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Research Article

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

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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 South-western 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 identification of the potential source of 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* (L.) T.Anderson	Akpuarachi (I)	FHI 109634	Leaves	Antihelmintic, asthma, astringent, diaphoretic, stomachic [15]
Annonaceae	*Xylopia aethiopica* (Dunal) A.Rich.	Kimba (H), Uda (I), Eruu, girinja (Y), Ethiopian pepper (E)	FHI 108978	Bark Root	Amenorrhea, biliousness, bronchitis, cough, dysentery, fibroid, malaria, rheumatism [16]
Araceae	*Anchomanes difformis* (Blume) Engl.	Igo langbodo, Ogirisako (Y), Oje (I), Chakara (H)	FHI 109638	Leaves Root	Asthma, diabetes, gastrointestinal disturbances, inflammation, microbial infections, pain, ulcerations [17]
	Culcasia scandens P.Beauv.	Oji azu ari nkwu (I),	FHI 110050	Leaves	Cancer, stomachache [18]
Compositae	*Aspilia africana* (Pers.) C.D.Adams	Jamajina (H), Oranjila (I), Yunyun, Ako yunyun (Y)	FHI 107511	Leaves	abortifacients, dysentery, hemorrhoid, hemostatic, nervous disorders, skin diseases, stomach disorders, tuberculosis, ulcers [18]
Leguminosae	*Crotalaria retusa* L.	Koropo, Alatunse, Saworo (Y), Rattle pea (E)	FHI 109052	Roots	cold, fever, flatulence, hemoptysis fever, leprosy, lung disease, skin infections [19]
	Tetrapleura tetraptera (Schum et Thonn) Taub.	Dawo (H), Uyayak (IB), Aridan/Aidan (Y)	FHI 110141	Bark Root	febrile convulsions, infantile flatulence, inflammation, rheumatic aches, stomach gripes [20]
	Pterocarpus osun Craib	Madubiya (H), Osun (Y)	FHI 108415	Leaves Bark	antipyretic, anti-sickling, asthma, blood supplement, candidiasis, eczema, skin infections[18]

Menispermaceae	*Triclisia subcordata* Oliv.	Alugbonron (Y)	FHI 109638	Leaves	Breast cancer [18]
Moraceae	*Treculia africana* Decne. 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), Awogbaarun, Arunyanyan (Y)	FHI 106992	Leaves Roots	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 wewe (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)	FH 107924	Leaves	candidiasis, cancer, diabetes, epistasis, 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 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:

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

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 ug/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 uL 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 uL of 2% (w/v) MTT dye in PBS and incubated at 37 °C for 2 h. DMSO (125 uL) 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;

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

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 IC_{50} and CC_{50} 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)	$4.66 \pm 0.23^{c,e}$
A. differmis (leaf)	0.17 ± 0.01^{d}
A. differmis (root)	$6.32 \pm 0.36^{b,e}$
A. gangetica (leaf)	$7.31 \pm 0.21^{a,b}$
C. frutescens (leaf)	11.64 ± 0.48^{k}
C. retusa (Root)	8.81 ± 0.78^{a}
C. scandens (leaf)	8.31 ± 0.46^{a}
M. lucida (leaf)	14.63 ± 0.56^{g}
N. tabacum (leaf)	58.35 ± 0.85^{h}
O. subscopoides (leaf)	7.98 ± 0.43^{a}
P. alliaceae (leaf)	3.33 ± 0.48^{c}
P. alliaceae (root)	1.66 ± 0.12^{d}
P. osun (Bark)	8.31 ± 0.60^{a}
P. osun (leaf)	56.35 ± 0.74^{l}
T. africana (leaf)	8.81 ± 0.34^{a}
T. subcordata (leaf)	5.15 ± 0.82^{e}
T. tetraptera (bark)	67.99 ± 0.67^{i}
T. tetraptera (root)	28.59 ± 0.60^{f}
X. aethiopica (bark)	28.59 ± 0.45^{f}
X. aethiopica (root)	63.84 ± 0.38^{j}

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. differmis, Morinda lucida*, and *Pterocarpus osun* had IC_{50} of 1.5, 20.0, 3.8, 16.9, and 4.1 against Salmonella typhi. Similarly, *A. differmis* 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_{50} of 0.9 µg mL^{-1}. Root extracts of *X. aethiopica* and leaf extract of *A. differmis* 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_{50} (µg/mL)			
	Salmonella typhi	***Pseudomonas aeroginosa***	***Escherichia coli***	***Candida albicans***
A. africana (leaf)	183.2 ± 7.5***	4.58 ± 0.47***	2.52 ± 0.17***	29.17 ± 0.74***
A. differmis (leaf)	3.8 ± 0.3	4.56 ± 0.21***	0.70 ± 0.05	28.01 ± 1.84***
A. differmis (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. tetraptera (bark)	238.9 ± 35.8***	ND	2.40 ± 0.15***	13.83 ± 3.47**
T. tetraptera (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. tetraptera*, and *N. tabacum* being the most cytotoxic with IC_{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. differmis (leaf)	284.2 ± 6.3***	62.0 ± 0.6***	ND
A. differmis (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. tetraptera (bark)	1.7 ± 0.2	14.9 ± 1.1***	14.0 ± 1.3***
T. tetraptera (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. tetraptera* 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. tetraptera* 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. aeroginosa, E. coli*, and *C. albicans*. Extracts of *X. aethiopica* root and leaf extract of *A. difformis* 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-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* [33].

This study also showed that bark and root extracts of *T. tetraptera* demonstrated cytotoxicity against all tested cancer cell lines. Fadeyi et al. [12] reported the cytotoxicity of extract of T. tetrapleura 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. tetraptera* 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. tetraptera* 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. Niyonizigiye 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. tetraptera* 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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