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Phytochemical and antimicrobial activities of the wild mango- *Irvingia gabonensis* extracts and fractions

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Summary
Crude methanol extracts obtained from the leaf, stem bark and root of the wild mango, *Irvingia gabonensis*, were screened for antimicrobial properties by agar well diffusion method at three different concentrations (100 mg/ml, 50 mg/ml and 25 mg/ml) against six human pathogenic microorganisms consisting of four bacteria and two fungi. The hexane, chloroform, ethyl acetate and methanol fractions of the leaf and root methanol extracts were also subjected to the same assay at concentrations of 100 mg/ml - 5mg/ml. Gentamicin and Tioconazole were used as positive and methanol as negative controls. Significant inhibitory activities were exhibited by the leaf and root extracts. The crude methanol extract of the root displayed the highest activity at a concentration of 100 mg/ml. It had a diameter of zone of inhibition of 19.7 mm while the reference drug had 19.3mm on *Pseudomonas aeruginosa*, the most sensitive bacteria. The fungi used in this study were also very sensitive to the leaf extract. All the active extracts and fractions exhibited concentration-dependent activities against all the test organisms. Diameter of zones of inhibition ranges from 10.0-30.0 mm. The stem bark was inactive against all the studied organisms. The most active fraction was the ethyl acetate soluble fraction of the leaf which showed a comparable antimicrobial activity against the organisms at concentrations 100mg/ml and 50mg/ml comparable to the reference standard drug Gentamicin and Tioconazole. The ethyl acetate soluble fractions of leaf and root were found to show the highest activity. At a concentration of 5mg/ml, the root ethyl acetate fraction inhibited the growth of all the bacteria tested. The phytochemical screening of the plant materials revealed the presence of tannins, saponins, alkaloids and anthraquinones and the absence of cardiac glycosides. Thin layer chromatography indicated the presence of phenolic compounds.

Keywords: *Irvingia gabonensis, wild mango, Antimicrobial properties, phytochemical analysis*

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Résumé
Les extraits méthanoïques brute obtenus des feuilles, écorce et racines des manguiers sauvages (*Irvingia gaboniensis*) étaient testés pour leurs propriétés antimicrobiennes par la méthode de diffusion d'Agar utilisant 3 différents concentrations : 100mg/ml ,50mg/ml et 25mg/ml contre six microorganismes humain pathogénique consistant à 4 bactéries et 2 algues. Les fractions des extraits d'hexane, de chloroforme, d'éthyle acétique et de méthanol des feuilles, de l'écorce et des racines étaient soumisse à l'analyse à des concentrations variant entre 100-5mg/ml. La gentamicine et la tioconazole étaient des contrôls positif et le méthanol comme control négatif respectivement. Les activités inhibitoires importantes étaient exposées par les extraits de feuilles et de racines. Les extraits éthanoïques des racines démontraient la plus grande activité à la concentration de 100mg /ml, Les algues utilisés dans cette étude étaient aussi très sensibles aux extraits des feuilles. Tous les extraits actifs et les fractions ont subit une concentration dépendant des activités contre tous les organismes testés. Le diamètre de la zone d'inhibition avait un rang de 10.0-30.0 mm. Les écorces étaient inactives contre tous les organismes étudiés. La fraction la plus active était l'extrait d'éthyle acétique des feuilles qui démontrait une activité antimicrobienne comparable contre les organismes à la concentration de 100mg /ml et 50mg/ml comparable à la référence standard, gentamicine et tioconazole. Les fractions solubles d'éthyle acétique et des racines avaient la plus grande activité. A la concentration de 5mg/ml, la fraction d'éthyle acétique des racines inhibait le développement tous les bactéries testées. Le test phytochimique des parties de la plante révélait la présence des tanines, saponines, anthracines et l'absence des glycosides cardiaques. La chromatographie a souche légère indiquait la présence des produits phénoliques.

Introduction

The wild mango, *Irvingia gabonensis* Aubry-Lecomte ex O'Rocke-Baill [Irvingiaceae], is botanically unrelated to cultivated mango; *Mangifera indica* L [Anacardiaceae] [1,2]. *Irvingia*

gabonensis seed is commonly referred to as dikanut and is a prominent indigenous plant in the Southern Nigeria and grows naturally in the forest habitat of several regions in Africa, extending from Senegal to the Sudan and south to Angola [3].

Traditionally, the stem bark is used for the treatment of colic, diarrhea and dysentery [4]. In Sierra Leone, the Mendes use the stem bark powder in water as a paste on the skin to ameliorate pain [5]. In other parts of Africa, the stem bark extract is ingested to produce analgesia. The water and ethanol extracts of the powdered stem bark have been reported to possess analgesic property and showed protective activity against pain in mice [6]. Seeds of *I. gabonensis* have been used in the management of obesity [7]. The aqueous leaf extract has been shown to protect mice against castor oil-induced diarrhea [8]. When dikanut, was fed to diabetic patients for 4 weeks, blood glucose became normal and the activities of the three ATPases increased significantly [9,10].

It is well known that infectious diseases account for a high proportion of health problems, especially in the developing countries. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problems in the treatment of infectious diseases [11]. This resistance has increased due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases. Given the evidence for the rapid global spread of resistant clinical isolates and the appearance of drug resistant strains among community acquired infections, the need for discovery or development of new antimicrobial agents active towards these resistant strains is of paramount importance [12]. For this reason the search for new antimicrobial drugs among plants becomes an important alternative and the situation has forced scientists to search for new antimicrobial substances from various sources, including medicinal plants [13].

In continuation of our study of Nigerian plants and food plants for anti-infective agents [14-16] the preliminary chemical composition and antimicrobial activities of the crude extracts and fractions of *I. gabonensis* plant parts is reported.

Materials and methods

Plant collection and authentication

Irvingia gabonensis leaves, stem bark and root were collected from a village at Ikire in Osun State of Nigeria in September 2005 The plant was authenticated at The Forestry Research Institute of Nigeria under FHI No.107517.

Extraction and fractionation of plant materials

The plant materials were sun dried over a period of 3-6 days. The dried samples were powdered with the hammer mill. Plant materials were macerated at room temperature in 80 % methanol for 72 hrs respectively. After removal of the solvent, extracts were stored in refrigerator at 4°C prior to use. Fractionation of crude methanol extracts of roots and leaves was done by solvent-solvent partitioning into hexane, chloroform, ethyl acetate and methanol extracts.

Phytochemical screening

Various phytochemical tests using the standard procedures [16] were carried out on the powdered samples to detect the presence of various secondary metabolites.

Antimicrobial assay

Microorganisms

Four bacteria and two fungi were used. This was made up of two Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and two Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria. The fungi consisted of one mold (*Aspergillus niger*) and one yeast (*Candida albicans*). They were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan.

Media

Nutrient broth no.2, pH 7.4; nutrient agar, pH 7.4; sabourand dextrose agar (SDA); tryptone soya; all products of Oxoid Laboratories, U. K. were used in the studies. The extracts were dissolved in methanol while methanol alone was used as a negative control.

Antimicrobial agents

Gentamicin, 0.5mg /ml (Lab.Oftalmiso, Spain) and Tioconazole cream, 1.0 mg/ml (Pfizer Inc., New York) were used in the assay as reference drugs.

Antimicrobial evaluation

The agar plate method was used to determine the antimicrobial properties of the test extracts with 24 hrs broth cultures. One fifth of a millilitre of the appropriate bacteria culture was inoculated into 20 ml quantities of molten and cooled nutrient agar. The agar was mixed for homogeneity and poured into 8.5 cm Petri dishes and allowed to set. Equidistant wells were bored into the agar using sterile cork borer (8mm diameter). Varying concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml) in methanol of each extract were

added into the wells and were incubated at 37°C for 24 h. Gentamicin (0.5 mg/ml) was used as positive control and methanol was used as negative control.

Assessment of antifungal activities was done by introducing 0.2 ml fungal hyphae on solidified Saboraud dextrose agar and spread evenly in the plates. Tioconazole cream (1 mg/ml) was used as reference antifungal drug. All plates were incubated at 37°C for 96 h. The experiments were carried out in triplicates and diameters of zones of inhibition measured.

Thin layer chromatography analysis was done using Merck silica gel GF_{254} and compounds were visualized using a UV lamp (254nm) and spraying was done with Dragendorff, Ferric chloride and 5% KOH in methanol respectively.

The results of the antimicrobial assays were expressed as means and standard errors. The statistical analysis of the antimicrobial result using the student T-test showed that the effect of the extracts is statistically significant compared to the control within 99% confidence level. P value less than 0.01.

Results

The phytochemical screening of the plant materials revealed that the plants contain secondary metabolites such as alkaloids, anthraquinone, tannins and saponins with the leaf having the highest concentrations. Cardiac glycosides were not detectable in the plant samples as displayed in Table 1. Also shown in Table 1 are the percentage yields of extracts and fractions of *I. gabonensis*.

Antimicrobial evaluation (Table 1) carried out on the crude aqueous methanol extract of the plants using agar plate method showed that they exhibited good inhibitory effects against the organisms tested with diameter of zones of inhibition ranging from 29.0 ± 1.7 to 13.7 ± 1.5 for the leaf extract and 19.7 ± 4.7 to 13.5 ± 2.1 mm for the root extract. The leaf and root extracts had a concentration dependent antimicrobial activity against Gram positive bacteria than Gram negative bacteria and also for the mold than the yeast used in the study. The diameter of zones of inhibition ranged from 25.7 ± 2.1 -10.0 ± 0.0 mm for the bacteria and 30.0 ± 2.8-10.0 ± 0.0 mm for the fungi. Highest activities was exhibited by *I. gabonensis* leaf extract against *Bacillus subtilis, Candida albicans* and *Aspergillus niger* with diameter of inhibition zones of 21.0 ± 1.7, 20.3 ± 0.6 and 29.0 ± 1.7 cm respectively. *I. gabonensis* stem bark showed no activity against any of the microorganisms tested in the study.

Table 1: Phytochemical and antimicrobial analysis of *Irvingia gabonensis* crude methanol extracts

Extracts[a] Constituents[b]	Yield (%)	Conc /mg/ml	Microorganisms					
			S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albican*	*A. niger*
Root	2.06	100	16.7 ± 1.2	18.0 ± 1.7	18.0 ± 0.0	19.7 ± 4.7	13.5 ± 2.1	18.5 ± 2.1
Alkaloid +		50	11.0 ± 0.0	12.5 ± 2.1	12.0 ± 0.0	13.0 ± 1.4	-	11.3 ± 1.5
Saponins +		25	10.0 ± 0.0	10.5 ± 0.7	10.0 ± 0.0	10.0 ± 0.0	-	10.0 ± 0.0
Tannin ++								
Anthraq ++								
Leaf	15.7	100	16.3 ± 1.5	21.0 ± 1.7	13.7 ± 1.5	13.7 ± 1.2	20.3 ± 0.6	29.0 ± 1.7
Alkaloid		50	15.3 ± 1.5	13.7 ± 2.3	-	12.0 ± 0.0	-	14.0 ± 1.0
Saponins ++		25	13.0 ± 2.0	10.0 ± 0.0	-	10.7 ± 1.2	-	11.3 ± 0.6
Tannin ++								
Anthraq +								
Stem bark	5.89	100	-	-	-	-	-	-
Alkaloid +		50	-	-	-	-	-	-
Saponins +		25	-	-	-	-	-	-
Tannin +								
Anthraq +								
Gentamicin		0.5	22.3 ± 1.5	26.5 ± 2.1	22.0 ± 1.4	19.3 ± 1.5	NT	NT
Tioconazole		1.0	NT	NT	NT	NT	21.3 ± 2.5	32.0 ± 1.5
Methanol[c]	-		-	-	-	-	-	-

[a] Methanol was used as negative control and the diameter of cork borer is 8.0mm
[b] phytochemical analysis ++ high concentration; + moderately present
NT – Not Tested

Table 2: Antimicrobial activities of the fractions of *Irvingia gabonensis*

fractions	Conc (mg/ml)	Diameters of zones of inhibition (mm) Microorganisms					
		S. aureus	*B. subtilis*	*P.aeruginosa*	*E. coli*	*C. albican*	*A. niger*
Root							
Hexane	100	17.5 ± 0.7	14.7 ± 0.6	13.3 ± 2.1	14.3 ± 1.5	29.7 ± 2.5	14.0 ± 3.5
	50	12.5 ± 0.7	12.0 ± 1.0	11.7 ± 1.5	11.3 ± 1.2	18.3 ± 1.5	12.5 ± 2.5
	20	10.5 ± 0.7	10.7 ± 0.6	10.7 ± 0.6	10.3 ± 1.2	13.7 ± 1.2	-
	10	-	10.0 ± 0.0	10.7 ± 1.2	-	-	-
	5	-	-	-	-	-	-
Chloroform	100	13.5 ± 0.7	12.5 ± 0.7	13.7 ± 1.5	13.7 ± 2.5	30.0 ± 1.4	13.7 ± 1.5
	50	12.0 ± 0.0	10.5 ± 0.7	11.7 ± 1.5	11.3 ± 1.2	25.3 ± 3.5	12.5 ± 2.5
	20	10.0 ± 0.0	-	11.3 ± 1.2	10.6 ± 1.2	-	12.0 ± 1.0
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
Ethyl acetate	100	25.7 ± 2.1	25.0 ± 1.0	22.7 ± 1.5	23.3 ± 2.9	24.5 ± 3.5	22.1 ± 3.2
	50	20.7 ± 0.6	20.3 ± 0.6	18.3 ± 0.6	20.3 ± 2.1	21.0 ± 0.0	18.0 ± 2.5
	20	18.5 ± 0.7	18.3 ± 0.6	16.5 ± 1.5	20.3 ± 0.6	16.5 ± 2.1	14.6 ± 2.6
	10	12.5 ± 0.7	16.5 ± 0.7	13.7 ± 1.5	14.0 ± 2.6	15.0 ± 2.8	-
	5	-	13.0 ± 0.	11.5 ± 0.7	11.3 ± 1.2	-	-
Methanol	100	18.0 ± 1.0	19.7 ± 2.1	21.0 ± 1.7	17.3 ± 0.6	21.2 ± 2.1	16.5 ± 2.1
	50	14.0 ± 1.0	15.3 ± 3.2	14.3 ± 1.5	13.3 ± 1.2	18.0 ± 2.6	13.5 ± 2.8
	20	11.3 ± 0.6	12.0 ± 1.0	11.0 ± 0.0	11.3 ± 0.6	-	-
	10	11.0 ± 1.0	12.3 ± 0.6	10.0 ± 0.0	11.0 ± 1.0	-	-
	5	-	10.7 ± 0.6	-	10.5 ± 0.7	-	-
Leaf							
Hexane	100	15.3 ± 1.2	21.0 ± 3.6	13.7 ± 1.2	12.3 ± 1.5	17.0 ± 3.5	16.2 ± 1.5
	50	12.5 ± 0.7	13.3 ± 2.9	12.3 ± 0.6	10.7 ± 1.2	13.5 ± 3.5	13.5 ± 2.1
	20	10.0 ± 0.0	11.0 ± 1.4	10.3 ± 0.6	-	-	12.0 ± 3.5
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
Chloroform	100	14.5 ± 0.7	16.0 ± 2.0	16.0 ± 1.0	14.3 ± 0.6	20.5 ± 3.5	14.2 ± 2.1
	50	13.0 ± 0.0	12.3 ± 1.5	13.7 ± 1.2	12.0 ± 0.0	17.5 ± 3.5	12.5 ± 2.6
	20	11.5 ± 0.7	10.3 ± 0.6	11.7 ± 1.5	12.0 ± 0.0	15.0 ± 4.2	-
	10	11.5 ± 0.7	10.7 ± 0.6	11.3 ± 0.6	-	12.0 ± 0.0	-
	5	10.7 ± 1.2	-	10.5 ± 0.7	-	-	-
Ethyl acetate	100	25.0 ± 0.0	17.3 ± 3.2	22.3 ± 2.5	23.3 ± 1.5	30.0 ± 2.8	18.5 ± 2.1
	50	18.5 ± 2.1	15.7 ± 2.1	16.3 ± 1.5	17.0 ± 1.7	22.0 ± 4.2	15.3 ± 2.5
	20	12.5 ± 2.1	13.0 ± 1.4	13.3 ± 1.2	11.7 ± 0.6	13.0 ± 2.8	14.0 ± 1.4
	10	11.0 ± 1.4	11.5 ± 0.7	12.3 ± 2.5	-	12.0 ± 1.4	12.2 ± 2.1
	5	-	11.0 ± 1.4	11.0 ± 1.4	-	-	-
Methanol	100	20.7 ± 3.5	19.0 ± 2.6	22.7 ± 2.5	21.0 ± 3.6	21.0 ± 1.4	18.3 ± 3.5
	50	17.7 ± 1.5	15.3 ± 0.6	17.7 ± 1.5	14.0 ± 0.0	15.5 ± 4.9	15.2 ± 2.1
	20	12.7 ± 3.1	12.7 ± 0.6	14.7 ± 1.5	11.3 ± 1.2	12.5 ± 3.5	12.8 ± 3.5
	10	-	11.3 ± 0.6	12.0 ± 1.0	11.0 ± 1.4	-	-
	5	-	-	-	-	-	-
Gentamycin	0.5	22.3 ± 1.5	26.5 ± 2.1	19.3 ± 1.5	22.0 ± 1.4	NT	NT
Tioconazole	1.0	NT	NT	NT	NT	21.3 ± 2.5	32.0 ± 1.5
Methanol		-	-	-	-	-	-

The antimicrobial properties of the fractions of the leaf and root extracts are shown in Table 2. The ethyl acetate soluble fraction, chloroform soluble fraction and hexane soluble fraction of leaf and root extracts exhibited good activity at all the 5 concentrations (100 -5 mg/ml) on all the test organisms. The diameter of inhibition zones ranges between 30.0 ± 2.8 and 10.0 ± 0.0 cm. The ethyl

acetate soluble fractions of the root extract exhibited the highest activity with inhibitory action against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* at concentrations as low as 5 mg/ml. It had diameters of zones of inhibition of 11.3 ± 1.2, 13.0 ± 0.0 and 11.5 ± 0.7 cm respectively.

Discussion

The results of this study showed that *I. gabonensis* leaf and root extracts inhibited the growth of the organisms tested in a comparable manner to the reference drugs; Gentamicin and Tioconazole. Gentamicin and Tioconazole were included in the experiment as reference antibacterial and antifungal drugs respectively while methanol was used as a negative control.

All the root and leaf fractions of *I. gabonensis* exhibited outstanding antimicrobial activities. Diameters of zones of inhibition range from 30.0 ± 1.4 to10.0 ± 0.0 mm while that produced by Gentamicin was found to be 26.5 ± 2.1 to 19.3 ± 1.5 and Tioconazole, 32.0 ± 1.5 mm to 21.3 ± 2.5 mm. Of all the fractions, the ethyl acetate fractions of the leaf and root exhibited the highest activities against all the organisms; inhibiting the growth of three out of the four bacteria and the yeast much more than the reference drugs, while the hexane soluble fractions showed the least activity. The most sensitive bacterium is the *Staphylococcus aureus*. The polarity of the solvent seems to play an important role in exhibiting potential antimicrobial activity.

At 100 mg/ml ethyl acetate fraction of the root displayed higher activity than the reference drugs. At the same concentration, it had an activity of 25.7 ± 2.1, 22.7 ± 1.5, 23.3 ± 2.9 and 24.5 ± 3.5 mm with *S. aureus, P.aeruginosa, E. coli* and *C. albican* respectively.

Thin layer chromatography analysis of the ethyl acetate fractions of the root and leaf extracts using chloroform: ethyl acetate solvent system (90:10) and spraying with 5% KOH in Ethanol indicated the presence of phenolic compounds. Three spots gave positive test (pink colouration after activation) for phenolic compounds with R_f values of 0.24, 0.58 and 0.87 for the root and 0.38, 0.52 and 0.83 for the leaf fractions respectively. Two spots tested positive (orange colouration) for alkaloids in the ethyl acetate fraction of the root extracts after spraying with Dragendorff reagent. The presence of tannins, saponins and anthraquinones could be responsible for the antimicrobial activity displayed by these fractions.

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