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Identification and bioactivity of Gmelina species (*Gmelina arborea*) commonly grown in some selected communities within Toro L.G.A of Bauchi State, Nigeria

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Abstract
Gmelina Species (*Gmelina arborea*) is described as fast-growing deciduous tree, occurring naturally throughout greater part of India at altitudes up to 1,500 meters. It also occur naturally in Nigeria, Sierra Leone, Malaysia, Cambodia etc. It belongs to the family Lamiaceae. The wood of *Gmelina arborea* is pale yellow to cream coloured or pinkish-buff when fresh. The tree is commonly planted as a garden and an avenue tree; growing in villages along agricultural land and on village community lands and water lands. It also has some medicinal uses. The tree provides medicine which used in the treatment of different ailments, both the bark and the roots. It also plays an important role in the health maintenance in developing countries, and higher plants continue to be promising sources of new medicines. The leaf is taken as a demulcent to treat gonorrhea, and cough and it is applied to wounds and ulcers (Bacaloni *et at.*, 2010).

Keywords: *Gmelina arborea*, deciduous tree, bioactivity

Introduction
Medicinal plants constitute one of the major 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 *Gmelina arborea* Vahl, an aromatic member of the Aristolochiaceae is known to originate from Brazil, Central America and the Caribbean. The plant is commonly known by the Yoruba in the south-western part of Nigeria as 'akogun*'*. It is a rhizomatous aromatic climbing shrub. *Lamiaceae* species are known to contain alkaloids and aristolochic acid 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) festering wounds, and tumors, and they are still in use majorly in Chinese herbal medicine *Gmelina arborea* Roxb is used to treat cholera, fever, bowel troubles, ulcers, leprosy, and poisonous bites. The leaf, stem, and floral essential oil compositions of *Gmelina arborea* have been previously reported.
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. It is widely distributed.

Materials and Methods
Plant Materials
The aerial parts of *Gmelina arborea and* the stem bark of *Gmelina* Roxb. were procured from a local market, in Toro community of Bauchi State; the mature stems of *Gmelina candahar* were procured from different locations within Toro L.G.A. of Bauchi State, mature leaves of *Gmelina philippensis* and stem bark of were procured from a local market at Magama Toro of Bauchi state. All plant materials were identified at the Herbarium unit of the Department of Forestry Technology, College of Agriculture, Bauchi. 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.

Determination of total alkaloids
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 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
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

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, 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

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, Staphylococcus *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 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 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: F12containing 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 todissolve 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 Spectra Max plus 384 microplate 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 tinge none (100 µg/mL) served as negative and positive controls, respectively. Solutions were added to wells in eight replicates. Average absorbance's, standard deviations, and percent kill ratios (% killcompound / % killcontrol) were calculated. Median inhibitory concentrations (IC50) were determined using the Reed-Muench method.

Results and Discussion

Extraction

The chloroform extraction yields and descriptions of the Gmelina species in Toro L.G.A of 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 different Gmelina trees plants.

Plat Extract	Extract color	Extract texture	Extract yield
Gmelina arborea Roxb.	Dark green	Semi-solid	66.00g
Gmelina arborea White Teak *bark*	Pale	Semi-sticky powder	78.00g
Gmelina arborea Candahar leaves	Yellow	Solid powder	57.00g
Gmelina arborea philippensis bark	Pale	Solid powder	69.00g
Gmelina arborea bark	Dark green	Semi-solid	84.00g

Plant Extract color, Extract texture, Extract yield

Gmelina arborea aerial parts Dark brown Sticky semi-solid powder 78.00 g (7.0%), *Gmelina arborea* philippensis bark Dark green Semi-sticky powder 84.00 g (10.0%) *Gmelina arborea* Roxb Brown Solid powder 75.32 g (5.94%) *Gmelina arborea leaves* Brown Solid powder 78.00 g (5.84%)

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
Gmelina arborea aerial parts	-	-	-	+++	++	-	+	-	+
Gmelina roxb bark	-	-	-	+++	++	-	+	-	+
Gmelina philippensis leaves	++	++	++	+++	++	-	-	-	+
Gmelina candahar bark	+	++	+	+++	+	-	+	+	-
Gmelina white T. bark	-	-	+	+++	-	-	+	-	-

Gmelina philippensis

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 *G. philippensis* bark extracts have shown the ethanol bark extract to be weakly antibacterial, while the hexane bark extract showed antifungal activity. 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 *G. philippensis.*

Gmelina roxb.

Gmelina roxb. 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 *Gmelina roxb* have shown marginal activity against several bacterial [89] and fungal [90] strains (MIC ≥ 50 mg/mL).

The dichloromethane bark extract of *Gmelina roxb* showed *in vitro* antiplasmodial activity. Sterols, triterpenoids, and flavonoids have been isolated.

Conclusions

The results of this research suggest that the use of various species of Gmelina in traditional herbal medicines can be scientifically justified. Traditional herbal medicines play a vital significant 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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