Evaluation of Selected Nigerian Medicinal Plants for Phenolic Content, Antimicrobial, and Cytotoxic Activities

Samuel Ayoolu Oguntimehin1*, Edith Oriabure Ajaiyeoba1, Omonike Oluymemisi Ogbole1, Hannah Odunola Dada-Adegbola2, Bosede Bolaji Oluremi3, Adekunle Johnson Adeniji4

1Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria
2Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria
3Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria
4Department of Virology, University of Ibadan, Ibadan, Nigeria

*Corresponding author: Samuel Ayoolu Oguntimehin, Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria
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Abstract
Increased exposure to pathogens and free radicals contributes to the high incidence and mortality rate of various types of cancers in Nigeria and globally. This study aimed to screen twenty extracts from fifteen selected Nigerian medicinal plants used traditionally for cancer treatment for phenolic content, antimicrobial, and cytotoxic activities. The phenolic content was determined by Folin – Ciocalteu method, and antimicrobial potentials of the extracts was evaluated using spectrophotometric growth inhibition method while MTT assay was used to assess their cytotoxicity to cancer cell lines. Bark and root 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 ethnomedicinal uses of most of the tested medicinal plants.

Keywords: MTT assay; Cytotoxicity; Ethnomedicine; Antioxidants; Antimicrobial

Introduction
Cancer remains one of the leading causes of death worldwide with an estimated 19.3 million new cancer cases and 10 million cancer deaths [1]. Several factors are associated with the development of cancer, but notable is the actions of free radicals and pathogenic microorganisms [2]. Studies have shown that reactive free radicals interact with macromolecules such as DNA in the cell, leading to damage to cell structure and functions [3]. In addition, pathogenic microbes are associated with cancer [4].

Cancer treatment faces challenges of resistance, toxicities to normal cells, and ineffectiveness of some anticancer agents [5]. The use of an agent with antioxidant and antimicrobial activities will be a good strategy in the treatment of cancer. Medicinal plants are a veritable source of anticancer agents [6], they are a rich source of phenolic compounds which are known to attenuate the actions of free radicals [7] and also showed antimicrobial activities [8].
The dependence of not less than 80% of the African populace on medicinal plants for healthcare needs [9] informed the inquiry into plants used traditionally in the treatment of cancer in Southwestern Nigeria. More so, various studies demonstrated the antioxidant [10], antimicrobial [11], and anticancer [12] activity of some Nigerian medicinal plants. Retrieving information on the pharmacological relevance of medicinal plants is often achieved through the conduction of ethnomedicinal surveys [13]. An ethnomedicinal survey was carried out in Ile-Ife, Osun State, Nigeria between June to December 2017. From the survey, twenty extracts from fifteen plant species were selected for this study based on how they are frequently mentioned. This study aims at identifying plant extracts with antioxidant, antimicrobial, and cytotoxic activities towards the potential identification of the new anticancer agents.

### Methods

#### Chemicals

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium salt (purity ≥ 97%), vincristine sulphate (purity ≥ 95%), nutrient agar, sabouraud dextrose agar, ketoconazole (purity = ≥ 99%), streptomycin (purity = ≥90%), gallic acid (purity ≥ 90%). All chemicals and media are purchased from Sigma-Aldrich (Germany), Folin – ciocalteu was purchased from Loba Chemie (India).

#### Plant material

Following the methods of Olorunniola et al. [14], an ethnobotanical survey was previously conducted in Ile-Ife, Osun state Nigeria. The plant parts of frequently mentioned plants were used for this study (Table 1). Plants were collected on the campus of the University of Ibadan, Nigeria, and authenticated at the herbarium of the Forest Research Institute of Nigeria, where voucher specimens were also deposited. The plant materials were dried at room temperature and pulverized into powder.

| Family            | Name                        | Local name                  | Identification number | Part used | Ethnomedicinal use                                    |
|-------------------|-----------------------------|-----------------------------|-----------------------|-----------|--------------------------------------------------------|
| Acanthaceae       | *Asystasia gangetica*       | Akpuarachi (I)              | FHI 109634            | Leaves    | Anti-helmintic, asthma, astringent, diaphoretic, stomachic |
|                   | *(L.) T.Anderson*            |                             |                       |           | [15]                                                   |
| Annonaceae        | *Xylopia aethiopica*        | Kimba (H), Uda (I), Eruu, girinja (Y), Ethiopian pepper (E) | FHI 108978            | Bark Root | Amenorrhea, biliousness, bronchitis, cough, dysentery, fibrilod, malaria, rheumatism |
|                   | *(Dunal) A.Rich.*            |                             |                       |           |                                                        |
| Araceae           | *Anchomanes diformis*       | Igo langbodo, Ogirisako (Y), Oje (I), Chakara (H) | FHI 109638            | Leaves Root | Asthma, diabetes, gastrointestinal disturbances, inflammation, microbial infections, pain, ulcerations |
|                   | *(Blume) Engl.*             |                             |                       |           |                                                        |
| Compositae        | *Culcasia scandens*         | Oji azu ari nkwu (I),       | FHI 110050            | Leaves    | Cancer, stomachache                                    |
|                   | *P.Beauv.*                  |                             |                       |           | [18]                                                   |
|                   |                             |                             |                       |           |                                                        |
|                   | *Aspilia africana*          | Jamajina (H), Oranjila (I), Yunyun, Ako yunyun (Y) | FHI 107511            | Leaves    | abortifacients, dysentery, hemorrhoid, hemostatic, nervous disorders, skin diseases, stomach disorders, tuberculosis, ulcers |
|                   | *(Pers.) C.D.Adams*         |                             |                       |           |                                                        |
| Leguminosae       | *Crotalaria retusa*         | Koropo, Alatunse, Savoro (Y), Rattle pea (E) | FHI 109052            | Roots     | cold, fever, flatulence, hemoptysis fever, leprosy, lung disease, skin infections |
|                   | L.*                        |                             |                       |           |                                                        |
| Tetrapleura tetraptera | *(Schum et Thonn) Taub.* | Dawo (H), Uuyak (IB), Aridan/Aidan (Y) | FHI 110141            | Bark Root | febrile convulsions, infantile flatulence, inflammation, rheumatic aches, stomach gripes |
|                   | *(Schum et Thonn) Taub.*    |                             |                       |           |                                                        |
| Pterocarpus osun | *Crab*                      | Madubiya (H), Osun (Y)      | FHI 108415            | Leaves Bark | antipyretic, anti-sickling, asthma, blood supplement, candidiasis, eczema, skin infections |
|                   | Craib*                      |                             |                       |           | [18]                                                   |
Menispermaceae  | *Trichlis subcordata* Oliv. | Alugbonron (Y) | FHI 109638 | Leaves | Breast cancer [18]

Moraceae  | *Trecolia africana* Deene. ex Trécul | Barafuta (H), Ukwa (I), Afon (Y) | FHI 106992 | Leaves | anemia, cough, guinea worm infections, hemorrhoid, malaria, ulcer, venereal disease [18]

Olacaceae  | *Olax subscorpioidea* Oliv. | Gwano kurmi (H), Aziza (I), Ifon (Y) | FHI 109983 | Leaves | abscess, antisickling, breast cancer, diabetes, hemorrhoid, jaundice, mental disorders, scalp infection in children, yellow fever [18]

Phytolaccaceae  | *Petiveria alliacea* L. | Guinea Hen weed (E), Awogbaaruun, Arunyanyan (Y) | FHI 106992 | Leaves | Anticancer, guinea worm infections [18]

Rubiaceae  | *Morinda lucida* Benth. | Oruwo (Y), Morinda, Indian mulberry (E) | FHI 110086 | Leaves | anticancer, candidiasis, diabetes, female infertility, malaria, vaginitis [18]

Solanaceae  | *Capsicum frutescens* L. | Ata weye (Y), Chilli pepper (E) | FHI 108325 | Leaves | anticancer, antisickling, breast cancer, diabetes, dysentery, fever, stimulant, measles [18]

| *Nicotiana tabacum* L. | Ewe taba (Y), Taba (H), Anwere (I) | FHI 107924 | Leaves | candidiasis, cancer, diabetes, epistaxis, gonorrhea, hemorrhoid, typhoid fever [18]

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

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

### Extraction

Each plant material (200 g) was macerated in 80% methanol for 78 h at room temperature. Extracts were filtered through filter paper (Whatman No. 1) and concentrated to dryness *in vacuo*.

### Total phenolic content (TPC) assay

The total phenolic content of the extracts was determined using Folin – Ciocalteu (FC) reagent following the method of Karakas et al. [21] with slight modification. The extracts were made into concentrations of 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% Na2CO3 (125 µL) was added to each well and afterward incubated in the dark for 2 h at 25 ± 2°C. The absorbance was recorded with a Thermo Fisher Scientific microplate reader at 758 nm. The experiment was carried out in triplicates. The total phenolic content was expressed as Gallic acid equivalents (GAE) [22].

### 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 used include *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, and *Salmonella typhi* ATCC 24683 while the fungi strain used was *Candida albicans* ATCC 24433. Nutrient broth and sabouraud dextrose broth were used for the maintenance of the bacterial and fungal strains respectively at 4°C.

### Preparation of inoculums

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

### Spectrophotometric growth inhibition method

The method of Ahmad et al. [23] was adopted with some modifications. Extracts and standard drugs (streptomycin and ketoconazole) were made into concentrations of 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 with 75 µL of the inoculum in 96-well plates. Sterile distilled water was used as the control. 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 indexes. The experiment was carried out in triplicates. The concentration at which there is 50% microbial inhibition (IC_{50}) was determined using Graph pad prism (5.0) while the percentage of microbial inhibition was calculated by using the equation:
% Inhibition = ∆Absorbance of control - ∆Absorbance of test sample x 100

∆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), University of 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% NaHCO3 (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 by Ogbole et al. [24]. Each cell line was seeded into a 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 using AmScope 40X-600X 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 to each well and left on a shaker for 30min to ease the solubility and evenness of the color formed. Absorbance at 492 nm was recorded with a Thermo Fisher Scientific microplate reader. The experiment was performed in triplicate while the CC50 was determined using graph pad prism 5.0. Percentage cytotoxicity of the extracts at various concentrations was calculated using the formula;

% Cytotoxicity (CC) = (A - B) x 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 5.0 was used for the statistical analysis of data. The data obtained were expressed as Mean ± SD (Standard deviation) values of three independent assessments. The IC₅₀ and CC₅₀ values of all test samples were determined with a nonlinear regression plot of log (cytotoxic concentration) against normalized percentage cytotoxicity. One-way at P < 0.05 followed by Tukey’s test was used to test for the significant difference between the extracts and the standard drugs.

**Results**

Among all tested extracts, leaf extracts of *N. 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.

| Plant extract            | TPC (mg GAE/g) |
|--------------------------|----------------|
| *A. africana* (leaf)     | 4.66 ± 0.23   |
| *A. difformis* (leaf)    | 0.17 ± 0.01   |
| *A. difformis* (root)    | 6.32 ± 0.36   |
| *A. gangetica* (leaf)    | 7.31 ± 0.21   |
| *C. frutescens* (leaf)   | 11.64 ± 0.48  |
| *C. retusa* (Root)       | 8.81 ± 0.78   |
| *C. scandens* (leaf)     | 8.31 ± 0.46   |
| *M. lucida* (leaf)       | 14.63 ± 0.56  |
| *N. tabacum* (leaf)      | 58.35 ± 0.85  |
| *O. subscopoides* (leaf) | 7.98 ± 0.43   |
| *P. alliaceae* (leaf)    | 3.33 ± 0.48   |
| *P. alliaceae* (root)    | 1.66 ± 0.12   |
| *P. osun* (Bark)         | 8.31 ± 0.60   |
| *P. osun* (leaf)         | 56.35 ± 0.74  |
| *T. africana* (leaf)     | 8.81 ± 0.34   |
| *T. subcordata* (leaf)   | 5.15 ± 0.82   |
| *T. tetraptera* (bark)   | 67.99 ± 0.67  |
| *T. tetraptera* (root)   | 28.59 ± 0.60  |
| *X. aethiopica* (bark)   | 28.59 ± 0.45  |
| *X. aethiopica* (root)   | 63.84 ± 0.38  |

Data obtained were expressed as means ± standard deviation (SD), n = 3, for TPC, samples with different superscripts are significantly different (P<0.05) from one another.

**Table 2:** Total phenolic content of crude plant extracts
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. diffusa*, *Morinda lucida*, and *Pterocarpus osun* had IC\textsubscript{50} of 1.5, 20.0, 3.8, 16.9, and 4.1 against Salmonella typhi. Similarly, *A. diffusa* and bark extract of *X. aethiopica* produced comparable activities against *E. coli*. All tested extracts were active against *Pseudomonas aeruginosa*; however, none was comparable (P<0.05) to the activity of Streptomycin with IC\textsubscript{50} of 0.9 µg mL\textsuperscript{-1}. Root extracts of *X. aethiopica* and leaf extract of *A. diffusa* showed a broad spectrum of antibacterial activities. Antifungal studies against *Candida albicans* showed that root extracts of *C. retusa*, *T. tetraptera*, and Ketoconazole exhibited comparable (P<0.05) antifungal activities.

| IC\textsubscript{50} (µg/mL) | Salmonella typhi | Pseudomonas aeruginosa | Escherichia coli | Candida albicans |
|-------------------------------|------------------|------------------------|------------------|------------------|
| *A. africana* (leaf)          | 183.2 ± 7.5***   | 4.58 ± 0.47***         | 2.52 ± 0.17***   | 29.17 ± 0.74***  |
| *A. diffusa* (leaf)           | 3.8 ± 0.3        | 4.56 ± 0.21***         | 0.70 ± 0.05      | 28.01 ± 1.84***  |
| *A. diffusa* (root)           | 337.5 ± 6.5***   | 4.58 ± 0.67***         | 4.41± 0.72***    | 14.19 ± 2.57**   |
| *A. gangetica* (leaf)         | 280.9 ± 7.4***   | 4.06 ± 0.89***         | 1.99 ±0.04***    | 28.94 ± 3.22***  |
| *C. frutescens* (leaf)        | 146.0 ± 2.3***   | 3.97 ± 0.38***         | 3.03 ± 0.048***  | 24.21 ± 0.52***  |
| *C. retusa* (Root)            | 296.7 ± 20.4***  | 4.73 ± 0.37***         | 3.14 ± 0.47***   | 4.52 ± 0.32      |
| *C. scandens* (leaf)          | 318.0 ± 25.7***  | 4.22 ± 0.36***         | 4.13 ± 0.19***   | 24.19 ± 4.29***  |
| *M. lucida* (leaf)            | 16.9 ± 2.9       | 3.32 ± 0.44***         | 3.18 ± 0.15***   | 46.82 ± 0.37***  |
| *N. tabacum* (leaf)           | 324.8 ± 5.1***   | 4.18 ± 0.30***         | 2.39 ± 0.08***   | 41.20 ± 0.53***  |
| *O. subscopoides* (leaf)      | 427.7 ± 17.3***  | 3.26 ± 0.24***         | 1.95 ± 0.05***   | 29.36 ± 0.38***  |
| *P. alliaceae* (leaf)         | 242.1 ± 23.9***  | 4.42 ± 0.24***         | 2.75 ± 0.10***   | 22.69 ± 1.56***  |
| *P. alliaceae* (root)         | 307.2 ± 15.5***  | 4.12 ± 0.23***         | 3.24 ± 0.51***   | 79.71 ± 4.21***  |
| *P. osun* (Bark)              | 310.4 ± 18.7***  | ND                      | 15.89 ± 0.31***  | 17.05 ± 1.98***  |
| *P. osun* (leaf)              | 4.1 ± 0.6        | 7.44 ± 0.23***         | 2.74 ± 0.14***   | 15.21 ± 1.85***  |
| *T. africana* (leaf)          | 401.8 ± 20.3***  | 4.78 ± 0.31***         | 3.00 ± 0.17***   | 35.89 ± 2.56***  |
| *T. subcordata* (leaf)        | 177.5 ± 2.1***   | 3.58 ± 0.39***         | 2.28 ± 0.39***   | 53.78 ± 3.26***  |
| *T. tetraperta* (bark)        | 238.9 ± 35.8***  | ND                      | 2.40 ± 0.15***   | 13.83 ± 3.47**   |
| *T. tetraperta* (root)        | 153.7 ± 18.8***  | ND                      | 3.02 ± 0.56***   | 10.62 ± 2.56    |
| *X. aethiopica* (bark)        | 20.0 ± 2.6       | 3.59 ± 0.05***         | 0.75 ± 0.09      | 20.29 ± 3.06***  |
| *X. aethiopica* (root)        | 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      |

Data obtained were expressed as means ± 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 the standard drug.

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

Extracts were also evaluated for their cytotoxicity on Hep 2, RD, and 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, with extracts of *A. africana*, *T. tetraperta*, and *N. tabacum* being the most cytotoxic with IC\textsubscript{50} 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 *C. scandens*, *C. frutescens*, and root extract of *X. aethiopica* with IC$_{50}$ 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 the HeLa cell line when compared with the standard drug. Extracts of *C. frutescens*, *A. africana*, *X. aethiopica*, *T. tetraptera*, and *C. retusa* showed a broad spectrum of cytotoxic activities against the tested cell lines.

| **CC$_{50}$ (µg/mL)** | **Hep 2** | **RD** | **HeLa** |
|------------------------|-----------|--------|---------|
| *A. africana* (leaf)   | 1.3 ± 0.3 | 8.3 ± 0.2* | 12.9 ± 0.6*** |
| *A. difformis* (leaf)  | 284.2 ± 6.3*** | 62.0 ± 0.6*** | ND |
| *A. difformis* (root)  | 65.3 ± 4.3*** | 5.1 ± 0.3 | 89.7 ± 1.6*** |
| *A. gangetica* (leaf)  | 31.6 ± 1.6*** | 8.7 ± 0.2* | ND |
| *C. frutescens* (leaf) | 5.1 ± 0.4 | 1.6 ± 0.0 | 5.1 ± 0.4*** |
| *C. retusa* (Root)     | 8.0 ± 1.2 | 8.6 ± 0.2* | 14.7 ± 0.4*** |
| *C. scandens* (leaf)   | 14.4 ± 0.9*** | 0.9 ± 0.0 | 92.4 ± 0.6*** |
| *M. lucida* (leaf)     | 100.5 ± 9.0*** | 14.7 ± 0.7*** | 85.4 ± 0.3*** |
| *N. tabacum* (leaf)    | 2.9 ± 0.4 | 54.0 ± 0.3*** | ND |
| *O. subscopoides* (leaf)| 5.3 ± 0.7 | 62.0 ± 0.9*** | ND |
| *P. alliaceae* (leaf)  | 5.3 ± 1.0 | 237.0 ± 2.3*** | 9.0 ± 0.7*** |
| *P. alliaceae* (root)  | 8.5 ± 0.2 | 31.6 ± 4.3*** | 253.7 ± 0.7*** |
| *P. osun* (Bark)       | 5.4 ± 0.1 | 14.3 ± 1.5*** | 144.1 ± 1.1*** |
| *P. osun* (leaf)       | 18.5 ± 2.5*** | 35.2 ± 6.2*** | 161.4 ± 0.6*** |
| *T. africana* (leaf)   | 100.5 ± 3.7*** | 5.2 ± 0.6 | ND |
| *T. subcordata* (leaf) | 54.0 ± 0.9*** | 62.0 ± 6.3*** | 58.1 ± 0.5*** |
| *T. tetraperta* (bark) | 1.7 ± 0.2 | 14.9 ± 1.1*** | 14.0 ± 1.3*** |
| *T. tetraperta* (root) | 3.4 ± 0.5 | 14.1 ± 0.6*** | 12.9 ± 0.9*** |
| *X. aethiopica* (bark) | 31.6 ± 0.7*** | 3.3 ± 0.9 | 0.2 ± 0.0 |
| *X. aethiopica* (root) | 8.6 ± 0.2 | 1.6 ± 0.2 | 10.0 ± 0.6*** |
| Vincristine sulfate     | 0.01 ± 0.0 | 0.6 ± 0.0 | 0.6 ± 0.0 |

Data obtained were expressed as means ± standard deviation (SD), ND = Not determined, n = 3, level of significant difference from vincristine sulfate (P<0.05) is represented by *,**,*** while samples without asterisks are not significantly different from vincristine sulfate.

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

**Discussions**

In developing nations, medicinal plants are very important in health care delivery [9]. The prevalence and rising mortality rate of cancers, and related diseases necessitated sourcing treatment alternatives from medicinal plants [12]. Bioactive compounds in extracts of medicinal plants are responsible for the diverse pharmacological activities demonstrated by these plants [25]. Identifying potential medicinal plants for cancer treatment is often achieved through information retrieved from traditional health practitioners (TMPs) [13]. This present study seeks to scientifically justify the ethnomedicinal use of fifteen (15) medicinal plant species used in the treatment of...
cancer and microbial infections.

Free radicals are proven contributors to the development and progression of most diseases via their damaging effects on macromolecules including proteins, DNA, and RNA [26]. Antioxidants, on the other hand, inhibit the actions of free radicals and therefore could potentially prevent the onset and progression of these diseases [27]. Previous reports showed that Nigerian medicinal plants have antioxidant activities [22,27].

Phenols are known to contribute to the antioxidant activities of medicinal plants [20]. Extract of *T. tetraplera* was shown to have high phenolic content and was demonstrated to have antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, and antilipidemic activities. Other extracts including bark and root extracts of *X. aethiopica* and root extract of *T. tetraplera* also showed high total phenolic contents.

In addition, evidences linking free radicals and chronic microbial infections with cancer have been reported. *Salmonella typhi* for example has been linked to the development of cancer of the gall bladder [28] while *Helicobacter pylori* have been linked to gastric cancer [4]. Phenolic compounds in extracts of medicinal plants contribute to their antimicrobial activities due to their damaging effect on the cell membrane and disruption of metabolism and synthesis of nucleic acids [29].

We observed that Extracts used in this study demonstrated antimicrobial activities against *S. typhi, P. aeruginosa, E. coli,* and *C. albicans*. Extracts of *X. aethiopica* root and leaf extract of *A. africana* produced a broad spectrum of antibacterial activities. Fruit extract of *X. aethiopica* and derivative of its major constituent had earlier been demonstrated to have antimicrobial activities [30]. Our findings demonstrated that the root and bark extracts of *X. aethiopica* equally hold antimicrobial properties. The activities observed in this study may be attributed to the high phenolic content as a result of the phenolic constituents of the test samples.

Varying cytotoxic activities against tested cell lines were reported for our study plants as observed in previous reports [12,24,31]. According to the National Cancer Institute (NCI) on screening of medicinal plants for cytotoxic activities, plant extracts with CC50 < 30 μg/mL are considered active [12]. Based on the NCI standard, nineteen (19) extracts were active against at least one (1) cancer cell line, while ten (10) extracts were active against at least two (2) cancer cell lines. Only six (6) extracts were cytotoxic to all the cancer cell lines used.

We are reporting perhaps the first cytotoxicity studies on the root and bark extract of *X. aethiopica*. The root extract of *X. aethiopica* produced 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), and cervix through the induction of apoptosis and arrest of the cell cycle [32]. Ent-15-oxoakaur-16-en-19-oic acid, 3,4,5-trihydroxy-6,6-dimethylpyranol[2,3-g] flavone, and isoteretrandrine are some of the cytotoxic constituents in the fruit extract of *X. aethiopica* [33].

This study also showed that bark and root extracts of *T. tetraplera* demonstrated cytotoxicity against all tested cancer cell lines. Fadeyi et al. [12] reported the cytotoxicity of extract of *T. tetraplera* against breast (BT-549) cancer cell line. Similarly, *in vitro*, and *in vivo* models of Ozaslan et al. [34] also demonstrated the cytotoxicity of fruit extract of *T. tetraplera* against Ehrlich Ascites tumor cells. Bioactive coumarin, saponins, terpenes, and some phenolics have been isolated from extracts of the plants [20]. The high phenolic content of *T. tetraplera* extract might contribute to its cytotoxicity, however, further works will aim at identifying the active cytotoxic compounds.

Stem extract of *C. retusa* was found to be more cytotoxic than leaf, seed, pod, and flower extracts of the plant although in an un-selective manner [35]. We report the cytotoxicity of its root extract to Hep 2, RD, and HeLa cell lines. Similarly, leaf extract of *A. africana* was found to be cytotoxic against the three cell lines used. Niyonzigaie et al. [36] reported the cytotoxicity of extracts of *A. africana* obtained using various green methods of extraction against AGS, A549, and HeLa cell lines. Gallic acid, chlorogenic acid, syringic acid, ferulic acid, and quercetin were detected in the most active extract of *A. africana*.

In this study, *C. frutescens* elicited a broad spectrum of cytotoxicity against all tested cell lines. Its fruits, commonly used in African cuisine are widely studied for their nutritional and health benefits [37]. Although a report suggests that capsaicin, a constituent of the fruits is a human carcinogen [38], however, the same compound has been reported to demonstrate cytotoxic and cancer prevention potentials [37]. A more recent report demonstrated that capsaicin and piperine from *Piper nigrum* could reverse the resistance of cancer cells to doxorubicin [39].

**Conclusion**

In this study, we evaluated the antioxidant, antimicrobial, and cytotoxicity of extracts of medicinal plants used traditionally in the treatment of cancer in Southwestern Nigeria. Results from the study justify the traditional use of these extracts in the treatment of cancer and accompanying conditions. Extracts of *T. tetraplera* and *X. aethiopica* was active in all the biological tests which could be linked to their high phenolic contents. Further purification might potentiate their pharmacological effects which will favor their pharmaceutical application in the development of anticancer and antimicrobial therapeutics. Our current endeavor is devoted to identifying the active constituents present in the extracts of these plants.
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References
1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71: 209-249.
2. Shahab L, McGowan JA, Waller J, Smith SG (2018) 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 103:308-316.
3. Vineis P, Wild CP. (2014) Global cancer patterns: causes and prevention. The Lancet 383:549-557.
4. Maggio-Price L, Treuting P, Zeng W, Tsang M, Bielefeldt-Ohmann H, Iritani BM (2006) Helicobacter infection is required for inflammation and colon cancer in SMAD3-deficient mice. Cancer Res 66:828-838.
5. Liu GF, Li GJ, Zhao H (2018) Efficacy and Toxicity of Different Chemotherapy Regimens in the Treatment of Advanced or Metastatic Pancreatic Cancer: A Network Meta-Analysis. J Cell Biochem 119:511-23.
6. Morsy N (2019) Anticancer agents from plants. Main Group Chemistry 18:169-191.
7. Rolim PM, Fidelis GP, Padiha CE, Santos ES, Rocha HA, et al. (2018) Phenolic profile and antioxidant activity from peels and seeds of melon (Cucumis melo L. var. reticulatus) and their antiproliferative effect in cancer cells. Braz J Med Biol Res 51:e6069.
8. Romulo A, Zuhud EA, Rondevaldova J, Kokoska L (2018) Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. Pharm Biol 56:287-293.
9. Abo KA, Lawal IO, Ogunkammi A (2011) Evaluation of extracts of Trichilia suboardata Oliv and Heinsia crinita (Afz) G. Taylor for antimicrobial activity against some clinical bacterial isolates and fungi. African Journal of Pharmacy and Pharmacology 5:125-131.
10. Akinmoladan AC, Obuotor EM, Farombi EO (2010) Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food 13:444-451.
11. Ugboko HU, Nwinyi OC, OnuruSU, Fatoki TH, Omonhinmin CA (2020) Antimicrobial importance of medicinal plants in Nigeria. The Scientific World Journal 2020:7059323.
12. Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL. (2013) In vitro anticancer screening of 24 locally used Nigerian medicinal plants. BMC Complement Altern Med 13:79.
13. Segun PA, Ogbole OO, Ajaiyeoba EO (2018) Medicinal plants used in the management of cancer among the Ijebus of Southwest Nigeria. J Herb Med 14:68-75.
14. Olotunrisola OS, Adetutu A, Balogun EA, Afolayan AJ. (2013) Ethnobotanical survey of medicinal plants used in the treatment of malaria in Ogbomoso, Southwest Nigeria. J Ethnopharmacol 150:71-78.
15. Mugabo P, Raji IA (2013) Effects of aqueous leaf extract of Asystasia gangetica on the blood pressure and heart rate in male spontaneously hypertensive Wistar rats. BMC Complement Altern Med 13:1-7.
16. Fetse JP, Kofie W, Adosrauk RK (2016) Ethnopharmacological importance of Xylopia aethiopica (DUNAL) A. RICH (Annonaceae)-A review. J Pharmaceutical Res Int 11:1.
17. Alabi TD, Cheguo NN, Brooks NL, Oguntimehin OA (2020) Effects of Anchomanes diffirons on inflammation, apoptosis, and organ toxicity in STZ-induced diabetic cardiomyopathy. Biomedicines 8:29.
18. Ajao AA, Mukaila YO, Sabiu S. (2021) Wandering through southwestern Nigeria: An inventory of Yoruba useful angiosperm plants. Helyon 8:e09868.
19. Sinan Ki, Saffič L, Peršurč Z, Pavlčík SK, Etienne OK, et al. (2020) A comparative study of the chemical composition, biological and multivariate analysis of Crotalaria retusa L. stem barks, fruits, and flowers obtained via different extraction protocols. South African Journal of Botany 126:101-108.
20. Adesina SK, Iwalewa EO, Johnny II (2016) Tetrapleura tetraptera Taub-ethnopharmacology, chemistry, medicinal and nutritional values-a review. British Journal of Pharmaceutical Research 12:1-22.
21. Karakas FP, Turker AU, Karakas A, Mstiviladze V, Pichette A, et al. (2017) 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 8:31-39.
22. Adeniran AA, Sonibare MA (2017) In vitro antioxidant activity, brine shrimp lethality and assessment of bioactive constituents of three wild Dioscorea species. Journal of Food Measurement and Characterization 11:685-695.
23. Ahmad S, Hassaan T, Rehman T, Basit A, Tahir A, et al. (2019) In vitro bioactivity of extracts from seeds of Cassia abrus L. growing in Pakistan. J Herb Med 16:100258.
24. Ogbole OO, Segun PA, Adeniji AJ (2017) 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 17:494.
25. Casuga FP, Castillo AL, Corpuz MJ (2016) GC–MS analysis of bioactive compounds present in different extracts of an endemic plant Broussonetia luzonica (Blanco) (Moraceae) leaves. Asian Pacific Journal of Tropical Biomedicine 6:957-961.
26. Oguntimehin SA, Oriola AO, Obuotor EM, Aladeseami AJ (2019) Bioassay guided phytochemistry of aerial part of Laportea aestuans (L.) chew (urticaceae). Niger J Nat Prod Med 23:6-12.
27. Aladeseami AJ, Oriola AO, Oguntimehin SA, Akinkunmi EO, Igbeneghu OA, et al. (2019) Comparative antimicrobial and antioxidant activities of four medicinal plants. Ife Journal of Science 21:59-66.
28. Tewari M, Mishra RR, Shukla HS (2010) Salmonella typhi and gallbladder cancer: report from an endemic region. Hepatobiliary Pancreat Dis Int 9:524-530.
29. Campos JF, Santos UPD, Rocha PDSD, Damiao MJ, Balestieri JBP, et al. (2015) Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless bee Tetragonisca fiebrigii (Jatai). Evid Based Complement Alternat Med 2015:296186.
30. Kofie W, Fetse JP, Adosrauk RK (2019) Antimicrobial activities of novel xylopic acid derivatives. JAMB 14:1-6.
31. Aladesanmi AJ, Famuyiwa FG, Oriola AO, Oguntimehin SA, Aiyedun PO, et al. (2020) Cytotoxic activity of selected Nigerian medicinal plants. Journal of Herbs, Spices & Medicinal Plants 26:203-217.

32. Adaramoye OA, Sarkar J, Singh N, Meena S, Changkija B, et al. (2011) Antiproliferative action of Xylopia aethiopica fruit extract on human cervical cancer cells. Phytother Res 25:1558-1563.

33. Kuete V, Sandjo LP, Mbaveng AT, Zeino M, Efferth T (2015) Cytotoxicity of compounds from Xylopia aethiopica towards multi-factorial drug-resistant cancer cells. Phytomedicine 22:1247-1254.

34. Ozaslan M, Karagoz ID, Lawal RA, Kilic IH, Cakir A, et al. (2016) Cytotoxic and anti-proliferative activities of the Tetrapleura tetraptera fruit extract on ehrlich ascites tumor cells. Int J Pharmacol 12:655-662.

35. Anim MT, Larbie C, Appiah-Opong R, Tuffour I, Owusu KB, et al. (2016) Phytochemical, antioxidant and cytotoxicity of hydroethanolic extracts of Crotalaria retusa L. World J Pharm Res 5990:162-179.

36. Niyonizigiye I, Nkurunziza D, Ngabire D, Gitachew AT, Chun BS, et al. (2020) Characterization and in vitro cytotoxicity of phytochemicals from Aspilia africana obtained using green extraction techniques. South African Journal of Botany 128:231-238.

37. Chapa-Oliver AM, Mejia-Teniente L (2016) Capsaicin: From plants to a cancer-suppressing agent. Molecules 21:931.

38. Archer VE, Jones DW (2002) Capsaicin pepper, cancer and ethnicity. Medical Hypotheses 59:450-457.

39. Li H, Krstin S, Wang S, Wink M (2018) Capsaicin and piperine can overcome multidrug resistance in cancer cells to doxorubicin. Molecules 23:557.