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Short Communication

Antimicrobial Activities of Irvingia gabonensis Leaf and Cyperus esculentus Extracts on Some Selected Isolates

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Abstract

Bacterial resistance to antibacterial drugs in the treatment of some bacterial infections have become a menace that is causing untold health challenges to patients. The antibacterial activity of ethanolic extract of tiger nut (*Cyperus Esculentus*) and Bush mango (*Irvingia Gabonensis*) against candida albicans, Echerichia coli, Pseudomonas sp, Salmonella sp and Staphylococcus aureus was evaluated using agar-well diffusion, Minimum Inhibitory Concentration (M.I.C) and Minimum Bacteriocidal Concentrations (M.B.C) methods. Echerichia coli had the highest total colony count of 1.3 X 10^{11} Colony forming unit/ml(Cfu/ml) followed by *Pseudomonas sp* (8.8 X 10^{10}Cfu/ml) and *Staphylococcus aureus* (6.7 X 10^{10}) at 1.0E-09 dilution, but in the 1.0E-10 dilution, Pseudomonas sp was highest with 9.7 X 10^{11}Cfu/ml followed by E. coli (9.0 X 10^{11}Cfu/ml).The result from the comparative agar well diffusion assay of Tiger nut and *Irvingia* crude extracts showed that the extracts have antimicrobial activity against some of the test isolates. *Pseudomonas spp.* were susceptible to the tiger nut extract (10% and 20%) with the diameter of Zone of Inhibition (ZOI) of 18.0±0mm and 18.5±1.5mm. While *Echerichia coli, Pseudomonas sp, Salmonella sp* and *Staphylococcus aureus* showed susceptibility to the *Irvingia* (10%) crude extract with ZOI diameters of 13±2mm, 15.5±0.5mm, 16.5±1.5mm and 16.5±0.5mm respectively. Pseudomonas also exhibited susceptibility to the Tiger nut (20%) crude extract having a ZOI diameter of 18.5±1.5 mm while *E.coli, Pseudomonas spp, Salmonella spp* and *Staphylococcus aureus* were susceptible to Irvingia (20%) crude extract, revealing ZOI diameters of 15.5±0.5 mm ,19±0 mm,18±1 mm and 15±0 mm respectively The *Minimum Inhibitory Concentration* (*MIC*) of both extracts was 50% for all organisms, while the *Minimum Bacteriocidal Concentration* (*MBC*) of both crude extracts was 50% for Staphylococcus aureus only. The dilution factors and total colony counts from 1.0E-09 and 1.0E-10 dilutions of 5 test isolates. where statistically analyzed with ANOVA, and the P. values where 0.13 and 0.4 respectively were significant.

Keywords: Bush mango; Tiger nut; antibacterial activity; Zone of inhibition; Ethanolic extract

Background

The rise of antibiotic resistant microorganism is one of the severe problems in health care system of the world and infectious diseases are the second most serious causes of death worldwide. Thus, it is essential to find new compounds that have antimicrobial properties by screening plant species to detect those that can synthesize new drugs [1]. Thus, this study is carried out to determine the antimicrobial activity of bush mango and tiger nut extracts on some selected microorganisms.

Tiger nut (*Cyperus esculentus*) is a tuber that is consumed widely in Nigeria and in various other parts of West and East Africa [2]. It is eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage [3].Tiger nuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate and are rich in sucrose (17.4-20.0%), fat (25.5%), protein (8.0%). Tiger nut is also rich in mineral elements such as sodium, calcium, potassium, magnesium, zinc and traces of copper [4].

Bush mango (*Irvingia gabonensis*) belongs to the *Irvingiaceae* plant family [5]. Bush mango leaf/root extracts have documentary inhibitory activity against several bacteria and fungi. It possesses antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus* [6]. Tiger nuts and bush mango have antibacterial activity against *Salmonella, Escherichia coli. Candida albicans, Staphylococcus aureus* and *Pseudomonas* [7].

Methods

Two hundred and twenty-five (225 grams) each of processed plant materials (*Irvingia gabonesis* and Tiger nut powders) were

soaked differently in 400ml of 80% absolute ethanol in 500ml conical flasks. The preparation was processed further using the model of [8] with slight modification. Plant extract filtrate of 10%, 20% and 50% v/v of *Irvingia gabonesis* and Tiger nut was prepared using method by [9] with slight modification.

Bacterial test colonies were introduced into 5mls of tryptic soy broth, incubated overnight at 37°C and centrifuged. Followed by standardization and serial dilution using stardard microbiological procedures. Agar well diffussion assay was done using double strenght Mueller Hinton Agar (MHA), and five wells were made with the aid of a steriized cork borer 6mm in diameter. Molten nutrient agar was dispensed to prevent plant extract diffusion to the next well, 200 microlitres of the (*Irvingia* a. extract 10%, 20%, & 50%) were dispensed into the various holes and *Cyperus esculentus* (Tiger nut 10%, 20%, & 50%) was also pipetted into various holes in another MHA plate. Distilled water was used as Negative control and Augumentin was used as positive control. The experiment was done in duplicates and the plates were kept for about 1hour at room temperature and incubated at 37% for 18-24hours. The diameter of zones of inhibition was measured after incubation [8].

The model of [8] was adopted with slight modification for minimum inhibitory concentration. Ten milliliter (10ml) volume of double strength molten Mueller Hinton agar after autoclaving at 45°C was dispensed into sterile universal bottle and 10ml of the *Irvingia gaboonesis* plant extract was added. Equal volume of the test plant extract in graded concentration of 20%, 30%, 50% and 100% was made. These were poured aseptically into sterile petri-dishes and were allowed to solidified at room temperature, for one hour with the lid of the petri-dishes slightly raised.

Twenty microlitre of standardized test bacteria were impregnated/ inoculated aseptically on the sterilized Whatman no 1 filter paper discs, placed on the agar surface at equidistance in duplicate for each concentration of the test plant extracts.

These were incubated at 37°C for 18-24hours. The minimum inhibitory concentration value was taken as the least concentration of the plant extract at 50%, showing no detectable growth.

Augmentin was used as standard antibiotic. The minimum bacteriocidal was determined by transferring inoculated bacterial discs into a sterile 3ml recovery of Luria Bertani broth from the plant extract concentration that showed no visible growth from the M.I.C determination.

These were incubated at 37^{oc} for 72hours. The least concentration of the plant extracts that showed no bacterial growth in the recovery liquid medium were 50% was taken as the M.B.C.

Results

(Figure 1) shows Total Colony Count of Isolated organisms. The graph depicts the highest total colony count by *E. coli* (1.3 X 10^{11} Colony forming unit/ml) followed by *Pseudomonas spp* (8.8 X 10^{10}Cfu/ml) and *Staphylococcus aureus* (6.7 X 10^{10}) at 1.0E-09 dilution, but in the 1.0E-10 dilution, *Pseudomonas spp* was highest with 9.7 X 10^{11}Cfu/ml followed by *E. coli* (9.0 X 1011Cfu/ml).

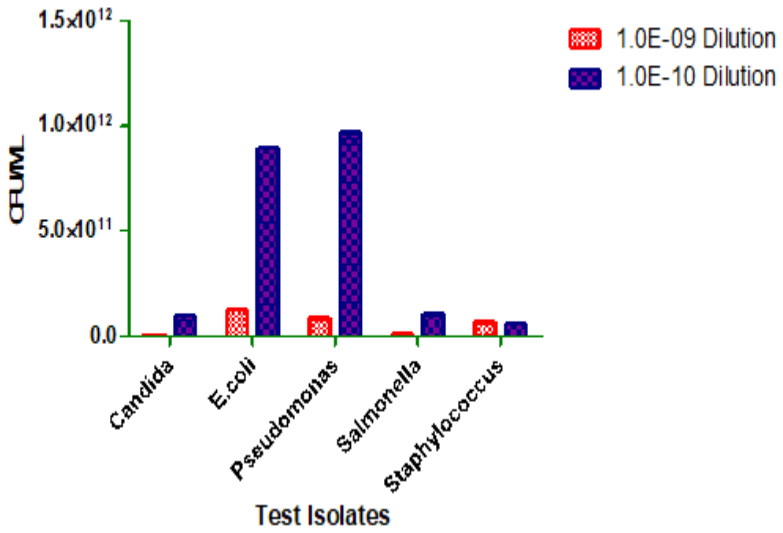

Figure 1: Total Colony Count of Test Isolates.

(Table 1) shows the Comparative Agar well diffusion assay of Tiger nut and *Irvingia* Crude Extracts against Test Isolates. The result of the susceptibility test of the organisms to the extracts showed that the extracts had antimicrobial activity against some of the test isolates. *Pseudomonas spp* showed antimicrobial susceptibility to the Tiger nut (10%) crude extract with a zone of inhibition (ZOI)

diameter of 18.0±0 mm while *Echerichia coli*, *Pseudomonas spp*, *Salmonellaspp* and *Staphylococcus aureus* showed susceptibility to the *Irvingia* (10%) crude extract with ZOI diameters of 13±2mm, 15.5±0.5mm, 16.5±1.5mm and 16.5±0.5mm respectively. *Pseudomonas* also exhibited susceptibility to the Tiger nut (20%) crude extract having a ZOI diameter of 18.5±1.5 mm while *E.coli*, *Pseudomonas spp*, *Salmonella spp* and *Staphylococcus aureus* were susceptible to *Irvingia* (20%) crude extract, revealing ZOI diameters of 15.5±0.5 mm ,19±0 mm,18±1 mm and 15±0 mm respectively. All test isolates showed resistance to the both 50% crude extracts of Tiger nut and *Irvingia*.

Table 1: Comparative Agar well diffusion assay of Tiger nut and *Irvingia* Crude Extracts against Test Isolates.

Organism	Tiger nut (10%)	Tiger nut (20%)	Irvingia (50%)	Irvingia (10%)	Irvingia (20%)	Irvingia (50%)	Augumentin	D/H_2O (50%)
Candida	5.0±5 **(R)**	0 **(R)**	0.5±0.5 **(R)**	11.5±0.5 **(I)**	12.5±0.5 **(I)**	5±0 **(R)**	30±0 **(S)**	0 **(R)**
E. coli	0 **(R)**	0 **(R)**	1.5±0.5 **(R)**	13±2 **(S)**	15.5±0.5 **(S)**	7.5±0.5 **(R)**	22.5±2.5 **(S)**	0 **(R)**
Pseudo	18.0±0 **(S)**	18.5±1.5 **(S)**	6.0±1 **(R)**	15.5±0.5 **(S)**	19±0 **(S)**	6.5±1.5 **(R)**	17±0 **(R)**	0 **(R)**
Salmonella	0 **(R)**	10.0±0 **(I)**	.0±1.4 **(R)**	16.5±1.5 **(S)**	18±1 **(S)**	5±0 **(R)**	0 **(R)**	0 **(R)**
Staphylococcus	0 **(R)**	0 **(R)**	2.5±2.5 **(R)**	16.5±0.5 **(S)**	15±0 **(S)**	4.5±0.5 **(R)**	20±0 **(R)**	0 **(R)**

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC).

Test Organisms	MIC (vol/vol)		M.C.B (vol/vol)	
	Tiger Nut	*Irvingia*	Tiger Nut	*Irvingia*
Candida	50	50	growth	growth
E.coli	50	50	growth	growth
Pseudomonas	50	50	growth	growth
Salmonella	50	50	growth	growth
Staphylococcus	50	50	growth	growth

(Table 2) shows the Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC). The Minimum inhibitory concentration value from the results obtained was the 50% concentration of the Tiger nut and *Irvingia* crude extracts. The Minimum bacteriocidal concentration of both crude extracts was also the 50% concentration for only *Staphylococcus aureus* as other isolates.

Values are inhibition zone diameters (ZOI) expressed as mean ± Standard deviation; Resistant(R) =ZOI ≤ 8mm; Intermediate (I) =ZOI =9-12mm; Susceptible(S) =ZOI ≥ 13mm; %= volume per volume concentration; Augumentin= positive control; Distilled water= negative control. *Pseudo=Pseudomonas sp*

The Minimum inhibitory concentration value from the results obtained was the 50% concentration of the Tiger nut and *Irvingia* crude extracts. The Minimum bacteriocidal concentration of both crude extracts was also the 50% concentration for only Staphylococcus aureus as other isolates grew in recovery broth.

Data between the groups were analyzed using Graph pad Prism software with ANOVA from results of total plate counts of 1.0E-09 and 1.0E-10 dilutions of 5 test isolates. The Dilution factors and Total colony counts for the isolates accounted for 27.77% and 42.63% of the total variance respectively; and a P value of 0.13 and 0.4 respectively that are considered not to be significant.

Discussion

Tiger nut (*Cyperus esculentus*) and *Irvingia gabonensis* (bush mango) have medicinal properties which have been harnessed by traditional medicine practitioners, but only a few of these properties have been proven scientifically. However, in this present study, only the tubers and leaves of *Cyperus esculentus* and *Irvingia* gabonensis were utilized. The results from the Comparative Agar well diffusion assay of Tiger nut and *Irvingia* Crude Extracts revealed that the extracts have antimicrobial activity against some of the test isolates. *Pseudomonas spp* showed antimicrobial susceptibility to the Tiger nut crude extract concentration of 10% and 20% with a Zone of Inhibition (ZOI) diameter of 18.0±0mm and 18.5±1.5mm respectively. *Echerichia coli*, *Pseudomonas spp*, *Salmonella spp* and *Staphylococcus aureus* showed susceptibility to the *Irvingia gabonensis* crude extract concentration of 10% and 20% with ZOI diameters of 13±2mm, 15.5±0.5mm, 16.5±1.5mm, 16.5±0.5mm and 15.5±0.5mm, 19±0mm, 18±1mm and 15±0mm respectively. The findings are in agreement with the study conducted by (1) who reported that *Escherichia coli* and *Staphylococcus aureus* were susceptible to *Irvingia gabonensis* crude extracts with the diameter of zones of inhibition ranging between 8mm-23mm for ethanolic ex-

tract. The result also confirms the study by [10] who reported Pseudomonas aeruginosa and Staphylococcus aureus are susceptible to tiger nut from concentrations of 10-250mg/mL and 125-250mg/ml respectively.

The study further revealed that the Minimum Inhibitory Concentration (MIC) value for *Candida*, *E.coli*, *Pseudomonas spp*, and *Salmonella spp* was 50% concentration of the Tiger nut and *Irvingia* crude extracts. While the Minimum bacteriocidal concentration (MBC) of both crude extracts was 50% concentration for *Staphylococcus aureus* only [1] reported that the Minimum Inhibitory Concentration (MIC) of *Escherichia coli* and *Staphylococcus aureus* ranged between 6.25mg/ml-50mg/ml, while the Minimum Bactericidal Concentration (MBC) ranged between 12.5mg/ml-50mg/ml (Seukep et al. 2013) reported that the *Cyperus esculentus* exhibited antimicrobial activities depending of bacteria strains, with minimal inhibitory concentrations (MICs) values ranging from 64 to 1024 µg/ml.

Conclusion

Tiger nut (*Cyperus esculentus*) and bush mango (*Irvingia gabonensis*) contains chemical constituents which possess antibacterial activity against Candida, albicans, *Echerichia coli*, *Pseudomonas spp*, *Salmonella spp* and *Staphylococcus aureus* the causative agents of several infections. The chance to find antimicrobial drugs were apparent on both extracts, therefore, the plant could be a source of new antibiotics for treatment of diseases. Exploration of Bush mango and Tiger nut can have an economic impact in marketing and employment in Africa. Tiger nut and Bush mango is a source of raw materials for fruit juices, and it can also improve the treatments of microbes that are resistant to antibiotic currently in use.

The abundance of bush mango and tiger nut plants in Nigeria, and Africa continent should be explored because both plant extracts possess antimicrobial activity and may reduce the drug resistant and cost of treatment diseases. It is therefore recommended.

Pharmaceutical companies should explore it, not only for Antimicrobial activity. But also, food and beverages companies should explore it's for the production of fruit juices because of it nutritional benefits to humans. More research work should be done on Bush mango and Tiger nut.

Conflict of Interest

There was no conflict of interest in the course of carrying or reporting this study.

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