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Alpha Amylase Inhibition and Antioxidant Activities of Bicyclic Diterpenoid Lactones from *Andrographis paniculata*

Olukayode S. Ajayi[1]*, Olaoye S. Balogun[1], Idowu J. Olawuni[2], Natasha October[3], Rasheed Adigun[3], Ibukunoluwa G. Akinlade[1]

[1]*Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria*
[2]*Department of Biochemistry & Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria*
[3]*Department of Chemistry, University of Pretoria, South Africa*

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ABSTRACT

Andrographis paniculata is an important medicinal plant reported for its efficacy in the treatment and management of various medical conditions in different parts of the world. This study evaluates the α-amylase inhibitory and antioxidant activities of the crude extracts, solvent fractions and isolated compounds from aerial parts of *A. paniculata*. Chromatographic fractionation of the aqueous methanol fraction led to isolation of 14-deoxyandrographolide (**1**) and andrographolide (**2**). The isolated compounds were characterized by set of spectroscopic techniques such as nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, mass spectrometry (MS) and their structures were confirmed by x-ray crystallography and by comparison of data with literature. The extract, fractions and isolated compounds were evaluated for anti-diabetic and antioxidant activities. Aqueous methanol fraction displayed the highest amount of total phenolic (TPC) and flavonoid content (TFC) of 9.42 ± 0.95 mg gallic acid equivalent/g (mg GAE/g) and 40.08 ± 17.93 mg quercetin equivalent/g (mg QUE/g) respectively amongst the extract and fractions. The aqueous methanol fraction, crude methanol extract and n-hexane fraction exhibited better α-amylase inhibitory activity with an inhibitory concentration (IC_{50}) of 0.057 ± 0.012, 0.0610 ± 0.035 and 0.080 ± 0.021 mg/mL respectively than acarbose, a standard reference drug with an IC_{50} value of 0.127 ± 0.013 mg/mL. Compound **1** and **2** exhibited a lower inhibitory activity with an (IC_{50}) values of 0.637 ± 0.003 and 0.761 ± 0.013 mg/mL respectively. The results of these study on *A. paniculata* may justify its traditional use in the management of diabetes mellitus.

Keywords: *Andrographis paniculata*, Acanthaceae, Amylase, Antioxidant, Diterpenoid, *Diabetes mellitus*.

Introduction

Diabetes mellitus is defined as a chronic metabolic disorder that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.[1] Diabetes mellitus is identified by hyperglycemia due to insulin insufficiency and/or insulin resistance contributing to excess blood glucose. According to WHO, diabetes mellitus affected an estimated 422 million people all around the world in the year 2014. It is also projected that diabetes will be the 7th leading cause of death in 2030. Management of blood glucose level is an essential approach in the control of diabetes complications.[1]

Natural products have been a good source of enzyme inhibitors.[2,3] Phytocompounds reported to have exhibited α-amylase inhibitory activity include; terpenoids, polyphenols, steroid and saponins.[4,5] Inhibitors of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) have been helpful as oral hypoglycemic medicines for the control of hyperglycemia exclusively in patients with type-2 diabetes mellitus.[6-9]

*Corresponding author. E mail: osajayi@oauife.edu.ng
Tel: +234 806 269 8338

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Inhibition of these enzymes holds carbohydrate breakdown and extends the total carbohydrate digestion time, leading to a decrease in the rate of glucose absorption and therefore reducing the postprandial rise in plasma glucose.[10,11] However, it has been observed that the commonly used synthetic antidiabetic drugs such as acarbose and miglitol possessed negative effects, such as diarrhoea, abdominal pain, flatulence, and serum levels of transaminases increases at higher doses.[12,13]

Andrographis (family Acanthaceae) is an important medicinal genus in the Indian system of medicine and is a large genus of herbs distributed in Asia, Africa, South, and Central America.[14,15] Extracts of *A. paniculata* Nees and its constituents have been reported to exhibit a wide spectrum of biological activities which include antihyperglycemic, hypoglycemic activities, antimicrobial, anti-inflammatory, antimalarial, antithrombotic, cardioprotective, hepatoprotective, and immune-stimulant properties.[16-18] It is known to be therapeutic, with the mostly used part being the roots, leaves, and aerial part of mature twig.[19] Today, various pharmaceuticals are using the extracts of *A. paniculata* in their preparations for treating liver disorders.[20] Previous phytochemical studies of *A. paniculata* revealed that it is a rich source of labdane diterpenoids[17,20] and 2′-oxygenated flavonoids[17,21]

Other species of *Andrographis* used as substitutes or as adulterants of *A. paniculata*, include *A. alata* and *A. lineate*.[22] As part of our search for novel inhibitors of carbohydrates hydrolyzing enzymes from natural sources, we have investigated the aerial extracts of *A. paniculata* for potential bioactive secondary metabolites.

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Materials and Methods

General experimental procedure
Silica gel (Merck 230–400 mesh) was used as a stationary phase for column chromatography. Sephadex LH-20 (Sigma -Aldrich) for purification. Analysis of column fractions was achieved by thin layer chromatography (TLC), using pre-coated plates (MERCK, silica gel 60 F_{254} 0.2 mm). Spots were detected at 254 and 366 nm. FTIR data was obtained from Shimadzu IR Prestige Spectrophotometer. Nuclear magnetic resonance (NMR) spectra data were obtained from a Brucker DMX Avance 300 MHz NMR Spectrometer. Chemical shifts are expressed in parts per million (ppm). Mass spectrum (TOF MS ES+) was determined on a Waters GCT-Premier Mass Spectrometer.

Plant material
The aerial parts of *A. paniculata* were collected in June 2016 at the Medicinal Farm, Faculty of Pharmacy Obafemi Awolowo University, Ile-Ife, Nigeria and were authenticated by Mr I.I. Ogunlowo, a taxonomist in the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, with voucher number FPI 2109. The aerial parts, which included leaves and twigs, were diced, and air-dried for two weeks and pulverized.

Extraction procedure
Powdered material (1.0 kg) was exhaustively extracted with 8.5 L of methanol in a 10 L extraction glass jar at room temperature with occasional shaking. The extract was filtered and the resultant filtrates were thereafter concentrated to dryness *in vacuo* at 40^{o}C. This afforded the crude extract of the plant (107.43 g, 10.74%). This was stored in amber bottles and kept under refrigeration until required.

Solvent partitioning of the crude methanol extracts
The crude methanol extract of *A. paniculata* (105.43 g) was dissolved in methanol and was partitioned with *n*-hexane (3 x 800 mL) in a 5 L separating funnel to give two fractions; the *n*-hexane and methanol fractions. Then, both fractions were concentrated using the rotary evaporator to give 19.95 g of *n*-hexane fraction and 73.02 g of the methanol fraction. Afterwards, total phenolic and total flavonoid content were determined, antioxidant and antidiabetic activities were determined for the crude methanol extract, n-hexane and aqueous fractions using appropriate assays.

Total phenolic and flavonoid content of the extract and fractions of A. paniculata
A method and procedure of determining the total phenolic content was employed by Singleton & Rossi [23] as described by Olawuni *et al.*,[24] using the Folin-Ciocalteu's phenol reagent which is an oxidizing reagent.

Determination of total phenolic content (TPC)
Briefly, 0.1 mL of the sample was diluted to 1 mL with distilled water followed by addition of 0.2 mL of Folin-ciocalteu's phenol reagent, mixed and allow to stand for 5 minutes. This is followed by addition of 1 mL of 7% (w/v) Na_2CO_3 solution and make up to 2.5 mL before incubating for 90 minutes at room temperature. The absorbance was read at 750 nm. Gallic acid (0,1 mg/mL) was used as standard in order to determine the Gallic acid Equivalent (GAE) of the sample after preparing a calibration curve (Figure 8). Distilled water was used as blank.

Determination of total flavonoids content (TFC)
The TFC were determined by preparing standard quercetin with varying concentrations to prepare a standard curve in comparison to the sample extract and fractions (Figure 9). This was carried out based on the aluminum chloride colorimetric assay method according to Zhilen[25] as described by Olawuni *et al.*,[24] Briefly, 0.1 mL of extract/standard was added 0.4 mL of distilled water. This was followed by 0.1 mL of 5% sodium nitrite. After 5 minutes, 0.1 mL of 10% Aluminum Chloride and 0.2 mL of 1 M sodium hydroxide was added and the volume was made up to 2.5 mL with distilled water. The absorbance at 510 nm was measured against the blank. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the sample.

Antioxidant activity of the crude extract and fractions and isolated compounds from A. paniculata
The crude extract, solvent fractions, and isolated compounds were tested for antioxidant activity using FRAP, TAC and DPPH free radical scavenging assays.

Determination of total antioxidant content (TAC)
The total antioxidant contents of the extract, fractions, and isolated compounds were determined based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex in an acidic pH.[26] 0.1 mL of the crude extract, the fractions and isolated compounds and standard solution of Ascorbic acid (20, 40, 60, 80, 100 µg/mL) was added to 1 mL of the reagent solution which consists of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95^{o}C for 90 minutes. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695 nm against a blank which consist of the reacting mixture containing distilled water in place of the extract and the fractions. The antioxidant activities of the extract and the fractions were expressed as an Ascorbic Acid Equivalent.

Determination of ferric reducing antioxidant power (FRAP)
The ferric-reducing antioxidant power of the extract, fractions, and isolated compounds was evaluated according to the method of Benzie & Strain.[27] The FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method with absorbance measured with a spectrophotometer. The principle of this method is based on the reduction of a colorless ferric-tripyridyltriazine complex to its blue ferrous colored form owing to the action of electron-donating in the presence of antioxidants. A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio 10:1:1 respectively, to give the working FRAP reagent. A 50µL aliquot of the extract, solvent fractions, and the isolated compounds at 0.1 mg/mL and 50 µL of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/mL) was added to 1 mL of FRAP reagent. Absorbance measurement was taken at 593 nm exactly 10 minutes after mixing against reagent blank containing 50 µL of distilled water. All measurement were taken at room temperature with samples protected from direct sunlight. The assay was carried out in three replicates. And the antioxidant activity of the extract was expressed as the number of mg equivalents of ascorbic acid (AAE)/g extract.

Determination of DPPH free scavenging assay
The free radical scavenging ability of the crude methanol extract, fractions, and the isolated compounds was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazylhydrate) as described by Brand-Williams *et al*.[28] Antioxidant compound, which can donate hydrogen on reaction with DPPH, leads to its reduction.[29] The change in color from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of the extract, fractions, isolated compounds or Standard (ascorbic acid) in a test tube was added 1 mL of 0.3 mM of DPPH in methanol. The mixture was mixed and incubated in the dark for 30 minutes after which the ab*sor*bance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract.
The percent of inhibition was calculated in the following way:

$$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$$

Where A_{blank}: is the absorbance of the control reaction (containing all reagents except the test compound and A_{sample}: is the absorbance of the test compound. Sample concentration containing or providing 50% inhibition (IC_{50}) is calculated from the graph plotting inhibition percent against exact concentration.

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In vitro α-amylase inhibitory assay

The crude methanol extract, fractions, and isolated compounds were tested for α-amylase inhibitory assay. This was carried out according to the method of Giancarlo *et al*. with some modifications.[30] The starch solution (1% w/v) was prepared by dispersing 1.0 g of potato starch in about 60 mL sodium phosphate buffer with pH of 6.9 (prepared by mixing 56.3 mL 0.2 M solution of NaH_2PO_4 and 69 mL 0.2 Na_2HPO_4 and then made up to 250 mL in a standard volumetric flask) inside a 200 mL beaker. The suspended solution obtained was boiled for 30 minutes and transferred into 100 mL standard volumetric flask and made up to the mark with the buffer. The α-amylase enzyme solution (50 unit/mL) was prepared by mixing 0.01 g of α-amylase in 10 mL of sodium phosphate buffer (pH 6.9) prepared as described above but contained 0.0006 mM sodium chloride. The extracts were dissolved in distilled water to give concentrations between 1000, 500, 400, 300, 200 and 100 µg/mL. The dinitrosalicylic acid reagent was prepared by weighing 0.1 g of 3,5-dinitrosalicylic acid, 2.99 g of sodium potassium tartrate and 0.16 g o f sodium hydroxide into 25 mL beaker and then dissolved with about 5 mL of the buffer prepared above. The solution obtained was transferred into 10 mL standard volumetric flask and beaker rinsed with small portion of in the buffer and then made up to the mark with the phosphate buffer. 50 µL microliter of crude methanol extract, n-hexane, aqueous methanol fractions and isolated compounds and 150 µL of starch solution as well as 10 of the α-amylase enzyme were mixed in a 96 well microplate and incubated at 37^{o}C for 30 mins, then, 20 µL of sodium hydroxide and 20 µL of dinitrosalicylic acid reagent were added and the closed micro titre plate was placed in a water bath set at 100^{o}C. After 20 mins, the mixture was removed from the water bath and cooled; thereafter, α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm using spectrophotometer (Elisa stat fax 2100, Awareness Technology Inc., USA). Blank samples were used to correct the absorption of the mixture, in which the α-amylase enzyme was replaced with buffer solution. Also, a control reaction was conducted, in which the extract, fractions, and isolated compounds were replaced with 50 µL of distilled water, and the maximum enzyme activity was determined. By removing the extract, fractions, and isolated compounds from solution, all the interferences from extract, fractions, and isolated compounds such as color or self-inhibitory interference with the assay were removed.

The % inhibition was calculated according to the following equation:

$$\% \text{ Inhibition} = \left\{\frac{Acontrol - Asample}{Acontrol}\right\} \times 100$$

$\mathbf{A}_{control}$ = absorbance of control sample and $\mathbf{A}_{sample}$ = absorbance of tested samples.

IC_{50} was calculated by plotting % inhibition against concentration and estimating the concentration at 50% of inhibition. The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

Statistical analysis

All the assays were performed in triplicate and a concentration-response curve was obtained by plotting inhibition percentages against concentrations. IC_{50} value of sample was obtained graphically by an inhibition curve. Data were subjected to descriptive statistical calculation using GraphPad® Instat Statistical Package (Prism 5) and expressed as mean values ± standard error of the mean.

Isolation of Compounds from Aqueous Methanol Fraction of Andrographis paniculata

The methanol fraction (70 g) was subjected to open column chromatography (8 cm internal diameter and 75 cm length) using 60-200 mesh Silica gel as stationary phase and eluted with a gradiently starting with n-hexane/ethyl acetate, ethyl acetate/methanol. Eluates were collected in 109 conical flasks (100 mL each). The content of the conical flasks was each analyzed on pre-coated aluminium TLC plates with appropriate solvent systems. Sub-fractions with similar TLC profile were subsequently bulked together into 11 sub-fractions (AP-1 – AP-11). Sub-fractions AP-3 – AP-5 were combined together (1755 mg) and further purified on a column (2 cm internal diameter and 40 cm length) of 25g of Sephadex LH-20 eluted isocratically with *n*-hexane/ethyl acetate (3:2). The crystals obtained from the combined sub-fractions above (AP-3 – AP-5) were re-crystallized from ethanol to give colourless needle-like crystals (1464 mg), as compound **1**. Sub-fraction AP-6 (204 mg), a yellowish-white crystalline solid was subjected to repeated recrystallization with ethanol to give white crystals (167 mg) as compound **2.**

Results and Discussion

The structures of compounds **1** and **2** were determined by 1D-NMR (^{1}H, ^{13}C and DEPT 135), Mass Spectrometry and the structures were confirmed by X-ray crystallographic analysis (Supplementary material).

*Compound **1***

Compound **1 (AP-A1)** was obtained as colorless needle-like crystal with m.p. 168-171^{0}C. It yielded a quasimolecular ion peak $[M+Na]^{\pm}$ at m/z 357.2047 (Calc. 357.2042, $C_{20}H_{30}O_4Na$) in the TOF MS ESI+ (positive mode) thus suggesting a molecular formula $C_{20}H_{30}O_4$ and six degree of unsaturation. Other fragment ions were at m/z 317.2117 $[M+H-OH]^+$ and 669.4377 $[2M+H]^+$. The IR spectrum of compound **1** showed absorption bands; O-H group (3284 cm^{-1}), α, β-unsaturated γ-lactone group (1755 and 1637 cm^{-1}), and *exo*-methylene group (902 cm^{-1}). The ^{1}H NMR spectrum (Supplementary material) (300 MHz, in DMSO-d_6) of compound **1** showed signals at (δ ppm): 1.30 (2H, m, H-1), 2.29 (1H, H-2), 1.71(1H, H-2), 3.38 (1H, H-3), 1.16 (1H, s, H-5), 7.45 (1H, m, H-14), 4.83 (2H, m, H-15), 4.80 (1H, s, H-17), 4.59 (1H, s, H-17), 4.15 (1H, s, H-19), 4.12 (1H, s, H-19), 1.59 (3H, s, CH_3-18) and 0.60 (3H, s, CH_3-20). The ^{13}C NMR spectrum (75 MHz, in DMSO-d_6) also shows a signal for 20 carbon atoms (Table 2). A carbonyl signal at 174.3 ppm was ascribed to the C-16 of the lactone part of the diterpenoid. An exo-methylidene double bond C-17 signal at 106.7 ppm. DEPT 135 showed 9 CH_2 and 4 CH and 2 CH_3 groups suggesting the presence of 5 quarternary carbons (Supplementary material). Based on the ^{1}H, ^{13}C and DEPT 135 NMR data (Table 2) and comparison of the data given in the literature (Figure 1).[31,32] The structure of compound **1** was identified as 14-deoxyandrographolide. The x-ray crystallographic data analysis also agreed with Bhattacharyya *et al*.[33] (Figure 2 and other supplementary material).

Compound 2

Compound **2 (AP-A2)** was also obtained as colourless plate-like crystals with a melting point of 228-230˚C. This also, yielded a quasimolecular ion peak $[M+Na]^{\pm}$ at m/z 373.1991 (Calc. 373.1993, $C_{20}H_{30}O_5Na$) in the TOF MS ESI+ (positive mode) thus revealing a molecular formula $C_{20}H_{30}O_5$ and six degree of unsaturation. Other fragment ions include m/z 701.4261 $[2M+H]^+$, 723.4084 $[2M+Na]^+$. The IR spectrum of compound **2** also showed absorption bands; O-H group (3400 cm^{-1}), α, β-unsaturated γ-lactone group (1728 and 1674 cm^{-1}) and *exo*-methylene group (908 cm^{-1}). The ^{1}H NMR spectrum (Supplementary material) (300 MHz, in DMSO-d_6) of compound **2** showed signals at (δ ppm): 1.22 (2H, m, H-1), 2.47 (2H, H-2) 4.39 (1H, H-3), 1.88 (1H , s, H-5), 4.62 (1H, m, H-14), 4.42 (2H, d, H-15), 4.81 (1H, s, H-17), 4.62 (1H, s, H-17), 4.59 (1H, s, H-19), 4.15 (1H, s, H-19), 0.66 (3H, s, CH_3-20) and 1.08 (3H, s, CH_3-18). The ^{13}C NMR spectrum revealed a carbonyl signal at 170.0 ppm which was ascribed to the C-16 of the lactone part of the diterpenoid. An exo-methylidene double bond, a C-17 signal at 108.3 ppm. DEPT 135 also revealed 8 CH_2 and 5 CH and 2 CH_3 groups and 5 quarternary carbons. Based on the ^{1}H, ^{13}C and DEPT 135 NMR data (Table 2) and comparison of the data given in the literature (Figure 1).[34] The structure of compound **2** was identified as andrographolide. The x-ray crystallographic data obtained is in agreement with that of Reddy *et al*. [35] (Figure 3 and other supplementary material).

*X-Ray crystallographic analysis of compounds **1** and **2***

The single crystals of compounds **1** and **2** were separately obtained from a solution of the compounds in methanol by slow evaporation at room temperature (Table 3).

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Qualitative phytochemical screening of *A. paniculata* showed the presence of saponins, tannins, alkaloids, flavonoids, resins, glycosides and terpenoids while phlobatannins, sterol, carbohydrate and phenols were found absent. This is in agreement with previous reports on *A. paniculata*. [36-38] However, this present study showed the presence of resins (Table 1). Phenolic compounds are one of the main classes of phytochemicals that can be found ubiquitously in many plants, they are very potent anti-oxidants and free radical scavengers which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers.[39] The total phenol content (TPC) of the crude and solvent fractions of *Andrographis paniculata* expressed in mg gallic acid equivalent/g (GAE/g) varied from 3.69 ± 0.17 to 9.42 ± 0.95. The methanol fraction with a TPC value of 9.42 ± 0.95 displayed the highest amount of TPC amongst the extract and fractions and this agrees with the work of Moure and co-workers which demonstrated that high polarity solvent will usually yield high amounts of polyphenolics.[40] The methanol fraction has also shown the highest amount of the Total Flavonoid Content (TFC) expressed in mg quercetin equivalent/g (QUE/g) with a value of 40.08 ± 17.93 followed by the *n*-hexane fraction then the crude extract (Figure 4).

It has been reported that oxidative stress plays a major role in the development of diabetes complications as a result of the formation free radicals which are disproportionate during diabetes due to oxidation of glucose and subsequent oxidative degradation of glycated proteins.[41] That the diabetic individual also has a pronounced cellular oxidative stress and reduced antioxidant potential leads to defective antioxidant status.[42] The aqueous methanol fraction exhibited the highest activity in the DPPH free radical scavenging assay with IC_{50} of 0.37 ± 0.01 mg/mL compare to the standard drug, ascorbic acid with IC_{50} of 0.02 ± 0.01 mg/mL while *n*-hexane fraction showed the least activity (Figure 5). Also, the methanol fraction exhibited the highest ferric reducing anti-oxidant power (FRAP) expressed in mg ascorbic acid equivalent/g (mg AAE)/g with a value of 20.42 ± 1.08 (Figure 6). However, unexpectedly, the *n*-hexane fraction showed highest activity in total antioxidant capacity (TAC), which probably may be due to the high concentration of chlorophyll in the *n*-hexane fraction (Figure 6). It has been earlier reported that there is a correlation between chlorophyll content and total antioxidant capacity and that chlorophyll possess antioxidant activities.[43] α-Amylase is a salivary or pancreatic enzyme which usually plays a major role in the metabolism or breakdown of complex carbohydrates into simple sugars such as glucose, fructose, galactose etc. The inhibitory activities of the crude extract, solvent fractions, and the isolated compounds from *A. paniculata* were investigated on the α–amylase enzyme and IC_{50} values were calculated. The crude extract, n-hexane, and methanol fractions exhibited better α-Amylase inhibitory activity (lower IC_{50}) than the standard reference drug, acarbose, which is in turn, better than the isolated compounds from *A. paniculate* (Figure 5). The test samples demonstrated inhibitory concentration-dependent effects on α-amylase activity. The strongest activity (1 mg/mL) was shown by the methanol fraction followed closely by crude methanol extract (Figure 7). They all possess high inhibitory activities at high extract/fraction concentrations. The isolated compounds displayed varying degree of *α*-amylase inhibitory potentials at different concentrations. Compound **1** and **2** exhibit a lower inhibitory activity with an IC_{50} 0.637 ± 0.003 and 0.761 ± 0.013 mg/mL respectively compared with acarbose, a standard reference drug, which showed α-amylase inhibitory activity with an IC_{50} of 0.127 ± 0.013 mg/mL. However, Compound **2**, Andrographolide, has been reported to exhibits a dose-dependent inhibition in the treatment diabetes associated with cognitive deficits in rats. It was also reported that andrographolide reduces the activity of acetyl-cholinesterase enzyme during diabetes progression and also inhibits oxidative stress, which improves diabetic hyperglycemia and insulin deficiency.[44] It has been also reported that an ethanolic extract of *A. paniculata* and andrographolide showed an alpha-glucosidase inhibitory effect in a concentration-dependent manner, these may justify its potential use for the management of type 2 diabetes mellitus.[45]

The presence of *α*,*β*-unsaturated *γ*-lactone and a furan ring system may probably be involved in inhibiting both *α*-amylase and α-glucosidase activities of *A. paniculata* and isolated compounds.

Table 1: Phytochemical Screening of the Crude Methanol Extract of *A. paniculata*

Phytochemicals	Inference
Tannins	+
Glycosides	+
Resins	+
Saponins	+
Phlobatannins	-
Flavonoids	+
Sterols	-
Carbohydrates	-
Alkaloids	+
Terpenoids	+

Key: – Not detected, + present

Table 2: ^{1}H (300 MHz, DMSO-d_6) and ^{13}C (75 MHz, DMSO-d_6) NMR data of Compound **1** and **2**

	Compound 1		Compound 2	
Position	^{1}H (ppm) *J* (Hz)	^{13}C (ppm)	^{1}H (ppm) *J* (Hz)	^{13}C (ppm)
1	1.30 (m, 2H)	37.5	1.22 (m, 2H)	37.5
2	2.29 (1H), 1.71(1H)	27.9	2.47	27.9
3	3.38 (1H)	78.5	4.39	78.5
4	-	42.3	-	42.3
5	1.16	55.4	1.88 (s, 1H)	55.5
6	1.87	24.2	1.18 (m, 2H)	23.1
7	6.46 s	38.9	1.67 (m, 2H)	38.6
8	-	147.5	-	147.6
9	3.48	54.6	2.51(br, 1H)	54.4
10	-	39.0	-	38.9
11	2.49 (2H)	21.6	2.49 m	24.0
12	2.51 (2H)	23.9	6.62 m	146.4
13	-	132.2	-	129.0
14	7.46 (br, 1H)	147.0	4.62 m	62.7
15	4.83 (m, 2H)	70.5	4.42 d	74.4
16	-	174.3	-	170.0
17	4.80, 4.59 (s, 1H)	106.7	4.81, 4.62 (s, 1H)	108.3
18	1.59 (s, 3H)	23.1	1.08 (s, 3H)	23.1
19	4.15, 4.12 (s, 1H)	62.7	4.59, 4.15 (s, 1H)	64.5
20	0.60 (s, 3H)	14.9	0.66 (s, 3H)	14.8

s – singlet, d - doublet, m – multiplet, br – broad

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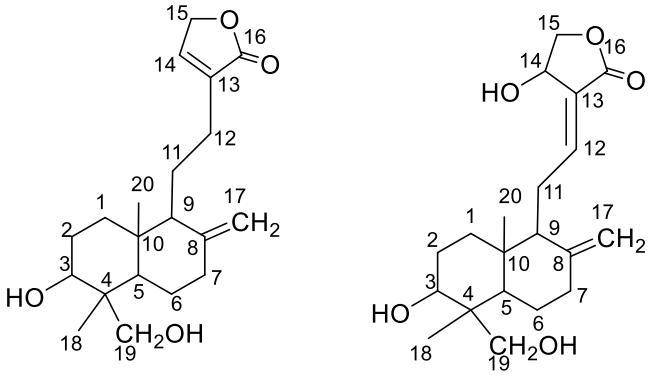
Table 3: X-Ray Crystallographic Data of Compounds **1** and **2**

	Compound 1	**Compound 2**
Formula	$C_{20}H_{30}O_4$	$C_{20}H_{30}O_5$
Molecular Weight	334.2144	350.2093
Space Group	$P2_1$	$P2_1$
Cell parameters (Å, deg)		
a	6.744(2)	6.533(3)
b	6.988(2)	10.636(2)
c	19.377(3)	17.946(8)
α	90.00˚	90.00˚
β	93.19(3)˚	97.47(< 1)˚
γ	90.00˚	90.00˚
V(Å^3)	911.766	931.007
Z	2	2
T(K)	283-303	283-303
CCDC	282628	264212



1 **2**

Figure 1: Structures of Compounds Isolated from *Andrographis paniculata*

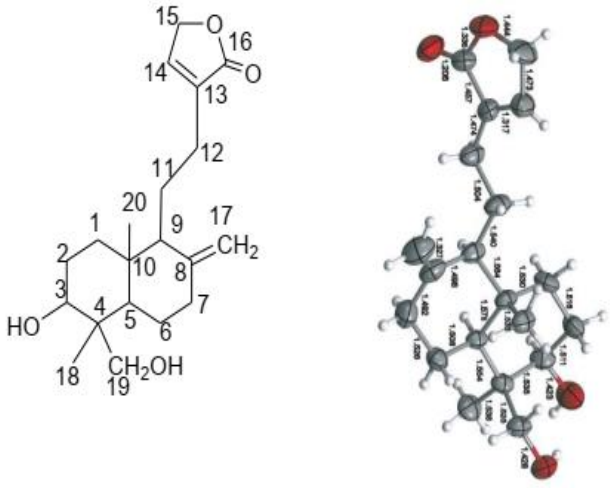


Figure 2: Structure and ORTEP Drawing of Compound **1** from X-ray Crystallographic Analysis

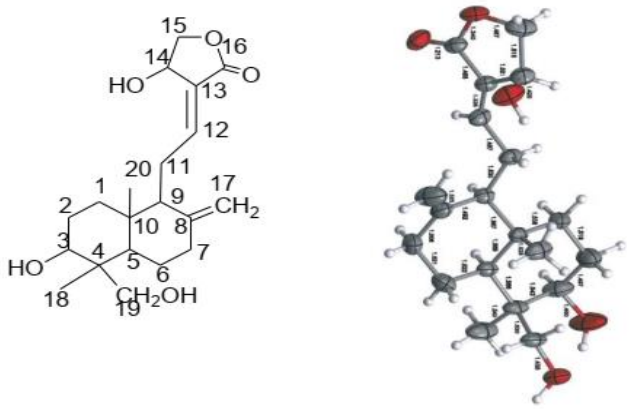


Figure 3: Structure and ORTEP Drawing of Compound **2** from X-ray Crystallographic Analysis

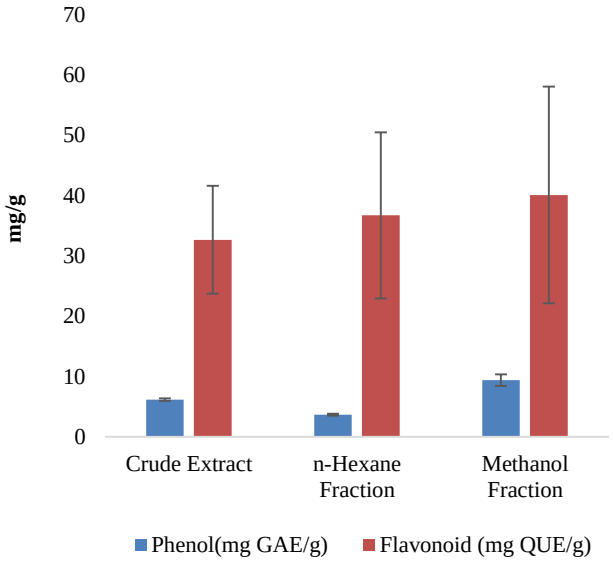


Figure 4: Total Phenolic and Flavanoids Contents of the Extract and Solvent Fractions

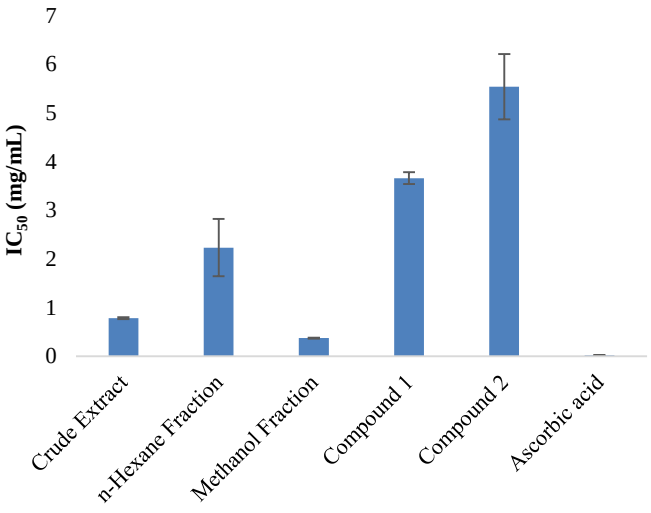


Figure 5: IC_{50} of DPPH Free Radical Scavenging Activity of Extract, Solvent Fractions and Isolated Compounds

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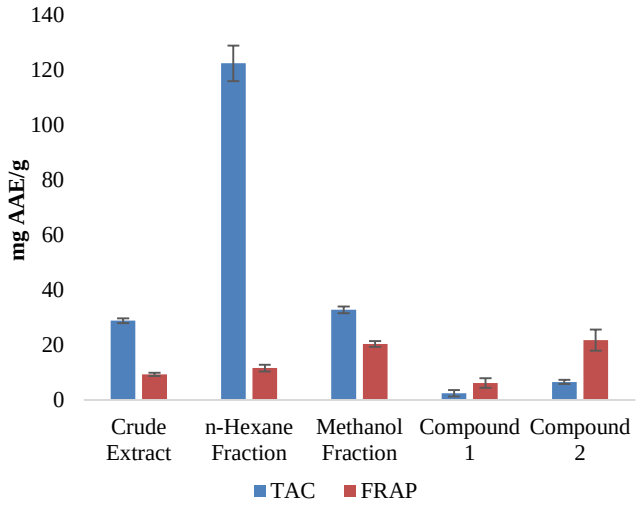

Figure 6: Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP) of Extract, Solvent Fractions and Isolated Compounds

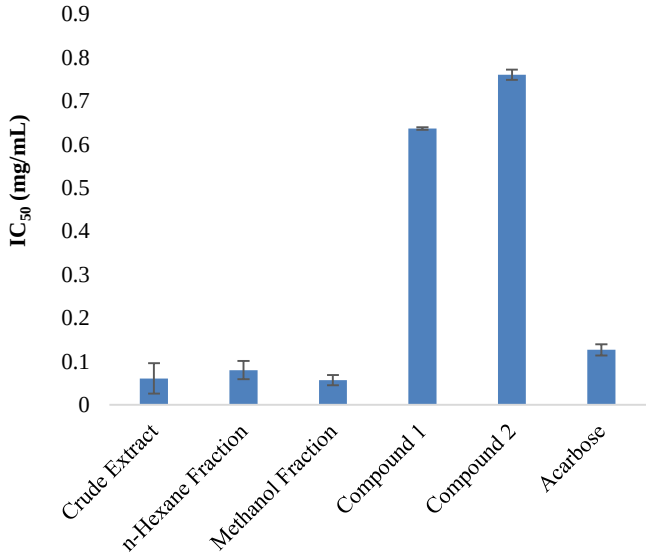

Figure 7: IC_{50} of *In vitro* α-Amylase Inhibitory Assay of Extract, Solvent Fractions and Isolated Compounds

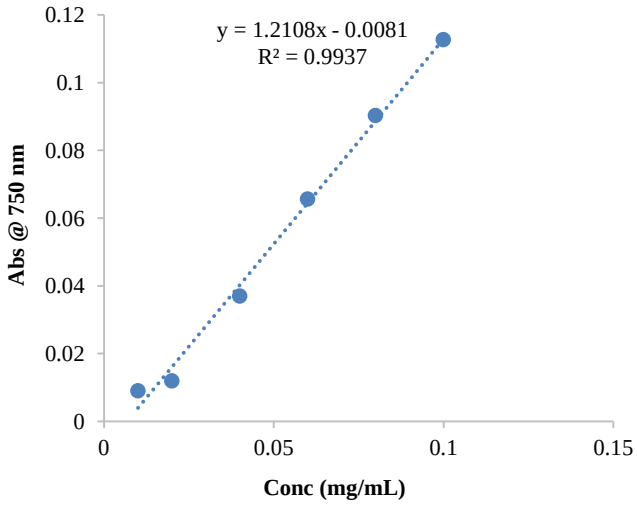

Figure 8: Gallic acid Calibration Curve for Total Phenol Content

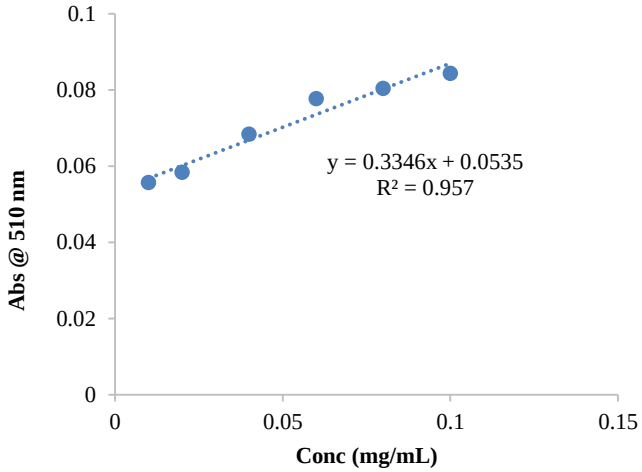

Figure 9: Quercetin Calibration Curve for Total Flavonoid Content

Among all, methanol extract has shown best enzyme inhibitory activity with an IC_{50} value 0.057 ± 0.012 mg/mL which is better than that of the standard drug, acarbose (Figure 7). The phytochemical screening on the extract of *A. paniculata* carried out in this study has revealed the presence of saponins, terpenoids, tannins, alkaloids, flavonoids, glycosides and resins which may contribute to the activities. It was reported in a previous study that lupeol, a pentacyclic triterpenoid isolated from stem bark of *C. macroptera* have the potential to exhibit α-amylase inhibitory activity.[5,44] The study also suggested the mechanism by which this fruit extract exerted this effect may be due to its action on carbohydrate binding regions of α-amylase enzymes that catalyse hydrolysis of the internal α-1,4 glucosidic linkages in starch and other related polysaccharides have also been targeted for the suppression of postprandial hyperglycemia.[5] Saponins also have been reported to be a potential probable α-amylase inhibitor.[8]

Conclusion

This study revealed that *Andrographis paniculata* extracts and isolated compounds had relative strong antidiabetic but moderate antioxidant activities. The result of the α–amylase activity inhibitory assay may give credence to the use of *Andrographis paniculata* extract/isolated compounds in the management of diabetes mellitus. This may lead to the development of a new source of antidiabetic agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Global Report on diabetes – (WHO). [Online]. 2016 [cited 2019 August 13]. Available from: https://www.who.int/health-topics/diabetes#
2. Ajayi OS, Aderogba MA, Obuotor EM, Majinda RRT. Acetylcholinesterase inhibitor from *Anthocleista vogelii* leaf extracts. J. Ethnopharmacol. 2019; 231:503-506.
3. Rauf A and Jehan N. Natural Products as a Potential Enzyme Inhibitors from Medicinal Plants (Murat Senturk). [Online]

2017 [cited 2021 March 15]. Available from: https://www.intechopen.com/books/enzyme-inhibitors-and-activators/natural-products-as-a-potential-enzyme-inhibitors-from-medicinal-plants.
4. Sulaimon LA, Anise EO, Obuotor EM, Samuel TA, Moshood AI, Olajide M, Fatoke T. In vitro antidiabetic potentials, antioxidant activities and phytochemical profile of african black pepper (*Piper guineense*). Clin Phytosci. 2020; 6:90.
5. Uddin G, Rauf A, Siddiqui BS, Muhammad N, Khan A, Shah SUA. Anti-nociceptive, anti-inflammatory and sedative activities of the extracts and chemical constituents of *Diospyros lotus* L. Phytomedicine. 2014; 21:954-959.
6. Bailey CJ. New approaches to the pharmacotherapy of diabetes. In: Pickup, J.C., William, G. (Eds.), Textbook of Diabetes. 2003; 3:1-73.
7. Maryam Y, Seyed MA, Farideh T. Effect of ultrasonic power on the activity of barley's alpha-amylase from post-sowing treatment of seeds. World Appl Sci J. 2008; 3(1):91-95.
8. Kumar DJM, Jayanthisiddhuraj B, Amutha B, Devi DM, Kumaran MDB, Kalaichelvan PT. Purification and characterization of -amylase and -galactosidase from *Bacillus Sp*. MNJ23 produced in a concomitant medium. American-Eurasian J Agric & Environ Sci. 2012; 12(5):566-573.
9. Poovitha S and Parani M. *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Compl. Altern Med. 2016; 16(S1):1-8.
10. Selfayan M and Namjooyan F. Inhibitory Effect of *Capparis spinosa* Extract on Pancreatic Alpha-Amylase Activity. Zahedan J Res Med Sci. 2016; 18(4):e6450.
11. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(5):1047-1053.
12. Oguntibeju O. Antioxidant-Antidiabetic Agents and Human Health. In: Oyagbemi AA, Salihu M, Oguntibeju OO, Esterhuyse AJ, Farombi EO. Some Selected Medicinal Plants with Antidiabetic Potentials. United Kingdom: IntechOpen Limited; 2014. 95-113 p.
13. Mohammed SA, Yaqub AG, Sanda KA, Nicholas AO, Arastus W, Muhammad M, Abdullahi, S. Review on diabetes, synthetic drugs and glycemic effects of medicinal plants. J Med *Plants* Res. 2013; 7(36):2628-2637.
14. Rafi M, Devi AF, Syafitri UD, Heryanto R, Suparto IH, Amran MB, Rohman A, Prajogo B, Lim LW. Classification of *Andrographis paniculata* extracts by solvent extraction using HPLC fingerprint and chemometric analysis. BMC Res Notes. 2020; 13(56):1-6.
15. Ghosh BK, Datta AK, Mandal A, Dubey PK, Halder S. An overview on *Andrographis paniculata* (Burm. F.) Nees. *Int J* Res Ayurveda Pharm. 2012; 3(6):752-760.
16. Polash SA, Saha T, Hossain MS, Sarker SR. Investigation of the phytochemicals, antioxidant, and antimicrobial activity of the *Andrographis paniculata* leaf and stem extracts. Adv. Biosci. Biotechnol. 2017; 8:149-162.
17. Joseph SM and Joseph JT. Bicyclic Diterpenoid and Phytosterol Constituents from *Andrographis Paniculata* (NEES). Orient. J. Chem. 2016; 32(4):2231-2233.
18. Pramanick S, Banerjee S, Achari B, Das B, Sen AK, Mukhopadhyay S, Neuman A, Prange T. Andropanolide and Isoandrographolide, minor diterpenoids from *Andrographis paniculata*: Structure and X-ray crystallographic analysis. J. Nat. Prod. 2006; 69:403-405.
19. Kumar A, Dora J, Singh A, Tripathi R. A review on King of Bitter (Kalmegh). Int. j. res. pharm. Chem. 2012; 2(1):116-124.
20. Toma GS, Tiwari SK, Chaturvedi GN. Kalmegh (*Andrographis paniculata* Nees) and its medicinal status. Nagarjun. 1982; 26:76-78.
21. Zhao HY and Fang WY. Antithrombotic effects of *Andrographis paniculata* Nees in preventing myocardiac infarction. Chin Med J. 1991; 104(9):770-775.
22. Balu S and Alagesaboopathi C. Anti-inflammatory activities of some species of *Andrographis* Wall. Anc Sci Life. 1993; 13:180-184.
23. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. Am. J. Enol. Vitic. 1965; 16:144-158.
24. Olawuni IJ, David OA., Bamidele FS and Ogundepo GE. (2019). Antioxidants and radical scavenging activities of Nigerian Soybeans (Glycine max (L.) Merr.). Eur J Med Plants. 27(1):1-8.
25. Zhilen J, Mengeheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64:555-559.
26. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum Complex: specific application to the determination of vitamin E. Anal Biochem. 1999; 269:337-341.
27. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. Anal Biochem. 1996; 239:70-76.
28. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol. 1995; 28:25-30.
29. Blois M. Antioxidant Determinations by the use of a stable free radical. Nature. 1958; 181**:**1199-1200.
30. Giancarlo S, Rosa LM, Nadjafi F. Francesco, M. Hypoglycaemic activity of two spices extracts: *Rhus coriaria* L. and *Bunium persicum* Boiss. Nat Prod Res. 2006; 20:882-886.
31. Zhou KL, Chen LX, Zhuang YL, Wang NL, Yao XS, Qiu F. Two new ent-labdane diterpenoid glycosides from the aerial parts of *Andrographis paniculata*. J Asian Nat Prod Res. 2008; 10:939-943.
32. Poonam K, Tiwari UK, Shukla A, Gaur AK. Chemical constituents isolated from *Andrographis paniculata*. Indian J Chem. 2010; 49: 356-359.
33. Bhattacharyya K, Kar T, Bocelli G, Cantoni A, Pramanick S, Banerjee S, Mukhopadhyay S. Redetermination of 14-deoxyandrographolide. Acta Crystallographica Section E: Struct Rep Online. 2005; E61:02743-02745.
34. Wei XH, Yang SJ, Liang N, Hu DY, Jin LH, Xue W, Yang S. Chemical Constituents of *Caesalpinia decapetala* (Roth) Alston. Molecules 2013; 18(1):1325-1336.
35. Reddy VLN, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV, Rao TP, Ram TS, Gonnade RG, Bhadbhade M, Venkateswarlu Y. A new BIS-Andrographolide Ether from *Andrographis paniculata* Nees and evaluation of anti-HIV activity. Nat Prod Res. 2005; 19:223-230.
36. Singh A, Meena AK, Sudeep Meena, Pant P, Padhi MM. Studies of Standardization of *Andrographis paniculata* Nees and identification by HPTLC using andrographolide as marker compound. Int J Pharmacol. 2012; 4(2):197-200.
37. Lalitha G, Nazeema TH, Sharmila L. Phytochemical screening and evaluation of antimicrobial activity, antioxidant activity, anticoagulant activity and fibrinolytic activity of leaves of *Andrographis paniculata* (leaf). *Int J Pharma. Bio. Sci.* 2015; 6(2):475-484.
38. Tandon C, Mathur P, Sen M. *Andrographis paniculata* Nees (Kalmegh): A review on its antibacterial activities and phytocompounds. *European* J Med Plants. 2015; 8(1):1-10.
39. Zohre MD, Foroogh N, Mohamad EA. Alpha amylase inhibition activity of some plants extract of *Teucrium* Species. European J Biol. Sciences. 2015; 7(1):26-31.
40. Moure A, Cruz J, Franco D, Domínguez J, Sineiro J, Domínguez H, José Núñez M, Parajó J. Natural antioxidants from residual sources. Food Chem. 2001; 72:145-171.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

41. Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. Int. J. Biochem Cell Biol. 2006; 38:794-803.
42. Keerthana G, Kalaivani MK, Sumathy A. *In vitro* alpha amylase inhibitory and antioxidant activities of ethanolic leaf extract of *Croton bonplandianum*. Asian J Pharm Clin Res. 2013; 6(4):32-36.
43. Cuong DX. Antioxidant chlorophyll purification from maize leaves by liquid-to-liquid extraction method. J *drug* deliv ther. 2020; 10(3):152-158.
44. Thakur AK, Rai G, Chatterjee SS, Kumar V. Beneficial effects of an *Andrographis paniculata* extract and andrographolide on cognitive functions in streptozotocin-induced diabetic rats. Pharm Biol. 2016; 54(9):1528-1538.
45. Subramanian R, Asmawi MZ, Amirin Sadikun. Effect of Andrographolide and Ethanol Extract of Andrographis paniculata on Liver Glycolytic, Gluconeogenic, and Lipogenic Enzymes in a Type 2 Diabetic Rat Model. Pharma Biol. 2008; 46(11):772-780.