



# COMPARATIVE STUDIES OF THE PHYTOCHEMICAL, ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF ETHANOL EXTRACTS FROM THE STEM, LEAVES AND TUBERS OF *Anchomanes difformis*



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**Abstract:** The phytochemical composition, antioxidant and antidiabetic activities of ethanol extract of the stem, leaves and tuber of *A. difformis*, were studied. The antioxidant contents were determined using Ferric-ion Reducing Antioxidant Power (FRAP), Total Antioxidant Capacity (TAC), and Trolox Equivalent Absorbance Capacity (TEAC) assays. The *in vitro* antidiabetic activities were determined spectrophotometrically, by measuring the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. The phytochemical results showed that the stem, leaves and tuber of *A. difformis* contain alkaloids, tannins, saponins, and flavonoids. Steroid was found only in the tuber while cardiac glycosides, anthraquinones and phlobatannins were absent in all the samples. The leaves, stem and tuber of *A. difformis* have antioxidant potentials with the highest antioxidant potential present in the tuber; FRAP ( $291.62 \pm 6.45$  mg/100g), TAC ( $381.00 \pm 7.22$  mg/100g), and TEAC ( $59.78 \pm 6.50$  mg/60g). The antidiabetic results revealed that the stem, leaves and tuber of *A. difformis* inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes *in vitro* but the stem possessed the highest antidiabetic potentials. This research provides evidence that *A. difformis* extracts could be a potential source of natural antioxidant and antidiabetic that may be used to manage oxidative stress and diabetes. The results from this research further indicated that the tuber would be a better antioxidant, while the stem may be more useful as an antidiabetic agent. The phytochemicals present support the extensive ethno-medicinal usage of the leaves, stem and tuber of *A. difformis*.

**Keywords:** *Anchomanes difformis*, antioxidant, diabetes,  $\alpha$ -amylase,  $\alpha$ -glucosidase, ROS, RNS

## Introduction

Medicinal plants remain the primary form of medicine in most countries. A very large number of the earth's population depends primarily on raw plant materials for their daily health care needs (Barrett and Kieffer, 2001). Most of the plant materials are used freshly, to obtain the extract from the whole plant or parts of it, which could be leaves, roots, flowers or fruit. In case of the woody forms, mostly the bark, roots, stems are used. Medicinal plants may be produced for immediate consumption or as basis for herbal products. It usually has some chemical substances that produce a definite physiological action in the human and animal body. The therapeutic values of these plants have been linked to their biologically active secondary metabolites such as flavonoids, alkaloids, tannins, polyphenols, saponins, etc. These bioactive agents have been reported to possess diverse biological roles in combating various diseases (Okwu, 2005).

Oxidative stress describes a physiological state in which the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) attains unbalanced levels, either by excess production or reduced removal due to the overwhelming antioxidant capacity of the system (Chonthida *et al.*, 2013). These highly reactive molecules are products of normal cellular metabolism, and they play crucial roles in most signaling pathways. The mitochondrion in cells is the site where most of these highly reactive species are generated (Chonthida *et al.*, 2013).

Diabetes mellitus is a metabolic disorder depicted by hyperglycemia (elevated levels of blood glucose) and glucose intolerance, which brings about defects of insulin secretion or insulin's action to boost glucose uptake (Cho *et al.*, 2018). This disorder causes a burden worldwide because of its high rate of morbidity, mortality, and higher health costs for management and treatment. Report of International Diabetes Federation (IDF, 2017), stated that about 451 million adults were reported to be living with diabetes worldwide. Also a prediction of about 693 million cases was projected to occur by 2045. On a global level, this disorder is prevalent more in the low-income and middle-income countries with almost 50% of the cases undiagnosed. In Africa, there is a high

incidence of undiagnosed cases of diabetes (69.2%) with 73.7% of all deaths due to diabetes occurring before the age of 60, thus showing the extent to which diabetes is destroying the labour force population (Cho *et al.*, 2018).

*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, Senegal and Togo (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 (Burkill, 1994). Quantitative and qualitative analyses of *A. difformis* have shown the significant presence of proximate, minerals and phytochemical constituents: carbohydrates, crude proteins, fats, fibres, calcium, magnesium, manganese, copper, iron, zinc, alkaloids, saponins, flavonoids and steroids. These phytochemical constituents may be responsible for the many functions ascribed to *A. difformis* (Egwurugwu *et al.*, 2016).

The various medical uses of *A. difformis* include antibacterial, anti-inflammatory, analgesic and hypothermic effects, diuresis and purgative, anti-hemolytic and anti-oxidant effects, anti-diabetic, anti-filariasis, insecticidal and antidiarrheal properties (Egwurugwu *et al.*, 2016). 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 (Chonthida *et al.*, 2013; Faleye *et al.*, 2018). Our previous report on the plant had demonstrated a moderate antidiabetic activity of *A. difformis* tuber extract (Faleye *et al.*, 2018); then, it was observed that the percentage yield was low in methanol, therefore, ethanol was chosen as the extraction solvent to compare the various plant parts for antioxidant and antidiabetic activities.

## Materials and Method

### *Plant collection, identification, preparation and extraction*

The plant materials were collected from a farm at Ikere-Ekiti and identified at the herbarium section of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-

Ekiti by Mr. Femi Omotayo. The leaves and the stems collected, were washed and air-dried at room temperature to constant weight; after which they were blended and extracted with 100% ethanol for 72 h. The tubers were peeled, washed, pulverized, dried, blended and extracted with 100% ethanol for 72 h (Faleye *et al.*, 2018). The crude extracts were obtained by filtration followed by evaporation using rotatory evaporator at 40°C. The extracts obtained were kept in air tight vials and refrigerated pending analyses.

#### Phytochemical analysis

The phytochemical analysis of the ethanol extracts of the leaves, stems and tubers of *A. difformis* were performed using the methods described by Harborne (1998), Evans (2002) and Sofowora (2008) with slight modifications. The analysis was carried out in triplicate.

#### Test for Flavonoids

1.0 g of each extract was dissolved with 5 cm<sup>3</sup> of distilled water in a test tube and 2.0 cm<sup>3</sup> of 10% (w/v) sodium hydroxide solution was added. The formation of yellow coloured solution indicates the presence of flavonoids (Evans, 2002).

#### Test for saponins

1.0 g of each extract was dissolved with 5.0 cm<sup>3</sup> distilled water in a test tube and shaken vigorously for about 1 min. The formation of a persisting honey comb indicates the presence of saponins (Evans, 2002).

#### Test for alkaloids

1.0 g of each extract was dissolved with 5.0 cm<sup>3</sup> distilled water in a test tube and 10.0 cm<sup>3</sup> of 1% HCl was added followed by the addition of two drops of Wagner's reagent (solution of iodine in potassium iodide). The formation of amorphous or crystalline precipitates or coloured precipitate indicates the presence of alkaloids (Sofowora, 2008).

#### Test for steroids

0.5 g of each extract was mixed with 2.0 cm<sup>3</sup> of acetic anhydride and shaken vigorously, followed by 2.0 cm<sup>3</sup> of H<sub>2</sub>SO<sub>4</sub>. The formation of violet to blue or green colour indicated the presence of steroids (Sofowora, 2008).

#### Test for phlobatannins

0.5 g of each extract was boiled with 1% aqueous HCl. The formation of red precipitate indicated the presence of phlobatannins (Harbone, 1998).

#### Test for tannins

1.0 g of each extract was dissolved with 5 cm<sup>3</sup> distilled water in a test tube and 2 drops of 10% ferric chloride was added. Instant formation of blue-black coloured solution indicates the presence of tannins (Evans, 2002).

#### Test for cardiac glycosides (Keller-Kiliani's test)

1.0 g of each extracts was dissolved with 5 cm<sup>3</sup> distilled water in a test tube and 2.0 cm<sup>3</sup> of glacial acetic acid containing 1 drop of 10% ferric chloride solution was added with 1.0 cm<sup>3</sup> concentrated sulphuric acid added down the wall of the test tube to form a layer underneath. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides, indicative of cardiac glycosides (Harborne, 1998).

#### Test for anthraquinones

1.0 g of each extract was shaken with 10.0 cm<sup>3</sup> of benzene. The mixture was filtered and 5.0 cm<sup>3</sup> of 10% ammonia were added, then shaken and observed. A pinkish solution indicates a positive test (Evans, 2002).

#### Antioxidant assays

##### Ferric-ion reducing antioxidant power (FRAP) assay

Ferric ions reducing power was measured according to the method of Oyaizu (1986) with a slightest modification. The ethanol extract of the leaves, stems and tubers of *A. difformis* in different concentrations ranging from 100 to 500 µl were mixed with 2.5 cm<sup>3</sup> of 20 mM phosphate buffer and 2.5 cm<sup>3</sup> 1%, w/v potassium ferricyanide, and then the mixture was

incubated at 50°C for 30 min. Afterwards, 2.5 cm<sup>3</sup> of 10%, w/v trichloroacetic acid and 0.5 cm<sup>3</sup> 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate and averaged (Oyaizu, 1986).

##### Total antioxidant capacity (TAC) assay

Molybdate reagent was prepared by measuring 1.0 cm<sup>3</sup> of 0.6 mol/dm<sup>3</sup> sulphuric acid, weighing 28 mM (better to use mole) sodium phosphate and 4 mM ammonium molybdate into 20 cm<sup>3</sup> distilled water in 50 cm<sup>3</sup> volumetric flask and made it up to the mark. The extracts of *A. difformis* in different concentration ranging from 100 to 500 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard (Prieto *et al.*, 1999).

##### Trolox equivalent absorbance capacity (TEAC) assay

TEAC was done according to the methods previously described by Pellegrini *et al.* (1999) and Re *et al.* (1999). The stock solutions which contains 7 mM ABTS (2,2'-Azino-bis (3-ethylbenzo thiazoline-6-sulphonic acid) (diammonium salt) and 140 mM potassium-peroxodisulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) was prepared and kept at -2°C. The working solution was then prepared by adding 88 µL K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution to 5 mL ABTS solution. The two solutions were well mixed and allowed to react for 24 hours at room temperature in the dark.

Trolox (6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard with concentrations ranging between 0 and 500 µM. After 24 h, the ABTS mixture was diluted with ethanol to read a start-up absorbance (control) of approximately 2.0 (± 0.1). The ethanol extracts of the leaves, stems and tuber of *A. difformis* were allowed to react with 300 µL ABTS in the dark at room temperature for 30 min. The absorbance was read at 734 nm at 25°C in the plate reader. The results were expressed as µM Trolox equivalents per milligram dry weight (µM TE/g) of the test samples.

##### Anti-diabetic screening

##### Determination of α-glucosidase inhibitory activity

The inhibitory effect of the ethanol extract of the leaves, stems and tubers of *A. difformis* on α-glucosidase activity was determined according to the chromogenic method described by Faleye *et al.* (2018). 5 units of α-glucosidase were pre-incubated with 20 µg/ml of the ethanol extract of the leaves, stems and tubers of *A. difformis* for 15 min. Paranitrophenylglucopyranoside (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 0.1M Na<sub>2</sub>CO<sub>3</sub>. The α-glucosidase activity was determined by measuring the yellow coloured p-nitrophenol released from PNPG at 400 nm. Each test was performed in triplicates and the mean absorption was used to calculate percentage α-glucosidase inhibition.

$$\% \alpha\text{-glucosidase inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \dots\dots\dots \text{Eqn 1}$$

Where A<sub>0</sub> = the absorbance of the control (blank without extract) and A<sub>1</sub> = the absorbance in the presence of the extract. The IC<sub>50</sub> value was determined from plots of percent inhibition versus log inhibitory concentration and calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference α-glucosidase inhibitor.

##### Determination of α-amylase inhibitory activity

*In vitro* amylase inhibition was studied by Bernfeld (1955) method. 100 µL of the extracts were allowed to react with 200

μL of α-amylase enzyme (Hi media Rm 638) and 100 μL of 2 mM of phosphate buffer (pH 6.9). After 20 min incubation, 100 μL of 1% starch solution was added. The same was performed for the controls where 200 μL of the enzyme was replaced by buffer. After incubation for 5 min, 500 μL of dinitrosalicylic acid reagent was added to both control and extracts. They were kept in boiling water bath for 5 min. The absorbance was recorded at 540nm and the percentage inhibition of α-amylase enzyme was calculated as:

$$\frac{A_0 - A_1}{A_0} \times 100 \dots \dots \dots \text{Equation 2}$$

Where A<sub>0</sub> = absorbance of the control (blank without extract) and A<sub>1</sub> = absorbance in the presence of the extract. The IC<sub>50</sub> value was determined from plots of percent inhibition versus log inhibitory concentration and calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference α-amylase inhibitor. All tests were carried out in triplicate (Bernfeld, 1955).

**Statistical analysis**

The results were expressed as mean values and standard deviation (SD) of three replicates. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with α=0.05. This treatment was carried out using SPSS v.16.0 (Statistical Program for Social Sciences) software.

**Results and Discussion**

**Determination of phytochemicals present in the ethanol extracts of the stem, leaves and tuber of *A. difformis***

Table 1 shows the qualitative analysis of the phytochemical constituents of the stem, leaves and tuber of *A. difformis*. It shows that the stem, leaves and tuber of *A. difformis* contain alkaloids, tannins, saponins and flavonoids. Steriod is only present in the tuber and not present in the leaves and stem. Cardiac glycosides, anthraquinones and phlobatannins were absent in all the samples.

Flavonoids and saponins have been reported to possess antioxidants, hepato-protective and anti-inflammatory activities and are used as antimicrobial, anticancer and antiallergic remedies. Flavonoids have also been suggested to play a protective role in liver diseases, cataracts and cardiovascular diseases (Tapas *et al.*, 2008). Tannins are a diverse group of polyphenols. Condensed tannins (syn. Proanthocyanidins), gallotannins and ellagitannins are the most widely occurring tannins. Procyanidins are particularly abundant in the human diet and are responsible for the sensation of astringency (drying and puckering of the oral mucosa) by interacting with salivary proteins and found potent to act as α-amylase inhibitors (Goncalves *et al.*, 2011). Condensed tannins have been demonstrated to exhibit numerous biological and pharmacological activities that are of interest in human and veterinary medicine such as inhibition of lipid oxidation, antioxidant, anti-viral, mutagenicity of carcinogens and tumor promotion (Amarowicz *et al.*, 2010).

Plant sterols (phytosterols) which chemically resemble cholesterol have the ability to block the absorption of dietary and endogenously derived cholesterol from the gut. They are not synthesized by the human body and are minimally absorbed by the human intestine. Phytosterols are bioactive components of all vegetable foods. They are 28- or 29-carbon alcohols and resemble cholesterol in vertebrates in terms of both functions (stabilization of phospholipid bilayers in plant cell membranes) and structure (steroid nucleus, 3β-hydroxyl group, 5, 6 double bonds) (Goncalves *et al.*, 2011).

**Table 1: Phytochemicals present in the ethanol extracts of the stem, leaves and tuber of *A. difformis***

Phytochemicals	Tuber	Leaves	Stem
Alkaloids	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phlobatannins	-	-	-
Steriods	+	-	-

+ = Present - = Absent

**Evaluation of the anti-oxidant activity of the ethanol extracts of the stem, leaves and tuber of *A. difformis***

The reducing capacity of antioxidant was coined in a single measure as "Total Antioxidant Capacity" (TAC). TAC of the ethanol extracts of the stem, leaves and tuber of *A. difformis* was estimated based on its reducing capacity by different methods such as FRAP, TEAC, and TACassays. Absorbance reflects directly to the reducing power in the methods of FRAP, TEAC, and TAC (McCord, 2000). Antioxidant capacity assays may be broadly classified as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. Majorities of HAT assays are kinetics based and involve a competitive reaction scheme in which antioxidant and substrate compete for free radicals thermally generated through the decomposition of azo compounds. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. SET assays are easier than HAT assays. SET assays like TAC, TEAC, and FRAP were selected to analyze the reduction capacity. These methods are involving the mechanism of single electron transfer system. In this system electron from oxidized antioxidant transferred to the substrate by inhibiting oxidation of oxidant. FRAP, TEAC and TAC methods are based on the redox antioxidant reaction (McCord, 2000).

**Ferric-ion reducing antioxidant power (FRAP) assay**

FRAP measures the ferric oxide reducing potency of extracts. Higher absorbance indicates higher reducing potency. Table 2 shows that the tuber extract has the highest absorbance indicating that it has the highest ferric oxide reducing potency. There was a significant difference (p < 0.05) between the absorbance of the extracts of the stem, leaves and tuber. The stem had the least ferric oxide reducing potency (25.37 ± 3.21 mg/100g) and the highest ferric oxide reducing potency was found in the tuber (291.62 ± 6.45 mg/100g), hence, the tuber is adjudged to have the best antioxidant activity.

**Table 2: Ferric ion reducing antioxidant power (FRAP) capacities of ethanol extracts of the stem, leaves and tuber of *A. difformis***

Sample	FRAP (Mean ± S.D) mg/100g
Stem	25.37 ± 3.21
Leaves	62.72 ± 4.50
Tuber	291.62 ± 6.45

**Table 3: Total antioxidant capacities (TAC) of the ethanol extracts of the Stem, leaves and tuber of *A. difformis***

Sample	TAC (Mean ± S.D) mg/100g
Stem	176.00 ± 0.95
Leaves	218.34 ± 5.93
Tuber	381.00 ± 7.22

**Total antioxidant capacity (TAC) assay**

TAC assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of a bluish green coloured phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is routinely applied in

the laboratory to evaluate the total antioxidant capacity of plant extracts. Table 3 shows that the tuber extract has the highest absorbance indicating that it has the highest antioxidant potency.

There was a significant difference ( $p < 0.05$ ) between the reducing potency of the stem ( $176.00 \pm 0.95$  mg/100g) and the tuber ( $381.00 \pm 7.22$  mg/100g); this is an indication that the tuber possessed the best antioxidant activity. However, no significant difference ( $p < 0.05$ ) was observed between the reducing potency of the leaves compared to the stem and tuber.

**Trolox equivalent absorbance capacity (TEAC) assay of ethanol extracts of the stem, leaves and tuber of *A. difformis***

This assay involves the direct production of the blue/green ABTS<sup>+</sup> chromophore from the reduction of ABTS. Fig. 1 shows the absorbance and the reducing potency of the stem, leaves and tuber of *A. difformis* at different concentrations. There was no significant difference ( $p < 0.05$ ) between the absorbance of the stem, leaves and tuber at different concentrations indicating that there was no significant difference between the reducing potency of the stem, leaves and tuber but there was a significant difference in the absorbance/reducing potency of the stem, leaves and tuber as compared with the standard (vitamin C), which implies that the tuber may be more useful as an antioxidant.

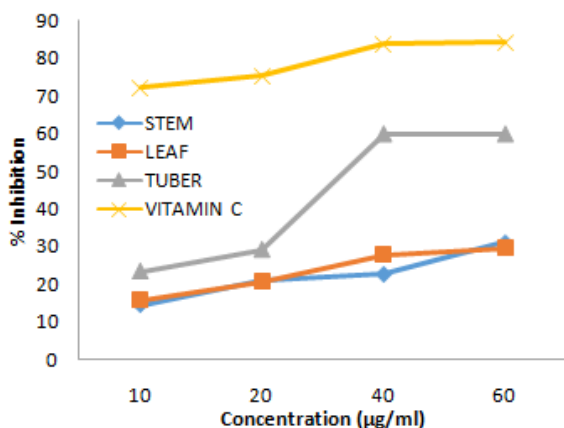


Fig. 1: Trolox equivalent absorbance capacity (TEAC) of the ethanol extracts of the stem, leaves and tuber of *A. difformis*, vitamin C was used as reference(values are expressed as mean  $\pm$  SD, n=3)

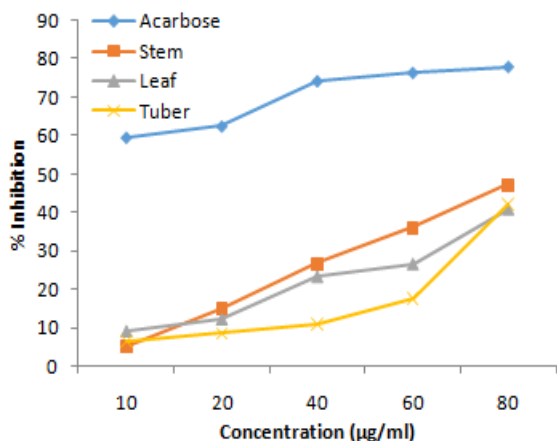


Fig. 2: % inhibition of  $\alpha$ -glucosidase enzyme by ethanol extract of the stem, leaves and tuber of *A. difformis*and reference Acarbose (values are expressed as mean  $\pm$  SD, n=3)

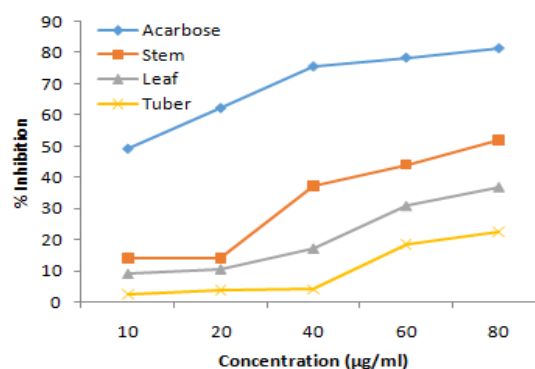


Fig. 3: % inhibition of  $\alpha$ -amylase enzyme by ethanol extract of the stem, leaves and tuber of *A. difformis*and reference Acarbose (values are expressed as mean  $\pm$  SD, n=3)

**Evaluation of the anti-diabetic property of the ethanol extracts of the stem, leaves and tuber of *A. difformis***

Diabetic mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin. A therapeutic approach to treat diabetes is therefore to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase (Evans, 2002). Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha amylase is involved in the breakdown of long chain carbohydrates while alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (Tapas *et al.*, 2008). Natural products from plants have been used for the treatment of diabetes for a long time especially in developing countries where the resources are limited and affordability and access to modern treatment is a problem. Extensive research has been carried out to screen the bioactivity of inhibitors because of their significant importance in health care and medicine. Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues. Studies have also shown that the bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycemic properties (Goncalves *et al.*, 2011). In animals, alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Hence, diabetes tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defense mechanism against insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behaviour. Therefore, alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection (Amarowicz *et al.*, 2010). Alpha glucosidase inhibitors are used as antidiabetic drugs for treating type 2 diabetes mellitus. They act by preventing the digestion of carbohydrates such as starch. Carbohydrates are normally converted into simple sugars which can be absorbed through the intestine. Intestinal alpha glucosidases hydrolyze complex

carbohydrates to glucose and other monosaccharides in the small intestine. The inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates. Less amounts of glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating, etc. (Re *et al.*, 1999). Therefore, natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating postprandial hyperglycemia with minimal side effects.

This study was carried out to investigate the inhibitory potentials of the ethanol extracts of the stem, leaves and tuber of *A. difformis* on alpha amylase and alpha glucosidase which are the key enzymes responsible for carbohydrate hydrolysis. The percentage  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition of the ethanol extracts of the stem, leaves and tuber of *A. difformis* were plotted as a function of concentrations in comparison with Acarbose as shown in Figs. 2 and 3. The results revealed that the ethanol extracts of the stem, leaves and tuber of *A. difformis* inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme *in vitro*. The highest activity was observed in the stem while the least was in the tuber. This is typically a reversal of what was obtainable in the antioxidant activity. Although, this is not in accordance with the general assertion that the bioactivity of polyphenols in plants may be linked to their antioxidant activity and hence, such plants may also possess hypoglycemic properties (Goncalves *et al.*, 2011). Our result is in agreement with findings by Dehghan *et al.* (2016), where it was observed that *Convolvulus persicus* showed a very strong antioxidant activity and was not listed among the plants that exhibited a very strong antidiabetic activity using  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition assays. The present study indicates that ethanol extracts of the stem, leaves and tuber of *A. difformis* possess mild antioxidant activity. The stem would be considered as a better antidiabetic agent than the leaves and tuber, hence may be useful in the management of postprandial hyperglycemia. A moderate antioxidant activity has also been reported for the methanol extract of the tuber (Faley *et al.*, 2018).

### Conclusion

This study examined the comparative analysis of the antioxidant and antidiabetic activities of the ethanol extracts of the leaves, stem and tuber of *A. difformis*. The results showed that the extracts possessed moderate antioxidant and antidiabetic properties. The tuber extract had the best antioxidant activity, while the stem showed the best antidiabetic activity. This might be as a result of natural bioactive compounds such as alkaloids and flavonoids which have also been reported in this work as part of its phytochemical compositions. In addition, steroid was present only in the tuber and absent in the leaves and stem of the plant. The extracts may be used in the management of diseases such as diabetes and oxidative stress.

### Conflict of Interest

There are no conflicts of interest.

### Acknowledgement

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