https://dx.doi.org/10.4314/bajopas.v19i1.19

BAYERO JOURNAL OF PURE AND APPLIED SCIENCES

Volume 19 Issue 1, June, 2026

Received: 10th February, 2026 **Accepted:** 7th May, 2026

PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *Amblygonocarpus andongensis* LEAF EXTRACTS

Umar, G. N.[a, b], Sallau, M. S., [b] Ibrahim, H.[b], Atta, H. I. [c] and Kamal, N. [d]

[a] Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria.

[b] Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

[c], Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

[d] Institute of Systems Biology (INBIOSIS), Universiti Kabangsaan Malaysia (UKM), Bangi, 43600, Malaysia.

Correspondence Email: gnumar.chm@buk.edu.ng

Abstract

Amblygonocarpus andongensis is a vascular plant native to tropical regions of Africa used in traditional medicine. Ethnomedicinal studies have revealed the use of its leaf extract in the treatment of stomach-ache without scientific validations. The aim of this study was to investigate phytoconstituents and antioxidant activity of *Amblygonocarpus andongensis* leaf extracts. The plant material was percolated in methanol, extracted and dried to yield a green coloured crude extract which was later fractionated. Metabolite profiling of the extracts was conducted using Thin Layer Chromatography. Phytochemical screening of the crude extract was carried out using standard methods. Antioxidant activity of the extracts was evaluated in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using quercetin and ascorbic acid as positive controls. The TLC analysis displayed several compounds with different profiles. Phytochemical screening of the crude extract revealed presence of alkaloids, anthraquinones, carbohydrates, carotenoids, emodins, flavonoids, fixed oils and fat, glycosides, phenolics, saponins, steroids, tannins and terpenoids while coumarins were found to be absent. The crude, n-hexane, dichlomethane and ethyl acetate extracts demonstrated antioxidant activity of IC_{50} value 29.545, 57.352, 20.357 and 15.962 µg/ml respectively relative to quercetin (IC_{50} value 2.717 µg/ml) and ascorbic acid (IC_{50} value 9.303 µg/ml). The antioxidant activity exhibited by the extracts could be associated with presence of phytochemicals such as phenols, tannins and flavonoids among others. This research for the first time reported the phytochemicals and antioxidant activity of *Amblygonocarpus andongensis* leaf methanolic extracts. The research also validates the traditional use of the plant leaf extract in the treatment of diseases associated with oxidative stress.

Keywords: *Amblygonocarpus andongensis*; Phytochemicals; Antioxidant; DPPH

INTRODUCTION

Illness and disease are among the most disturbing challenges facing humanity resulting from many and different factors including aging (Hochberg, 2023). However, multiple solutions to these numerous challenges are offered by nature in form of sustainable bioactive natural products that can treat and manage diseases and disorders (Singla *et al.*, 2023). For almost the entire existence of mankind, medicinal herbs have been used to treat various diseases (Mitra *et al.*, 2022), and the healing effectiveness of medicinal plants is due to the presence of natural phytochemicals in them (Gizaw *et al.*, 2022). Phytochemicals are bioactive compounds produced by plants for their defense, and they are found in grains, fruits and vegetables among others (Kumar *et al.*, 2023). Moreover, they have been reported for their pharmacological properties including antioxidant, antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activity (Gonfa *et al.*, 2023; Kumar *et al.*, 2023). Phytochemicals are broadly classified as terpenes and terpenoids, alkaloids, and phenolic compounds, and their specific structural characteristics depend on the reaction pathway from which they were bio-synthesized (Bitwell *et al.*, 2023).

Free radical, an atom, molecule or ion with unpaired valence electron (Adriana *et al.*, 2012; Clifford, 2016) is produced by cells through breathing and cell-mediated immune systems (Baliyan *et al.*, 2022). The overproduction of free radicals results in damaging macromolecules such as nucleic acids, proteins and lipids leading to tissue damage in various chronic and degenerative diseases (Martemucci *et al.*, 2022).

In addition, it also contributes to intestinal diseases such as inflammatory bowel disease (IBD), enteric infections, ischemic intestinal injuries, and colorectal cancer (Wang *et al.*, 2020) that could lead to stomach discomfort. Antioxidants are natural or synthetic compounds that protect living systems from oxidative damage caused by free radicals in order to minimize the development of diseases (Halliwell, 2024).

Amblygonocarpus andongensis otherwise known as kolon iche (Hausa), Aidotoro (Yoruba) and Jigaree-hi (Fulfulde) is a tall tree belonging to the family *Mimosaceae* (Oluranti *et al.*, 2012; Ugwah *et al.*, 2014; Kayode *et al.*, 2016). Ethnomedicinal studies have revealed the use of its stem bark extracts in the treatment of ulcers, diarrhea, diabetes, hypertension, wounds and inflammation among others (Ebbo *et al.*, 2020; Baponwa *et al.*, 2022). The leaf extract has also been reported to treat stomach-ache (Ugwah *et al.*, 2014) without scientific validations**.** Researches have reported the anti-nociceptive (Nwinyi *et al.*, 2006), anti-psychotic (Ebbo *et al.*, 2008), anti-diarrhoeal (Ugwah *et al.*, 2014) and toxicological (Ebbo *et al.*, 2020) properties of the stem bark extracts of the plant. In another research, Baponwa *et al.*, (2022) investigated the phytochemical content and

antioxidant activity of the stem bark extracts of the plant. However, there were no similar reports on the leaf extract of the plant. Therefore, the aim of the present study is to carryout phytochemical screening and antioxidant activity of *Amblygonocarpus andongensis* leaf extracts to establish a relationship between stomach-ache and antioxidant activity.

MATERIALS AND METHODS

Materials

Dichloromethane and n-hexane were purchased from Chemiz Chemicals, Malaysia, ethyl acetate and methanol were purchased from Systerm and R & M Chemicals Malaysia respectively. Corning 96 well plates**,** Aluminium precoated TLC plates with silica gel (GF254, 20×20 cm, 0.25±0.03 mm thickness and 8±2 µm≥80% particles size) were purchased from Shanghai Shengya Chemical Co. Ltd, Gonghe New Road, Shanghai. Weighing balances (Mettle Toledo AB54) and (Mettler- Toldeo PB303-S/FACT TOP-PAN) were used for weighing purposes at Microbiology Departments ABU, Zaria and Metabolomic Laboratory, INBIOSIS, UKM, Malaysia. Absorbance readings for DPPH assay were taken using micro plate reader (iMark™/13390) at Metabolomic Laboratory, INBIOSIS, UKM, Malaysia.

Sampling and Authentication of Plant Material

Fresh and healthy plant leaves were collected in a sterile plastic bag from Bakori local government zone, Katsina State, (GPS coordinates: 11° 33′ 20.12″ N, 7° 25′ 27.08″ E), brought to the laboratory and processed within 24 h (Kuo *et al*., 2021). The fresh leaf was authenticated by Mal. Namadi, a botanist at the Herbarium Unit, Biological Sciences Department, Ahmadu Bello University, Zaria where the Voucher Number (ABU4167) was deposited for future reference.

Extraction of Plant Material and Fractionation

Extraction

The shade dried and powdered plant leaf material (450 g) was percolated in methanol (2,250 ml) at room temp and allowed to stay for 18h. The leaf extract was then filtered, dried under vacuum on a rotary evaporator and kept in refrigerator until use for analysis (Ebbo *et al*., 2020).

Fractionation

A 100 mL solution of 90% aqueous methanol (v/v) was prepared and used to dissolve the F1 extract (10 g). The solution was transferred into a 1 L separatory funnel and mixed thoroughly. Hexane (100 mL) was then added, and the mixture was shaken and allowed to separate into layers. The upper hexane layer was collected into a round-bottom flask and evaporated to dryness. Subsequently, the methanol–water concentration was adjusted to a 60:200 ratio (v/v; total volume 260 mL, equivalent to approximately 23% methanol) and transferred into the separatory funnel. Dichloromethane (100 mL) was added, the mixture was shaken, and the layers were allowed to separate. The organic layer (dichloromethane) was collected into a separate round-bottom flask and evaporated to dryness. The remaining aqueous methanol layer was then transferred into a new round-bottom flask, and the methanol was evaporated under reduced pressure. Ethyl acetate (100 mL) was added to the remaining aqueous phase, mixed thoroughly in a separatory funnel, and left to stand for phase separation. The ethyl acetate layer was collected and evaporated to dryness (Di *et al*., 2023).

Phytochemical Screening

Presence or absence of phytochemicals such as alkaloids, anthraquinones, carbohydrates, carotenoids, coumarins, emodins, flavonoids, fixed oils and fat, glycosides, phenolics, saponins, steroids, tannins and terpenoids in the crude extract were tested in accordance with standard methods.

Test for Alkaloids (Dragendorff Test)

Plant material (200 mg) was boiled in 10 mL methanol and filtered. Then, 1% HCl was added followed by 6 drops of Dragendroff reagent. The formation of brownish-red precipitate indicated alkaloids presence

Test for Anthraquinones (Ammonium Hydroxide Test)

Extract (10 mg) was dissolved in 1 mL isopropyl alcohol followed by a drop of conc. ammonium hydroxide solution. The formation of red colour after 2 min indicated anthraquinone presence (Shaikh and Patil, 2020).

Test for Carbohydrates (Barfoed's Test)

Extract (100 mg) was dissolved in 5 mL distilled water and filtered. Filtrate (1 mL) was added to 1 mL Barfoed's reagent and heated for 2 min. The formation of red precipitate indicated carbohydrates presence (Shaikh and Patil, 2020).

Test for Carotenoids (Carr-Price Reaction)

Drops (2-3) of saturated solution of antimony trichloride in chloroform were added to dried extract. The formation of blue-green colour which eventually changed to red indicated carotenoids presence (Shaikh and Patil, 2020).

Test for Coumarins (NaOH Test)

Extract (500 mg) was mixed with 1 mL 10% NaOH followed by 1 mL chloroform. The formation of yellow colour indicated the presence of coumarins (Shaikh and Patil, 2020). However, the test was found to be negative as there was no formation of yellow colour.

Test for Emodins

Extract (20 mg) was mixed with 2 mL ammonium hydroxide followed by 3 mL of benzene. The formation of red colour indicated emodins presence (Shaikh and Patil, 2020).

Test for Fixed Oils and Fat (Stain Test)

Little quantity of the extract was pressed in between two filter papers. Oil stain on the paper indicated fixed oils and fat presence (Shaikh and Patil, 2020).

Test for Flavonoids (Alkaline Reagent Test)

Extract (10 mg) was dissolved in 1 mL methanol followed by successive addition of 2% NaOH solution (2 mL) and few drops of dil. HCl. The change of intense yellow colour formed to colourless on addition of diluted acid indicated flavonoids presence (Shaikh and Patil, 2020).

Test for Glycosides (**Aqueous NaOH Test**)
Extract (10 mg) dissolved in 1 mL methanol was reacted with 1 mL of water followed by few drops of aqueous NaOH solution. The formation of yellow colour indicated glycosides presence (Shaikh and Patil, 2020).

Test for Phenolics (**Ferric Chloride Test**)
Extract (5 mg) was dissolved in 0.5 ml of 1% ferric chloride solution. The formation of bluish-black color indicated the presence of phenolic compounds (Kebede *et al*., 2021).

Test for Saponins (**Frothing Test**)
Extract (2.5 mg) was dissolved in 5 ml distilled water and shaken well in a test tube followed by warming. The formation of persistent foam indicated the presence of saponins (Kebede *et al*., 2021).

Test for Steroids
Extract (5 g) was mixed with 10 mL chloroform followed by 10 mL conc. sulphuric acid. The formation of bilayer (red top layer and greenish bottom layer) indicated the presence of steroids (Dubale *et al*., 2023).

Test for Tannins (Braymer's Test)
Extract (2.5 mg) was boiled in 5ml of distilled water in a test tube and then filtered through Whatman's no. 1 filter paper followed by addition 2-3 drops of 0.1% ferric chloride to the filterate. The formation blue-black precipitate indicated tannins presence (Kebede *et al*., 2021).

Test for Terpenoids
Extract (1 g) was mixed with 2 mL chloroform followed by 3 mL conc. sulphuric acid. The formation of reddish-brown colour indicated the presence of terpenoids (Dubale *et al*., 2023).

Thin Layer Chromatography
TLC was conducted according to the method describe by Ginovyan *et al*., (2020). A spot of the prepared sample extract was placed on aluminium pre-coated thin layer chromatography plate using capillary tube and allowed to dry. The plate was developed in appropriate solvent involving n-hexane (100%), dichloromethane (100%) and ethyl acetate (100%). The retention factor (R_f) value was calculated using the following relation:

$$R_f \text{ value} = \frac{Distance\ moved\ by\ the\ sample}{Distance\ moved\ by\ the\ solvent}$$

DPPH Radical Scavenging Assay
The radical scavenging activity of the extracts was evaluated using the method described by Liu *et al*. (2020), with slight modifications. Test samples were prepared in methanol at serial concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL. Aliquots of 100 µL from each concentration were dispensed in triplicate into wells of a 96-well microplate. Subsequently, 200 µL of 0.2 mmol/L DPPH solution in methanol (equivalent to 78.9 mg/L) was added to each well. The plate was then incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured again at 517 nm. A solution of 0.2 mmol/L DPPH in methanol without extract served as the control. Ascorbic acid and quercetin were employed as standard antioxidants (positive controls). The percentage of radical scavenging activity (RSA%) for each sample was calculated using the following equation:

$$RSA\% = \frac{A0 - A1}{A0} \times 100$$

where RSA% represents the percentage antioxidant activity, A1 is the absorbance of the sample while and A0 is the absorbance of the control. The RSA% values were plotted against the corresponding concentrations (µg/mL), and the half-maximal inhibitory concentration (IC_{50}) was determined using SPSS statistical software.

RESULTS AND DISCUSSION

Extraction and Fractionation

The percentage yield and appearance of the extracts are presented in Table 1 whereas the fractionation result of the crude extract is presented in Figure 1.

Table 1: Yield, Percentage Yield and Appearances of the Extracts

S/N	Fraction	Appearance	Yield (mg/g)	% Yield
1	F1	green solid	89,390/450	19.86
2	F1-1	oily green	1,880/10	18.80
3	F1-2	green solid	1,080/10	10.80
4	F1-3	brown solid	760/10	7.60

F1: crude extract; F1-1: n-hexane extract; F1-2: dichloromethane extract; F1-3: ethyl acetate extract

The percentage yield of n-hexane extract was found to be the highest among the extracts followed by dichloromethane extract then ethyl acetate extract. This indicated the decrease in quantities of the extracts with increase in solvent polarity, suggesting presence of more nonpolar compounds than the polar ones in the extract. Fraction F1 and F1-2 appeared as green solids while fractions F1-1 and F1-3 appeared as oily green and brown solid respectively (Figure 1).

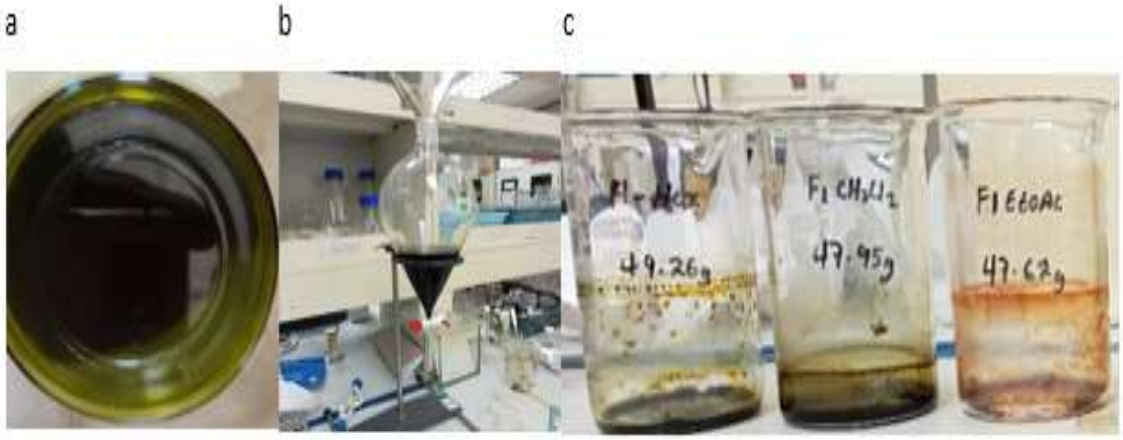

Figure 1: *Amblygonocarpus andongensis* crude leaf extract (a), fractionation stage (b) and fractionated extracts (c).

Phytochemical Screening

The detected phytochemicals present in the crude extract were presented in Table 2.

Table 2: Phytochemical Screening of *Amblygonocarpus andongensis* Crude Leaf Extract.

S/N	Phytochemical	Inference
1	Alkaloids	+
2	Anthraquinones	+
3	Carbohydrates	+
4	Carotenoids	+
5	Coumarins	-
6	Emodins	+
7	Flavanoids	+
8	Fixed oils and fat	+
9	Glycosides	+
10	Phenolics	+
11	Saponins	+
12	Steroids	+
13	Tannins	+
14	Terpenoids	+

+ implies present while – implies not detected

The phytochemical screening result indicated that, of all the phytochemicals screened only coumarins were found to be absent. This revealed the richness of *Amblygonocarpus andongensis* leaf in phytochemicals showcasing its diversity in bioactive compounds as well as connecting its uses in traditional medicine. Phytochemicals such as saponins, tannins, alkaloids, terpenoids, anthraquinones, phenolics, glycosides, and flavonoids have been suggested to possess pharmacological properties (Kebede *et al.*, 2021).

Thin Layer Chromatography (TLC)

The TLC result presented in Table 3 below shows the number of spots and R_f values in different solvent system used while spots appearances are presented in Figure 2.

Table 3: TLC Analysis of the Fractions in Different Solvent System.

Fraction	Solvent system (100%)	No. of spot (s)	R_f value(s)
F1	n-Hexane	1	0.15
F1-1		2	0.02, 0.37
F1	Dichloromethane	6	0.08, 0.10, 0.19, 0.27, 0.37, 0.47
F1-2		8	0.08, 0.12, 0.19, 0.28, 0.47, 0.56, 0.63, 0.98
F1	Ethyl acetate	2	0.38, 0.94
F1-3		2	0.38, 0.95

F1: crude extract; F1-1: n-Hexane extract; F1-2: dichloromethane extract; F1-3: ethyl acetate extract

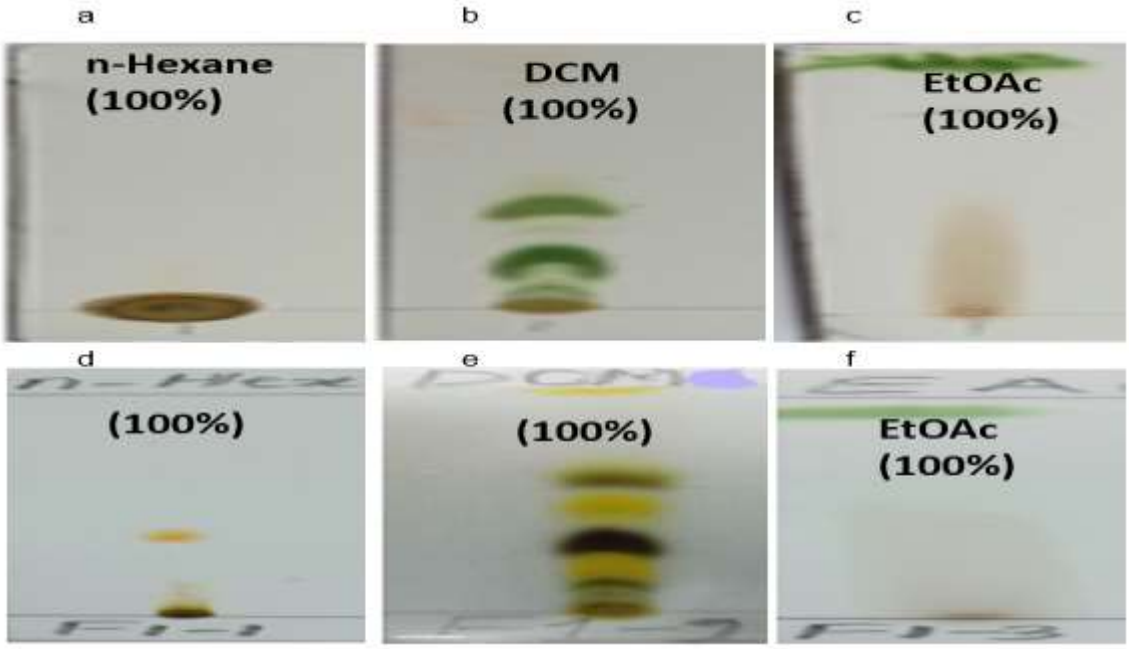

Figure 2**:** Comparative TLC analysis of fractions F1 and F1-1 in n-Hexane (a and d), F1 and F1-2 in dichloromethane (b and e) and F1 and F1-3 in ethyl acetate (c and f) showing different compounds with different TLC profiles. Fractions F1 and F1-1 were found to produce one (1) and two (2) spots respectively in n-Hexane solvent system while fractions F1 and F1-2 produced six (6) and eight (8) spots in dichloromethane solvent system respectively. This indicated that there is correlation between purification of the extracts and number of compounds i.e. as the extracts are getting purer more compounds are coming out. Moreover, similar compounds were observed in the case of fractions F1 and F1-3 in ethyl acetate solvent system.

DPPH Radical Scavenging Assay

The antioxidant result of the samples are presented in Table 4 and Figure 3. The higher the IC_{50} value the lower the activity of the sample and vice versa.

Table 4: Comparative Percentages DPPH Radical Scavenging Activity of F1 Fractions and Positive Controls.

Conc. (µg/ml)	F1	F1-1	F1-2	F1-3	Ascorbic acid	Quercetin
1000	93.9497	95.3149	94.3066	94.2445	97.9971	94.0428
500	93.3137	94.3531	93.5309	93.8101	96.4629	93.9032
250	92.9724	77.3348	90.0189	92.8483	96.2147	93.7015
125	92.6001	51.8616	85.9414	92.7242	95.3078	93.3913
62.5	69.9504	40.6454	72.4294	90.6311	95.0751	89.1015
31.25	40.8781	35.8517	61.6227	71.9020	84.7099	82.7707
15.625	33.0438	29.2119	51.3869	40.8626	63.0500	72.5070
7.8125	22.7428	24.1390	21.2194	26.2581	33.0841	54.9054
IC_{50}	29.5450	57.3520	20.3570	15.9620	9.3030	2.7170

All the fractions displayed antioxidant activity at various concentrations in a dose dependent manner. On the over all, fraction F1-3 due to its least IC_{50} value was found to be the most active among the fractions followed by fraction F1-2 (Table 4 and Figure 3). These 2 fractions displayed activity even higher than that of the crude extract (F1) though lower compared to the positive controls. Fraction F1-1 revealed the least activity in comparison to all fractions. The trend in which the antioxidant activity was exhibited by the fractions indicated increase in activity with increase in solvent polarity which could be associated with type of phytochemicals present in each extract. This suggested that there was distribution of phytochemicals from nonpolar to medium and highly polar ones across the extracts. Since antioxidant activity proceeds through transfer of H atoms or electrons (Gulcin and Alwasel, 2023), the polarity-dependent increase in radical scavenging activity observed in the fractions may be attributed to the presence of a majority of the polar compounds in more polar solvents.

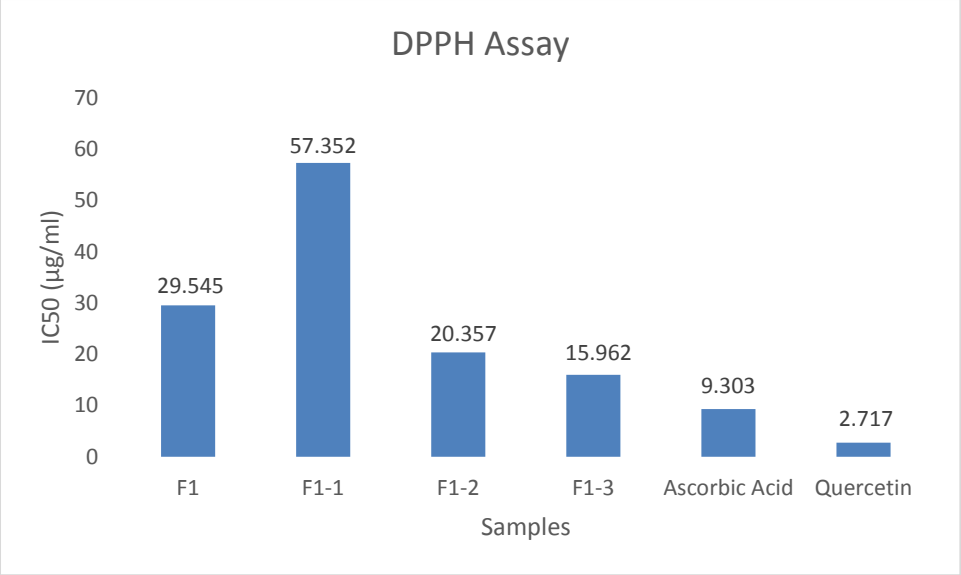

Figure 3: DPPH radical scavenging activity of fractions F1, F1-1, F1-2 and F1-3 in comparison positive controls.

CONCLUSION

All the extracts were found to possess radical scavenging activity with ethylacetate extract being the highest. The antioxidant activity demonstrated by the extracts could be due to the presence of phytochemicals such as phenolics, tannins and flavonoids among others. TLC analysis of the extracts revealed the presence of several phytochemicals with different TLC profiles. This research for the first time reported the phytoconstituents and DPPH radical scavenging activity of *Amblygonocarpus andongensis* leaf extracts. It also established the metabolite profiling of the extracts using TLC. This research also validates the traditional use of *Amblygonocarpus andongensis* leaf extract in the treatment of diseases and challenges associated with oxidative stress.

Research Future Direction

The research is currently focusing on LCMS analysis and column chromatographic techniques towards isolation of pure compounds.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Tertiary Education Trust Fund (TETFund) Nigeria Institution Based Research for sponsoring a research conducted at Institute of Systems Biology (INBIOSIS) University Kebangsaan Malaysia (UKM), Malaysia. We also acknowledge the laboratory support of the Chemistry Department, ABU Zaria, Microbiology Department of ABU Zaria and the Department of Pure and Industrial Chemistry, BUK, Kano.

Declaration of Generative AI in Scientific Writing

During the preparation of this work, the authors used ChatGPT 3.5 in order to assist in refining the clarity, coherence, and improve academic tone to polish sentence structure where appropriate. All content was carefully reviewed and edited by the authors to ensure originality, accuracy, and adherence to the academic standards of the field. The final responsibility for the content in publication remains entirely with the authors.

Credit Authorship Contribution Statement

Gali Nuhu Umar: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing- original draft, Writing- review & editing. **Muhammad Sani Sallau**: Methodology, Validation, Resources, Supervision, Writing- review & editing. **Hamisu Ibrahim**: Methodology, Validation, Resources, Supervision, Writing- review & editing. **Habiba Iliyasu Atta**: Methodology, Validation, Resources, Supervision, Writing- review & editing. **Nurkhalida Kamal**: Conceptualization, Methodology, Validation, Resources, Supervision, Funding acquisition, Writing-review & editing.

REFERENCES

Adriana M. F., Lilian S. P., Charlane K. S., Wemerson N. M., Roosevelt A. G., Otemberg S. C., Maria-de F. V., Reinaldo N. A. and Temilce S. A. (2012)."Total Phenolic Content and Antioxidant Activity of Some *Malvaceae* Family Species". *Antioxidants*, 1, 33-43.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, 27(4), 1326.

Baponwa, O., Amang, A. P., Mezui, C., Koubala, B. B., Siwe, G. T., Vandi, V. L., & Tan, P. V. (2022). Antioxidant Mechanism of Renal and Hepatic Failure Prevention Related to Paracetamol Overdose by the Aqueous Extract of *Amblygonocarpus andongensis* Stem Bark.

BioMed Research International, 2022(1), 1846558.

Bitwell, C., Indra, S. S., Luke, C., & Kakoma, M. K. (2023). A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Scientific African*, *19*, e01585.

Clifford W. F. (2016). "Platinum Anti-cancer Drugs: Free Radical Mechanism of Pt-DNA Adduct Formation and Anti-neoplastic Effect". *Free Radical Biology and Medicine*, 95, 216–229.

Di, X., Hardardottir, I., Freysdottir, J., Wang, D., Gustafson, K. R., Omarsdottir, S., & Molinski, T. F. (2023). Geobarrettin D, a Rare Herbipoline-Containing 6-Bromoindole Alkaloid from *Geodia barretti*. *Molecules*, *28*(7), 2937.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*, 51-62.

Ebbo, A. A., Elsa, A. T., Etuk, E. U., Ladan, M. J., & Saganuwan, S. A. (2008). Antipsychotic Effect of Aqueous Stem Bark Extract of *Amblygonocarpus andongensis* in Wistar Albino Rats. *Animal Research International*, *5*(2).

Ebbo, A. A., Elsa, A. T., Etuk, E. U., Ladan, M. J., & Saganuwan, S. A. (2020). Phytochemical Screening and Toxicological Effects of *Amblygonocarpus andongensis* Aqueous Stem Bark Extract in Wistar Albino Rat. *African Journal of Pharmacy and Pharmacology*, 14(5), 107-112.

Ginovyan, M., Ayvazyan, A., Nikoyan, A., Tumanyan, L., & Trchounian, A. (2020). Phytochemical Screening and Detection of Antibacterial Components from Crude Extracts of Some Armenian Herbs Using TLC-bioautographic Technique. *Current Microbiology*, 77(7), 1223-1232.

Gizaw, A., Marami, L. M., Teshome, I., Sarba, E. J., Admasu, P., Babele, D. A., ... & Abdisa, K. (2022). Phytochemical Screening and In-vitro Antifungal Activity of Selected Medicinal Plants Against *Candida albicans* and *Aspergillus niger* in West Shewa Zone, Ethiopia. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022(1), 3299146.

Gonfa, Y. H., Tessema, F. B., Bachheti, A., Rai, N., Tadesse, M. G., Singab, A. N., ... & Bachheti, R. K. (2023). Anti-inflammatory activity of Phytochemicals from Medicinal Plants and Their Nanoparticles: A Review. *Current Research in Biotechnology*, *6*, 100152.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, 11(8), 2248.

Halliwell, B. (2024). Understanding Mechanisms of Antioxidant Action in Health and Disease. *Nature Reviews Molecular Cell Biology*, 25(1), 13-33.

Hochberg, M. E. (2023). Addressing Challenges in Evolutionary Medicine: Three Priorities. *Frontiers in Science*, 1, 1123806.

Kayode, J., & Sanni, P. O. (2016). Survey of Barks Used for Medicine in the Central Zone of Lagos State, Nigeria. *Journal of Botanical Papers*, 1(1), 1-7.

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial Activities Evaluation and Phytochemical Screening of Some Selected Medicinal Plants: A Possible Alternative in the Treatment of Multidrug-resistant Microbes. *PloS one*, 16(3), e0249253.

Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., ... & Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules*, 28(2), 887.

Kuo, J., Chang, C. F., & Chi, W. C. (2021). Isolation of Endophytic Fungi with Antimicrobial Activity from Medicinal Plant *Zanthoxylum simulans* Hance. *Folia microbiologica*, *66*(3), 385–397.

Liu, D., Guo, Y., Wu, P., Wang, Y., Golly, M. K., & Ma, H. (2020). The Necessity of Walnut Proteolysis Based on Evaluation after In-vitro Simulated Digestion: ACE Inhibition and DPPH Radical-Scavenging Activities. *Food Chemistry*, 311, 125960.

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*, 2022; 2: 48–78.

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., ... & Simal-Gandara, J. (2022). Impact of Heavy Metals on the Environment and Human Health: Novel Therapeutic Insights to Counter the Toxicity. *Journal of King Saud University–Science*, *34*.

Oluranti, A. C., Michael, U. O., Jane, U. O. C., & Ayembe, N. A. (2012). Ethno Botanical Studies of Medicinal Plants Used in the Management of Peptic Ulcer Disease in Sokoto State, North Western Nigeria. *Int. Res. J. Pharm. Pharmacol*, 2(9), 225-230.

Singla, R. K., De, R., Efferth, T., Mezzetti, B., Uddin, M. S., Ntie-Kang, F., ... & Ahmad, J. (2023). The International Natural Product Sciences Taskforce (INPST) and the Power of Twitter Networking Exemplified Through# INPST Hashtag Analysis. *Phytomedicine*, *108*, 154520.

Shaikh, J. R., & Patil, M. (2020). Qualitative Tests for Preliminary Phytochemical Screening: An Overview. *International Journal of Chemical Studies*, 8(2), 603-608.

Ugwah, O. M., Ugwah-Oguejiofor, C. J., Abubakar, K., Okorie, N. A., & Njan, A. A. (2014). Anti-diarrhoeal Activities of Aqueous Stem Bark Extract of *Amblygonocarpus andongensis* (welw. exoliv.) Exell&torre. *Nigerian Journal of Pharmaceutical Sciences*, 13(2), 1-11.

Wang, Y., Chen, Y., Zhang, X., Lu, Y., & Chen, H. (2020). New Insights in Intestinal Oxidative Stress Damage and the Health Intervention Effects of Nutrients: A Review. *Journal of Functional Foods*, *75*, 104248.