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Antimicrobial activities of *Irvingia gabonensis* Leaf against diarrhoea Causing Agents

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Abstract*—Antimicrobial effect of Irvingia gabonensis leaf extracts was tested against bacteria and fungal agents that causes diarrhoea (Salmonella typhi, E.coli, Staphylococcus aureus, Shigella dysenteriae and Candida albican). Fresh tender leaves of Irvingia gabonensis was collected, air-dried, grounded and soarked in n-hexane, methanol and aqueous solvents. The Antimicrobial activities were determined using agar well diffusion assay, then the MIC, MBC and MFC were determined by agar dilution assay. The results revealed that the crude methanol and n- hexane extracts of Irvingia gabonensis produced the highest antifungal effects against Candida albicans with the MIC of 25 mg/ml and MFC of 50 mg/ml. The crude aqueous extract of I.gabonensis had no antifungal activity on Candida albicans. The crude methanol extracts of Irvingia gabonensis produced the highest antibacterial effects with MIC ranged of 6.25 mg/ml to 25 mg/ml and MBC of 12.5 mg/ml to 50 mg/ml. These results suggest that Irvingia gabonensis leaf extract is recommended as a diarrhoea disease remedy.*

***Keywords*— *Antimicrobial activity, Irvingia gabonensis, diarrhoea*.**

I. INTRODUCTION

The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to synthetic drugs (1). Emergence of resistant strains of pathogenic microorganism has also continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use (2). The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (3).

Irvingia gabonensis (Ugiri) is a genus of African and south-east Asian trees in the family Irvingiaceae. Other names commonly attributed to it are Wild Mango or Bush Mango (4). In Nigeria, where both the seeds are well consumed, it is commonly called Ugiri or Ogbono by Igbo (5), Mbukpabuyo by the Efik and Ibibo, Aapon by the Yoruba, Ogwi by the Benin area, Apioro by the Deltians and Goronor by the Hausas(Oral communication). The genus,Irvingia comprises of seven species which include *Irvingia gabonensis, Irvingia excels, Irvingia gradifolia, Irvingia malayaria, Irvingia giorobur, Irvingia smithii and Irvingia wombolu* (6). The plant *Irvingia gabonensis* bears edible mango- like fruits, and is especially valued for their fat and protein rich nut (7). Ethno medicinal treatments utilize the bark, kernels, leaves, or roots for a variety of ailments(8).The bark is mixed with palm oil for treating diarrhoea and for reducing the breast-feeding period. The shavings of the stem bark are consumed by mouth to treat hernias, yellow fever, and dysentery, and the boiled bark relieves tooth pain. Also, in certain parts of Africa, the bark extract is ingested to produce an analgesic effect (9). The seed have been found to reduce fasting blood glucose levels in obese subjects(10). The powdered kernels act as an astringent and are also applied to burns(5). The stems of the tree have been used as chewing sticks to help

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clean teeth(11). In Nigeria and Cameroun, the seeds of *Irvingia gabonensis* are used as a condiment in soup(12). Preliminary phytochemistry screening of the aqueous leaf extract of *Irvingia gabonensis* revealed the presence of saponins, tannins, phenols and phlobatanins. It has been reported that saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, diuretics, steroids, vitamins D and cardiac glycosides(13).

Antimicrobial is a substance that acts to inhibit the growth of harmful microorganisms or acts to destroy them, such as bacteria, virus, fungi, and protozoa. The discovery and development of antibiotics are among the most influential and successful achievements of modern science and technology for the

control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used anti-microbial agents is increasing with an alarming frequency (14,15 and 16).

However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively affordable treatment, profound therapeutic benefit, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their concentration to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains. Herbal medicine is still the stronghold of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (17).

In the work of Unaeze et al [18] they observed that the inhibitory action of the plant extracts could be attributed to the presence of the phytochemical constituents in the plant extracts such as alkaloid, flavonoid and saponin.

The objective of the study was to determine the antimicrobial activity, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of this plant extract on the tested organisms causing diarrhoea.

II. MATERIALS AND METHODS

2.1 Plant collection:

Irvingia gabonensis leaf was collected from Lilu town in Ihiala L.G.A of Anambra State, Nigeria in March, 2017. The plant was identified and authenticated in the Department of botany, Nnamdi Azikiwe University, Awka Nigeria where the sample was deposited. The leaf spread out and dried on a clean surface under a shade at room temperature to exclude direct Sunlight in order to prevent the active constituents of the leaf from being degraded due to photochemical reactions. It was air dried for about eight days after which, the dried leaves were gathered, and crushed with grinder. The powder was weighed using an electric weighing balance by Kern ALS 220 – 4. The powder was then stored in an air tight bag at room temperature and used for further extraction.

2.2 Preparation of plant extract

The ground leaf was prepared in three ways to get the extracts.

2.2.1 Aqueous extract (*Maceration Method*)

Maceration method was used for aqueous extraction and powdered leaf of *Irvingia gabonensis* was used. Then,150 g of the plant was weighed and put in 375 ml of distilled water and allowed to stand for 48 hrs, agitate or shake for 45 mins. The extract was filtered using British standard mesh filter and first muslin cloth and concentrated by using air drying under constant air current and water bath at 50 ^{o}C. The extract was then transferred into a clean container and stored in the refrigerator at -4^{o} C for 10 days , until required for use.

2.2.2 Organic solvent extraction by maceration

This was carried out at Pharmacognosis Department, Faculty of Pharmaceutical Sciences, Agulu. Then,150 g of the plant leaf powder was transferred into 1000 ml volumetric flask, then 375 ml of solvent (methanol and n-hexane) were added. This was covered and allowed for 48 hrs with continuous shaking, filtered and transferred to rotary evaporator for concentration. The extract was then transferred into a clean container and stored in the refrigerator until required for use.

2.2.3 Extraction by Soxhlet method

This method was carried out by continuously extracting a sample with a non polar organic solvent (hexane) for about 4-6 hrs.

2.3 Antimicrobial Screening of Plant Extracts.

From the stored extract in the refrigerator, the concentrated aqueous extract of the plant was weighed 1200 mg of extract (1.2 g) as the stock. The extract was dissolved in 3 ml of distilled water to obtain 400 mg /ml as our interest. This was done for aqueous extract of the plant.

1200 mg (1.2 g) of methanol and n-hexane extracts of the plant were weighed and dissolved in 3 ml of DMSO to make a concentration of 400 mg /ml.

2.3.1 Control Organisms used for Antimicrobial screening of Plant.

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Standard organisms were used for the antimicrobial / antifungal sensitivity testing.

Four of these organisms were typed organisms and were collected from Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical science, Agulu, Nnamdi Azikiwe University. The organisms were subcultured in different selective media for colony morphology confirmation of the typed organisms. All the organisms were re-confirmed through biochemical tests: catalase, coagulase, motility, indole, urease and Triple sugar iron agar (TSI).

Salmonella typhi	NCTC 10950
E.coli	NCTC 10418
Staphylococcus aureus	NCTC 6571
Shigella dysenteriae	ATCC 14420
Candida albican	

These organisms were control organisms and were stored in agar slants in a refrigerator at 2-4^{0}C until used.

Prior to use, these organisms were sub-cultured on Nutrient agar plates, or Sabouraud dextrose agar plates at 37^{0}C for 24 h.

2.3.2 Determination of Susceptibilities of Organisms to Crude Extract

Prior to testing, each organism was sub cultured from the nutrient agar slope (storage system) into a nutrient agar plate. This was incubated at 37^{o}C for 24 hrs. After 24 hrs incubation, a colony of each tested organism was inoculated into 5 ml of sterile Nutrient broth and incubated at 37^{o}C for another 24 hrs. Thereafter, turbidity was checked.

The turbidity was adjusted to 0.5 Macfarland standard and diluted to obtain a final turbidity in approximately 1×10^8 cfu / ml. The agar diffusion method was employed for this procedure.

Muller Hinton agar was used for bacteria while Sabouraud dextrose agar was used for fungal cultivation. These media were sterilized in an autoclave at 121^{0}C (15 ibs pressure) for 15 min before use. Petri dishes were sterilized in a hot air oven at 175^{0}C for 1 hr and was labelled appropriately.

2.3.3 Agar Diffusion Method:

From the first concentration (400 mg /ml) that was gotten from the stock i.e 1200 mg extract dissolved in 3 ml, further doubling dilution was prepared to give 1:200, 1:100,1:50,1:25,1:12.5,1:6.25,1:3.125. Then, 0.1 ml of broth culture of each tested organism or fungi was placed at the centre of a sterilized petri dish and 20 ml of prepared Muller Hinton Agar or Sabourand's dextrose agar poured into it. The dish was swirled gently to ensure even distribution of the bacteria or fungi and the mixture was then allowed to gel. When gelled, six wells of 7 mm in diameter were bored in each petri dish using a sterile cork borer and each well was labeled appropriately for each crude extract or dilution of crude extract, the wells were carefully filled with 2 drops of a 2 ml pipette of both stock solutions(crude extract) and different dilutions of the extract, which is equivalent to 0.04 ml starting with the highest dilutions, the control drugs were added. DMSO, Methanol and conventional antibiotic (ciprotab) were used as controls. Ciprotab was used at a concentration of 200 mg/ml. This was achieved by dissolving 500 mg of the tablet in 2.5 ml of sterile water. The plates were kept for 30 mins on the bench for diffusion of the extract to take place before incubation. The dishes were incubated at 37^{o}C for 24 hrs and observed for inhibition. The fungi were inoculated in Sabourand dextrose agar and incubated at room temperature (25^{0}C) for 24 - 48 hrs. The zones of inhibition were measured and the results noted. This was done for aqueous, n-hexane and methanol extracts of the plant in the tested organisms. The whole process was repeated in triplicate.

3.3.4 Agar Dilution Method:

Then, 1200 mg /ml(1.2 g) of the extract each was weighed as stock solution, Muller Hinton agar and Sabourand dextrose agar was prepared. Then, using formula:

$C_1V_1 = C_2V_2$

Because we want to get 400mg /ml as first dilution.

Where ,

C_1 = Concentration of stock (1200 mg/ml)

V_1 = Unknown

C_2 = 400 mg / ml (our interest)

V_2 = Final volume of agar to prepare (5 ml)

It was allowed to gelled, the petri dish was divided, then from the adjusted 0.5 Macfarland broth culture of tested organism, with a loopful of diluted tested organism was streak with wireloop on top of the gelled mixture of extract and agar. Incubated at 37^{0}C for 24 h for bacteria and at room temp for fungi. Observed for growth or absence of growth.

Presence of growth was indicated using positive (+) sign or negative (-) sign. From here, the tentative minimum inhibitory concentration (MIC's) was obtained, that was the last or minimum dilution of the extract which inhibits the visible growth of organisms. Also the tentative minimum bactericidal concentration (MBC) was obtained, that was the last or minimum dilution of the extract in which there was no growth after subculture onto fresh media. These were indicated using (-) sign.

3.3.5 Minimum Bactericidal Concentration (MBC)

From the tubes showing no visible sign of growth in MIC determination, test microorganisms were inoculated onto sterile nutrient agar plates by streak plate method. The plates

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were then incubated at 37^0C for 24 hrs. The least concentration that did not show growth of test organisms after subculture was considered as the MBC.

III. RESULTS

Results of fig 1 to 3 showed the antimicrobial activities of crude n-hexane, methanol and aqueous extracts of *Irvingia gabonensis* against test organisms. In Fig 1, Methanol crude extract of *Irvingia gabonensis* exhibited higher mean± standard deviation(22±1) zone of inhibition against *Shigella dysenteriae* at 400 mg/ml concentration. Methanol and n-hexane crude extracts of *Irvingia gabonensis* exhibited antifungal activities against *Candida albicans* (10.0±1.0 and 12.0±1.0 respectively). In Fig 2, N-hexane crude extract of *Irvingia gabonensis* had no effect on *E.coli* and *Salmonella typhi* (0.0±0.0). In Fig 3, Aqueous crude extract of *Irvingia gabonensis* had no antifungal activity(0.0±0.0).

Table 1 shows the minimum bactericidal and fungicidal concentration(MBCs/MFCs) of different extracts of *Irvingia gabonensis* on test organisms.The Aqueous extract show that the MIC of *E.coli* was 25 mg/ml with MBC of 50 mg/ml, the MIC of *Shigella dysenteriae* was 25 mg/ml with MBC of 50 mg/ml. The Methanol extract of *Irvingia gabonensis* also show that the MIC of *S.aureus* was 12.5 mg/ml with MBC of 25 mg/ml, MIC of *E.coli* was 6.25 mg/ml with MBC of 12.5 mg/ml, MIC,s of *Shigella dysenteriae* and *Salmonella typhi* were 6.25 mg/ml with MBC,s of 12.5 mg/ml. *Candida albicans* had MIC of 25 mg/ml with MFC of 50 mg/ml.

The n-hexane extract of *Irvingia gabonensis* also show that the MIC of *Candida albicans* was 25 mg/ml with MFC of 50 mg/ml. The MIC,s of *S.aureus* and *Shigella dysenteriae* were 25 mg/ml with MBC,s of 50 mg/ml.

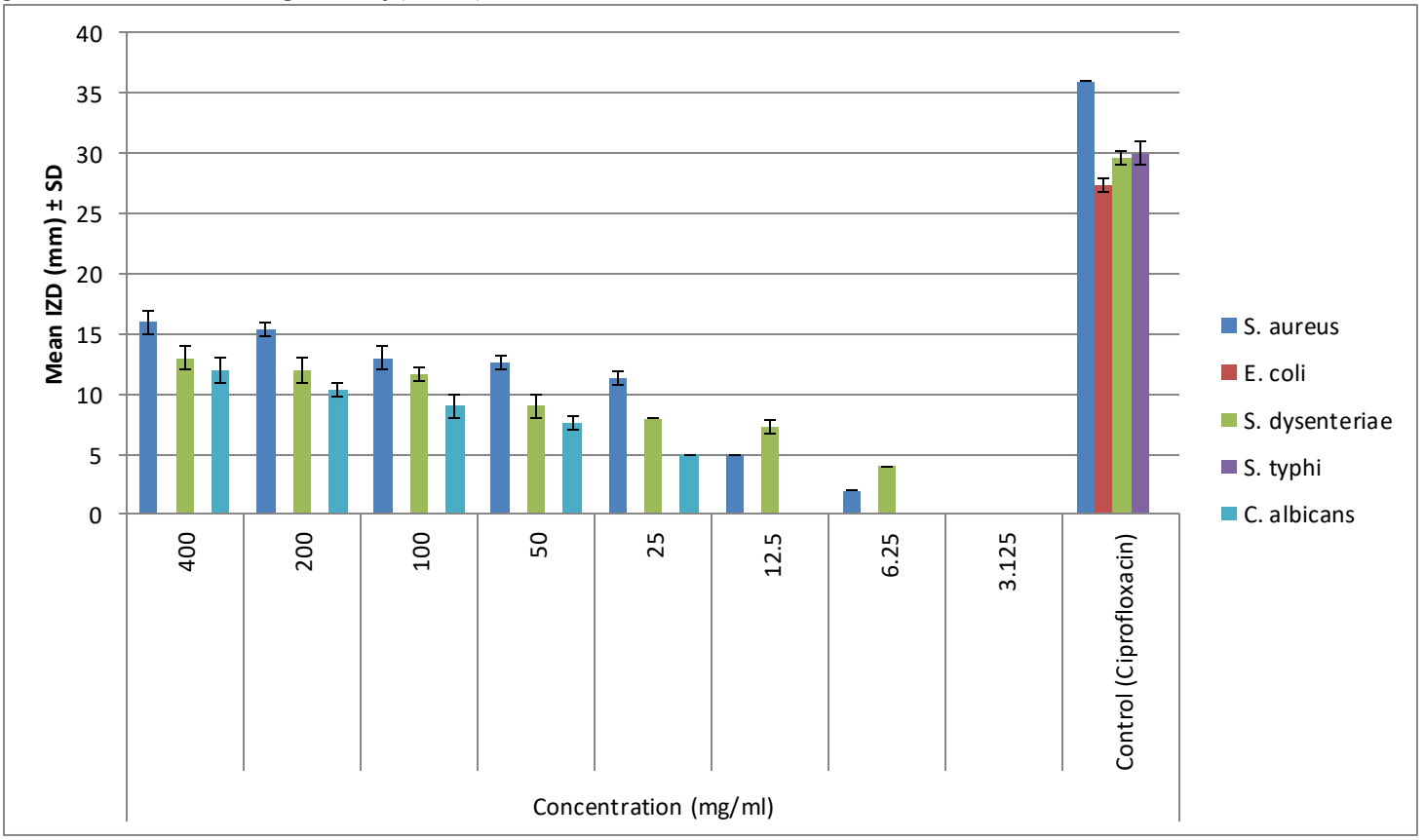

Fig 1: Antimicrobial activity of the crude n-hexane extract of Irvingia gabonensis leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

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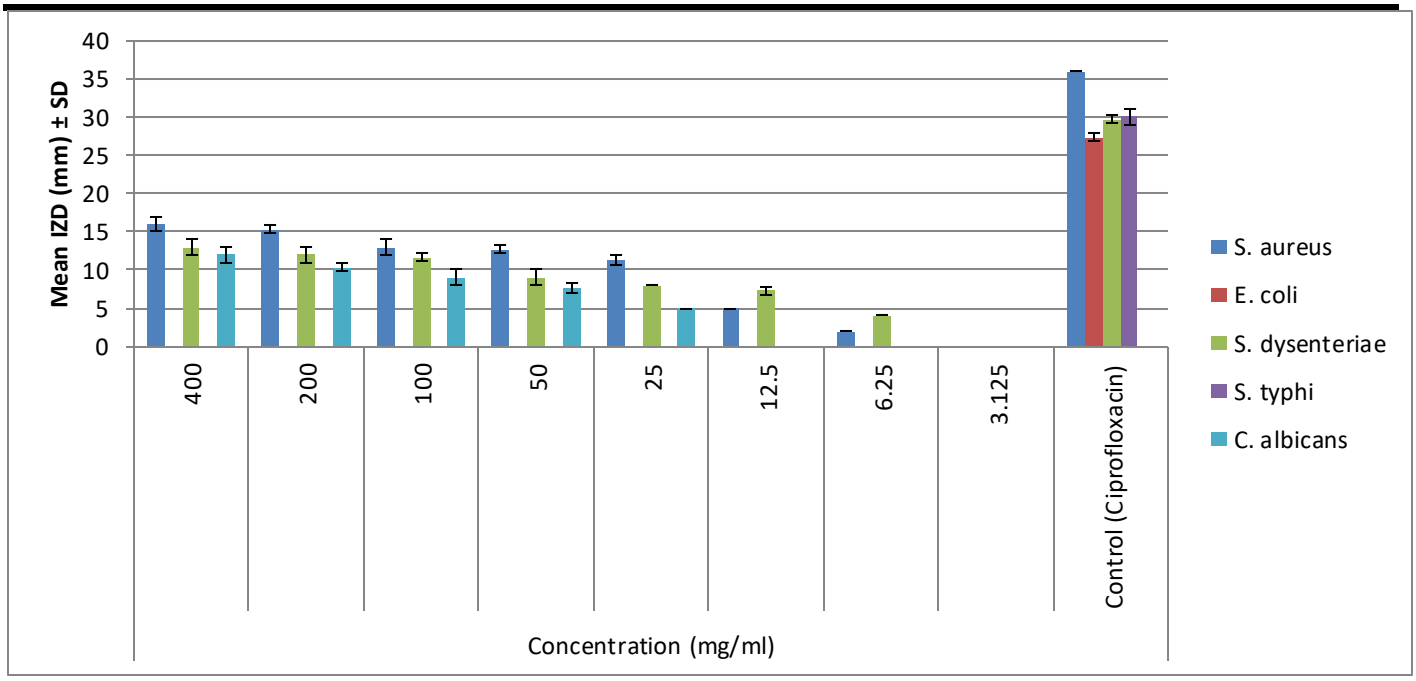

Fig.2: Antimicrobial activity of the crude n-hexane extract of Irvingia gabonensis leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

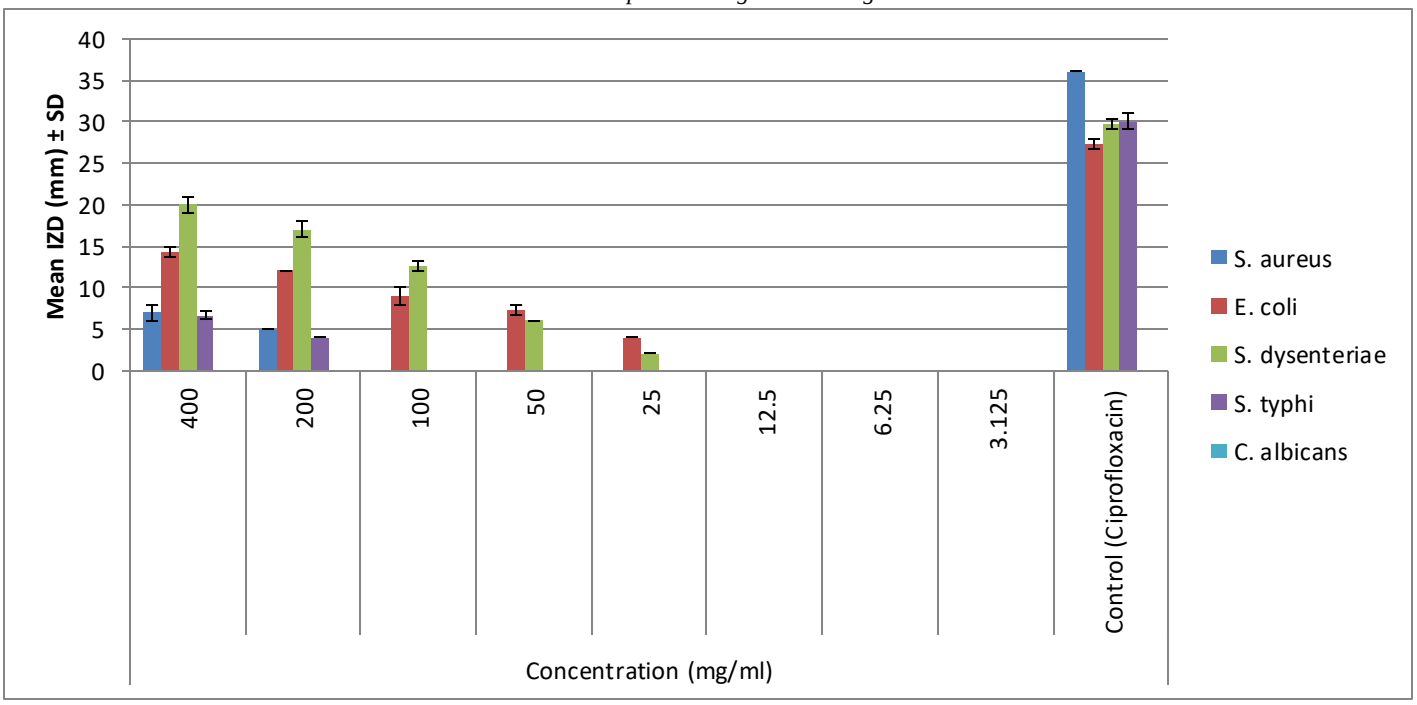

Fig 1: Antimicrobial activity of the crude aqueous extract of Irvingia gabonensis leaves showing the mean inhibition zone diameters and standard deviation produced against test organisms.

Table.1: Minimum inhibitory concentrations (MICs) and Minimum Bactericidal/Fungicidal Concentrations (MBCs/MFCs) of extracts of I. gabonensis on test organisms.

Test Organisms	*Aqueous*		*Methanol*		*N-Hexane*	
	MICs	MBCs/MFCs	MICs	MBCs/MFCs	MICs	MBCs/MFCs
S. aureus	200	400	12.5	25	25	50
E. coli	25	50	6.25	12.5	50	100
S.dysenteriae	25	50	6.25	12.5	25	50
S. typhi	200	400	6.25	12.5	50	100
C. albicans	-	-	25	50	25	50

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IV. DISCUSSION

This study evaluated the antimicrobial effect of n-hexane, methanol and aqueous extracts of *Irvingia gabonensis* (ugiri) leaf against bacterial and fungal agents that causes diarrhoea. The results of the present study showed that the methanol extract of *Irvingia gabonensis* had significant antimicrobial effects.

The antimicrobial effect observed against the test organisms may also be as a result of these bioactive components present in the crude extract as reported by Haslam et al (19). The crude methanol extracts of *Irvingia gabonensis* produced the highest antibacterial effects, where as the n-haxane extract of *Irvingia gabonensis* produced the highest antifungal effects against *Candida albicans*.The crude aqueous extract of *Irvingia gabonensis* had no antifungal activity on *Candida albicans*. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the plant on the test organisms varied, showing that the effect of the plant extracts differed from one organism to the other. This agrees with the work of Rabe, (20) that also showed good effect with methanol extract of *Irvingia gabonensis*. However, the present study revealed that methanol was the best extracting solvent for *Irvingia gabonensis* and in line with the work of Bipul *et al*, (21). Kordali *et al*, (22) had ealier reported that the percentage recovery from plants were dependent on the type of solvent used. These results clearly confirm that *Irvingia gabonensis* leaf is effective alternative therapy against microbial agents that cause diarrhoea disease.

V. CONCLUSION

We conclude that the *Irvingia gabonensis* leaf extract have a significant antimicrobial activity against diarrhoea causing agents. The demonstration of antimicrobial activity of *Irvingia gabonensis* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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