South Asian Research Journal of Natural Products

Volume 7, Issue 2, Page 102-122, 2024; Article no.SARJNP.116748

In vitro Antioxidant and Biological Activities of Extract and Fractions from *Telfairia occidentalis* Stems

Godwin Ndarake Enin [a*], Basil Nse Ita [a], Blessing Jumbo [a], Mfoniso Udofot James [a], Sarah Etim Joseph [a], Bassey Sunday Antia [a], Paul Sunday Thomas [b] and Jude Efiom Okokon [c]

[a] Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Uyo, Nigeria.
[b] Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author GNE designed the study and wrote the manuscript. Authors MUJ, SEJ and BJ performed the laboratory work. Authors JEO, PST and BNI analysed the data. Authors BSA and BNI edit the manuscript. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/116748

Original Research Article

Received: 26/02/2024
Accepted: 30/04/2024
Published: 04/05/2024

ABSTRACT

Telfairia occidentalis (TO) Hook is a widely consumed vegetable in Nigeria. This study investigated the antioxidant and antidiabetic activities of stem ethanol extract (ETTO) and fractions (hexane, HTO; dichloromethane, DTO; ethylacetate, ETO; methanol, MTO and aqueous, Aq.TO) from *Telfairia occidentalis* by measuring the scavenging activity, reducing power, total phenolic content (TPC), total flavonoid content (TFC), and the *in vivo* alpha amylase and glucosidase inhibitory effects. The ethanol stem extract exhibited significant ($p < 0.05$) reduction in

**Corresponding author: Email: enin.godwin@gmail.com;*

blood glucose levels of treated rats. ETO exhibited the highest radical scavenging (EC_{50} = 116.256 µg/mL) and reducing (EC_{50} = 55.218 µg/mL) activity. Contents of total phenolics and flavonoids were highest in ETO (22.17 mg GAE/ g and 129.56 RE/ g respectively), while HTO showed the lowest radical scavenging and reducing activity (EC_{50} = 249.159 µg/mL and 97.781 µg/mL respectively). Gass chromatography-mass spectrum (GC-MS) analysis of the fractions revealed that saturated fatty acids, polyunsaturated fatty acids, amino acids, alcohols, amides and straight chain hydrocarbons were the major constituents. This research demonstrates that the stem extract and fractions of TO may alleviate oxidative-stress related diseases and exhibit antidiabetic activity, potentially through the inhibition of alpha amylase and alpha glucosidase enzymes, which may be attributed to its phytochemical constituents.

Keywords: Telfairia occidentalis stem; antioxidant assay; total phenolics; flavonoids; alpha amylase and glucosidase; GC-MS.

1. INTRODUCTION

"Plants have been used for a long time now as sources of food, nutrients, shelter, medicine and nutraceuticals in rural and urban centers of the world. The sales of plant-based products have increased bountifully with annual turn-over of about 7.2%; patronage is received from over 80% of the world population, utilizing for medicinal and other purposes" [1,2]. On daily basis, new diseases and infections are discovered, spread of old ones are on the rise posing serious concerns and burden on the global health facilities and institutions [2]. Research efforts are now directed to the use of herbal medicines for the treatment and management of these diseases because of their low cost, high efficacy, nonnarcotic nature, and lesser side effects [3]. Although massive progress has been made in this area, a continuous search for therapeutically active substances is inevitable as the world population continues to grow. *Cucurbitaceae* family of herbs is one of the herbs-family with documented history in phytotherapy [4].

Telfairia occidentalis Hook is a member of the *Cucurbitaceae* family [5]. It is a popular Nigerian vegetable, especially in the Niger-Delta and the Eastern regions of the country; the leaves, stem and seeds are used to prepare different kinds of meals [6]. "The seeds are very nutritious and are eaten roasted or boiled. Leaves are good sources of proteins, vitamins (B-complex), minerals, fatty acids (linoleic and oleic acids), and fibers" [7]. Biological activities reported in the seeds and leaves include: haematological [8], antidiabetic [9], anticancer [10], anti-inflammatory [11] and anti-plasmodial [4]. "The median lethal dose of the seed extract was estimated to be 3.46g/kg" [12]. "Phytochemical studies of the root extracts have shown the presence of alkaloids, flavonoids, tannins, terpenes, saponins, and cardiac glycosides" [13]. "Palmitoleic acid (16.62 %) and elaidic acid (0.85 %) were the major omega 9 fatty acids reported in hexane leaf extract" [14]. "Bioactive α-amyrin and β-amyrin (Figs 1 and 2) have also been reported from the fruit pericarp of the plant" [15]. "Anti-sickling activities of 78.84 ± 1.34% and 95.4 ± 0.81% reversal have been reported in the ethanol leaf extract" [16]. "Defatted ethanol leaf extract inhibits phenyl hydrazine-induced anaemia" [17]. Despite the reported studies on the seeds and leaves of *T. occidentalis*, records available show that there is paucity of information on the antioxidant and biological activities of the stems of this plant. In this study, we investigated the chemical composition, antioxidant, alpha amylase and alpha glucosidase inhibition activities of the extract and fractions of *T. occidentalis* stems.

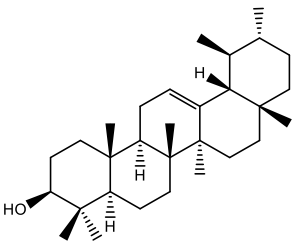


Fig. 1. α-amyrin

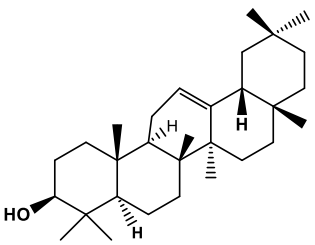


Fig. 2. β-amyrin

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

The materials used include *T. occidentalis* powdered stems, oral gastric gavage, weighing balance, gloves, scissors, glucometer and strips (fine test), distilled water, acarbose (Aldrich sigma, USA; standard drug), stirrer, beakers, 1mL syringe, starch, sucrose, maltose (Aldrich sigma, USA).

2.2 Plant Collection, identification and authentication

Fresh plant of *T. occidentalis* were harvested from a farmland in Mkpat Enin L.G.A of Akwa Ibom State, Nigeria, in June 2023. The plant was identified and authenticated by Professor Margaret E. Bassey of the Department of Botany and Ecological Study, University of Uyo, Uyo, Nigeria. Herbarium specimen (UUH 4413) of the sample was deposited in the Faculty of Science Herbarium.

2.3 Preparation and Extraction of Sample

T. occidentalis stems were carefully detached from the leaf stalk, chopped into smaller sizes, washed with clean water and dried under shade for two weeks. This was further reduced to powdered form using laboratory mill. Exactly 600 g of the powdered sample was weighed into an extraction jar and macerated in 4500 mL of ethanol. After 72 hours of intermittent shaking, the solution was filtered and the filtrate was concentrated to obtain the ethanol crude extract.

2.3.1 Fractionation of samples

Fractionation was carried out using Vacuum Liquid Chromatography technique as described by Pelletier et al. [18]. One hundred and twenty-six grammes (126 g) of the extract was carefully introduced on to the packed column and was successively eluted with hexane, dichloromethane, ethyl acetate, methanol, and water to obtain their respective fractions. Each fraction was re-filtered and the filtrates were concentrated to obtain the respective fractions.

2.4 Animals

Albino Wistar rats (120 -135 g) of either sex maintained at the Animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria were used for the study. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Approval for animal studies protocols were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/AE/21/023).

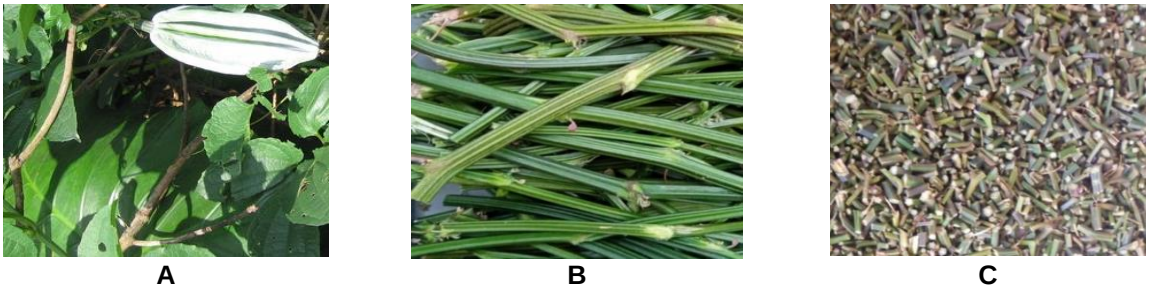
2.5 Phytochemical Screening

2.5.1 Qualitative phytochemical screening

Tests for the presence of saponins, tannins, flavonoids, alkaloids and anthraquinones were conducted according to standard procedures [19,20].

2.5.2 Total phenolic content

Total phenolic content was determined spectrophotometrically according the method of Kim et al. [21], with slight modification. Briefly, 0.5 mL (1 mg/mL) of sample was mixed with 2 mL of 10% Folin-ciocalteu reagent, 2 mL of 7% Na_2CO_3 and 2 mL of distilled were added to form a solution. The resulting mixture was left for 15 seconds and incubated at 40 °C for 30 minutes for a colour development. The absorbance of the sample was measured at 765 nm and total phenolic content was determined from gallic acid calibration curve. Results were expressed as gallic acid equivalents per 100g of dry weight (mg GAE/100 g).



A B C

Fig. 3. (A) *T. occidentalis* plant, (B) Detached stem and (C) Chopped stems

2.5.3 Total Flavonoid Content

The total flavonoid content was obtained using the procedure described by Subhashini et al. [22]. Sample (1 mg/mL) was diluted with 200 µL distilled water followed by the addition of 150 µL of 5% sodium nitric solution. This mixture was incubated for 5 minutes and added to 150 µL of 10% $AlCl_3.6H_2O$. Six minutes later, 2 mL of 1 M NaOH was added and made up to 5 mL with distilled water. The spectrophotometric reading was taken at 510 nm and the total flavonoid content was quantified from rutin calibration graph. Results were expressed as rutin equivalents per 100g of dry weight (mg RE/ 100 g).

2.6 Antioxidant Evaluation

The antioxidant evaluation was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric ion Reducing Antioxidant Power (FRAP) assays.

2.6.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH free radical scavenging activity of the *T. occidentalis* stem extract, fractions and ascorbic acid prepared in methanol at various concentrations (20, 40, 60, 80 and 100 µg/mL) were evaluated according to the method of Shekhar and Anju [23]. DPPH (0.1 M, 1 mL) was added to 3 mL of the solution prepared with the extract, fractions and ascorbic acid and stirred for 1 minute, each mixture was incubated in the dark for 30 minutes and the absorbance was measured at 517 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard error of mean. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.
The percentage DPPH scavenging effect was calculated as shown below:

$$\text{DPPH percentage scavenging effect} = [(A_0 - As) / A_0] \times 10$$

Where A_0 is the absorbance of control reaction, and as is the absorbance of the standard (ascorbic acid).

2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of the plant was determined using a standard procedure [24]. Various concentrations (20, 40, 60, 80, 100 µg/mL) of sample (2 mL) were mixed with a solution containing 2 mL of 0.2 M sodium phosphate buffer (pH 6.6.) and 2 mL of 1% w/v potassium ferricyanide. The mixture obtained was incubated at 50 ºC for 20 minutes, 2 mL trichloroacetic acid (10% w/v) was added and the mixture was centrifuged at 650 rpm for 10 minutes before taking the absorbance reading at 700 nm.

2.7Alpha-Amylase Inhibitory Study

“Thirty Wistar rats were divided into 6 groups of 5 rats each. The rats in all the groups were fasted for 18 h and fasting blood glucose concentration was first taken at 0 min before administration. Group I, as the normal control, received distilled water (10 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight orally with distilled water (10 mL/kg) as vehicle, simultaneously. Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Groups IV, V and VI were administered simultaneously, starch (2 g/kg) and *T. occidentalis* stem extract at 150, 300 and 450 mg/kg respectively. All administrations were done orally and blood glucose concentrations were monitored at 30, 60, 120 and 180 min” [25]. The blood glucose level was used to assess the effect of extract on the enzyme activity.

2.8 Alpha Glucosidase Inhibition Study

2.8.1 Maltase inhibition study (using maltose as substrate)

“Thirty Wistar rats were divided into 6 groups of 5 rats each. The rats in all the groups were fasted for 18 hours and fasting blood glucose concentration was first taken at 0 minutes before administration. Group I, as the normal control, received distilled water (10 mL/kg). Group II rats were orally administered starch at 2 g/kg body weight (orally with distilled water as vehicle) and distilled water (10 mL/kg) simultaneously. Rats in group III were administered starch (2 g/kg) and the standard drug (acarbose) at 100 mg/kg simultaneously. Groups IV, V and VI were administered simultaneously, maltase (2 g/kg) and *T. occidentalis* stem extract at 150, 300 and 450 respectively. All administrations were done orally and blood glucose concentrations were monitored at 30, 60, 120 and 180 minutes” [25]. The blood glucose level was used to assess the effect of extract on the enzyme activity.

2.8.2 Sucrose inhibition study (using sucrose as substrate)

"The procedure as described above was used for this study but with sucrose used as a substrate" [25,26].

2.9 Blood Glucose Determination

"Drops of blood from tip of rat tails were dropped on stripes and glucose concentrations were measured using a glucometer according to manufacturer's specifications (fine test). The glucometer works with the following principle; the blood sample is exposed to a membrane covering the reagent pad (strip), which is coated with an enzyme (glucose oxidase, glucose dehydrogenase). The reaction causes a colour change and the intensity of this change is directly proportional to the amount of glucose in the blood sample. Light from an LED strikes the pad surface and is reflected to a photodiode, which measures the light intensity and converts it to electrical signals. An electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The resulting current is directly proportional to the amount of glucose in the sample" [26].

2.10 GC-MS Analysis

A GC (Agilent 19091S-433U0) interfaced with a mass spectrometer and a capillary column (length, 30 m; thickness, 0.25 m; diameter, 0.25 mm) were employed to analyse the samples. Helium gas (99.999%) was the carrier gas at 1 mL/min and sample injection volume of 1µL was at split ratio (10:1). The oven temperature progressed from 80 °C, with an increase of 5 °C/min, to 250 °C and maintained at 325 °C. The ion source was set at 230 °C and the ionization voltage at 70 eV. Interpretation of GC-MS was conducted using the database of National Institute Standard Technology. The mass spectrum of the unknown compound was compared with the spectrum of the known compound available in the NIST library [27].

2.11 Statistical Analysis

Each test was conducted in triplicates and the results expressed as mean values and standard deviation. Data obtained were analysed statistically using One–way ANOVA followed by Tukey-Kramer multiple comparison post-hoc test using InstatR Graphpad software, (San Diego, USA). Differences between means were considered significant at $p< 0.05$ and very significant at $p < 0.001$.

3. RESULTS

3.1 Extraction Yield

Table 2 summarizes the yield (%) of extract ad fractions from *T. occidentalis* stems using solvents of varying polarities. The weight of the crude extract was 139.5 g and the percentage yield of fractions ranged from 0.01% to 3.25%. The highest and lowest yield (%) were recorded for the aqueous (3.25%) and the hexane (0.1%) fractions respectively. White amorphous powder, light green marsh and dirty-green oil were the colours and appearances of the hexane, dichloromethane and the ethyl acetate fraction respectively. The methanol and the aqueous fractions were green and dark-green viscous oil respectively while the ethanol crude extract was a dense green material.

3.2 Phytochemical Analysis

3.2.1 Qualitative phytochemical screening

The presence of alkaloids, flavonoids, saponins, and tannins were detected in the fractions. Phytochemical screening carried out on all the fractions revealed that anthraquinones were not present (Table 1).

3.2.2 Total phenolic content

The values for the phenolics ranges from 3.07 to 22.17 mg GAE/g, the ethylacetate fraction had the highest phenolic content while the lowest phenolic content was obtained from the hexane fraction (Table 2).

3.2.3 Total flavonoid content

Fig. 17 (see the appendix) showed the rutin standard calibration curve ($y = 0.0023x + 0.0318$), the total flavonoid content was represented as rutin equivalents (mg RE/g of extract). The values for the TFC were in the order: ethyl acetate > DCM > methanol > aqueous > hexane (Table 2). The ethyl acetate fraction had the highest TFC value (129.56 mg RE//g) followed by the dichloromethane fraction (103.16 mg RE/g) while the least value was observed in the hexane fraction (22.36 mg RE/g).

Table 1. Phytochemical screening of *T. occidentalis* stem

Extract	alkaloids	flavonoids	Saponins	tannins	anthraquinones
Ethanol	+	+	+	+	–

Key: + = Present; – = absent. Hagger's, alkaline reagent, frothing and lead acetate for the detection of alkaloids, flavonoids, saponins and tannins respectively. Bontrager's test for anthraquinone

Table 2. Extraction yield, total phenolic and flavonoid contents in *T. occidentalis* stem fractions

Fraction	Weight (g)	Yield (%)	Appearance	TPC (mg GAE/g)	TFC (mg RE/g)
HTO	0.1	<1 (0.01)	White powder	3.07±0.001	22.36±0.002
DTO	0.9	<1 (0.15)	Light-green marsh	8.15±0.002	103.16±0.001
ETO	0.6	<1 (0.10)	Brown oil	22.17±0.001	129.56±0.001
MTO	12.8	2.13	Green marsh	16.41±0.001	34.76±0.002
Aq.TO	19.5	3.25	Dark-green marsh	11.43±0.003	25.56±0.001

Data represent mean ±standard deviation (SD) of triplicate values

3.3 *In vitro* Antioxidant Assay

3.3.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging activity

Antioxidant evaluation revealed that *T. occidentalis* stems possess antioxidant activity in both assays, although both the fractions and the extract exhibited lower antioxidant activity (14–39%) compared to the standard drug, ascorbic acid (84–96%). ETTO (EC_{50} = 183.003 µg/mL), HTO (EC_{50} = 249.15 µg/mL), DTO (EC_{50} = 181.367 µg/mL), ETO (EC_{50} = 116.256 µg/mL), MTO (EC_{50} = 231.78 µg/mL), Aq.TO (EC_{50} = 222.387 µg/mL), AA (EC_{50} = 15.713 µg/mL), The percentage inhibition was therefore in the order ascorbic acid > ethyl acetate fraction > dichloromethane fraction > methanol > aqueous fraction (Fig. 4A).

3.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

In FRAP assay, ascorbic acid (standard drug) exhibited the highest ferric reducing antioxidant power (1.745 nm). The reducing power of the extract and fractions were in the following order: ethyl acetate (EC_{50} = 55.218 µg/mL), extract (EC_{50} = 60.468 µg/mL), methanol (EC_{50} = 65.60 µg/mL), dichloromethane (EC_{50} = 77.589 µg/mL) and aqueous (EC_{50} = 93.63 µg/mL), HTO (EC_{50} = 97.781 µg/mL). The standard drug, ascorbic acid AA (EC_{50} = 16.662 µg/mL). It was observed that the FRAP assay was concentration-dependent. (Fig. 4B).

3.4 Alpha Amylase Glucosidase Inhibition Assay

Administration of maltose (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after 30 mins. The percentages were maltose (75.92%), *T. occidentalis* stem extract-treated groups (32.14 - 43.36%), and acarbose-treated group (3.37%). These increases were reduced after 60 minutes with only the low dose (150 mg/kg) treated group having Blood Glucose Level (BGL) increment of 4.12%. All the extract-treated groups had their BGL reduced to a normal level at 120 minutes and sustained throughout the duration of the study. Also, co-administration of the starch with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 3).

Administration of starch (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after 30 mins. The percentages were starch (55.07%), *T. occidentalis* stem extract-treated groups (26.75 - 40.65%), and acarbose-treated group (17.97%). These increases were reduced after 120 minutes with all the groups treated with the stem extract had their BGL reduced to a normal level and this was sustained throughout the study. Also, co-administration of the maltose with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 3).

Administration of sucrose (2 g/kg) produced a 64.37% increase in blood glucose concentration

30 minutes after administration of the sucrose in the control group. BGL increments of 44.04-58.19 % were also recorded in groups treated with 150,300 and 450 mg/kg of *T. occidentalis* stem extract and no increment for acarbose treated group. At 180 min, group treated with the high dose (450 mg/kg) had the BGL reduced to normal, while 150 and 300 mg/kg treated groups had BGL of 0.65 and 4.50% respectively. At 180 min, all the extract-treated groups had their BGL reduced to normal (Table 3).

3.5 GC-MS Analysis

The results of the GC-MS analyses of the hexane, dichloromethane, ethyl acetate, methanol and aqueous fractions are presented, respectively, in Table 5-7. Peak area (%) values of fractions in each case, are enclosed in a bracket. In the hexane fraction, the prominent peaks were matched with their respective compounds as follows: 2,3-Diphenylcyclopropylmethyl phenyl sulfoxide, trans- (14.87% and 4.80%), 1H-Indole, 5-methyl-2-phenyl- (19.46%), 1-Bromo-11-iodoundecane (11.95%), stearic acid hydrazide (5.01%), 2-Piperidinone N-[4-bromo-n-butyl]- (3.36%), 3-Phenylthiane, S-oxide (3.03%). In the dichloromethane fraction, % peak area of the following compound were prominent at their respective retention time: *trans*-13-Octadecenoic acid (18.09%), n-Hexadecenoic acid (16.21% & 14.53%), linoelaidic acid (12.83%), 9,12-Octadecadienoic acid (4.99%), oleic acid (4.52%) and *cis*-Vaccenic acid (3.02%). The ethylacetate fraction had prominent peaks matched with thiosulphuric acid ($H_2S_2O_3$)-S-[2-(tetrahydro-1,1-dioxido-3-thienyl) amino] ethyl] ester (70.44%); phenylephrine (4.46%), benzenemethanol, α-[(methylamino)methyl]- (3.74%), 1,2-Benzenediol, 4-[2-(methylamino) ethyl]- (2.36%), 1,3,4-Oxadiazol-2-amine, 5-(1-phenyl-5-tetrazolyl)- (2.28%). For the methanol fraction, cystine (24.04%), phenylephrine (17.74%), hexadecane (8.23% & 5.99%), cystamine (5.03% & 3.03%), S-[Tri-t-butoxysilyl]-2-mercaptoethylamine (2.01 & 3.89%), eicosane (3.52%) were present. Pentafluorochlorodimethyl trisulfide (34.71%), furazano[3,4-b]pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)- (25.76%), metaraminol (9.08%), heneicosane (5.09%) an cystamine at 8.17%, 8.19% and 3.51% were confirmed in the aqueous fraction. Fig. 7-13 show the mass spectrum of Benzenemethanol, α-[1-(methylamino)ethyl]-; S-[Tri-t-butoxysilyl]-2-mercaptoethylamine; 1,3,4-Oxadiazol-2-amine, 5-(1-phenyl-5-tetrazolyl)-: 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; 9,12-Octadecadienoic acid (E, E)-; *trans*-13-Octadecenoic acid and 2-Piperidinone, N-[4-bromo-n-butyl]. -butyl]-. GC-MS Chromatogram of *T. occidentalis* stem hexane, dichloromethane, ethyl acetate and methanol fractions are also shown (Fig.12-16).

4. DISCUSSION

Edible leafy vegetables are important sources of nutrients, vitamins and mineral elements to the body. The presence of bioactive constituents such as alkaloids, saponins, tannins, flavonoids and phenolics (Table 1) in the *T. occidentalis* stem extract confirmed the biological usefulness of this plant. Alkaloids have antibacterial, antiviral, anticancer, antifungal, and antimalarial properties, while saponins have insecticidal, anthelmintic, anticancer, antiviral, antibacterial, and antifungal properties. Similarly, flavonoids also have antibacterial, antiaging, antiallergenic, anti-inflammatory, anticancer and antiviral activities, while phenolic compounds have antioxidant and antibacterial properties [3]. Previous wok reported that saponin and tannins were not detected [19]. The results of the phenolic and flavonoids contents of the stem revealed that the ethylacetate fraction possess the highest phenolic (22.17 mg GAE/g**)** and flavonoids (129.56 mg RE/g) contents. The dichloromethane fraction had a flavonoid content of 103.16 mg RE/g but we observed low phenolic content (8.15 mg GAE/g) (Table 2). The percentage antioxidant activity of the stem of *T. occidentalis* showed that the ethylacetate fraction demonstrated a higher antioxidant activity than other fractions (Fig. 4A). In FRAP assay, the highest reducing power was exhibited by the standard drug (ascorbic acid). The higher antioxidant activity exhibited by ethylacetate fraction also corroborated the results of the flavonoids and phenolic contents of the fraction and, could possibly be linked to the bioactive constituents detected in this fraction. A similar trend was also observed with the dichloromethane fraction. Flavonoids, apart from being antioxidants are reported to have antibacterial, antiaging, antiallergenic, anti-inflammatory, anticancer, and antiviral activities, while phenolic compounds have antioxidant and antibacterial properties [3].

Table 3. Effect of ethanol stem extract of *T. occidentalis* on Blood Glucose Level of rat after oral administration of maltose, starch and sucrose load

Treatment	Dose mg/kg	Blood Glucose Level mg/dL in				
		0 min	30 min	60 min	120 min	180 min
Control (Normal saline)	-	100.00±4.25	88.33±1.85	92.33±4.25(1.80)	89.0±4.35(1.55)	87.33±3.84(3.98)
Maltose						
Starch	2000	81.0±3.52	142.50±7.13^{c}(75.92)	118.75±2.56^{a}(46.60)	91.25±7.13(12.65)	88.75±4.30(9.56)
Acarbose	100	85.34±1.36	88.22±1.10(3.37)	86.0±2.20(0.77)	84.26±1.14^{a}	82.28±2.26
Extract	150	84.75±4.07	121.5±22.06(43.36)	88.25±4.54(4.12)	89.75±2.00(5.89)	87.0±2.42(2.65)
	300	92.25±2.68	129.25±7.79^{b}(40.10)	88.50±9.04^{a}	77.0±3.02()	64.50±2.75
	450	85.50±4.83	126.0±10.57^{b}(32.14)	72.50±2.90^{b}	66.50±2.87^{b}	56.25±2.75^{a}
Starch						
Starch	2000	81.25±3.22	126.0±2.97^{c} (55.07)	136.75±10.42^{a}(67.38)	101.0±6.46(24.30)	91.25±4.78(12.30)
Acarbose	100	72.33±2.69	85.33±12.97(17.97)	80.33±7.21(11.06)	74.0±1.00(2.30)	72.33±8.68(0)
Extract	150	96.25±7.49	122.0±6.41^{a}(26.75)	127.0±12.38^{a}(30.75)	90.50±11.16^{a}	91.75±2.13^{a}
	300	92.25±8.04	129.75±13.25^{b}(40.65)	132.50±9.71^{a}(43.63)	87.25±6.89^{a}	89.25±4.44
	450	97.50±4.48	126.25±6.23^{b}(29.48)	130.75±10.40^{b}(34.10)	85.75±11.57^{b}	91.50±5.79^{a}
Sucrose						
Starch	2000	83.50±4.51	137.25±13.91^{c}(64.37)	118.0±4.49^{a}(41.31)	96.75±6.48(15.86)	86.75±2.56(3.89)
Acarbose	100	90.33±2.48	86.66±2.90	82.0±6.00	71.66±3.75	78.0±3.78
Extract	150	76.75±5.76	120.75±4.66^{c}(57.32)	91.0±3.46^{a}(18.56)	77.25±7.59(0.65)	76.75±4.46(0)
	300	77.75±2.68	123.0±19.78^{c}(58.19)	92.50±6.51^{a}(18.97)	81.25±0.75(4.50)	74.0±0.91
	450	84.0±3.46	121.0±13.46^{b}(44.04)	90.0±2.61^{b}(7.14)	77.0±4.06^{b}	78.25±3.42^{a}

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p< 0.01$, when compared to control. (n=6). Values in brackets are percentage increases in blood glucose concentrations compared to 0 min in the same group

Furthermore, we subjected the ethanol crude extract of the stem to biological assay and we found the stem extract to inhibit increases in blood glucose concentration following starch administration independent of the dose (Table 3). "It has been reported that complete digestion of dietary polysaccharides like starch is achieved by the combined action of α-amylases and α-glucosidase enzymes" [28]. "The α-amylase enzyme digests α-bonds of the α-linked polysaccharides yielding disaccharides, like maltose, which are further reduced to monosaccharides by membrane bound α-glucosidase enzymes" [28,29]. "Inhibitions of these enzymes delay the digestion of ingested carbohydrates thereby resulting in a small rise in blood glucose concentrations following carbohydrate meals as was observed in this study. As a target for managing Type 2 diabetes mellitus, many medicinal plants have been reported to possess α-amylase and α-glucosidase inhibitory potential" [30,31]. "Similarly, the stem extract significantly inhibited blood glucose rises when co-administered with maltose and sucrose. Acarbose, the standard drug used in this study significantly inhibited blood glucose rise when co-administered with starch, maltose and sucrose. The results of this study suggest that *T. occidentalis* stem may possess antidiabetic activity and support similar reports for the leaf extract" [4]. "Also, our result suggests the involvement of inhibitory effects on alpha glucosidase and amylase as one of the modes of antidiabetic activity of the stem extract. The inhibitory activities of plant extracts could be linked to their phytochemical constituents. These compounds have been variously reported to inhibit alpha glucosidase and alpha amylase activities" [32,33]. "Phenols have been reported to inhibit alpha amylase and alpha glucosidase" [34]. "Also, polyphenolic compounds from plants known to cause several effects on the biological systems which include enzymes inhibitions" [35,36].

"The phenolic compounds are known to be strong metal ion chelators and protein precipitation agents forming insoluble complexes with proteins as well as acting as biological oxidants" [37]. "The presence of the phenolic compounds in the stem ethanolic extract of this plant may suggest their inhibitory potential on α-amylase and the membrane-bound intestinal α-glucosidase enzymes" [38]. The presence of these compounds in the extract may have contributed to the observed activity in this study and therefore explains the antidiabetic activity of the stem extract of *T. occidentalis*. To confirm the chemical constituents, GC-MS profiling was carried out on the fractions, the results showed that saturated fatty acids, unsaturated omega-9 fatty acids, omega-6-*trans* fatty acids, amino acids, alcohols, phenols and hydrocarbons are the main constituents in the stem and selected structures are shown (Table 4). Pentadecanoic acid 14-methyl-, methyl ester; hexadecanoic acid; oleic acid; (E)-9-Octadecenoic acid ethyl ester; stearic acid hydrazide was found in the hexane fraction (Table 5). *trans*-13-Octadecenoic acid; n-hexadecenoic acid; linoelaidic acid; 9,12-Octadecadienoic acid; oleic acid and *cis*-Vaccenic acid were found in the dichloromethane fraction (Table 6). In ethylacetate fraction, thiosulphuric acid ($H_2S_2O_3$)-S-[2-(tetrahydro-1,1-dioxido-3-thienyl)amino]ethyl] ester, phenylephrine, 1,2-Benzenediol, 4-[2-(methylamino)ethyl]-; 1,3,4-Oxadiazol-2-amine; 5-(1-phenyl-5-tetrazolyl)- were the prominent peaks (Table 7).

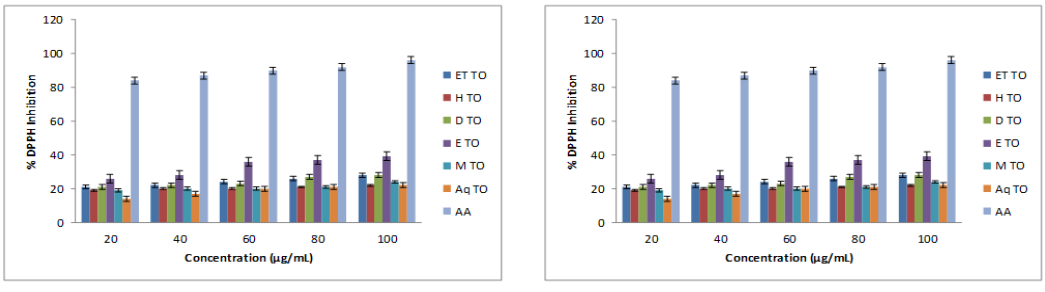


Fig. 4. Antioxidant activities of *T. occidentalis* stems extract and fractions at various concentrations. Data represent mean ± standard deviation (SD) of thee replicates. (A) DPPH radical scavenging activity, (B) Ferric Reducing Power (FRAP). For DPPH, ETTO (EC_{50} = 183.003 µg/mL), HTO (EC_{50} = 249.15 µg/mL), DTO (EC_{50} = 181.367 µg/mL), ETO (EC_{50} = 116.256 µg/mL), MTO (EC_{50} = 231.78 µg/mL), Aq.TO (EC_{50} = 222.387 µg/mL), AA (EC_{50} = 15.713 µg/mL), For FRAP, ETTO (EC_{50} = 60.468 µg/mL), HTO (EC_{50} = 97.781 µg/mL), DTO (EC_{50} = 77.589 µg/mL), ETO (EC_{50} = 55.218 µg/mL), MTO (EC_{50} = 65.60 µg/mL), Aq.TO (EC_{50} = 93.63 µg/mL), AA (EC_{50} = 16.662 µg/mL).

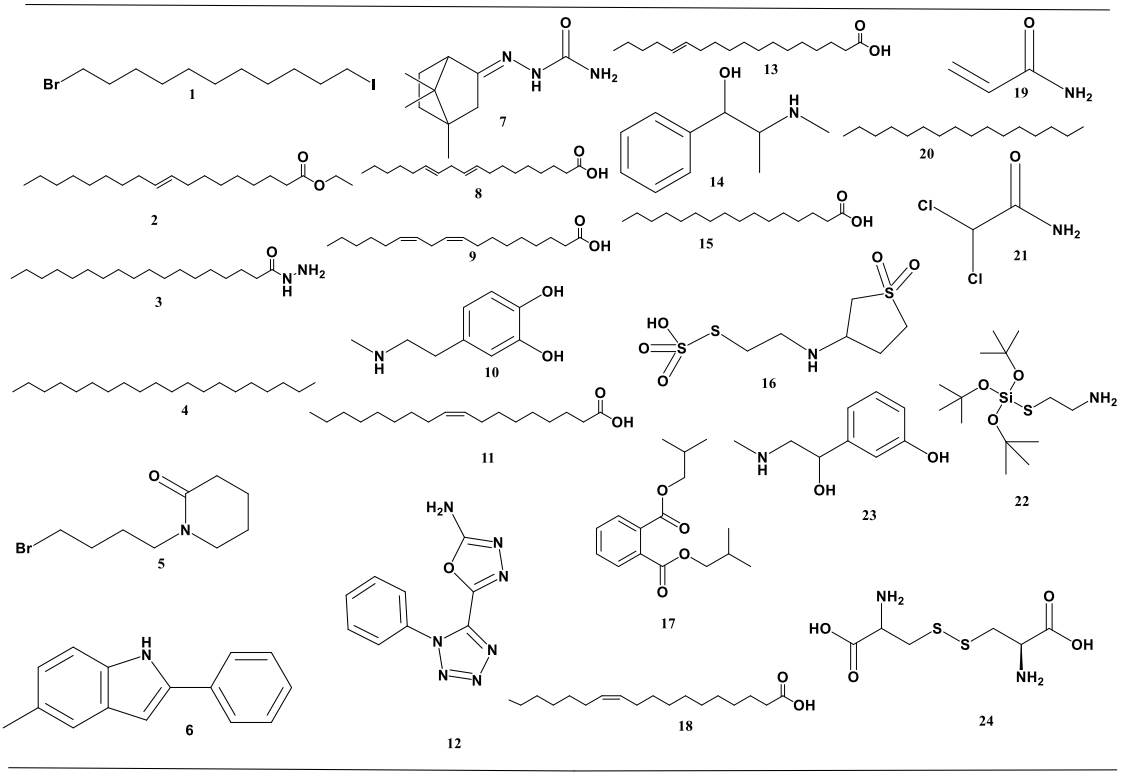


Fig. 5. Selected compounds from GC-MS analysis of *T. occidentalis* stem

1. 1-Bromo-11-iodoundecane, 2. (E)-9-Octadecenoic acid ethyl ester, 3. Stearic acid hydrazine, 4. Eicosane, 5. 2-Piperidinone, N-[4-bromo-n-butyl]-, 6. 1H-Indole, 5-methyl-2-phenyl-, 7. Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1α,2β,5α)]-, 8. 9,12-Octadecadienoic acid (E, E)-, 9. 9,12-Octadecadienoic acid (Z, Z)-, 10. Benzenemethanol, α-[1-(methylamino)ethyl]-, 11. Oleic acid 12. 1,3,4-Oxadiazol-2-amine, 5-(1-phenyl-5-tetrazolyl)-, 13. trans-13-Octadecenoic acid, 14. Benzenemethanol, α-[1-(methylamino)ethyl]-, 15. Hexadecenoic acid (palmitic acid), 16. Thiosulfuric acid ($H_2S_2O_3$), S-[2-[(tetrahydro-1,1-dioxido-3-thienyl)amino]ethyl] ester 17. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, 18. cis-Vaccenic acid, 19. 2-Propenamide, 20. Hexadecane 21. Acetamide, 2,2-dichloro-, 22. S-[Tri-t-butoxysilyl]-2-mercaptoethylamine, 23. Phenylephrine 24. Cystine

Table 4. Major Phytochemical Constituents Identified in *T. occidentalis* Stems Hexane Fraction

S/NO	RT	Compound	MF	MW	Composition (%)
1.	22.0371	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	1.29
2.	22.4314	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.46	1.04
3.	23.787	Ethyl 15-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298.50	0.94
4.	23.9393	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296.53	1.36
5.	24.0422	Oleic Acid	$C_{18}H_{34}O_2$	282.46	1.69
6.	24.3209	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.51	2.79
7.	24.4325	i-Propyl 9-tetradecenoate	$C_{17}H_{34}O_2$	270.50	1.71
8.	24.5246	Octacosanal	$C_{28}H_{56}O$	408.74	1.92
9.	24.7351	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.13	3.36
10.	26.1391	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53	0.32
11.	28.0142	Eicosane, 9-cyclohexyl-	$C_{26}H_{52}$	364.69	0.67
12.	28.4639	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.13	9.94
13.	28.5366	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298.51	5.01
14.	29.561	Tetracosane, 1-bromo-	$C_{24}H_{49}Br$	417.55	1.87
15.	29.8086	Eicosane	$C_{20}H_{42}$	282.55	2.87
16.	30.2453	1-Bromo-11-iodoundecane	$C11H_{22}BrI$	361.10	11.95
17.	32.0633	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	207.27	19.46

S/NO	RT	Compound	MF	MW	Composition (%)
18.	32.1469	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-	$C_{21}H_{22}$	274.39	1.38
19.	33.730	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332.46	20.53

RT- retention time; MF- molecular formular; MW- molecular weight

Table 5. Major Phytochemicals Identified in *T. occidentalis* Stems Dichloromethane Fraction

S/NO	RT	Compound	MF	MW	Composition (%)
1.	18.0262	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	1.15
2.	19.2806	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1α,2β.,5α)]-	$C_{10}H_{18}$	138.25	0.25
3.	19.4477	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	1.01
4.	22.0339	Oleic Acid	$C_{18}H3_4O_2$	282.46	5.48
5.	22.3775	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	30.75
6.	23.818	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O$	298.50	0.38
7.	24.067	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	0.56
7.	24.4659	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.50	0.32
8.	25.5472	Linoelaidic acid (9,12-Octadecadienoic acid (E, E)-	$C_{18}H_{32}O_2$	280.40	12.83
9.	25.6258	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.40	4.99
10.	25.6976	*trans*-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	31.42
11.	26.098	Octadecanoic acid	$C_{18}H_{36}O_2$	284.46	2.85
12.	27.2971	*cis*-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296.50	1.85
13.	27.5126	5-Eicosene, (E)-	$C_{20}H_{40}$	280.53	0.53
14.	27.6953	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282.48	3.02

RT- retention time; MF- molecular formular; MW- molecular weight

Table 6. Major Phytochemicals Identified in *T. occidentalis* stems Ethylacetate Fraction

S/NO	RT	Compound	MF	MW	Composition (%)
1.	9.4594	Benzeethamine,4-methoxy-alpha-methyl-	$C_{10}H_{15}NO$	165.23	0.98
2.	11.075	1,2-Benzenediol, 4-[2-(methylamino)ethyl]-	$C_9H_{13}NO_2$	167.09	2.36
3.	11.369	Paradrine	$C_9H_{13}NO$	151.21	1.67
4.	11.777	3-Propoxyamphetamine	$C_{12}H_{19}NO$	193.28	1.73
5.	12.275	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	1.57
6.	13.898	Amphetamine	$C_9H_{13}N$	135.21	0.58
7.	21.865	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34	1.16
8.	21.977	n-Hexadecanoic acid	$C_{16}H_{32}O$	256.43	0.49
9.	25.291	Oleic Acid	$C_{18}H_{34}O$	282.46	1.64
10.	25.723	Octadecanoic acid	$C_{18}H_{36}O$	284.48	1.27
11.	28.496	Benzenemethanol, α-[1-(methylamino)ethyl]-	$C_{10}H_{15}NO$	165.23	3.74
12.	28.67	1,3,4-Oxadiazol-2-amine, 5-(1-phenyl-5-tetrazolyl)-	$C_8H_7N_3O$	161.16	2.28
13.	28.873	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, semicarbazone	$C_{11}H_{19}N_3O$	209.29	2.28
14.	29.481	Phenylephrine	$C_9H_{13}NO_2$	167.20	4.46
15	30.938	Thiosulfuric acid ($H_2S_2O_3$), S-[2-[(tetrahydro-1,1-dioxido-3-thienyl)amino]ethyl] ester	$C_6H_{13}NO_5S_3$	275.37	70.42

RT- retention time; MF- molecular formular; MW- molecular weight

GC-MS analysis of the methanol showed the presence of S-[Tri-t-butoxysilyl]-2-mercaptoethylamine; p-Hydroxyamphetamine; benzeneethanamine-4-methoxy-α-methyl-; (-)-Norephedrine; cystine; phenylephrine and 2-Propenamide in the methanol fraction (Table 7). Phenolic compounds, alcohols, fatty acids including their esters have shown antioxidative properties [39]. For example, hexadecenoic acid, oleic acid, eicosane, acetamide and butyl phenol have shown antioxidant activity [4,40]. The presence of some of these compounds in the stem extract, especially, in the ethyl acetate fraction [41] and methanol fraction could be also linked to the observed α-amylase and α-glucosidase inhibition [42,43,44].

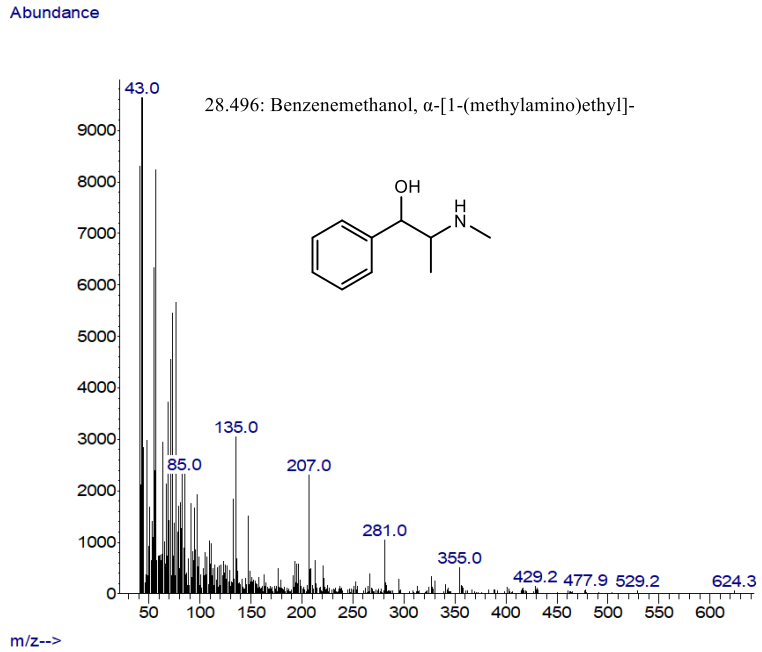


Fig. 6

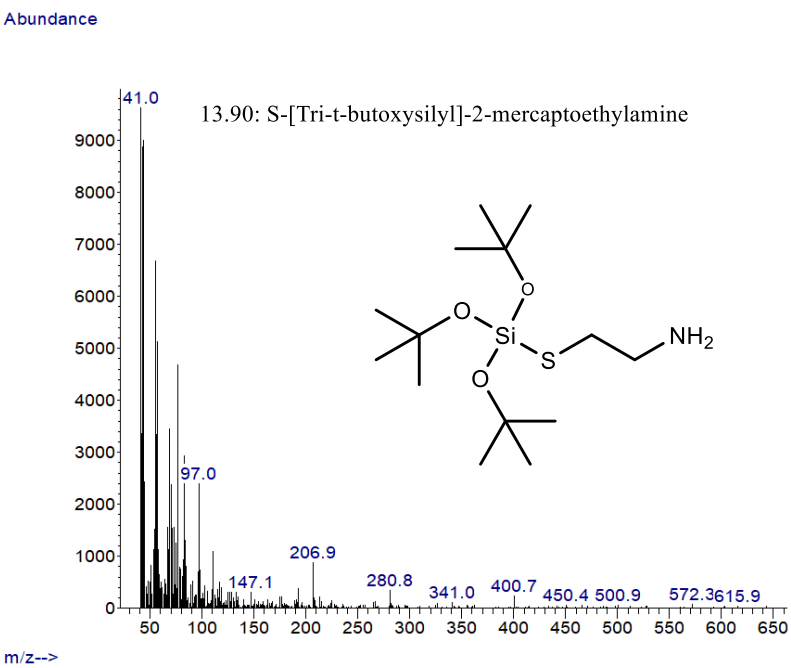


Fig. 7

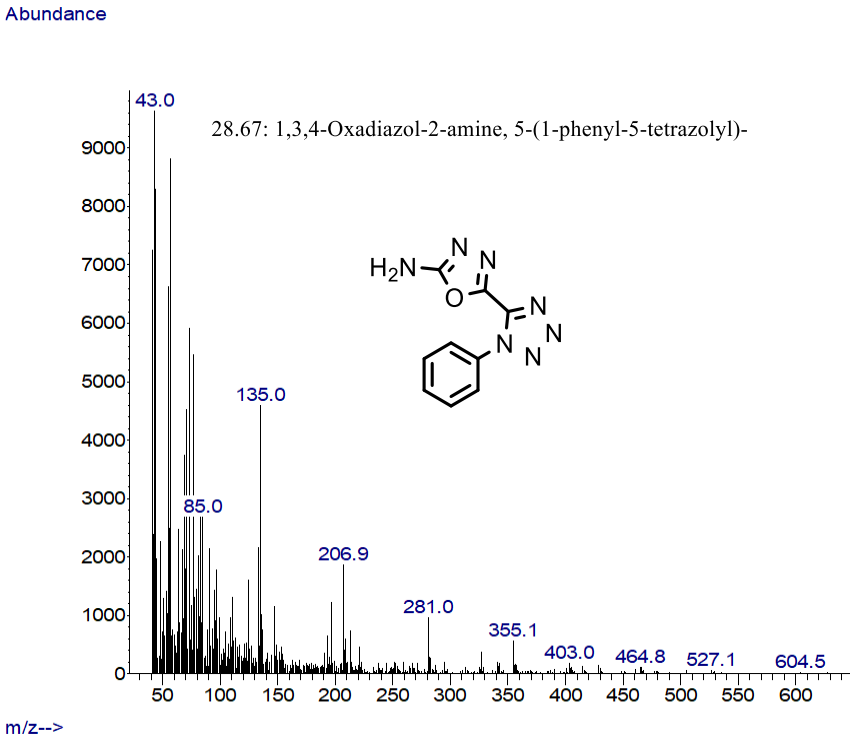

Abundance
28.67: 1,3,4-Oxadiazol-2-amine, 5-(1-phenyl-5-tetrazolyl)-
43.0
135.0
85.0
206.9
281.0
355.1
403.0
464.8
527.1
604.5
m/z-->

Fig. 8

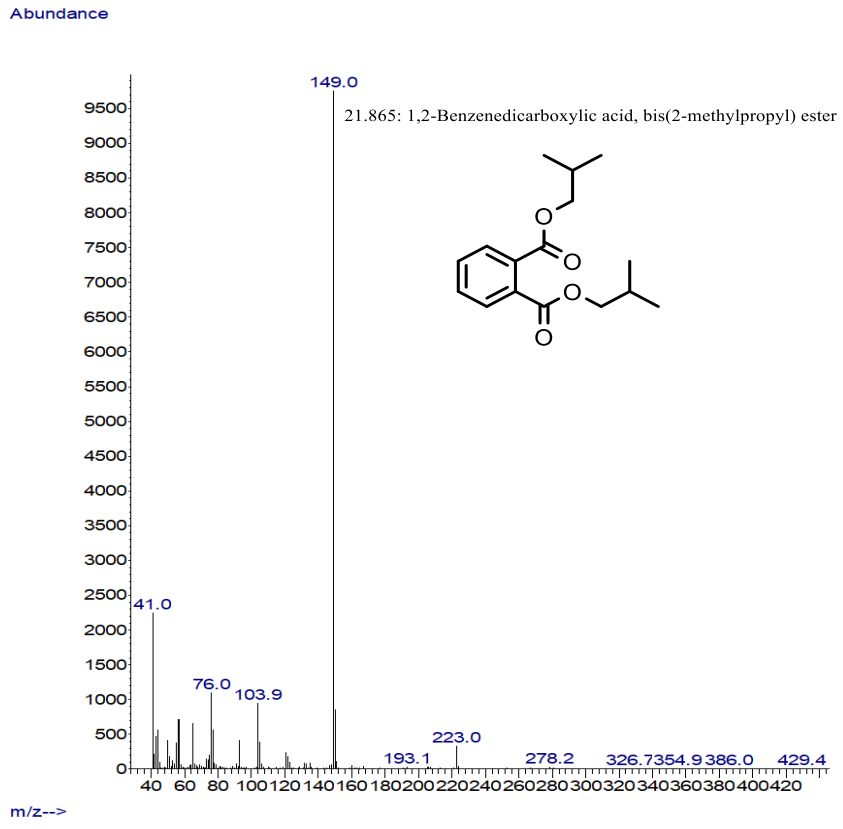

Abundance
21.865: 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
149.0
41.0
76.0
103.9
223.0
193.1
278.2
326.7
354.9
386.0
429.4
m/z-->

Fig. 9

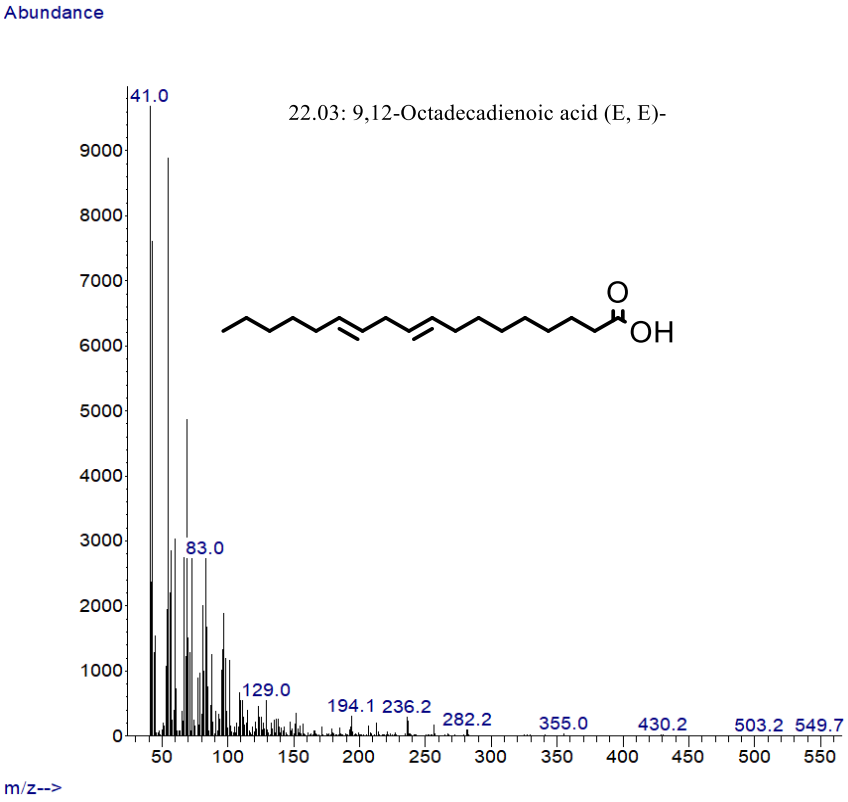

Abundance
22.03: 9,12-Octadecadienoic acid (E, E)-
41.0
83.0
129.0
194.1
236.2
282.2
355.0
430.2
503.2
549.7
O
OH
m/z-->

Fig. 10

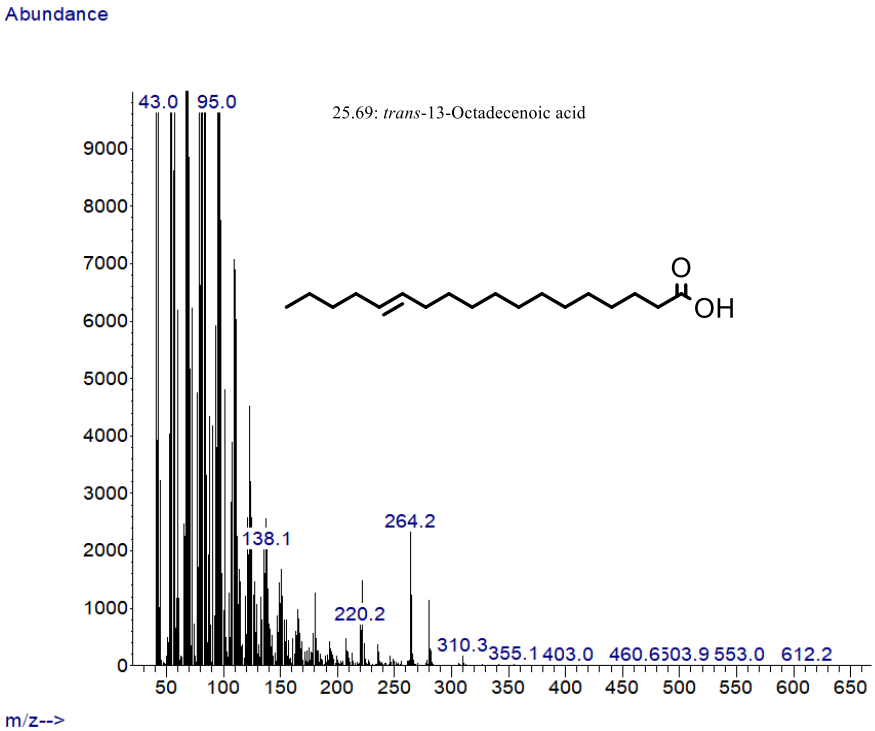

Abundance
25.69: *trans*-13-Octadecenoic acid
43.0
95.0
138.1
220.2
264.2
310.3
355.1
403.0
460.6
503.9
553.0
612.2
O
OH
m/z-->

Fig. 11

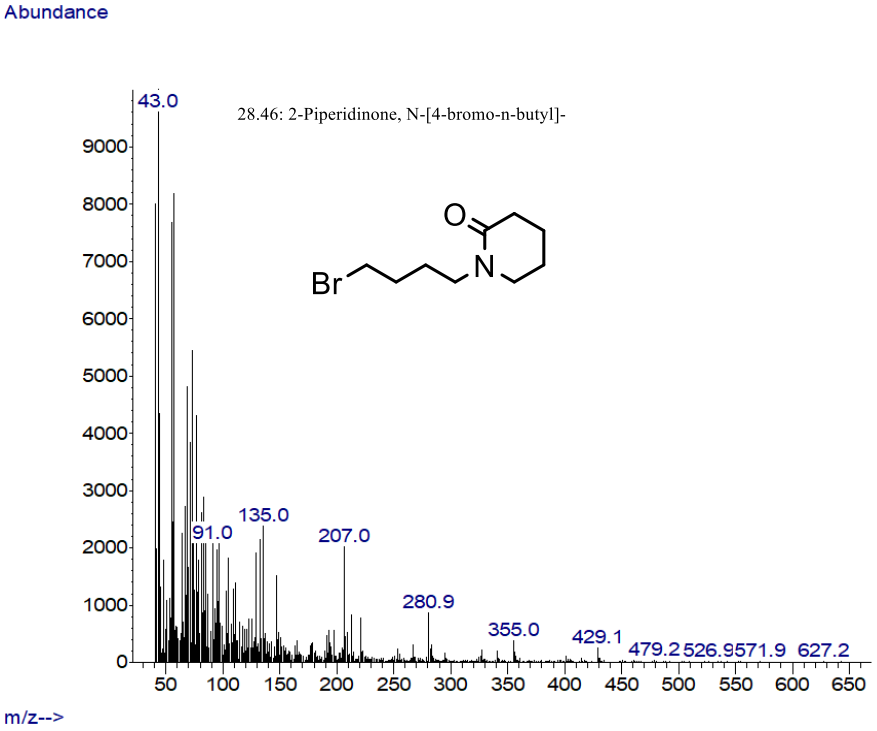


Fig. 12

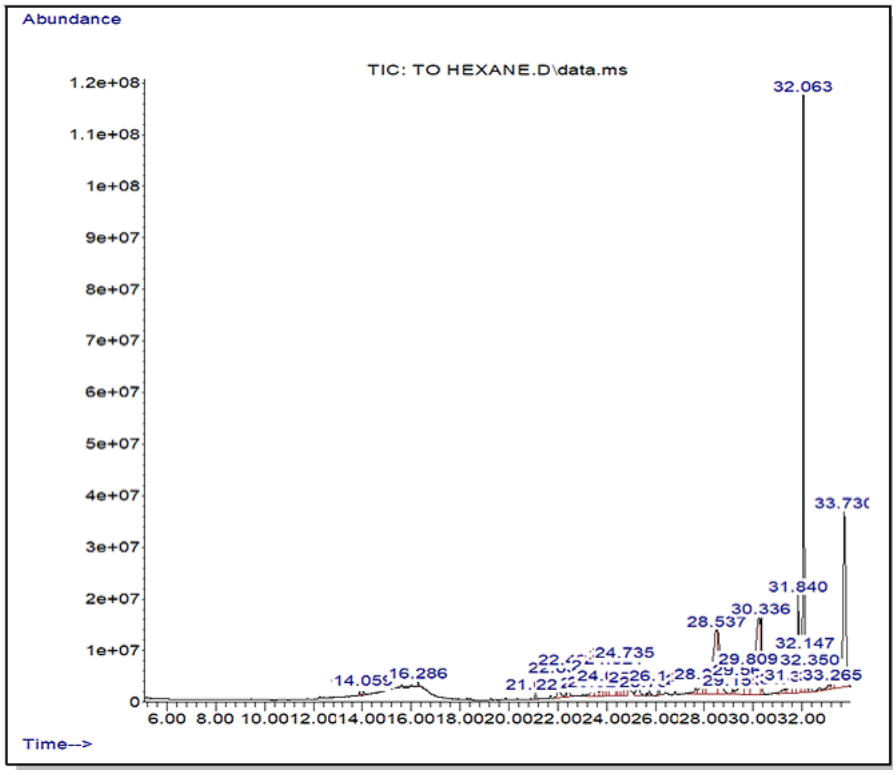


Fig. 13

Fig. 6-13. GC-MS Chromatogram of *T. occidentalis* stem hexane fraction

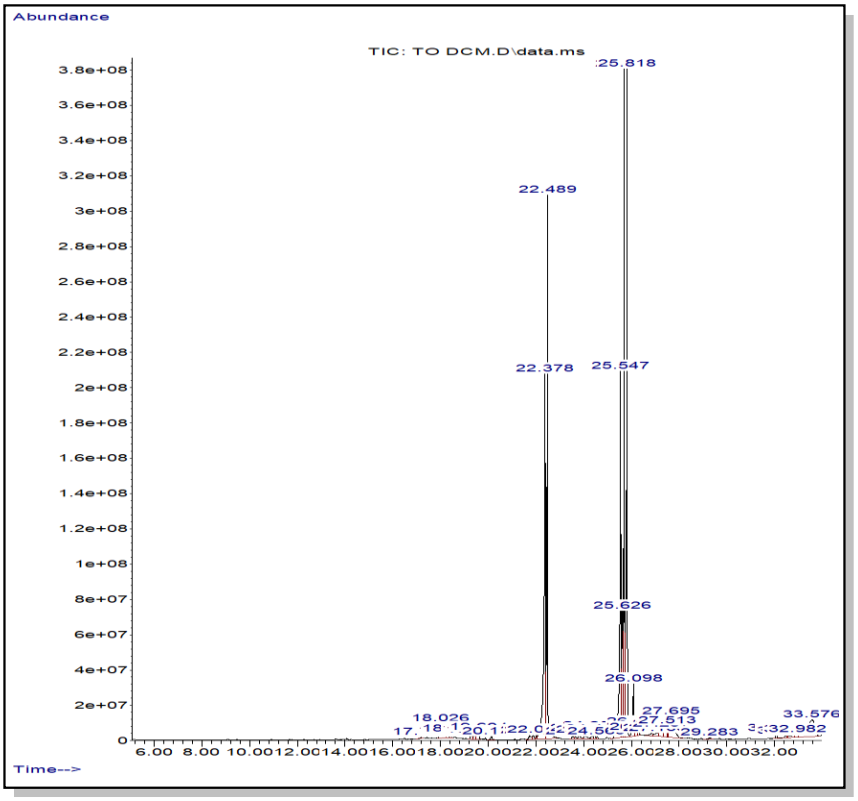


Fig. 14. GC-MS Chromatogram of *T. occidentalis* stem dichloromethane fraction

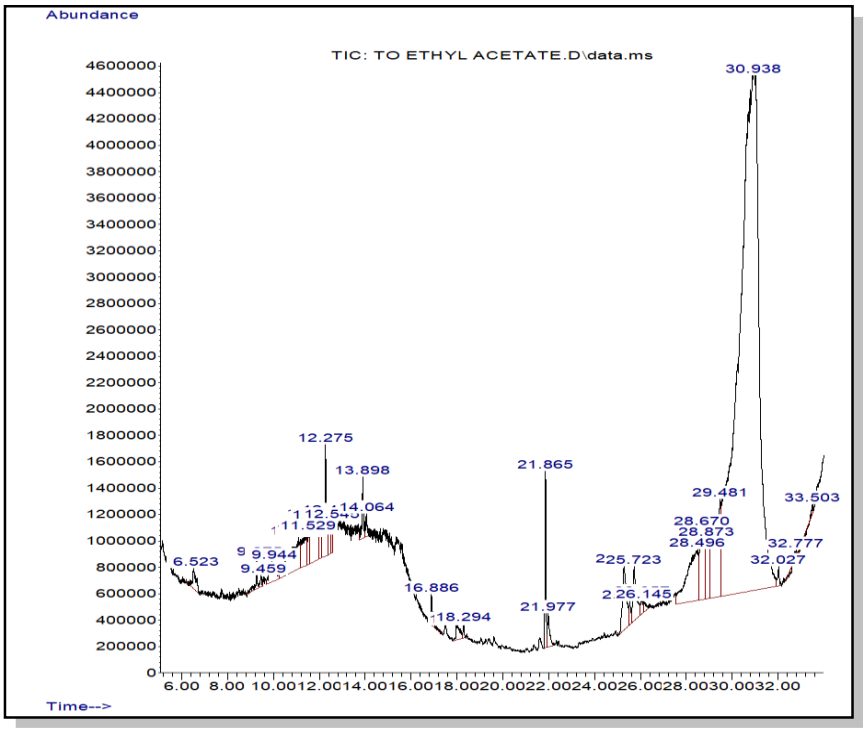


Fig. 15. GC-MS Chromatogram of *T. occidentalis* stem ethylacetaate fraction

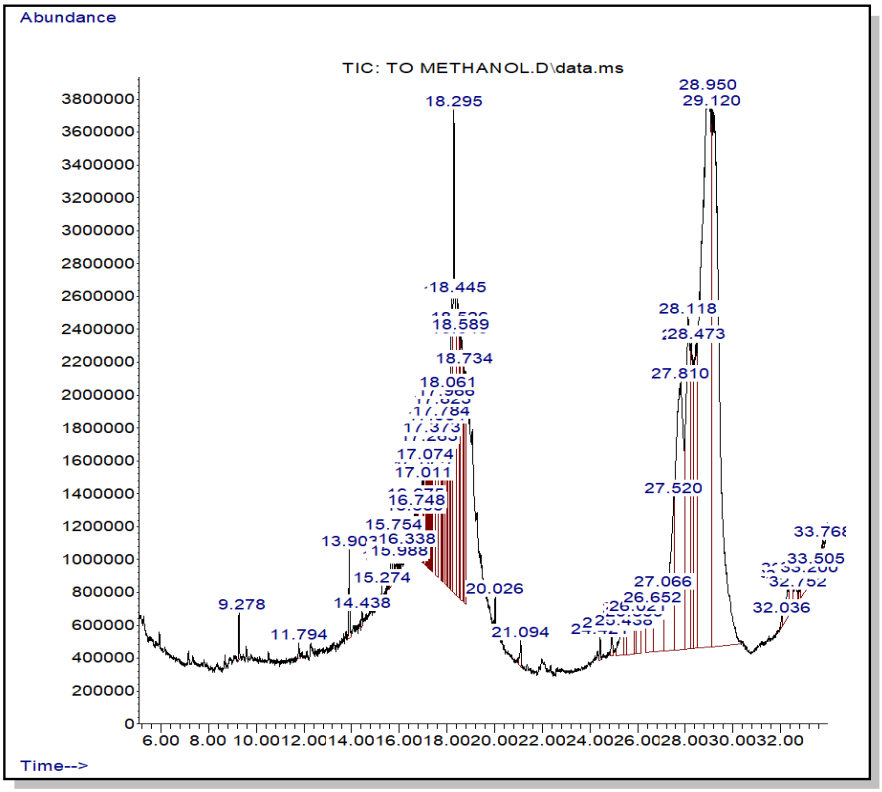


Fig. 16. GC-MS Chromatogram of *T. occidentalis* stem methanol fraction

Table 7. Major Phytochemicals Identified in *T. occidentalis* Stems Methanol Fraction

S/NO	RT	Compound	MF	MW	Composition (%)
1.	13.9025	S-[Tri-t-butoxysilyl]-2-mercaptoethylamine	$C_{14}H_{33}NO_3SSi$	323.57	11.5563
2.	14.4383	Paradrine (p-Hydroxyamphetamine)	$C_9H_{13}NO$	151.21	0.3450
3.	16.675	Benzeneethanamine, 4-methoxy-α-methyl-	$C_{10}H_{15}NO$	165.23	0.8770
4.	17.3186	(-)-Norephedrine	$C_9H_{13}NO$	151.21	1.4234
5.	26.6515	1-Octadecanamine, N-methyl-	$C_{10}H_{15}NO$	283.53	1.5028
6.	27.0659	Acetamide, 2,2-dichloro-	$C_2H_3C_{l2}NO$	127.95	1.8607
7.	27.5196	Eicosane	$C_{20}H_{42}$	282.55	3.525
8.	27.8104	Hexadecane	$C_{16}H_{34}$	226.44	14.2245
9.	28.2265	2-Propenamide	C_3H_5NO	71.08	3.0284
10.	28.9503	Cystine	$C_6H_{12}N_2O_4S_2$	240.29	30.1540
11.	29.1201	Phenylephrine	$C_9H_{13}NO_2$	167.20	17.7355

RT- retention time; MF- molecular formular; MW- molecular weight

5. CONCLUSION

The results of this study suggest that *T. occidentalis* stem possess antioxidant activity which, may be attributed to the presence of the phytochemical constituents. It also suggests that inhibition of alpha amylase and alpha glucosidase enzymes maybe one of the modes of antidiabetic activity of the stem extract of *T. occidentalis* which may in turn be attributed to the activities of its phytochemical constituents.

ETHICAL APPROVAL

Approval for animal studies protocols were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/AE/21/023).

ACKNOWLEDGEMENTS

The authors are grateful to the University of Uyo, Uyo for the provision of the laboratory space. Professor (Mrs.) Margaret E. Bassey of Botany and Ecological studies who identified and authenticated the plant and Mr. Nsikak Malachy of the Department of Pharmacognosy who provided technical support during the experimental are acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yusupova ZA, Baratjon ogli SF, Abduqunduzovna MZ. Medicinal plants growing in our republic medicinal properties. *Periodica Journal of Modern Philosophy, Social Sciences and Humanities*. 2023; 7(15):5-7.
2. Karahan F, Ozyigit II, Saracoglu IA, Yalcin IE, Ozyigit AH, Ilcim A. Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern Mediterranean region of Turkey. Biological Trace Element Research. 2020;197:316-329.
3. Ouandaogo HS, Diallo S, Odari E, Kinyua J. Phytochemical Screening and GC-MS Analysis of Methanolic and Aqueous Extracts of *Ocimum kilimandscharicum* Leaves. *ACS omega*. 2023;8(50):47560-47572.
4. Eseyin OA, Sattar MA, Rathore HA. A review of the pharmacological and biological activities of the aerial parts of *Telfairia occidentalis* Hook. f. (Cucurbitaceae). Tropical Journal of Pharmaceutical Research. 2014;13(10): 1761-1769.
Available:http://dx.doi.org/10.4314/tjpr.v13i10.28
5. Agbede, JO, Adegbenro M, Onibi GE, Oboh C, Aletor VA. Nutritive evaluation of *Telfairia occidentalis* leaf protein concentrate in infant foods. African Journal of Biotechnology. 2008;7(15): 2721-2727.
Available:http://www.academicjournals.org/AJB
6. Okpiabhele UUA, Adegbegi AJ. *Telfairia occidentalis* Hook f. Mitigates Carbon-Tetrachloride-Induced Nephrotoxicity in Wistar Rats. Journal of Science and Technology Research. 2003;5(2).
Available:https://doi.org/10.5281/zenodo.8010075
7. Oboh G, Akindahunsi AA. Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutrition and Health. 2004;18(1):29-36.
8. Aiyeloja AA, Bello OA. Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. Educational Research and Reviews. 2006;1(1):16.
9. Eseyin OA, Ebong P, Ekpo A, Igboasoiyi A, Oforah E. Hypoglycaemic effect of the seed extract of *Telfairia occidentalis* in rats. Pak. J. Biol. Sci. 2007;10:498-501.
10. Ejike CE, Ezeanyika LU. Inhibition of the Experimental Induction of Benign Prostatic Hyperplasia: A Possible Role for Fluted Pumpkin (*Telfairia occidentalis* Hook f.) Seeds. Urologia Intern. 2011;87(2):218-224.
11. Okokon JE, Dar A, Choudhary MI. Chemical constituents and analgesic activity of *Telfairia occidentalis*. *Phytopharmacol*. 2012;3:359-3.
12. Eseyin, AO, Oforah E, Dooka BD. Preliminary study of the hypoglycemic action of the extract of leaf of *Telfairea occidentalis* in normoglycemic guinea pigs. Global Journal of Pure and Applied Sciences. 2000;6(4):639-641.
13. Ogbonnaya EA, Uadia OP. Short term effect of aqueous extracts of root, pod, and stem of *Telfairia occidentalis* on some biochemical parameters in rats. Journal of Applied Sciences and Environmental Management. 2014;18(1):113-118.
14. Inuwa HM, Aimola IA, Muhammad A, Habila N, Okibe P, Latayo M, Ahmed Z. Isolation and determination of omega-9 fatty acids from *Telfairia occidentalis*. International Journal of Food Nutrition and Safety. 2012;1(1):9-14.
15. Eseyin OA, Sattar MA, Rathore HA, Aigbe F, Afzal S, Ahmad A, Lazhari M, Akthar S. GC-MS and HPLC proiles of phenolic fractions of the leaf of *Telfairia occidentalis*. Pakistan Journal OF Pharmaceutical Sciences. 2018;31(1).
16. Cyril-Olutayo C, Agbedahunsi J, Elufioye O. Antisickling properties of some indigenous and exotic plant species in Nigeria. Planta Medica. 2012;78(11):I2-17.
17. Oladele JO, Oyeleke OM, Awosanya OO, Olowookere BD, Oladele, O.T. Fluted

Pumpkin (*Telfairia occidentalis*) protects against phenyl hydrazine-induced anaemia and associated toxicities in rats. Advances in Traditional Medicine. 2021;21(4):739-745.

18. Pelletier SW, Chokshi HP, Desai HK. Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. Journal of Natural Products. 1986;49(5):892-900.
19. Njinga NS, Bakare-Odunola MT, Abdullahi ST, Okonkwo JA, Oladimeji OH. Comparative phytochemical analysis and antioxidant activity of the stem and seed of *Telfairia occidentalis* Hook. F. (Curcurbitaceae). J. Pharm. Res. Dev. & Pract. 2016;1(1):69-78
ISSN:2579-0455.
20. Enin GN, Antia BS, Shaibu SE, Nyakno I. Comparison of the chemical composition, nutritional values, total phenolics and flavonoids content of the ripe and unripe *Solanum nigrum* Linn. Fruits from Nigeria. World Journal of Pharmacy and Pharmaceutical Sciences. 2023;12 (8)1-18.
DOI: 10.20959/wjpps20238-25410.
21. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry*. 2003; 81(3):321-326.
22. Subhashini R, Rao UM, Sumathi P, Gunalan G. A comparative phytochemical analysis of cocoa and green tea. Indian Journal of Science and Technology. 2010;3(2):188-192.
23. Shekhar TC, Anju G. Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. Leaves, American Journal of Ethnomedicine. 2014;1(4):244-249.
24. Oyaizu M. Evaluation of antioxidant activity of extract of natural origin. Journal of Nutrition. 1986;44:107-112.
25. Gidado A, Watafua M, Sa'ad RS, Tagi HS, Abdullahi S. Alpha-amylase, maltase and sucrase inhibitions by aqueous leaf extracts of *Anacardium occidentale* (Anacardiacea) and *Piliostigma reticulatum* (Caesalpiniaceae) in rats. Tropical Journal of Natural Product Research. 2019;3(6): 210-215.
Available:https://doi.org/10.26538/tjnpr/v3i6.5
26. World Health Organization (2011). Glucose analyser. Core medical equipment information.
Available:www.who.int/medical_devices/en/index.html
Accessed on: 5 June, 2019.
27. Kadhim MJ, Mohammed GJ, Hussein H. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016;8(7):655-670.
28. Kalra S. Alpha Glucosidase Inhibitors. The Journal of Pakistan Medical Association. 2014; 64(4):474-476.
29. Alongi M, Anese M. Effect of coffee roasting on in vitro α-glucosidase activity: Inhibition and mechanism of action. Food Research International. 2018;111:480-487.
Available:https://doi.org/10.1016/j.foodres.2018.05.061
30. Ibrahim MA, Koorbanally NA, Islam MS. Anti-oxidative activity and inhibition of key enzymes linked to type 2 diabetes (α-glucosidase and α-amylase) by *Khaya senegalensis*. *Acta* Pharmaceutica. 2014; 64(3):311-324.
Available:https://doi.org/10.2478/acph-2014-0025
31. Esimone CO, Okonta JM, Ezugwu CO. Blood sugar lowering effect of Anacardium occidentale leaf extract in experimental rabbit model. Journal of Natural Remedies. 2001;1(1):60–63.
32. Proença C, Freitas M, Ribeiro D, Oliveira EF, Sousa JL, Tome SM, Ramos MJ, Silva A, Fernandes PA, Fernandes E. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017; 32(1):1216-1228.
Available:https://doi.org/10.1080/14756366.2017.1368503
33. Su J, Tang Z. Effects of (-)-epigallocatechin gallate and quercetin on the activity and structure of α-amylase. Tropical Journal of Pharmaceutical Research. 2019;18(3):585-590.
Available:http://dx.doi.org/10.4314/tjpr.v18i3.20
34. Oboh G, Olasehinde TA, Ademosun AO. Inhibition of enzymes linked to type-2 diabetes and hypertension by essential oils from peels of orange and lemon. International Journal of Food Properties. 2017; 20(sup1):S586-S594.
Available:https://doi.org/10.1080/10942912.2017.1303709

35. Kalita D, Holm DG, LaBarbera DV, Petrash JM, Jayanty SS. Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds. PloS One 2018; 13(1): e0191025. Available:https://doi.org/10.1371/journal.pone.0191025
36. Funke I, Melzig MF. Effect of different phenolic compounds on α-amylase activity screening by microplate-reader based kinetic assay. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2005;60(10):796–797.
37. Ishnava KB, Metisariya DM. In vitro study on α-amylase inhibitory activity of selected ethnobotanical plant extra its and its herbal formulations. International Journal of Pharmacognosy and Chinese Medicine. 2018;2(3):01-10.
38. Irondi EA, Akintunde JK, Agboola SO, Boligon AA, Athayde ML. Blanching influences the phenolics composition, antioxidant activity, and inhibitory effect of Adansonia digitata leaves extract on α-amylase, α-glucosidase, and aldose reductase. Food science & nutrition. 2017; 5(2):233-242.
39. Shori AB. Screening of antidiabetic and antioxidant activities of medicinal plants. Journal of Integrative Medicine. 2015;13(5):297-305.
40. Eseyin OA, Ebong P, Eyong EU, Umoh E, Agboke A. Hypoglycaemic activity of ethyl acetate fraction of the leaf extract of *Telfairia occidentalis*. Pakistan Journal of Pharmaceutical Sciences. 2010;23(3): 341-344.
41. Siswadi S, Saragih GS. Phytochemical analysis of bioactive compounds in ethanolic extract of Sterculia quadrifida R. Br. In AIP Conference Proceedings. 2021; 2353(1).
42. Enin GN, Okokon JE, Odokwo BO, Antia BS. Phytochemical Screening and in vivo Inhibitory Study of *Telfaira occidentalis* Hook f. Seeds Extract on Alpha Amylase and Alpha Glucosidase of Rats. Journal of Science and Technology Research. 2023;5(4).
43. Enin GN, Okokon JE, David EM, Emmanuel SE, Ekanem EM, Antia BS. 2023. *In vivo* Alpha-amylase and Alpha-glucosidase Inhibitory Potentials of *Panicum maximum* Jacq. (Guinea grass) Leaf Extract on Wister Rats. Biology, Medicine, & Natural Product Chemistry. 2023;12(2):681-685.
44. Kumar S, Ritika. A. brief review of the biological potential of indole derivatives. Future Journal of Pharmaceutical Sciences. 2020;6(121):1-19.

APPENDIX

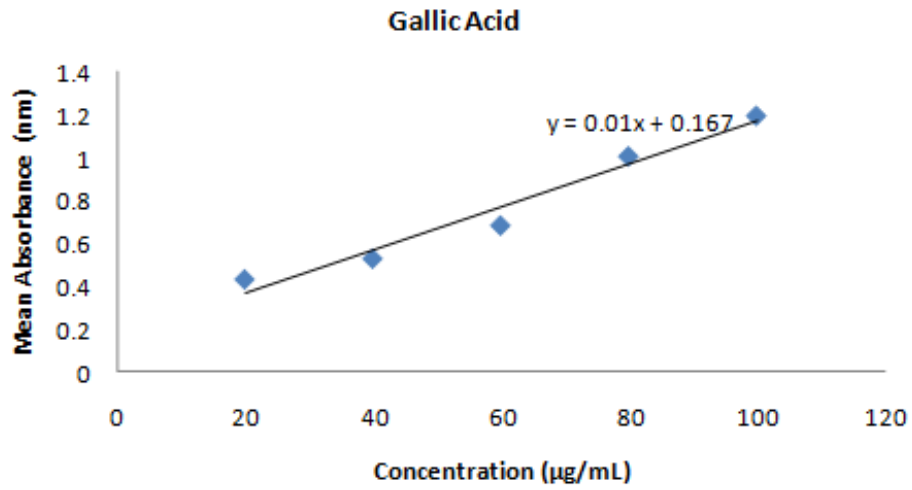


Fig. 17. Total phenolic content for standard gallic acid

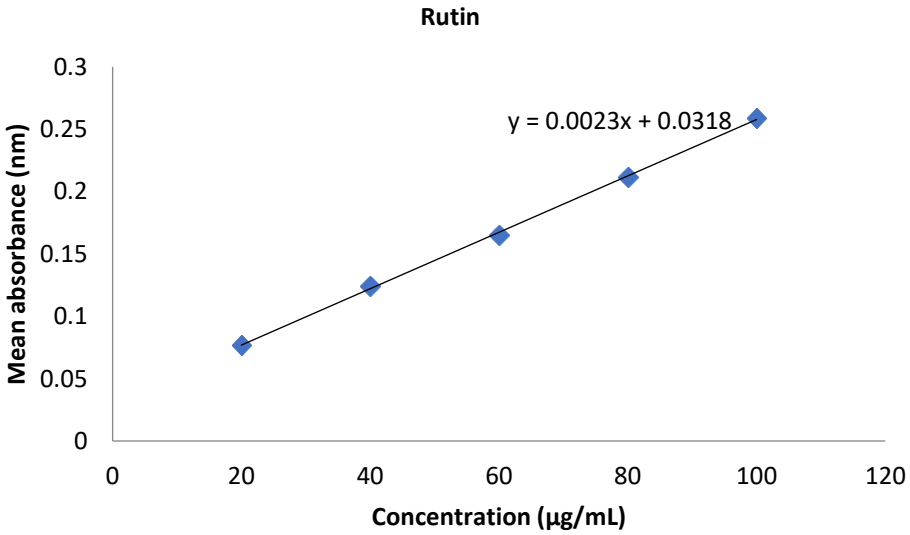


Fig. 18. Total phenolic content for standard gallic acid

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/116748