

# Evaluation of Selected Nigerian Medicinal Plants for Antioxidant, Antimicrobial and Cytotoxic activities

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## Research Article

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# Abstract

**Background:** Increased exposure to pathogens and free radicals contributed to the high incidence and mortality rate of various cancers in Nigeria and globally. Promotion of scientific research on medicinal plants in collaboration with traditional health practitioners to validate claims made on safety, efficacy and quality of traditional medicinal plants in the treatment of cancer is imperative. This study aims at screening extracts of selected Nigerian medicinal plants used traditionally for cancer treatment for antioxidant, antimicrobial and cytotoxic activities towards identification of potential source of new anticancer agents.

**Methods:** Twenty-one extracts from sixteen medicinal plants species were screened for their cytotoxicity on RD, HeLa and Hep-2 cancer cell lines using MTT assay. The DPPH radical scavenging activity of the extracts as well as their Total Phenolic Content (TPC) were evaluated. The extracts were also evaluated for their antimicrobial activity using spectrophotometric growth inhibition method.

**Results:** Extracts of *Tetrapleura tetrapleura* and *Xylopia aethiopica* showed high DPPH inhibitory activity and phenolic content. Extracts of *X. aethiopica* and *Anchomanes difformis* showed broad spectrum of antibacterial activities while root extracts of *Crotolaria retusa* and *T. tetraptera* exhibited antifungal activities comparable ( $P < 0.05$ ) to Ketoconazole. Extracts of *Capcicum frutescens*, *Aspilia africana*, *X. aethiopica*, *T. tetraptera* and *C. retusa* showed broad spectrum of cytotoxic activities.

**Conclusions:** Extracts of *T. tetraptera* and *X. aethiopica* demonstrated satisfactory activities in all the biological tests which could be linked to their high phenolic contents. The findings support the ethno-medicinal uses of most of the tested medicinal plants.

## Background

Cancer remains one of the leading cause of death worldwide with an estimate of 18.1 million new cancer cases and 9.6 million cancer deaths (1). Several factors have been found to be associated with the development of cancer but notable are the actions of free radicals and pathogenic microorganisms (2, 3). Studies have shown that reactive free radicals interact with macromolecules such as DNA in the cell, leading to damages to cell structure and functions (4). In addition pathogenic microbes have been found to be associated with some kind of cancer (5).

Cancer treatment faces challenges of resistance, toxicities to normal cells and ineffectiveness of some anticancer agents (6–9). This has necessitated the search for new anticancer agents from medicinal plants. Studies have shown that plants are rich in phenols which are able to attenuate the actions of free radicals (10) and are able to elicit antimicrobial activities (11, 12). In addition, plants are also veritable source of anticancer agents (13–15).

The dependence of vast majority of African populace on medicinal plants for healthcare needs (16) formed the basis for the inquiry into plants used traditionally in the treatment of cancer in South Western

Nigeria. Retrieving information on the pharmacological relevance of medicinal plants is often achieved through the conduction of ethnomedicinal surveys (17). An ethnomedicinal survey was carried out in Ile-Ife, Osun state, Nigeria between June to December 2017. From the survey, twenty-one extracts from sixteen plants species were selected for this study. This study aims at screening the selected plant extracts for antioxidant, antimicrobial and cytotoxic activities towards identification of potential source of new anticancer agents.

## Methods

### Chemicals

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium salt, DPPH [2,2-diphenyl-1-picrylhydrazyl], ascorbic acid (purity = 99%), vincristine sulphate (purity =  $\geq$  95%), nutrient agar, Folin – ciocalteu, sabouraud dextrose agar, ketoconazole (purity =  $\geq$  99%), streptomycin (purity = 90%), gallic acid (purity = 90%).

### Plant material

The plant parts used for this study (Table 1) were collected and identified at the botanical garden of the University of Ibadan, Ibadan, Nigeria. Authentication of the plant material was done at the herbarium of the Forest Research Institute of Nigeria, Ibadan Nigeria, where voucher specimens were also deposited. The plant materials were dried at room temperature and pulverized into powder. Freshly crushed endosperm of *Cocos nucifera* was used in this study.

### Extraction

Each plant material (200 g) was macerated in 80% methanol for 78 h at room temperature. Crushed endosperm of *Cocos nucifera* (200 g) was extracted in *n*-hexane. Extracts were filtered through filter paper (Whatman No. 1) and concentrated to dryness *in vacuo*.

### DPPH radical scavenging assay

The free radical scavenging activities of the extracts against 2,2-diphenyl-1, picrylhydazyl (DPPH) was evaluated following the method of (18) with slight modification. Extracts and ascorbic acid were made into various concentrations (1.56, 3.13, 6.25, 12.5, 25, 50 and 100  $\mu$ g/mL) in methanol while a 0.004% (w/v) solution of DPPH in methanol was freshly prepared. Each concentration of the extract (1 mL) was mixed with 3 ml of the DPPH solution and incubated in the dark for 30 min at  $27 \pm 2$  °C. In the control experiment, 1 mL of methanol was used in the place of the extracts. Absorption of the reaction was measured at 517 nm. The experiment was repeated three times. The concentration at which there is 50% inhibition of the DPPH' (IC<sub>50</sub>) was determined using Graph pad prism (5.0) while the percentage inhibition of the extracts was calculated using the formula;

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

## *Absorbance of control*

### **Total phenolic content (TPC) assay**

The total phenolic content of the extracts was determined using Folin – Ciocalteu (FC) reagent following the method of (19) with slight modification. The extracts were made into 100 µg/mL while 10% FC (v/v) in methanol was freshly prepared. The FC reagent (25 µL) was added to 50 µL of the extracts in 96 – well plates and allowed to stand for 3 min. For the blank, methanol was used in place of the extracts. A solution of 7.5% Na<sub>2</sub>CO<sub>3</sub> (125 µL) was added into each well and afterwards incubated in the dark for 2 h at 25 ± 2 °C. The absorbance was recorded with Thermo Fisher Scientific microplate reader at 758 nm. The experiment was carried out in triplicates. The total phenolic content was therefore expressed as Gallic acid equivalents (GAE) (18).

### **In vitro antimicrobial assay**

#### **Test organisms**

Reference bacterial and fungi strains were obtained from the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan Nigeria. The bacterial strains include: *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, and *Salmonella typhi* ATCC 24683 while the fungi strain used was *Candida albicans* ATCC 24433. Nutrients broth and sabouraud dextrose broth was used for the maintenance of the bacterial and fungal strains respectively at 4 °C.

#### **Preparation of inoculums**

A small piece of colony from a day-old cultures of each test organisms was adjusted to cell density of 1x10<sup>8</sup> CFU/mL in sterile distilled water using McFarland Standard No. 0.5.

#### **Spectrophotometric growth inhibition method**

The method of (20) was adopted with some modifications. Extracts and standard drugs (streptomycin and ketoconazole) were made into concentrations 1000, 500, 250, 125, 62.50, 31.25 and 15.63 µg/mL in freshly prepared nutrient or sabouraud dextrose broth. An aliquot of 75 µL of each test concentration was gently mixed together with 75 µL of the inoculum in wells of 96 – well plate. Sterile distilled water was used in the control experiment. The absorbance at 540 nm was taken before and after 24 h of incubation at 37 °C. Differences in optical densities were taken as microbial growth index. The experiment was carried out in triplicates. The concentration at which there is 50% microbial inhibition (IC<sub>50</sub>) was determined using Graph pad prism (5.0) while the percentage microbial inhibition was calculated by using the equation:

$$\% \text{ Inhibition} = \frac{\Delta \text{Absorbance of control} - \Delta \text{Absorbance of test sample}}{\Delta \text{Absorbance of control}} \times 100$$

$$\Delta \text{Absorbance of control}$$

## Cytotoxicity assay

### Cell culture

Culture of human larynx epithelioma (Hep 2), Human Rhabdomyosarcoma (RD) and cervical adenocarcinoma (HeLa) cell lines were obtained from the Department of Virology, University College Hospital (UCH) affiliated to the University of Ibadan, Ibadan, Nigeria. The cells were maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) (v/v), 100 units/mL of penicillin, 100 µg/mL of streptomycin, 0.07% NaHCO<sub>3</sub> (w/v), 2 mM L-glutamine and 1% non-essential amino acids.

### MTT Assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] viability assay was carried out following a mildly modified method of (21). Each cell line was seeded into different 96 well plate and incubated at 37°C for 24 h. Extracts and positive control (vincristine sulphate) were freshly made into concentrations of 1000, 100, 10, 1, 0.1 and 0.01 µg/mL with 5% (v/v) DMSO in maintenance medium. At the expiration of 24 h, medium in wells with confluent monolayer cells were carefully replaced with 200 µL of various concentrations of the extracts and were further incubated at 37 °C for 72 h. Cytopathic effects of the extracts at various concentrations after 72 h was evaluated and scored under microscope. Medium in wells was carefully replaced with 25 µL of 2% (w/v) MTT dye in PBS and incubated at 37 °C for 2 h. DMSO (125 µL) was added into each well and left on a shaker for 30min to ease solubility and evenness of the colour formed. Absorbance of each well at 492 nm was recorded with a spectrophotometer. The experiment was performed in triplicate while the CC<sub>50</sub> was determined using graph pad prism 5.0. Percentage cytotoxicity of the extracts at various concentrations was calculated using the formula;

$$\% \text{ Cytotoxicity (CC)} = \frac{(A - B)}{A} \times 100$$

A

Where: A = the optical density of untreated cells

B = the optical density of cells treated with plant extracts/ control drug

### Statistical analysis

Graphpad Prism, version 7.01 was used for the statistical analysis of data. The data obtained were expressed as Mean ± SD (Standard deviation) values of three independent assessments. The IC<sub>50</sub> and CC<sub>50</sub> values of all test samples were determined with nonlinear regression plot of log (cytotoxic concentration) against normalized percentage cytotoxicity. One-way at P < 0.05 followed by Tukey's test was used test for the significant difference between the extracts and the standard drugs.

## Results

The antioxidant activities of the extracts were assessed by evaluating their inhibitory activities against DPPH. Except for bark extract of *T. tetrapleura* with an IC<sub>50</sub> of 4.59 µg/mL and root extract of *X. aethiopica* with an IC<sub>50</sub> of 6.01 µg/mL, the inhibitory activities of other extracts are significantly (P<0.05) different from ascorbic acid (Table 2).

Phenols are class of compounds with proven antioxidant activities, therefore samples with high phenol contents often exhibit antioxidant activities. Among all tested extracts, leaf extracts of *Nicotiana tabacum* and *P. osun*, bark extract of *T. tetraptera* and root extract of *X. aethiopica* had the highest phenolic contents (Table 2) with gallic acid equivalences of 58.35, 56.35, 67.99, 63.84 mg GAE/g, respectively. However, these four extracts are statistically different (P<0.05) in their phenolic contents.

The antimicrobial study showed that the extracts exhibit inhibitory activity against tested bacterial and fungal strains (Table 3). The root and bark extracts of *X. aethiopica* and leaf extracts of *A. difformis*, *Morinda lucida* and *Pterocarpus osun* displayed comparable (P<0.05) antibacterial inhibitory activity against *Salmonella typhi* when compared to Streptomycin. All tested extracts were active against *Pseudomonas aeruginosa*, however, none was comparable (P<0.05) to the activity of Streptomycin with IC<sub>50</sub> of 0.9 µg mL<sup>-1</sup>. Similarly, *A. difformis*, bark extract of *X. aethiopica* elicit comparable activities against *E. coli*. Root extracts of *X. aethiopica* and leaf extract of *A. difformis* showed broad spectrum of antibacterial activities. Antifungal studies against *Candida albicans* showed that root extracts of *Crotalaria retusa*, *Tetrapleura tetraptera* and Ketoconazole exhibited comparable (P<0.05) antifungal activities.

Extracts were evaluated for their cytotoxicity effects on human larynx epithelioma (Hep 2), Human Rhabdomyosarcoma (RD) and cervical adenocarcinoma (HeLa) cell lines. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used to monitor viable cells after treatment with extracts. Most extracts were active against Hep 2, yet, extracts of *Aspilia africana*, *T. tetraptera* and *N. tabacum* were the most cytotoxic with IC<sub>50</sub> of 1.3, 1.7 and 2.9 µg/mL respectively which were comparable (P<0.05) to the standard drug (Table 4). On RD cell line, the cytotoxicity of the standard drug was comparable to that of *Culcasia scanden*, *Capsicum frutescens* and root extract of *X. aethiopica* with IC<sub>50</sub> of 0.9, 1.6 and 1.6 µg/mL, respectively. Only the bark extract of *X. aethiopica* demonstrated comparable (P<0.05) cytotoxic activity against HeLa cell line when compared with standard drug. Extracts of *C. frutescens*, *A. africana*, *X. aethiopica*, *T. tetraptera* and *Crotalaria retusa* showed broad spectrum of cytotoxic activities against the tested cell lines.

## Discussions

Medicinal plants are very important in health care delivery in developing nations of the world (16). Rising prevalence and mortality of cancer and related diseases necessitated sourcing for treatment alternatives from medicinal plants (22). Bioactive compounds in extracts of medicinal plants are responsible for the

diverse pharmacological activities demonstrated by these plants (23, 24). Identifying potential medicinal plants for cancer treatment is often achieved through information retrieved from traditional health practitioner (TMPs) (17). This present study seeks to scientifically justify the ethnomedicinal use of sixteen medicinal plant species used in the treatment of cancer and microbial infections.

Free radicals are proven contributor to the development and progression of most disease conditions via their damaging effects on macromolecules including proteins, DNA and RNA (25). Antioxidants on the other hand inhibits the actions of free radicals and therefore could potentially prevent the onset and progression of these diseases (26). Previous reports showed that Nigerian medicinal plants elicit antioxidant activities (18, 26). In our study, the bark extract of *T. tetraptera* had the highest inhibitory activity against the DPPH radical as well as very high total phenolic content. Phenolic compounds have been widely studied for their biological activities and are known to contribute to the antioxidant activity of extracts (10, 18, 19, 27). It could be safely posited that phenolic compounds of *T. tetraptera* are partly responsible for its antioxidant activity as well as other reported biological activities such as hypoglycemic, antimicrobial, anti-inflammatory and antilipidemic activities (28). Other extracts including bark and root extracts of *X. aethiopica* and root extract of *T. tetraptera* showed high total phenolic contents which also correspond to their high inhibitory activities against DPPH radical.

In addition to free radicals, there are various evidences linking chronic microbial infections with cancer. *Salmonella typhi* for example has been linked to the development of cancer of the gall bladder (29) while *Helicobacter pylori* has been linked to gastric cancer (5). Several reports demonstrated the antimicrobial potentials of medicinal plants. Phenolic compounds in extracts of medicinal plants contribute to their antimicrobial activities going by their damaging effect on the cell membrane and disruption of metabolism and synthesis of nucleic acids (30).

Results showed that the study plants elicited antimicrobial activities against *S. typhi*, *P. aeruginosa*, *E. coli* and *C. albicans*. Root extracts of *X. aethiopica* and leaf extract of *A. difformis* elicit broad spectrum of antibacterial activities. Fruit extract of *X. aethiopica* and derivative of its major constituent had earlier been demonstrated to elicit antimicrobial activities (31, 32). Our findings demonstrated that the root and bark extracts of *X. aethiopica* equally hold antimicrobial properties, and considering their high phenolic content, phenolic compounds might be responsible for the observed activities.

As with the reports of (22), (26) and (21), extracts of this study showed varying cytotoxic activities against tested cell lines. According to National Cancer Institute (NCI) on screening of medicinal plants for cytotoxic activities, plant extracts with  $CC_{50} < 30 \mu\text{g/mL}$  are considered active (22). Based on the NCI standard, nineteen extract were active against at least one cancer cell line, while ten extracts were active against at least two cancer cell lines. Only six extracts were cytotoxic to all the cancer cell lines.

We are reporting perhaps the first cytotoxicity studies on the root and bark extract of *X. aethiopica*. The root extract of *X. aethiopica* elicit the most pronounced cytotoxicity against Hep 2, RD and HeLa cell lines. Earlier studies showed that fruit extract of *X. aethiopica* was cytotoxic to cancer cell lines of the prostate

(DU-145), breast (JIMT-1), pancreatic (MIA-PaCa 2) ( Aladesanmi et al., 2020) and cervix through the induction of apoptosis and arrest of the cell cycle (34). Ent-15-oxokaur-16-en-19-oic acid, 3,4,5-trihydroxy-6,6-dimethylpyrano [2,3-g]flavone and isotetrandrine are some of the cytotoxic constituents in the fruit extract of *X. aethiopica* (35, 36).

Our study also showed that bark and root extracts of *T. tetraptera* demonstrated cytotoxicity against all tested cancer cell lines. (22) reported the cytotoxicity of extract of *T. tetrapleura* against breast (BT-549) cancer cell line. Similarly, *in vitro* and *in vivo* models of (37) also demonstrated the cytotoxicity of fruit extract of *T. tetraptera* against Ehrlich Ascites tumour cells. Bioactive coumarin, saponins, terpenes and some phenolics have been isolated from extracts of this plants (28). The high phenolic content of *T. tetraptera* extract as shown in this study might contribute to its cytotoxicity, however, further works will aim at identifying the active cytotoxic compounds.

Stem extract of *Crotolaria retusa* was found to be more cytotoxic than leaf, seed, pod and flower extracts of the plant although in an un-selective manner (38). We report the cytotoxicity of its root extract to Hep 2, RD and HeLa cell lines. Similarly, we found leaf extract of *Aspilia africana* to be cytotoxic against the three tested cell lines. (39) reported the cytotoxicity of leaf extracts obtained using various green methods of extraction against AGS, A549, and HeLa cell lines. Gallic acid, chlorogenic acid, syringic acid, ferric acid, and quercetin were detected in the most active extract of *A. africana*.

*Capsicum frutescens* elicited broad spectrum of cytotoxicity against all tested cell lines. Its fruits, commonly used in African cuisine is widely studied for its nutritional and health benefits (40). Although evidences suggest that capsaicin, a constituent of the fruits is a human carcinogen (41), however the same compound has been reported to demonstrate cytotoxic and cancer prevention potentials (40). A more recent report demonstrated that capsaicin and piperine from *Piper nigrum* could reverse resistance of cancer cells to doxorubicin (42). Other notable cytotoxic extracts are the bark extracts of *Pterocarpus osun*, leaf extract of *Culcasia scandens* and *Pativera alliaceae* which were cytotoxic to at least two cancer cell lines.

## Conclusions

In this study, we evaluated the antimicrobial, antioxidant and cytotoxicity of extracts of medicinal plants used traditionally in the treatment of cancer in South Western Nigeria. Results from the study justifies the traditional use of these extracts in the treatment of cancer and accompanying conditions. Extracts of *T. tetraptera* and *X. aethiopica* demonstrated satisfactory activities in all the biological tests which could be linked to their high phenolic contents. Further purification might potentiate their pharmacological effects which will favour their pharmaceutical application in the development of anticancer and antimicrobial therapeutics. Our current endeavour is devoted towards unraveling mechanism of action as well as identifying the active constituents present in the extracts of these plants.

## Declarations

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** All

**Availability of data and materials:**

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

**Competing interests**

The authors declare that they have no competing interest.

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**Authors' contributions**

SAO, EOA and OOO designed the study which was carried out under the supervision of EOA. SAO and HOD carried out the antimicrobial studies, SAO, BBO and AJA carried out the cytotoxicity studies, SAO and OOO analyzed and interpreted the results. SAO prepared the final manuscript which was read and approved by all authors.

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## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
2. Reagan M. Causes Of Cancer: Genetic, Epigenetic, Viral, Microenvironmental, and Environmental Contributions to Cancer. *Cancer.* In Gary SS, Kimberly PL editors. Prevention, Early Detection, Treatment and Recovery. Wiley; 2019. p. 53–74.
3. Shahab L, McGowan JA, Waller J, Smith SG. Prevalence of beliefs about actual and mythical causes of cancer and their association with socio-demographic and health-related characteristics: Findings from a cross-sectional survey in England. *Eur J Cancer.* 2018;103:308–16.  
<https://doi.org/10.1016/j.ejca.2018.03.029>
4. Vineis P, Wild CP. Global cancer patterns: Causes and prevention. *Lancet.* 2014;383(9916):549–57.  
[http://dx.doi.org/10.1016/S0140-6736\(13\)62224-2](http://dx.doi.org/10.1016/S0140-6736(13)62224-2)

5. Maggio-Price L, Treuting P, Zeng W, Tsang M, Bielefeldt-Ohmann H, Iritani BM. Helicobacter infection is required for inflammation and colon cancer in Smad3-deficient mice. *Cancer Res.* 2006;66(2):828–38.
6. Sougiannis AT, VanderVeen BN, Enos RT, Velazquez KT, Bader JE, Carson M, et al. Impact of 5 fluorouracil chemotherapy on gut inflammation, functional parameters, and gut microbiota. *Brain Behav Immun.* 2019;80:44–55. <https://doi.org/10.1016/j.bbi.2019.02.020>
7. Liu GF, Li GJ, Zhao H. Efficacy and Toxicity of Different Chemotherapy Regimens in the Treatment of Advanced or Metastatic Pancreatic Cancer: A Network Meta-Analysis. *J Cell Biochem.* 2018;119(1):511–23.
8. Nurgali K, Jagoe RT, Abalo R. Editorial: Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae? *Front Pharmacol.* 2018;9:1–3.
9. Florea AM, Büsselberg D. Cisplatin as an anti-tumor drug: Cellular mechanisms of activity, drug resistance and induced side effects. *Cancers (Basel).* 2011;3(1):1351–71.
10. Rolim PM, Fidelis GP, Padilha CEA, Santos ES, Rocha HAO, Macedo GR. Phenolic profile and antioxidant activity from peels and seeds of melon (*Cucumis melo L. var. reticulatus*) and their antiproliferative effect in cancer cells. *Brazilian J Med Biol Res.* 2018;51(4):1–14.
11. Akhtar N, Ihsan-ul-Haq, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arab J Chem.* 2018;11(8):1223–35. <https://doi.org/10.1016/j.arabjc.2015.01.013>
12. Romulo A, Zuhud EAM, Rondevaldova J, Kokoska L. Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. *Pharm Biol.* 2018;56(1):287–93. <https://doi.org/10.1080/13880209.2018.1462834>
13. Morsy N. Anticancer agents from plants. *Main Gr Chem.* 2019;18(3):169–91.
14. Seca AML, Pinto DCGA. Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. *Int J Mol Sci.* 2018;19(1).
15. Fridlender M, Kapulnik Y, Koltai H. Plant derived substances with anti-cancer activity: From folklore to practice. *Front Plant Sci.* 2015;6:1–9.
16. Abo KA, Lawal IO, Ogunkanmi A. Evaluation of extracts of *Trichlisia subcordata* Oliv and *Heinsia crinita* (Afz) G. Taylor for antimicrobial activity against some clinical bacterial isolates and fungi. *African J Pharm Pharmacol.* 2011;5(2):125–31.
17. Segun PA, Ogbole OO, Ajaiyeoba EO. Medicinal plants used in the management of cancer among the ijebus of southwestern Nigeria. *J Herb Med.* 2018;14:68–75. <https://doi.org/10.1016/j.hermed.2018.04.002>
18. Adeniran AA, Sonibare MA. In vitro antioxidant activity, brine shrimp lethality and assessment of bioactive constituents of three wild *Dioscorea* species. *J Food Meas Charact.* 2017;11(2):685–95.
19. Karakas FP, Turker AU, Karakas A, Mshvildadze V, Pichette A, Legault J. In vitro cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers

- of common daisy—A medicinal plant. *J Herb Med.* 2017;8:31–9.  
<http://dx.doi.org/10.1016/j.hermed.2016.11.003>
20. Ahmad S, Hassan A, Rehman T, Basit A, Tahir A, Arshad MA. In vitro bioactivity of extracts from seeds of *Cassia absus* L. growing in Pakistan. *J Herb Med.* 2019;16(July 2017):100258.  
<https://doi.org/10.1016/j.hermed.2019.100258>
  21. Ogbole OO, Segun PA, Adeniji AJ. In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC Complement Altern Med.* 2017;17(1):1–10.
  22. Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL. In vitro anticancer screening of 24 locally used Nigerian medicinal plants. *BMC Complement Altern Med.* 2013;1;13(1):79.
  23. Casuga FP, Castillo AL, Corpuz MJAT. GC–MS analysis of bioactive compounds present in different extracts of an endemic plant *Broussonetia luzonica* (Blanco) (Moraceae) leaves. *Asian Pac J Trop Biomed.* 2016;6(11):957–61. <http://dx.doi.org/10.1016/j.apjtb.2016.08.015>
  24. Rahmatullah M, Jahan R, Khatun MA, Jahan FI, Azad AK, Bashir ABMA, et al. A pharmacological evaluation of medicinal plants used by folk medicinal practitioners of station Purbo Para village of Jamalpur Sadar Upazila in Jamalpur District, Bangladesh. *Am J Sustain Agric.* 2010;4(2):170–95.
  25. Oguntimehin SA, Oriola AO, Obuotor EM, Aladesanmi AJ. Bioassay guided phytochemistry of aerial part of *Laportea Aestuans* (L.) Chew (Urticaceae). *Niger J Nat Prod Med.* 2020;23:6–12.
  26. Aladesanmi AJ, Oriola AO, Oguntimehin SA, Akinkunmi EO, Igbeneghu OA, Obuotor EM. Comparative antimicrobial and antioxidant activities of four medicinal plants. *Ife J Sci.* 2020;21(3):59.
  27. Faten AE, Eman AH, Yahaya G. Determination of antioxidant and anti-inflammatory activities, as well as in vitro cytotoxic activities of extracts of *Anastatica hierochuntica* (Kaff Maryam) against HeLa cell lines. *J Med Plants Res.* 2016;10(7):77–87.
  28. Adesina S, Iwalewa E, Johnny I. *Tetrapleura tetraptera* Taub- Ethnopharmacology, Chemistry, Medicinal and Nutritional Values- A Review. *Br J Pharm Res.* 2016;12(3):1–22.
  29. Tewari M, Mishra RR, Shukla HS. Salmonella typhi and gallbladder cancer: Report from an endemic region. *Hepatobiliary Pancreat Dis Int.* 2010;9(5):524–30.
  30. Campos JF, Das Santos UP, Da Rocha PDS, Damião MJ, Balestieri JBP, Cardoso CAL, et al. Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of Propolis from the Stingless Bee *Tetragonisca fiebrigi* (Jataí). *Evidence-based Complement Altern Med.* 2015;2015.
  31. Kofie W, Fetse JP, Adosraku RK. Antimicrobial Activities of Novel Xylopic Acid Derivatives. *J Adv Microbiol.* 2019;14(1):1–6.
  32. Ogbonna CN, Nozaki, K, Yajima, H. Antimicrobial activity of *Xylopiya aethiopicum*, *Aframomum melegueta* and *Piper guineense* ethanolic extracts and the potential of using *Xylopiya aethiopicum* to preserve fresh orange juice. *African J Biotechnol.* 2013;12(16):1993–8.
  33. Aladesanmi AJ, Famuyiwa FG, Oriola AO, Oguntimehin SA, Aiyedun PO, Arthur G. Cytotoxic Activity of Selected Nigerian Medicinal Plants. *J Herbs, Spices Med Plants.* 2020;26(2):203–17.  
<https://doi.org/10.1080/10496475.2019.1706214>

34. Adaramoye OA, Sarkar J, Singh N, Meena S, Changkija B, Yadav PP, et al. Antiproliferative action of *Xylopiya aethiopic*a fruit extract on human cervical cancer cells. *Phyther Res.* 2011;25(10):1558–63.
35. Kuete V, Sandjo LP, Mbaveng AT, Zeino M, Efferth T. Cytotoxicity of compounds from *Xylopiya aethiopic*a towards multi-factorial drug-resistant cancer cells. *Phytomedicine* [Internet]. 2015;22(14):1247–54. <http://dx.doi.org/10.1016/j.phymed.2015.10.008>
36. Choumessi AT, Danel M, Chassaing S, Truchet I, Penlap VB, Pieme AC, et al. Characterization of the antiproliferative activity of *Xylopiya aethiopic*a. *Cell Div.* 2012;7:1–8.
37. Ozaslan M, Karagoz ID, Lawal RA, Kilic IH, Cakir A, Odesanmi OS, et al. Cytotoxic and anti-proliferative activities of the *Tetrapleura tetraptera* fruit extract on ehrlich ascites tumor cells. *Int J Pharmacol.* 2016;12(6):655–62. <http://dx.doi.org/10.3923/ijp.2016.655.662>
38. Anim MT, Larbie C, Appiah-Opong R, Tuffour I, Baffour-Awuah Owusu K, Aning A. Phytochemical, Antioxidant and Cytotoxicity of Hydroethanolic Extracts of *Crotalaria Retusa* L. Larbie al. *World J Pharm Res World.* 2016;5(2):163.
39. Niyonizigiye I, Nkurunziza D, Ngabire D, Gitachew AT, Chun BS, Kim G D. Characterization and in vitro cytotoxicity of phytochemicals from *Aspilia africana* obtained using green extraction techniques. *South African J Bot.* 2020;128:231–8. <https://doi.org/10.1016/j.sajb.2019.11.013>
40. Chapa-Oliver AM, Mejía-Teniente L. Capsaicin: From plants to a cancer-suppressing agent. *Molecules.* 2016;21(8):1–14.
41. Archer VE, Jones DW. Capsaicin pepper, cancer and ethnicity. *Med Hypotheses.* 2002;59(4):450–7.
42. Li H, Krstin S, Wang S, Wink M. Capsaicin and piperine can overcome multidrug resistance in cancer cells to doxorubicin. *Molecules.* 2018;23(3):1–11.

## Tables

**Table 1: List of study plants, ethnomedicinal uses and identification numbers**

S/N	Family	Name	Local name	Identification number	Part used	Sample code
1	Acanthaceae	<i>Asystasia gangetica</i> (L.) T.Anderson	Akpuarachi (I)	FHI 109634	Leaves	AgL
2	Annonaceae	<i>Xylopia aethiopica</i> (Dunal) A.Rich	Kimba (H), Uda (I), Eruu, girinja (Y), Ethiopian pepper (E)	FHI 108978	Bark	XaB
					Root	XaR
3	Araceae	<i>Anchomanes difformis</i> (Blume) Engl.	Igo langbodo, Ogirisako (Y), Oje (I), Chakara (H)	FHI 109638	Leaves	AdL
					Root	AdR
4	Araceae	<i>Culcasia scandens</i> P.Beauv.	Oji azu ari nkwu (I),	FHI 110050	Leaves	CsL
5	Arecaceae	<i>Cocos nucifera</i> L.	Agbon (Y), Coconut (E)	FHI 109041	Fruits oil	CnO
6	Asteraceae	<i>Aspilia africana</i> (Pers.) C.D.Adams	Jamajina (H), Oranjila (I), Yunyun, Ako yunyun (Y)	FHI 107511	Leaves	AaL
7	Leguminosae	<i>Crotalaria retusa</i> L.	Koropo, Alatunse, Saworo (Y), Rattle pea (E)	FHI 109052	Roots	CrR
8	Leguminosae	<i>Tetrapleura tetraptera</i> (Schum et Thonn) Taubert	Dawo (H), Uyayak (IB), Aridan/Aidan (Y)	FHI 110141	Bark	TtB
					Root	TtR
9	Leguminosae	<i>Pterocarpus osun</i> Craib	Madubiya (H), Osun (Y)	FHI 108415	Leaves	PoL
					Bark	PoB
10	Menispermaceae	<i>Triclisia subcordata</i> Oliv	Alugbonron (Y)	FHI 109638	Leaves	TsL
11	Moraceae	<i>Treculia africana</i> Decne. ex Trécul	Barafuta (H), Ukwa (I), Afon (Y)	FHI 106992	Leaves	TaL
12	Olacaceae	<i>Olax subscoipoidea</i> Oliv.	Gwano kurmi (H), Aziza (I), Ifon (Y)	FHI 109983	Leaves	OsL
13	Petiveriaceae	<i>Petiveria alliacea</i> L.	Guinea Hen weed (E), Awogbaarun, Arunyanyan (Y)	FHI 106992	Leaves	PaL
					Roots	PaR

14	Rubiaceae	<i>Morinda lucida</i> Benth.	Oruwo (Y), Morinda, Indian mulberry (E)	FHI 110086	Leaves	MIL
15	Solanaceae	<i>Capsicum</i> <i>frutescens</i> L.	Ata wewe (Y), Chilli pepper (E)	FHI 108325	Leaves	CfL
16	Solanaceae	<i>Nicotiana</i> <i>tabacum</i> L.	Ewe taba (Y), Taba (H), Anwere (I)	FH 107924	Leaves	NtL

E – English, H – Hausa, I – Igbo, IB – Ibibio, Y – Yoruba

**Table 2: Total phenolic content and DPPH inhibitory activities of crude plant extracts**

Plant extract	TPC(mg GAE/g)	DPPH IC <sub>50</sub> (µg/mL)
AaL	4.66 ± 0.23 <sup>c,e</sup>	55.78 ± 1.14 <sup>***</sup>
AdL	0.17 ± 0.01 <sup>d</sup>	147.09 ± 3.34 <sup>***</sup>
AdR	6.32 ± 0.36 <sup>b,e</sup>	2058.19 ± 0.56 <sup>***</sup>
AgL	7.31 ± 0.21 <sup>a,b</sup>	190.49 ± 1.45 <sup>***</sup>
CfL	11.64 ± 0.48 <sup>k</sup>	1454.19 ± 0.87 <sup>***</sup>
CnO	2.83 ± 0.05 <sup>c,d</sup>	9128.35 ± 1.97 <sup>***</sup>
CrR	8.81 ± 0.78 <sup>a</sup>	1021.39 ± 3.30 <sup>***</sup>
CsL	8.31 ± 0.46 <sup>a</sup>	65.97 ± 3.57 <sup>***</sup>
MIL	14.63 ± 0.56 <sup>g</sup>	21.09 ± 0.2 <sup>***</sup>
NtL	58.35 ± 0.85 <sup>h</sup>	51.33 ± 0.61 <sup>***</sup>
OsL	7.98 ± 0.43 <sup>a</sup>	ND
PaL	3.33 ± 0.48 <sup>c</sup>	ND
PaR	1.66 ± 0.12 <sup>d</sup>	59.83 ± 2.93 <sup>***</sup>
PoB	8.31 ± 0.60 <sup>a</sup>	10.68 ± 0.74 <sup>***</sup>
PoL	56.35 ± 0.74 <sup>l</sup>	19.32 ± 0.79 <sup>***</sup>
TaL	8.81 ± 0.34 <sup>a</sup>	48.17 ± 0.35 <sup>***</sup>
TsL	5.15 ± 0.82 <sup>e</sup>	16.86 ± 0.94 <sup>***</sup>
TtB	67.99 ± 0.67 <sup>i</sup>	4.59 ± 0.23
TtR	28.59 ± 0.60 <sup>f</sup>	7.10 ± 0.44 <sup>*</sup>
XaB	28.59 ± 0.45 <sup>f</sup>	12.06 ± 1.06 <sup>***</sup>
XaR	63.84 ± 0.38 <sup>j</sup>	6.01 ± 0.36
Ascorbic acid		1.65 ± 0.06

*Aspilia africana* (leaf) = AaL, *Anchomanes difformis* (leaf) = AdL; *Anchomanes difformis* (root) = AdR, *Asystasia gangetica* (leaf) = AgL, *Capsicum frutescens* (leaf) = CfL, *Cocos nucifera* (fruit) = CoO, *Crotolaria retusa* (Root) = CrR, *Culcasia scandens* (leaf) = CsL, *Morinda lucida* (leaf) = MIL, *Nitotiana*

*tabacum* (leaf) = NtL, *Olax subscopoides* (leaf) = OsL, *Pativera alliaceae* (leaf) = PaL, *Pativera alliaceae* (root) = PaR, *Treculia africana* (leaf) = TaL, *Triclisia subcordat* (leaf) = TsL, *Tetrapleura tetraptera* (bark) = TtB, *Tetrapleura tetraptera* (root) = TtR, *Xylopi aethiopica* (bark) = XaB, *Xylopi aethiopica* (root) = XaR, data obtained were expressed as means  $\pm$  standard deviation (SD), ND = Not determined, n = 3, for TPC, samples with different superscripts are significantly different ( $P < 0.05$ ) from one another, for DPPH, level of significant difference from ascorbic acid ( $P < 0.05$ ) is represented by \*, \*\*, \*\*\* while Samples without asterisks are not significantly different from ascorbic acid.

**Table 3: Antibacterial and antifungal activities of crude plant extracts**

IC <sub>50</sub> (µg/mL)				
	<i>Salmonella typhi</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
AaL	183.2 ± 7.5***	4.58 ± 0.47***	2.52 ± 0.17***	29.17 ± 0.74***
AdL	3.8 ± 0.3	4.56 ± 0.21***	0.70 ± 0.05	28.01 ± 1.84***
AdR	337.5 ± 6.5***	4.58 ± 0.67***	4.41 ± 0.72***	14.19 ± 2.57**
AgL	280.9 ± 7.4***	4.06 ± 0.89***	1.99 ± 0.04***	28.94 ± 3.22***
CfL	146.0 ± 2.3***	3.97 ± 0.38***	3.03 ± 0.048***	24.21 ± 0.52***
CrR	296.7 ± 20.4***	4.73 ± 0.37***	3.14 ± 0.47***	4.52 ± 0.32
CsL	318.0 ± 25.7***	4.22 ± 0.36***	4.13 ± 0.19***	24.19 ± 4.29***
MIL	16.9 ± 2.9	3.32 ± 0.44***	3.18 ± 0.15***	46.82 ± 0.37***
NtL	324.8 ± 5.1***	4.18 ± 0.30***	2.39 ± 0.08***	41.20 ± 0.53***
OsL	427.7 ± 17.3***	3.26 ± 0.24***	1.95 ± 0.05***	29.36 ± 0.38***
PaL	242.1 ± 23.9***	4.42 ± 0.24***	2.75 ± 0.10***	22.69 ± 1.56***
PaR	307.2 ± 15.5***	4.12 ± 0.23***	3.24 ± 0.51***	79.71 ± 4.21***
PoB	310.4 ± 18.7***	ND	15.89 ± 0.31***	17.05 ± 1.98***
PoL	4.1 ± 0.6	7.44 ± 0.23***	2.74 ± 0.14***	15.21 ± 1.85***
TaL	401.8 ± 20.3***	4.78 ± 0.31***	3.00 ± 0.17***	35.89 ± 2.56***
TsL	177.5 ± 2.1***	3.58 ± 0.39***	2.28 ± 0.39***	53.78 ± 3.26***
TtB	238.9 ± 35.8***	ND	2.40 ± 0.15***	13.83 ± 3.47**
TtR	153.7 ± 18.8***	ND	3.02 ± 0.56***	10.62 ± 2.56
XaB	20.0 ± 2.6	3.59 ± 0.05***	0.75 ± 0.09	20.29 ± 3.06***
XaR	1.5 ± 0.7	4.90 ± 0.05***	1.52 ± 0.51**	30.78 ± 4.07***
Streptomycin	0.1 ± 0.00	0.98 ± 0.12	0.24 ± 0.04	-
Ketoconazole	-	-	-	3.95 ± 0.36

*Aspilia africana* (leaf) = AaL, *Anchomanes difformis* (leaf) = AdL; *Anchomanes difformis* (root) = AdR, *Asystasia gangetica* (leaf) = AgL, *Capsicum frutescens* (leaf) = CfL, *Cocos nucifera* (fruit) = CoO, *Crotalaria retusa* (Root) = CrR, *Culcasia scandens* (leaf) = CsL, *Morinda lucida* (leaf) = MIL, *Nicotiana tabacum* (leaf) = NtL, *Olox subscopoides* (leaf) = OsL, *Pativera alliaceae* (leaf) = PaL, *Pativera alliaceae* (root) = PaR, *Treculia africana* (leaf) = TaL, *Triclisia subcordat* (leaf) = TsL, *Tetrapleura tetraptera* (bark) =

TtB, *Tetrapleura tetraptera* (root) = TtR, *Xylopia aethiopica* (bark) = XaB, *Xylopia aethiopica* (root) = XaR, data obtained were expressed as means  $\pm$  standard deviation (SD), ND = Not determined, n = 3, level of significant difference from positive control/standard drug (P<0.05) is represented by \*,\*\*,\*\*\* while samples without asterisks are not significantly different from standard drug.

**Table 4: Cytotoxic activities of crude plant extracts**

CC <sub>50</sub> ( $\mu$ g/mL)			
	Hep 2	RD	HeLa
AaL	1.3 $\pm$ 0.3	8.3 $\pm$ 0.2*	12.9 $\pm$ 0.6***
AdL	284.2 $\pm$ 6.3***	62.0 $\pm$ 0.6***	ND
AdR	65.3 $\pm$ 4.3***	5.1 $\pm$ 0.3	89.7 $\pm$ 1.6***
AgL	31.6 $\pm$ 1.6***	8.7 $\pm$ 0.2*	ND
CfL	5.1 $\pm$ 0.4	1.6 $\pm$ 0.0	5.1 $\pm$ 0.4***
CnO	100.5 $\pm$ 2.9***	284.2 $\pm$ 1.5***	ND
CrR	8.0 $\pm$ 1.2	8.6 $\pm$ 0.2*	14.7 $\pm$ 0.4***
CsL	14.4 $\pm$ 0.9***	0.9 $\pm$ 0.0	92.4 $\pm$ 0.6***
MIL	100.5 $\pm$ 9.0***	14.7 $\pm$ 0.7***	85.4 $\pm$ 0.3***
NtL	2.9 $\pm$ 0.4	54.0 $\pm$ 0.3***	ND
OsL	5.3 $\pm$ 0.7	62.0 $\pm$ 0.9***	ND
PaL	5.3 $\pm$ 1.0	237.0 $\pm$ 2.3***	9.0 $\pm$ 0.7***
PaR	8.5 $\pm$ 0.2	31.6 $\pm$ 4.3***	253.7 $\pm$ 0.7***
PoB	5.4 $\pm$ 0.1	14.3 $\pm$ 1.5***	144.1 $\pm$ 1.1***
PoL	18.5 $\pm$ 2.5***	35.2 $\pm$ 6.2***	161.4 $\pm$ 0.6***
TaL	100.5 $\pm$ 3.7***	5.2 $\pm$ 0.6	ND
TsL	54.0 $\pm$ 0.9***	62.0 $\pm$ 6.3***	58.1 $\pm$ 0.5***
TtB	1.7 $\pm$ 0.2	14.9 $\pm$ 1.1***	14.0 $\pm$ 1.3***
TtR	3.4 $\pm$ 0.5	14.1 $\pm$ 0.6***	12.9 $\pm$ 0.9***
XaB	31.6 $\pm$ 0.7***	3.3 $\pm$ 0.9	0.2 $\pm$ 0.0
XaR	8.6 $\pm$ 0.2	1.6 $\pm$ 0.2	10.0 $\pm$ 0.6***
vincristine sulphate	0.01 $\pm$ 0.0	0.6 $\pm$ 0.0	0.6 $\pm$ 0.0

*Aspilia africana* (leaf) = AaL, *Anchomanes difformis* (leaf) = AdL; *Anchomanes difformis* (root) = AdR, *Asystasia gangetica* (leaf) = AgL, *Capsicum frutescens* (leaf) = CfL, *Cocos nucifera* (fruit) = CoO, *Crotolaria retusa* (Root) = CrR, *Culcasia scandens* (leaf) = CsL, *Morinda lucida* (leaf) = MIL, *Nicotiana tabacum* (leaf) = NtL, *Olax subscopoides* (leaf) = OsL, *Pativera alliaceae* (leaf) = PaL, *Pativera alliaceae* (root) = PaR, *Treculia africana* (leaf) = TaL, *Triclisia subcordat* (leaf) = TsL, *Tetrapleura tetraptera* (bark) = TtB, *Tetrapleura tetraptera* (root) = TtR, *Xylopia aethiopica* (bark) = XaB, *Xylopia aethiopica* (root) = XaR, data obtained were expressed as means  $\pm$  standard deviation (SD), ND = Not determined, n = 3, level of significant difference from vincristine sulphate (P<0.05) is represented by \*,\*\*,\*\*\* while samples without asterisks are not significantly different from vincristine sulphate.