Evaluation of Antioxidant, Antimicrobial Activity, and GC-MS analysis of methanol extract of *Andrographispaniculata*(Burm.f.) Wall. ex Nees, growing wild in South-West Nigeria

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Abstract

Plants are generally of great importance because they contain phytochemicals, which have significant pharmacological importance and are the basis for drug synthesis. *Andrographis paniculata* is an herbaceous plant from the family Ancanthaceae. Literature on the bioactivity of the Nigerian-grown species is not common. The present research was carried out to investigate the radical scavenging and antimicrobial activity of the methanol extract of a Nigerian variety growing without cultivation in south-west Nigeria. *In vitro* antioxidant activity of the methanol extract of the whole plant was tested by four different assays: DPPH, ABTS, FRAP and NO. The methanol extract showed promising antioxidant activity compared with the reference (ascorbic acid). It was observed that the activity was concentration dependent, and values of 86.12±0.03, 90.18±0.03, 80.20±0.04 and 92.15±0.06 were obtained for DPPH, ABTS, FRAP, and NOx, respectively, under the same condition. All the tested bacteria (gram positive and gram negative) were inhibited in their growth except *Proteus mirabilis*, which showed a resistance at lower concentrations (0.25 mg/ml and 0.50 mg/ml). The gas chromatography-mass spectrometry analysis revealed the presence ofneophthadiene which has the highest peak area of 29.42%, followed by ergost-5-en-3-ol and methyl sterate with 10.57% and 7.29%, respectively. Also, 9-octadecadienoic acid methyl ester, pentadecanoic acid, and squalene have percentage peak areas of 7.89%., 6.66%, and 4.20%, respectively. These bioactive compound will nevertheless contribute to the bioactivities experience with the Nigerian grown variety of *Andrographispaniculata*(Burm.f.) Wall. ex Nees.

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Keyword: ***Andrographis paniculata*, GC- MS, antioxidant. Antimicrobial activity**.

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Introduction

Plants have been known to be great therapeutic agents in the history of mankind, and they have been believed and well accepted as the habitual treatment to cure various diseases.Nearly 80% of the human population in developing countries and about 25% in developed countries are estimated to depend on therapeutics of botanical origin (Abera et al.,2017)). This is because plants have phytochemicals with a wide range of therapeutic functions, such as antimicrobial, anti-inflammatory, and anti-diabetic properties (Obasi et al., 2017).Research has shown that individual parts of plants, such as leaves, flowers, fruits, bark, roots, and even seeds, have their own medicinal applications (Petrovska**,**2012). Also, the geographic locations of the plant and the weather conditions of plant cultivation go a long way in determining the phyto-constituents of the plant, resulting in differences in phytochemicals present in plants of the same species grown in different climates (Owokotomo et al.,2016).*Andrographispaniculata*(Burm.f.) Wall. ex Neesis believed to be of Asian origin, which includes India, Thailand, Sri Lanka, and Malaysia. It is also found on the African continent, including Nigeria, and in the United States of America. *Andrographis paniculata*(*A. paniculata)* found in a family of Acanthceae, Kingdom Plantae, and Genus *Andrographis. A. paniculata is* commonly called the “King of Bitters." It contains over 40 species. From the historical periods, it has been used as source of therapeuticagant to cure various ailments such as diabetes mellitus, oxidative stress, general inflammation, and microbial mediated diseases (Abas et al.,2016). In south-west Nigeria, the medicinal value of the plant is recognized, and it is known by the Yoruba people as “Jogbo” or "Mejemeje.” The whole parts of the plant, which include the stem, leaf, flower, fruits, seeds, and roots, have been used as therapeutic agents in natural medicine. It is also reported that the plant has been used to treat snakebite and other poisonous bites. The root parts of *A.paniculata* is used to treat malaria, high blood pressure, urinary tract infections, and also to treat respiratory infections (Okhuarobo et al.,2014). The plant has been reported to contain some phytochemicals, which include flavonoids, tannins, and alkaloids, which have

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antiviral properties (Dirar et al.,2019). There is an important phyto-constituent named Andrographolide, which has been discovered to have a potential to inhibit activity against various viral diseases by discouraging DNA replication (Özçelik, et al., 2011).Oxidative stress is the root cause of much disorderliness in the body, such as inflammatory cancer, cardiovascular diseases, diabetes mellitus, and so on (Paemanee et al.,2019). The situation is caused by uncontrolled generation of free radicals (reactive oxygen and nitrogen species) or their insufficient chain termination reactions in the cell metabolisms.(Ferry and Roussel.,2011)Free nitrogen and oxygen species are not stable and are situated in the external environment, and they are also generated in the body during the normal metabolic processes in the body (Arika et al.,2019).Naturally occurring free radicals scavengerssuch as phenolics and flavonoids are found abundantly both in edible and non-edible plants that exhibit medicinal importance (Bhat et al.,2015). Excessive production of these radicals results in progressive damage as well as degeneration of the cell. A cell is said to be in a state of oxidative stress when the level of production of ROS overcomes the inbuilt defense mechanisms in the body (Sengul et al.,2009). Using the cutting-edge GC-MS technology, the current study examined the phytochemical contents of *Andrographispaniculata*(Burm.f.) Wall. ex Nees, growing wild in south-west Nigeria,and their antibacterial and radical scavenging properties.

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MATERIALS AND METHODS

Sample Collection, Identification, Treatment

The samples of *A. paniculata* were collected from Ado, Ekiti State, Nigeria. It was taken to the Department of Plant and Crop Science, Federal University of Technology Akure, for identification. The collected plant sample was properly cleaned, air dried at room temperature (25 ^{o}C), pulverized into powder form, and packed into a sterile container for designated analysis.

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Extraction

About 120 g of dried pulverized *A. paniculata* was placed in a clean round-bottom flask and extracted with 600 mL methanol, and left for 72 hours with intermittent stirring. Filter paper (Whatman No. 1) was used to filter through to get a

solution.Then the solvent was eliminated using a rotary evaporator at 39 ^{o}C. The concentrated extract was preserved at 4 ^{o}C.

GC-MS Analysis of the Extracts

Agilent Technologies' 7890A GC and 5977B MSD were used to analyze the sample. The following were the experimental settings for the GC-MS system: The Hp5-MS capillary standard non-polar column measures 30 mm in length, 0.25 mm in ID, and 0.25 mm in film thickness. The planned temperature (oven temp) in the gas chromatography section was 40 °C, rising to 250 °C at a rate of 5 °C/mm. The injection volume was 1 ml. Helium gas served as the carrier gas, and the mobile phase flow rate was fixed at 1.0 ml/min. Samples dissolved in methanol were comprehensively scanned at a range of 40-650 m/z using the NIST mass spectral library reference.

.**Determination of *in vitro* Antioxidant Activity of the Plant extract**

Ferric Reducing Antioxidant Assay.

The plant extract's capacity to scavenge radicals was evaluated using a slightly modified version of Oyaizu's (Noctor et al.,1998) methodology. Ascorbic acid, which functions as a reference, and five different quantities of methanolic extracts (25, 50, 100, 200, and 400 g/ml) were employed at the same concentrations and combined with roughly 2 ml of phosphate buffer (pH6.6, 2M) and 2 ml of 1% potassium ferricyanide $K_3Fe(CN)_6$. The mixture was allowed to incubate at 50 °C for 20 minutes. After adding 10% and 2 milliliters of trichloroacetic acid, the mixture was centrifuged for 10 minutes at 1000 revolutions per minute. Two milliliters of distilled water and one milliliter of 0.1% ferric chloride were used to aspirate the resultant supernatants. The absorbance was taken with a UV-visible spectrophotometer at 700nm and the concentration equivalent was recorded..

Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activities. The procedure was adopted as mordified by Duru et al.,2017. The 2, 2-diphenyl-1-picryl-hydrazyl radical was used to evaluate the antioxidant capacity of the plant extract. There were six different concentrations of the extract (12.5, 25, 50,

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100, 200, and 400 ug/ml). The same concentration was made for ascorbic acid, the standard reference. The percentage (%) inhibition of free radical was calculated using the following formula to determine the radical scavenging activity:

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$$\% \text{ inhibition} = \frac{(A - A_1)}{A} \times 100$$

Where A= absorbance of the blank (DPPH);
A_1= absorbance of the extract (DPPH+ extract)

2,2-Azinobis-(3-ethylbenzothiazolin-6-sulphonic acid(ABTS) radical scavenging assay Using scorbic acid as the reference, the plant extract's capacity to lower ABTS at different concentrations was examined (Sulekha et al 2009). 2.45 mMpotassium persulfate(1/1, v/v) and 7 mM ABTS stock solution were used to prepare the ABTS radical. The mixture was then left for 10–16 hours at 250 °C in a dark area until the reaction was finished. An ABTS solution was mixed with distilled water to create a diluted solution that had an absorbance of 734 nm. Next, 1 mL of various sample solutions was mixed with 3.0 ml of ABTS.After 6 minutes of incubation, the absorbance was measured at 734 nm. The scavenging rate was calculated using the formula:

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$$\text{ABTS radical scavenging rate (\%)} = \frac{A_0 - A}{A_0} \times 100$$

where "A_0" (control) was the absorbance of ABTS blank solution, and
A" was the final absorbance of the tested sample after 6 min of incubation.

Nitrous oxide (NO) scavenging assay Using the Nitric Oxide Scavenging Assay, the radical scavenging ability of A. paniculata was evaluated. The methodology was applied in accordance with Shukla *et al.*'s protocol. A 2 mL of sodium nitropruside were dissolved in 0.5 mL of phosphate buffer and combined with 0.5 mL of the sample at different concentrations. The mixture was combined with Griess reagent after being incubated at 250 °C for 150 minutes. After an additional half-hour of incubation, the absorbance was measured. The standard of reference was ascorbic

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acid. Using the following formula, the degree of nitric oxide radical inhibition was determined: Percent (%) inhibition of NO radical = $\frac{A^0 - A}{A^0} \times 100$

Where A^0 is the absorbance of the blank control (NO radical solution without test sample), and A is the absorbance of the test sample.

Antimicrobial activity assay

Nutrient agar media were prepared according to manufacturer instructions and autoclaved at 121 °C. The agar it was allowed to cool to about 45 °C and for 15 minutes. After sterilizing the agar, it was poured into the sterilized petri dishes in a uniform thickness of approximately 20 mL, and the agar was allowed to set at ambient temperature. After solidifying the media, the sterile cotton swab was used to spread the inoculums throughout the medium uniformly. The agar plate was allowed to rest for 1 hour under the laminar hood and incubated later at 37°C for one day. In this method, a 0.5-mm sterile filter paper disc was soaked in extract solution for 2 hours and then placed on the surface of the agar plate. The plates were kept at room temperature for 2 hours pre-ditusionas described by Murray et al. (2002) and modified by Olurinola (2004).

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Statistical analysis

One-way analysis of variance (ANOVA) version 17.0 was used to analyze the quantitative phytochemicals, antioxidant and antimicrobial activities of the plant extract; this was to check the significant difference among the means of different groups. This was followed by Tukey's tests for pairwise comparisons and separation of means. $P < 0.05$ was considered statistically significant.

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RESULT AND DICUSSION

The determination of bioactive compounds by GC-MS (Table 1) revealed the presence of about 39 compounds in the methanol extract of *Andrographis paniculata*. The identification of the compounds was ascertained by the peak area and retention time.Of all the compounds identified, neophthadiene has the highest peak area of 29.42%, followed by ergost-5-en-3-ol and methyl sterate with 10.57% and 7.29%,

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respectively. Also, 9-octadecadienoic acid methyl ester, pentadecanoic acid, and squalene have percentage peak areas of 7.89%., 6.66%, and 4.20%, respectively. These compounds have been reported to exhibit anti-inflammatory, cancer-preventive(Rajeswar et al.,2013) hepatoprotective, antimicrobial, and antioxidant properties (Abebaw,2018). There was also the presence of 9,17-octadecadienoic acid (13.05%), which has been reported to have antiandrogenic, (Nishanthini et al.,2014)anticoronary, anti-inflammatory, hepatoprotective, hypocholesterolemic, antihistaminic, anti-arthritic, antiacne, and antieczemic properties (Sreeja, 2018). Squalene and neoclovene were reported to have antimicrobial, anti-diuretic, antioxidant, anticancer, neuroprotective, and anti-inflammatory activities (Abdelhaki et al., 2018). 9-octadecadienoic acid methyl ester and pentadecanoic acid were reported to have antioxidant and nematicide properties(Rajeswari et al.,2013). It also serves as flavoring agents, hemolytic, and 5-alpha-reductase inhibitor (Alves-Silva.et al.,2016).

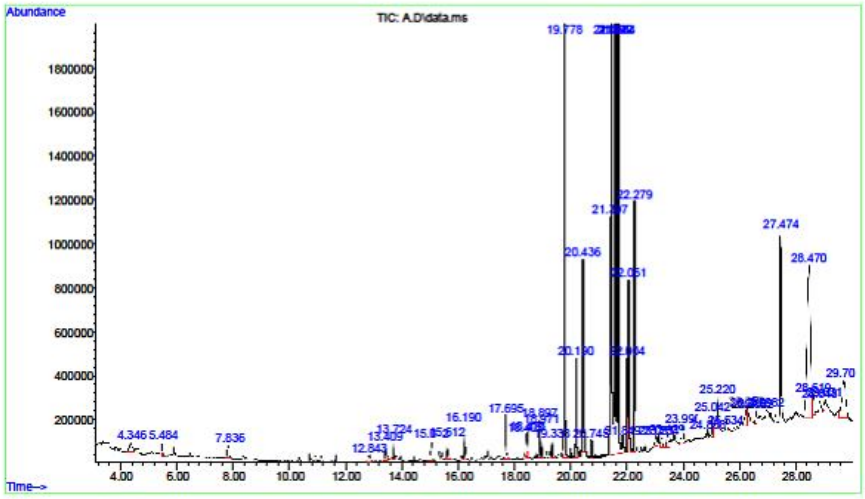

Figure 1: The GC-Ms total ion chromatogram (TIC)

Comment [WU31]: Figures should be less pixel with high contrast

Table 1: Result obtained from GC – MS analysis from the plant Extract

Peak No	Retention time	% Area	Name of compound	Molecular formula (g/mol	Molecular weight
2	5.484	0.24	Benzene, 1-	C_8H_7NO	133.15

			isocyanato-3-methoxyl		
4	12.843	0.25	Cyclotetrasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444.92
5	13.409	0.48	Eugenol	$C_{10}H_{12}O_2$	164.20
8	15.612	0.39	2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a trimethyl	$C_{11}H_{16}O_2$	180.24
9	16.190	0.49	9-octadecene(E)	$C_{18}H_{36}$	252.5
10	17.695	0.73	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.40
11	18.405	0.40	1-octadecene	$C_{18}H_{36}$	252.5
12	18.473	0.39	10-methyl nonadecane	$C_{20}H_{42}$	282.5
13	18.897	0.47	Phytol	$C_{10}H_{18}$	138.25
14	18.971	0.51	2-pentadecanone	$C_{18}H_{36}O$	268.50
15	19.338	0.24	3-Eicosyne	$C_{20}H_{38}$	278.52
16	19.778	6.66	Pentadecanoic acid	$C_{17}HO_2$	270.45
17	20.190	1.55	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34

18	20.436	3.11	Hexadecanoic acid	$C_{18}H_{36}O_2$	284.47
9921	21.455	7.89	9-octadecenoic acid(z,z) methyl ester	$C_{19}H_{34}O_2$	296.48
22	21.592	29.42	Neophtadiene	$C_{20}H_{38}$	278.52
23	21.684	7.29	Methyl stearate	$C_{19}H_{38}O_2$	298.50
25	22.004	1.38	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.49
26	22.061	3.41	7,10,13-hexadecatrienoicacid methyl ester	$C_{17}H_{28}O_2$	264.403
27	22.279	4.03	Octadecanoic acid, ethyl ester		
28	23.429	0.60	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.55
29	23.990	0.47	Eicosane	$C_{21}H_{42}$	282.54
30	24.888	0.25	17-octadecynoic acid, methyl ester	$C_{18}H_{32}O_2$	280.44
31	25.220	0.63	Phthalic acid	$C_8H_6O_4$	166.13
32	26.250	0.84	Aciphyllene	$C_{15}H_{24}$	204.35
33	26.290	0.31	11,13-dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.46
34	26.982	0.44	Naphthalenon	$C_{10}H_8O$	144.17

			e		
35	27.474	4.22	Squalene	$C_{30}H_{50}$	410.70
36	28.470	10.57	Ergost-5-en-3-ol	$C_{30}H_{50}O_2$	442.71
37	28.619	0.51	1,1,3trimethyl-2-hydroxylmethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	222.37
38	28.848	0.50	2-cyclohexene-1-carboxaldehyde	$C_{10}H_{16}O$	152.23
39	29.706	2.77	Neoclovene	$C_{15}H_{24}$	204.35

The surge in demand for effective antimicrobial drugs against drug-resistant microorganisms has led to an unrelenting search for alternatives, especially from plant species(Stalin, 2019). In the field of natural medicine, numerous plant species have been exploited to unfold their potentials as a source of drugs to treat and prevent human diseases. The present investigation was motivated by ethnophamacology-based information by the traditional healers in south-west Nigeria. According to local sources, *A. paniculata* is used in the management of many diseases (Ruto et al.,2010). Hence, the antimicrobial evaluation of the methanol extract of *A. paniculata* was tested against eight standard bacteria: *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus pneumonia.* It was observed that the extract showed remarkable activity against all the tested organisms, with the zone of inhibition of diameter ranging from 7 mm to 20 mm.The results showed that *A. paniculata* was active against all the bacterial strains, and the activity is directly

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proportional to the concentration of the extract (Table 2). A highest zone of inhibition was observed with *Klebsiella pneumonia* (20.5±0.15) and *Staphylococcus pneumonia* (20.0±0.12) at a higher concentration of 1.25 mg/mL. The values of the diameter of the zone of inhibition of 16.0±0.12 , 17.5±0.13 were observed with *Pseudomonas aeruginosa* , *Staphylococcus aureus,* respectively. A total resistance was observed with *Proteus mirabilis,* as no zone of inhibition was recorded at a lower concentration of *A. paniculata*(0.25mg/ml amd 0.50mg/ml), but a slight activity was recorded at an increased concentration of the extract (0.75 mg/ml, 1.0 mg/ml, and 1.25 mg/ml).

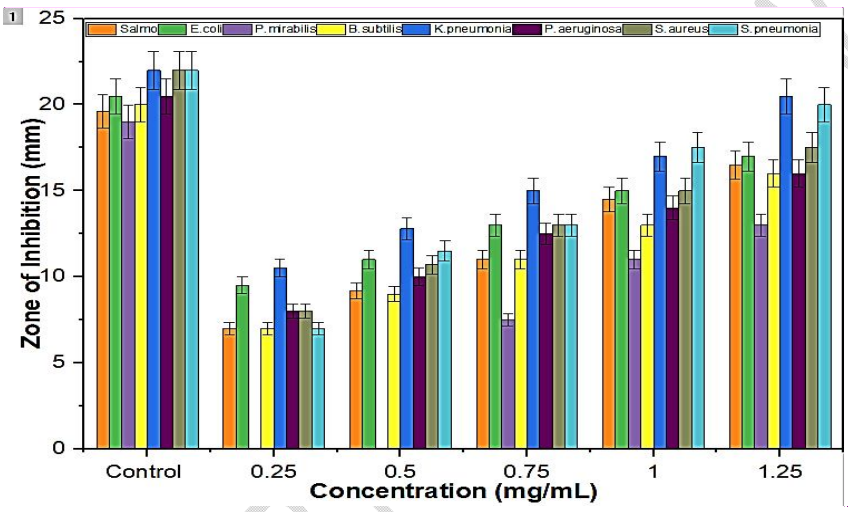

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Figure 2: Antimicrobial activity of methanol extract of *Andrographis paniculata*

Increased generation of reactive nitrogen/oxygen species and decreased activity of radical scavenging in the body results to oxidative stress (Wang et al.,2012). Although , generation of free radicals(reactive oxygen/nitrogen species (ROS/RNS)) is unavoidable for aerobic organisms and in functioning cells, and it occurs at a controlled rate (Saeed et al.,2012). In this investigation, the radical scavenging activity of *A. paniculata* was analyzed with four different assay (DPPH, FRAP, ABTS and NO) with ascorbic acid as a reference, to ascertain its efficacy as antioxidant ABTS and DPPH are typical radical scavenger which are very efficient in determining antioxidant ability (Stoclet and Schmini et nal., 2011). At 400µg/ml, *A.paniculata* showed 86.12±0.03of DPPH radical scavenging activity, while at the same concentration the reference (ascorbic acid) reads 94.03±0.03. the same trend was observed with ABTS and FRAP scavenging activity which also showed a concentration dependent antioxidant activity of .Meanwhile, aremarkable antioxidant

activity was observed in nitric acid (NO) assay where the scavenging activity (92.15±0.06)was higher than the reference (91.07±0.01). It is generally accepted that the presence of some phytochemical such as phenols and flavonoids contribute to radical scavenging ability (Salah et al.,2010). The result were expressed as percentage of concentration as depicted in the figures below Increased generation of reactive nitrogen/oxygen species and decreased activity of radical scavenging in the body results in oxidative stress (Soni and Sosa.,2013). Although, generation of reactive oxygen/nitrogen species (ROS/RNS) is unavoidable for aerobic organisms and in functioning cells, it occurs at a controlled rate (shukla et al.,2012). In this investigation, the radical scavenging activity of *A. paniculata* was analyzed with four different assays (DPPH, FRAP, ABTS, and NO) with ascorbic acid as a reference, to ascertain its efficacy, as antioxidant ABTS and DPPH are typical radical scavengers that are very efficient in determining antioxidant ability(Sohal and Orr, 2012) At 400µg/ml, *A. paniculata* showed 86.12±0.03of DPPH radical scavenging activity, while at the same concentration the (ascorbic acid) reads 94.03±0.03. The same trend was observed with ABTS and FRAP scavenging activity, which also showed concentration-dependent antioxidant activity.Meanwhile, aremarkable antioxidant activity was observed in the nitric acid (NOx) assay, where the scavenging activity (92.15±0.06)was higher than the reference (91.07±0.01). It is generally accepted that the presence of some phytochemical such as phenols and flavonoids contribute to radical scavenging ability (40).

The result were expressed as percentage of concentration as depicted in the figures below

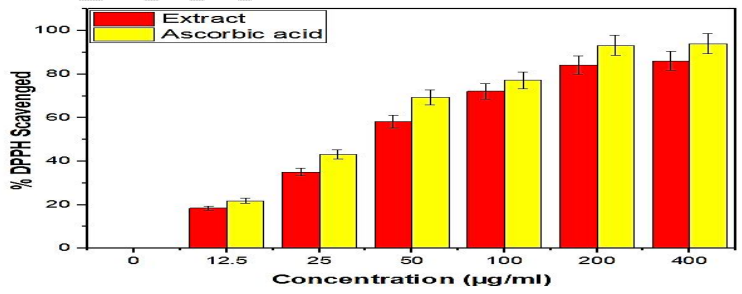

Figure 3: DPPH radical scarvenging activity of methanol extract of *Andrographis paniculata*

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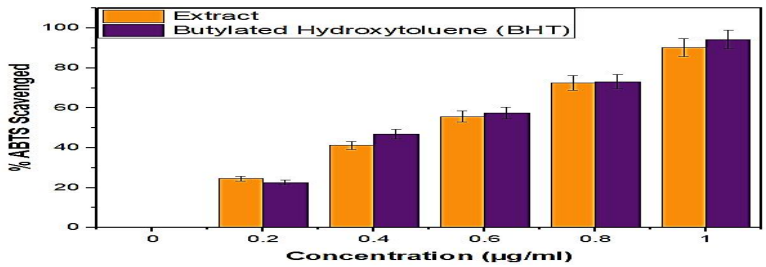

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Figure 4: ABTS radical scarvenging activity of methanol extract of *Andrographis paniculata*

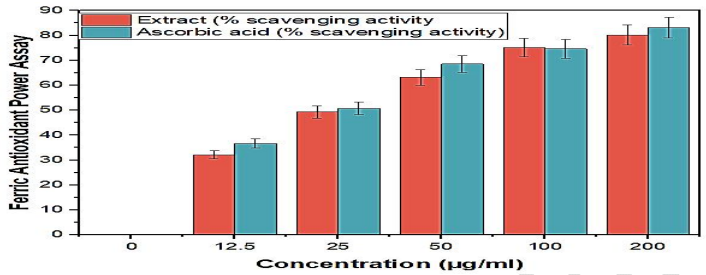

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Figure 5: FRAP radical scarvenging activity of methanol extract of *Andrographis panicula99ta*

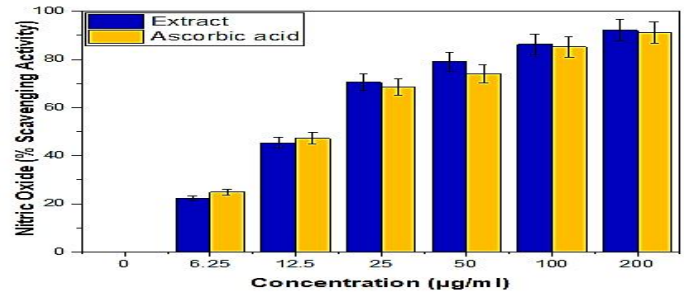

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Figure 6: NO radical scarvenging activity of methanol extract of *Andrographis paniculata*

Conclusion

This study looked into the volatile component, radical scavenging ability, and antibacterial properties of the *A. peniculaata* variant found in Nigeria. Phytochemicals with potential for use in medication development, such as phytol, ergost-5-en-3-ol, neophthadiene, and neoclovene, are found in the GC-MS study results. The results of the antimicrobial assay demonstrated the plants' potential to combat medically significant pathogens such as *Staphylococcus aureus*. Furthermore, a number of

antioxidant bioassays point to the extract's potential application in the management of diseases brought on by oxidative stress. This study backs the use of *Andrographispaniculata(Burm.f.) Wall. ex Nees* found in Nigeria in medicine as long as the necessary standardization is carried out.

Comment [WU39]: Highlight the future out comes of the study

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