



Phytochemical Analysis and Biological Evaluation of *Andrographis paniculata* (Burm.f.) Wall. ex Nees, from Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The medicinal plant, *Andrographis paniculata* (Burm.f.) Wall. ex Nees, used in Nigeria's ethno-medicine, was investigated for its radical scavenging and antimicrobial properties, as well as its phytochemical composition, using gas chromatography-mass spectrometric (GC-MS) analysis. *In vitro* antioxidant activity of the methanol extract of the whole plant was tested through four different assays: DPPH, ABTS, FRAP, and NOx. The GC-MS analysis revealed the presence of neophthadiene (29.42%), followed by ergost-5-en-3-ol (10.57%), and methyl sterate (7.29%) as the major compounds present in the methanol extract of *Andrographis paniculata*. 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

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condition. All the tested bacteria (gram positive and gram negative) were inhibited by the methanol extract of *Andrographis paniculata*, except *Proteus mirabilis*, which showed a resistance at lower concentrations (0.25 mg/ml and 0.50 mg/ml). These findings suggest the bioactive potentials of *Andrographis paniculata* (Burm.f.) Wall. ex Nees, supporting its traditional use in Nigerian ethnomedicine and highlighting potential applications in pharmaceuticals.

Keywords: *Andrographis paniculata*; GC- MS; antioxidant; antimicrobial activity.

1. INTRODUCTION

Plants have been known to be great therapeutic agents in the history of mankind, and they have been 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). *Andrographis paniculata* (Burm.f.) Wall. ex Nees is 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 Acanthaceae, Kingdom Plantae, and Genus *Andrographis*. The plant is commonly called the "King of Bitters." From the historical periods, it has been used as source of therapeutic agents 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 (Okhwarobo et al., 2014).

The plant has been reported to contain some phytochemicals, which include flavonoids, tannins, and alkaloids, which have 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) In addition to being produced in the body during regular metabolic processes, free nitrogen and oxygen species are unstable substances found in the external environment (Arika et al., 2019). Naturally occurring free radicals scavengers such as phenolics and flavonoids are found abundantly in plants (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 *Andrographis paniculata* (Burm.f.) Wall. ex Nees, growing wild in south-west Nigeria, and their antibacterial and radical scavenging properties.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification, Treatment

The samples of *A. paniculata* were collected from Ado-Ekiti, Ekiti State, Nigeria. It was taken to the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, where it was identified by Mr Adejobi and was given the voucher number: FUTA 398. The collected plant samples were properly cleaned, air dried at room

temperature (25 °C), pulverized into powder form, and packed into a sterile polythene container before extraction.

2.2 Extraction

Approximately 120 g of dried pulverized sample of *A. paniculata* was placed in a clean round-bottom flask and extracted with 600 mL methanol, and left for 72 hours with intermittent stirring. Then, the crude methanol extract was obtained through filtration of the decoction using a Whatman No. 1 filter paper, and the crude extract was concentrated using a rotary evaporator (Bibby Scientific Limited, Stone, Staffordshire, ST15 0SA, UK) at 39 °C. The concentrated extract was stored in the refrigerator at 0 °C prior to analysis.

2.3 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 dimensions of the Hp5-MS capillary column are 30 mm in length, 0.25 mm in ID, and 0.25 mm in film thickness. The initial oven temperature was 40 °C, rising to 250 °C at a rate of 5 °C/min. The volume injected was 1 mL. Helium 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.

2.4 Determination of *In vitro* Antioxidant Activity of the Plant Extract

2.4.1 Ferric reducing antioxidant assay

The plant extract's capacity to scavenge free radicals using the ferric reducing bioassay was evaluated based on the method described by Noctor et al. (1998). Ascorbic acid, which functions as a reference, and five different concentrations of the extracts (25, 50, 100, 200, and 400 g/ml) were employed at the same concentrations and combined with roughly 2 ml of phosphate buffer (pH 6.6, 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, 2 mL of 10% trichloroacetic acid was added, and 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. (Bibby Scientific Limited, Stone, Staffordshire, ST15 0SA, UK) at 700nm and the concentration equivalent was recorded.

2.5 Determination of 1,1, Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activities

The procedure was adopted as described by Duru et al.,2017. The 2, 2-diphenyl-1-picrylhydrazyl radical was used to evaluate the antioxidant capacity of the plant extract. There were six different concentrations of the extract (12.5, 25, 50, 100, 200, and 400 ug/ml). The same concentration was made for ascorbic acid, the standard reference. The wavelength of the absorbance is 700nm. The percentage (%) inhibition of free radical was calculated using the following formula to determine the radical scavenging activity:

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

Where A= absorbance of the blank (DPPH);

A₁= absorbance of the extract (DPPH+ extract)

2.6 2,2-Azinobis-(3-ethylbenzothiazolin-6-sulphonic acid (ABTS) Radical Scavenging Assay

Using ascorbic acid as the reference, the plant extract's capacity to lower ABTS at different concentrations was examined (Sulekha et al 2009). About 2.45 mM potassium 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:

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

where "A₀" (control) was the absorbance of ABTS blank solution, and

A" was the final absorbance of the tested sample after 6 min of incubation.

2.7 Nitrous Oxide (NOx) 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.* (2012)'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 acid. Using the following formula, the degree of nitric oxide radical inhibition was determined: Percent (%) inhibition of NOx.

$$\text{radical} = \frac{A_0 - A}{A_0} \times 100$$

Where A⁰ is the absorbance of the blank control (NOx radical solution without test sample), and A is the absorbance of the test sample.

2.8 Antimicrobial Activity Assay

The antimicrobial assay followed previous methods described by Murray *et al.* (2002) and Olurinola (2004). Nutrient agar media were prepared and autoclaved at 121 °C. The agar was allowed to cool to for 15 minutes until temperature of about 45 °C. After sterilizing the agar, it was poured into the sterilized petri dishes (60 mm) 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 an incubator.

2.9 Statistical Analysis

All the analysis was performed in triplicate and statistical analysis was done using 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.

3. RESULTS AND DICUSSION

The results from the GC-MS analysis of the methanol extract of *Andrographis paniculata* (Table 1) revealed the presence of 36 compounds. The major compounds identified were neophthadiene (29.42%), followed by ergost-5-en-3-ol (10.57%) and methyl sterate (7.29%). Other notable compounds were 9-octadecadienoic acid methyl ester (7.89%), pentadecanoic acid (6.66%), and squalene (4.20%). These compounds have been reported to exhibit anti-inflammatory, anti-cancer, hepatoprotective, antimicrobial, and antioxidant properties (Rajeswar *et al.*, 2013; Abebaw, 2018). There was also the presence of 9,17-octadecadienoic acid (13.05%), which has been reported to have antiandrogenic, anticorony, anti-inflammatory, hepatoprotective, hypocholesterolemic, antihistaminic, anti-arthritis, antiacne, and antieczemic properties (Nishanthini *et al.*, 2014; Sreeja, 2018). Squalene and neoclovene have been 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 nematicidal properties (Rajeswari *et al.*, 2013). It also serves as flavoring agents, and 5-alpha-reductase inhibitors (Alves-Silva *et al.*, 2016).

The antimicrobial evaluation of the methanol extract of *A. paniculata* was tested against eight bacteria species: *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Staphylococcus pneumonia*. The results (Fig. 2) showed that the extract had 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 proportional to the concentration of the extract (Fig. 2). The 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 mm were observed with *Pseudomonas aeruginosa*,

Staphylococcus aureus, respectively. Some resistance was observed with *Proteus mirabilis*, as no inhibition was recorded at low concentrations of 0.25mg/ml, and 0.50mg/ml, but a slight activity was recorded at higher concentrations of the extract (0.75 mg/ml, 1.0 mg/ml, and 1.25 mg/ml).

Furthermore, in this investigation, the radical scavenging activity of *A. paniculata* was analyzed with four different assays (DPPH, FRAP, ABTS and NOx) with ascorbic acid as a reference. At 400 µg/ml, *A.paniculata* showed 86.12±0.03% of DPPH radical scavenging

activity, while at the same concentration the reference radical scavenging agent, ascorbic acid elicited 94.03±0.03% activity. The same trend was observed with ABTS and FRAP scavenging activity which also showed a concentration dependent antioxidant activity (Figs. 3,4,5 and 6). Meanwhile, a remarkable antioxidant activity was observed in nitric acid (NOx) assay where the scavenging activity (92.15±0.06 %) was recorded compared with the reference (91.07±0.01%). It is generally accepted that the presence of some phytochemicals such as phenols and flavonoids contribute to radical scavenging ability (Salah et al., 2010).

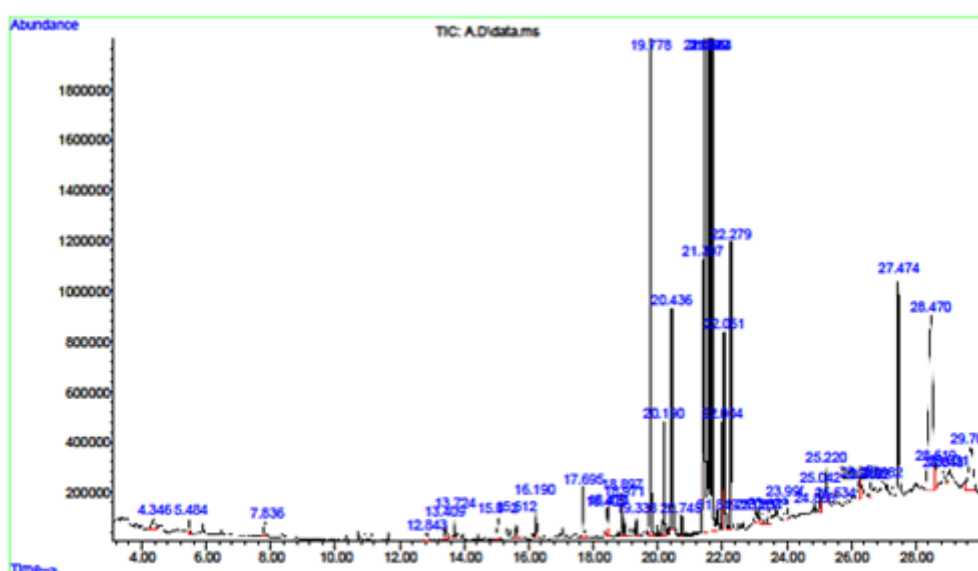


Fig. 1. The GC-MS total ion chromatogram (TIC)

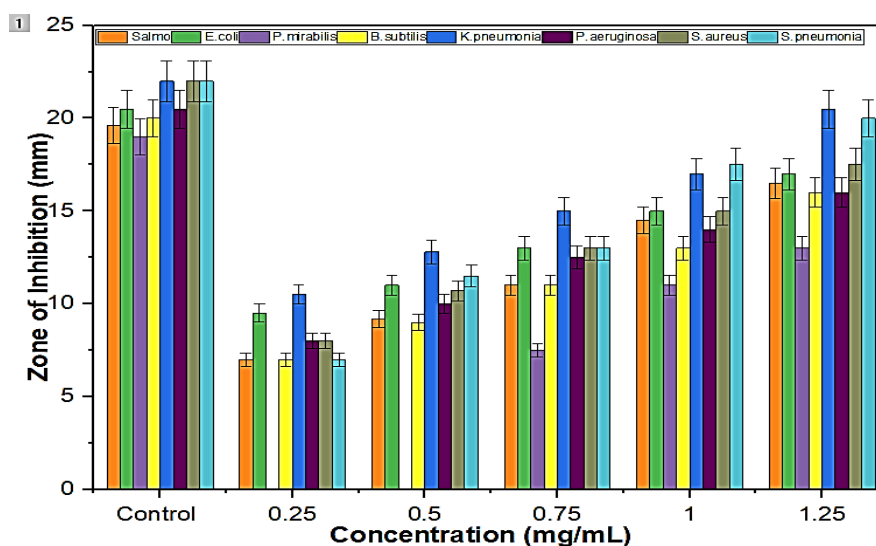


Fig. 2. Antimicrobial activity of methanol extract of *Andrographis paniculata*

Table 1. Result obtained from GC – MS analysis from the plant extract

S/N	Retention time	% Area	Name of compound	Molecular formula (g/mol)	Molecular weight
1	5.484	0.24	Benzene, 1-isocyanato-3-methoxyl	C ₈ H ₇ NO	133.15
2	13.409	0.48	Eugenol	C ₁₀ H ₁₂ O ₂	164.20
3	15.612	0.39	2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C ₁₁ H ₁₆ O ₂	180.24
4	16.190	0.49	9-octadecene(E)	C ₁₈ H ₃₆	252.5
5	17.695	0.73	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂	242.40
6	18.405	0.40	1-octadecene	C ₁₈ H ₃₆	252.5
7	18.473	0.39	10-methyl nonadecane	C ₂₀ H ₄₂	282.5
8	18.897	0.47	Phytol	C ₁₀ H ₁₈	138.25
9	18.971	0.51	2-pentadecanone	C ₁₈ H ₃₆ O	268.50
10	19.338	0.24	3-Eicosyne	C ₂₀ H ₃₈	278.52
11	19.778	6.66	Pentadecanoic acid	C ₁₇ HO ₂	270.45
14	20.190	1.55	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.34
15	20.436	3.11	Hexadecanoic acid	C ₁₈ H ₃₆ O ₂	284.47
16	21.455	7.89	9-octadecenoic acid(z,z) methyl ester	C ₁₉ H ₃₄ O ₂	296.48
17	21.592	29.42	Neophtadiene	C ₂₀ H ₃₈	278.52
18	21.684	7.29	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.50
19	22.004	1.38	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308.49
20	22.061	3.41	7,10,13-hexadecatrienoicacid methyl ester	C ₁₇ H ₂₈ O ₂	264.403
21	22.279	4.03	Octadecanoic acid, ethyl ester		
22	23.429	0.60	Eicosanoic acid, methyl ester	C ₂₁ H ₄₂ O ₂	326.55
23	23.990	0.47	Eicosane	C ₂₁ H ₄₂	282.54
24	24.888	0.25	17-octadecynoic acid, methyl ester	C ₁₈ H ₃₂ O ₂	280.44
25	25.220	0.63	Phthalic acid	C ₈ H ₆ O ₄	166.13
26	26.250	0.84	Aciphyllene	C ₁₅ H ₂₄	204.35
27	26.290	0.31	11,13-dimethyl-12-tetradecen-1-ol acetate	C ₁₈ H ₃₄ O ₂	282.46
28	26.982	0.44	Naphthalenone	C ₁₀ H ₈ O	144.17
29	27.474	4.22	Squalene	C ₃₀ H ₅₀	410.70
30	28.470	10.57	Ergost-5-en-3-ol	C ₃₀ H ₅₀ O ₂	442.71
31	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
32	28.848	0.50	2-cyclohexene-1-carboxaldehyde	C ₁₀ H ₁₆ O	152.23
33	29.706	2.77	Neoclovene	C ₁₅ H ₂₄	204.35

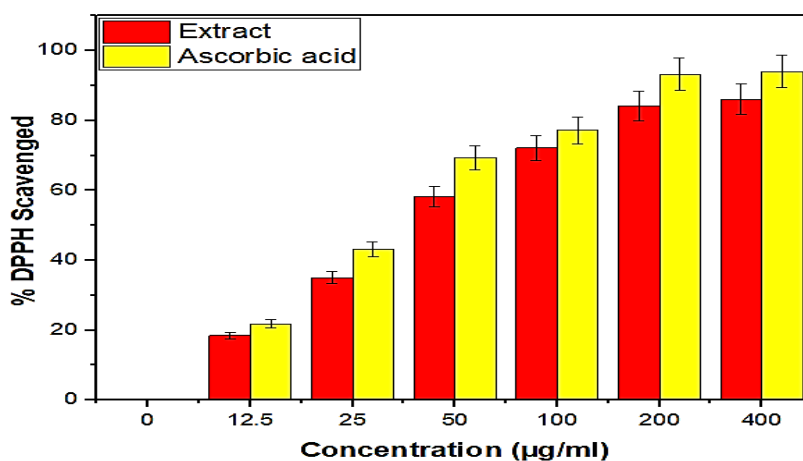


Fig. 3. DPPH radical scavenging activity of methanol extract of *Andrographis paniculata*

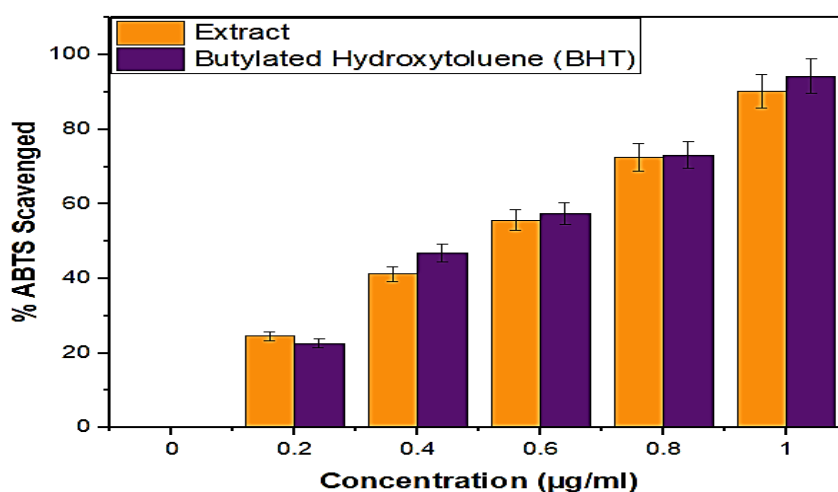


Fig. 4. ABTS radical scavenging activity of methanol extract of *Andrographis paniculata*

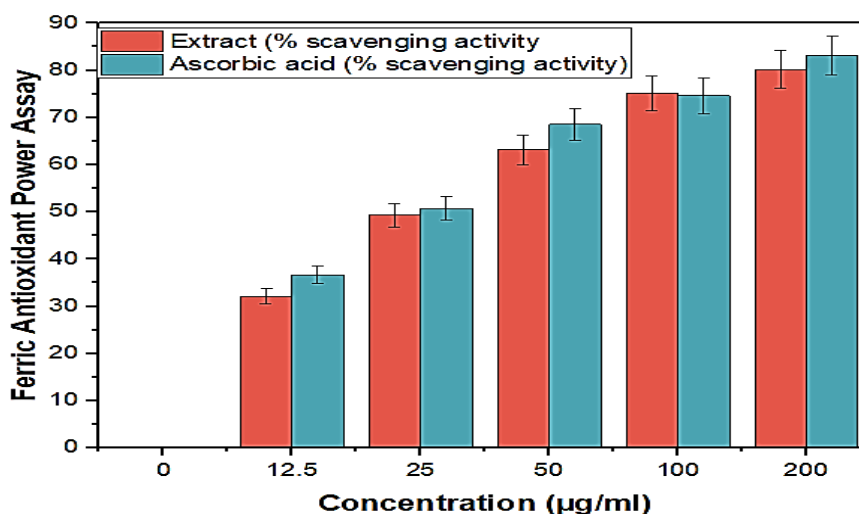


Fig. 5. FRAP radical scavenging activity of methanol extract of *Andrographis paniculata*

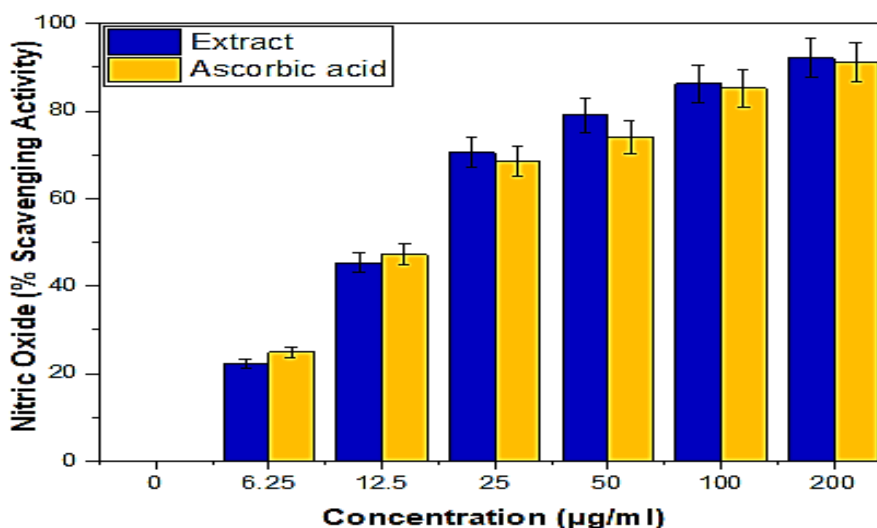


Fig. 6. NO radical scavenging activity of methanol extract of *Andrographis paniculata*

4. 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, were 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. These findings warrant further investigation into the plant's therapeutic potential, particularly in the development of natural antioxidants and antimicrobials. Additionally, our study contributes to the scientific understanding of Nigeria's medicinal plants resources and highlights opportunities for phyto-pharmaceutical innovations

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Abas, F., Ismail, A., Lajis, N. H., & Shaari, K. (2016). Anti-Diabetic Activity and Metabolic Changes Obese Diabetic Rats. *Molecules*, 21(8), 1026.
- Abdelhak R, Soraya B (2018): Phytochemical characterization, anti inflammatory and anti-ulcer activity of a spontaneous succulent *Delosperma* species. *Univers J Agr Res* 6(3):113–117
- Abebaw M, Mishra B, Gelayee DA (2018). Evaluation of anti-ulcer activity of the leaf extract of *Osyris quadripartita* Decne (Santalaceae) in rats. *J Exp Pharmacol* 9:1–11.
- Abera B (2017) Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. *J Ethnobiol Ethnomed*.10(1):40.
- Alves-Silva JM, Zuzarte M, Marques C, Ligia S, Girao H (2016) Protective effects of terpenes on the cardiovascular system: current advances and future perspectives. *Curr Med Chem* 23(40):1–42
- Arika, W.C., Kibiti, M., Njagi J.M and Ngugi M.P., (2019). *In vitro* antioxidant properties of dichloromethanolic leaf extract of *Gnidia glauca* (Fresen) as a promising antiobesity drug," *Journal of Evidence-Based Integrative Medicine*, 24, 2019.
- Bahat, A.H., Dar K.B., Anees S (2015). "Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight,"

- Biomedicine & Pharmacotherapy*, vol. 74, pp. 101–110, 2015.
- Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid content and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, 120, 261–267.
- Duru MKC, Akubugwo EI, Chinyere GC, Alisa CO, Nwaogwugwu JC(2017). Effect of seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum (A case study of Nche stream). *Academic Journal of Chemistry*. 2(8):2519-7045.
- Ferry M., and Roussel, A-M (2011) "Micronutrient status and cognitive decline in ageing," *European Geriatric Medicine*, 2(1):15–21.
- Murray, P.R., Baron, E.J., Tenover, F.C. and Tenover, H.R (2002). Manual of clinical microbiology. ASM press DC 6th Ed. Pp15-18.
- Nishanthini A, Mohan VR, Jeeva S (2014). Phytochemical, FT-IR, and GC-MS analysis of stem and leaf of *Tiliacora acuminata* (Lam.) Hook f and Thomas (menispermaceae). *Int J Pharm Sci Res* 5(9):3977–3986.
- Noctor G, Foyer CH. Ascorbate and glutathione(1998). Keeping active oxygen under
- Obasi I.O., Uzoечи C.C., Ezenwa A.U., Anumodu CK.(2017). *In-vitro* phytochemical characterization and antibacterial activity of *Newbouldia laevis* (boundary tree) on *Escherichia coli* and *Staphylococcus aureus*. *Asian Journal of Microbiology and Biotechnology*. ;2(1):30-36.
- Okhuarobo, A., Falodun, J. E., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*, 4(3), 213–222.
- Oluriola, P.F. (2004) A laboratory manual of pharmaceutical microbiology, Abuja Nigeria. Pp69-105
- Owokotomo, I.A., Jabar, J.M., Abata, E.O. (2016): Radical scavenging activity of essential oil from some Nigerian Medicinal plant and spices. *Applied Tropical Agriculture*. 21(1) 196-200
- Özçelik, B., Kartal, M., & Orhan, I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology*, 49(4), 396–402.
- Paemane, A., Hitakarun, A., Wintachai, P., Roytrakul, S., & Smith, D. R. (2019). Biomedicine & Pharmacotherapy A proteomic analysis of the anti-dengue virus activity of andrographolide. *Biomedicine & Pharmacotherapy*, 109, 322–332.
- Petrovska B.B., Historical review of medicinal plants' usage (2014). *Phcog. Rev.* 6 (11) (2012) . *Pharmacother* 69:78–90.
- Rajeswari G, Murugan M, Mohan VR (2013). GC-MS analysis of bioactive components of *Hugonia mystax* L. bark (Linaceae). *J Pharm Biomed Sci* 29: 818–824
- Ruto, M. C.; Ngugi, C. M.; Kareru, P. G.; et al. (2010). Antioxidant activity and antimicrobial properties of *Entada leptostachya* and *Prosopis juliflora* extracts. *J. Med. Plants Econ. Dev.*, 2,(31).
- Saeed N, Khan MR, Shabbir M (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC complement Altern. Med* 12(1):221–232.
- Salah-Fatnassi K B., Slim-Bannour A., Harzallah-Skhiri F., Mahjoub M A., Mighri Z., Chaumont J.P., Aouni M. (2010). *Acta Botanica Gallica*. 157(3);433-444.
- Sengul M., Yildiz H., Gungor, N., Cetin B., Eser Z., Ercisli S.(2009): Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants, *Pak. J. Pharm. Sci.* 22 (1);102–10.
- Shukla, S., Mehta, A.M., Mehta M., Bajpai V.K(2012). Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert, *Exp. Toxicol. Pathol.* 64 (7-8) (2012) 807–811.
- Sohal R.S. and W. C. Orr W.C "Redox stress hypothesis of aging(2012)," *Free Radical Biology and Medicine*, 52(3), 539–555.
- Soni A, Sosa S (2013): Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *J Pharmacogn Phytochem* 2(4):22–24
- Stoclet JC, Schini-Kerth V (2011) Dietary flavonoids and human health. Ann Sulekha M, Satish Y, Sunita Y, Rajesh N. Antioxidants: a review. *Journal of Chemical and Pharmaceutical Research*. 2009;1(1): 102-104.

Wang Y., Huang S., Shao S., Qian L., Xu P., (2012): Studies on bioactivities of tea (*Camellia sinensis* L.) fruit peel extracts: antioxidant activity and inhibitory potential against α -glucosidase and α -amylase in vitro, *Ind. Crop. Prod.* 37(1): 520–526.

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