INVESTIGATION OF THE METHANOL EXTRACT OF *Anchomanes difformis* TUBER EXTRACT FOR *IN VITRO* ANTIOXIDANT, α- AMYLASE AND α- GLUCOSIDASE INHIBITORY ACTIVITIES

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Abstract: The primary stage of diabetes mellitus type 2 is linked with postprandial hyperglycemia. This is believed to increase the production of free radicals and reactive oxygen species, leading to oxidative tissue damage. In an effort to identify herbal drugs which may be useful in the prevention or mitigation of diabetes, the antioxidant activities, *in vitro* α-amylase and glucosidase inhibition of methanol extract from the tuber of *Anchomanes difformis* was studied. The extract showed total phenol content (TPC) 442.7 ±2.28 GAE mg/100g and ferric-reducing antioxidant power (FRAP) 176.6 ±3.31 AAEmg/100g. At the maximum concentration 100 µg/ml, the metal ion inhibition, DPPH radical scavenging activity and nitric oxide scavenging activity are 43.3, 34.9 and 58.6 µg/ml, respectively. The plant did not show prominent α- amylase and glucosidase inhibitory activity (IC_{50}>100 µg/ml). This study shows that *A. difformis* contains phenolic compounds that can be useful in the prevention or mitigation of cellular damages linked to diabetic conditions.

Keywords: *Anchomanes difformis*, Araceae, α-amylase, α-Glucosidase, Antioxidant

Introduction

Around the globe, virtually all cultures have used medicinal plants as sources of medicine (Baquar, 1995; Gilani and Atta, 2005). There is renewed and increased interest in natural medicines for reasons such as high cost of producing patentable chemicals and drugs, burden and high cost of modern healthcare in developing countries (Schneider, 2001; Hack, 2006; Sofowora, 2008). Diabetes mellitus is a chronic non-communicable disease. The World Health Organization (WHO) predicted that the global prevalence of the disease would increase from 2.8 to 4.4% by 2030 with the most substantial increase predicted to occur in developing countries. Various ailments like cardiovascular diseases, Alzheimer, diabetes, cancer and other anti-inflammatory conditions are associated with oxidative stress (Shorinwa and Aghanya, 2015).

Anchomanes difformis commonly known as forest *Anchomanes* in English, belongs to the family, *Araceae*. It is a native plant of the African continent, particularly the following countries: Nigeria, Cameroon, Ghana, Cote d`ivoire, Sierra Leone, Senegaland Togo (Akah and Njike, 1990; Ataman and Idu, 2015). It is a tropical herb that grows in shady terrestrial areas and can grow up to 2 m high. It has a stem and spathe that arises from a horizontal tuber (Oliver, 1960; Burkill, 1994). *A. difformis* is known by different names in Nigeria including "Olumahi" by the Igbos (Umuahia), "Ebaenan" by the Efik, "Chakara" by the Hausas, "boubekeodu" by the Ijaws (Burkill, 1994), "Abrisoko" by the Yorubas in the South West, Nigeria (Oyetayo, 2007), and "Olikhoror" by the Bini tribe of Edo State (Ataman and Idu, 2015).

Quantitative and qualitative analyses of the significant presence of the following phytochemical/ mineral constituents: carbohydrates, crude proteins, fats, fibres, calcium, magnesium, manganese, copper, iron, zinc, alkaloids, saponins, flavonoids and steroids have been reported (Egwurugwu *et al*., 2016). These phytochemical constituents may be responsible for the many functions ascribed to *A.difformis*. The various medicinal uses of *A. difformis* are; anti-inflammatory (Oyetayo, 2007), analgesic and hypothermic effects, diuresis and purgative, anti-hemolytic and anti-oxidant effects, anti-diabetic, anti-filariasis, insecticidal and antidiarrheal properties (Akah and Njike, 1990). It has also been found to reduce serum concentrations of some sex hormones implicated in the pathogenesis of uterine fibroids, suggesting its possible role in the management of uterine myomata (Egwurugwu *et al*., 2016). In Zaria (Nigeria), the decoction of the root is used to treat diabetes, cough and throat related problems (Aliyu *et al*., 2013).

The rhizome has been used for the treatment of many disease conditions in various parts of the world; it is used as rubefacients vesicant for external application in Guinea. In Ivory Coast it is considered to be a powerful purgative and is used to treat Oedema, difficult child birth, as poison antidote as well as a strong diuretic for treating urethal discharge, jaundice and kidney pains (Burkill, 1985). Phytochemical analysis of the leaf, stem and tuber of *A. difformis* revealed the presence of saponins, tannins and alkaloids (Adegoke *et al*., 1968). It was discovered that the tuber extract is an effective inhibitor of *Salmonella* species and *Bacillus substillis*. This confirms the local use of the extract in the treatment of dysentery by herbal practitioner (Oyetayo, 2007, Adeyemi *et al*., 2015).

It appears there is limited information on the medicinal uses of the tuber of *A. difformis* is scarce; therefore the purpose of the study is to assess the antioxidant potential of the tuber extract from *A. difformis* and its ability to inhibit α-amylase and glucosidase enzymes. The study was further prompted by the claims of some traditional health practitioners in some parts of Nigeria that the tubers of *A. difformis* are effective remedies for the management and or control of diabetes. This work will also provide medicinal justification for the use of *A. difformis* in the management, control and or treatment of diabetes.

Materials and Methods

Collection and preparation of plant material

Anchomanes difformis tubers were collected from Ikere-Ekiti, Ekiti State, Nigeria in the month of February 2017. The plant was identified by Mr. Omotayo of the Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado Ekiti. The tubers were sun-dried for 2 weeks and then broken into smaller particles using mortar and pestle, it was then pulverized into fine powder using a mechanical grinder and weighed. The representation of the plant and the tuber is shown in Fig. 1. 2.8 kg of the powder plant material

was extracted with methanol for 72 h; the extract was filtered and allowed to evaporate to dryness with the aid of a rotary evaporator at 50ºC. The concentrated extract (28.2 g) was stored in an air tight sample vial pending analysis.


A


B

Fig. 1: The photograph of *Anchomanes difformis* plant (A) and its tuber (B)

Determination of antioxidant activity of A. difformis methanol extract

Total phenolic content

The total phenolic content of the extract was determined using the method of Macdonald *et al*(2001) with slight modifications. The calibration curve was prepared by mixing methanol solution of gallic acid (1 ml; 0.025-0.400 mg/ml) with 5 ml of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7 M). Absorbance values were measured at 765 nm using a UV-VIS spectrophotometer (UVmini-1240, Shimadzu Corporation, Kyoto, Japan) and the standard curve was plotted. One milliliter of each of the extract solutions in methanol (5 g/L) was also mixed with the reagents above and after 30 min the absorbance was measured to determine the total phenolic contents. All determinations were carried out in triplicate. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the following formula: T = C×V/M; where T = total phenolic contents, milligram per gram of sample extract, in GAE; C = the concentration of gallic acid established from the calibration curve, mg/ml; V = the volume of extract, milliliter; M = the weight of sample extract (g).

Ferric-reducing antioxidant power assay (FRAP)

A stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 0.3M acetate buffer of pH 3.6 was prepared (Govindan and Muthukrishnan, 2013). The ferric-reducing antioxidant power (FRAP) reagent consist of 2.5 ml TPTZ solution, 2.5 mL ferric chloride solution and 25 ml acetate buffer. The reagent was freshly prepared and warmed to 37^0C. 900 mlFRAP reagent was mixed with 90 ml water and 30 mL test methanolic extract of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37^0C for 30 minutes and the absorbance was recorded at 595 nm. An intense blue colour complex was formed when ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex was reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded.

Metal ion chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.* (1994). 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of 2 mM $FeCl_2$ was added. Then, after about 30s, 0.1 ml of 5 mM ferrozine was added to the mixture. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+} Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as:

Chelating (%) = (Ao - A1) / Ao × 100

Where: Ao was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

Scavenging ability towards DPPH radical (DPPH assay)

The DPPH assay was performed as described by Shirwaikar *et al.* (2006). This method depends on the reduction of purple DPPH to a yellow coloured diphenyl picrylhydrazine and the remaining DPPH which showed maximum absorption at 517 nm was measured. About 2 ml of various concentrations of each extract were added to 2 ml solution of 0.1 mM DPPH. An equal amount of methanol and DPPH served as control. After 20 min of incubation at 37ºC in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

% inhibition = [A of blank – A of sample] / A of blank x 100.

The inhibition curve was plotted for triplicate experiments and represented as % of mean inhibition ±standard deviation. IC_{50} values were obtained using Graph Pad prism 5.0.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which reacts with oxygen to produce nitrite ions; this can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25ºC for 150 min. From the incubated mixture 0.5 ml was added to 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min, lastly, 1.0 ml naphthylethylene diaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min and the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was then calculated.

α-amylase inhibition assay

Inhibition of alpha amylase activity was carried out using starch-iodine method as reported by Xiao *et al.* (2006). Total assay mixture composed of 120 µl 0.02M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 1.5 ml of salivary amylase and plant extracts of concentration range

from 20-100 µg/ml were incubated at 37ºC for 10 min. Soluble starch (1%, w/v) was added to each reaction mixture and were incubated at 37°C for 15 min. Subsequently, 1M HCl (60 µl) was added to stop the enzymatic reaction, followed by the addition of 300 µl of iodine reagent (5 mM I_2 and 5 mM KI). Colour change was observed and the absorbance was recorded at 620 nm. The % of inhibition was calculated using the formular:

$$[1-(Ao - A1)/Ao] \times 100$$

Where: Ao was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

α-glucosidase inhibition assay

The inhibitory effect of the extract on α-glucosidase activity was determined according to the chromogenic method described by Kim *et al*. (2005). Briefly, 5 units of α-glucosidase were pre-incubated with 20-100 µg/ml of the extract for 15 min. Para-nitrophenylglucopyranoside (PNPG) (3 mM) dissolved in 20 mM phosphate buffer, pH 6.9 was added to start the reaction. The reaction mixture was further incubated at 37°C for 20 min and stopped by addition of 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from PNPG at 400 nm. Each test was performed three times and the mean absorption was used to calculate percentage α-glucosidase inhibition. Percentage α-glucosidase inhibition was calculated according to the following formula:

$$(Ao - A1)/Ao \times 100$$

Where: Ao was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract.

Results and Discussion

Total phenolic content

Anchomanes difformis is commonly used traditionally to treat many diseases whose pathogenesis are among other factors linked to oxidative stress (Shorinwa and Aghanya, 2015). In this study, we report the antioxidant potentials of *A. difformis* tuber extracts. The extract was found to have phenolic contents of 442 ± 2.28 mg/100 g GAE (Table 1). The high phenolics content of the extract indicates high antioxidant potentials since the phenolics compounds have been reported to possess antioxidant activities (Akinwunmi and Faleye, 2017).

Table 1: TPC and FRAP (mean ± SEM) measured in the tuber extract of leaves of *A. difformis*

TPC (GAE mg/100g)	FRAP (AAE mg/100g)
442.7 ±2.28	176.6 ±3.31

FRAP assay

The Ferric reducing antioxidant power in the ADM tuber extract is presented in Table 1. The ferric reducing ability of the ADM extract was found to be 176 AAE mg/100g). The FRAP assay is based on the capability of antioxidant to reduce TPTZeFe (III) complex to the TPTZeFe (II) complex, thereby forming a blue Fe^{2+}TPTZ complex with an absorption maximum at 593 nm. The reduction capacity of a compound may serve as a major indicator of its potential antioxidant activity. A higher absorbance also indicates a higher ferric reducing power (Govindan and Muthukrishnan, 2013).

Metal chelation assay

The result of metal ion chelation of the methanol tuber extract of *A difformis* is presented in Fig 2. At the lowest concentration of 20 µg/ml, it shows 23.4% inhibition while at the highest concentration of 100 µg/ml, 43.3% inhibition was observed. Fe chelation may be one of the possible mechanisms through which antioxidant phytochemicals in *A. difformis* prevent lipid peroxidation in tissue by forming a complex with Fe, thus preventing the initiation of lipid peroxidation.

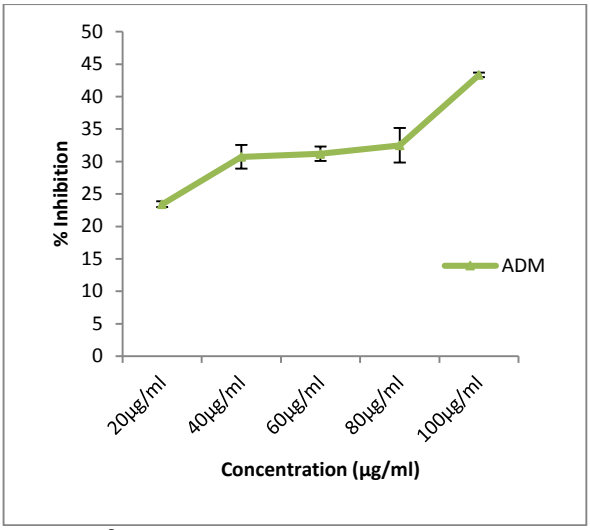

Fig. 2: Fe^{2+} chelating ability of the tuber extract of A. *difformis*. Values are expressed as mean±SEM, n=3

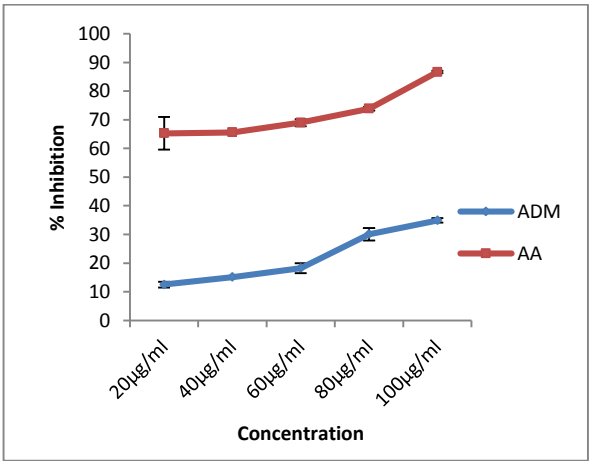

Fig. 3: DPPH radical scavenging activity of extract of *Adifformis*. Data are represented as the mean value ± SEM (n=3)

DPPH free radical scavenging activity

DPPH radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Do *et al*., 2013). Fig. 3 shows the DPPH scavenging activities of the extract in a concentration-dependent manner. At the highest concentration 100 µg/ml, ADM extract had the % inhibition 39.2 as against ascorbic acid (86.7%). The free radical chain reaction is generally accepted as a common mechanism of lipid peroxidation. The result suggest that the extract possess a mild antioxidant activity and probably have the ability to inhibit autoxidation of lipids and could thus be beneficial in the treatment of various diseases where lipid peroxidation is an important mechanism for pathogenesis (Fig 3).

Nitric oxide scavenging activity

The result of nitric oxide scavenging activity of *A. difformis* tuber extract is presented in Fig 4. At the lowest concentration of 20 µg/ml, the extract shows a 15.2% inhibition and 62.5% at the highest concentration of 100 µg/ml with IC_{50} value of 74.8 µg/ml, while ascorbic acid used as standard was found to show IC_{50} value < 20 µg/ml, the extract showed concentration dependent inhibition. However, the activity of ascorbic acid was more pronounced than that of the extract.

Nitric oxide plays a vital role in several inflammatory processes. Higher levels of these radicals are toxic to tissue and contribute to the vascular collapse, many carcinoma and ulcerative colitis. The toxicity of nitric oxide increases when it reacts with superoxide radical forming highly reactive peroxy nitrate anion (Suresh and Suriyavathana, 2012). *A. difformis* extract decreases the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principle in the extract, which competes with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation *in vivo* (Govindan and Muthukrishnan, 2013).

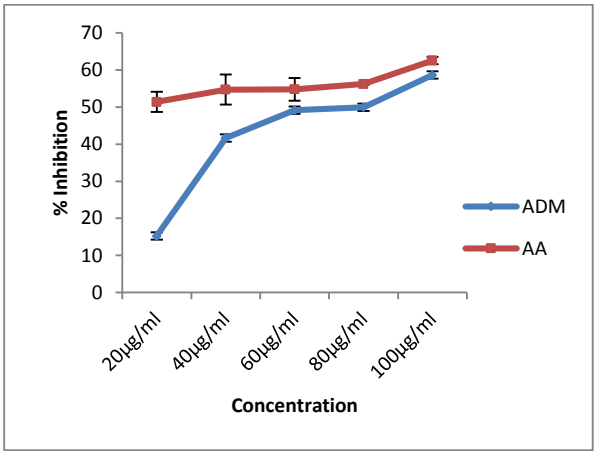

Fig. 4: Nitric oxide scavenging activity of methanolic extract of *Anchomanes difformis tuber* and ascorbic acid. Values are expressed as mean ± SEM (n=3)

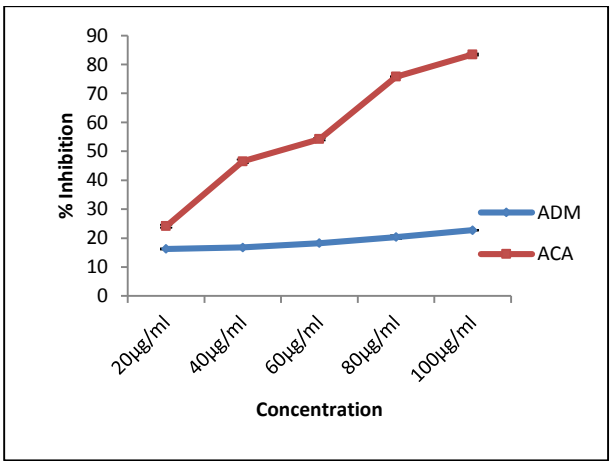

Fig. 5: α-amylase inhibition activity of *ADifformis tuber* extract. Values are expressed as mean±SEM, n=3

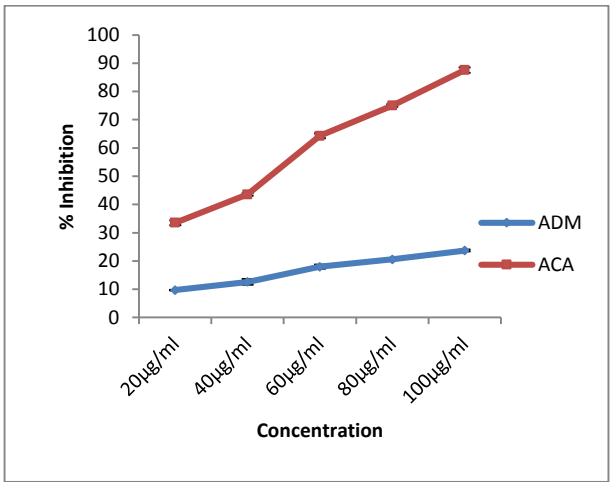

Fig. 6: α-glucosidase inhibition activity of *ADifformis tuber* extract. Values are expressed as mean±SEM, n=3

α-amylase and α-glucosidase inhibition activity

The result of α-amylase and α-glucosidase inhibition activity of methanol extract of *A. difformis* and acarbose is presented in Figs. 5 and 6. Methanol extract of *A. difformis* exerted its *in vitro* inhibitory potential as against α-amylase and α-glucosidase with IC_{50} values >100 µg/ml, as compared to acarbose with IC_{50} values of 45.9 µg/mg and 42.5 µg/ml. At the lowest concentration of 20 µg/ml, *A. difformis* showed 16.2% inhibition of α-amylase and 22.7% at the highest concentration, while on α-glucosidase, it displayed 9.69% inhibition at lowest concentration and 23.7% inhibition at the highest concentration. The result above indicates a weak inhibition of the enzymes, the activity of acarbose is more pronounced.

Conclusion

Free radicals play a significant role in the development of tissues and pathological events in living organisms. There are proofs that illustrate that increased uptake of fruit and vegetables reduce the risk of cancer. This is credited to the existence of antioxidants in fruits and vegetables. Most of the natural antioxidants active compounds are phenolics and flavonoids. Plant phenolics mainly function as chain breakers, free radical scavengers and electron donors capable of reducing the oxidative damage connected with cardiovascular diseases, cancer and atherosclerosis. Furthermore, flavonoids are well known as powerful antioxidants. Finally, *A. difformis* tuber extract contains some antioxidant agents that could be relevant in the therapeutic action of this plant part. These findings deserve further studies on the isolation and characterization of the bioactive compounds responsible for the antioxidant/antidiabetic activities observed therein.

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Conflicts of Interest

There are no conflicts of interest.

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