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Antioxidant Properties and Invitro Carbohydrate Digestive Enzyme Inhibition of *Anchomanes difformis* Roots in Diabetic Rat Models

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Abstract

Objective: This study investigated the antioxidant activities and antidiabetic potentials of Anchomanes difformis root extract by evaluating its enzymatic antioxidant activities (catalase, superoxide dismutase, and glutathione peroxidase), malondiadehyde and non-enzymatic antioxidant activities (DPPH, ABTS and hydroxyl radical scavenging). Additionally, the extract's ability to inhibit alpha-amylase and alpha-glucosidase enzymes was assessed.

Materials and Methods: Twenty-four (24) adult male albino rats (Wistar strain) weighing within the ranges of 150–250 g were used for the antidiabetic study. All rats were randomly divided into four groups. All groups were treated with the following regimen for twenty-one (21) days. The normal control (NC) Group 1 and diabetic control (DC) Group 11 were fed on normal feed and water ad libitum throughout the period. Diabetic rats Groups III and IV received aqueous and ethanol extracts of A. difformis at a dose of 500mg/kg body weight respectively.

Results: The extract showed significant (P<0.05) increase in activities of SOD, GPX and CAT and significant (P<0.05) reduction in MDA level of treated diabetic rats. The extract also exhibited significant antioxidant activity, as demonstrated by its ability to scavenge DPPH, ABTS, and hydroxyl radicals. Additionally, the extract showed potential inhibition of alpha glucosidase activity in a concentration dependent manner and moderate inhibition of alpha amylase activity, suggesting its potential as a natural antidiabetic agent. These findings highlight the potential of Anchomanes difformis root extract as a natural therapeutic agent for managing diabetes mellitus and related oxidative stress disorder.

Keywords: Values are mean ± standard deviation, Values (represented as Bar charts) are mean, while error bars are standard deviations, Values in a group with different alphabetical superscript are significantly different at P<0.05

Introduction

One of those native medicinal plants, Anchomanes difformis, has been used by traditional herbalists to cure a variety of ailments and disorders. It is a member of the ARACEAE family. In English, it is referred to as woodland Anchomanes. It is a prickly-stemmed herbaceous plant [1,2]. The genus Anchomaness difformis a large herbaceous plant is a member of the Aracaea family. It is a significant medicinal plant that grows in tropical regions throughout Africa, but it is particularly prevalent in West African forests [3]. It is a substantial herbaceous plant. According to Dalziel [4], the plant root is also referred to as Chakara (Hausa) in Northern Nigeria, Oje in Eastern Nigeria, and Ishu agan (Yoruba) in South Western Nigeria. Locals frequently refer to it as bush cocoyam [5]. A root decoction is used to cure diabetes mellitus, according to ethno-medicinal information from herbal practitioners in Zaria, Nigeria. The root or tuber is used as a diuretic and to treat diabetes mellitus in the Republic of Benin. There is currently no scientific evidence to back up this assertion. Additionally, it has been stated that the powdered root of Anchomanes difformis combined with palm oil is used as a treatment for respiratory illnesses in children in Zaire (DRC) and South Western Nigeria, respectively. The herb is also said to possess anti-microbial qualities [6,7]. The oldest kind of medicine known to humans is the usage of herbs, which has been used throughout history in all civilizations [8]. About 80% of the world's population, particularly in developing and undeveloped nations, uses herbal medicine as their major source of health care due to poverty and restricted access to modern medicine [9]. Herbal remedies are not available in government healthcare facilities, despite the widespread usage of herbal medicine in the delivery of healthcare in nations like Nigeria and the accessibility of medicinal herbs in local markets. This may be partially caused by a lack of sufficient knowledge and thorough scientific investigation of the most widely utilized herbal remedies. Anchomanes difformis is a plant that has long been employed as an herbal remedy in traditional medicine. A decoction of the herb is used to cure diabetes among other illnesses, according to ethno-medicinal information from herbal practitioners in Zaria City, Nigeria [10]. This assertion cannot be verified or supported scientifically.

A series of metabolic illnesses known as diabetes mellitus are defined by hyperglycaemia caused by deficiencies in insulin secretion, action, or both. According to Adams et al, [11], long-term damage, dysfunction, and failure of many organs, particularly the eyes, kidneys, nerves, heart, and blood arteries, are linked to the chronic hyperglycaemia of diabetes. An estimated 537 million persons worldwide are projected to have diabetes mellitus, with a global prevalence of 10.5 percent among adults aged 20 to 79. Diabetes Atlas, IDF, 2021. Approximately 90% of diabetes diagnoses worldwide are for type 2 diabetes mellitus (T2DM) [12]. The World Health Organization (WHO) estimates that 3% of the world's population currently has diabetes; by 2025, this percentage is projected to quadruple [13]. Rapid urbanization, westernization, and the ensuing lifestyle modifications are some of the factors contributing to this expected increase. Another component causing this threat is genetic predisposition [14]. In DM, oxidative stress has a number of detrimental impacts on cellular physiology. For the islet, one of the tissues with the lowest levels of intrinsic antioxidant defence, this is particularly significant and harmful. Numerous biochemical routes and mechanisms of action have been connected to the deleterious effects of chronic hyperglycemia and oxidative stress on the functionality of vascular, retinal, and renal tissues [15]. Cellular damage brought on by hyperglycaemia is significantly influenced by oxidative stress. Free radical generation can be stimulated by high glucose levels. A situation of imbalance between ROS and their protection occurs as a result of the body's weak defence system's inability to counteract the increased ROS creation, which leads to the dominance of the condition of oxidative stress [16]. Since oxidative stress and ROS have a variety of regulatory functions in cells, they are required in small amounts for proper metabolic activities. In order to get rid of antigens, neutrophils and macrophages create ROS during the respiratory burst process [17]. Additionally, they serve as signals that promote the expression of several genes that code for transcription factors, differentiation, and development as well as genes that stimulate fibroblast growth, cellular signalling, cell-cell adhesion, involvement in vaso-regulation, and increased levels of antioxidant enzymes [18]. However, excessive and/or unregulated ROS generation is harmful. The metabolic anomalies of diabetes

lead to an overproduction of mitochondrial superoxide in both large and small artery endothelial cells as well as in the myocardium due to oxidative stress [19]. According to Oguntibeju, [20], oxidative stress is a mediator of insulin resistance, which leads to glucose intolerance and the establishment of diabetes mellitus, favours the development of atherosclerotic complications, and contributes to the rise in many micro- and macro-vascular complications. Through a variety of mechanisms, including increased intracellular formation of advanced glycation end products (AGEs), increased expression of the AGEs receptor and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway, hyperglycemia damages tissue [21]. High fatty-acid flux causes oxidative damage in insulin-sensitive tissues like the muscle, liver, and heart while exposing non-insulin-sensitive tissues like the eye, kidney, and nervous system to high circulating glucose and fatty acid levels and, consequently, ROS-induced diabetic complications [22].

Materials and Methods

Plant Collection

Four kilogrammes of fresh tubers of Anchomanes difformis plant were collected from a natural habitat at Okpella, Etsako East LGA of Edo State and authenticated in the Department of Botany, Ambrose Alli University, Ekpoma, Edo State.

Preparation of Extraction

Extraction was done at the University of Lagos (UNILAG), Department of Pharmacology, College of Medicine, Lagos state. The fresh tubers of Anchomanes difformis plant were obtained, washed, chopped and air dried for two weeks. The size was reduced with mortar and pestle into fine powder. About 2.0 kg of the powder was extracted with distilled ethanol (7000ml) by soaking for three days with periodic stirring. The samples were filtered with sintered glass funnel to eliminate particles. The filtrates collected were then concentrated using a rotary evaporator to give brownish viscous pastes which were then weighed directly. The aqueous extract was treated the same way, although distilled water (7000ml) was used in place of ethanol. The brownish pastes (both extracts) were kept in the freezer at -21^{o}C prior to use. The yields were 23.6gm and 21.3gm for ethanol and aqueous extracts respectively.

Animal Model

Twenty-four (24) adult male albino rats (Wistar strain) weighing within the ranges of 150–250 g were used for the antidiabetic study. The rats were procured from the Animal House Department, College of Medicine, Ambrose Alli University Ekpoma, Edo State and transferred to the experimental Laboratory at Health Affairs Ventures, Ekpoma, Edo State where they were allowed two (2) weeks of acclimatization, then weighed again and housed in wooden cages with wire-mesh at the top and sides. They were kept under controlled environmental conditions of temperature (28±20C), relative humidity (50±5%) and a twelve-hour light/dark cycle. The animal facility was adequately ventilated. Tap water and feeds (Top Feed) were provided ad libitum throughout the experimental period.

Determination of Antioxidant Activity of Extracts of A. difformis using DPPH

The radical scavenging activities of the plant extracts against 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH) Sigma-Aldrich were determined by the method of Ohkawa [23] using UV spectrophotometer at 517 nm [24].

Procedure: Different concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 mg/ml) of the extracts were prepared in methanol (Analytical grade). Vitamin C was used as the antioxidant standard at concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 mg/ml. Exactly 1.0 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1mM DPPH in methanol and thereafter the absorbance was determined on a UV-Visible spectrophotometer. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula.

Calculation: % Inhibition = (Ab - Aa) / Ab X 100

Where Ab is the absorbance of the blank sample (without the extract) and Aa is the absorbance
Of the extract.

Determination of ABTS Radical Scavenging Assay of Anchomanes Difformis

ABTS assay was based on the slightly modified method of Huang [25].

Procedure: ABTS radical cation (ABTS+) was made by reacting 7 mM ABTS solution with 2.45 mM potassium sulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. The ABTS+ solution was concentrated with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 100 µl of sample or trolox standard to 3.9 ml of diluted ABTS solution, absorbance was read on the spectrophotometer at 734 nm immediately after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were executed in triplicates and the end results were averaged.

Determination of Hydroxyl Radical Scavenging Activity of Root Extract of Anchomanes difformis

The modified method of Halliwell [26] was used for assay of the scavenging ability of the hydroxyl radicals. Using dilute deionized water, Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), H_2O_2 (10 mM), Ascorbic Acid (1 mM), and Deoxyribose (10 mM) were made [27].

Procedure: The assay was performed by adding 0.1 ml EDTA, 0.36 ml of deoxyribose 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 1.0 ml of the extract of different concentrations (50, 100, 200, 400 and 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence into a test-tube. The mixture was then incubated at 37ºC for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging action of the extract is reported as % inhibition of deoxyribose. The degradation is determined by using the subsequent equation.

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

Inhibition of Activities of Carbohydrate Digestive Enzymes by Extract of Anchomanes difformis

Alpha-Amylase Inhibition Assay

Alpha-amylase inhibitory ability of the extract was assayed as reported by Kwon [28].

Procedure: Appropriate dilutions of the extract (0 - 500 µL), and 500 µL of 0.02 M sodium phosphate buffer [pH 6.9; containing 0.006 M NaCl and 0.5 mg/mL of porcine pancreas α-amylase (EC 3.2.1.1)] were incubated at 37 °C for 10 minutes. Thereafter, 500 µL of starch solution (1 % starch in 0.02 M sodium phosphate buffer) was added. The reaction mixture was then incubated at 37 °C for 15 minutes, and the reaction was terminated with 1.0 mL of 3,5-dinitrosalicylic acid (DNSA) colour reagent (1 % DNSA and 12 % sodium potassium tartrate, in 0.4 M NaOH). The reaction mixture was then incubated in a boiling water bath for 5 minutes, and cooled to room temperature. The absorbance was measured at 540 nm, and the percentage α-amylase inhibition was then calculated.

Calculation: % Inhibition = (A control- A sample) / A control X 100

Where, A control is the absorbance of the control,A sample is the absorbance of the test sample.

Alpha-Glucosidase Inhibition Assay

The ability of the extract to inhibit α-glucosidase was determined as reported by Kashtoh & Baek, [29].

Procedure: About 50 ml of B. stearothermophillus α-glucosidase (0.1 U/mL in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2000 mg/ml), was pre-incubated with appropriate dilutions of the extract for 15 minutes. Thereafter, 50 µl of 3 mM para-nitrophenylglucopyranoside (PNPG), dissolved in 20 mM phosphate buffer (pH 6.9) was added as a substrate to start the reaction. The reaction mixture was further incubated at 37 °C for 20 minutes, and the reaction was terminated by addition of 2 mL of 0.1 M Na2CO3. The α-glucosidase activity was determined by measuring the yellow-coloured p-nitrophenol released from PNPG at 400 nm. The percentage α-glucosidase inhibition was then calculated.

Calculation: % Inhibition = (A control- A sample) / A control X 100

Where, A control is the absorbance of the control,A sample is the absorbance of the test samples

Anti-Diabetic Study

Induction of Diabetes Mellitus

Prior to induction, the experimental animals (rats) were fasted overnight. A single dose of 60mg/kg body weight of Streptozotocin (Sigma- Aldrich Corp. St Louis, MO, USA) dissolved in saline solution was administered intra-peritoneally. After injection, the rats had access to food and water. The rats that had blood glucose levels of between 250 -300 mg/100ml after 5 days were selected for the study.

Experimental Design

Plasma activities of Antioxidant Enzymes and MDA Levels of Diabetic Rats 21 Days after Administration of 500mg/Kg Body Weight of Extracts of A. difformis

Feed was withdrawn from the rats and they were fasted overnight but with free access to water. The administration of extracts of A. difformis was performed through the oral route.

Group I: Normal Control rats (NC) + distilled water

Group 11: Diabetic control (DC) received normal feed and water, daily for 21 days

Group III: Diabetic rats received aqueous extract of A. difformis at a dose of 500mg/kg body weight plus normal feed, daily for 21 days.

Group IV: Diabetic rats received ethanol extract of A. difformis at a dose of 500mg/kg body weight plus normal feed, daily for 21 days.

Sample Collection

At day 21, blood samples were collected through the saphenous vein into appropriately- labelled lithium heparin bottles for

biochemical analyses. Plasma samples were obtained after centrifugation for 5 minutes at 3000 Revolution per Minute (rpm). The plasma obtained was stored frozen at -200 C until the time of analysis.

Determination of Catalase Activity

Catalase (CAT) activity was determined by the method of Claiborne [30,31].

Principle: The disappearance of peroxide is followed spectrophotometrically at 240 nm. One enzyme unit decomposes one micromole of H2O2 per minute at 25oC and pH 7.0.

Procedure: Exactly 50 µl of sample was mixed with 2.95 ml of 19 mM H2O2 in 50 mM potassium phosphate buffer (pH 7.0) at room temperature. The decrease in absorbance at 240 nm was monitored for 3 min at 15 sec interval. A unit of the enzyme activity is defined as the amount of enzyme catalyzing the decomposition of 1 µmol of H2O2 per minute at 25 ^{o}C and pH 7.0. Catalase activity was expressed as U/mg protein.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was assayed according to the method of Suttle [32], using Randox Laboratories Kit (Cat. No. SD 125) [33].

Principle: Xanthine oxidase (XOD) oxidizes xanthine to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Procedure: Exactly 30 µl of sample or standard was mixed with 1000 µl substrate reagent (containing 0.05 mM xanthine and 0.025 mM 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride). To this, 150 µl of xanthine oxidase was added. Then the initial and final absorbance at 505 nm was read after 30 seconds and 3 minutes respectively, against air. Percentage inhibition of standard was plotted against log10 (standard conc. in SOD U/mL), and then the SOD units of sample was estimated from the standard curve

Determination of Glutathione Peroxidase (GSH-Px) Activity

GSH-PX activity was determined following the method of Kraus and Ganther [34] using Randox Laboratories Kit (Cat No. RS 504) [35].

Principle: GSH-PX catalyses the oxidation of GSH by cumene hydrperoxide. The GSSH produced is immediately converted to GSH in the presence of glutathione reductase and NADPH, with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance is measured at 340 nm.

Procedure: Exactly 20 µl of sample was mixed with 1000 µl of reagent containing (4 mM glutathione, glutathione reductase and 0.34 mM NADPH in 0.05 M phosphate buffer, pH 7.2 and 4.3 mM EDTA) and 40 µl of 0.18 mM cumene hydroperoxide. The initial absorbance at 340 nm against reagent blank was read after 1 minute; then the absorbance was read again after 1 and 2 minutes. One unit of GSH-Px was defined as the amount of enzyme required to oxidize 1 mM of NADPH per minute at 25 °C. GSH-Px activity was expressed as U/mg protein.

Determination of Plasma Malondialdehyde

The modified method of Gutteridge [36] was used for the assay [37]

Principle: MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce red compound, which has a maximum absorption peak at 532 nm.

Procedure: Exactly 200 µl of plasma was dispensed into a test tube containing 1000 µl of glacial acetic acid. About 100 µl of 1% thiobarbituric acid (TBA) in 0.05N NaOH was added to the mixture. The test tube was placed in a boiling water bath for 15 minutes and then allowed to cool. The absorbance of the red coloured product formed was read in a spectrophotometer at 532nm against a reagent blank.

$$\text{MDA content} = \frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs Std} - \text{Abs blank}} \times [\text{Std}] \text{ nmol/ml}$$

Data Analysis

Statistical analysis was done using SPSS version 21.0. All values were expressed as mean ± standard deviation. The ANOVA was the chosen Statistical tool. Values were significant at $p \leq 0.05$

Results

Plasma activities of Antioxidant Enzymes and MDA Levels of Diabetic Rats 21 Days after Administration of 500mg/Kg Body Weight of Extracts of A. difformis. Figure 1 showed a significant ($P<0.05$) increase in activities of SOD, GPX and CAT in diabetic rats administered with aqueous extract Group III (3131.40 ± 16.93, 43.00 ± 1.34, 0.88±00) and ethanol extract Group IV (3235±42.10, 47.78±1.52 and 0.88±0.4) when compared with the diabetic control Group II (1903±29.60, 32.97±0.73 and 0.53±0.05) respectively. In Figure 2, there was a progressive and significant ($P<0.05$) increase in plasma MDA levels of diabetic control rats (Group II) from day 0 to day 21 when compared to the normal control rats (Group I) which showed no significant increase ($P>0.05$). Significant ($P<0.05$) reduction was also observed in MDA levels of the treated diabetic rats in groups III and IV.

Key: Values are mean ± standard deviation. Values (represented as Bar charts) are mean, while error bars are standard deviations.

Values in a group with different alphabetical superscript are significantly different at P<0.05.

Group I: Normal control rats, Group II: Diabetic control rats, Group III: Diabetic rats plus aqueous extract, Group IV: Diabetic rats plus ethanol extract.

Non-Enzymatic Antioxidant activities of Root Extracts of Anchomanes difformis

In Figure 3, the radical scavenging effect of extracts of A. difformis and the standard (Vit C) exhibited increases in percentage inhibition in a dose-dependent manner. The ethanol extract showed the highest DPPH radical scavenging activity (% inhibition) of 93.62±0.85 at a concentration of 2.0mg/ml while the standard, vitamin C and aqueous extract showed % inhibitions of 92.31±0.30 and 91.65±0.41 respectively at 2.0mg/ml concentration. The scavenging power of the ethanol and aqueous extracts of A. difformis for ABTS radicals were analysed and compared (Figure 4).

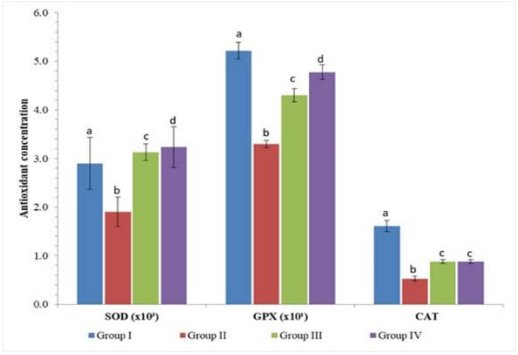

Figure 1: *Mean plasma activities of SOD, GPX and CAT of control and diabetic rats 21 days after administration of 500mg/kg body weight of root extracts A. difformis.*

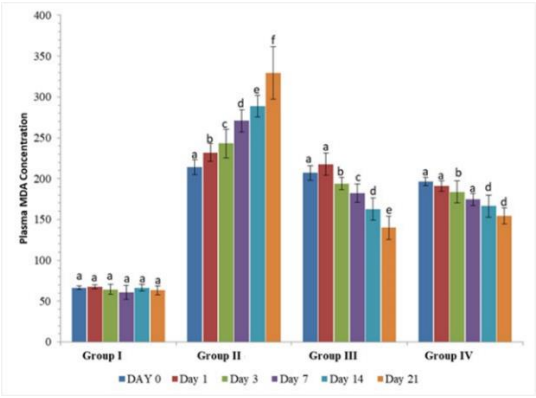

Figure 2*: Mean plasma levels of MDA of controls and diabetic rats 21 days after administration of 500mg/kg body weight of root extracts of A. difformis.*

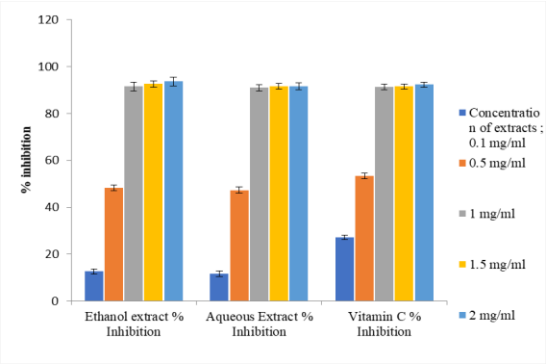

Figure 3: *Antioxidant capacity of the root extracts of Anchomanes difformis using DPPH.*

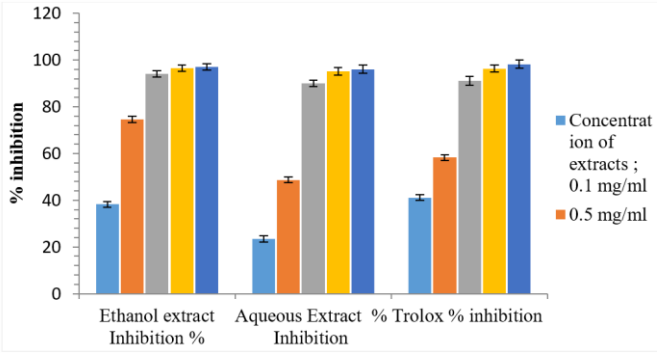

Figure 4*: ABTS Radical Scavenging Assay of Root Extract of Achomanes difformis.*

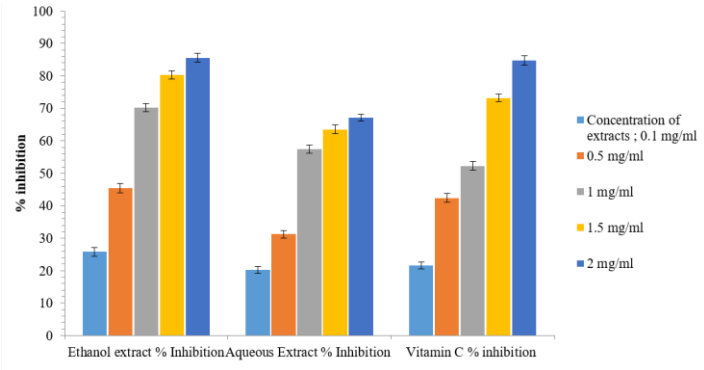

Figure 5*: Hydroxyl Radical Scavenging Activity of Root Extract of Anchomanes difformis.*

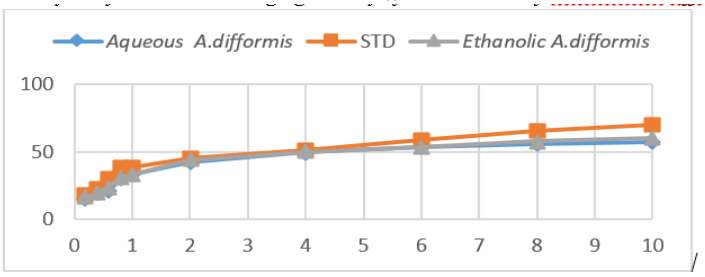

***Figure 6**: α- amylase inhibition activity of ethanol and aqueous root extracts of Anchomanes difformis and the Control, Acarbose.*

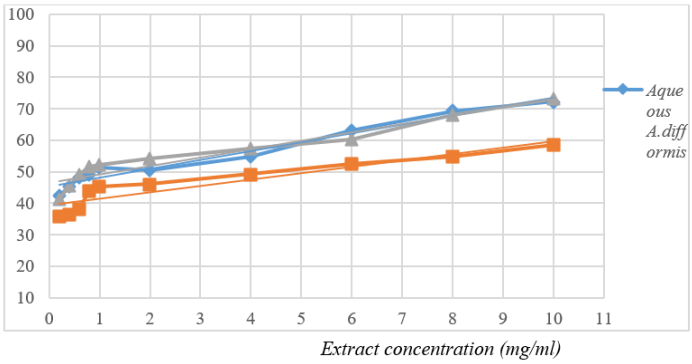

***Figure 7**: α-glucosidase inhibition activity of ethanol and aqueous root extracts of Anchomanes difformis and the Control (Acarbose).*

The scavenging capacity of the extracts and standard (Trolox) increased with an increase in concentrations. The % inhibition of the standard was 98.1±0.71 at 2.0mg/ml concentration while the % inhibition for ethanol and aqueous extracts were 97.1±0.30 and 96.10±0.70 respectively at same concentration. In Figure 5, both ethanol and aqueous extracts showed strong concentration-dependent scavenging abilities for hydroxyl radicals. The ethanol extract was the most powerful radical scavenger of the hydroxyl radicals, with an inhibition of 85.60% at a concentration of 2-0mg/ml while the aqueous extract demonstrated moderate scavenging abilities (inhibition of up to 67.11% at 2.0mg/ml concentration) when compared to the standard (vitamin C) which exhibited radical scavenging abilities (inhibition) of 84.71% at 2.0mg/ml concentration.

In-Vitro Inhibition of Carbohydrate Digestive Enzymes

In Figure 6, moderate inhibition of alpha amylase activity was observed with IC50 of 5.911 and 6.259 for ethanol and aqueous extracts respectively when compared with the control (acarbose) with IC50 of 4.571. Figure 7 showed that both ethanol and aqueous root extracts of A. difformis significantly inhibited alpha glucosidase activity in a concentration dependent manner as shown by the lower IC50 of 1.323 and 1.656 respectively and better inhibitory activity when compared to the control, Acarbose with IC50 of 5.226. Aqueous A. difformis (IC50=6.259), Ethanol A. difformis (IC50=5.911) and Control (IC50=4.571). Aqueous A. difformis (IC50=1.656), Ethanol A. difformis (IC50=1.323) and Control (IC50=5.226)

Discussion

In our study, we discovered that the extracts of A. difformis exhibited significant increases in percentage inhibition that are dose-dependent in their ability to scavenge free radicals. However, the ethanol extract exhibited higher DPPH radical scavenging activity (inhibition) than aqueous extract. This result is at variance to those of Faleye et al [10], who noted a weak antioxidant potential of A. difformis root extract. We also noted that the ethanol and aqueous extracts of A. difformis for ABTS radicals had greater scavenging power when concentrations were raised. The extracts' anti-radical qualities compared favourably with the standard. This result is consistent with a study by Sivagamasundari. [38], which found that the leaf extract of Capparis zeylanica had similar effects on free radical scavenging and antioxidant activity. The flavonoids and phenolic content in the tuber of A. difformis may be responsible for the plant extracts' ability to scavenge DPPH and ABTS radicals. According to reports, one of the main groups of chemicals serving as principal antioxidant free radical terminator or scavenger are plant phenolics [39]. Both ethanol and aqueous extracts exhibit potent, concentration-dependent hydroxyl radical scavenging properties. Ethanol extract demonstrated stronger ability to scavenge hydroxyl radicals than aqueous extract. According to the study's findings, the ethanol root extract of A. difformis has outstanding hydroxyl radical scavenging properties. Since hydroxyl radicals are extremely reactive oxygen species, the human body lacks an enzyme that can specifically defend against them. This study demonstrates that the plant's ethanol extract has potent and excellent hydroxyl radical scavenging properties, making it a potent lipid peroxidation chain reaction terminator.

The inhibition of the activity of the enzymes that break down carbohydrates (alpha-amylase and alpha-glucosidase) and delaying the absorption of carbohydrates from the small intestine are two of the principal treatments for diabetes mellitus, especially type 2 [40]. The study's findings, demonstrated that A. difformis root extracts in both ethanol and aqueous forms, significantly inhibited alpha glucosidase activity in a concentration-dependent manner. This is demonstrated by the lower IC50 values and by the extracts' superior inhibitory activity when compared to the standard, acarbose. Moderate suppression of alpha amylase activity was noted in ethanol and aqueous extracts. This finding is consistent with that of Faleye, [10] who found that the alpha amylase activity was mildly inhibited by root extracts of A. difformis. Diabetes mellitus is commonly managed with acarbose. It can prevent the conversion of polysaccharides to monosaccharides by gastro-intestinal glucosidase, which delays the absorption of glucose and reduces postprandial hyperglycaemia. Alpha glucosidase is strongly inhibited by the ethanol and aqueous extracts of A. difformis, but very slightly by alpha amylase. The presence of flavonoids and phenolic compounds in root extracts, as well as their robust capacity to scavenge free radicals as shown by the DPPH, ABTS, and Hydroxyl radical scavenging assays, may be responsible for the inhibitory impact. In their respective research, Nemzer, [41], and Proença, [42] highlighted those flavonoids and phenolic compounds are efficient inhibitors of alpha glucosidase and alpha

amylase activities. So, with minimal to no adverse effects, the root extract of A. difformis could be employed as an anti-diabetic medication to treat postprandial hyperglycaemia. This result is consistent with a study by Etsassala, [43], which found that an ethanol extract of Orthosiphontamineus had similar effects on the activities of alpha glucosidase and alpha amylase. We also noted in this study that treatment of diabetic rats with A. difformis extracts both aqueous and ethanol, significantly ($p<0.05$) improved the activities of the antioxidant enzymes SOD, GPX and CAT when compared to the untreated diabetic rats. Reactive oxygen species (ROS) may be the cause for the decreased SOD and CAT activities in the diabetic control Group II. In diabetes mellitus, hyperglycaemia can easily inactivate antioxidant enzymes like SOD, CAT, and GPX by glycating these proteins and causing oxidative stress, which in turn leads to lipid peroxidation [44,45]. Decreased levels of antioxidant enzymes and increased levels of lipid peroxidation have been well documented in induced diabetes. In the enzymatic antioxidant defence system, SOD is one of the key enzymes that scavenges superoxide radicals by converting them to H2O2 and molecular oxygen. The observed decrease in SOD activity in Group II diabetic control rats could be the result of the inactivation by H2O2 or glycosylation of the enzymes which have been reported to occur in diabetes. H2O2 is eliminated by GPX and CAT [46,47]. The enhanced CAT activity in treatment Groups III and IV may be related to increased H2O2 generation. It is possible that increased CAT activity which in turn would protect SOD inactivation by H2O2, would cause an increase in SOD activity. Increase in SOD activity would protect GPX and CAT against inactivation by superoxide anions [48,49]. The decrease in antioxidant enzyme activity in diabetic control Group II and an increase in antioxidant enzyme activity in the treatment Groups obtained in this study, are consistent with the reports of Zhang [46] and Amevor [47].

The plasma malondialdehyde (MDA), a by-product of fatty acid peroxidation and an index of assessing oxidative damage, is used to detect lipid peroxidation. In this study, we observed that the plasma MDA levels in the diabetic control rats increased gradually and significantly ($p<0.05$) from day 0 to day 21 when compared to the normal control rats, which exhibited no significant increase ($p>0.05$). This result is consistent with the findings of Omolaoye [50], who in their investigation found that diabetic rats had significantly higher MDA levels. MDA concentrations were significantly ($p<0.05$) reduced after STZ-induced diabetic rats in Groups III and IV were given aqueous and ethanol extracts of A. difformis. This study's observation of lower MDA levels is a result of decreased lipid peroxidation. Reactive oxygen species (ROS) are produced during hyperglycaemia, which leads to oxidative damage and the emergence of diabetic complications [51]. MDA and other extremely reactive aldehydes are produced when lipids are peroxidised. MDA has been shown to be a key indicator of oxidative stress and lipid damage caused by free radicals [37]. Significant alterations in lipid metabolism and structure have been linked to diabetes, particularly in patients with vascular complications, according to Eid, [52]. The development of diabetes complications may be influenced by peroxidative injury, according to the increased level of MDA in diabetics. As seen in diabetic control (Group II), the rise in lipid peroxidation may also be a sign of decline in the defence mechanism of enzymatic and non-enzymatic antioxidants. It has been noted that diabetic subjects have plasma or serum elevated MDA levels [53,54]. According to Ito, [55], lipid peroxidation in diabetics causes a variety of secondary complications, such as arteriosclerosis and brain diseases. In hyperglycemic mice, Li, [56], found increased lipid peroxidation measured by MDA and connected it to the development of myocardial infarction. As a result of increased lipid peroxidation by ROS in the ocular membrane system, diabetic retinopathy and cataracts are the most common causes of irreversible micro-vascular complications [57,58]. The aqueous and ethanol extracts free radical scavenging potentials of this plant, as well as the phytochemical components of A. difformis, whose antioxidant effects contribute to protecting membrane lipid against peroxidation, may be responsible for the reduction in MDA levels after extracts of the root of A. difformis were administered to diabetic rats in Groups III and IV. According to multiple studies [50,59,60], glucose-lowering herbs decreased MDA levels in STZ or Alloxan-induced diabetic rats. By giving this plant extract to diabetic rats, it may lessen oxidative damage to organs, particularly pancreatic cells, so improving those cells' functioning and defending against diabetic complications.

Conclusion

This study shows that Anchomanes difformis tuber extracts have anti-diabetic properties. The plant extract's ability to suppress the activities of the carbohydrate-digesting enzymes (α-amylase and α-glucosidase) is also responsible for the plant's anti-diabetic potentials. According to the study, the plant's root extract is effective at scavenging free radicals, terminating lipid peroxidation, and quenching reactive oxygen species (ROS), which are linked to the pathophysiology of numerous diseases.

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Disclosure of Conflict of Interest

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Availability of Data and Materials

The authors declare consent for all available data present in this study.

Authors' Contribution

The entire study procedure was conducted with the involvement of all writers.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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