Tropical Journal of Pharmaceutical Research May 2025; 24 (5): 703-709
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v24i5.8

Original Research Article

Antiplasmodial and antitrypanosomal effects of some plant extracts utilized within Nigerian traditional healing practices

Ijeoma S Okoro[1,2]*, Sampson D Umoh[1], Anita K Asekunowo[3], Ahamefula A Ahuchaogu[4], Omotola M Fayomi[1], Peter O Onuwa[1]

[1]Department of Chemistry, Joseph Sarwuan Tarka University, Makurdi, Nigeria, [2]Department of Chemistry, Rhodes University, PO Box 94, Grahamstown 6140, South Africa, [3]Department of Chemistry, Faculty of Science, University of Lagos, [4]Department of Pure and Industrial Chemistry, Abia State University, Nigeria

For correspondence: **Email: Okoro.ijeoma@uam.edu.ng; **Tel:** +234-7036975804*

Sent for review: 8 January 2025 *Revised accepted: 20 May 2025*

Abstract

Purpose: *To investigate the antiplasmodial and antitrypanosomal properties of extracts from Anthocleista djalonensis A. Chev. (Gentianaceae), Vernonia cinerea Less (Asteraceae), and Pycnanthus angolensis Welw. (Myristicaceae).*
Methods: *Cell growth inhibitory activities were measured using Trypanosoma brucei (10^6 – 10^7 parasites/mL in HMI-9 or SDM-79 medium) and Plasmodium falciparum strain 3D7 (0.1 –10 % parasitemia and 10^8 parasites/mL in culture). An IC_{50} value less than 10 µg/mL was required for activity.*
Results: *Viability of P. falciparum and T. brucei was significantly reduced when exposed to extracts of A. djalonensis and P. angolensis at 25 µg/mL ($p < 0.05$), with extract of V. cinerea demonstrating less antiplasmodial activity. Furthermore, crude extracts of A. djalonensis, V. cinerea, and P. angolensis demonstrated strong anti-trypanosomal activity with IC_{50} values of 0.0036, 2.818, and 4.677 µg/mL, respectively.*
Conclusion: *The study reveals the antiplasmodial and antitrypanosomal properties of A. djalonensis, V. cinerea, and P. angolensis, highlighting the need for further investigation of their constituents.*

Keywords: *A. djalonensis, V. cinerea, P. angolensis, Antiplasmodial activity, Antitrypanosomal activity*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Search for scientific evidence and novel biologically active compounds remains a continuum and has not been thoroughly explored. Several Nigerian plants, such as *Combretum dolichopetalum* [1] and *Baphia nitida* [2] have been shown to possess antiplasmodial and anti-trypanosomal properties.

Anthocleista djalonensis Chev. (*A. djalonensis*) is one of the species in the family *Gentianaceae*. Previous studies had documented its antimicrobial and anti-HIV-1 properties [3]. The root infusion is used to treat pyrexia, constipation, gonococci, and chest pains [4]. *Vernonia cinerea* (*V. cinerea; Asteraceae*) is used as an indigenous medicine for stomach aches, colds, asthma, bronchitis, and intermittent

fever [5]. It is recommended by the Ayurveda Pharmacopoeia of India [6].

Pycnanthus angolensis (*P. angolensis*; Welw) Warb., a *Myristicaceae* plant, is used to cure or control toothaches, headaches, sore throats, ulcers, and wounds [7]. Only few biological or phytochemical studies have been conducted on extracts of *V. cinerea, P. angolensis*, and *A. djalonensis*. Therefore, this current study investigated the antiplasmodial and antitrypanosomal properties of three Nigerian medicinal herb extracts (*P. angolensis* stems, *V. cinerea* plant, and *A. djalonensis* root).

EXPERIMENTAL

Plant material

A. djalonensis roots were collected in Zaki Biam, Benue State, Nigeria, at 9° 36' 30.41 E longitude and 7° 30' 48.95 N latitude. While *V. cinerea* whole plant and *P. angolensis* stem barks were obtained from Arochukwu, Abia State, Nigeria, situated at latitude 5° 22' 59.99" N, Longitude 7° 54' 59.99" E, and 9° 36' 30.41" E. The plants were identified at the Taxonomy Unit of the Forestry Department at Michael Okpara University of Agriculture, Umudike (MOUAU) by a taxonomist, Mr. Ibe Ndukwe. A sample of a voucher with serial numbers AD/124, VC/125, and PA/126, respectively, was deposited at the same facility.

Extraction

The plant samples (3.50 kg of *A. djalonensis*, 1.95 kg of *V. cinerea*, and 3.78 kg of *P. angolensis*) were macerated in methanol (10, 5, and 13 L, respectively), for 48 h at room temperature (27 ± 2 °C) on three separate occasions [8]. The mixture was filtered and concentrated using a rotary evaporator to yield 12 g, 8 g, and 15 g of *A. djalonensis*, *V. cinerea*, and *P. angolensis* extracts, respectively.

Antiplasmodial activity

Previously established methods [9] were adopted in this study. The malaria parasite, *P. falciparum* isolates 3D7 (obtained from BEI Resources (NIAID, USA) and maintained in (Roswell Park Memorial Institute) RPMI 1640 medium supplemented with human erythrocytes under standard *in vitro* culture conditions), with 25 mM supplemented with 25 mM HEPES (Lonza, Switzerland), 2 mM L-glutamine (Gibco, USA), 20 mM glucose (Sigma-Aldrich, USA), 5 % Albumax II (Gibco, USA), 0.65 mM hypoxanthine (Sigma-Aldrich, USA), 2 – 4 % human hemoglobin (Sigma-Aldrich, USA), and 60 µg/mL gentamycin (Gibco, USA) was used. Parasitic organisms were grown at 37 °C in a mixture that included 5 % carbon dioxide, 5 % oxygen, and 90 % nitrogen in sealed T25 and T75 growth vessels. Using crude extracts at concentrations of 25 *µ*g/mL, the *Plasmodium falciparum* 3D7 strain was placed in 96-well plates at 1 % parasitemia and 2 % hematocrit to test the effects of the specimens. An incubator with CO_2 was then used to keep the mixture at 37 °C for 48 h. Following a 48 h incubation period, 20 µL of the culture medium was extracted from each well on the plate. Subsequently, 125 *µ*L of Malstat and Nitroblue Tetrazolium/Phenazine Ethosulfate solutions were mixed on a 96-well plate. A 96-well plate reader was used to detect the absorbance at 620 nm to assess activity of the parasite lactate dehydrating enzyme (pLDH) with Abs620 in each well acting as an indicator of parasite abundance and pLDH activity.

Antitrypanosomal activity

The antitrypanosomal ability of the extracts was investigated by cultivating *Trypanosoma bruce in vitro* in 96-well plates at 25 µg/mL. Following a 48 h incubation period, the incorporation of a reagent comprising resazurin allowed for the determination of the total number of parasites that survived drug exposure. Their activities were tested, according to established methods [9], using a multiwell illuminated surface reader to quantify emission at 590 nm and fluorescence at 560 nm.

Single concentration screening

The proportion of parasitic infections or cell survival was found for each medication concentration. The extracts were tested in three wells, and a standard deviation (SD) was established. Concentration of each extract used was 25 µg/mL. Positive controls were pentamidine (1 µM) and chloroquine (10 µM). Cells treated with 100 µL of distilled water served as the negative control to provide a comparison for the antitrypanosomal and antiplasmodial tests, respectively. A microplate reader (Biotek Synergy MX) was used for each measurement, and determinations were done in triplicates (n = 3) using a 96-well plate format.

Dose-response

The IC_{50} was determined by non-linear regression from the resulting dose-response curve following the computation of each sample's percent viability vs log (extract concentration). Depending on the kind of test, pentamidine or

chloroquine was used as a drug standard for comparison. Dose-response experiments were used to determine IC_{50} for the samples under certain criteria. The IC_{50} < 10 µg/mL testing range was 100 – 0.00001 µg/mL, comprising two different threefold serial dilutions (one for anti-trypanosomal testing and the other for antiplasmodial testing).

Statistical analysis

Data were analyzed using Statistical Packages for Social Sciences (SPSS version 22.0, IBM, Armonk, NY, USA). Results were expressed in mean ± standard deviation (SD) and differences were compared using one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Percentage viability

Antiplasmodial activity of MethAD (*A. djalonensis* root methanol extract) showed a negative PCV (-9.6000 %), meaning it significantly reduced *P. falciparum* viability ($p < 0.05$; IC_{50} of 0.00154 µg/mL indicating high potency). MethPA (*P. angolensis* stem methanol extract) and MethVC (*V. cinerea* plant methanol extract) exhibited moderate antiplasmodial activity, with PCV values of 42.382 and 66.408 %, respectively. The IC_{50} of *V. cinerea* was 3.5420 µg/mL, while that of *P. angolensis* was not determined because the viability (%; PVC) was more than 50 % (66.408 %), indicating a parasite reduction of less than 50 %. Furthermore, MethAD exhibited significantly stronger activity against *T. brucei* (PCV of 19 % and IC_{50} of 0.0036 µg/mL, indicating significant trypanocidal effects ($p < 0.05$; Table 1).

Furthermore, the *V. cinerea* sample, namely MethVC, of *A. djalonensis* sample, which induced parasite viability percentages less than 50 % (66.4080 %; antiplasmodial activity), was not tested for IC_{50} determination following the graphical regression method on the dose-response curve.

Table 1: Bioassay data showing percentage viability, IC_{50} values for antiplasmodial and anti-trypanosomal activity

Sample	Antiplasmodial activity		Antitrypanosomal activity	
	Percentage viability (PCV)	***IC₅₀***	***Percentage Viability (PCV)***	***IC₅₀***
MethAD	-9.6000	0.00154	19.0000	0.0036
MethVC	66.4080	ND	32.1000	2.8180
MethPA	42.3824	3.5420	45.5000	4.6770
Chloroquine	0.0125	-	-	-
Pentamidine	0.0012	-	-	-

Key: MethAD: *A. djalonensis* root methanol extract; MethVC: Whole *V. cinerea* plant methanol extract; MethPA: *P. angolensis* root methanol extract; ND: not determined; PCV: Percentage of viable cells; IC_{50} stands for 50 % inhibitory concentration or the sample concentration that, at µg/mL for extract in µM for reference medicines, decreases the proliferation and growth of cells by 50 %

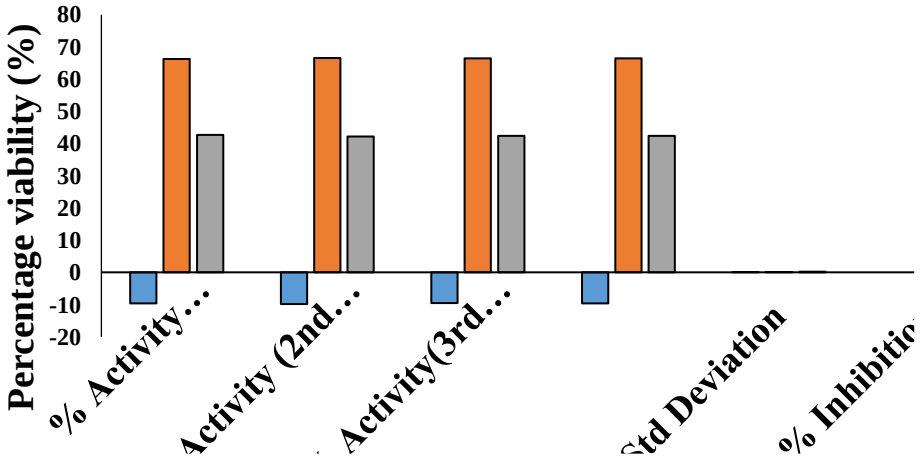

Figure 1: Effect of *A. djalonensis* roots, *V. cinerea* whole bark, and *P. angolensis* stem bark crude extracts on *P. falciparum* viability. Each bar in Figure 1 represents the means of three independent experiments with standard deviation bars. The blue bar is MethAD (-9.60 %), the orange bar is MethVC (42.38 %), and the grey bar is MethPA (66.41 %)

Antiplasmodial activity

Figures 1 and Table 1 show the *in vitro* antiplasmodial activity of the crude extract of *A. djalonensis*, *V. cinerea,* and *P. angolensis*.

Dose-response curve of antiplasmodial activity

The dose-response curves for MethAD, MethPA, and Chloroquine were obtained by plotting the logarithm of concentration (log_{10}) against the percentage inhibition of biological activity. This approach allows for better visualization and comparison of drug potency across a wide range of concentrations. For MethAD, the IC_{50} value, defined as the concentration required to inhibit 50 % of the biological target, was determined to be 0.01547 µg/mL, corresponding to $log_{10}(IC_{50})$ of -1.81. At very low concentrations, MethAD shows baseline activity with negligible inhibition. As the concentration increases, the inhibition also increases until it reaches the IC_{50}, beyond which a plateau is observed where further increases in concentration do not yield a significant increase in inhibition. For the methanol extract of *Pycnanthus angolensis* stem bark (MethPA), the IC_{50} value was found to be 3.5420 *µ*g/mL, which corresponds to a $log_{10}(IC_{50})$ of 0.55 on the dose-response curve. The curve characteristics were similar to MethAD. Inhibition profile of chloroquine showed a sigmoidal curve with low activity at minimal doses, increased inhibition near the IC_{50}, and a plateau at higher concentrations. Overall, comparison of the IC_{50} values showed that Chloroquine > MethAD > MethPA in terms of potency (Figure 2).

Antitrypanosomal activity

Methanol extracts of *A. djalonensis* (MethAD), *V. cinerea* (MethVC), and *P. angolensis* (MethPA) showed a high level of antitrypanosomal activity at 25 µg/mL with IC_{50} values of 0.0036, 2.8180, and 4.6770 µg/mL, respectively. The IC_{50} concentration for pentamidine, the standard medication, was 0.0012 µg/mL (Table 1, Figure 3).

Dose-response curve of antitrypanosomal activity

Antitrypanosomal activity of MethAD, MethVC, and MethPA was evaluated based on percentage viability and IC_{50} values. MethAD exhibited the highest antitrypanosomal activity with the lowest IC_{50} value (0.0036 *µ*g/mL) and the lowest percentage viability (19 %), indicating strong efficacy in inhibiting Trypanosoma parasites. Therefore, MethAD was the most potent compound, followed by MethVC and MethPA (Figure 4).

DISCUSSION

A. djalonensis, V. cinerea, and P. angolensis species are used in traditional Nigerian medicine. They were chosen for this investigation because of their traditional use in managing both malaria and typhoid [10]. Viability of *37D P. falciparum* was less than 1 % following exposure to crude extract of *A. djalonensis*. In a separate investigation, *A. djalonensis* leaf and stem bark extract showed considerable antiplasmodial action in both the 4-day initial infection test and the established infection [11].

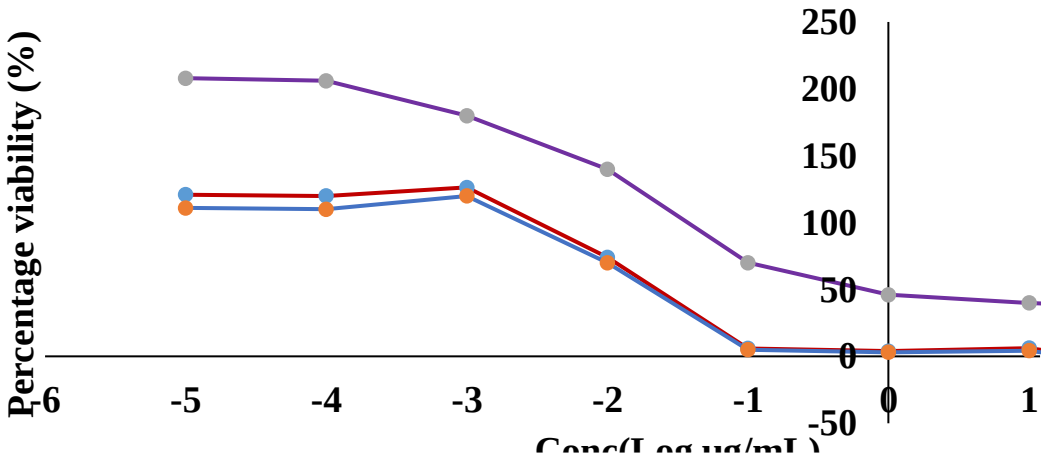

Figure 2: Dose-response curve of MethAD, MethPA, and Chloroquine

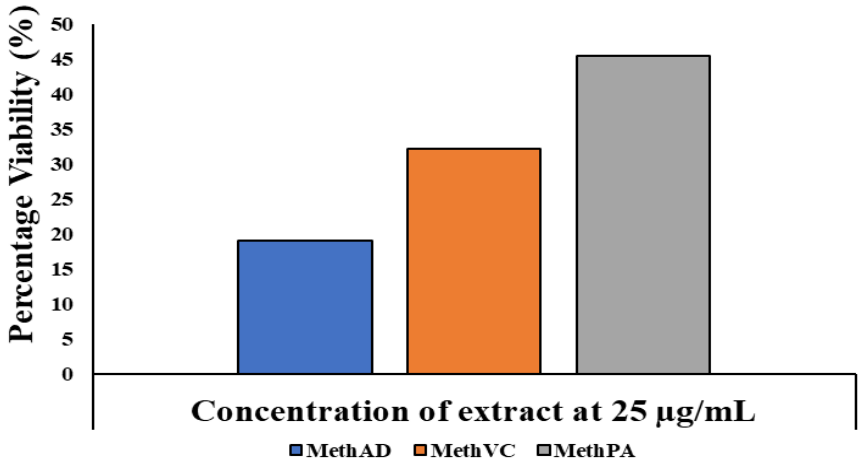

Figure 3: Effect of *A. djalonensis* roots *V. cinerea* whole bark, and *P. angolensis* stem bark crude extracts on *Trypanosoma brucei* viability. Each bar represents the means of 3 independent experiments with standard deviation bars. Blue bar-MethAD (19 %), Orange bar-MethVC (32.1 %), Grey bar-MethPA (45.5 %)

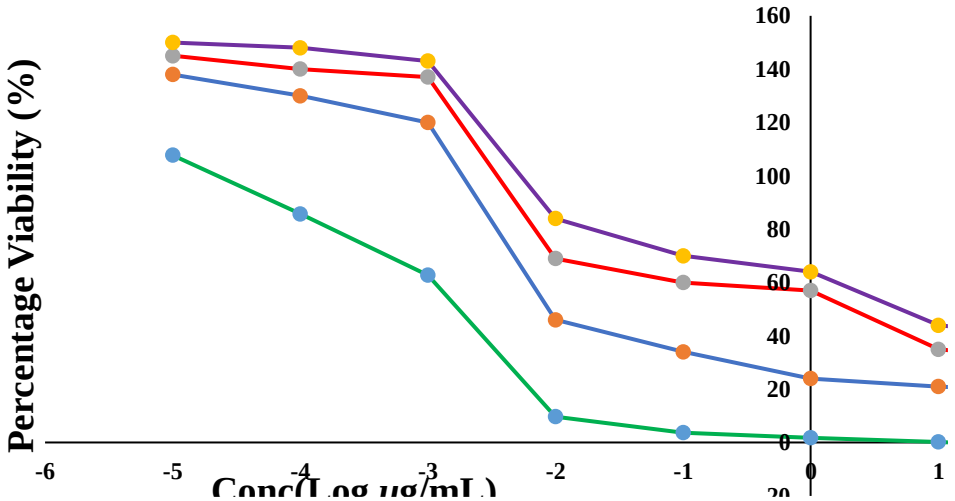

Figure 4: Antitrypanosomal assay dose-response curve of MethAD, MethVC, and MethPA compared to pentamidine

Furthermore, the viability of *37D P. falciparum* was significantly greater than 50 % following exposure to *V. cinerea* extract. *V. cinerea* subjected to phytochemical screening in another study has revealed the presence of steroids, glycosides, triterpenoids, and esters in the methanolic extract of the leaves and stem bark [12]. Thus, the low levels of these compounds observed in *V. cinerea* crude extracts may explain the modest parasiticidal activity of MethVC. Also, crude extract of *P. angolensis* had a *P. falciparum* viability percentage of less than 50 %, indicating a good antiplasmodial action. Such observations in *A. djalonensis* and *P. angolensis* crude extracts may be due to the phytochemical constituents such as alkaloids, tannins, flavonoids, cardiac glycosides, saponins, and terpenoids, which have previously been detected and shown to possess a variety of biological activities, including anti-parasitic activity [13].

The hunt for novel plant-derived antiplasmodials has been sparked by the extraordinary efficacy of quinine and related medications, as well as artemisinin. While several native plants have significant antiplasmodial and anti-malarial action, the antiplasmodial properties of plants are attributed to many groups of phytoconstituents, including flavonoids, terpenes, alkaloids, and steroids. In a previous study following *in vitro* investigations, the antiplasmodial activity of an extract was rated as significant if its IC_{50} was less than 5 µg/mL, good if it was 5 µg/mL and less than 10 µg/mL, and moderate if it was 10 µg/mL and less than 20 µg/mL [14]. This validates MethAD and MethPA as strong candidates for additional bio-guided fractionation and as

potential antiplasmodial extracts. The plants (*A. djalonensis*, *V. cinerea,* and *P. angolensis*) were chosen for this investigation due to their parasiticidal properties (antiplasmodial activity), although they have not been employed as an antitrypanosomal medication in traditional Nigerian medicine.

Traditionally, the use of these plant species for the treatment of skin infections, allergies, high temperature, diabetes, hepatitis, skin problems, and urinary tract infections has been reported [15,16]. With regards to *A. djalonensis*, neither the plant nor the *Gentianacea* plant family has been shown to possess any antitrypanosomal properties [17]. Possible compounds that give *A. djalonensis*, *V. cinerea,* and *P. angolensis* species their antiprotozoal action may also be highly concentrated in the crude extracts (MethAD, MethVC, and MethPA).

CONCLUSION

The findings of this study show that *A. djalonensis*, *V. cinerea*, and *P. angolensis extracts* exhibit significant antiplasmodial and antitrypanosomal properties. The extraction, purification, and characterization of the phytoconstituents are necessary to facilitate the identification of new antiplasmodial as well as anti-trypanosomal compounds. Studies on the isolation and characterization of these compounds would be required in further investigations.

DECLARATIONS

Acknowledgment and funding

The authors gratefully acknowledge the use of Professor Rui Krause's laboratory at Rhodes University's Department of Chemistry in Grahamstown. The South African Medical Research Council (MRC) and Rhodes University, with funding from the National Treasury's Economic Competitiveness and Support Package, supported this study through Sandisa Imbewu. For the Rhodes University Academic Fellowship, IS Okoro is appreciative.

The Economic Competitiveness and Support Package of the National Treasury provided funding to the South African Medical Research Council (MRC) for this research study and Rhodes University, Sandisa Imbewu.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Nnadi CO, Onuku R, Ayoka TO, Okorie NH, Nwodo NJ. Chemical constituents of Combretum dolichopetalum: Characterization, antitrypanosomal activities and molecular docking studies. Trop J Pharm Res 2022; 21(4): 801-808.*
2. *Nkwocha CC, Nduka FO, Eze C, Ogbuonye C, Azi C, Agbatai H, Nwafor N. Baphia nitida ethanol leaf extract restores haematological and biochemical status in Plasmodium berghei passaged rats. Trop J Pharm Res 2024; 23(3): 509-515.*
3. *Okoro IS, Umoh SD, Asekunowo AK, Etim EE, Ante IE, Ichoron N. Exploring the antimicrobial and anti-HIV-1 properties of Anthocleista djalonensis, Vernonia cinerea, and Pycnanthus angolensis extracts. Discov Chem 2024; 1(1): 72.*
4. *Togola A, Diallo D, Dembélé S, Barsett H, Paulsen BS. Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. J Ethnobiol Ethnomed 2005; 1: 1-9.*
5. *Sonibare MA, Aremu OT, Okorie PN. Antioxidant and antimicrobial activities of solvent fractions of Vernonia cinerea (L.) Less leaf extract. Afr Health Sci 2016; 16(2): 629-639.*
6. *Abubakker N. Evaluation of enzymic and non-enzymic antioxidant levels in vernonia cinerea extracts. Asian J Pharm Clin Res 2018; 11: 167.*
7. *Sofidiya MO, Awolesi AO. Antinociceptive and antiulcer activities of Pycnanthus angolensis. Rev Bras Farmacogn 2015; 25: 252-257.*

8. Mohamad S, Zin NM, Wahab HA, Ibrahim P, Sulaiman SF, Zahariluddin ASM, Noor SSM. Antituberculosis potential of some ethnobotanically selected Malaysian plants. J Ethnopharmacol 2011; 133(3): 1021-1026.
9. Teinkela JEM, Noundou XS, Nguemfo EL, Meyer F, Wintjens R, Isaacs M, Mpondo AEM, Hoppe HC, Krause RWM, Azebaze AGB. Biological activities of plant extracts from Ficus elastica and Selaginella vogelli: An antimalarial, antitrypanosomal and cytotoxity evaluation. Saudi J Biol Sci 2018; 25(1): 117-122.
10. Dalziel JM. The useful plants of west tropical Africa. 1937.
11. Bassey AS, Okokon JE, Etim EI, Umoh FU, Bassey E. Evaluation of the in vivo antimalarial activity of ethanolic leaf and stembark extracts of Anthocleista djalonensis. Indian J Pharmacol 2009; 41(6): 258-261.
12. Trang NM, Vinh LB, Phong NV, Yang SY: Traditional Uses, Phytochemistry, and Pharmacological Activities of Vernonia cinerea (L.) Less. An Updated Review. Nutrients 2024, 16(9): 1396.
13. Okoro IS, Tor-Anyiin TA. Evaluation of cytotoxicity and phytochemical screening of Pycnanthus angolensis (Welw.) Warb dichloromethane and ethyl acetate stem bark extracts against HeLa cells. Int J Cancer Res 2017; 13: 71-75.
14. Gathirwa JW, Rukunga GM, Njagi EN, Omar SA, Guantai AN, Muthaura CN, Mwitari PG, Kimani CW, Kirira PG, Tolo FM. In vitro anti-plasmodial and in vivo anti-malarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya. J Nat Med 2007; 61: 261-268.
15. Igoli J, Ogaji O, Tor-Ayiin T, Igoli N. Traditional medicine practice amongst the Igede people of Nigeria. Part II. Afr J Tradit Complement Altern Med 2005; 2(2): 134–152-134–152.
16. Dougnon V, Assogba P, Koudokpon H, Ayena C, Vissoh S, Akpaka G, Klotoe JR, Baba-Moussa L, Bankole H, Koudouvo K. Treatment of urinary tract infections with medicinal plants: Monographs of some plants species revealed by traditherapists. Infectious Dis Herb Med 2020; 1: 114
17. Anyanwu GO, Onyeneke CE, Rauf K. Medicinal plants of the genus Anthocleista—A review of their ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol 2015; 175: 648-667.