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RESEARCH ARTICLE

Antibacterial, Antifungal and Anti-tubercular Activities of Chloroform Fraction of the Leaf Extract of *Irvingia Gabonensis* (African Bush Mango)

Ifedolapo O. Olanrewaju[a*], Raphael C. Mordi[a] and JohnBull O. Echeme[b]

[a]*Department of Chemistry, Covenant University, KM. 10, Idiroko Road, Ota, Ogun State, Nigeria,* [b]*Chemical Sciences Department, Federal University of Technology, Umudike, Abia State, Nigeria*

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Abstract: ***Background*:** The prevalence of anti-drug resistance by disease causing microorganisms has necessitated the search for alternative sources of drugs for the treatment of the ailments caused by these microorganisms. This study examines the biological properties of extracts from the leaves of *Irvingia gabonensis* (bush mango).

***Objective*:** The objective of this study is to determine the anti-microbial activity of chloroform fraction of the leaf extract and compare it with that of clinical reference.

***Method*:** Antimicrobial activity of the chloroform fraction of the leaf extract of *Irvingia gabonensis* was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi*, *Klebsiella pneumonia*, *Salmonella paratyphi*, *Candida albicans* and *Trichophyton rubrum* by using the agar well diffusion method and *Mycobacterium tuberculosis* using agar proportion method on Lowenstein–Jensen medium. Preliminary phytochemical screening of the chloroform leaf fraction was done using qualitative standard methods.

***Result*:** This showed the presence of saponins, flavonoids, tannins, coumarin, phenol and alkaloids. Organisms were susceptible to chloroform fraction at different concentrations. The lowest MIC value obtained was 0.625mg/mL for *S. aureus* and *S. typhi*. While, five out of seven mycobacterial strains that were used, were susceptible.

***Conclusion*:** The antimicrobial activity is a result of the phytochemicals present in leaf. Therefore, we conclude that *Irvingia gabonensis* leaves can be used in the development of new pharmaceuticals research activities such as drug production.

Keywords: Bush Mango, *Mycobacterium tuberculosis,* Lowenstein–Jensen medium, anti-microbial, agar well diffusion method, anti-tubercular, M. tubercular strain H37Rv.

1. INTRODUCTION

In recent times, it has been observed globally that bacterial and fungal infections are of particular concern as a result of the increase in resistance to antimicrobial agents such as antibiotic drugs [1]. Organisms like *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Candida albicans*, *Trichophyton rubrum* and *Mycobacterium tuberculosis* are emerging in wide new varieties of strain which are Multidrug Resistant (MDR). Of course, this has made most of the drugs available ineffective. The resistance of these organisms poses a threat to the treatment of infectious diseases they cause [2]. It is, therefore, necessary that steps be taken to reduce the problems caused by the various strains of bacteria and fungi. Presently, for the development and discovery of newer drugs, many plant products are evaluated on the basis of their traditional uses. Hence, plant derived antimicrobial agents have received considerable attention from pharmacognosy because plants possess antimicrobial and antiviral activities that can combat diseases [2, 3].

Interestingly, *Irvingia gabonensis* is an ethno-medicinal plant that has been used for traditional therapeutic purposes [4, 5]. Various parts of *I. gabonensis* have found use in the treatment of a variety of ailments, for example, in the treatment of diarrhoea, gastrointestinal, liver conditions, yellow fever, relieve body pains, sterility, hernia, urethral discharge, as an antidote for poisoning and for reduction of breast-feeding period [5, 6]. It has also been found to fight obesity, reduce body fat, lower body cholesterol and control appetite. The seed has been known to reduce blood glucose levels in subjects with obesity while the bark has been reported to have analgesic effects [7, 8]. The seed extract has been reported to have diuretic effect comparable to that produced by

*Address correspondence to this author at the Department of Chemistry, Covenant University, P.M.B.1023, KM. 10, Idiroko Road, Ota, Ogun State, Nigeria, Tel: +234-803-943-3336; E-mails: ladychmife@gmail.com Ifedolapo.olanrewaju@covenantuniversity.edu.ng

acetazolamide and this effect was found to be dose dependent [9]. The leaf and root were documented to have inhibitory properties against microorganisms [10, 11].

This study therefore sought to investigate the antimicrobial properties of the chloroform fraction of the extract of I. gabonensis against human pathogens such as *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa*, *S. dysenteriae*, *K. pneumonia*, *S. paratyphi*, *C. albican*, *T. rubrum* and *M. tuberculosis*. Information from this research will assist pharmacology in pursuit of new bioactive compounds and improvement on antimicrobial drugs.

2. EXPERIMENTAL

The bush mango tree *(Irvingia gabonensis)* grows wild in the University premises and the required parts were harvested as needed.

2.1. Plant Material and Extraction

The extraction of *Irvingia gabonensis* leaves was carried out using known standard procedures [12]. Air-dried leaves were ground into powder with a mechanical grinder. The powder (300 g) was extracted in ethanol (2 L) by cold maceration for three (3) days. These were filtered out and concentrated with a rotary evaporator as crude ethanol extract. The crude extract was fractionated between chloroform and distilled water (1:1) with the aid of separating funnel. The organic chloroform layer was collected separately, concentrated as chloroform fraction and stored at 2 ^{o}C until further use.

2.2. Preliminary Phytochemical Screening

The chloroform fraction was subjected to preliminary qualitative phytochemical screening to identify the presence of saponins, tannin, phenol, alkaloids, flavonoids, triterpenoids, cardiac glycoside, steroids, anthraquinone, coumarins as described in literature [13-15].

2.3. Antimicrobial Studies

2.3.1. Collection and Maintenance of Test Organisms

The test organisms that were used for this study were all clinical isolates collected from the department of medical microbiology and parasitology, Sacred Heart Hospital, Lantoro, Abeokuta, Ogun State, Nigeria.

These isolates include; Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella paratyphi, Candida albicans and Trichophyton rubrum. Biochemical analysis such as sugar fermentation, citrate utilization, oxidase reaction, Voges Proskauer, methyl red, capsule staining, spore staining, motility, Gram staining, germ tube test, assimilation tests indole test, urease test, hydrogen sulphide test, gelatine liquefaction and gram staining reaction was carried out on each test organism. These were then kept as stock culture on slant in the refrigerator set at 4ºC [16, 17].

2.3.2. Turbidity Standard for Inoculums Preparation

McFarland standard provides research laboratory guidance for the standardization of numeral organisms for susceptibility testing or procedures requiring a standardization of the inoculums. To standardize the inoculums density for a susceptibility test using a modified method by BSAC [18].

2.3.3. Preparation of Inoculums

Inoculums are prepared according to the manufacturer's instruction for Mueller Hinton broth. This is comparable to that of the 0.5 McFarland standard or against a white background with conflicting dark line. The inoculums gave semi confluent growth of colonies after 18 hours' incubation. Denser inoculums result in a reduced zone of inhibition and lighter inoculums have opposite effect. The 0.5 McFarland standard provides turbidity comparable to 1.5 x 10^8 CFU/mL bacterial suspension [19].

2.3.4. Antimicrobial activity by agar well diffusion method

Agar well diffusion method was adopted for testing antibacterial activities of fractions. Microbial cultures of 0.5 McFarland turbidity standard was inoculated on Mueller Hinton agar plate with diameter 9 cm. Thereafter, the plates were incubated at 37 ^{o}C for 24 hours for bacteria at 28 ^{o}C and 72 hours for fungi. Antimicrobial activity was determined by measuring the zone of inhibition around each well. Duplicate test was conducted against each organism [19].

2.3.5. Determination of Minimum Inhibitory Concentration (MIC)

MIC is the lowest concentration of extracts that inhibited noticeable growth of the test organisms after 24 hours using the tube dilution method as described by the Clinical and Laboratory Standards Institute [19]. 1 mL of different concentrates (0.312 mg/mL, 0.625 mg/mL, 2.5 mg/mL, 5 mg/mL and 10 mg/mL) of chloroform fraction in nutrient broth is placed in different test tubes. A drop of the standardized bacteria was added in each test tube and was incubated at 37 ^{o}C for 24 hours. Test was carried out in triplicates for accuracy [19].

2.3.6. Anti-Mycobacterial Susceptibility Testing

Agar proportion method using Lowenstein Jensen media was employed for the susceptibility test [20, 21]. The clinical isolates of *Mycobacterium tuberculosis* (drug susceptible and drug resistant) were tested against chloroform fraction from leaves of *I. gabonensis*, alongside with two reference drugs, which are rifampicin and levofloxacin.

2.3.7. Drug Susceptibility Testing

Modified Susceptibility testing against Rifampicin, Levofloxacin and chloroform fraction of *I. gabonensis* leaf extract were performed on L–J medium, as described by Fujiki [20] and Canetti *et al*. [22].

3. RESULTS AND DISCUSSION

Several plant extracts have been reported to show activities against bacteria and fungi because extracts commonly possess antimicrobial activities as a result of the activities of plant toxicity to microorganisms. This has proven effective in the control and management of different organisms. Figs. (**1**, **2**, **3** and **4**) below show the charts obtained from the antibiotic susceptibility and MIC tests carried out on organisms with the chloroform fraction of the ethanol extracts of *I. gabonensis and its* efficacy at different concentration of fractions relative to the standards.

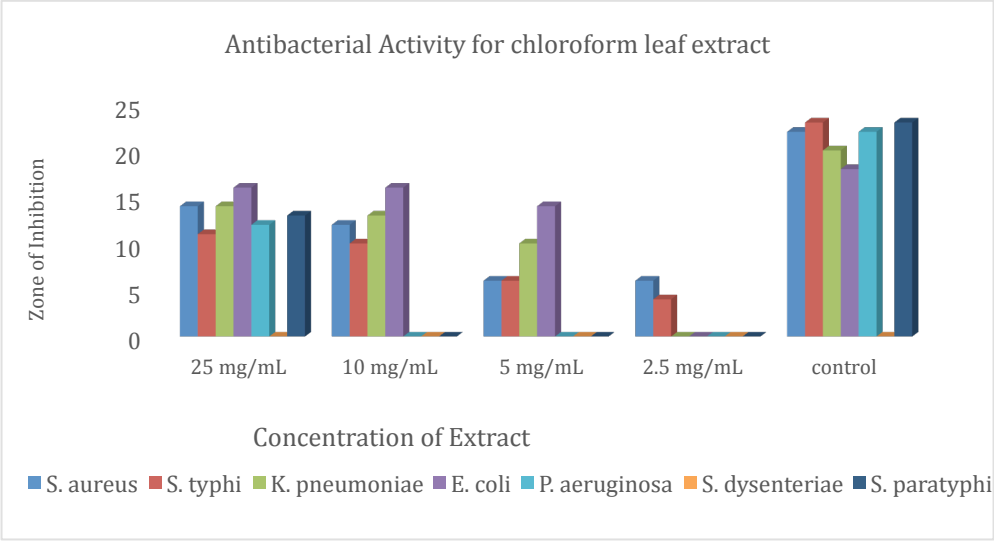

Fig. (1). Zone of inhibition for chloroform leaf extract of Irvingia gabonensis against bacteria at different concentrations.

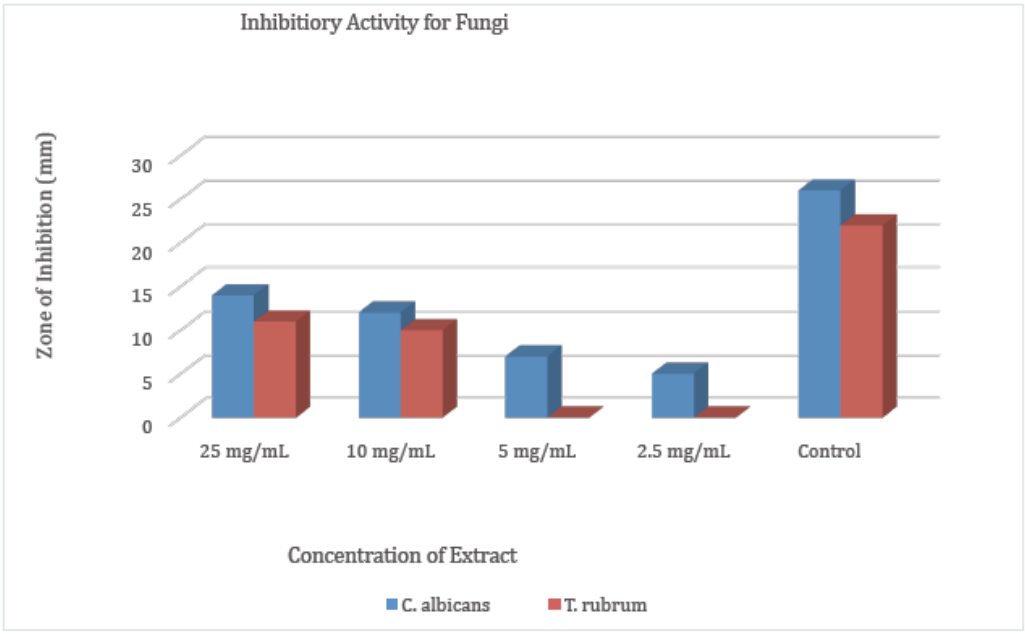

Fig. (2). Zone of inhibition of extract against C. albican and T. rubrum.

3.1. Phytochemical Analysis

The phytochemical analysis of the chloroform fraction revealed the presence of alkaloid, tannin, flavonoid, phenol, saponin and coumarin. The presence of these families of compounds in fraction confirms the results of previous studies on the extracts of *I. gabonensis* [11-29]. These phytochemicals confirm the pharmacological properties reported previously such as anti-ulcer and anti-diarrhoea [23], antioxidant [5-24], anti-inflammatory [6], anti- cancer [25] and anti-microbial activities [11- 29].

3.2. Antibacterial and Antifungal Activities

The *in vitro* antibacterial activity and MIC of chloroform fraction of the leaves of *I. gabonensis* shows activity against *S. aureus, S. typhi, K. pneumoniae, E. coli, P. aeruginosa and S. paratyphi* using agar well diffusion method Fig. (**1**). The result shows the highest antibacterial activity value against *S. typhi* at 2.5 mg/mL (4 mm) and lowest activity value for *E. coli* at 25 mg/mL (16 mm). Antifungal activity of the chloroform fraction from the leaf extract of *I. gabonensis* is reported in Fig. (**2**) against test organisms (*C. albicans* and *T. rubrum*). The result obtained reveals highest antifungal activity value against *C. albicans* at 2.5 mg/mL (5 mm) and lowest activity value for *C. albicans* at 25 mg/mL (14 mm). The MIC values obtained in this study are compared favourably with those reported in the literature for some of the test organisms [10- 29].

Figs (**3** and **4**) show the MIC charts for both bacteria and fungi against the chloroform fraction. The least concentration of the chloroform fraction that did not permit any visible growth of the inoculated test organism was regarded as the

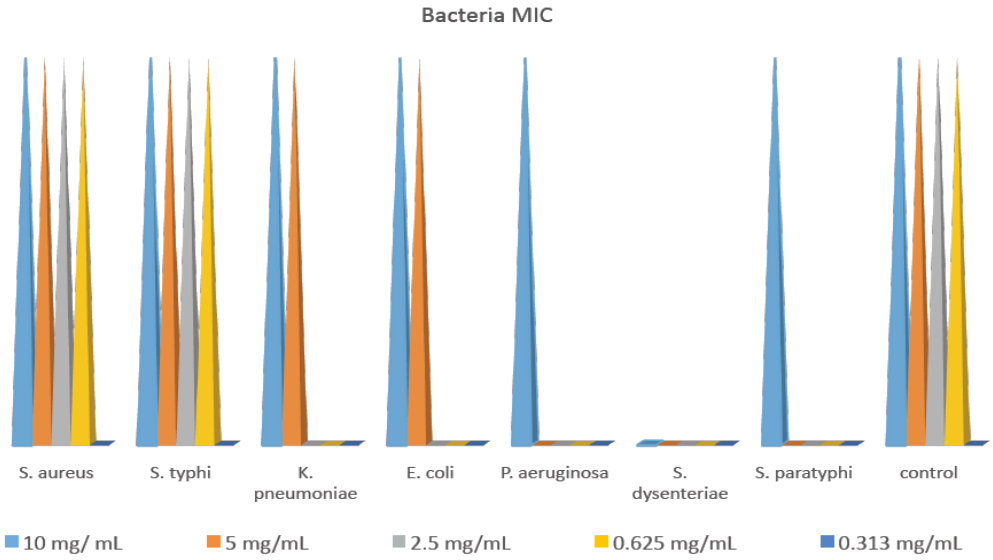

Fig. (3). MIC values at different concentration for chloroform leaf extract.

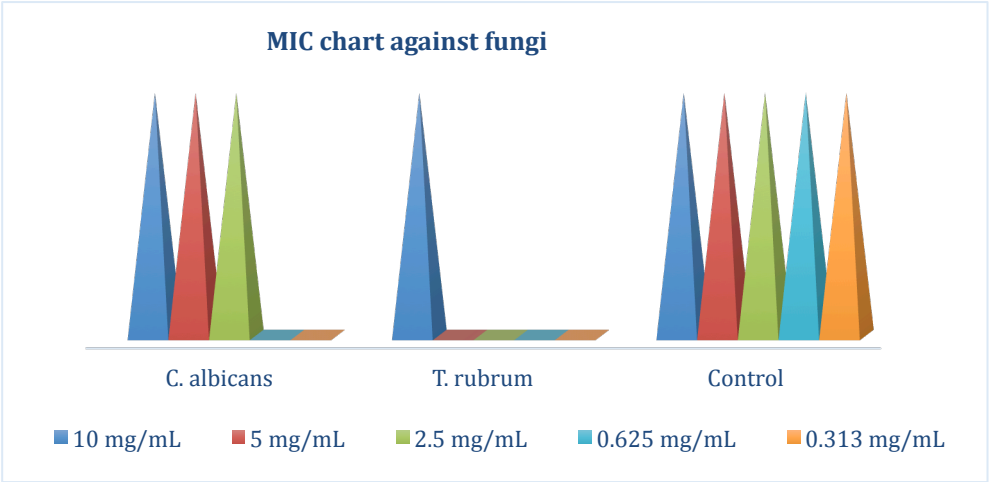

Fig. (4). MIC for extract against at different concentration standard drug, C. albican and T. rubrum.

Table 1. Anti-mycobacterial activity of chloroform Fraction.

DS-MTB 1	DS-MTB 2	DS-MTB 3	DS-MTB 4	DS-MTB 5	DR-MTB 1	DR-MTB 2	H37Rv	Rifampicin	Levoflaxacin	
S	S	S	S	S	R	R	S	S	S	-

Rifampicin 40 mg and Levofloxacin 200 mg was used as control; DS- Drug susceptible Strains; DR-Drug Resistant Strains.

minimum inhibitory concentration (MIC). Figure 3 shows that the least MIC for *S. aureus* and *S. typhi* is 0.625 mg/mL; for *K. pneumonia* and *E. coli* is 5 mg/mL and for *P. aeruginosa* and *S. paratyphi* is10 mg/mL. Also, MIC for fungi was seen at 10 mg/mL and 2.5 mg/mL for *T. rubrum* and *C. albicans* respectively as shown on Figure 4. We can safely suggest that chloroform fraction of the extract possesses toxic activity against bacteria and fungi, thereby confirming the reported claim by traditional users for the treatment of ailments caused by these organisms.

Chloroform fraction of leaf with low activity against test organism had a high MIC as in the case of *S. aureus* and *S. typhi* while active fraction gave a low MIC. This supports the claim that *I. gabonensis* contains chemical constituents that have antibacterial and antifungal activities against test organisms as reported in the literature [5-26]; making the plant a good source of antibiotic drugs.

3.3. Anti-Mycobacterial Activity

The result of anti-mycobacterial activity of the chloroform fraction on seven M. tuberculosis strains is shown in Table **1**. These strains were performed on standard L-J media method, and incubated for 42 days [20-22]. The Table shows that DS-MTB-1 to DS-MTB-5 strains and the H37Rv strain were all susceptible to the chloroform fraction of the leaf extract of *I. gabonensis*. This is favourable with the standard

drugs (Rifampicin and Levofloxacin) used. The result provides evidence that the leaves are a potential source of anti-TB agent, suggesting that the phyto-constituents responsible for the activity may be the presence in the chloroform fraction. [30]. Therefore, there is a need to isolate and elucidate the phyto-constituent(s) responsible for the anti-mycobacterial activity.

CONCLUSION

This study therefore observed that the in-vitro antimicrobial activity of the chloroform fraction against some selected organisms (*S. aureus*, *S. typhi*, *K. pneumonia*, *E. coli*, *P. aeruginosa*, *S. paratyphi C. albicans, T. rubrum* and *M. tuberculosis*) at different concentrations justifies the traditional claims and applications in disease management. To the best of our knowledge, this study reports the *in vitro* anti-mycobacterial activity of plant extract for the first time. *I. gabonensis* is used in the treatment remedy for yellow fever, diarrhoea, diabetes and wound healing [27]. Hence, with the result obtained from this investigations, the chloroform fraction of the methanolic extracts of *I. gabonensis* leaf was found to be active against some organisms, as compared to the standard drugs. This justifies the traditional use of the leaf in infectious conditions. However, before it is used by human beings, isolation of pure compound, toxicological study, and pharmacological activity should be carried out to better evaluate the potential efficiency of the chloroform fraction from ethanol crude extract as the antimicrobial agents.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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