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Full Length Research Paper

Antimicrobial potential of extracts and fractions of the African walnut – *Tetracarpidium conophorum*

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Antibacterial and antifungal evaluation of the leaf, stem bark, kernel and root methanol extracts as well as the hexane, chloroform, ethyl acetate and methanol fractions of the leaf of *Tetracarpidium conophorum*, the African walnut, were investigated using the agar cup diffusion and agar broth dilution techniques. Extracts and fractions were tested against four clinical strains of 2 Gram positive, 2 Gram negative bacteria and two of fungi. They exhibited concentration-dependent antimicrobial properties. The extracts displayed higher activities to the Gram positive organisms. The edible nut was devoid of any antimicrobial property. The leaf extract was most active and it inhibited the growth of all the microorganisms used in the study. This led to the bioassay-guided fractionation of the leaf methanol extract and the ethyl acetate fraction of the leaf extract, displayed higher activities with the bacteria and fungi used in the assay, at the five test concentrations (100 mg – 10 mg/ml). *Pseudomomas aeruginosa* and *Candida albicans* were most sensitive to the extracts. Ampicillin and tioconazole were used as positive control, and methanol, used as negative control. The plant materials were also screened for secondary metabolites and this indicated the presence of alkaloids, saponins and tannins and absence of cardiac glycosides. The thin layer chromatographic analysis of the ethyl acetate fraction of the leaf crude extract confirmed the presence of alkaloids and tannis. These could be responsible for observed activity in the leaf of the plant; thus justifying its traditional uses especially in the treatment of dysentery.

Key words: *Tetracarpidium conophorum*, African walnut, antimicrobial properties, phytochemical analysis.

INTRODUCTION

Tetracarpidium conophorum Mull. (Arg), family Euphorbiaceae, is also known as Conophor. In southern Nigerian ethnomedicine, it is used as a male fertility agent and the leaves are used for the treatment of dysentery and to improve fertility in males. It is known as *ukpa* (Igbo) and *awusa* or *asala* (Yoruba). *T. conophorum* is known in the littoral and the western Cameroon as *kaso* or *ngak* (Dalziel, 1937).

The oil from the nut has found use in the formulation of wood varnish, stand oil, vulcanized oil for rubber and leather substitute. Most of the studies on the plant have been on the nutritive value of the seeds which is a snack and delicacy (Oke and Fafunso, 1975; Adebona, 1988; Akpuaka, 2000). Two isolectins, Agglutin I and II were characterized from the seed extract (Animashaun et al., 1994). The presence of oxalates, phytates and tannins as well as proteins, fibre, oil and carbohydrates in conophor nut has been reported (Enujiugha, 2003; Enujiugha and Ayodele-Oni, 2003).

In continuation of our study of plants and plant foods grown in Nigeria and the west African sub-region for anti-infective agents and biologically useful natural compounds, based on ethnomedical uses (Ajaiyeoba, 2002; Ajaiyeoba et al., 2003), we present herein the antibacterial, antifungal studies and phytochemical analysis of the African walnut, which had not been investigated hitherto.

MATERIALS AND METHODS

Plant collection and authentication

The plant materials (leaves, stem bark roots and fruits) were collected from Ikire in Osun State, Nigeria. They

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were authenticated by Mr. A. Odewo at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515. The African walnut, *T. conophorum* Mull. (Arg) (conophor) is a West Equatorial perennial climber often found growing wild in the moist forest zones of sub – Saharan Africa. It belongs to the family Euphorbiaceae. It is widely distributed in the southern part of Nigeria (Dalziel, 1937).

Preparation and extraction of plant samples

The air-dried plant materials were each ground with a Hammer mill. The four plant samples were extracted by maceration at room temperature (30°C) in aqueous methanol (20:80) for 72 h respectively. After removal of solvents, yields of extracts were obtained and the extracts stored in the refrigerator. The crude CH_3OH extract of the leaf was fractionated, using liquid-liquid extraction into hexane, chloroform, ethyl acetate and methanol. Plant parts collected were screened for the presence of tannins, alkaloids, anthraquinones, cyanogenetic glycolsides, saponin glycosides, and steroidal nucleus using the method described previously (Ajaiyeoba, 2002).

Antimicrobial assay

Clinical strains of 4 human pathogenic bacteria made up of 2 Gram-positive (*Staphyloccocus aureus* and *Bacillus subtilis*) and 2 Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were used for the antibacterial assay, while for the antifungal assay, one yeast (*Candida albicans*) and one mold (*Aspergillus flavus*) were used for the studies. The microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. Nutrient broth, nutrient agar, sabourand dextrose agar (SDA), tryptone soya broth agar (Oxoid Laboratories,U.K) were used in the study. Methanol was used in dissolving the extracts and drugs and used as negative control in the assay. The agar cup (8 mm) diffusion and agar broth tube dilution procedures were utilized in the studies and similar to our previous studies (Ajaiyeoba, 2002; Ajaiyeoba et al., 2003). Gentamycin (0.5 mg/ml), as reference antibacterial, for fungi; tioconazole (1 mg/ml) was used. Thin layer chromatography were done using Merck silica gel GF_{254} and compounds were visualized using UV lamp (254 nm) and visual spray reagents (Dragendorf and Ferric chloride reagents) respectively.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the plant parts for secondary metabolites, showed the presence of saponins, alkaloids, tannins and anthraquinones in the plant samples. The concentration of these metabolites were higher in the leaves as shown in Table 1. Cardiac glycosides were not detected in leaf, stem bark, roots and kernel of *T. conophorum*. Percentage yields of extracts were determined after removal of solvents respectively and the results are displayed in Table 1. Also shown in Table 1 are the antibacterial and antifungal activities of crude methanol extracts of the 4 plant materials, presented as means of 3 replicates of diameters of zones of inhibition (standard errors on means, SEM ,are also included). The extracts displayed concentration-dependent antibacterial and antifungal properties. The root extract displayed intrinsic antibacterial properties. Of the 6 microorganisms used, *S. aureus* was most sensitive to the root and stem bark extracts. At 100 mg/ml, the extract had a diameter of zones of inhibition of 14.0±0.0 and13.0±1.2 cm; respecttively. Both extracts did not show any antifungal property in the present study. The leaf extract exhibited the highest activities with all the microorganisms investigated. At 100 mg/mml, it had an activity of 15.5±2.3 and 14.4±1.7 cm with *B. subtilis* and *P. aeruginosa r*espectively. The leaf extract also showed antifungal properties, inhibiting the growth of the *A. niger,* a normally resistant mold, much more than the reference drug, tioconazole (Table 1). The kernel did not show any activity with the microorga-nisms used in this study.

The antibacterial and antifungal properties of the fractions of the leaf extracts are presented in Table 2. The hexane, chloroform, ethyl acetate and methanol fractions of the leaf extracts displayed good antimicrobial activities which were concentration-dependent at the 5 concentrations (100–5 mg/ml) tested. The most sensitive bacteria to the four fractions were *P. aeruginosa*. The ethyl acetate fraction was the most active extract, while the hexane fraction showed least activity. The fractions also inhibited the growth of the 2 fungi used in the study. The yeast, *C. albicans* and the mold, *A. niger*, were inhibited even at a concentration of 10 mg/ml, comparable to tioconazole. In the antimicrobial analyses, gentamycin was included as reference antibacterial compound, tioconazole as the reference for antifungal. Methanol was included in the experiments as a negative control and it did not display any antimicrobial activity as shown in Tables 1 and 2.

The thin layer chromatographic analysis of the ethyl acetate fraction gave positive spot test (purple colouration, after spraying with $FeCl_3$) for acidic compounds, most probably tannins. The edible part of the plant, the kernel did not show any antimicrobial property in the assay. However, *T. comophorum* is an economic plant and it is widely cultivated for production of the nuts which are delicacies snack food. Results of this study have shown that *T. conophorum* has a high potential as an antimicrobial medicinal plant. This is therefore improving the value of the plant. It is reported to be useful in the folklore in the treatment of dysentery. This investigation therefore justifies its ethnomedical use, having displayed

Table 1. Phytochemical and antimicrobial analysis of *Tetracarpidium conophorum* crude methanol extracts.

Zones of inhibition (cm)								
Extracts[a] Constituents[b]	**Yield (%)**	**Conc. (mg/ml)**	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***	***C. Albicans***	***A. niger***
Roots	2.49							
Alkaloid ++		100	14.0 ± 0.0	12.3 ± 2.3	11.0 ± 1.4	11.0 ± 1.7	-	-
Saponins +		50	14.0 ± 0.0	12.0 ± 2.7	-	-	-	-
Tannin +		25	12.0 ± 0.0	11.0 ± 1.4	-	-	-	-
Anthraq +								
Stem bark	2.59							
Alkaloids +		100	13.0 ± 1.2	12.0 ± 0.0	10.5 ± 1.7	11.0 ± 0.0	-	-
Saponin +		50	13.0 ± 0.0	11.5 ± 0.7	-	-	-	-
Tannin +		25	-	11.0 ± 1.4	-	-	-	-
Anthraq. +								
Leaf	26.18							
Alkaloids ++		100	12.7 ± 1.2	15.5 ± 2.3	14.0 ± 1.7	12.0 ± 0.0	12.3 ± 0.4	16.3 ± 1.5
Saponin ++		50	11.5 ± 2.1	13.7 ± 1.5	12.0 ± 0.0	11.5 ± 0.0	-	10.0 ± 0.0
Tannin ++		25	-	10.0 ± 0.7	11.0 ± 0.0	-	-	-
Anthraq. +								
Kernel	0.12							
Alkaloids +		100	-	-	-	-	-	-
Saponin +		50	-	-	-	-	-	-
Tannin +		25	-	-	-	-	-	-
Anthraq. +								
Gentamycin		0.5	22.3 ± 1.5	26.5 ± 2.1	19.3 ± 1.5	22.0 ± 1.4	ND	ND
Tioconazole		1.0	ND	ND	ND	ND	18.5 ± 0.2	15.0 ± 0.0

[a]Methanol was used as Negative control and did not inhibit growth of the microorganisms

[b]Phytochemical analysis: ++high concentration; + normal.

Table 2. Antimicrobial activities of leaf Fractions of *Tetracarpidium conophorum*

Zones of inhibition (cm)							
Fractions	**Conce. (mg/ml)**	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***E. coli***	***C. albicans***	***A. niger***
Hexane	100	12.3 ±1.2	14.5 ± 1.5	13.0 ± 1.0	12.3 ± 1.5	12.0 ± 2.0	15.5 ± 1.2
	50	11.0 ± 1.0	12.0 ± 1.7	11.7 ± 0.7	11.7 ± 0.6	10.0 ± 0.0	13.3 ± 0.6
	20	-	10.3 ± 0.6	11.0 ± 1.0	10.0 ± 0.0	-	11.3 ± 1.5
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
Chloroform	100	12.7 ± 0.6	15.0 ± 2.6	12.7 ± 0.6	12.3 ± 0.6	17.5 ± 0.6	14.3 ± 0.6
	50	11.5 ± 0.7	11.3 ± 1.5	10.7 ± 0.6	10.7 ± 0.6	12.2 ± 0.6	12.5 ± 0.6
	20	10.5 ±0.7	10.0 ± 0.0	10.0 ± 0.0	9.7 ± 0.6		-
	10	10.4 ± 0.6	-	-	-	-	-
	5	-	-	-	-	-	-
Ethyl acetate	100	13.7 ± 1.2	11.5 ± 0.7	17.5 ± 3.5	13.0 ± 1.4	16.5 ± 0.7	18.2 ± 1.2
	50	12.3 ± 0.7	-	13.5 ± 0.7	11.5 ±1.2	13.0 ±0.0	13.3 ± 0.7
	20	11.5 ± 1.0	-	12.0 ± 1.4	-	12.0 ±0.0	12.7 ±1.2
	10	11.0 ± 0.7	-	11.5 ± 1.4	-	11.0 ±1.4	-
	5	-	-	11.0 ± 0.7	-	-	-

Table 2. Contd.

Methanol	100	16.0 ± 2.8	14.3 ± 0.6	14.3 ± 0.6	14.7 ± 0.6	12.0 ± 1.5	14.7 ± 1.5
	50	13.2 ± 1.5	12.0 ± 1.0	12.3 ± 0.6	12.0 ± 1.0	-	12.5 ± 1.2
	20	11.5 ± 0.7	10.7 ± 1.2	11.3 ± 1.2	9.5 ± 1.7	-	-
	10	-	10.0 ± 0.0	10.0 ± 0.0	-	-	-
	5						
Gentamycin	0.5	26.5 ±2.1	22.3 ± 1.5	19.3 ± 1.5	22.0 ± 1.4	NT	NT
Tioconazole	1.0	NT	NT	NT	NT	18.6 ± 0.3	15.0 ± 0.2
Methanol		-	-	-	-	-	-

activities with the human pathogenic microorganisms that were used in this study. The need for development of newer antimicrobial chemotherapeutic agents is imperative. This is because there is increasing treatment failure rates of microbial infections due to drug-resistant antibiotics (Selwyn et al., 1980). The most active fraction in the present study, the ethyl acetate fraction of the leaf methanol extract, has a very high potential as a source for drug discovery for antimicrobial agents. This is being investigated by our group and the results will be presented in due course.

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