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Phytochemical screening and antimicrobial activity of some medicinal trees grown in Bauchi state, north eastern, Nigeria

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Abstract
Traditional herbal medicines plays an important role in the health maintenance in most developing countries, and higher plants continue to be promising sources of new medicines. In this work, we have extracted and screened some medicinal tree 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 antimicrobial activity screens in this study.

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].

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. 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 sicknessand 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 CF. Gaertn. [Syn. *Butyrospermum paradoxum* (CF. 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.

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 drop wise to the extract until the precipitation was complete. The whole msolution 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% FeCl3 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% FeCl3 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% Na2CO3 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 micro broth 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 × 108 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 finalminimum 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 107 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% CO2 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 □ 105 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 × 104 cells/well and a volume of 100 µL in each well and incubated at 37 °C and 5% CO2 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% CO2 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 Spectra Max plus 384 micro plate reader). The plate was incubated at 37 °C and 5% CO2 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 (% killcompound/ % killcontrol) were calculated.

Median inhibitory concentrations (IC50) were determined using the Reed-Muench method [66].

Results and Discussion

Extraction

The chloroform extraction yields and descriptions of the Medicinal tree plants in Bauchi State 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 medicinal trees plants.

Plat Extract	Extract color	Extract texture	Extract yield
Aristolochia rengens aerial parts	Dark brown	Semi-solid	75.00g
Chryssophyllum albidum bark	Dark green	Semi-sticky powder	110.00g
Opilia celtidifolia leaves	Brown	Solid powder	67.00g
Taminalia catappa bark	Brown	Solid powder	65.00g
Vitellaria paradoxa bark	Dark brown	Semi-solid	64.00g

Plant extract extract color extract texture extract yield

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 medicinal tree plants.

Plant Extracts	Phenolics	Tannins	Phlobatannins	Alkaloids	Flavanoids	Cardiac glycosides	Steroids	Terpenoids	Antraquinones
Aristolochia rengens aerial parts	-	-	-	+++	++	-	+	-	+
Chryssophyllum albidum bark	-	-	-	+++	++	-	+	-	+
Opilia celtidifolia leaves	++	++	++	+++	++	-	-	-	+
Taminalia catappa bark	+	++	+	+++	+	-	+	+	-
Vitellaria paradoxa bark	-	-	+	+++	-	-	+	-	-

Chrysophyllum albidum

The crude chloroform bark extract of *C. albidum* showed selective *in-vitro* cytotoxicity to Hep-G2 cells (IC50=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 (LD50=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 (IC50=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 IC50 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 *invitro* 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 Tree species 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 chem otherapeuticagents for discovery and development as conventional singleentity drugs.

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