 0.2mg/ml concentration using similar procedure. The inhibition of α-amylase was calculated as follows:

$$\% \text{ inhibition} = \left[\frac{A_{control} - A_{extract}}{A_{control}} \right] X\ 100$$

Determination of Total Phenolic Content: The total phenolic content of the extract was determined in terms of gallic acid equivalent using Folin-Ciocalteu reagent (Adesegun, et al., 2009). The method depends on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes. Gallic acid equivalent was determined from a calibration concentration curve. The extract (100mg/ml, 1.0ml) was mixed thoroughly with 5ml Folin-Ciocalteu reagent (1:9 in distilled water) and after 5min, 4.0ml of sodium carbonate (0.7M) was added and the mixture was allowed to stand for 1h with intermittent shaking. The absorbance was measured at 765nm in a spectrophotometer. All determinations were carried out in triplicate.

***Determination of Radical Scavenging Activity*:** The method of Koleva, et al., (2002) with some modifications was used for the determination of free radical scavenging activity of the extract. The extract (1.0ml, 0.1-5.0mg/ml) was diluted to 20% of the original concentration with methanol and 1.0ml of methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (1mM) was added. The mixture was shaken vigorously and allowed to stand at 32°C in dark for 30min. The decrease in absorbance at 517nm was then measured using spectrophotometer. The radical scavenging ability of the hexane, chloroform and aqueous fractions were tested at 0.2mg/ml using similar procedure. Ascorbic acid was used as reference standard and deionized water in place of extract or the control was used as blank. All analysis was performed in triplicate and the ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging Effect (\%)} = [(Ao - A_1)/Ao] \times 100$$

- Ao was absorbance of the control; A_1 was absorbance in the presence of the sample of extract or standard.

Evaluation of Reducing Ability: The reducing power of the extract was determined by the method of Yen and Chen (1995) with some modifications. The ability of the extract and its fractions to reduce the ferric-ferricyanide complex to the ferrous-ferricyanate complex of Prussian blue was estimated by recording the absorbance at 700nm after incubation. The extract (1.0ml, 0.1-5.0mg/ml) was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of potassium ferricyanide (1%) was added then the mixture was incubated at 50°C for 20min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture and centrifuged at 1000g for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm against a blank in the spectrophotometer. The reducing power of hexane, chloroform and aqueous fractions were tested at 0.2mg/ml concentration using similar procedure Ascorbic acid was used as positive controls. Higher absorbance of the reaction mixture indicated increased reducing power.

Statistical Analysis: All data were expressed as Mean±Standard error of mean. Analysis of variance was performed by ANOVA procedures and $P<0.05$ was considered to be statistically significant.

RESULTS

Alpha-amylase inhibitory activity: The extract of *P. osun* possessed concentration dependent inhibitory effect on the starch breakdown *in vitro* as shown in Table 1. The highest inhibitory effect was at 2.0mg/ml and the IC50 was 0.6mg/ml compared to acarbose 0.04mg/ml used as positive control. The hexane, chloroform and aqueous fractions of *P. osun* showed α-amylase inhibitory effects 16.16±0.94, 14.34±0.48 and 71.87±3.41% respectively at 0.2mg/ml.

Total Phenolic content: of the *P. osun* leaf extract determined using Folin Ciocalteu reagent in terms of gallic acid equivalent (GAE) was found to be 97.01±0.02mg/g.

DPPH radical scavenging activity: The scavenging activity of the DPPH radical was tested by reduction of the stable radical DPPH to the yellow colored diphenyl picryl hydrazine. The scavenging effect of extract and ascorbic acid are depicted in Table 2. As the concentration increases, the scavenging potential of the crude extract increased by combating formation of the DPPH free radical. The extract and ascorbic acid scavenged the radical with IC50; 0.45 and 0.07mg/ml respectively. At 0.2mg/ml, the free radical scavenging ability of hexane, chloroform and aqueous fractions were 12.35±0.07, 46.20±1.23 and 94.6±3.45% respectively while ascorbic acid was 88.26±3.88%.

Reducing power: The crude extract showed a dose-dependent increase in reducing power (Table 3). The hexane, chloroform and aqueous fractions of *P. osun* gave 0.034, 0.234, 0.948 absorbance respectively at 0.2mg/ml. Ascorbic acid had the highest 0.987. The reducing power thus follows the order ascorbic acid> aqueous> chloroform > hexane.

DISCUSSION

Medicinal plants are natural sources of antioxidants and bioactive compounds useful to health. Postprandial hyperglycemia is one of the risk factors associated with type 2 diabetes mellitus. Digestion of dietary starch by α-glucosidases like pancreatic α-amylase plays significant role in elevated blood glucose thus inhibition of these enzymes is very useful tool in management of hyperglycemia (Kwon, et al., 2006).

The anti-diabetic activity of medicinal plants could be evaluated using several methods; *in vitro* α-amylase inhibitory assay is one of such techniques. The extract of *P. osun* inhibited α-amylase but its activity was significantly less than that of positive control acarbose ($P<0.05$). The inhibitory effect of aqueous fraction was comparable to than that of the positive control, indicating that the α-amylase inhibitory ability resides in this fraction. Alpha-amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions responsible for breaking down of α-1,4-glycosidic bonds in starch. Therefore, the enzyme increases the availability of glucose in the blood. *P. osun* extract could be useful in post-prandial hyperglyceamia by reducing the hydrolysis of carbohydrates. The observed activity may be due to the presence of chemical constituents such as phenolic compounds (tannins and flavonoids), terpenes and triterpenes in the extract and fractions (Ebi and Ofoefule, 2000; Kim, et al., 2000). Phenolics have been reported to inhibit α-amylase activities. They also have anti-hyperglycemic activity and inhibit the development of diabetes (Hanamura, et al., 2006; Zunino, et al., 2007).

Free radicals play a significant role in biological damages and DPPH is commonly used in evaluation free radical scavenging properties of natural antioxidants (Adesegun, et al., 2007). The crude extract of *P. osun* showed DPPH radical activity but this is significantly less ($P<0.05$) than that ascorbic acid used as positive control. The scavenging power of the aqueous fraction was significantly higher ($P<0.05$) than that of ascorbic acid and the crude. But chloroform and hexane fractions showed weaker activity compared to control. This suggested that the plant is natural source of antioxidants. The DPPH radical changes color from purple to yellow after reacting with antioxidants which scavenge the radical by hydrogen donation. The extent of the reaction depends on the hydrogen donating ability of the antioxidant.

The extract of *P. osun* has a high phenolic content which may be responsible for the observed α-amylase inhibitory and antioxidant properties. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators due to the ability of their hydroxyl groups to scavenge radicals. This activity is reported to be due to their redox properties which play a significant role in ion adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposition of peroxides (Tsao and Akhtar, 2005).

The results also showed *P. osun* leaf extract as a reducing agent although the activity was lower than ascorbic acid (Table 3). The reducing ability of aqueous fraction was comparable to ascorbic acid. The result suggest that the extract could react with free radicals and convert them to more stable compounds. In the reducing power assay, the antioxidants in the extract cause the reduction by conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. The phenolic compounds present in

extract as well as flavonoids and tannins reported in this genus may responsible for reported activity. Antioxidants are known to have reducing power (Duh, et al., 1999).

CONCLUSION

This study shows that the leaf extract of *Pterocarpus osun* possess α-amylase inhibitory activity and antioxidant properties. The fractionation of the extract revealed that the observed activities reside mainly in the aqueous fraction. The extract could be a veritable source of natural antioxidant and antidiabetic agent.

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Table -1: Alpha-amylase inhibitory activities of extract and acarbose.

Concentration (mg/ml)	Inhibitory Effect (%)*	
	Extract	Acarbose
0.020	-	22.00±1.23
0.050	-	59.50±4.37
0.075	-	68.90±3.87
0.100	12.20±0.07	78.00±3.24
0.200	16.01±1.01	88.10±4.76
0.500	37.43±1.87	77.36±5.24
1.000	65.44±4.45	-
2.000	74.98±4.35	-

- *Each value represents mean ± SEM (n = 3).

Table -2: Radical scavenging effect of extract and ascorbic acid.

Concentration (mg/ml)	Inhibitory Effect (%)*	
	Extract	Ascorbic acid
0.02	-	11.53±0.74
0.05	9.95±0.56	30.84±1.89
0.10	16.82±1.94	82.90±5.43
0.20	36.92±1.45	88.26±4.24
0.50	52.65±4.32	94.64±4.32
0.75	60.01±3.56	94.70±3.24
1.00	67.71±3.12	-
2.00	77.36±4.15	-

- *Each value represents mean ± SEM (n = 3).

Table -3: Reducing power of extract and ascorbic acid .

Concentration (mg/ml)	Absorbance (nm)	
	Extract	Ascorbic acid
0.05	0.124	0.332
0.10	0.213	0.563
0.20	0.380	0.987
0.50	0.913	1.776
1.00	1.433	2.906
2.00	2.013	3.441

- *Each value represents mean ± SEM (n = 3).

