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Research Paper:

Antioxidant and Inhibitory Activities of Enzymes Linked to Type II Diabetes Mellitus: The Novel Role of Chrysobalanus Orbicularis Leaf Extract

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ABSTRACT

Background: Chrysobalanus orbicularis is commonly used as food, the seeds as a spice for the popular pepper soup, and the leaves are traditionally utilized for managing Type II Diabetes Mellitus (T2DM) in the Niger Delta area of Nigeria. Due to the limited scientific evidence on C. orbicularis, this study investigated its antioxidant and inhibitory properties against major enzymes linked to T2DM.

Methods: The antioxidant activity was measured via methods for possible scavenging potentials. The α-amylase and α-glucosidase inhibitory activities were examined using a standard model.

Results: The C. orbicularis aqueous leaf extract compared well with the standard compounds, revealing the high antioxidant and inhibitory properties against α-amylase and α- glucosidase.

Conclusion: The findings validate the conventional application of C. orbicularis in the treatment of patients with type II diabetes mellitus and other oxidative stress-related disorders.

Keywords: Antioxidant activity; C. orbicularis; α-amylase and α-glucosidase; Diabetes mellitus; Medicinal plants

Introduction

Recently, there has been a significant growth in the use of plant-based phytochemicals in the management of diseases and are being propagated in various countries due to the natural origins and low side effects [1]. During oxidative stress, the human body produces free radicals that overwhelm the enzymatic antioxidants, such as Glutathione (GPx) [2]. The free radicals are mainly produced through biophysical and environmental processes, which cause cellular damages and in most cases result in apoptosis [3]. The quest for natural antioxidants as beneficial agents to inhibit free radicals in the pathogenesis of some human diseases is an important area of clinical interest [4]. Reactive Oxygen Species (ROS) generates hyperglycemia, leading to inequity in antioxidants in the body and results in oxidative stress. However, the blood sugar level is checked by digestive enzymes, like the α-amylase

and α-glucosidase. The enzyme, α-amylase, is responsible for breaking down long-chain carbohydrates while α-glucosidase converts carbohydrate to glucose in the small intestine. The inhibitory effect of α-glucosidase has been acknowledged as a beneficial target for the control of postprandial hyperglycemia and type II diabetes. Therefore, it is of clinical benefit to provide antioxidants, α-amylase and α-glucosidase inhibitors through nutrients that can help manage type II diabetes [5].

Chrysobalanus orbicularis (C. orbicularis) is an evergreen plant with a red apple-like fruit locally known as Omillo. The leaves are oval in shape, and the plant belongs to Chrysobalancea family. It grows mainly as shrub and is known for its management of diabetes mellitus traditionally but has not been fully studied. The whole plant is used locally; its dried fruits and seeds are used for preparing pepper soup, a delicacy in the Southern Nigeria and western Africa. Based on ethno-botanical usage, this study aimed to examine the antioxidant and inhibitory potentials of C. orbicularis aqueous leaf extract against α-amylase and α-glucosidase.

Materials and Methods

Plant materials: The leaves from C. orbicularis plant were collected from Coco, Itsekiri area in Delta State, Nigeria, and were identified at the Forestry and Research Institute, Ibadan, Oyo State, Nigeria, where they were authenticated and given a voucher Herbarium number (FHI:112232).

Preparation of plant materials: Preparation and extraction of the plant materials were achieved by the procedure defined previously by Nwozo, et al. [6]. The plant leaves were washed with clean water and air dried for three weeks and then pulverized into fine powder in a blender. The powdered sample (1000 g) was extracted in aqueous by cold maceration for 72 hours followed by periodic stirring. The material was freeze-dried and the yield of 23.345g was stored for further analysis.

Biochemical analyses: Assay of Ferric Reducing Antioxidant Potential (FRAP): The FRAP property of aqueous extract of C. orbicularis leaves was estimated by evaluating its ability to reduce FeCl3 solution based on the protocol explained by a previous study [7]. The reducing power was expressed as the gallic acid equivalent.

Assay of 1,1-Diphenyl-1-2-Picryl-Hydrazil (DPPH) radical scavenging ability: The DPPH radical scavenging ability was measured via a previously described procedure [8]. One mL of the extracts at varying concentrations (100-560 µg/mL) was incubated at 25°C at dark for 30 minutes with 500µL of 0.3 mM DPPH solution (prepared in methanol). Then the absorbance was read at 517nm against a blank test tube without the sample.

Assay of Nitric Oxide (NO) scavenging activity: The scavenging activity of the extract of C. orbicularis leaves against Nitric Oxide (NO) radical was evaluated by the procedure, as previously described [9]. A 250 µL of the extract at various concentrations (100–500µg/mL) was incubated with 250 µL of 10 mM sodium nitroprusside-sodium phosphate buffer (pH 7.4) for 2h at 37°C. Afterward, 250µL of Griess reagent was added to the reaction solution and the absorbance was read at 546 nm. The percent inhibition of the NO produced was obtained by comparing the absorbance of the extracts (sample) with that of the control in the absence of scavengers.

Determination of Hydrogen Peroxide (H_2O_2) scavenging activity: The hydrogen peroxide scavenging activity of the aqueous extract of C. orbicularis leaves was measured via a previously described procedure [10]. The hydrogen peroxide solution (40 mM) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm, using a UV spectrophotometer. A 1.5mL aliquot of the extract was added to the hydrogen peroxide solution and the absorbance was read at 560nm, using a UV spectrophotometer.

Assay of 2, 2-Azino-Bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS) scavenging activity: The ABTS activity of the extracts was achieved via the protocol described previously [11]. The stock solutions included 7mM ABTS and 2.4 mM potassium persulfate. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to mix for 14h at 25°C in the dark. The mixture was then diluted by adding 1mL ABTS solution to 60mL methanol, to obtain an absorbance of 0.706±0.01 units at 734nm on a spectrophotometer. Fresh ABTS solution was prepared for each assay. The aqueous extract (1.5mL) was allowed to react with 1mL of the ABTS solution and the absorbance was read at 734nm after 7 min, using a spectrophotometer.

Determination of α-glucosidase inhibitory activity: The inhibitory effect of the aqueous extract of C. orbicularis leaves against α-glucosidase activity was determined via a procedure as defined previously [12]. A 250µL of 1.0 U/mL of α-glucosidase, dissolved in phosphate buffer (100 mmol/L, pH 6.8), was incubated with 250µL of the extract or acarbose as the standard, for 20 min at 37°C. A 250µL of p-nitrophenyl-α-D-

glucopyranoside (pNPG) solution (5 mmol/L) that was prepared in the same phosphate buffer was added to the reaction mixture and incubated for another 30 min at 37°C. The absorbance of released p-nitrophenol was measured at 405 nm.

Assay of α-amylase inhibitory activity: The inhibitory action of the extract against α-amylase activity was evaluated based on the established procedure of a previous study [13]. Briefly, 250µL (1.5 mg/mL stock) of the extracts at varying concentrations (100–500 µg/mL) or acarbose was incubated with 250µL of the enzyme (porcine pancreatic amylase 2 U/mL) in phosphate buffer (100 mmol/L, pH 6.8) for 20 min at 37°C. Thereafter, 250µL of 1% starch prepared in the same buffer was added to the premixed solution and incubated further for 1h at 37°C. One mL of the color reagent, Dinitrosalicylate (DNS), was added and the mixture was boiled for 10 min. The absorbance was read at 540nm and the inhibitory activity was calculated as the proportion of sample versus the control.

Data analyses: The data were analyzed via GraphPad Prism 8.0 (Version 8, Software Program, GraphPad Prism Inc., San Diego, CA, USA) and presented as the Means±SD in triplicates.

Results

The FRAP scavenging activity of the aqueous extract of C. orbicularis leaves is shown in Figure 1. The activity showed a rise with increasing concentration of the extract. In contrast, gallic acid revealed a significant increase in reducing ability as compared to the extract ($P<0.05$). The DPPH scavenging activity of the extract exhibited inhibitory activity and compared well with butylated hydroxytoluene, used as the standard (Figure 2).

The nitric oxide scavenging activity rose progressively with increasing concentration and compared well with that of the standard (Figure 3). As presented in Figure 4, an upsurge in the concentration of the extract yielded an increase in hydrogen peroxide scavenging activity and compared well with that of the gallic acid as the standard. Figure 5 shows the result of the scavenging activity of ABTS. There was an upsurge with the increasing concentration, and compared well with the standard. Figure 6 shows the result of the inhibitory activities of the extract against α-amylase and α-glucosidase, and compared well with acarbose as the standard.

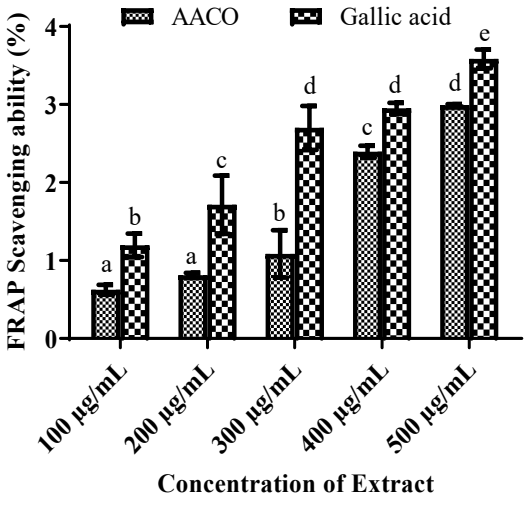

Figure 1. FRAP scavenging ability of aqueous extract of C. orbicularis leaves

Values are shown as Mean±SD; n=10;

Legend: AACO – Chrysobalanus orbicularis leaf extract; Gallic acid is the standard;

FRAP- Ferric reducing antioxidant potential; a-eSuperscrpted alphabets over the bars for a given concentration of each extract indicate significance of difference at $P<0.05$.

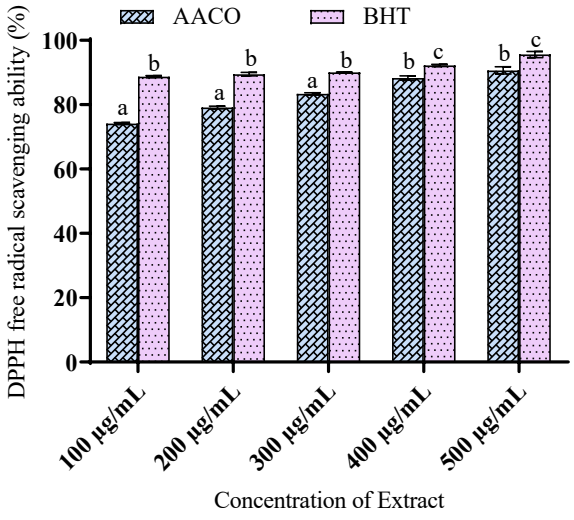

Figure 2. DPPH scavenging ability of aqueous extract of C. orbicularis leaves

Values are represented as Mean±SD; n=10;

Legend: AACO – Chrysobalanus orbicularis leaf extract; BHT is the standard (butylated hydroxyl toluene);

DPPH- 1, 1-diphenyl-2-picryl-hydrazil. a-cSuperscripted alphabets over the bars for a given concentration of each extract indicate significance of difference at $P<0.05$.

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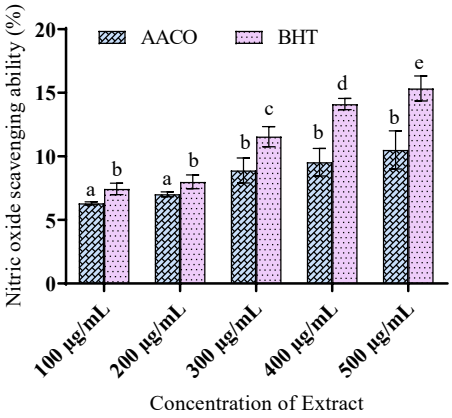

Figure 3. Nitric oxide scavenging ability of aqueous extract of C. orbicularis leaves

Values are represented as Mean±SD; n=10;

Legend: AACO = Chrysobalanus orbicularis leaf extract. BHT is the standard (butylated hydroxyl toluene);

a-eSuperscripted alphabets over the bars for a given concentration of each extract indicate significance of difference at P<0.05.

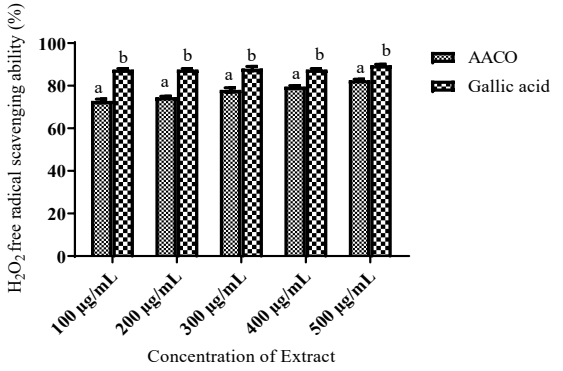

Figure 4. H_2O_2 scavenging ability of the aqueous extract of C. orbicularis leaves

Values are represented as Mean±SD; n=10;

Legend: AACO – Chrysobalanus orbicularis leaf extract. BHT is the standard (butylated hydroxyl toluene);

a-bSuperscripted alphabets over the bars for a given concentration of each extract indicate significance of difference at P<0.05.

Discussion

Free radicals have been involved in the development and progression of numerous diseases, such as diabetes and its complications [14-17]. It has been reported that the hyperglycemia produces free radicals and results in an imbalance of radical-antioxidant system in favor of radicals, leading to oxidative stress [18, 19]. The ability of a constituent to scavenge free radicals is linked to its electron transfer ability [20]. In the present study, the free radical scavenging and ferric reducing properties of the aqueous extract of C. orbicularis leaves were evaluated in vitro. The scavenging property was investigated to test the total antioxidant capacity, using ferric reducing power, 1,1-diphenyl-1-2-picryl-hydrazil (DPPH), hydrogen peroxide (H_2O_2), Nitric Oxide (NO) and 2, 2-Azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS). These methods are generally employed to assess the efficacy of the antioxidant activities of plants' extracts [8, 13]. The aqueous extract of C. orbicularis showed high levels of radical scavenging activities, thus this plant possesses antioxidant potentials. This property is likely to offer inhibitory effect and;

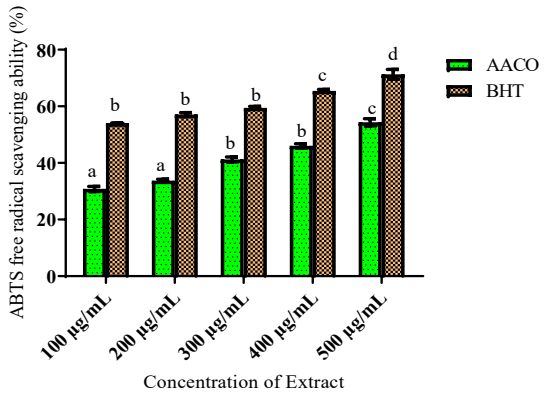

Figure 5. ABTS scavenging ability of the aqueous extract of C.orbicularis leaves

Values are represented as Mean±SD; n=10;

Legend: AACO: Chrysobalanus orbicularis leaf extract. BHT is the standard (butylated hydroxyl toluene);

a-dSuperscripted alphabets over the bars for a given concentration of each extract indicate significance of difference at p<0.05.

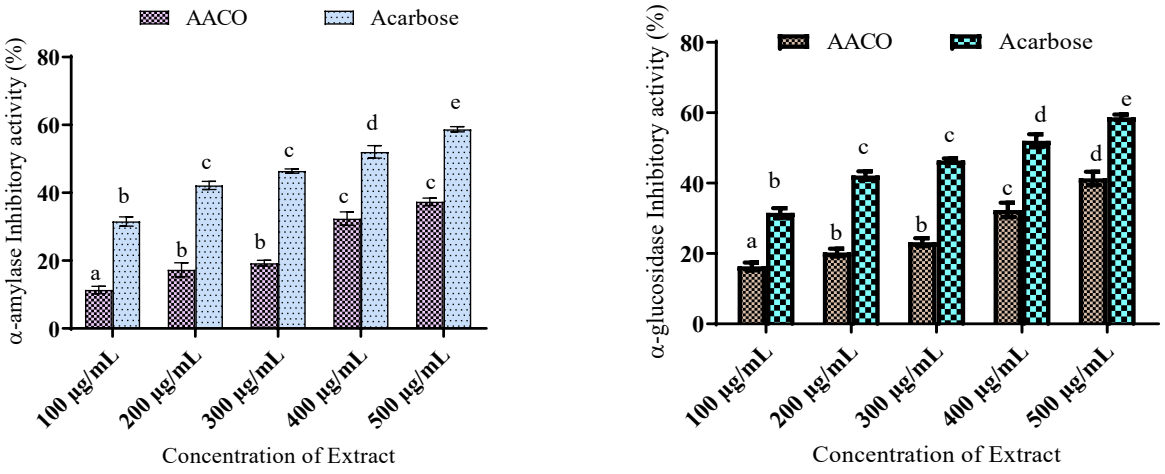

Figure 6. α-amylase and α-glucosidase inhibitory activity of C. orbicularis leaf extract

Legend: AACO – Chrysobalanus orbicularis leaf extract;

ACARBOSE: The standard values are represented as mean±standard deviation (SD); n=10;

a-eSuperscripted alphabets over the bars for a given concentration of each extract indicate significance of difference at P<0.05.

therefore, used clinically in the management of the oxidative stressors in such diseases, as diabetes mellitus type II.

Delaying the postprandial rise in the serum glucose level via the inhibition of α-amylase and α-glucosidase is critical to the management of patients with diabetes type II [21, 22]. Specifically, the inhibition of the enzymes that are implicated in the intermediary metabolism of carbohydrates is one of the most effective therapeutic approach in lowering postprandial blood glucose levels in diabetic patients [23, 24]. The enzyme, α-amylase, has been known to control and hydrolyze complex carbohydrates to disaccharides, while α-glucosidase breaks down oligosaccharides to monosaccharides, thereby leading to a rise in postprandial serum glucose level [25, 26]. The ability of C. orbicularis leaf extract to inhibit the two enzymes confirms the potential of the extract in the management of diabetes mellitus [18, 19]. Since the antioxidant activity of the extract has not been examined previously, this study attempted this investigation for the first time. The antioxidant activity of plants is primarily linked to their phenolic compounds content.

In a study by de Oliveira Barbosa, et al. [27], the extract was rich in phenolic compounds with high antioxidant properties. The results obtained by the current study are consistent with those reported by de Oliveira Barbosa, et al. [27]. Further, the high scavenging property has been attributed to the hydroxyl groups present in the chemical structure of the phenolic compounds, which are the essential components for scavenging free radicals [28, 29].

Conclusions

The aqueous extract of C. orbicularis leaves exhibited high antioxidant activity in vitro compared to the standard. In addition, the extract showed inhibitory property against α-amylase and α-glucosidase. These findings suggest that the extract of C. orbicularis leaves has the ability to inhibit the oxidative stress induced by free radicals in various human pathologic conditions, such as diabetes mellitus. This study is the first to report the in vitro antioxidant activity of the extract against α-amylase and α-glucosidase.

Ethical Considerations

Compliance with ethical guidelines

The study is an in vitro one which did not involve animals and human subjects. All ethical principles of chemicals and plants studies were applied in this research.

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Author's contributions

Conceptualization and methodology: Basiru Olaitan Ajiboye, Oluwafemi Adeleke Ojo; Data analysis: Lisa Ilobekemen Ekakitie; Writing of the original draft, review and ed-

iting of the manuscript: Lisa Ilobekemen Ekakitie, Basiru Olaitan Ajiboye, Babatunji Emmanuel Oyinloye.

Conflict of interest

The authors declare no conflict of interests with any entity in conducting this study.

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