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Phytochemical and bioactivity screening of six Nigerian medicinal plants

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Abstract
Traditional herbal medicines play an important role in the health maintenance in developing countries, and higher plants continue to be promising sources of new medicines. In this work, we have extracted and screened six Nigerian medicinal plants for biological activity. Chloroform extracts from the aerial parts of *Aristolochia ringens*, the bark of *Chrysophyllum albidum*, the stems of *Costus afer*, the leaves of *Opilia celtidifolia*, the bark of *Terminalia catappa*, and the bark of *Vitellaria paradoxa*, were obtained and screened for phytochemical constituents, antibacterial, antifungal, and cytotoxic activities. *Aristolochia ringens* showed excellent antibacterial activity against *Bacillus cereus* and good antifungal activity against *Candida albicans*. *A. ringens* was also cytotoxic to Hep-G2 and MCF-7 cells. *Chrysophyllum albidum* showed antibacterial activity against *Escherichia coli* and cytotoxic activity to Hep-G2 cells. *Costus afer* showed good cytotoxic activity against Hep-G2 cells and antibacterial activity against *B. cereus*. *Opilia celtidifolia* extract was cytotoxic to Hep-G2 and antibacterial to *E. coli*. Neither *T. catappa* nor *V. paradoxa* extracts showed activity in the bioactivity screens in this study. The bioactivities shown in this study underscore the importance of traditional herbal medicines.

Keywords: Herbal medicines, cytotoxicity, antibacterial, antifungal

Introduction
Medicinal plants constitute one of the most promising sources to obtain biologically active compounds which have been used for treatment of many human and animal diseases in various communities. Hence, natural products derived from herbs have continued to play a very important role in all divisions of human population either directly as folk medicines or indirectly in the preparation of recent drugs used as antioxidant, antibacterial, antiparasitic, antifungal, cytotoxic, anti-*Fusarium* activities for food safety and consumption [1–7].

Aristolochia ringens Vahl, an aromatic member of the Aristolochiaceae is known to originate from Brazil, Central America and the Caribbean [8]. The plant is commonly known by the Yoruba in the south-western part of Nigeria as 'akogun'. It is a rhizomatous aromatic climbing shrub. *Aristolochia* species are known to contain alkaloids and aristolochic acid [9]. Various *Aristolochia* species have been reported in herbal medicines since antiquity in obstetrics and in treatment of snakebite (e.g., *Echis carinatus*, saw-scaled viper) [10], festering wounds, and tumors, and they are still in use majorly in Chinese herbal medicine [11, 12]. *A. ringens* is used to treat cholera, fever, bowel troubles, ulcers, leprosy, and poisonous bites [13, 14]. The leaf, stem, and floral essential oil compositions of *A. ringens* have been previously reported [15]. Sesquiterpenoids were the main components in the leaf essential oil including β-caryophyllene (11.4%), *trans*-4(14),5-muuroladiene (13.0%), bicyclogermacrene (12.8%), spathulenol (8.0%), as well as the diterpenoid methyl copalate (10.3%).

Chrysophyllum albidum G. Don., commonly known as white star apple, is a forest fruit tree and belongs to the Sapotaceae family with about 800 species [16]. It is widely distributed in west and central tropical Africa [17–19]. In Nigeria, it is found along forest savannah transitional and coastal savannah zones. The plant often grows to a height of 25-37 m in height with a mature girth varying from 1.5 to 2 m [20]. The tree has dark green, pale tawny with silver-white when mature, flowers appear on the leaf axils and fruit spherical slightly pointed at the tip with about 3.2 cm in diameter, greenish-grey when unripe, turning orange-red when matured. Locally the plant is named 'agbalumo', (Yoruba) 'udara' (Igbo) according to the specific area. *C. albidum* has been used in traditional herbal medicine to treat malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruptions, stomach ache and diarrhea [21]. Earlier investigators have reported that extracts of the seeds and roots of *C.*

albidium have demonstrated good antimicrobial (e.g., *Escherichia coli*), anti-inflammatory, anti-diarrheal and anti-hemorrhoidal properties [22].

Costus (Ginger lily) is a genus of stout, perennial and rhizomatous herbs of the Costusaceae comprised of 150 species found in tropical belt of West African countries including Cameroon and South Africa [23–25]. *Costus afer* Ker Gawl. is an erect herbaceous plant growing up to 4 m with white and yellow flowers [26]. The succulent stem and root contain several bioactive metabolites as a remedy for headache, fever, rheumatism, cough, sleeping sickness and stomach ache [27]. *C. afer*, locally called bush cane, 'ireke omode' or 'teteregun' (Yoruba) and 'opete' (Igbo), is used in West African traditional medicine for ailments such as laxative, hepatic disorders and miscarriage. The plant has diverse pharmacological properties *viz.* anti-inflammatory, rheumatism, arthritis, helminthic, epileptic attack, hemorrhoids, as diuretics, and also had served as an antidote for food poisoning [28, 29]. Laboratory study of the stem of *C. afer* had shown that the plant possessed potent antioxidant activity [30]. Several groups have investigated the essential oil [29], topical anti-inflammatory activity [30] as well as *in vitro* and *in vivo* pharmacological activities of the methanol leaf extract [28].

Opilia celtidifolia (Guill. & Perr.) Endl. ex Walp. (Opiliaceae) is a spreading liana, heavily-branched shrub or tree up to a height of 10 m, common in savannah or fringing forest. It is prevalent in western Africa from Nigeria to Senegal and disseminated over the arid parts of tropical Africa [24]. Medicinally, the plant decoction is taken as a remedy for wound healing, malaria fever, abdominal pains, internal worms and as an appetite-enhancing agent [31–36]. The plant, locally called 'koyinsorun', is used in West African traditional medicine for treating sleeping sickness and leprosy [24].

Terminalia catappa (tropical almond) are trees or shrubs in the Combretaceae family. The genus is made up of nearly 200 species [9] and is native to tropics and sub tropics regions of Africa, India and Southeast Asia including Thailand and Malaysia. *T. catappa* is a tree, up to 35 m in height with an upright, symmetrical crown and produces fruits 5-10 cm long, broad, green at first, then yellow and finally red when ripe [37] with a flesh surrounding a large fibrous nuts. All parts of the plant had been studied extensively. Ethnobotanically, the aqueous bark extract is used traditionally for treatments of wounds, boils, dysentery and diarrhea [38, 39]. Leaf decoctions of *T. catappa* have been broadly used in local traditional medicine for the treatment of liver ailments, headache, colic, and as a cardiotonic and diuretic [40, 41]. Petroleum ether, methanol, and aqueous extracts of *T. catappa* have also demonstrated antidiabetic activity [42, 43].

Vitellaria paradoxa C.F. Gaertn. [syn. *Butyrospermum paradoxum* (C.F. Gaertn.) Hepper], belonging to the family Sapotaceae, is a small to medium-sized tree, 10-15 m high, much branched, dense, spreading, round to hemispherical crown. The tree grows naturally in the wild of the dry savannah belt of West Africa, from Senegal in the west to Sudan in the east and onto the foothills of the Ethiopian mountains. It is rich in oil and serves as a source of edible oil for many households in the northern part of Nigeria [44, 45]. *V. paradoxa* has many applications in folkloric medicine. It is commonly called 'shea butter' (English) and 'emi-emi' (Yoruba) in Nigeria. Shea butter contains high levels of UV-absorbing triterpene cinnamates, tocopherols (vitamin E), and phytosterols [46]. Badifu confirmed that shea butter contains high anti-oxidant properties and (5-15%) of unsaponifiable lipids, which include phytosterols such as campesterol, stigmasterol, β-sitosterol and α-spinosterol, as well as triterpenes such as cinnamic acid esters of α- and β-amyrin, parkeol, butyrospermol, and lupeol [47]. Its anti-oxidant properties have led to its use to protect the skin from sunburn, eczema and as a skin rejuvenator [47]. Additionally, shea kernels contain about 0.4% catechin polyphenolics such as gallic acid, catechin, epicatechin, epicatechin gallate, gallocatechin, epigallocatechin, epigallocatechin gallate, as well as quercetin and *trans*-cinnamic acid [48]. A phytochemical screen of the methanol bark extract of *V. paradoxa* has revealed the presence of carbohydrates, saponins, steroids, and alkaloids [45].

Materials and Methods

Plant Material

The aerial parts of *A. ringens* and the stem bark of *C. albidum* were procured from a local market at Idi Oro market, Lagos State (6.56° N, 3.3° E); the mature stems of *C. afer* (tropical almond) were procured from a local market at Lagos Island, Lagos State; mature leaves of *O. celtidifolia* and stem bark of *V. paradoxa*. (shea-butter tree) were procured from a local market at Agege, Lagos State; the stem bark of *T. catappa* was collected from the premises of Lagos State University central mosque. All plant materials were identified at the Herbarium unit of the Department of Botany, University of Lagos. The plant materials for each plant were dried in air for five days, pulverized using a grinding machine, and stored in polyethylene bags. For each plant, 750 g of plant material was soaked in 1.5 L chloroform for 24 h. Each crude extract was filtered using Whatman filter paper No 42 (125 mm). The filtrates were concentrated under reduced pressure at 80°C using a rotary evaporator and stored under refrigeration at 4°C. The extract yields of each extract were determined and are represented as percentages based on dry mass of the plant materials. All the solvents, chemicals, and reagents used were of analytical grade and were purchased either from Tunnex Chemicals, Alapere (Lagos) or from Olaolu Chemicals, Igbo Elerin, (Lagos).

Qualitative Phytochemical Screening

Phytochemical screening was performed on the extracts using standard protocols to identify chemical constituents [49–51].

Screening for Alkaloids [52]: The crude extract (0.5 g) was mixed with 5 mL of 1% HCl on a steam bath and filtered while hot. The residue was rinsed with distilled water and 1 mL of the filtrate was treated with a few drops of Wagner's reagent. A positive alkaloid test was revealed by a reddish brown precipitate.

Screening for Flavonoids [53]: The extract (0.2 g) was dissolved in water (2 mL) and filtered. Aqueous sodium hydroxide (2 mL, 10%) was added. The appearance of a yellow color indicated the presence of flavonoids.

Screening for Phenolics [53]: The chloroform extract (0.2 g) was diluted with 50% ethanol and 3 drops of 10% ferric chloride were added. A deep bluish-green solution indicated of the presence of phenols.

Screening for Tannins [54]: A portion of the extract was dissolved in water, after which the solution was clarified by filtration. A 10% ferric chloride solution was added to the filtrate. The appearance of a bluish-black color indicated a positive test for tannins.

Screening for Anthraquinones [55, 56]: Crude extract (0.5 g) was shaken with 10 mL of benzene and filtered. A 10%

aqueous ammonia solution was added to the filtrate and the mixture was shaken. The formation of a pink, red or violet color in the aqueous phase indicates the presence of anthraquinones.

Screening for Cardiac Glycosides [57]: Crude extract (0.5 g) was dissolved in glacial acetic acid (2 mL) containing 1 drop of 10% ferric chloride solution. The solution was under layered with 2 mL of concentrated sulfuric acid. Formation of a brown ring at the phase interface indicated the presence of deoxy sugars, characteristic of cardiac glycosides.

Screening for Phlobatannins [58, 59]: Crude extract (0.2 g) was mixed with 10 mL water. A few drops of 1% HCl were added and the mixture boiled. Formation of a red precipitate indicated the presence of phlobatannins.

Screening for Terpenoids [60]: Acetic anhydride (0.5 mL) was mixed with 0.2 g extract dissolved in 50% ethanol. A few drops of concentrated sulfuric acid were added. Formation of a bluish green precipitate indicated the presence of terpenoids.

Screening for Steroids [60, 61]: Crude extract (0.2 g) was dissolved in 2 mL of glacial acetic acid and the solution heated, allowed to cool, and filtered. The residue was dissolved in 0.5 mL of acetic anhydride followed by the addition of few drops of concentrated sulfuric acid. A color change from violet to blue or bluish-green was a positive indication for the presence of steroids.

Quantitative Phytochemical Screening

Determination of Total Alkaloids [51]: The crude extract (5 g) was weighed into a 250-mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated aqueous ammonia was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The solid alkaloid residue was dried and weighed.

Determination of Total Phenolics, Phenanthroline Method [62]: The chloroform extract (500 mg) was dissolved in methanol, 1 mL of 0.2% $FeCl_3$ solution in methanol, and 0.5 mL of 0.5% 1,10-phenanthroline solution in methanol were placed into a 10-mL volumetric flask and diluted to a final volume of 10 mL with methanol. The solution was mixed and left at room temperature in the dark. After 20 min, the absorbance of an orange-red solution was measured at 510 nm against a reagent blank (1 mL of 0.2% $FeCl_3$ and 0.5 mL of 0.5% 1,10-phenanthroline, diluted to 10 mL). The results are expressed as mg of gallic acid equivalents per 100 g plant extract.

Determination of Total Phenolics, Folin-Ciocalteu Method [63]: The total phenolic content (TPC) of the chloroform extracts was determined using the Folin-Ciocalteu method. Each extract (100 mg) was dissolved in 2 mL acetone to which was then added 2 mL of 50% Folin-Ciocalteu reagent [64], followed by addition of 5 mL of 20% Na_2CO_3 solution. The mixture was shaken vigorously and diluted to 10 mL. After 30 min the absorbance was measured at 730 nm. The total phenolic content is expressed as mg of gallic acid equivalents per 100 g plant extract.

Determination of Total Flavonoids [65]: Crude extract (10 g) was exhaustively extracted with 100 mL of 80% aqueous methanol at room temperature. The solution was filtered and the filtrate transferred into a crucible and evaporated to dryness over a water bath. Total flavonoid content is expressed as the mass of the residue after drying.

Antimicrobial Screening

The chloroform extracts were screened for antibacterial activity against Gram-positive bacteria, *Bacillus cereus, Staphylococcus aureus,* and *Staphylococcus epidermidis*, and Gram-negative bacteria, *Escherichia coli, Pseudomonas aeruginosa,* and *Serratia marcescens*. Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique. Dilutions of the chloroform extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of each crude extract in DMSO plus 50 µL CAMHB. The extracts were then serially diluted (1:1) in CAMHB in 96-well plates. Microorganisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 h; the lowest concentration without turbidity was defined as the final minimum inhibitory concentration (MIC). DMSO was used as a negative control and Gentamicin was used as a positive antibiotic control. Antifungal activity was determined as described above using *Candida albicans* in yeast-mold (YM) broth with approximately 7.5 x 10^7 CFU/mL. Antifungal activity against *Aspergillus niger* and *Botrytis cinerea* were determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture and grey mold horticulture, respectively, diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

Cytotoxicity Screening

Human hepatocarcinoma cell line (Hep-G2, CRL-11997™) cells were grown in complete medium (DMEM: F12 containing L-glutamine and sodium bicarbonate, 10% FBS, and 1% penicillin/streptomycin) incubated at 37°C in a 5% CO_2 environment. Once 80 - 90% confluent, the cells were washed with phosphate buffered saline (PBS), treated with 0.25% (w/v) of Trypsin/EDTA, counted and suspended in fresh complete media. About 5×10^5 cells/well (100 µL) were seeded into 96 well plates and incubated for 24 hours to attach. Cells were then treated with the extracts at a final concentration of 10 and 50 µg/mL in 200 µL medium for 72 hours. Thereafter, the medium was removed, and DMEM: F12 medium containing MTT (5 mg/mL in PBS) was added to the cells and incubated for 1 h. The MTT-containing medium was then carefully removed and replaced with DMSO (200 µL per well), the plate was then gently mixed to dissolve the formazan crystals. Absorbance was measured at 550 nm. All extracts were tested in triplicate.

MCF-7 (human breast tumor, ATCC No. HTB-22) cells were grown in RPMI 1640 supplemented with 10% Fetal bovine serum (FBS), 30 mM HEPES, sodium bicarbonate, and 100,000 units penicillin/streptomycin (10 mg/L) at pH=7.35. MCF-7 cells were plated into 96-well cell culture plates at a concentration of 1.2×10^4 cells/well and a volume of 100 µL in each well and incubated at 37°C and 5% CO_2 for 48 hours. After 48 hours, the cells reached 70-80% confluent growth. The supernatant fluid was carefully aspirated and replaced with 100 µL growth medium containing 1 µL of extract (1% in DMSO), giving a final concentration of 100 µg/mL (100 ppm). Assays were repeated at 10 µg/mL (10 ppm). The plate was then incubated at 37°C and 5% CO_2 for 48 hours, after which the supernatant liquid was gently aspirated from each well. Into each well, 100 µL of MTT solution was added and the pre-read absorbance was immediately measured spectrophotometrically at 570 nm (using a Molecular Devices

SpectraMax Plus 384 microplate reader). The plate was incubated at 37°C and 5% CO_2 for 4 h, after which the supernatant liquid was removed and DMSO (100 µL) was used to dissolve the purple formazan crystals. The amount of formazan produced was determined spectrophotometrically at 570 nm. DMSO, and tingenone (100 µg/mL) served as negative and positive controls, respectively. Solutions were added to wells in eight replicates. Average absorbances, standard deviations, and percent kill ratios (% $kill_{compound}$ / % $kill_{control}$) were calculated.

Median inhibitory concentrations (IC_{50}) were determined using the Reed-Muench method [66].

Results and Discussion

Extraction

The chloroform extraction yields and descriptions of the six Nigerian medicinal plants are summarized in Table 1. Bark extractions with chloroform gave larger yields than leaves or herbaceous plant parts, which suggests larger concentrations of relatively non-polar extractables in the barks.

Table 1: Nature and yield of crude chloroform extracts from six Nigerian medicinal plants.

Plant Extract	Extract color	Extract texture	Extract yield
Aristolochia ringens aerial parts	Dark brown	Sticky semi-solid powder	84.00 g (7.0%)
Chrysophyllum albidum bark	Dark green	Semi-sticky powder	120.00 g (10.0%)
Costus afer stems	Brown	Solid powder	75.32 g (5.94%)
Opilia celtidifolia leaves	Brown	Solid powder	70.00 g (5.84%)
Terminalia catappa bark	Dark brown	Semi-solid powder	153.55 g (14.00%)
Vitellaria paradoxa bark	Dark brown	Semi-solid powder	150.00 g (13.0%)

Phytochemical Screening

Qualitative phytochemical screening was carried out on the crude chloroform extracts, and the results are summarized in Table 2. Quantitative assessment of phytochemical constituents is summarized in Table 3. Not surprisingly, polar phytochemicals such as phenolics and tannins were found in only two crude chloroform extracts. Conversely, alkaloids were found in five of the six extracts, while flavonoids and terpenoids were found in four extracts.

Antimicrobial Screening

Each of the crude chloroform extracts was screened for antimicrobial activity against a panel of Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus,* and *Staphylococcus epidermidis*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Serratia marcescens*), and fungi (*Aspergillus niger*, *Botrytis cinerea*, and *Candida albicans*). Antimicrobial activities as minimum inhibitory concentrations are summarized in Table 4. The most susceptible organisms in this study were *B. cereus* and *E. coli* with two extracts showing MIC < 100 µg/mL and one extract with MIC = 156 µg/mL. *Staphylococcus epidermidis* and *Serratia marcescens* were particularly insensitive to the plant extracts. Likewise, neither *A. niger* nor *B. cinerea* were susceptible to the Nigerian extracts. *Candida albicans*, however, was somewhat sensitive to *A. ringens* extract (MIC = 156 µg/mL).

Table 2: Qualitative analysis of phytochemical constituents of six Nigerian medicinal plants.

Plant Extract	Phenolics	Tannins	Phlobatannins	Alkaloids	Flavonoids	Cardiac glycosides	Steroids	Terpenoids	Anthraquinones
Aristolochia ringens aerial parts	-	-	-	+++	++	-	+	-	+
Chrysophyllum albidum bark	-	-	-	+++	++	++	+	-	-
Costus afer stems	++	++	++	+++	++	+	-	+	-
Opilia celtidifolia leaves	-	-	+	+++	-	-	-	+	+
Terminalia catappa bark	+	++	+	+++	++	+	-	+	+
Vitellaria paradoxa bark	-	-	-	+++	-	-	-	+	-

+++ = Test strongly positive; ++ = Positive; + = Weakly positive; - = Negative

Table 3: Quantitative analysis of phytochemical constituents of six Nigerian medicinal plants.

Plant Extract	Phenolics (mg/100 g) 1,10-phenanthroline method	Phenolics (mg/100 g) Folin-Ciocalteu method	Alkaloids (mg/100 g)	Flavonoids (mg/100 g)	Cardiac glycosides (mg/100 g)
Aristolochia ringens aerial parts	-	-	45.82 ± 0.70	13.53 ± 0.30	-
Chrysophyllum albidum bark	-	-	52.50 ± 0.50	26.60 ± 0.80	27.10 ± 0.80
Costus afer stems	10.35 ± 0.50	12.00 ± 0.50	44.35 ± 0.50	16.25 ± 0.5	10.35 ± 0.33
Opilia celtidifolia leaves	-	-	43.30 ± 0.4	-	-
Terminalia catappa bark	10.55 ± 0.30	9.35 ± 0.40	45.00 ± 0.4	10.35 ± 0.70	9.35 ± 0.50
Vitellaria paradoxa bark	-	-	44.00 ± 03	-	-

Table 4: Antimicrobial activities (MIC, µg/mL) of six Nigerian medicinal plant extracts.

Plant Extract	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	B. cereus	***S. aureus***	***S. epidermidis***	***E. coli***	***P. aeruginosa***	***S. marcescens***	***A. niger***	***B. cinerea***	***C. albicans***
Aristolochia ringens aerial parts	< 19.5	> 2500	> 2500	> 2500	625	1250	625	625	156
Chrysophyllum albidum bark	313	2500	> 2500	< 19.5	313	> 2500	625	625	625
Costus afer stems	78	313	> 2500	156	313	> 2500	1250	625	> 2500
Opilia celtidifolia leaves	156	625	> 2500	78	156	> 2500	1250	625	> 2500
Terminalia catappa bark	> 2500	1250	> 2500	2500	313	> 2500	> 2500	625	> 2500
Vitellaria paradoxa bark	> 2500	1250	> 2500	2500	313	> 2500	> 2500	625	> 2500

Cytotoxicity Screening

The crude chloroform extracts were screened for *in-vitro* cytotoxic activity against Hep-G2 human hepatocellular carcinoma and MCF-7 human breast adenocarcinoma cells. The cytotoxic activities are summarized in Table 5.

Table 5: Cytotoxicity (IC_{50}, µg/mL) of six Nigerian medicinal plant extracts.

Plant Extract	Hep-G2	MCF-7
Aristolochia ringens aerial parts	16.4 ± 1.8	81.6 ± 12.8
Chrysophyllum albidum bark	23.1 ± 4.8	> 100
Costus afer stems	17.6 ± 1.3	> 100
Opilia celtidifolia leaves	24.7 ± 6.8	> 100
Terminalia catappa bark	> 50	> 100
Vitellaria paradoxa bark	> 50	129 ± 14

Aristolochia ringens

Phytochemical screening of *A. ringens* chloroform extract revealed the presence of alkaloids, flavonoids, steroids, and anthraquinones. This extract showed excellent antibacterial activity against *B. cereus* (MIC < 19.5 µg/mL) and antifungal activity against *C. albicans* (MIC = 156 µg/mL). *A. ringens* extract was also cytotoxic to both Hep-G2 and MCF-7 cell lines (IC_{50} = 16.4 and 81.6 µg/mL, respectively). Previous studies on *A. ringens* have shown that the root extracts of *A. ringens* have exhibited insecticidal activity [67] as well as broad spectrum cytotoxic activity [68], while the methanol extract of the plant showed *in-vivo* antitrypanosomal activity [69]. The volatile components from the stems and leaves of *A. ringens* have been identified [15]. The stems were dominated by the monoterpenoids limonene (20.0%), *p*-cymene (17.8%), α-phellandrene (16.1%), and linalool (6.5%), while the leaves were rich in sesquiterpenoids β-caryophyllene (11.4%), *trans*-4(14), 5-muuroladiene (13.0%), and spathulenol (8.0%), and the diterpenoid methyl copalate (10.3%). The diterpenoid *ent*-labd-8(17)-en-15-oic acid has also been identified in *A. ringens* [70].

Chrysophyllum albidum

The crude chloroform bark extract of *C. albidum* showed selective *in-vitro* cytotoxicity to Hep-G2 cells (IC_{50} = 23.1 µg/mL). In addition, the extract showed excellent antibacterial activity against *E. coli* (MIC < 19.5 µg/mL). In a previous study, the ethanol leaf extract of *C. albidum* showed antibacterial activity against Gram-positive *S. aureus* and *Streptococcus pyogenes*, but MIC values were not determined [71]. In addition, the methanol bark extract of *C. albidum* has shown *in-vivo* antiplasmodial activity in mice, but was relatively non-toxic to the mice (LD_{50} = 1850 mg/kg) [72]. Phytochemical screening of the chloroform bark extract of *C. albidum* showed positive results for alkaloids, flavonoids, cardiac glycosides, and steroids (Tables 2 and 3). Previous phytochemical analysis of the stem bark of *C. albidum* showed large quantities of alkaloids, flavonoids, cardiac glycosides, and saponins [22, 73], complementing the results of this current study. Leaf extracts of *C. albidum* have also tested positive for alkaloids, flavonoids, cardiac glycosides, and steroids [74].

Costus afer

The chloroform extract of the stems of *C. afer* tested positive for phenolics, tannins, phlobatannins, alkaloids, flavonoids, cardiac glycosides, and terpenoids. The phytochemical screening results corroborate previous studies of the stem extract that indicated alkaloids, saponins, triterpenoids and steroids, flavonoids, phlobatannins, and tannins [25, 28, 75]. The flavonoid glycoside kaempferol 3-*O*-α-L-rhamnopyranoside has been isolated from the aerial parts of *C. afer* [76]. Volatile constituents of the stem have included fatty acids and fatty acid derivatives (palmitic acid, (*Z*)-vaccenic acid, oleic acid, stearic acid) as well as phytosterols (campesterol and stigmasterol) [75, 77]. The chloroform stem extract in this study showed antibacterial activity against *B. cereus* (MIC = 78 µg/mL) and *E. coli* (MIC = 156 µg/mL), but no antifungal activity. In addition, *C. afer* extract showed good, selective cytotoxicity against Hep-G2 cells. The juice [78] and the leaf extract [79] of *C. afer* have shown moderate antiparasitic activity against *Entamoeba histolytica*.

Opilia celtidifolia

The chloroform leaf extract of *O. celtidifolia* showed selective *in-vitro* cytotoxicity to Hep-G2 cells (IC_{50} = 24.7 µg/mL) and moderate antibacterial activity to *B. cereus*, *E. coli*, and *P. aeruginosa* (MIC = 156, 78, and 156 µg/mL, respectively). *O. celtidifolia* methanol extract had previously shown cytotoxic activity to HeLa and A431 cells [80]. *Opilia celtidifolia* is used traditionally to treat malaria and the dichloromethane leaf extract has shown *in-vitro* antiplasmodial and antitrypanosomal activity [81]. Phytochemical screening of the extract indicated the presence of alkaloids, phlobitannins, terpenoids, and anthraquinones. Leaf extracts of *O. celtidifolia* have revealed triterpenoid saponins [82, 83].

Terminalia catappa

The crude chloroform bark extract of *T. catappa* showed positive phytochemical tests for polyphenolics, including tannins, phlobatannins, and flavonoids, as well as terpenoids and anthraquinones. The extract was, however, inactive in the bioactivity screens in this present study. Previous work with *T. catappa* bark extracts have shown the ethanol bark extract to be weakly antibacterial [84] while the hexane bark extract showed antifungal activity [85]. Minimum inhibitory concentrations were not determined in either of these previous studies, however. Sterols, triterpenoids, and saponins have been isolated and identified in the bark extracts of *T. catappa* [86, 87].

Vitellaria paradoxa

Vitellaria paradoxa chloroform bark extract tested positive for only alkaloids and terpenoids. Likewise, the bark extract was neither antimicrobial nor cytotoxic in our bioassays. In contrast, the methanol bark extract has shown *in-vitro*

cytotoxic activity against several tumor cell lines with IC_{50} ranging from 24 to 67 µg/mL [88]. Likewise, the ethanol bark extracts of *V. paradoxa* have shown marginal activity against several bacterial [89] and fungal [90] strains (MIC ≥ 50 mg/mL). The dichloromethane bark extract of *V. paradoxa* showed *in-vitro* antiplasmodial activity [91]. Sterols, triterpenoids, and flavonoids have been isolated and characterized from *V. paradoxa* bark [92].

Conclusions

The results of this investigation suggest that the use of some of these plants in traditional herbal medicines can be scientifically justified. Traditional herbal medicines play a major role in the health care in many developing nations and such herbal medicines may provide new chemotherapeutic agents for discovery and development as conventional single-entity drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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