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Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants

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Abstract

The antimicrobial activity of non-polar, moderately polar and polar extracts of the leaves of Alchornea laxiflora, Cnidoscolus acontifolius (Euphorbiaceae), Newbouldia laevis, (Bignoniaceae) and Adansonia digitata (Bombacaceae) against Escherichia coli NCIB 86, Staphylococcus aereus NCIB8588, Bacillus subtilis NCIB 3610, Pseudomonas aeruginosa NCIB 950 were investigated. There was generally no activity against the different strains of bacteria used. While the in vitro antioxidant activities of the hexane, ethyl acetate, butanol and aqueous extracts of the aerial parts of the plant materials determined by ferric thiocynate method showed that the species provided at least one fraction with highly promising antioxidant activity. However, phytochemical screening gave secondary metabolites that have been found to be of medicinal importance both in preventive and curative medicine.

Key Words: Medicinal plants, Phytochemicals, antimicrobial activity, antioxidant activity, ferric thiocynate.

INTRODUCTION

A medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purposes on which are precursors for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have preventive and curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants and are useful for humanity [1]. In view of many diseases defiling drugs, health practices are now changing from curative to preventive medicine. Phytochemicals popular in preventive medicine

are flavonoids, polyphenols, saponins, lignoids and vitamins. Also, a knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, which are precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [2].

Alchornea laxiflora belongs to Euphorbiaceae family. It is a shrub up to six metre in height. It is from Northern and Southern Nigeria and West Cameroon. It is also widely spread in central, eastern and Southern tropical Africa. The stems, especially the brachets are used in Nigeria as chew-sticks and its leaves are used to preserve kolanut in Nigeria. The decoctions of the leaves of *A. laxiflora* are also used in the management of inflammatory and infectious diseases [3]. *Cnidoscolus acontifolius* (Euphorbiaceae) is a shrub widely distributed throughout Africa especially in the drier regions and has been used in folk medicine as a remedy for numerous diseases ranging from inflammation to heart diseases. *Newbouldia laevis* (Bignoniaceae) is a sun-loving, fast-growing, drought-tolerant species from west tropical Africa. It is used traditionally for diarrhoea, dysentery, dropsy, swellings, oedema, and gout; and as febrifuges and genital stimulants/depressants. In both Yorubaland and Hausaland the tree is held in regard: a leaf is placed on the head of a new chief, and cutting the tree with an axe or burning as fuel is avoided. They are also used in Nigeria as a roundworm vermifuge and stomachic, and for migraine and earache [4]. *Adansonia digitata* (Bombacaceae) is a fruit-producing tree found in the savannas of tropical and southern Africa. The barks are used for the treatment of fever in Nigeria. Drinking of the aqueous extract of bark of *A. digitata* is used in Nigeria traditional medicine as treatment of sickle cell anaemia. The leaves are used medicinally as a diaphoretic and as astringent. The leaves have hypo-sensitive and antihistamine properties, which are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, guinea worm [4,5]. The baobab fruit pulp is therapeutically employed as febrifuge, analgesic, anti-dysentery and for the treatment of small pox and measles.

Many scientific research has been carried out on these plants and secondary metabolites of medicinal importance; alkaloids, flavonoids, terpenes etc have been reported, pure compounds have also been isolated and characterised [5-9]. But the objective of this research work is to relate the secondary metabolites found in these plants to their antioxidant and antimicrobial activities. The plants will be phytochemically screened for bioactive chemical substances [10]. The antioxidant property will be determined by another method; the ferric thiocyanate method not yet reported in literature for these plants. The antimicrobial activity will be determined by the agar well diffusion method. Oxygen reactive species have been found to participate in a growing number of diorders, they cause oxidative damage thus altering the structure and function of cells of biological macro-molecules, so any compound with anti-oxidant activity will prevent the formation of reactive species [11]. On the basis of this, natural anti-oxidant are been given much attention in recent times.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Alchornea laxiflora, Adansonia digitata, Newbouldia laevis,* and *Cnidoscolus acontifolius* were collected in June at different locations within Ibadan Metropolis in Oyo State, Nigeria. Specimens were identified and authenticated at Botany and Microbiology Dept of University of Ibadan. The leaves were air-dried and ground into fine powder and kept in non-absorptive nylon for subsequent use.

Reagents

The following chemicals and reagents were used: Hexane, ethyl acetate, methanol, ethanol, butanol, chloroform (all BDH chemicals), Fehling's solution A and B, 5% Ferric chloride, concentrated tetraoxosulphate VI acid, Dragendroff's reagent, hydrochloric acid, magnesium turnings, glacial acetic acid, ammonia solution, copper acetate, Molisch reagent, Silical gel F_{254} (Precoated aluminium sheets, Sigma, India), Mayer's reagent, linoleic acid, phosphate buffer, ammonium thiocynate, ferrous chloride, ferric thiocynate.

Materials

Test Organisms: *Escherichia coli* NCIB 86, *Staphylococcus aereus* NCIB8588, *Bacillus subtilis* NCIB 3610, *Pseudomonas aeruginosa* NCIB 950 (Micro organisms were collected from the stock of the Dept of Pharmaceutics, Faculty of Pharmacy of O.A.U.)

The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4^oC. 100ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37^oC for 24 hrs.

Stock solution of the extracts: Hexane, Ethylacetate, Butanol and Aqueous fractions.

Reference Standards: Streptomycin (1mg/ml) for antimicrobial activity and α-tocopherol for antioxidant activity.

Methods

Phytochemical Screening

The leaves of *A. laxiflora, A. digitata, N. leavis* and *C.acontifolius* were phytochemically screened for the presence of secondary metabolites. Fractions for phytochemical screening were obtained as follows:

100g of dried powdered leaves of plants were separately dissolved in 500ml of methanol and kept for 72 hrs before filtration. The solvent was distilled off leaving the methanol crude extracts. The methanol crude extracts were used to test for the presence of alkaloids, saponins, tannins, flavonoids, glycosides, phenols, steroids, anthraquinones, cardioactive-glycosides, resins and carbohydrates [10,12].

Extraction and Partitioning Procedures

1kg of dried-powdered leaves of *A. laxiflora A. digitata, N. leavis and C. acontifolius* were first defatted with hexane to obtain the non-polar extract. Methanol was added to the marc obtained and kept for 72 hrs before filtration. The combined filterates were decanted and were evaporated to dryness in a rotary evaporator at 37^0C and stored in a desiccator prior to further analysis. Thin Layer Chromatography was employed using silica gel 60 F_{254} precoated plates and solvent system: Ethylacetate/methanol (8:2) to detect antioxidant activity by using DPPH as a spray reagent. Yellow coloration on some of the spots on the TLC plates indicates that the methanolic extracts of these plants have antioxidant activity after which it was subjected to spectrophotometric experiments using UV-Visible (HE λ 10S Perkin-elmer α RIOOA Recorder, Switzerland). Ethylacetate was successfully added to aqueous solution of the crude methanolic extract in order to obtain the moderately polar fractions while butanol was added to the mother liquor to remove the polar fractions leaving behind an aqueous solution (highly polar fraction). All these fractions were subjected to antimicrobial (except the aqueous extract) and antioxidant activities.

Antimicrobial Screening Method

Antimicrobial activities of hexane, ethylacetate and butanol fractions of *A. laxiflora, A. digitata, N. leavis* and *C. acontifolius* were carried out using the agar well diffusion method. 0.2ml of an overnight broth culture of test micro-organisms was added to 20ml of cooled molten agar. It was mixed well and then poured into a sterile petri-dish and allowed to set. The stock was maintained on nutrient agar slant and subcultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Grade No. 1 Whatman filter paper. 100 discs were obtained by punching and putting in vials-bottles and sterilizing in an oven at 150°C for 15 min. Thereafter the cups (9mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions of extracts (50µL) at concentration of 40g/ml were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents were introduced into the remaining cups on each plate to act as positive and negative controls respectively. The plates were left at room temperature for 1 hour, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37^{o}C for 24 hrs in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimetre [13,14].

Determination of antioxidant activity

The antioxidant activities of hexane, ethyl acetate, butanol and aqueous extract of the plant materials were determined by ferric thiocynate method [15]. 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (50, 100, 250, 500 µg/mL) were prepared. A mixture of a 2 mL of sample in 99.5% ethanol, 2.0 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05 M phosphate buffer (PH 7.0) and 2 mL of water was placed in a vial with a screw cap and placed in an oven at 60^{o}C in the dark. To 0.1 mL of this sample solution, 10 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocynate was added. After the addition of 0.1 mL of 2 x 10-2 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour developed was measured in 3 min at 500 nm [16]. The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of α-tocopherol (reference compound) [17]. The inhibition of lipid peroxidation in percentage was calculated by this equation:

$$\% \text{ Inhibition} = 1 - (A1/A2) \times 100$$

Where A1 was the absorbance of the test sample and A2 was the absorbance of control reaction.

The results are shown in Table 3.

RESULTS AND DISCUSSION

These results of the phytochemical screening shown below in table 1, showed that *A. laxiflora, A. digitata, N. leavis* and *C. acontifolius* are generally rich in phytochemicals, alkaloids, flavonoids, saponins, tannins, etc. which may have justified their use in ethnomedicine. Several flavonoids of medicinal importance have been isolated from *A. laxiflora* while *A. digitata* is known to be rich in terpenoids. These classes of compound are known to show curative activity against several pathogens and therefore could explain the use, traditionally of these plants for the treatment of wide array of illnesses [17]. These extracts, however do not show appreciable

microbial activities against the different strains of microorganism used (table 2). In those that seem to have, the activity may have been boosted by the solvent. The results of this analysis therefore suggest that these secondary metabolites found in all these four medicinal plants have little or no antimicrobial effect and where there was it may have been due to synergistic effect.

Table 1: Phytochemical Screening of *A.laxiflora, A digitata, N.laevis and C.acontifolius*

Compounds	***A. laxiflora***	***A. digitata***	***N. leavis***	***C. acontifolius***
Alkaloids	+ve	+ve	-ve	+ve
Flavonoids	+ve	-ve	-ve	-ve
Saponins	+ve	-ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve	+ve
Cardio-Active Glycosides	+ve	+ve	+ve	+ve
Steroids	+ve	+ve	-ve	+ve
Phenols	+ve	+ve	+ve	-ve
Resins	-ve	+ve	+ve	+ve
Anthraquinones	-ve	-ve	-ve	+ve
Reducing Sugars	+ve	+ve	-ve	+ve

Key: *+ve =Positive -ve =Negative*

Table 2: Antimicrobial Activities of *A.laxiflora, A digitata, N.laevis and C.acontifolius*

	Zones of inhibition(mm) ± Standard error			
Extracts	***S. aereus***	***E. coli***	***B. subtilis***	***P. aeruginosa***
A.laxiflora				
Hexane	R	R	R	R
Ethylacetate	14.5±0.3	10.4±0.2	10.1±0.1	R
Butanol	13.3±0.2	16.1±0.1	17.1±0.3	13.4±0.1
A.digitata				
Hexane	R	R	R	R
Ethylacetate	R	R	R	R
Butanol	13.2±0.1	17.5±0.3	18.4±0.1	R
N.leavis				
Hexane	R	R	R	R
Ethylacetate	R	R	R	R
Butanol	12.5±0.3	17.3±0.2	18.2±0.1	R
C. acontifolius				
Hexane	R	R	R	R
Ethylacetate	R	R	R	R
Butanol	12.4±0.1	16.4±0.3	18.4±0.1	R
Controls				
Hexane	10.3±0.2	R	R	R
Ethylacetate	R	R	R	R
Butanol	15.5±0.1	17.4±0.3	18.3±0.1	R
Streptomycin (1mg/ml), (control)	27.1±0.1	23.2±0.1	27.3±0.2	R

***Key:** Results: Mean of three trials ± Standard error, R → Resistance .(Antimicrobial activities of hexane, ethylacetate and butanol fractions of A. laxiflora, A. digitata, N. leavis and C. acontifolius were carried out using the agar well diffusion method).*

The antioxidant activities of the hexane, ethyl acetate, butanol and water extracts of *A. laxiflora, A. digitata*, *N. leavis* and *C. acontifolius* were determined by ferric thiocynate (FTC) and the values are presented in Table 3. FTC method was used to determine the amount of peroxide

which oxidized ferrous chloride ($FeCl_2$) to a reddish ferric chloride ($FeCl_3$) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases.

Table 3: Antioxidant Activities of *A.laxiflora, A digitata, N.laevis and C.acontifolius*

Extracts	**Percentage inhibition**			
	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL
A.digitata				
Hexane	10	12	16	18
Ethylacetate	14	17	18	20
Butanol	32	50	70	78
Water	11	14	14	16
N. laevis				
Hexane	10	11	13	16
Ethylacetate	15	17	19	22
Butanol	28	50	64	72
Water	08	10	11	12
C. aconitifolius				
Hexane	12	15	18	20
Ethylacetate	18	22	29	33
Butanol	34	49	68	76
Water	10	12	14	15

***Key:** Each value represent % inhibition against control. The antioxidant activities of hexane, ethyl acetate, butanol and water extract of the plant materials as determined by ferric thiocynate method*

Hexane, ethyl acetate, butanol and water extract at various concentration (50, 100, 250 and 500 in µg/mL), showed antioxidant activities in a concentration dependent manner. However, butanol extract at the concentration of 500µg/mL for all the medicinal plants showed an antioxidant activity (70-78%) very close to that of 500 µg/mL of α-tocopherol (82%), the reference compound. It has been observed that the extract exhibited strong activity with the increase in polarity (with reference to organic solvent), indicating that polyphenols or flavanone or flavonoids may play important roles in the activities. The present findings are in agreement with the report of Tepe *et al*. [18]. In spite of water being very polar the aqueous extract had less activity which might be due to the fact that most of the polar compounds are extracted with butanol itself. The antioxidant properties of plants have been linked to their therapeutic and protective effects in many diseases such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections and inflammation [12,17,20].

CONCLUSION

The Phytochemical screening of the methanol extract of the leaves of *A. laxiflora, A. digitata, N. leavis* and *C. acontifolius* showed the presence of secondary metabolites such as alkaloids, flavonoids, tannins, glycosides and phenols. These secondary metabolites have been found useful in both preventive and curative medicine. Also, the present study has been able to establish that these medicinal plants though commonly used in folk medicine are not effective against the following strains of bacteria *Escherichia coli, Staphylococcus aereus, Bacillus subtilis* and *Pseudomonas aeruginosa* . The antioxidant activity of the hexane, ethyl acetate, butanol and water extracts of *Adansonia digitata, Newbouldia laevis, Alchornea laxiflora* and *Cnidoscolus acontifolius* determined by ferric thiocynate (FTC) method showed that the hexane, ethyl acetate, butanol and water extracts at various concentrations (50, 100, 250 and 500 in µg/mL), showed antioxidant activities in a concentration dependent manner, the butanol extract however at the concentration of 500µg/mL for all the medicinal plants showed 70-78% antioxidant activity which is very close to that of 500 µg/mL of α-tocopherol (82%), the reference compound. It was

also observed that the extracts exhibited strong activity with the increase in polarity (with reference to organic solvent), indicating that polyphenols or flavanone or flavonoids may play important roles in the activities. This current study reports on the phytochemical screening, antimicrobial and antioxidant capacity of products derived from these plants which are known for their centenary use in traditional African medicine. This study was conducted as an initial step to elucidate the therapeutical, nutriceutical and cosmeceutical potential of these plant products until all the active components of these plants will be clearly established.

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