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Phytochemical and antimicrobial screening of constituents of some medicinal plants

***Salihu L. and Ado K.**

Department of Chemistry, Nigerian Defense Academy Kaduna, Nigeria

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ABSTRACT

Various parts of plants, namely, Daniella oliveri, Hymenocardia acida, Taminalia mollis, Cussonia arborea, Mangifera indica, Schwenkia americana and Carissa edulis, mostly used for herbal medicine in Northern Nigeria were screened for secondary metabolites and antimicrobial activities. Phytochemical investigations of their extracts revealed the presence of alkaloids, flavonoids, steroids, glycosides, cardiac glycosides, anthraquinones, tannins and saponins. Cytotoxicity tests carried out on the extracts indicate high BST (LC_{50} 3 - 196 µg/cm^3) and AGT (LC_{50} 27-269 µg/cm^3) activities. The extracts showed low to moderate activities against the bacteria Escherichia coli, Staphylococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, and the fungi Candida albicans, Aspergillus flavus and Aspergillus niger.

Keywords: Medicinal plants, cytotoxicity, phytochemical, antimicrobial

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INTRODUCTION

Medicinal plant is any plant in which one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs [10]. It has now been established that any plant which naturally synthesizes and accumulates some secondary metabolites such as alkaloids, glycosides, tannins, volatile oils, phenols and contains minerals and vitamins possesses medicinal properties [21]. All plants produce chemical compounds as part of their normal metabolic activities. The compounds include primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites found in a smaller range of plants. Some useful ones are found only in a particular genus or species [4]. The useful antimicrobial phytochemicals include phenolics and polyphenols, quinones, flavonoids and flavones, tannins, coumarins, alkaloids, terpenoids and essential oils.

The use of plant and animal parts for medicinal purposes has long been in existence and has been widely documented [18]. These ancient indigenous practices were discovered by a series of “trial and error” which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies [3]. In recent times herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations [5].

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular for 80% of world population in Asia, Latin America and Africa [1]. In recent years pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce cost effective remedies that are affordable to the population [17,11]. Similarly, there has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of

antimicrobial chemical additives [2,19] The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

The chemical constituents and antimicrobial activities of seven plants locally used as traditional remedies were investigated to establish their efficacies. Their antimicrobial activities were determined by screening their extracts against certain species of bacteria and fungi.

MATERIALS AND METHODS

Sample collection

The entire plant parts of *Schwenkia americana,* rhizome of *Aristolochia albida*, root bark of *Taminalia mollis, Hymenocardia acida* and *Carissa edulis,* stem bark of *Daniella oliveri*, *Cussonia arborea* and *Mangifera indica* were collected from Rigachikun, Giwa Local Government Area, Kaduna State, Nigeria. The plants were identified and authenticated by Mr U.S Gallah of Herbarium Unit in the Department of Biology, Ahmadu Bello University Zaria. The plant materials were air-dried, pulverized and stored in clean polythene bags at ambient temperature. Clinical isolates of *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa*, were obtained from the Microbiology Section, Ahmadu Bello University Teaching Hospital, Zaria. Fungal isolates of C*andida albicans, Aspergillus flavus and Aspergillus niger* were obtained from the Department of Biology, Nigerian Defence Academy, Kaduna.

Extraction

A portion (50g) each of the respective parts of *D. oliveri, H. acida, C. edulis, S. americana, M. indica, T. mollis and C. arborea* was separately percolated in 200 cm^3 each of methanol, ethyl acetate and n-hexane for two weeks. Each extract was filtered and evaporated to dryness at 40°C using rotary evaporator. Each residue was then allowed to cool, weighed and stored in refrigerator until needed.

Phytochemical screening

The extracts were screened for the presence of alkaloids, flavonoids, steroids, glycosides, cardiac glycosides, anthraquinones, tannins and saponins.

Alkaloids

A quantity (1cm^3) of 1% aqeous HCl was added to 3cm^3 of each extract in a test-tube and the mixture heated for 20 min, cooled and filtered. 1cm^3 portion of the filtrate was treated with two drops of Wagner's reagent. Formation of cream or brown precipitate respectively indicated the presence of alkaloids [22].

Flavonoids

A portion (1g) of the extract was added to 1cm^3 of 10% NaOH. Formation of a yellow coloration indicated the presence of flavonoids [22].

Glycosides

A portion (0.5g) of the extract was dissolved in 2.5M H_2SO_4 (2.5cm^3), boiled, allowed to cool and neutralized with 20% KOH. 5cm^3 of Fehling's solutions A and B (1:1) was added to the neutralized mixture and then boiled. Formation of brick-red precipitate indicated the presence of glycosides [22].

Cardiac glycosides

To a portion (0.5g) of each extract in a test-tube, 2cm^3 of chloroform and 1cm^3 of concentrated H_2SO_4 were added to form a lower layer. Formation of a reddish-brown ring at the interface indicated the presence of aglycone portion of cardiac glycosides [22].

Steroids

A portion (1g) of each extract was dissolved in 1cm^3 of ethanol. Then 1cm^3 of concentrated H_2SO_4 was added to the solution. Formation of a red coloration indicated the presence of steroids [24].

Saponins

A portion (1g) of each extract was added to 5cm^3 of distilled water and vigorously shaken for 2 min. Formation of froth indicated the presence of saponins [22].

Tannins
A quantity (10cm^3) of distilled water was added to 2g of each extract, stirred and filtered. 1cm^3 of ferric chloride was then added to the filtrate. Formation of a blue-green precipitate indicated the presence of tannins [24].

Antibacterial assay
The antibacterial activities of the plant extracts were determined using the methods described by [15]. Solutions of varying concentrations, 7 x 10^2, 6 x 10^2, 5 x 10^2 and 4 x 10^2 µg/cm^3 were prepared for each extract using the respective solvents of extraction. Filter paper was carefully labeled and cut into small sizes (0.5 cm) and separately introduced into a beaker containing the dilute extract solution. They were dried at 50°C. A control was similarly set up using distilled water and respective solvents of extraction.

Molar Hilton agar was used as the growth medium for the microbes. Each medium was prepared by dissolving 38g of the agar in 1000cm^3 of distilled water, heated to dissolve, autoclaved at 121^{o}C for 15 min, cooled and transferred into sterile petri dishes to solidify. Isolates of *S. aureus, S. pneumoniae, E. coli and P. aeruginosa* were separately cultured on each plate and the sterile paper discs were incubated at 37°C for 24hr. The zones of inhibition were measured with the aid of plastic ruler.

Brine shrimp lethality test (BST)
Fractions obtained were evaluated for lethality to brine shrimp using standard methods [14,13]. In this test a drop of DMSO was added to vials of the test and control substances to enhance the solubility of test materials.

Aphyosemion gardneri test (AGT)
A portion (0.5g) of each extract was dissolved in 5cm^3 of solvent of extraction to give a stock solution of 100,000 µg/cm^3. A serial dilution was made by taking 0.5, 0.05 and 0.005 cm^3 of the solution and diluting to 50 cm^3 to give concentrations of 1000, 100 and 10 µg/cm^3 respectively. Ten (5 day old) *Aphyosemion gardneri* fingerlings were introduced into each beaker containing the test solution and left for 24 h. Each beaker was examined and the number of surviving *A. gardneri* fingerlings determined and recorded. A control consisting of 10 *A. gardneri*, 2 drops of DMSO and 50 cm^3 of water were similarly set up. The procedure was repeated in triplicate and the concentration that kills 50% of the test organisms (LC_{50}) was computed using Finney probit analysis programme [6].

Antifungal assay
The antifungal activities of the extracts were determined by using the method described by [15]. Potato dextrose agar was used as a growth medium. Isolates of *C. albicans, A. niger and A. flavus* were used as test organisms.

RESULTS AND DISCUSSION

Results of the phytochemical screening of the extracts indicate that the methanol and ethyl acetate extracts of the plants were richer in phytocompounds than the n-hexane fractions (see Table 1). For instance, alkaloids were detected in both the methanol and ethyl acetate fractions of *D. oliveri, H. acida, C. arborea, T. mollis* and *M. indica*. Alkaloids have been known to have antiviral and antitumor activities [20]. Flavonoids which have been found to have broad spectrum activities [7-9] were detected in the methanol and ethyl acetate fractions of *C. edulis, S. americana* and *C. arborea*. They were also detected in ethyl acetate and n-hexane fractions of *T. mollis* and *M. indica*. Glycosides and cardiac glycosides were detected in methanol extracts of *D. oliveri, H. acida* and *C. edulis*. Glycosides were also detected in the ethyl acetate fraction of *M. indica*. Glycosides and cardiac glycosides have been reported to have antimicrobial properties [20]. Anthraquinones were detected in both methanol and ethyl acetate extracts of *H. acida*. Anthroquinones have been reported to have great antimicrobial properties, provide a source of stable free-radicals [12] and complex irreversibly with nucleophilic amino acids in protein, leading to inactivation of the protein and loss of function. They also render substrate unavailable to the microorganisms [12]. Tannins were detected in methanol and ethyl acetate extracts of *T.mollis, M. indica, S. americana* and *C. arborea*, methanol extracts of *D. oliveri* and *H. acida* and ethyl acetate extract of *C. edulis*. Alkaloids and tannins have been reported to be effective against diarrhoea as well as intestinal infections associated with AIDS [12,23]. Saponins were detected in all the extracts of *S. americana*, n-hexane extracts of *D. oliveri, T. mollis, M. indica* and *C. arborea* and methanol extract of *H. acida*. Saponins are effective in the treatment of syphilis and certain skin diseases [16,24].

Results of the cytotoxicity tests indicate that the methanol extracts of all the plants tested were very active (BST LC_{50} 62 – 196 µg/cm^3 and AGT LC_{50} 28 - 269 µg/cm^3) (see Table 2). Similarly, with the exception of *C. edulis* and *T. molis* all the extracts of ethyl acetate tested were very active (BST LC_{50} 52 – 178 µg/cm^3 and AGT LC_{50} 66 - 171 µg/cm^3). Extracts of n-hexane tested were inactive except that of *S. americana* which was moderately active (BST LC_{50} 455 µg/cm^3).

Table 1: Results of phytochemcial screening of extracts

Plant	Parts	Solvent of Extraction	Alkaloids	Flavonoids	Steroids	Glycosides	Cardiac Glycosides	Tannins	Saponins	Anthra-quinones
D. oliveri	Stem-bark	Methanol	+	-	+++	+++	+++	++	-	-
		Ethyl acetate	+	-	+++	-	+++	-	-	-
		n-hexane	-	-	-	-	-	-	+	-
H. acida	Root	Methanol	++	-	+++	+	+++	++	+	+
		Ethyl acetate	+	-	+++	-	++	-	-	+
		n-hexane	-	-	-	-	-	-	-	-
T. mollis	Root	Methanol	+++	-	+++	-	-	+++	-	-
		Ethyl acetate	-	+++	-	-	++	+++	-	-
		n-hexane	-	-	-	-	-	-	++	-
C. edulis	Root	Methanol	-	+++	+	+++	+	-	-	-
		Ethyl acetate	-	++	+++	-	+	+	-	-
		n-hexane	-	-	-	-	-	-	-	-
M. indica	Stem-bark	Methanol	-	-	+++	+++	+++	++	-	-
		Ethyl acetate	++	-	+++	+++	-	+	-	-
		n-hexane	-	++	-	-	+++	-	++	-
S. americana	Whole plant	Methanol	-	+++	-	-	-	++	+	-
		Ethyl acetate	-	++	-	-	-	+	+	-
		n-hexane	-	-	-	-	-	-	+	-
C. areborea	Stem-bark	Methanol	++	++	-	-	-	+	-	-
		Ethyl acetate	++	++	+	-	-	+	-	-
		n-hexane	+	-	-	-	+	-	-	-

+++ :Present in large quantity, ++ :Present in moderate quantity, + :Present in small quantity, - :Absent

Table 2: Results of Brine shrimp lethality test (BST) of extracts

Plant	Solvent of Extraction	BST LC_{50} (µg/cm^3)*
D. oliveri	methanol	66.5446 111.9554/38.0214
	ethyl acetate	52.2051 83.0607/32.5159)
	n-hexane	3988.95 187234/1329.60501)
H. acida	methanol	93.4756 144.7298/60.0193
	ethyl acetate	149.7237 238.9873/98.7794
	n-hexane	3988.95 187234/1329.60501)
T. mollis	methanol	122.4134 191.7965/79.6399
	ethyl acetate	3988.95 187234/1329.60501)
	n-hexane	4555.7020 %2817182.00/1352.0410)
C. edulis	methanol	196.4870 302.9393/133.6443
	ethyl acetate	3988.95 187234/1329.60501)
	n-hexane	3988.95 187234/1329.60501)
M. indica	methanol	68.7815 111.9992/41.7068
	ethyl acetate	122.4134 191.7965/79.6399)
	n-hexane	1625.4870 10652.2000/831.7991)

**Upper/Lower Limit 95% Confidence Interval*

Generally, the antimicrobial screening of the methanol and ethyl acetate extracts showed low to moderate activities (see Tables 3 and 4). For instance, the methanol extracts of *C. edulis*, *C. arborea*, *H. acida* and *T. mollis* showed moderate antibacterial activities against *E.coli*. The extract of *T. mollis* also showed moderate activities against the fungi *C. albicans* and *A. flavus* (see Table 5). The ethyl acetate extracts of *C. arborea*, *M. indica* and *T. mollis* exhibited moderate antibacterial activities against *S. pneumoniae* and *S. aureus*. Furthermore, the ethyl acetate extract of *C. arborea* showed moderate antibacterial activity against *S. aureus* and *P. aeruginosa* and antifungal activities against *S. flavus* and *A. niger*. However, the extracts of n-hexane were inactive.

Table 3: Zone of inhibition diameter (mm) of bacterial and Fungal growth in methanol extracts of plants

Plant	Part	Conc (x10^2 µg/cm^3)	*E. coli*	*S. pneumoniae*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. flavus*	*A. niger*
D. oliveri	Stem-bark	7	7	12	7	11	NI	NI	9
		6	NI	12	5	8	NI	NI	7
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
H. acida	Root	7	15	12	10	13	11	NI	NI
		6	13	8	8	10	9	NI	NI
		5	10	7	NI	NI	9	NI	NI
		4	9	NI	NI	NI	8	NI	NI
T. mollis	Root	7	15	NI	NI	10	9	NI	NI
		6	12	NI	NI	8	7	NI	NI
		5	9	NI	NI	7	NI	NI	NI
		4	8	NI	NI	7	NI	N	N
C. edulis	Root	7	12	NI	NI	9	NI	NI	NI
		6	9	NI	NI	8	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
M. indica	Stem-bark	7	10	10	14	NI	8	NI	NI
		6	NI	9	12	NI	8	NI	NI
		5	NI	7	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
S. americana	Whole plant	7	5	NI	NI	NI	8	NI	NI
		6	8	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
C. areborea	Stem-bark	7	13	NI	14	12	NI	12	14
		6	10	NI	NI	9	NI	10	12
		5	8	NI	NI	NI	NI	8	NI
		4	NI	NI	NI	NI	NI	NI	NI

NI - No Inhibition

Table 4: Zone of inhibition diameter (mm) of bacterial and fungal growth in ethyl acetate extracts of plants

Plant	Part	Conc (x10^2 µg/cm^3)	*E. coli*	*S. pnemonae*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. flavus*	*A. niger*
D. oliveri	Stem-bark	7	NI	NI	12	8	8	11	NI
		6	NI	NI	9	7	7	9	NI
		5	NI	NI	8	NI	NI	9	NI
		4	NI	NI	NI	NI	NI	7	NI
H. acida	Root	7	10	7	NI	8	NI	9	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
T. mollis	Root	7	NI	15	NI	13	12	13	NI
		6	NI	11	NI	11	11	10	NI
		5	NI	9	NI	10	8	9	NI
		4	NI	8	NI	8	7	8	NI
C. edulis	Root	7	NI	NI	8	8	NI	NI	NI
		6	NI	NI	Ni	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
M. indica	Stem-bark	7	10	12	NI	14	NI	NI	NI
		6	8	10	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
S. americana	Whole plant	7	10	NI	NI	NI	NI	NI	NI
		6	7	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
C. areborea	Stem-bark	7	10	14	NI	12	8	9	NI
		6	8	12	NI	12	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI

NI - No Inhibition

This is expected in view of the absence of phytocompounds tested for and the resulting BST and AGT inactivity. It was generally observed that most of the antifungal activities recorded were low. However, *C. albicans* and *A. flavus* were sensitive to many of the extracts.

Table 5: Zone of inhibition diameter (mm) of bacterial and fungal growth in n-hexane extracts of plants

Plant	Part	Conc ($x10^2$ $\mu g/cm^3$)	*E. coli*	*S. pnemonae*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. flavus*	*A. niger*
D. oliveri	Stem-bark	7	NI	NI	NI	7	NI	NI	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
H. acida	Root	7	NI	7	NI	NI	NI	NI	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
T. mollis	Root	7	NI	NI	NI	7	NI	9	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
C. edulis	Root	7	NI	NI	NI	NI	NI	NI	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
M. indica	Stem-bark	7	8	NI	NI	9	NI	8	NI
		6	NI	NI	NI	NI	NI	7	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
S. americana	Whole plant	7	NI	NI	NI	6	NI	NI	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI
C. areborea	Stem-bark	7	NI	8	NI	NI	NI	NI	NI
		6	NI	NI	NI	NI	NI	NI	NI
		5	NI	NI	NI	NI	NI	NI	NI
		4	NI	NI	NI	NI	NI	NI	NI

NI - No Inhibition

CONCLUSION

Methanol and ethyl acetate extracts of all the plants were very active against the shrimp larvae. However, some of them exhibited moderate antibacterial activities against *E. coli, S. pneumoniae and S. aureus* and most of them exhibited low antifungal activities against *C. albicans.*

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