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Phytochemical Characterization and Antioxidant Bioactivity of *Andrographis paniculata* (Nees)

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Abstract

Background: Medicinal plants are natural sources of antioxidants effective in the treatment of radical-mediated diseases. This study evaluated the *in-vitro* antioxidant and phytochemical constituents of the methanolic leaves extract of *Andrographis paniculata*.

Methods: Fresh *A. paniculata* leaves were harvested from a local farm, air-dried and extracted with methanol. Chemical composition, antioxidant activities, and α -amylase enzyme inhibitory potentials of the extract were determined.

Results: The extract of *A. paniculata* concentration-dependently scavenges 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acids) (ABTS) radicals. It scavenges nitric oxide radicals with IC₅₀ of 145.99 μ g/ml compared to 167.17 μ g/ml of standard ascorbic acid and has 41% activity of standard ascorbic acid ferric reducing power. The extract also inhibited the induction of lipid peroxidation and α -Amylase activity in a concentration-dependent manner. The phytochemical assays employed revealed the presence of various phytochemicals in the extract. Further analysis with gas-chromatography revealed the possible presence of Andrographolide, Deoxyandrographolide, Apigenin, Kaempferol, Quercetin, Methyl vanillate, Methyl Caffeate, Beta-sitosterol, Vanillic acid in the extract. The total phenolics content was found to be 29.11 mg GAE/g, and proximate analysis revealed the moisture content, crude protein, crude fat, crude fiber, total ash, and Nitrogen free extract to be 21.89%, 5.66%, 8.74%, 0.95%, 6.87%, and 55.89% respectively.

Conclusion: The plant *A. paniculata* demonstrated good antioxidant potentials and contain various phytochemicals. Therefore, it could be inferred that the effectiveness of *A. paniculata* as a medicinal plant could be due to the presence of various phenolics and antioxidant compounds in the plant.

Keywords: Phytochemical constituents, *Andrographis paniculata*, Antioxidant, Lipid peroxidation, Gas-chromatography

1.0 INTRODUCTION

Plants are enriched with several phytochemical compounds such as tannins, coumarins, quinones, anthraquinone, amine, triterpenoids, lignans, flavonoids and many other secondary metabolites, which are a great source of reactive oxygen species scavengers [1,2]. They also contain antioxidant compounds and have been reported to have wound healing, anticancer, anti-mutagenic, anti-inflammatory and antihypertensive properties in many studies [1,3]. Consumption of a rich diet in natural antioxidants has been shown to decrease cardiovascular disease and reduce the risk of cancer, age-related disease among others [4].

Researchers globally in the last decade are working towards the employment of natural phytochemicals present in herbs, leafy vegetables, beans, berry, cherries and crops in the management of diseases [5]. Many secondary plant metabolites with limited research studies are being subjected to extensive investigation to determine their suitability as a pharmacological agent [1]. *Andrographis paniculata* also called the king of bitters is a traditional herbaceous plant of the family Acanthaceae. It is widely cultivated across Europe, Asia, Africa, and used to manage various ailments [6,7]. Several studies have demonstrated the anticancer, anti-inflammatory, hypotensive property, antiangiogenic, antihyperglycemic and antimalarial properties of *A. paniculata* extract [8-10].

Many bioactive compounds such as andrographolide, 14-deoxy-11,12-didehydroandrographolide, kaempferol, quercetin, 14-deoxy andrographolide and many others have been isolated from *A. paniculata* [11]. The role of andrographolide (major bioactive compound of *A. paniculata*) was highlighted in a study to be (i) abatement of acute brain injury in Wistar rats [12], (ii) amelioration of permanent middle cerebral artery occlusion (pMCAO), (iii) reduced neurological deficits in mice and (iv) good antiviral potency [13,14]. Therefore, this study aims to determine the phytochemical composition and antioxidant efficacy of *A. paniculata* methanolic extract in the quest to strengthen scientific knowledge.

2.0 METHODOLOGY

2.1 Reagents

Ferric chloride, dinitro salicylic acid reagent, butanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ethanol, Folin-Ciocalteu reagent, starch, thiobarbituric acid, sodium carbonate, sodium chloride, sodium nitroprusside, naphthyl ethylenediamine dichloride, α -amylase, glacial acetic acid and potassium hexacyanoferrate, and trichloroacetic acid were obtained from Sigma-Aldrich Chemical Co. Ltd. (England).

2.2 Plant Materials and Extract Preparation

A. paniculata leaves were obtained from a local farm at Ibadan, Oyo State. The identification and authentication of the plant were done at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso by Prof A.J. Ogunkunle and a specimen was deposited in the herbarium with voucher number LH0538. The leaves were air-dried at room temperature and grounded into a powder. One hundred gram (100g) of the powdered *A. paniculata* leaves were soaked in 500ml of methanol and shaken for 72 hours. Afterwards, it was filtered and the supernatant was concentrated and evaporated to dryness at 50°C with a rotary evaporator under reduced pressure.

2.3 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The assay was performed as previously described by Schelesier *et al.*, [15]. In the DPPH assay, the radical solution is prepared by dissolving 2.4mg DPPH in 100mls of ethanol. Antioxidants reduce the free radical 2,2-Diphenyl-1-picrylhydrazyl, which has an absorption maximum at 517nm. 1.95ml DPPH was measured as blank, for the photometric assay, 1.95ml DPPH solution and 50 μ l antioxidant solution (plant extract or standard gallic acid) were mixed. The reaction was measured after 30 minutes until $\Delta A = 0.003 \text{ min}^{-1}$. The anti-oxidative activity was calculated using the following equation.

$$\% \text{ Inhibition activity} = \frac{A(\text{DPPH}) - A(\text{Extract})}{A(\text{DPPH})} \times 100$$

Where A= Absorbance

2.4 Trolox Equivalent Antioxidant Capacity Assay (ABTS)

1ml of freshly prepared ABTS solution and various concentrations of extract ranging from 20-100 μ L were mixed for 45 seconds and measured immediately after 1 minute at 734nm. The extract antioxidant activity was determined using the following equations;

% Inhibition activity= ((A(ABTS+)-A(Extracts)) / (A(ABTS) X 100

Where A= Absorbance

2.5 Ferric Reducing Antioxidant property (FRAP) Assay

The Fe³⁺ reducing power of the extract was determined by the method of Oyaizu [16]. Various concentrations of extract at 0.75 mL was mixed with 0.75 mL of potassium hexacyanoferrate [K₃Fe(CN)₆] (1%, w/v) and 0.75 mL of phosphate buffer (0.2 M, pH 6.6). The mixture was incubated for 20 min at 50°C in a water bath. The 10% trichloroacetic acid (TCA) solution 0.75 mL was then added to stop the reaction, this was centrifuged for 10 min at 3000 r/min. The supernatant (1.5 mL) was mixed with 0.1 mL of ferric chloride (FeCl₃) solution (0.1%, w/v) and 1.5 mL of distilled water for 10 min. The absorbance at 700 nm was measured as the reducing power, the higher the absorbance the greater is the reducing power.

2.6 Nitric Oxide Scavenging Activity

This was carried out using the method described by Gar rat [17]. 2ml of 10mM sodium nitroprusside was dissolved in 0.5ml phosphate buffer of PH 7.4. The dissolved mixture was mixed with 0.5ml of *A. paniculata* extract at ranging concentrations. The mixture was incubated at room temperature for two and a half hours. 0.5ml of incubated sample plus 0.5ml of Griess reagent were then re-incubated for thirty minutes and the absorbance was measured at 546nm. Percentage inhibition was calculated as;

$$\text{Inhibition of NO radical} = [A_0 - A_1 / A_0 \times 100]$$

Where A₀ is the absorbance before reaction and A₁ is the absorbance after reaction has taken place with Griess reagent.

2.7 Lipid Peroxidation Inhibition Assay

This is a modified thiobarbituric acid reactive substance (TBARS) assay (18). The lipid source is egg yolk homogenate and Fenton Reagent (FeSO₄/ H₂O₂) was the source of free radicals. The reaction mixture containing 0.5ml egg yolk homogenate (10% in distilled water V/V), 0.05ml FeSO₄ (0.07M), and 0.1ml of the extract was incubated for 30min. The absorbance was read at 532nm and the percentage induction of lipid peroxidation is 100% in the control which is compared to the reduction in the plant extract samples.

$$\text{Induction of lipid peroxidation (\%)} = 100 - [(A_0 - A_1) / A_0 \times 100]$$

Where A₀ is the absorbance of the control and A₁ is the absorbance of the sample

2.8 Inhibition of Alpha-amylase

The determination of α-amylase inhibition was carried out using a modified dinitrosalicylic acid (DNS) method previously described by Bernfeld [19]. 1mL of methanolic extracts of *A. paniculata* were pre-incubated with α-amylase 1 U/mL for 30 min. The starch solution 1 mL (1% w/v) was added to the mixture and incubated for 10 min at 37°C. The 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) was then added to the reaction to stop it. This was followed by heating in a boiling water bath for 5 min. A control was prepared by using buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20°C) instead of plant extracts. The absorbance was read at 540 nm.

$$\% \text{ inhibition} = [A_0 - A_1] / A_0 \times 100$$

Where A₀ is the absorbance of the control and A₁ is the absorbance of the sample

2.9 Phytochemical Composition Screening

Qualitative analysis of *S. siamea* methanolic extract was carried out by testing for the presence of flavonoids, terpenoids, tannins, phenols, saponins, phytosterol, alkaloids, phlobotannins and cardiac glycoside. Each assay was carried out following the method of Sofowora *et al.*, [20] for flavonoids, Ejikeme *et al.*, [21] for terpenoids and tannins, Santhi *et al.*, [22] for phenol, Harbone *et al.*, [23] for saponins, phytosterol and alkaloids, Ajiboye *et al.*, [24], for phlobotaninns and cardiac glycoside

2.10 Determination of total phenolic compound in methanolic extract of *A. paniculata*

Total phenolics of *A. paniculata* were determined by the Folin-Ciocalteu method (Folin Ciocalteu *et al.*) [25]. For the preparation of the calibration curve, 1ml Folin-Ciocalteu reagent (diluted ten-fold) and 4ml (75g/L) sodium carbonate were mixed with 1ml of aliquots of 0.24, 0.075, 0.0105 and 0.3mg/ml ethanol gallic acid solutions. The absorbance was read at 765nm after 30 minutes and the calibration curve was drawn. 1ml Folin-Calceus reagent and 4ml (75g/L) sodium carbonate was mixed with 1ml of *A. paniculata* and after 30 minutes the absorbance

was read for the determination of plant phenolic. The total content of phenolic compounds in *A. paniculata* (Gallic acid equivalent) was calculated using the following formula

$$C = c \cdot v/m$$

Where C is the total content of phenolic compound (mg/g plant extract, in GAE)

c is the concentration of Gallic acid established from the calibration curve in mg/ml

v is the volume of extract in ml

m is the weight of pure plant extract.

2.11 Gas Chromatography Analysis

The gas chromatography (GC) study was done using Shimadzu GC-17A gas chromatography fitted with a Flame Ionization Detector (FID) and an autosampler. GC column used was, fused silica capillary column OV-1, DB-1 (30 m x 0.53 mm, 0.5 µm film thickness), at 75 °C and programmed to 75 °C at 240 °C/min and 5 min hold. Injector and detector were at 240 and 250 °C respectively. About 1 µL of each sample was injected, and the relative quantity of the chemical compounds present in the extract of *A. paniculata* was expressed as a percentage based on the peak area produced in the chromatogram. The identification of *A. paniculata* constituents was carried out by comparison of GC retention times of *A. paniculata* with GC retention times of desired standards compounds.

2.12 Proximate Analysis of *Andrographis paniculata* leaves

The proximate composition of *A. paniculata* leaf powder samples was determined using standard procedures. Moisture content was determined as described by Udo and Ogunwele [26] with slight modification. Ash was determined by incineration (550 °C) of known weights of samples in a muffle furnace [27]. The crude lipid content was determined using the Soxhlet method described by Udo and Ogunwele [26]. The Crude fiber was determined after digesting a known weight of fat-free sample with sulfuric acid and sodium hydroxide as described by Udo and Ogunwele [26]. The crude protein percentage was evaluated using the Miro-Kjeldahl method described by AOAC [28], while nitrogen-free extract (NFE) was determined by addition of all percent of moisture, fat, crude protein, ash and crude fiber subtracted from 100% [29]

2.13 Statistical Analysis

Results are expressed as means ± SEM. Statistical analyses were performed using one-way analysis of variance followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and $p < 0.05$ was considered statistically significant.

3.0 RESULTS

3.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Figure 1. Effect of Time on Different Concentration of Methanolic extract of *A. paniculata* on Inhibition of DPPH Radical

The *A. paniculata* demonstrated a concentration and time-dependent scavenging activity by quenching DPPH radicals and was compared with gallic acid, as a positive control. The IC₅₀ values (defined as the concentration of test compound required to produce 50% inhibition) obtained are 326.37 µg/dL and 16.13 µg/dL for *A. paniculata* and gallic acid respectively (Figure 1 and 2)

3.2 ABTS Radical Cation Scavenging Ability

The scavenging abilities of *A. paniculata* leave against ABTS radicals were evaluated and compared with gallic acid. The scavenging capabilities were found to increase with the increasing concentration of *A. paniculata* and gallic acid. Comparatively, the scavenging abilities of *A. paniculata* were 25% of that of standard gallic acid at the same concentration and experimental conditions.

Figure 2. The Effects of Different Concentrations of *A. paniculata* and Gallic on The Inhibition of the DPPH Radical.

Figure 3. The Effects of Different Concentrations of *A. paniculata* and Gallic on the Inhibition of the ABTS Radical.

3.3 Ferric Reducing Antioxidant Property (FRAP) Assay

The reducing abilities of *A. paniculata* leaves were evaluated and compared with standard ascorbic acid. The reductive capabilities were found to increase with the increase in the concentration of standard ascorbic acid and *A. paniculata*. Comparatively, standard ascorbic acid has greater reducing power than *A. paniculata* with the reducing abilities of *A. paniculata* about 41% of that of standard ascorbic acid (Figure 4).

Figure 4. Ferric Reducing power of *A. paniculata* and Ascorbic Acid at Different Concentrations

3.4 Nitric Oxide Scavenging Activity

The nitric oxide scavenging ability of *A. paniculata* extract and standard ascorbic acid was compared. The *A. paniculata* extract and standard ascorbic acid scavenge nitric oxide radicals in a concentration-dependent manner with IC₅₀ values (known as the concentration needed to caused 50% inhibition) for *A. paniculata* and ascorbic acids given as 145.99 µg/ml and 167.17µg/ml respectively (Figure 5).

Figure 5. The eEffects of Different Concentrations of *A. paniculata* on the Inhibition ofNitric Oxide Radical Formation.

3.5 Lipid Peroxidation Inhibition Assay (TBARS)

The ability of *A. paniculata* extract to inhibit the induction of lipid peroxidation was compared with the control sample which has 100% lipid peroxidation induction. The inhibition of lipid peroxidation induction by *A. paniculata* extract was found to be concentration-dependent with 100µg/mL of the extract inhibited lipid peroxidation induction by 31.85% and 500µg/mL of the extract inhibited lipid peroxidation induction by 74.48% (Figure 6)

Figure 6. The Effects of Different Concentrations of *A. paniculata* on the Induction of Lipid Peroxidation

3.6 Inhibition of α - Amylase

The methanolic extract of *A. paniculata* significantly inhibited α -amylase activity in this study. The level of inhibition was found to be concentration-dependent and the maximum percentage inhibition of α -amylase activity of 81.82% was obtained at 250µg/dL of the extract (Figure 7).

Figure 7. The Effects of Different Concentrations of *A. paniculata* on Inhibition of α -amylase Activity.

3.7 Qualitative Phytochemical Analysis of Powder Sample of *A. paniculata*

The result of the qualitative analysis of the phytochemicals in *A. paniculata* extract is presented in Table 1. The study revealed the presence of alkaloids, flavonoids, tannins, phenol, terpenoids, phlobotanins, cardiac glycosides, and saponin, in the *A. paniculata* extract while phytosterols was absent

Table 1: Phytochemical Composition of the Methanolic Leaves Extract of *A. paniculata*

Constituents	Test	Observation	Inference
Terpenoid	Chloroform test	Brown ring formation	+
Saponins	Foam test	Foam for some minute on the addition of olive oil	+
Phenols	Ferric Chloride Test	Bluish Black	+
Phlobotanins	Hydrochloric acid	Deposit of red precipitate	+
Phytosterols	Chloroform test	No brown ring formation	-
Alkaloids	Mayer's test	Cream colour precipitate	+
Cardiac glycosides	Acetic acid test	Violet-green ring	+
Flavonoids	Alkaline reagent	Yellow colouration formation	+
Tannins	Ferric chloride test	Brownish green colouration	+

3.8 Determination of Total Phenolic Compounds and Proximate Analysis of *A. paniculata*

The proximate analysis result reveals the percentage composition of moisture, crude protein, crude fat, crude fiber, total ash content and nitrogen-free fat content of methanolic extract of *A. paniculata* to be 21.89, 5.66, 8.74, 0.95, 6.87, 55.89 respectively as shown in Table 2 below. The phenolic content of *A. paniculata* extract was also determined in this study. The total amount of phenolic compounds present in the methanolic extract of *A. paniculata* was 29.11mg in gallic acid equivalent (GAE) (Table 2).

3.9 GC Analysis

The compounds likely present in the methanolic extract of *A. paniculata* leaves are presented in Table 3. The elution order in a GC column was used for the characterization and identification of the compounds. The elution time and the amount of these compounds were also presented.

Table 2: The phenolic content and proximate analysis of *A. paniculata*

Sample	<i>A. paniculata</i>
Moisture	21.89
Crude protein % (%n x 6.25)	5.66
Crude fat %	8.74
Crude fibre %	0.95
Total ash %	6.78
NFE %	55.89
Total phenolic content	29.11

From the result obtained quercetin (9.88%) is the most abundant compound possibly present in the methanolic extract of *A. paniculata*. Also presented is the GC chromatograms (Fig 8), which show the detected peaks and their retention time in the column that correspond to the compounds present in the extract.

4. DISCUSSION

The imbalance between free radical generating and scavenging systems ultimately leads to oxidative stress which can be regarded as a multifactorial disease causative agent, with several known diseases including but not limited to cardiovascular disorders, neurodegenerative tauopathies, mitochondrial dysfunction, diabetes

Table 3. Biologically Active Chemical Compounds Present in *A.paniculata* Leaves Extract

Name of compounds	Retention time (min)	Relative abundance (%)
p-Hydroxybenzoate	2.63	8.31
Caffeic acid	3.53	1.53
Methylcaffeate	4.20	1.83
Onysilin	4.68	2.93
Vanillic acid	5.13	1.53
Methyl vanillate	6.05	5.83
Cinnamic acid	6.58	7.70
Apigenin	7.30	5.93
Beta-sitosterol	7.76	6.46
Quercetin	8.41	9.88
Deoxygrapholide	9.13	7.20
Andrographolide	9.73	4.87
Kaempferol	10.13	5.83
Kalmegin	10.85	8.49
Ferulic acid	11.38	3.82
Adipic acid	12.05	6.71
Cosmosilin	12.05	8.84
Tetracosylferul	13.25	1.45
Skullcapflavone	13.66	0.84

mellitus among others [30]. Plants are vastly known to contain several bodily essential phytochemicals and metabolites, having a great impact in not only ameliorating

Figure 8. GC Chromatogram of *A. paniculata* Leaves Methanolic Extract.

the derailing impact of oxidative stress but as well functioning as a defence system in protecting cells. Studies have, however explored several plants potentials, yielding a considerable amount of positive results [31,32].

In evaluating the antioxidant status of medicinal plants, several biochemical cascades have come to prominence. These assays include DPPH, ABTS, lipid peroxidation, nitric oxide assay and ferric reducing power of such plants. This study, results revealed that *A. paniculata* possess strong antioxidant scavenging potentials on DPPH and ABTS radicals in a concentration and time-dependent manner. This result indicated that *A. paniculata* leaves extract has hydrogen donating ability, this agrees with previously reported study [33]. A similar antioxidant study (FRAP assay) evaluated in this study, *A. paniculata* showed great activity in reducing Fe^{3+} to Fe^{2+} in a highly reproducible and concentration-dependent manner. This thus indicates that *A. paniculata* has an embedded potential to transform free radicals into stable and unharmed products. This result is similar to a previous study carried out by Adedoyin *et al.*, [34].

Under physiological condition, Nitric oxide radicals are generated and naturally reacts with oxygen to produce stable products. In this study, *A. paniculata* methanolic extract compared with the standard ascorbic acid demonstrated strong antioxidant potential against nitrite ion formation in a concentration-dependent manner. The nitric oxide radical scavenging activities of *A. paniculata* has been reported previously [35]. Lipid peroxidation/malondialdehyde formation is generally believed to be a strong signal in free radicals impounded cells. This, however, triggers a cycle of toxicological cascades which if not put to check could disrupt biomembranes and wreak havoc [36]. Employing TBARS assay, maximum induction of MDA (100%) was observed in the control while *A. paniculata* methanolic extract in a concentration-dependent manner successfully prevented MDA formation. Similarly, Bajpai *et al.*, [35] reported the protective effects of *A. paniculata* against Fe^{3+} -induced lipid peroxidation.

As earlier pointed, oxidative stress triggers the onset of diabetes mellitus. However, inhibitors of α -amylase; a hydrolyzing enzyme, have been revealed as a hypoglycemic drug for the control of excessive blood glucose levels in humans [36]. The Cell-free antidiabetic assay carried out in this study showed that *A. paniculata* is effective

in inhibiting α -amylase activity which might be attributed to the presence of phenolic compounds in the plant extract [38].

Plants have pharmacological activities attributed to the secondary metabolites which are responsible for essential bioactivities. Screening of the leaves of methanolic extract of *A. paniculata* revealed terpenoid, saponins, phenols, Phlobatanins, alkaloids, cardiac glycoside and flavonoid. These phytoconstituents have been reported to be associated with several nutritional bioactivities. Phytochemical composition screening of *A. paniculata* leaves shows the presence of saponins, which are triterpenoid glycosides responsible for the bitter taste and as well known for their hemolytic effect on red blood cells [39]. They possess cholesterol-reducing abilities and exhibit structure-dependent bioactivities [40]. The saponins content of plants also helps in fighting pathogens and boosting the immune system.

The presence of terpenoids indicates that steroidal compounds could be present, which are of great use/ importance in synthesizing sex hormones synthetic compounds [41]. Phlobatanins as well are researched for their analgesics and wound healing capabilities [41]. Flavonoids and phenols are responsible alongside carotene for the colouration of vegetables and herbs. They possess health-promoting benefits not limited to their antioxidant, anti-inflammatory and vaso-protective abilities. They act as diuretics and could possess anti-plasmodial properties. Thus, potentially making *A. paniculata* leaves a great medicinal herb to large varieties of diseases [42].

Alkaloids is present in several medicinal plants, and it constitutes an appreciable percentage in many available drugs, hence highly essential in diseases management. Moreso, cardiac glycosides possess an effective and direct action on the cardiac system, supporting the strength of the heart and the rate of contraction when failing [43].

The total phenolic content in methanolic leaves extract of *A. paniculata* determined in this study was found to be high. Therefore, it was considered that the high antioxidant potential of leaves extract of *A. paniculata* could be attributable to its high amount of phenolic compounds content. The nature of phenolic compounds present in *A. paniculata* extract was carried out using gas chromatography. GC analysis revealed hydroxybenzoate, caffeic acid, Methylcaffeate, onysillins, vanillic acid, methyl vanillate, cinnamic acid, apigenin, beta-sitosterol, quer-

cetin, andrographolide, deoxy andrographolide, kaempferol, kalmegin, ferulic acid, adipic acid, cosmosilins, tertacosylferyl and skulcapflavon in the extract. Many of these compounds have been identified in *A. paniculata* in the previous study [11].

Proximate analysis of *A. paniculata* leaves revealed that the plant has a substantial amount of moisture, crude protein, crude fat, crude fibre, total ash content, and nitrogen-free extract. From the result, nitrogen-free fat has the highest value, while crude fibre has the smallest value. The ash content of *A. paniculata* leaves shows that the plant is rich in the mineral element, while the moisture content of the leaves would prevent microbial growth and increase the storage span [44]. The crude fibre in *A. paniculata* has the potential to reduce serum cholesterol levels, preventing coronary heart diseases, lowering constipation, and reduce the risk of hypertension [45]. Thus, the result obtained in this study offers a scientific basis that methanolic leaves extract from *A. paniculata* contains certain nutritional values that could be significant in managing malnutrition and multifactorial disorders.

The results obtained in this study indicate that *A. paniculata* extract, through its actions has good antioxidant properties. The bioactive compounds present in *A. paniculata* methanolic extract (majorly andrographolide, 14-deoxy-11,12-didehydroandrographolide and 14-deoxy andrographolide) have been mentioned in previous studies to prevent the toxicity cascade usually triggered by radicals [46-48]. The anticancer, hepatoprotective activity, hypotensive property, antiangiogenic, anti-hyperglycemic and antimalarial potentials of *A. paniculata* have been reported in previous studies which can be attributed to the diverse group of phytochemicals such as diterpenoids, diterpene glycosides, lactones, flavonoids and flavonoids glycosides present in *A. paniculata* leaves [49,50]. In the present study, *A. paniculata* was shown to have an antioxidant effect. This could be attributed to the presence of phenols, terpenoids, cardiac glycoside, tannins, flavonoids and saponins found in the plant extract in this study. Also, flavonoids and terpenoids, in particular, have been speculated to be responsible for great antioxidant and anti-inflammatory potentials of *A. paniculata* in the previous study [51], and these constituents may account for good pharmacological properties of *A. paniculata* extract obtained in this study.

This study concludes that the phytochemical composition and proximate analysis of compounds naturally present in *A. paniculata* leaves possess fat and proteins, with an appreciable quantity of phytochemicals like alkaloids, phenols, and flavonoids that provide essential nutritional supplements in food and enhances body-building. In addition, *A. paniculata* methanolic extract demonstrated strong antioxidant and antidiabetic properties which could be attributed to the various phytochemicals present in the extract.

Conflict of Interest

Authors declare that there is no conflict of interest.

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Authors' Contribution

AOO conceived and designed the study, collected data, performed data analysis and contributed to manuscript writing. **AOA** contributed to data collected, data analysis tools and manuscript writing. **AA** contributed to study design and data analysis. **OSO, AWO** contributed to data analysis tools and analysis of data. All authors approved the final version of the manuscript

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