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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *IRVINGIA GABONENSIS* (AUBRY-LECOMTE EX O'RORKE) BAILL

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ABSTRACT

Irvingia gabonensis is an African deciduous tree species that bear edible mango-like fruits. The phytochemical screening and *in vitro* antimicrobial activity of the plant part extracts were evaluated. The methanol extracts of the plant were used for all the analyses. Both the qualitative and the quantitative analyses of the plant extracts were carried out using standard techniques. The susceptibility of the test organisms to the herbal extracts was done using the determination of the minimum inhibitory concentration (MIC). The significant difference was measured using Duncan's Multiple Range Test. Alkaloids, flavonoids, saponins, tannins, and terpenoids occurred in high levels in the leaf, stem bark, and ripe fruit peel of *I. gabonensis* where as low values of anthraquinones, phenols, and steroids were also found in all the parts. The plant extracts exhibited dose-dependent effects on the microorganisms tested. The methanol extracts of *I. gabonensis* parts effectively inhibited the growth of *Staphylococcus aureus, Streptococcus viridians, Escherichia coli, Pseudomona saeruginosa, Salmonella enterica, Shigella sonnei, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Fusarium oxysporum,* and *Rhizopus stolonifer*. These extracts, therefore, showed good antibacterial and antifungal activities at different concentrations *in vitro*. Hence, the pharmaceutical application of these plant parts' antimicrobial properties is suggested.

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Introduction

Irvingia is a dicot genus of the family, Irvingiaceae. This is a family of flowering plants, consisting of 13 species distributed in three genera, *Allantospermum, Irvingia,* and *Klainedoxa* [1]. The genus comprises seven species, *Irvingia gabonensis* (Aubry-Lecomte Ex O'rorke) Baill., *I. grandifolia* (Engl.) Engl., *I.malayana* Oliver ex. Bennett, *I. robur* Mildbr., *I. smithii* Hook.f., *I. tenuinucleata* Tiegh. and *I. wombolu* Vermoesen [2]. The botanic description of *I. gabonensis* was documented in Hutchinson and Dalziel [3]. The flowers are short, clustered, mostly axillary racemes, or subpaniculate. The pedicel is up to 10 mm long. The leaves are obovate-elliptic or, more or less cuneate or narrowly rounded at the base, shortly and broadly acuminate. The fruits are broadly, somewhat flattened, about 5–6 cm long with smooth skin, fibrous exocarp, and hard endocarp.

Plants serve as medicine since ancient days. A wide range of phytochemicals that are traditionally classified as primary and secondary metabolites occur naturally in plants [4]. They dissolve in an array of solvents based on their nature [5]. Among the diverse uses of secondary metabolites is their function as a pharmacological active compound and they occur in various structural classes. The type and level of the biological active compounds in plants are responsible for their medicinal properties. Their concentrations in various plant parts vary [6, 7]. Their syntheses and accumulations are influenced by the environment and defense against herbivory [8].

Moreover, when bacteria form a parasitic association with other organisms, they are classified as pathogens. Pathogenic microorganisms cause human diseases and subsequent death. The plant extracts exhibit dose-dependent effects on the microorganisms [9, 10]. The level of inhibitory activity of a plant extract against pathogenic microorganisms determines the degree of its potency [11, 12]. Phytochemicals are the mechanisms that plants use to protect themselves against the effects of their pathogens [13]. Hence, constitutes the natural source of antimicrobial substances.

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Furthermore, the tropical rainforest zone is rich in medicinal plants possessing a wide range of therapeutic potentials that are underutilized. *Irvingia gabonensis* is one of the medicinal plants that are underexploited. In addition, medicinal plants have been proven efficacious in the treatment of various diseases, and this has led to a boost in their search over the last two decades. The objectives of this study, therefore, were to screen the *I. gabonensis* leaf, stem bark, and ripe fruit peel for the presence of phytochemicals and as well as determine their antimicrobial activity.

Materials and Methods

Collection of Plant Material

The leaf, stem bark, and ripe fruit peel of *I. gabonensis* used in this work were collected in June from Ihioma, Imo State Nigeria. The samples were authenticated at the Herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, where the voucher specimen was deposited.

Preparation of Samples

The ripe fruits of *I. gabonensis* were peeled with a table knife. The leaf, stem bark, and ripe fruit peel were sliced with a table knife and then oven-dried (LDD906MF, Australia) at a temperature of 70^{o} C for 12 hours. The samples were then ground in a mortar with a pestle, and later into powdered form with an electric blender (Omega, USA). The powdered samples were then kept in an air-tight container before use.

Extraction of Plant Material

The methanol extracts of the plant were prepared by adding the powdered samples of the leaf, stem bark, and ripefruit peel in 100ml of methanol. The concentrations of the extracts were determined by adding 50g, 75g, 100g, and 150g to100ml of methanol. The whole setup was left for 24hours at room temperature and thereafter filtered using Whatman filter paper. The extract was then concentrated to 50ml, stored in an air-tight container, and kept in a refrigerator at 40^{o} C before use.

Qualitative Phytochemical Analysis

Qualitative tests were conducted using the standard methods described by Harborne [14], and their presence was denoted by a sign (+).

Quantitative Phytochemical Screening

The quantitative phytochemical determinations of the samples were carried out using standard procedures. Alkaloid, flavonoid, and steroid contents were determined by the gravimetric methods of Harborne [15]. The method of Ezeabara and Egwuoba [16] was used to determine the anthraquinone content. The tannin level of the samples was determined using the Folin-Dennis colorimetric method described by Kirk and Sawyer [17]. The method of AOAC [18] was used to determine the saponin content. Concentrations of phenols were determined using the Folin-cio Caltean colorimetric method [19]. The total terpenoid content of the plant specimen was determined by the method described by Ferguson [20].

Microbial Analysis

Preparation of Microorganisms for the Experiment

The pure culture of the microorganisms was obtained from the Pathology Department of the National Root Crop Research Institute, Umudike, Abia State, Nigeria. The bacteria isolates include Gram-positive: *Staphylococcus aureus, and Streptococcus viridans,* and the Gram-negative bacteria are *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica,* and *Shigella sonnei*. The fungi were *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Fusarium oxysporum* and *Rhizopus stolonifer*. The stock cultures of bacteria were sub-cultured in nutrient agar (NA) slants while mould on Sabour and Dextrose Agar (SDA) slants and stored at 4^{o}C.

Antimicrobial Test Procedures Preparation of Stock Solution

The initial concentration of each plant extract (5 g) was diluted with 50 ml of methanol to obtain the stock culture. Moreover, 100, 150, 200, and 250mg/ml concentrations were obtained from the stock culture and stored at room temperature before use.

Antimicrobial Susceptibility Testing

The test organisms were checked for susceptibility to the herbal extracts by carrying out antimicrobial screening using the extracts and by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A measured 20ml of sterile nutrient agar was poured into the sterile Petridish and allowed to gel. The surface was flooded with 2ml of 18 hours broth culture standardized according to the National Committee for Clinic Laboratory Standard [21] by gradually adding normal saline to compare its turbidity to the McFarland standard of 0.5 which is approximately1.0x10 cfu/ml. The surface was allowed to dry and a sterile No.4 Cork borer was used to bore six holes of 2.5cm equal in size on the surface. A measured 0.1ml of the extracts at different concentrations of 6.25%w/v, 12.5%w/v, 25%w/v, 50%w/v, and 100%w/v was dropped into each hole and the plate was kept for an hour at room temperature and incubated at 37°C for 18hours. The diameter of zones of inhibition was measured after incubation to the nearest millimeter (mm). The experiment was repeated three times and the mean diameter was taken. The effects of the extracts on bacteria and fungi pathogens were compared with those of the standard antibiotics, amoxicillin, and fungabacter for bacteria and fungi respectively as standard controls.

Statistical Analysis

Analysis of Variance (ANOVA) using SPSS version 21 was used in analyzing the data collected from the study. Duncan's Multiple Range Test (DMRT) was used to measure the test of significance, and the data were expressed as mean±standard deviation of triplicate determinations.

Results and Discussion

All the phytochemicals tested were present in the *I. gabonensis* leaf, stem bark, and ripe fruit peel **(Table 1)**. Hence, the plant parts are filled with bioactive compounds that have powerful health benefits. Alkaloids, flavonoids, saponins, tannins, and terpenoids occurred athigh levels in all the plant parts while low concentrations of anthraquinones, phenols, and steroids were detected, especially in the ripe fruit peel. This finding allied with the report of the previous study, where relatively high levels of alkaloids, flavonoids, saponins, tannins, and terpenoidsas well as low values of phenols and steroids were also detected in the leaf and stem bark of this plant [22]. The highest percentage of saponins (1.67±0.03) and tannins (1.41±0.04) were found in the leaf. The alkaloid concentrations followed a pattern that the ripe fruit peel>leaf>stem bark. Alkaloids are found in approximately 20% of plant species [23]. Traditionally, the stem bark of this plant is used as a pain relieverin Sierra Leone [24]. This is probably a result of the analgesic property of alkaloids. Hence, the *I.gabonensis* stem bark extract could be regarded as an effective painkiller.

Table 1. Mean quantitative phytochemical composition of the leaf, stem bark, and fruit peel of *Irvingia gabonensis*

Composition (mg/100g)	Plant Parts		
	Leaf	Stem bark	Ripe fruit peel
Alkaloids	1.33±0.01^{b}	1.10±0.03^{a}	1.64±0.02^{c}
Anthraquinone	0.49±0.04^{b}	0.51±0.02^{b}	0.32±0.05^{a}
Flavonoids	1.62±0.01^{b}	1.39±0.06^{a}	1.61±0.02^{b}
Phenols	0.35±0.06^{b}	0.55±0.02^{c}	0.21±0.03^{a}
Saponins	1.67±0.03^{c}	0.53±0.02^{a}	1.04±0.02^{b}
Steroids	0.41±0.02^{c}	0.23±0.02^{b}	0.16±0.02^{a}
Tannins	1.41±0.04^{b}	0.90±0.04^{a}	1.33±0.04^{b}
Terpenoids	1.61±0.02^{b}	1.17±0.03^{a}	1.73±0.02^{c}

Results are in Mean±Std of triplicate determinations. Means with the same letter in a column are not significantly different (p>0.05)

On the antimicrobial activity investigation, the crude extracts showed varied levels of activity against the microorganisms tested **(Table 2)**. The degree of the inhibitory activity of the leaf and ripe fruit peel wasintenser when compared with that of the stem bark against all the test microorganisms. The extent of the effectiveness was dependent on the level of the concentrations of the plant extracts. *Escherichia coli*, *P. aeruginosa, Salmonella enterica*, *Shigella sonnei*, *Staphylococcusaureus,* and *Streptococcus viridians* are disease-causative agents. *Pseudomonas* and *E. coli* are among the most critical group of threatening multidrug-resistant bacteria in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters [25]. Besides, severe and often lethal infections such as bloodstream infections and pneumonia can be attributed to them. Traditionally, the decoction of the *I. gabonesis* stem bark is used in treating gonorrhea, liver, and gastrointestinal disorders, in Senegal [26]. Moreover, decreased gastrointestinal motility and protection against diarrhea were reported in animal studies administered with both aqueous and methanol leaf extracts of *I. gabonesis* [27, 28]. Therefore, the effectiveness of the leaf extract of this plant against *E. coli* (12.24±0.03 mm) and *P. aeruginosa* (12.56±0.03 mm) at the concentration of 250 Mg/mm could be the confirmation for the traditional use of the leaf for diarrhea and gastrointestinal disorder treatments. Furthermore, the antidiarrhoeal potentials of plant-based tannins have extensively been reported [29, 30]. Therefore, the high tannin level (1.41±0.04%) of the leaf extract could presumably be responsible for the antidiarrhoeal effect. The leaf extract of this plant was most effective against *Salmonella enterica* at 11.81±0.02 mm, *Staphylococcusaureus* at 13.22±0.02 mm, *Streptococcus viridians* at 13.63±0.01 mm when compared with the stem bark and ripe fruit peel extracts at 250Mg/ml concentration. *Salmonella enterica* is the cause of life-threatening systemic enteric fever [31]. *Staphylococcus aureus* was reported to be the second main pathogen for deaths associated with antimicrobial resistance in 2019 [32]. Besides, an estimated 20% to 30% of the human population are long-term carriers of *S. aureus* [33, 34]. *Streptococcus viridians* are associated with sepsis and pneumonia in the neutropenic host, and sepsis and meningitis in the neonate [35]. In addition, the *I. gabonensis* leaf extract showed the highest level of inhibition against *Shigella sonnei* at all the concentrations, except at150Mg/ml, where the highest value of the inhibition was 7.67±0.01mm in the ripe fruit peel extract. Alkaloids extracted from *Sanguisorba officinalis* L. also had antimicrobial qualities against *P. aeruginosa* and *E. coli* [36]. Moreover, the extracts of *Trema guineensis* (Schumach. & Thonn.) Ficalho, *Phyllanthus discoideus* (Baill) Mull. Arg. and *Acalypha wikesiana* Mull. Arg. that are traditionally used in South-West Nigeria also showed antimicrobial effects against *E.*

coli and *S. aureus* [37]. Moreover, alkaloids, anthraquinones, flavonoids, saponins, and tannins were reported present in these plants. Similarly, the high activity of the *I. gabonensis* leaf extract against *E. coli*, *P. aeruginosa*, *Salmonella enterica*, *Shigella sonnei*, *Streptococcus viridians*, and *Staphylococcus aureus* may be attributed to the high concentrations of alkaloids (1.33±0.01%), flavonoids (1.62±0.01%), saponins (1.67±0.03%), tannins (1.41±0.04%) and terpenoids (1.61±0.02%). Hence, the effective antibacterial action of *I.gabonensis* leaf could be hugely due to the synergistic actions of these phytochemicals.

Table 2. Effects of methanol extracts of *Irvingia gabonensis* leaf, stem, and ripe fruit peel on bacterial pathogens

Concentration (Mg/ml)	Bacterial Strains	Mean Zone of Inhibition (mm) ± SD				
		Control	Leaf	Stem bark	Ripe fruit peel	p-value
100	*Staphylococcus aureus*	12.53±0.06^{d}	5.77±0.03c	3.42±0.01a	5.32±0.24b	0.001
	Salmonella enterica	11.32±0.05^{d}	4.34±0.01c	2.96±0.02a	3.91±0.11b	0.001
	Escherichia coli	11.66±0.05^{d}	4.36±0.03c	2.40±0.04a	3.86±0.03b	0.000
	Pseudomonas aeruginosa	12.03±0.05^{d}	5.52±0.021b	3.43±0.000a	5.24±0.622b	0.019
	Shigella sonnei	13.54±0.05^{d}	5.28±0.02c	3.20±0.04a	5.05±0.06b	0.000
	Streptococcus viridians	12.19±0.62b	6.31±0.01c	4.57±0.01a	6.10±0.04b	0.000
150	*Staphylococcus aureus*	14.81±0.05^{d}	7.77±0.01c	4.76±0.03a	7.54±0.03b	0.000
	Salmonella enterica	13.22±0.08^{d}	7.32±0.01c	4.45±0.01a	6.65±0.05b	0.000
	Escherichia coli	13.73±0.06^{d}	6.23±0.01c	4.04±0.02a	5.51±0.03b	0.000
	Pseudomonas aeruginosa	14.04±0.01^{d}	7.98±0.02c	5.44±0.04a	7.78±0.02b	0.000
	Shigella sonnei	14.64±0.01^{d}	7.55±0.02b	5.67±0.01a	7.67±0.01c	0.000
	Streptococcus viridians	13.22±0.6d	8.01±0.01b	5.14±0.03a	8.63±0.01c	0.000
200	*Staphylococcus aureus*	15.84±0.06^{d}	10.36±0.03c	6.40±0.01a	9.58±0.01b	0.000
	Salmonella enterica	15.42±0.09^{d}	9.38±0.01c	6.31±0.02a	8.66±0.02b	0.000
	Escherichia coli	16.77±0.06^{d}	9.26±0.00c	5.45±0.01a	7.41±0.00b	0.000
	Pseudomonas aeruginosa	15.03±0.05^{d}	11.36±0.01c	7.23±0.01a	10.01±0.08b	0.000
	Shigella sonnei	16.73±0.06^{d}	10.12±0.01c	8.66±0.02a	10.01±0.08b	0.000
	Streptococcus viridans	15.64±0.05^{d}	9.87±0.01a	9.88±0.02a	10.05±0.04b	0.013
250	*Staphylococcus aureus*	17.87±0.06^{d}	13.22±0.02c	9.32±0.02a	11.89±0.01b	0.000
	Salmonella enterica	17.52±0.09^{d}	11.81±0.02c	10.33±0.01b	11.56±0.05a	0.000
	Escherichia coli	16.77±0.05^{d}	12.24±0.03c	8.22±0.01b	10.14±0.03a	0.000
	Pseudomonas aeruginosa	17.03±0.05^{d}	12.56±0.03c	10.87±0.04b	10.87±0.04b	0.000
	Shigella sonnei	17.45±0.06^{d}	11.56±0.01b	10.54±0.03a	11.21±0.01b	0.000
	Streptococcus viridans	17.55±0.06^{d}	13.63±0.01c	11.92±0.69a	13.43±0.01b	0.041

Results are in Mean± Std of triplicate determinations. This means with the same letter in a column is not significantly different (p>0.05)

However, on the antifungal test, the effectiveness of *I. gabonensis* extracts against *A. niger, P. chrysogenum, R. stolonifer, F. oxysporum, and A. favus* was dose-dependent **(Table 3)**. All the setest microorganisms are among the disease-causing fungi. The ripe fruit peel extract of *I. gabonensis* was the most active against *A. niger* at 12.32±0.00 mm, in comparison with the other parts. The aggressive nature of *A. niger* as a causative agent of pneumonia has been demonstrated [38]. In addition, the effectiveness of the extracts of the *I. gabonensis* parts against *F. oxysporum* followed a sequence that the leaf (12.27±0.01mm)>ripe fruit peel (12.18±0.00 mm)>stem bark (11.07±0.02 mm) at 250Mg/ml concentration. *Fusarium* species have long been associated with localized infections in immunocompetent individuals [39] and circulated infections among those who are severely immunocompromised [40]. *Fusarium* species infections regularly involve the skin, either as the primary or the metastatic site [41]. The inhibitory effect of the ripe fruit peel extract (14.60±0.03 mm) at 250Mg/ml concentration was most pronounced against *P. chrysogenum* while the least was the leaf extract (12.31±0.01mm). *Penicillium chrysogenum* is often identified in immunosuppressed patients, either due to human immunodeficiency virus or from immunosuppressant medications post-transplantation [42]. Therefore, it is a rare cause of infection in immunocompetent patients. The leaf and stembark extracts of *I. gabonensis* at 250Mg/ml, inhibited the growth of *A. flavus* (13.45±0.01 mm; 13.89±0.01 mm) and *R. stolonifer* (13.21±0.03 mm; 13.40±0.02 mm) respectively. The methanol leaf extract of *I. gabonensis* at 200mg/ml had a lesser inhibitory effect against *A. flavus* at 9.95±0.01 mm and *R. stolonifer* at 9.52±0.01 mm, when compared with the effect of the ethanol leaf extract of *Dacryodes edulis* (G. Don) H. J. Lam. in previous work [43]; where the antifungal activity against *A. flavus* and *R. stolonifer* were 13.58±0.0 mm and 13.60±0.00 mm respectively. *Aspergillus flavus* is the second most common (approximately 15–20%) causative agent of invasive *Aspergillus* infections [44]. The test organisms exhibited different patterns of susceptibility to the *I. gabonensis* extracts at different concentrations. The methanol leaf, stem bark, and ripe fruit peel extracts of this plant could, therefore, be regarded as effective antifungals.

Table 3. Effects of methanol extracts of Irvingia gabonensis leaf, stem, and ripe fruit peel on fungal pathogens

Concentration (Mg/ml)	**Fungal Strains**	**Mean Zone of Inhibition (mm) ± SD**				
		Control	**Leaf**	**Stem bark**	**Ripe fruit peel**	
100	*Aspergillus niger*	16.03±0.05^{d}	5.84±0.00b	3.57±0.02a	5.92±0.04b	0.000
	Penicillum chrysogenum	17.02±0.05^{d}	5.72±0.02a	5.71±0.01a	6.29±0.01b	0.000
	Rhizopus Stolonifer	16.04±0.06^{d}	5.23±0.02a	5.59±0.01a	6.65±0.04b	0.000
	Fusarium oxysporum	17.06±0.05^{d}	4.59±0.01b	3.59±0.01a	6.20±0.14c	0.000
	Aspergillus flavus	16.43±0.06^{d}	6.00±0.03c	4.45±0.00a	5.03±0.03b	0.000
150	*Aspergillus niger*	17.83±0.05^{d}	7.56±0.021	5.45±0.01a	7.25±0.01b	0.000
	Penicillum chrysogenum	18.32±0.09^{d}	6.35±0.02a	8.05±0.03b	9.02±0.01c	0.000
	Rhizopus Stolonifer	17.76±0.05^{d}	7.23±0.00b	7.04±0.01a	9.65±0.03c	0.000
	Fusarium oxysporum	18.02±0.05^{d}	8.13±0.01b	5.14±0.01a	8.25±0.01c	0.000
	Aspergillus flavus	17.34±0.09^{d}	7.31±0.01b	5.87±0.03a	8.15±0.01c	0.000
200	*Aspergillus niger*	18.86±0.06^{b}	9.12±0.03b	8.78±0.01a	10.67±0.02c	0.000
	Penicillum chrysogenum	19.54±0.06^{d}	8.37±0.01a	11.17±0.01b	11.95±0.01c	0.000
	Rhizopus Stolonifer	18.86±0.05^{d}	9.52±0.007a	10.33±0.01b	12.40±0.01c	0.000
	Fusarium oxysporum	19.82±0.05^{d}	11.35±0.03c	7.58±0.01a	10.79±0.02b	0.000
	Aspergillus flavus	18.68±0.06^{d}	9.95±0.01a	10.64±0.02b	11.60±0.02c	0.000
250	*Aspergillus niger*	19.88±0.06^{d}	11.26±0.01a	11.52±0.01b	12.32±0.00c	0.000
	Penicillum chrysogenum	20.48±0.05^{d}	12.31±0.01a	13.35±0.02b	14.60±0.03c	0.000
	Rhizopus Stolonifer	19.78±0.06^{d}	13.21±0.03b	12.13±0.00a	13.40±0.02c	0.000
	Fusarium oxysporum	21.42±0.06^{d}	12.27±0.01c	11.07±0.02a	12.18±0.00b	0.000
	Aspergillus flavus	22.84±0.05^{d}	13.45±0.01b	12.71±0.01a	13.89±0.01c	0.000

Results are in Mean±Std of three different determinations. The same letter in a column is not significantly different (p>0.05).

Conclusion

The levels of alkaloids, flavonoids, saponins, tannins, and terpenoids were high in these parts of *I. gabonensis*; hence, this plant could be regarded as a rich source of them. The findings of this study suggested that all the compounds detected in the parts of *I. gabonensis* have antimicrobial effects. Hence, the extracts displayed various degrees of antibacterial and antifungal effects against all the test microorganisms, *in vitro*. Moreover, the effectiveness of these plant extracts increases with the increase in concentrations. The anti-staphylococcal of the leaf extract and the anti-streptococcal activities of the leaf and ripe fruit peel extracts of this plant can be further explored.

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