ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2024.25

The Nigerian Journal of PHARMACY

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THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

# Bioactivity of the ethanol *extracts of Flabellaria paniculata, Rhapiostylis beninensis* roots and *Khaya ivorensis* Bark Against Multidrug-Resistance Bacteria

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ARTICLE INFO

*Article history:*

Received 28th June 2024
Revised 16th September 2024
Accepted 20th September 2024
Online Published

*Keywords:*

*Khaya ivorensis,*

*Flabellaria paniculata*,

*Rhaphiostylis beninensis,*

multidrug resistance (MDR),

phytochemical screening,

antibacterial activity.

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ABSTRACT

**Background:** Antimicrobial resistance is a global health problem that has led to the loss of effectiveness of many antimicrobial drugs, thus necessitating the continuous search for alternatives, especially from natural sources. *Flabellaria paniculata*, *Rhapiostylis beninensis,* and *Khaya ivorensis* are plants that have been used in traditional medicine to treat microbial infections, but documented evaluations of their effectiveness in scientific literature are few **Methods:** The roots of *Flabellaria paniculata*, *Rhapiostyl beninensis,* and bark of *Khaya ivorensis* were extracted by cold maceration method in 70% ethanol. The extracts were analyzed for their phytochemical content. Clinically isolated bacteria were screened for their multidrug resistance status. The antimicrobial activity of the individual plant extracts against clinical multidrug resistance (MDR) bacterial isolates consisting of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* strains was evaluated by the agar-well diffusion technique on Mueller Hinton agar. The solid dilution method was used to assess the MIC and MBC of susceptible strains.

**Results**: The phytochemical screening showed that the three plant extracts contain terpenoids, steroids, and saponins in similar quantities, while flavonoids were more abundant in *K. ivorensis* (109mg/100g), *F. paniculata* (52mg/100g), and *R. beninensis* (38mg/100g) respectively. Eleven, ten, and two of the MDR strains of bacteria tested were susceptible to the inhibitory activity of the individual extracts of *K. ivorensis, F. paniculata*, and *R. beninensis* respectively in a concentration-dependent manner. The range of MIC for *K. ivorensis* (1.56-12.5 mg/mL) was lower than for *F. paniculata* (6.25-50 mg/mL) and *R. beninensis* (12.5-50 mg/mL).

**Conclusion:** The ethanol extracts of the studied plants showed antimicrobial activity against tested MDR bacterial strains, providing evidence to justify their traditional use, and demonstrating their potential in treating bacterial infections and as a future source for newer chemotherapeutic agents.

## Introduction

Infectious diseases are responsible for 90% of the health problems globally with an annual mortality of 14million people, 90% of whom are in developing countries with Africa accounting for 68% of the deaths.[1]. Bacterial antimicrobial resistance (AMR), which happens when changes in bacteria reduce the effectiveness of the medications used to treat infections, is one of the biggest risks to public health in the twenty-first century [2,3]. The continued rise in antibiotic resistance has limited patients' options to fewer therapeutic alternatives and is responsible for the significant increase in morbidity and mortality[4]. This has been compounded by the fact that, the development of novel antibiotics has not kept up with the growth of multi-drug resistant pathogenic and environmental bacterial strains [3]. To choose the most effective antimicrobial agent to utilize against an infection agent, it is vital to ascertain the pathogen's susceptibility [5]. In many developing countries, especially African societies, the practice of using plants as medicine is widely spread. Previous studies have shown

that several African medicinal herbs possess effective antibacterial properties against Gram-negative MDR phenotypes[6]. The global demand for medicinal plants has been rising due to the increasing popularity of natural products[7]. Consequently, scientists have been focusing on the study of medicinal plants to develop as an indigenous source of novel chemicals with therapeutic potential[8].

The plant *Rhaphiostylis beninensis* known in Igbo, Yoruba, Edo, Itsekiri, and Urhobo as *Oke ikpokrikpo*, *Itá para*, *Usuende/Usumede*, *Kumeri*, and *Umeni* respectively is found primarily in Southern region of Nigeria[9], where the plant's root is used locally as a soup seasoning and condiments. The root decotion of *R. beninensis* is purportedly used in South-Western Nigeria to treat the ailment known as *Afun (vitiligo)*, which causes the entire body to turn white[10], as well as a tonic for young children between the ages of two and three [11,10]. The root has also been shown to possess anti-inflammatory, analgesic, antibacterial, cytotoxic, growth inhibition, anti-sickling, antioxidant, and aphrodisiac properties[9]. The phytochemical screening of the plant has shown that it contains flavonoids, tannins, alkaloids, phenols, saponins, phytate, and oxalate[12]. The plant *Khaya ivorensis* of the family Meliaceae commonly referred to as African mahogany is known as *Oganwo* in Yoruba and *Madaci* in Hausa. A tropical hardwood species that is native to West and Central Africa and commonly found in Angola, Gabon, Liberia, Cote d'Ivoire, Ghana, Nigeria, and Cameroon, *K. ivorensis,* has been designated as a threatened tree species[13]. The phytochemical constituents of *K. ivorensis* stem bark include alkaloids, flavonoids, phenol, saponins, and tannins [14.] The bitter bark of *K. ivorensis* which is used as a treatment regimen for coughs and whooping cough in Northern Nigeria is also combined as a mixture with black peppercorns in the treatment of diarrhoea and dysentery[15]. The bark decoction is applied topically as a lotion for rheumatic and back discomfort [15]. and to treat chest infections and coughs in the northern region of Nigeria. In contrast, seed oil is used to treat colds, stomach aches, and *Plasmodium falciparum* parasites that are multi-drug resistant[16].

*F. paniculata Cav. (Malpighiaceae*) has common names as Panicle Flabellaria and Flabellaria Vine and locally known as *Tinupogbe* in Yoruba. Previous studies conducted revealed the presence of saponins, terpenoids, and tannins in the root of *F. paniculata,* while alkaloids and flavonoids were absent[17]. *F. paniculata* is used traditionally in Nigeria for the treatment of skin infections, wound dressing, ulcers and conditions associated with pain and swelling[18].

At present, there have been cases of multi-drug resistant bacteria from blood, urine, and stool samples in the hospitals[19-21]. This is alarming and requires the right drug to be administered, hence the need for researchers to explore newer therapies that can address it. The concoction prepared from the bark of *K. ivorensis*, roots of *R. beninensis*, and *F. paniculata* is now used in the Western part of Nigeria for healing different bacteria-related diseases. There is a need to establish its antibacterial activities to utilize it as a novel therapy.

## Methods

### Collection and identification of plant materials

The roots of *F. paniculata*, *R. beninensis,* and the bark of *K. ivorensis* were collected from Olosha market, Lagos State in January, 2022. The plants were authenticated by Dr. Nodza George of the Department of Botany, University of Lagos. Specimens of the samples were deposited at the herbarium and assigned the voucher numbers LUH 9076, LUH 10056, and LUH 9055 for the bark of *K. ivorensis,* roots of *F. paniculata* and *R. beninensis* respectively.

### Preparation of Extracts

The roots of *F. paniculata*, *R. beninensis,* and the bark of *K. ivorensis* collected were air-dried at room temperature and ground into coarse powder using a miller. *K. ivoriensis* (3kg), *F. paniculata* (3kg) and *R. beninensis* (2.2kg) were individually macerated in 5, 17.5 and 9.5 L of 70% ethanol for 72 h through percolation at room temperature. The extract was filtered through cotton wool and Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator at 40 ºC

### Ethical approval

Ethical approval was obtained from the Health Research Ethics and Committee of the College of Medicine of the University of Lagos, Nigeria, in April 2022 with CMULHREC approval number:
CMUL/ACUREC/04/22/978.

### Collection of multidrug-resistant (MDR) bacterial strains

A total of twenty (20) different clinical bacterial strains isolated from patient samples with wound, blood culture, and urinary tract infections were collected from the Microbiology Laboratory Unit of Lagos University Teaching Hospital (LUTH). They comprise of

*Staphylococcus aureus (*LN001, LN029, LN033, ML477)*, Pseudomonas aeruginosa (*UR596, UR589, ML797, UR978, ML478 & ML605), *Klebsiella pneumoniae (*BC406, BC512, UR589, ML602 & BC565),*Esherichia coli (*ML475, BC 554, ML476 & UR979) and *Aeromonas spp.* (ML 225). These multidrug-resistant (MDR) bacterial isolates came from unidentified patients. All isolates were identified by GP colorimetric identification card from Vitek MS (bioM'erieux, Marcy l'Etoile, France), using automated biochemical testing[22].

**Antibiotic sensitivity testing**

Antibiotic sensitivity testing on all bacterial strains isolated from patients and reference control strains was conducted by using Kirby Bauer's disc diffusion method. As indicated by the Clinical and Laboratory Standards Institute Guidelines 2020, these investigations utilized 16 antibiotics from 6 different groups [23]. They were then transported in slant of Tryptone soy agar to the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy, College of Medicine University of Lagos, where they were subcultured on plates of sterilized Mueller Hinton agar.

**Preparation of standardized bacterial suspension**

MDR *S. aureus*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* strain bacterial colonies from an overnight culture were used to make a standardized bacterial solution. Each test organism's isolated colonies were selected using a sterile inoculating loop and suspended in 2 mL of sterile saline. The saline tube was well mixed, and the suspension's turbidity was set to 0.5 McFarland standard. After 15 minutes of preparation, the suspension was put to use[24].

**Evaluating the antibacterial properties of extracts**

The evaluation of the antibacterial activity of plant extracts was conducted using the agar well diffusion method. Each petri dish was filled with a 20 mL aliquot of Mueller-Hinton agar, which had been prepared as recommended by the manufacturers. A prepared, standardized inoculum was swabbed onto Mueller Hilton agar. A sterile cork borer with a 10 mm diameter was used to drill wells into each plate. Using a tiny pipette, 0.2 mL of variously diluted extract concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, respectively) were added to the wells. The plates were then left to stand for 15 minutes to allow the extracts to pre-diffuse before being incubated for 24 hours at 37 °C. Using a transparent plastic ruler, the inhibition zone diameter was measured and properly documented.

**Determination of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentrations (MBC) of the extracts**

Double-strength Mueller-Hinton agar and the solid dilution method were used to determine the Minimum Inhibitory Concentration (MIC) of each sample of extract. From each stock solution of the extracts, 5 mL was taken and diluted aseptically with the same volume of sterile water in a petri dish to create a stock solution of 100 mg/mL, 50 mg/ml, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL of each of the extracts. Then 100 µL of each of the standardized test bacterial isolates was added to the petri dishes as an inoculant. Additionally, each of the standardized test bacterial isolates was inoculated into double-strength Muller Hinton agar in a petri dish as the experimental control. All the petri dish cultures, including the experimental controls, were incubated at 37 °C for 24 hours and their growth was monitored. The least inhibitory concentration of the extract is defined to be the lowest concentration at which the bacterial growth was inhibited (MIC).

A minimum bactericidal concentrations (MBC) assay was performed by inoculating new nutrient agar plates with one loop-full of culture from each cultured plate that showed no growth, including the MIC plates. The plates were incubated for 24 hours at 37 °C after which the MBC values for each extracted sample were calculated using the lowest concentration of extract that killed the bacteria on the solid medium25.

**Results**

The yields of ethanol extraction of *K. ivorensis*, *R. beninensis,* and *F. paniculata* were 4.3%, 2.85%, and 4.46% respectively. The results of the antibiotic sensitivity test shown in Table 1 revealed that many of the isolates were multidrug-resistant (MDR). Specifically, six strains consisting of two *E. coli* (ML475, UR979), two *K. pneumoniae* (BC 406, UR 589), and two *P. aeruginosa* (UR978 and ML 478) were resistant to five classes of antibiotics. In addition, eleven strains made up of four *S. aureus* (LN001, LN029, LN033 and ML477), two *K. pneumoniae* (BC 512, BC 565), two *E. coli* (BC 554, ML 476), one *Aeromonas spp.* (ML 225) and two *P. aeruginosa* (ML 605, UR 424) were resistant to four classes of antibiotics. Three bacterial strains consisting of two *P. aeruginosa* (ML 797, UR 596) and one *K. pneumoniae* (ML602) were resistant to three classes of antibiotics.

**Antibacterial activity of the plant extracts**

The ethanol extracts of stem bark of *K. ivorensis* and *F. paniculata* exhibited a concentration-dependent broad-spectrum inhibition against multiple strains of test MDR bacteria (Table 2) with a pattern of the increasing zone of inhibition as the concentration increases. However, the extracts of the 2 plants had no inhibitory effect on any strain of *K. pneumoniae* (k1-k5) or *E. coli* (E1-E4). In contrast, the concentration-dependent inhibitory activity of ethanol extracts of *R. beninensis* was observed only in 2 strains of the bacterial isolates while all others were resistant to it.

The antibacterial effect of the extracts in different combinations is shown in Table 3. The zones of inhibition obtained were also concentration-dependent and reduced as the concentration of the combined extracts decreased. Combined ethanol extracts of (*K. ivorensis* + *F. paniculata*) have a broad spectrum of activity that is similar to the combination of (*K. ivorensis*+ *R. beninensis*) extracts, in terms of the number of isolates inhibited. The spectrum of activity of combined ethanol extracts of (*F. paniculata* + *R. beninensis*) was narrow, limited to only 3 of the test bacterial isolates.

**Table 1: Pattern of Antibiotic susceptibility**

| SN | | Bacterial strains | Pattern of Antibiotic susceptibility | | |
|---|---|---|---|---|---|
| | | | Antibiotics that isolates were resistant to | Antibiotics that isolates were susceptible to | Number of classes of resistant antibiotics |
| 1. | LN029 | *S. aureus* | CIP,SXT, E,DA, AZM | CN | 4 |
| 2. | LN001 | *S. aureus* | CIP,SXT, E, DA | AZM | 4 |
| 3. | ML 477 | *S. aureus* | CIP,SXT, E, DA | CN | |
| 4. | LN033 | *S. aureus* | CIP,SXT,E, DA,AZM | CN | 4 |
| 5. | ML 225 | *Aeromonas* spp | CIP,AMC,CXM,LEV | CT,MRP | 4 |
| 6. | ML 476 | *E. coli* | ETP,FEP,CIP,AMC, CAZ,CRO,CXM | CN,SXT,CT | 4 |
| 7. | UR 979 | *E. coli* | CN,CIP,CFM,AMC,CAZ,CRO,SXT | ETP,FEP,AK | 5 |
| 8 | 554 BC | *E. coli* | FEP,CN,CIP,AMC,CAZ,CRO,CXM | ETP,SXT,CT | 4 |
| 9 | ML 475 | *E. coli* | ETP,CN,CIP,AMC,CAZ,ZXT,CXM | CT | 5 |
| 10. | UR 589 | *K. pneumoniae* | CIP,AMC,CAZ,CRO,SXT,CXM | AK,CN,CT | 5 |
| 11. | BC 512 | *K. pneumoniae* | FEP,AK,AMC,CAZ,CRO,CXM,UNZ | CT | 4 |
| 12. | BC406 | *K. pneumoniae* | ETP,AK,CN,CIP,CFM,AMC,CAZ,SXT | Nil | 5 |
| 13. | ML 602 | *K. pneumoniae* | CIP,CFM,AMC,CRO,SXT,CXM,CT | ETP,AK | |
| 14. | BC 565 | *K. pneumoniae* | CN,AMC,CAZ,SXT,CXM | ETP,AK | 4 |
| 15. | ML 797 | *P. aeruginosa* | ETP,CN,CAZ | FEP,AK,CRO,CT | 3 |
| 16. | UR 978 | *P. aeruginosa* | ETP,FEP,AK,CN,CIP,CAZ | CT | 5 |
| 17. | ML 605 | *P. aeruginosa* | AK,CN,CIP,CRO | CT | 4 |
| 18. | UR 424 | *P. aeruginosa* | AK,CN,CIP,CAZ | CT | 4 |
| 19 | ML 478 | *P. aeruginosa* | ETP,AK,CN,CIP,CAZ | CT | 5 |
| 20 | UR 596 | *P. aeruginosa* | ETP,MRP,FEP,CIP | F,AK,CN | 3 |

Keys: R-resistant I-intermediate S-susceptible ( F- Nitrofurantoin, ETP- Ertapenem, MRP-Meropenom, FEP-Cefipime, AK-Amikacin, CN-Gentamycin, CIP-Ciprofloxacin, CRO-Ceftriaxone, CXM-cefuroxime, CFM-Cefixime, AMC-Amoxicillin/clavulanic acid, CAZ-Ceftazidime, SXT-Sulphamethaxazole/Trimethoprim, UNZ-Sulbactam/ampicillin, CT-Colistin, LEV-Levofloxacin, E-Erythromycin, DA-Clindamycin, LZD-Linozilid AZM-Azithromycin

**Table 2: Antibacterial activity of ethanol extracts of *K. ivorensis bark*, roots of *F. paniculata,* and *R. beninensis* against MDR bacterial strains**

| Plant extract | Concentration (mg/mL) | Inhibition zone diameter in mm | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | P1 | P2 | P3 | P4 | P5 | P6 | A | E1-E4 | K1-K5 |
| *R. beninensis* | 100 | 21 | _ | _ | _ | 19 | _ | _ | _ | _ | _ | _ | _ | _ |
| | 50 | 19 | _ | _ | _ | 17 | _ | _ | _ | _ | _ | _ | _ | _ |
| | 25 | 17 | _ | _ | _ | 14 | _ | _ | _ | _ | _ | _ | _ | _ |
| | 12.5 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| | Water | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| *K. ivorensis* | 100 | 27 | 22 | 23 | 25 | 28 | 21 | 24 | 26 | 22 | 22 | 20 | _ | _ |
| | 50 | 26 | 20 | 21 | 24 | 22 | 20 | 22 | 24 | 21 | 20 | 19 | _ | _ |
| | 25 | 23 | 18 | 19 | 23 | 20 | 18 | 18 | 21 | 19 | 19 | 15 | _ | _ |
| | 12.5 | 21 | 17 | 18 | 20 | 18 | 15 | 16 | 18 | 18 | 18 | _ | _ | _ |
| | Water | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| *F. paniculata* | 100 | 23 | 20 | 20 | 24 | 24 | 22 | 21 | 22 | 20 | 20 | _ | _ | _ |
| | 50 | 20 | 18 | 18 | 23 | 17 | 19 | 19 | 18 | 18 | 18 | _ | _ | _ |
| | 25 | 18 | 17 | 17 | 22 | 15 | 15 | 16 | 16 | 16 | 16 | _ | _ | _ |
| | 12.5 | 16 | 16 | 15 | 18 | 13 | _ | _ | _ | 15 | 15 | _ | _ | _ |
| | Water | _ | | _ | _ | _ | _ | _ | _ | _ | _ | _ | | _ |

Keys: P- *P. aeruginosa,* K- *K. pneumoniae,* E- *E. coli,* S- *S. aureus,* A- *Aeromonas spp.*
(-)- No inhibition

Table 3: Antibacterial activity of combined ethanol extracts from the bark of *K. ivorensis*, roots of *F. paniculata,* and *R. beninensis* against MDR bacterial strains

| Plant extracts | Concentration (mg/mL) | Inhibition zone diameter (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | P1 | P2 | P3 | P4 | P5 | P6 | A | E1-E4 | K1-K5 |
| *K. ivorensis + F. paniculata* | 50/50 | 26 | 24 | 23 | 27 | _ | _ | 19 | _ | 22 | 19 | 17 | _ | _ |
| | 25/25 | 24 | 22 | 20 | 24 | _ | _ | 15 | _ | 20 | 18 | 15 | _ | _ |
| | 12.5/12.5 | 21 | 17 | 19 | 20 | _ | _ | 13 | _ | 18 | 17 | _ | _ | _ |
| | 6.25/6.25 | 19 | 15 | 18 | 19 | _ | _ | 12 | _ | 16 | 15 | _ | _ | _ |
| | Water | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| *F. paniculata +R.beninensis* | 50/50 | 19 | 19 | 21 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| | 25/25 | 15 | 17 | 20 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| | 12.5/12.5 | 14 | _ | 18 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| | 6.25/6.25 | _ | _ | 16 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| | Water | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| *K. ivorensis + R. beninensis* | 50/50 | 25 | 20 | 17 | 24 | _ | _ | 19 | _ | 20 | 20 | 19 | _ | _ |
| | 25/25 | 23 | 18 | 16 | 20 | _ | _ | 16 | _ | 19 | 16 | 14 | _ | _ |
| | 12.5/12.5 | 22 | 16 | 13 | 19 | _ | _ | 13 | _ | 18 | 15 | 12 | _ | _ |
| | 6.25/6.25 | 19 | _ | _ | 17 | _ | _ | _ | _ | 16 | 14 | _ | _ | _ |
| | Water | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |

Keys: P- *P. aeruginosa*, K- *K. pneumoniae*, E-*E. coli*, S- *S. aureus*, A- *Aeromonas spp*.
(-)- No inhibition

**Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanol extracts from the bark of *K. ivorensis*, roots of *F. paniculata* and *R. beninensis***

The minimum inhibitory concentration (MIC) of the ethanol extracts of the 3 plants and the combined extracts against the different MDRs tested are reported in Table 4. The MIC values range from 1.56 to 25mg/ml for *K. ivorensis,* 6.25 to 50mg/ml for *F. paniculata,*12.5 to 50mg/ml for *R. beninensis*, 3.125 to 25mg/ml for combined (*K. ivorensis* + *F. paniculata*), 6.25 to 25mg/ml for combined (*K. ivorensis*+ *R. beninensis*) and 25 to 50mg/ml for combined (*F. paniculata* + *R. beninensis*). The minimal bactericidal concentration (MBC) against various MDR organisms, was computed following the MIC determination, and the findings are shown in Table 5. The MBC values obtained varied from 1.56 to 12.5mg/ml for *K. ivorensis*, 6.25 to 50mg/ml for *F. paniculata*, 12.5 to 50mg/ml for *R. beninensis*, 3.125 to 25mg/ml for combined (*K. ivorensis* + *F. paniculata*), 6.25 to 25mg/ml for combined (*K. ivorensis*+ *R. beninensis*) and 25 to 50mg/ml for combined (*F. paniculata* + *R. beninensis*)

**Table 4: Minimal Inhibitory Concentration (MIC) of ethanol extracts of bark of *K. ivorensis*, roots of *F. paniculata,* and *R. beninensis***

| Bacterial strains | Plant extracts and MIC (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | *F. paniculata* | *K. ivorensis* | *R. beninensis* | *F. paniculata* + *K. ivorensis* | *F. paniculata* + *R. beninensis* | *R. beninensis*+ *K. ivorensis* |
| P1 | 12.5 | 6.25 | 25 | 12.5 | 50 | 12.5 |
| P5 | 12.5 | 3.125 | 25 | 6.25 | 25 | 6.25 |
| P6 | 6.25 | 1.56 | 25 | 3.125 | 25 | 6.25 |
| *S2* | 6.25 | 3.125 | 12.5 | 6.25 | 25 | 6.25 |
| S3 | 12.5 | 25 | 50 | 12.5 | 50 | 25 |
| S1 | 50 | 12.5 | 25 | 25 | 50 | 25 |
| A | 25 | 3.125 | 50 | 25 | 50 | 12.5 |

Keys: P= *P. aeruginosa*, S= *S. aureus*, A= *Aeromonas Spp*

**Table 5: Minimum bactericidal concentration (MBC) of ethanol extracts from the bark of *K. ivorensis*, roots of *F. paniculata,* and *R. beninensis***

| Bacterial strains | Plant extracts and MBCs (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | *F. paniculata* | *K. ivorensis* | *R. beninensis* | *F. paniculata*+ *K. ivorensis* | *F. paniculata* +*R.beninenss* | *R. beninensis*+ *k. ivorensis* |
| P1 | 50 | 6.25 | 25 | 25 | 50 | 25 |
| P5 | 12.5 | 3.125 | 50 | 6.25 | 50 | 6.25 |
| P6 | 6.25 | 1.56 | 50 | 3.125 | 25 | 6.25 |
| S2 | 50 | 6.25 | 50 | 12.5 | 25 | 6.25 |
| S3 | 12.5 | 25 | 50 | 25 | 50 | 25 |
| S1 | 25 | 12.5 | 12.5 | 25 | 50 | 25 |
| A | 50 | 6.25 | 25 | 25 | 50 | 12.5 |

Keys: P= *P. aeruginosa*, S= *S. aureus*, A= *Aeromonas Spp*

**Discussion**

The outcome of the susceptibility testing of the test clinical isolates to the various conventional antibiotics confirmed that many of them are multidrug resistant. Six of the isolates (2 each of *E. coli*, *K.pneumoniae* and *P. aeruginosa* strains) were resistant to five classes of antibiotics, eleven isolates (4 *S. aureus*, 1 *Aeromonas spp* and 2 each of *E. coli,K. pneumonia* and P. aeruginosa strains) to four classes and three isolates (1 *K.pneumoniae*,and 2 *P. aeruginosa* strains) were resistant to three classes. This finding highlights the problem of multidrug resistance among bacterial strains causing clinical infections and emphasizes the need to search for alternative effective therapeutic agents, especially from plant sources.

Despite the reported use of the extracts of the studied plants in traditional medicine as a treatment regimen for bacterial infections, few studies have been carried out to evaluate their antibacterial effects scientifically. The antibacterial activity study showed that individual ethanol extracts of *K. ivorensis* and *F. paniculata* had inhibitory activity on all the bacterial strains tested. All the bacterial strains were susceptible to extracts of *K. ivorensis* and *F. paniculata* in a concentration-dependent manner as reflected by the zones of inhibition which increase with increasing concentration of the extracts. The ethanol extracts of *K. ivorensis and F. paniculata* thus exhibited a broad spectrum of antibacterial activity against multiple MDR bacterial strains tested. These findings agree with that of26 where medicinal plants exhibited a wide range of antibacterial activity against bacterial pathogens known to cause both skin and wound infections. However, the ethanol extracts of *R. beninensis* showed a narrow spectrum of antibacterial activity because only two of the seven bacterial strains were susceptible to it while five were resistant. The antibacterial potency of the extracts of the 3 plants was also reflected in their MIC values against susceptible organisms which range from 1.56 to 25mg/ml for *K. ivorensis,* 6.25 to 50mg/ml for *F. paniculata,* and 12.5 to 50mg/ml for *R. beninensis*. The MIC values indicate that the order of antibacterial activity of the extracts is *K. ivorensis* > *F. paniculata* > *R. beninensis* which shows that the lowest concentration required to inhibit bacterial growth increases from extracts of *K. ivorensis* to *F. paniculata* to *R. beninensis*. The extracts of *K. ivorensis* had MBC values ranging from 1.56 mg/ml to 6.25 mg/ml in six out of the seven bacterial strains used which signifies that it is not only bacteriostatic at low concentration but also bactericidal. The antibacterial effect of *K. ivorensis* observed was in agreement with the studies by27 on *S. aureus* which gave the best activity as compared with other organisms used. The extracts of *F. paniculata* with MBC ranges from 6.25 mg/ml to 50 mg/ml showed moderate bactericidal activity compared to *R. beninensis* with MBC ranges from 12.5 to 50 mg/ml in all the susceptible bacterial strains tested, indicating that it has bactericidal effects only at high concentrations. The result of *F. paniculata* is in concord with the studies conducted by 28 which had antibacterial activity on *P. aeruginosa* and *S. aureus*, but was inert against *E. coli* and *K. pneumonia* while the results of studies conducted on *R. beninensis* by 29 was in partial agreement as the extract was active on both *S. aureus* and *E. coli* while in this current studies, its only effective on *S. aureus*. To determine the value of using the extracts of the plants together as an herbal potion as it is sometimes done in folk medicine, the antibacterial activity of the combined extracts was evaluated. The zones of inhibition observed for a combination of the extracts of *K. ivorensis* and *F. paniculata* compared to individual extracts of the plants did not show that the combined extracts produced a synergistic effect as expected but rather a concentration-dependent activity on only three strains of *S. aureus* (S2, S3, and S4). However, a combination of either extract of *R. beninensis* and *K. ivorensis* or *F. paniculata* and *R. beninensis* produced zones of inhibition that showed less inhibitory activity on all the tested bacterial strains. The MIC of the combination *K. ivorensis* and *F. paniculata* extracts against three out of seven bacterial strains (one *S. aureus* and two *P. aeruginosa*) used, decreased by half when compared with the MIC of *F. paniculata* alone, suggesting that there may be synergism between *K. ivorensis* and *F. paniculata* against those 3 isolates. Against three of the seven bacterial strains (one *P. aeruginosa* and two *S. aureus*), the MIC of a combination of *K. ivorensis* and *R. beninensis* extracts was reduced by half, and by a quarter in another three of the seven strains (two *P. aeruginosa* and one *S. aureus*). However, it was still higher than the MIC for *K. ivorensis* extracts alone against all the bacterial strains. This may suggest that the reduction in the MIC values for the combined extracts is being driven by the strength of the antibacterial activity of *K. ivorensis* extracts. The enhanced antibacterial effect expected when two antibacterial agents are combined was not evident in this study. This may suggest that there may be no benefit in combining the extracts of the two plants in a single herbal concoction to treat bacterial infections. On the other hand, the MIC of combined extracts of *F. paniculata* and *R. beninensis* against the bacterial isolates increased when compared to the MIC of their extracts. This may depict that an antagonistic antibacterial effect exists between the

phyto-constituents of the extracts of *F. paniculata* and *R. beninensis*. This evidence put in doubt the value of using the extracts of the two plants together in an herbal remedy for the treatment of bacterial infections. The antagonistic report of extract combination in this study did not concur with several documentations on the synergistic activities of several extract combinations against both human and plant pathogens 21,30,31. This could be attributed to the geographical factor and the presence of an antagonistic compound(s) in one of the extracts.

The MBC values for combined extracts of *K. ivorensis* and *F. paniculata (*ranges from 3.125 mg/ml to 25 mg/ML), combined extracts of *R. beninensis* and *K. ivorensis (*6.25 mg/ml to 25 mg/ml), and combined extracts of *F. paniculata* and *R. beninensis (*25 mg/ml to 50 mg/ml), imply that, in terms of bactericidal activity, the extracts of combined *K. ivorensis* and *F. paniculata* > combined extracts of *R. beninensis* and *K. ivorensis* > combined extracts of *F. paniculata* and *R. beninensis* in that order. The most significant bioactive components of plants are obtained from alkaloids, tannins, flavonoids, and phenolic compounds which are secondary metabolites, in line with other studies 32,33. The present study reveals that *K. ivorensis* and *F. paniculata* have tannins, flavonoids and phenol which may explain why the antibacterial activity of *K. ivorensis* and *F. paniculata* extracts was better compared to *R. beninensis* which has only flavonoids.

Compared to contemporary antibiotics, the extracts of *K. ivorensis* displayed broad-spectrum bactericidal activity against both Gram-negative and Gram-positive bacteria (*P. aeruginosa*, *S. aureus*, and Aeromonas) more than the extracts of *F. paniculata*, which was active against *P. aeruginosa* and *S. aureus* and extracts of *R. beninensis* with less activity on only one strain of *S. aureus* and one strain of *P. aeruginosa*.

**Conclusion**

The antimicrobial assay of the extracts reveals that *K. ivorensis* has better antibacterial activity, followed by *F. paniculata,* and then *R. beninensis.* The combined extracts of *K. ivorensis and F. paniculata* produce synergistic effects while *R. beninensis* plus *F. paniculata* show antagonism in the tested organisms. The overall result suggests that combining the extracts of *R. beninensis* with either of the other plants does not have significant synergistic or additive antibacterial effect. This overall result presents baseline information for the potential development of novel therapeutic antimicrobial agents from extracts of *K. ivorensis* and *F. paniculata* capable of dealing with specific diseases of MDR *S. aureus, Aeromonas spp.,* and *P. aeruginosa*.

**Conflict of Interest**

The authors declare that there is no conflict of interest for the publication of this article.

**Acknowledgement**

The authors want to acknowledge the support of the Staff of the Microbiology Department at Lagos University Teaching Hospital (LUTH) Lagos, Technologist in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Lagos, Idi-Araba.

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