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GC-MS-Based Phytochemical Characterisation and in vitro Evaluation of the Antioxidant and Antidiabetic Activity of *Andrographis paniculata* (Burm.f.) Nees

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ABSTRACT

The present study evaluated the phytochemical constituents, GC-MS profile, and in vitro antioxidant and antidiabetic activities of the methanol extract of Andrographis paniculata (Burm.f.) Nees. Phytochemical constituents were quantified using standard analytical methods, while volatile compounds were identified by gas chromatography-mass spectrometry (GC-MS). The antidiabetic activity of the extract was evaluated using in vitro α-amylase and α-glucosidase inhibitory assays, whereas its antioxidant potential was assessed using DPPH and ABTS radical scavenging assays. Phytochemical analysis revealed the presence of appreciable amounts of total phenols (19.10 ± 0.00), total flavonoids (18.53 ± 0.06), total alkaloids (23.15 ± 0.56), and total tannins (4.64 ± 0.11). GC-MS analysis identified more than 30 volatile compounds, among which neophytadiene (29.42%), ergost-5-en-3-ol (10.57%), and 9-octadecenoic acid (7.89%) were the predominant constituents. The methanol extract exhibited remarkable antioxidant activity, comparable to that of the standard antioxidant (ascorbic acid). Furthermore, the extract demonstrated concentration-dependent inhibitory activities against both α-amylase and α-glucosidase enzymes, indicating promising antidiabetic potential. The presence of both volatile and non-volatile phytochemicals in A. paniculata may contribute to its observed biological activities and supports its traditional medicinal use. These findings suggest that the methanol extract of A. paniculata represents a promising source of natural antioxidant and antidiabetic agents. However, further in vivo studies and comprehensive toxicological evaluations are required to establish its efficacy, safety, and therapeutic potential.

***Keywords*— *Andrographis paniculata*, alpha amylase, antioxidant, antidiabetic, GC-MS**

I. INTRODUCTION

Since ancient times, nature has offered a wealth of resources that are utilized in ethnomedicine to combat a whole lot of ailments [1]. Numerous phytochemicals, also known as secondary metabolites, have been found useful either as individuals or as additives for the treatment of diseases [2]. The discovery of new drugs originally stemmed from traditional remedies and the folk knowledge of indigenous peoples [3]. As

a result, plants constitute an important source of medications, either as crude drugs for the general public or as isolated active chemicals given in standardized dosages [4]. Notably, 80% of these natural compounds are employed for the same or similar ethnomedical purposes in different cultures [5]. *Andrographis paniculata* (AP) is an herbaceous plant from the Acanthaceae family [6] (Figure 1). It is also known as "Kalmegh" in hindi. It is native to Taiwan, Mainland China, and India [7] and widely grown in Southeast Asian countries, including Malaysia. It has a strong background history of therapeutic usage in traditional and Ayurvedic medicine, as well as Chinese medicines [8].

In Ayurveda, *Andrographis paniculata* is commonly used to treat sore throat, flu, liver disorders, jaundice, antispasmodic, malaria, eczema, intestinal worm infestation, and gonorrhoea [8]. On the other hand, the plant is used to remove body heat such as fevers and toxins, besides treating diarrhea, laryngitis, gastric infections, and inflammation in Chinese Medicine [9]. In contemporary pharmacology, AP acts as an immune system stimulant, treats pharyngotonsillitis, myocardial ischemia, and is anticancer, antimicrobial and anti-inflammatory [10].

The present study aimed at the determination of phytochemical composition and bioactivities of *Andrographis paniculata* which also has a local name (Yoruba: Jogbo) variety that is found in Nigeria. The review of literatures showed paucity in the antidiabetis and antioxidant potential of *Andrographis paniculata* especially in its local variety. It has been proven that the geographical location always determines the phytochemical constituent and their corresponding bioactivities. Hence the need to explore the bioactivities and phytochemical compositions.

Figure 1: *Andrographis paniculata* before harvest.

II. MATERIALS AND METHODS

The whole plant of *Andrographis paniculata* was collected from a private floral garden at the Federal Housing estate Ado-Ekiti, Ekiti State, Nigeria. The plant sample was authenticated at the Department of Crop, Soil and pest Management, Federal university of Technology Akure, Nigeria, where voucher specimens have been deposited in the herbarium with identification number: FUTA 398.

A. Sample preparation and Extraction

The collected plant sample was cleaned of the dust and soil. It was air dried at room temperature (250^0C) for six-weeks until constant weight was obtained. The dried sample was pulverized and stored in an airtight container prior to extraction. A 250 g of pulverized sample was soaked with 2.5-liter methanol for 72 hours. The separation was done with filter paper (Whattman No 1) and the extract was concentrated under a reduced pressure to a small volume by means of rotary evaporator.

B. Phytochemical Screening of Methanol Extract of A. paniculata

A phytochemical component of the extract was evaluated by the same methodologies [11] outlined. Four different phytochemicals (phenol, alkaloids, flavonoids and tannis) were identified quantitatively.

C. Determination of bioactive compounds by Gas Chromatography mass spectrophotometry

The sample was analyzed using an Agilent Technologies 7890A Gas Chromatograph and 5977B Mass Selective Detector. The identity of the volatile components was determined by comparing their mass spectra with literature reports and performing computer matching against NIST and WILEY libraries.

D. In vitro antioxidant activity

Methods used to assess the antioxidant properties of the plant extract include 2,2- Azinobis-3-ethylbenzothiazolin-6-sulphonic acid (ABTS) [12] radical scavenging assay and 2,2-Dipheny-1-picrylhydroxyl (DPPH) [13] radical scavenging assay.

E. Antidiabetic activity of Andrographis paniculata methanol extract

The in vitro antidiabetic assay was carried out by utilizing two different techniques: alpha-amylase inhibitory assay and alpha-glucosidase enzyme inhibition assay [14].

F. α-Amylase Inhibition Assay

A reaction mixture containing 1 mL of plant extract at varying concentrations and 1 mL of 1% starch solution was incubated with α-amylase enzyme at 37 °C for 30 minutes. The reaction was terminated with DNS reagent and boiled for 5 minutes. Absorbance was measured at 540 nm, and percentage inhibition was calculated relative to control.

G. α-Glucosidase Inhibition Assay

A mixture of plant extract and α-glucosidase enzyme was incubated at 37 °C for 15 minutes before adding p-nitrophenyl-α-D-glucopyranoside (PNPG) as substrate. The reaction was terminated by sodium carbonate solution, and absorbance was measured at 405 nm. Inhibition percentage was calculated relative to control.

III. RESULTS

Table 1: Quantitative phytochemicals screening of *Andrographis paniculata methanol extract*

Parameters	Percentage (%)
Alkaloid	23.15±0.56
Flavonoid	18.53±0.06
Tannin	4.64±0.11
Phenol	19.10± 0.00

Table 2: Some of the major bioactives compounds identified with GC-MS analysis of the methanol extract of *Andrographis paniculata*

Peak No	Retention time / min	% Area	Name of compound	Molecular formula	Molecular weight (g/mol)
1	22.279	4.03	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53
2	19.778	6.66	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40
3	21.397	3.75	9,12-octadecadienoic acid	$C_{19}H_{34}O_2$	294.47
4	21.455	7.89	9-octadecenoic acid(z,z) methyl ester	$C_{19}H_{34}O_2$	296.48
5	21.592	29.42	Neophtadiene	$C_{20}H_{38}$	278.52
6	21.684	7.29	Methyl stearate	$C_{19}H_{38}O_2$	298.50
7	27.474	4.22	Squalene	$C_{30}H_{50}$	410.70
8	28.470	10.57	Ergost-5-en-3-ol	$C_{28}H_{48}O_2$	400.68

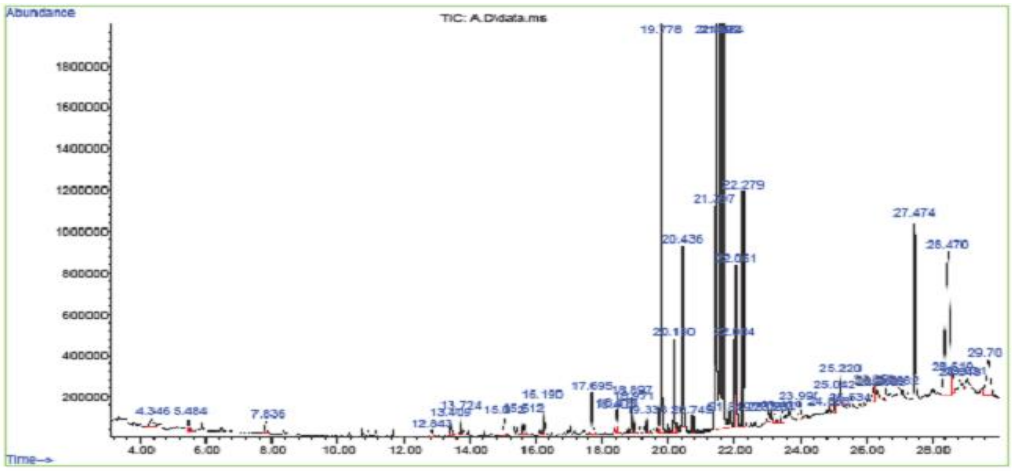

Figure 2: Chromatogram of GC-MS analysis of the methanol extract of *Andrographic paniculata*

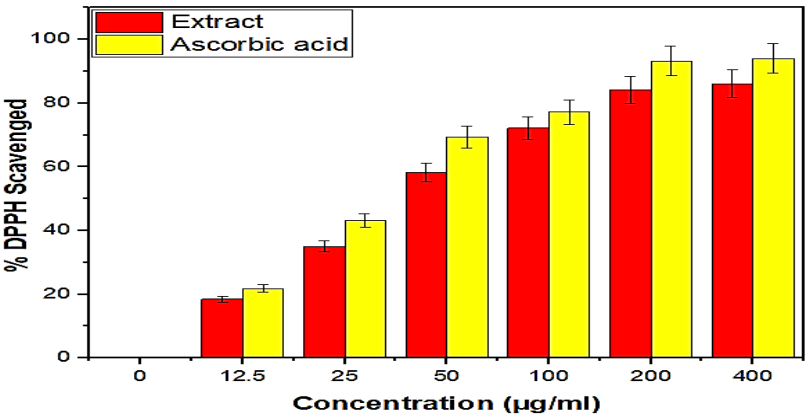

Figure 3: DPPH free radical scavenging ability of the extract of *Andrographic paniculata*

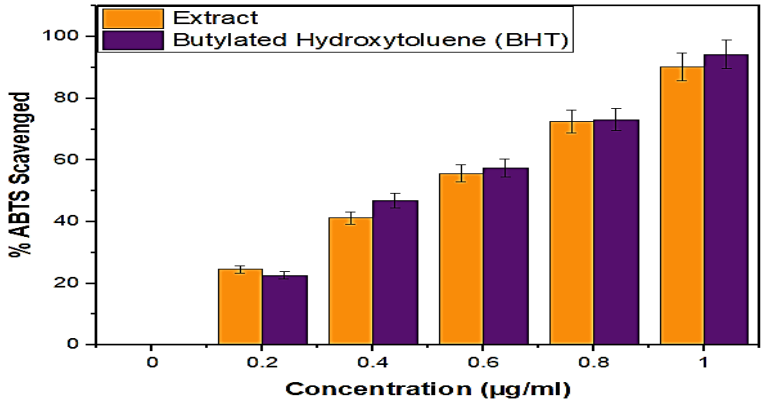

Figure 4: ABTS free radical scavenging ability of the extract of *A. paniculata*

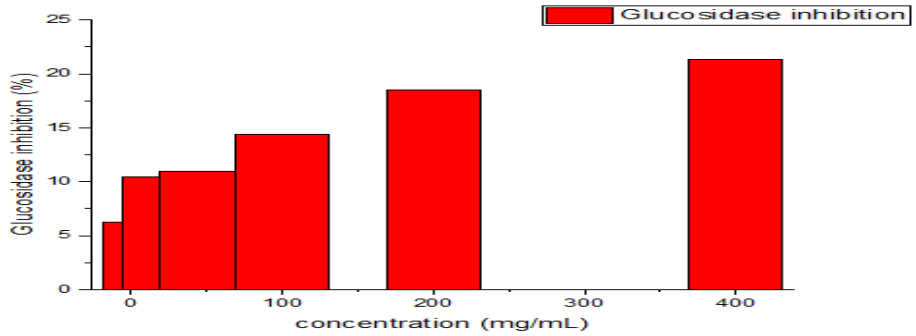

Figure 5: Percentage of alpha glucosidase inhibition

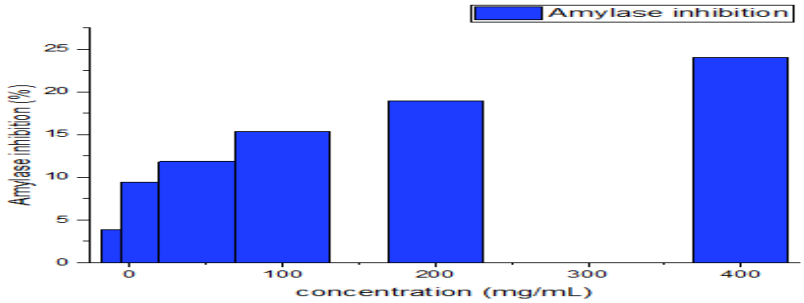

Figure 5: Percentage of alpha Amylase inhibition

IV. DISCUSSION

The quantitative phytochemical screening of *Andrographis paniculata* revealed the presence of some important secondary metabolites at varying concentrations (Table 1), which is the major determinant in the therapeutic activities of the plant extract. The various compounds detected are recognized for their medicinal significance. These natural compounds can exert a protective measure on liver (hepatoprotective) through multiple mechanisms, such as reducing, scavenging free radicals to protect liver cells from oxidative damage and inflammation [15]. Flavonoids are often referred to as nature's biological response modifiers due to their ability to influence the body's reactions to allergies and viruses, demonstrating anti-allergic, anti-inflammatory, antimicrobial, and anticancer properties [16]. Flavonoids isolated from ethanol extract of *A. paniculata* was found to be active against hepatitis and anti-allergic reaction [17]. Alkaloids are also utilized in treating diseases like malaria, as pain relievers, and in managing heart conditions [18]. It also modulates the immune system, enhancing the defense mechanisms of body against infections and reducing excessive immune responses that can lead to chronic inflammation and protect cell from oxidative damage [19].

Andrographis paniculata also contain another compound named 14-deoxyandrographolide, which was found to decreases inflammation by inhibiting the production of pro-inflammatory cytokines and enzymes, it also possesses antimicrobial properties that help to fight numerous bacterial and viral infections [20, 21]. The methanol extract of the whole plant of *A. paniculata* as analyzed by GC-MS (Table 2) show the presence of 35 different bioactive compounds and they were identified along with their retention times, molecular weight, and percentage area composition in the extract. The analysis was able to reveal about eight bioactive compounds with prominent peak areas. They include 9,12-octadecadienoic acid (3.75%), Octadecanoic acid, ethyl ester (4.03%), squalene (4.22%), Pentadecanoic acid (6.66%), Methyl stearate (7.29%), 9-octadecenoic acid(z,z) methyl ester (7.89%), Ergost-5-en-3-ol (10.57%), and Neophtadiene (29.42%).

Neophtadiene which happen to be the major compounds is an organic compound that belong to diterpenes, and it was reported to have anti-oxidant, anti-inflammatory, anti-pyretic and anti-microbial activity [22]. Neophtadiene was also detected in dichloromethane extract of *A. paniculata*. Ergost-5-en-3-ol also known as campsterol, a good reducing agent which function as cholesterol absorption and has anticancer and antioxidant activity. It is also potent against inflammation, arthritic, pyretic and ulcer [23]. Squalene, a 30-carbon isoprenoid compound was also identified in the methanol extract. Squalene is very important bioactive compound as it is an intermediate metabolite in the formation of cholesterol which is not suspectable to lipid peroxidation [24]. Ethanolic extract of *A. paniculata* was also reported to contain squalene and it brings about neutralization of different xenobiotics and act as anti-inflammatory, anti-aging and anti-neoplastic [25]. For example, for example phytol and Hexadeconoic acid, methyl ester is an antimicrobial, anti-inflammatory and anti-cancer [26].

The DPPH free radical assay (fig 3) and the ABTS assay (fig. 4) were carried out at different concentration base on the ability of the methanol extract to scavenge DPPH free radical showed a remarkable scavenging activity on a dose dependent trend. This is in agreement with the outcome when methanol extract of *Andrographis paniculata* inhibited the formation of reactive oxygen species [27]. The pharmacological significance of these phytoconstituent lies in their ability to provide multiple therapeutic effects, flavonoids and phenolic acids contribute powerful antioxidant activities that help neutralize harmful free radicals [27]. The significant presence of squalene and phenols in *Andrographis paniculata* make it a potential remedy for hepatoprotective, anti-inflammatory, antioxidant, immunomodulatory, and antifibrotic properties [18]. The ABTS assay also prove the potential of the extract as a promising antioxidant as compared to the standard (ascorbic acid). The antidiabetic activity was analyzed via alpha-amylase and alpha-glucosidase.

Alpha-amylase is an enzyme responsible for the breakdown of larger carbohydrate into small monosaccharide and alpha – glucosidase is the one that ensure the absorption of glucose into the body. The inhibitory effect demonstrated by the extract against the enzymes reveal the antidiabetic potential of the plant extract, although the inhibition is concentration dependent.

V. CONCLUSION

The phytochemical and GC-MS analysis of methanol extract of Andrograhis paniculata revealed significant bioactive compounds such as Neophtadiene, squalene, neoclovene alkaloids, flavonoids, which probably play active roles in the bioactivities examined. The extract was able to neutralize the free radicals which are the root of many diseases. Also, the inhibition of alpha amylase and alpha glucosidase by the extract showed its potential as antidiabetic. Meanwhile, it is suggested that further biochemical investigation should be done on the extract to ascertain its efficacy and safety.

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