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EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE STEM BARK OF *MAESOBOTRYA DUSENII* (PAX) HUTCHINSON (EUPHORBIACEAE)

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ABSTRACT: Introduction: Infection due to antimicrobial resistance is currently one of the major threats to mankind. This study investigates the antimicrobial activity of the various extracts of the stem bark of *Maesobotrya dusenii* (Pax) Hutchinson (Euphorbiaceae). **Methods:** Pulverized 100 g of stem bark of *M. dusenii* extracted with methanol. Another 400 g of the plant material was also successively macerated with *n*-hexane, ethyl acetate, and methanol. These extracts were assessed for antimicrobial activity on clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* using agar diffusion method and minimum inhibitory concentration was determined. Ciprofloxacin was used as a standard antibacterial drug, while ketoconazole was used as a standard antifungal drug. **Results:** The ethyl acetate extract was the most active extract on *E. coli* with a minimum inhibitory concentration (MIC) of 0.025 mg/mL. The *n*-hexane extract also exhibited activity on *E. coli* with MIC of 0.196 mg/mL. Only *n*-hexane extract was active against *Candida albicans* with a MIC of 12.5 mg/mL. **Conclusion:** The ethyl acetate extract was active against *E. coli*, while *n*-hexane extract was active against *C. albicans*. Thus, suggesting that the extracts could be of great value in the development of a potent antimicrobial agent.

INTRODUCTION: Infectious diseases have remained the leading cause of death accounting for one-quarter of all death in the world [1]. Infections due to antimicrobial resistance (AMR) are on the increase [2]. Antimicrobial resistance is currently one of the major threats facing mankind. The emergence and rapid spread of multi-drug resistant organisms (vancomycin, methicillin, extended-spectrum β-lactam, carbapenem, and colistin) have put the world in a dilemma.

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The health and economic burden associated with AMR on a global scale are dreadful. Available antimicrobials have been misused and are almost ineffective while some of the drugs are associated with dangerous side effects in some individuals [3]. Therefore, there is a need for discovery of novel antimicrobial agents to combat the growth of resistant microbes [4].

Plant-derived medicines have made large contributions to human health and well-being. They provide key chemical compounds for the development of new antimicrobial drugs. The most interesting bioactive constituents of a plant in this regard are phenolic compounds, alkaloids, tannins and flavonoids [5]. An antimicrobial agent is a compound or substance that kills or inhibits the growth of microorganisms.

They could be antibacterial, antifungal, anti-protozoal or antiviral. *Maesobotrya dusenii* belong to the family Euphorbiaceae. It is mostly found in the rain forest of Southern Nigeria, Wetland East Cameroun, Equatorial Guinea. It is a tree or shrub of about 15 m high with a dense crown. The fruit contained an edible pulp of a sourish taste and used to make jam [6]. The root and the stem bark of *Maesobotrya dusenii* have been used locally to treat skin infections, spots, gonorrhea, dysentery in Akwa Ibom [7]. The anti-hyperglycemic and anti-hyperlipidemic effect of the leaves on Albino rat has also been reported [8]. The antimicrobial effect of the ethanolic extract of the stem bark has been reported [9]. This study, therefore, investigates the anti-microbial effect of various extracts of the stem bark of *Maesobotrya dusenii*.

MATERIALS AND METHODS:

Sample Collection and Identification: *M. dusenii* stem bark was collected from farmland in Omuike, Aluu, Ikwerre Local Government Area of Rivers State. The plant has earlier been identified and authenticated in the previous study by the author with voucher specimen number UPF301 in the herbarium of Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The stem bark was air-dried at room temperature, pulverized and preserved for further use.

Extraction: Powdered stem bark of *M. dusenii* (100 g) was macerated with methanol for 3 days. The obtained extract was named crude methanol (CME). A 400 g of the powdered bark was also successively macerated with *n*-hexane, ethyl acetate, and methanol for 3 days consecutively. The combined filtrate of each of the solvent was evaporated in vacuo in a rotary evaporator at 40 °C and weighed. The three extracts were named NHE, EAE and ME respectively.

Antimicrobial Assay Method:

Preparation of Test Extracts: A 500 mg (0.5 g) of each of the dried extract was weighed out and dissolved in 10 ml of appropriate solvents (Dimethyl sulphoxide (DMSO) for *n*-hexane and ethyl acetate extracts, while water for methanol extracts) to give stock solutions of 50 mg/mL concentration each. Two-fold serial dilutions of the 50 mg/mL stock solutions were made.

Preparation of Standard Drugs: Ciprofloxacin was used as a standard antibacterial agent. A 500 mg of ciprofloxacin was dissolved in 100 ml of water to obtain a concentration of 5 mg/mL stock solution. A 5 mg/mL of ketoconazole was also prepared as a standard antifungal agent. Lesser concentrations (2.5, 1.25, 0.625, 0.3125 mg/mL) were also obtained from the 5 mg/mL stock solutions of the ciprofloxacin and ketoconazole standard agents by two-fold serial dilutions.

Test Microorganisms: The clinical isolates of the test organisms; *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium), *Pseudomonas aeruginosa* (Gram-negative bacterium) and *Candida albicans* (Fungus) obtained from University of Port Harcourt Teaching Hospital (UPTH) were standardized to 0.5 Mcfarland standard.

Antimicrobial Susceptibility Test Using Agar Diffusion Method: A 0.1 ml of each of the test microorganisms was added aseptically to the prepared Muller Hinton agar [10] in the universal bottles and properly mixed. The mixture was then poured into corresponding sterile Petri dishes which were labeled in duplicate for each of the test microorganism and allowed to solidify. Five wells were made on each of the plates using sterile cork borer. The wells were labeled for the four (4) extracts during the fifth well at the center for the standard antibiotic. A drop of 50 mg/mL of each extract was carefully and aseptically added to the wells accordingly. 5 mg/mL of the standard antibiotic (Ciprofloxacin) was also aseptically added into the fifth well. The Petri dishes were allowed to stay for 15 min for proper diffusion. The plates (Petri dishes) were incubated at 37 °C for 24h. The diameter of the resulting zones of inhibition was measured in millimetre (mm) through the base of the plates using a metre rule. The procedure was repeated for the fungal organism, *C. albicans*, but using sabouraud dextrose agar as nutrient medium [11] and 5 mg/mL of ketoconazole as a standard anti-fungal agent.

Determination of Minimum Inhibitory Concentration (MIC) of Extracts on Various Micro-organisms: Agar layers containing susceptible microorganisms from the above test for activity of extracts were prepared in various Petri

dishes and labeled. Duplicate plates (dishes) were made for each organism. Using sterile cork borers, 5 wells were made and labeled for various concentrations of the serial dilutions made. A drop of the respective concentrations of the extracts and standard drugs was added to the well labeled for each and kept for 15 min to allow diffusion of the extracts into the agar layer. The plates were incubated at 37 °C for 24 h. The diameter of any resulting zone of inhibition was measured in millimeter. MICs of extracts on various micro-organisms were obtained as less than or equal to the concentrations of the extracts which on further dilution produced no zone of inhibition (activity) on the microorganisms.

RESULTS AND DISCUSSION: From the antimicrobial susceptibility test result shown in **Table 1**, the antimicrobial activity of the extracts at 50 mg/mL on *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* were obtained by their zone of inhibition of growth of these microorganisms. It is obvious that all the extracts exhibited antimicrobial activity against *E. coli* though ethyl acetate extract has the highest zone of inhibition of 6 mm. the *n*-Hexane extract did not show activity against *S. aureus*, but other extracts exhibited activity with the methanol extract (ME) being the most active with 12 mm zone of inhibition. Only ethyl acetate extract exhibited inhibition of 4 mm in *P. aeruginosa* which is not as wide as the standard drug 24 mm. *Candida albicans* was inhibited (9 mm) by *n*-hexane extract. It could, therefore, be said that the antifungal activity of the stem bark of *M. dusenii* lies in its *n*-hexane extract although not as high as the standard drug (24 mm). The table also showed that the dissolution solvent does not affect the activity of the extracts as it did not exhibit any inhibition on the organisms.

TABLE 1: ANTIMICROBIAL SUSCEPTIBILITY TEST FOR ACTIVITY OF EXTRACTS AT 50 mg/mL

Microorganism	Plate	Inhibition zone diameter (mm)					
		CME	NHE	EAE	ME	Standard Drug	DMSO
S. aureus	1	2	0	9	10	14	0
	2	1	0	9	12	10	0
E. coli	1	3	4	6	4	24	0
	2	3	5	5	4	25	0
P. aeruginosa	1	0	0	4	0	9	0
	2	0	0	4	0	24	0
C. albicans	1	0	9	0	0	24	0
	2	0	8	0	0	23	0

CME = Crude methanol extract (50 mg/mL); N-HE = *n*-hexane extract (50 mg/mL); EAE = Ethyl acetate extract (50 mg/mL); ME = Methanol extract (50 mg/mL); Standard drugs = Ciprofloxacin 5 mg/mL (for bacteria), Ketoconazole 5 mg/mL (for fungi)

TABLE 2: THE SUSCEPTIBILITY OF CLINICAL ISOLATES USING COMMERCIALLY PREPARED ANTIBIOTIC DISCS

Standard Antibiotics (For gram-positive organism)	Inhibition zone diameter (mm) *S. aureus* (Clinical isolates)		Standard Antibiotic (For gram-negative organisms)	Inhibition zone diameter (mm) *E. coli* (Clinical isolates)		*P. aeruginosa* (Clinical isolates)	
	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2
S	0	0	S	20	19	0	0
SXT	0	0	SXT	20	15	0	0
E	0	0	CH	25	24	0	0
PEF	13	14	SP	20	18	0	0
CN	0	0	CPX	27	29	0	0
APX	18	15	AM	0	0	0	0
Z	0	0	AU	0	0	0	0
AM	0	0	CN	0	0	0	0
R	0	0	PEF	21	22	0	0
CPX	13	10	OFX	0	0	0	0

S= Streptomycin (30 µg); CN= Gentamicin (10 µg); SXT= Septrin (30 µg), APX= Ampiclox (30 µg); E= Erythromycin (10 µg); Z= Zinnacef (20 µg); PEF= Perfloxacin (10 µg); SP = Sparfloxacin (10 µg); CH= Chloramphenicol (30 µg); AM= Amoxacillin (30 µg); OFX= Tarivid (10 µg); CPX= Ciprofloxacin (10 µg); AU= Augmentin (30 µg)

The susceptibility of clinical isolates on commercially prepared antibiotic discs was determined on a large number of standard antibiotics to compare the activity of the available

antibiotics to that of the extracts on same organisms, **Table 2**. None of the commercial antibiotics at the concentrations used produced activity against *P. aeruginosa* (a very resistant organism to most antibiotics) whereas the EAE at 50 mg/mL showed activity against it. However, *E. coli* also showed to be the most susceptible to a larger number of commercial antibiotics.

TABLE 3: DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF EXTRACTS

Extract	Concentration mg/mL	Average zone of inhibition diameter (mm)			
		S. aureus	***P. aeruginosa***	***E. coli***	***C. albicans***
CME	25.00	-		3 ± 0.01	
	12.50	-		2 ± 0.01	
	6.25	-		1 ± 0.02	
	3.125	-		1 ± 0.02	
	*2.50			*29 ± 0.03	
N-HE	25.00			5 ± 0.71	9 ± 0.02
	12.50			4 ± 0.71	3.5 ± 0.35
	6.25			4 ± 0.71	-
	3.125			3.5 ± 0.35	-
	*2.50			*24 ± 0.02	*32 ± 0.04
EAE	25.00	-	-	5 ± 0.71	
	12.50	-	-	4 ± 0.71	
	6.25	-	-	4 ± 0.03	
	3.125	-	-	3.5 ± 0.35	
	*2.50			*29 ± 0.03	
ME	25.00	-		-	
	12.50	-		-	
	6.25	-		-	
	3.125	-		-	

[-] = No activity at present; [ ] = No previous activity; CME = Crude methanol extract; NHE = *n*-Hexane extract; EAE = Ethyl acetate extract; ME = Methanol extract; * Standard drugs: Ciprofloxacin (for bacteria) and Ketoconazole (for fungi)

TABLE 4: CONTINUATION OF THE DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF EXTRACTS ON *ESCHERICHIA COLI*

Extract	Average zone of inhibition diameter (mm) of various concentrations								
	1.563 mg/mL	**0.782 mg/mL**	**0.391 mg/mL**	**0.196 mg/mL**	**0.098 mg/mL**	**0.049 mg/mL**	**0.025 mg/mL**	**0.0125 mg/mL**	***0.625 mg/mL**
CME	0	0	0	0	0	0	0	0	29.5 ± 0.35
N-HE	8 ± 0.71	7 ± 0.01	5 ± 0.01	4 ± 0.71	0	0	0	0	24 ± 0.02
EAE	6 ± 0.01	6 ± 0.71	5 ± 1.41	5 ± 0.71	3.5 ± 0.35	3 ± 0.01	2 ± 0.01	0	24 ± 0.02

* Ciprofloxacin (0.625 mg/mL); CME = Crude methanol extract; NHE = *n*-Hexane extract; EAE = Ethyl acetate extract

The minimum inhibitory concentrations (MICs) of extracts which produced antimicrobial activity against specific microorganisms were determined. The antibacterial activity of all the extracts on *S. aureus* and *P. aeruginosa* were lost on dilution to 25 mg/mL, **Table 3**. However, the antibacterial activity of the CME, N-HE, and EAE on *E. coli* continued to a concentration of 3.125 mg/mL as presented in **Table 4**. Only ME lost activity on *E. coli* on dilution to 25 mg/mL on **Table 3**.

This may be because most of the plant potent phytoconstituents would have been extracted by *n*-hexane and ethyl acetate unlike in the crude methanol extract where they would all be present since it was a polar solvent on crude plant material. The antifungal activity of the NHE on *C. albicans* was lost at a concentration of 6.25 mg/mL. The MIC of the *n*-hexane extract on *C. albicans* was therefore taken as ≤12.5 mg/mL since the lowest concentration that produced activity was 12.5 mg/mL. Similarly, the MICs of other extracts were taken as less than or equal to the lowest concentration at which the last antimicrobial activity was recorded on the microorganisms.

Thus, the MICs of the *n*-hexane and ethyl acetate extracts on *E. coli* are ≤ 0.196 mg/mL and ≤ 0.025 mg/mL respectively although the zone of inhibition was not as wide as that of the standard drug at 0.625 mg/mL. The graphical determination of the MIC of ethyl acetate extract on *E. coli* also gave 0.025 mg/mL [12, 13]. The result of this research thus justifies the use of *Maesobotrya dusenii* stem back

in the treatment of diarrhea and skin infections as *E. coli,* and *C. albicans* are known to be causative organisms for diarrhea and skin infections (vaginal candidiasis) respectively.

TABLE 5: SUMMARY OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE EXTRACTS

Extract	**Crude methanol extract**		***n*-hexane extract**		**Ethyl acetate extract**			**Methanol extract**	
Organism	*S. aureus*	*E. coli*	*E. coli*	*C. albicans*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. coli*
MIC (mg/mL)	≤500.0	≤ 3.125	≤0.196	≤12.50	≤50.0	≤0.025	≤50.0	≤ 50.0	≤50.0

CONCLUSION: The ethyl acetate extract has the highest antibacterial activity against *E. coli* with MIC of 0.025 mg/mL followed by *n*-hexane extract which has antifungal activity against *C. albicans* with a minimum inhibitory concentration of 12.5 mg/mL. The study shows that stem bark of *Maesobotrya dusenii* has an antibacterial and anti-fungal activity which justifies its use in folk medicine in treating diarrhea and skin diseases.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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