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Comparative Evaluation of the Antimicrobial Activities of some plants used in Natural Medicine – *Spondias mombin, Calliaidra portoricensiss, Dennettia tripetala, Anthocleista djalonensis and Cronton zambesicus*

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Abstract

The bioactive constituents of five Nigerian plants in use in Traditional medicine namely, *Spindias mombin* (family: *Anacardinaceae*), *Calliandra portoricensis* (family: *Fabaceae*), *Dennettia tripetala* (family: *Annonaceae*), *Anthocleista djalonensis* (family: *Loganiaceae*) and *Croton zambasicus* (family: *Euphoirbiaceae*), were investigated for antimicrobial activities. The morphological parts of the plants evaluated were the leaf and root. The dried and pulverized samples were subjected to successive extraction using organic solvents; n-hexane, ethylacetate and 70% aqueous methanol. The respective extracts were concentrated *en vacuo* using a rotatory evaporator at less than 40° C. Seven human pathologically viable and clinical strains of microganisms comprising the G+ve, G-ve, fungi and nocosomial strains were used as test organisms, ciprofloxacin and fluconazole solutions served as the control reference standards. Agar well diffusion assay method was used and the Inhibition Zones of growth were measured to assess activities for all the extracts. The Minimum Inhibitory Concenyrations (MIC) and Total Activity (TA) were also determined. *C. zambasicus* exhibited a broad antibacterial activity whereas *C. portoricensis* showed the best spectrum of both antibacterial and antifungal activities. The extract of *D. tripetala* ranked lowest in activity of all the test samples. Ethyl acetate extracts ranked the highest of all the three organic solvents used for the study.

Keywords: Nigerian plants, extracts in different solvents, susceptibility antimicrobial testing with human pathogenic microorganisms.

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INTRODUCTION

A greater proportion of natural products used medicinally are derived from plants. Admittedly, the poisonous and medicinal properties of plants were discovered by man while in search of food. It has been estimated by the World Health Organization (WHO) that about 80 % of the world's inhabitants rely mainly on traditional medicine for their primary health care [1]. In recognition of this, Alma Ata declaration provided for the accommodation of proven traditional medicines in National Drug Policies and Regulatory Measures [2]. Prescription data analysis also indicated that, "About 25% contained plant extracts or active components derived from high plants. At least 119 chemical substances derived from 90 plants species can be considered as important drugs commercially in use in one or more countries" [3]. Natural medicine has been embraced largely because of perceptions of it having no adverse effects, availability, affordability and the fact that many ailments have defied the orthodox medicine: Additionally, the world market for herbal medicines including herbal products and raw materials has been estimated to reach $43 billion with an annual growth rate of between 5 and 15% [4].

The terms antimicrobial and antibiotic have been applied interchangeably. Antibiotics being natural substances that inhibit the growth of bacteria or kill them directly, however in practice, most commercial antibiotics have been chemically altered or enhanced producing antimicrobials [5].

Deriving from the above, there is then a justification for investigation of various plants from biodiversity as potential and veritable sources of antimicrobial agents. While exploring these, the concentrations of the prospective medicinal antimicrobial candidates must be small and causes little or no host damage [6].

The antibiotics of popular usage are; beta lactam group such as the peniallins and the Cephalosphorins; the non-lactam ones include tetracyclines, erythromycins, aminoglycosides such as gentamycin and kanamycins; the macrolides, polyenes, chloramphenicol, fucidic acid, norobicin and the relatively new group – the fluorinated-4-quinolone methanol such as the ciprofloxacin with its broad based activity spectrum [7].

Table-1: Summary of the selected plants for the invagination

S/N	Plant Species	Family	Medicinal uses	Distribution	References
1.	*Spondias mombin*	*Anacordiacea*	Antibacterial (Gonorrhea) inflammation	West Africa including Nigeria	[8]
2.	*Dennettia tripetala*	*Annonaceae*	Antibacterial hypolipidemic	Tropics Nigeria	[9]
3.	*Calliandra portoricensis*	*Fabaceae*	Antimicrobial, tonsillitis, ulcer	Central America,– Mexico, Panama, West Africa	[10]
4.	*Anthocleista djaalonensis*	*Loganiaceae*	Microbial infections., Anti-inflammatory	Tropics such as West Africa	[11]
5.	*Croton zambasicus*	*Euphorbiaceae*	Antimicrobial	Tropics,West Africa, Nigeria	[12]

Available Extraction Methods Include

Repeated maceration with agitation, percolation or by continuous extraction such as with soxhlet extractor. Prior to these, the extraction process involves rupture of cells in order to remove contents and optimize surface area of material with extraction solvent which increases the amount of leaching cells. These end can be achieved by grinding tissues to the smallest particles possible [13].

Plant extracts usually used in antimicrobial bioassay are prepared by maceration or percolation of fresh green plants or dried powdered plant material with water or organic solvents. Fractions of the total extracts are carried out prior to the testing in order to separate polar from non-polar compounds acid and neutral from basic substances [14]. Further, the listrd conditions must also be fulfilled;

1. The plant products must be brought into contact with the microorganisms that have been selected for the test.
2. Conditions must be adjusted such that microorganisms are able to grow when no antimicrobial agents are present.
3. There must be some means of judging the amount of growth, if any is made by the test organisms during the period of time chosen for the test.

Conforming to the above three criteria therefore are the difusion, dilution and bio-autographic methods whereas the method requiring sterile samples may not be suitable [15].

The selection of the test microorganisms should be as diverse as possible and preferably, representatives of all important groups of pathogenic microorganisms, especially when the investigation is of general character [16]. The same report further highlighted that many crude extracts exhibited aspecific inhibitory activity against *staphylococcus aureus* due to a synergistic effect of several plant components.

THE AIM OF THIS RESEARCH

- To evaluate the antimicrobial activities of crude extracts from five different plants used in Nigerian natural medicine and
- To identify the most promising of all five plants for isolation and characterization of the antibacterial and antifungal compounds of the same plant.

MATERIALS AND METHODS

MATERIALS

Anthocleista djalonensis,*Dennittia tripetala*and,*Calliandra portoricensis* were harvested from Osisioma Ngwa Local Government Area of Abia State Nigeria whereas *Spondias mombin* and *Croton zambasicus* were collected from the University of Port Harcourt community in Obiakpo Local Government Area of Rivers State, Nigeria. They were all authenticated at the Herbarium of the Department of Plant Science and Biotechnology of University of Port Harcourt, Nigeria.

METHODS

Preparation of Crude Plant extracts:

100 g each of the dried and pulverized plant samples were macerated and subjected to successive extraction for 24 x 2 hours using organic solvents of varying polarities,: n-hexane,, ethyl acetate and methanol. The extracts were filtered and the air-dried husks were re-packed for successive maceration with the next solvent. The filtrate was concentrated *en vacuo* using a rotary evaporator at temperature not exceeding 45°C. The yield was noted and the residue-sample labeled accordingly for the bio-assays. The above

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procedure was repeated for the rest of the solvents for all the plant samples obtaining samples; Sm-L; Dt-L; Cp-R; Cz-L and Ad-R.

Test Micro-Organisms
Test microorganisms were selected to reflect a fairly wide spread of human pathogenically viable and clinical isolates, some of which are also nocosomial strains. The microorganisms used were; *Staphylococcus aureus* (gram +ve cocci), *Streptococcus fecalis*(gram +ve cocci),), *Klebsiella pneumoneae (gram –ve rod) Aspergillus niger* (fungus).

The clinical isolates were from the Department of Microbiology of University of Port Harcourt Teaching Hospital and authenticated by Dr. Martins Oluleye of same Department.

Preparation of Test Microorganisms
For bacterial suspension, a loopful of isolated microbial colony from the slant was sub-cultured into 10ml of peptone water. This was incubated at 37°C for 18 h prior to the susceptibility testing. Then 0.5ml of the actively growing test bacterial suspension was again sub-cultured into 9.50ml of peptone water, the turbidity of which was matched with that of standard of 0.5 McFarland units [17] ($1.5x10^8$) c.f.u. per ml.

For preparation of fungi, the isolated fungal test organisms were maintained on Sabouraud Dextrose Agar (SDA) at room temperature (25°C) for 72 h and thereafter sub-cultured as for bacteria for the test.

Invitro Susceptibility testing of the plant extracts against the selected microorganisms
The cup-plate agar diffusion method [18] was adopted in this research for the susceptibility testing of the prepared extracts and the standard reference samples.

All the glass wares and petri dishes were sterilized using the autoclave at 21°C under pressure of 15 pounds per square inch (psi) and for 20 minutes. Following standard microbiological procedure [19]. 1 ml of the stock sub-culture suspension 1.50 x 10^8cfu / ml [20] was carefully seeded into Muller Hinton molten nutrient agar in aliquot of 20ml each. This was distributed into sterile petri-dishes and allowed to solidify.

AT each of the quadrants of the petri-dish containing nutrient agar, a cup is made with an 8mm gauge sterile cork-borer. Opposite cups were filled with 0.2ml of (40 and 20)mg/ml respectively of 10% aqueous Dimethyl sulphoxide (DMSO) solutions of each of the crude extracts (Sm-L, Dt-L, Cp-R, Cz-L, Ad-R). Micro-pipettes were used to deliver the test samples. The remaining two cups were loaded with 0.2ml of ciprofloxacillin solution of 4 microgram per ml (bacteria) and 0.2ml Flucmazole solution of 200 microgram per ml (fungi). The plain 10% aq. DMSO, Ciprofloxacin solution and fluconazole solution were used as controls.

The petri-dishes in triplicate containing test microorganisms, crude plant extracts and the controls were allowed to stand for 1h at room temperature for diffusion before loading in the incubator in upright position. This is incubated at 37°C for 18 h (bacteria) and at room temperature (25°C) for 72 h (Fungi).

At the end of the susceptibility testing period, the diameters of the growth inhibition zones were measured.

Determination of Minimum Inhibitory Concentration (MIC)
This was determined by a modification of standard agar- well diffusion method [22, 23]. The active crude plant samples were dissolved in 10 % aqueous DMSO by serial two-fold dilution to concentrations of; (40,20,10 and 5) mg/ ml. These were loaded in the nutrient agar wells as described in above

RESULTS AND DISCUSSION

Table-2: The percentage yield of the plant extracts in different solvents

S/N	Plant Species	n-hexane	Ethyl acetate	Methanol
1.	Sm-L	1.41	1.86	1.45
2. .	Cz-L	2.06	1.96	2.28
3.	Cp-R	1.43	2.30	4.98
4.	Dt-L	0.62	3.48	2.25
5.	Ad-R	0.30	1.96	1.71

Sm-L (*Spondias mombin* leaf),; Cz-L (*Croton zambasi*cus; leaf), Cp-R (*Calliandra portoricensis* – Root), Dt-L (*Dennettia tripetela* - leaf) and Ad-R (*Anthocleista djalonensis* - Root);

From the above table, most of the phytoconstituents were extractable in polar solvents.

Table-3: The susceptibility testing results for the sample extracts and controls against the selected microorganisms

S/N	Plant Species	Solvent of extraction	Sa EXT.		Sa CTR.	Ec EXT.		Ec CTR.	Bs EXT.		Bs CTR.	Kp EXT.		Kp CTR.	Sf EXT.		Sf CTR.	Ca EXT.		Ca CTR.	An EXT.		An CTR.
			40	20		40	20		40	20		40	20		40	20		40	20		40	20	
1	Cp (Root)	Hexane	15.00±0.40	10.00±0.30	23.00±0.15	12.00±0.25	5.00±0.70	23.00±0.23	20.00±0.60	8.00±0.60	30.00±0.25	13.00±10	10.00±0.50	15.00±0.85	8.00±0.30	-	17.00±0.45	17.00±0.48	10.00±0.25	15.00±0.40	7.00±0.60	-	13.00±0.50
		Ethyl acetate	17.00±0.35	11.00±0.80	21.00±0.15	13.00±0.75	5.00±0.70	15.00±0.75	15.00±0.20	7.00±0.65	22.00±0.30	10.00±0.10	5.00±0.40	14.00±0.90	16.00±0.85	9.00±0.20	30.00±0.35	25.00±0.50	15.00±0.80	15.00±0.40	15.00±0.15	5.00±0.20	11.00±0.60
		Methanol	14.00±0.50	5.00±0.40	15.00±0.70	8.00±0.10	6.00±0.80	15.00±0.25	21.000±0.20	11.00±0.25	17.00±0.35	-	-	14.00±0.65	18.00±0.60	10.00±0.40	35.00±0.25	28.00±0.55	15.00±0.70	16.00±0.15	-	-	13.00±0.70
2	Sm (Leaf)	Hexane	20.00±0.25	9.00±0.80	35.00±0.35	-	-	21.00±0.20	12.00±0.15	-	21.00±0.70	-	-	13.00±0.85	-	-	23.00±0.40	10.00±0.65	-	11.00±0.80	-	-	12.00±0.30
		Ethyl acetate	20.00±0.35	11.00±0.50	24.00±0.20	-	-	16.00±0.10	5.00±0.80	-	10.00±0.35	7.00±0.25	-	12.00±0.60	11.00±0.15	-	35.00±0.50	-	-	13.00±0.45	-		11.00±0.25
		Methanol	-	-	17.00±0.60	-	-	21.00±0.75	8.00±0.85	-	30.00±0.45	-	-	10.00±0.20	-	-	8.00±0.30	-	-	15.00±0.20	-	-	12.00±0.60
3	Cz (Leaf)	Hexane	20.00±0.50	9.00±0.75	35.00±0.75	-	-	28.00±0.80	20.00±0.55	5.00±0.35	27.00±0.15	11.00±0.25	-	12.00±0.60	-	-	25.00±0.10	11.00±0.20	-	14.00±0.70	7.00±0.30	-	11.00±0.45
		Ethyl acetate	-	-	21.00±0.50	-	-	28.00±0.65	-	-	7.00±0.15	10.00±0.40	-	23.00±0.65	20.00±0.20	11.00±0.80	30.00±0.30	-	-	11.00±0.10	-	-	13.00±0.40
		Methanol	-	-	21.00±0.25	-	-	10.00±0.35	10.00±0.60	-	27.00±0.35	-	-	100.00±0.75	-	-	9.00±0.80	5.00±0.30	-	12.00±0.70	-	-	11.00±0.45
4	Dt (Leaf)	Hexane	12.00±0.85	-	35.00±0.25	8.00±0.55	-	23.00±0.40	-	-	26.00±0.60	-	-	10.00±0.35	-	-	23.00±0.15	-	-	10.00±0.90	-	-	13.00±0.25
		Ethyl acetate	15.00±0.35	5.00±0.70	33.00±0.20	-	-	25.00±0.40	-	-	10.00±0.15	15.00±0.75	-	10.00±0.20	12.00±0.45	-	28.00±0.50	-	-	14.00±0.70	-	-	12.00±0.35
		Methanol	9.00±0.30	-	21.00±0.50	-	-	8.00±0.45	17.00±0.65	12.00±0.75	37.00±0.20	-	-	11.00±0.35	-	-	8.00±0.60	-	-	11.00±0.85	-	-	14.00±0.10
	Ad (Root)	Hexane	12.00±0.70	-	22.00±0.35	-	-	15.00±0.25	-	-	30.00±0.80	-	-	9.00±0.35	-	-	10.00±0.70	-	-	15.00±0.25	9.00±0.65	-	13.00±0.35
		Ethyl	--	-	20.00±0.45	-	-	25.00±0.70	-	-	8.00±0.15	-	-	11.00±0.30	-	-	32.00±0.60	-	-	12.00±0.40	-	-	14.00±0.80
		Methanol	-	-	10.00±0.25	-	-	11.00±0.75	11.00±0.10	-	25.00±0.40	-	-	13.00±0.30	-	-	28.00±0.55	11.00±0.20	-	12.00±0.50	-	-	11.00±0.75

Test Organisms used – MDIZ (mm)

Sm-L (*Spondias mombin* leaf),; Cz-L (*Croton zambasi*cus; leaf), Cp-R (*Calliandra portoricensis* – Root), Dt-L (*Dennettia tripetela* - leaf) and Ad-R (*Anthocleista djalonensis* - Root);

MDIZ = Mean Diameter of Inhibition Zone (mm); EXT. = extracts and standard reference samples in parenthesis.

(-) = No inhibition of growth field. Values were expressed as mean ± SEM; n=3; Analysis was processed with (SPSS-version 20.0) software and a one way (ANOVA) at $P \leq 0.05$.

Table-5: Minimum Inhibitory Concentration (MIC) AND (TA) for the sample extracts and controls against the selected microorganisms

S/N	Plant Species	Solvent of extract	Test Microorganisms													
			Sa		Ec		Bs		Kp		Sf		Ca		An	
			MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA
	C.p (Root)	n-Hx Eto Ac MeOH	10.00±0.15 5.00±0.45 5.00±0.60	1.4 4.6 10.	10.00±0.75 5.00±0.30 10.00±0.60	1.4 4.6 4.9	5.00±75 5.00±0.50 10.00±0.25	2.8 4.6 4.9	10.00±0.80 5.00±0.45 10.00±0.15	1.4 4.6 4.9	10.00±0.45 5.00±0.85 10.00±0.20	1.4 4.6 4.9	5.00±0.15 5.00±0.50 5.00±0.85	2.8 4.6 10	10.00±0.45 5.00±0.20 10.00±0.60	1.4 4.6 4.9
	C.z (Leaf)	n-Hx Eto Ac MeOH	5.00±0.75 - -	4.2 - -	- 10.00±0.80 20.00±0.45	- 2.1 1.2	10.00±0.40 - -	2.1 - -	- 10.00±0.60 -	- 2.1 -	- 10.00±0.40 -	- 2.1 -	10.00±0.35 - -	2.1 - -	20.00±0.45 - -	1.1 - -
	Sm (Leaf)	n-Hx Eto Ac MeOH	5.00±0.80 5.00±0.35 -	2.8 3.8 -	- - -	- - -	- - -	- - -	- - -	- - -	- 10.00±0.75 -	- 1.9 -	- - -	- - -	- - -	- - -
	Dt (Leaf)	n-Hx Eto Ac MeOH	20.00±0.50 10.00±0.80 20.00±0.25	3.1 3.5 1.2	10.00±0.60 10.00±0.40 -	6.2 3.5 -	- - 10.00±0.75	- - 2.3	- 10.00±25 -	- 3.5 -	- 10.00±0.10 -	- 3.5 -	- 10.00±0.20 -	- 3.5 -	- - -	- - -
	Ad (Root)	n-Hx Eto Ac MeOH	10.00±0.15 - -	3.0 - -	- - 20.00±0.25	- - -.9	- - 20.00±0.20	- - 0.9	- - -	- - -	- -	- - -	- - 20.00±0.15	- - 0.9	20.00±0.10 - -	1.5 - -

Sm-L (*Spondia smombin* leaf),;Cz-L (*Croton zambasi*cus; leaf), Cp-R (*Calliandra portoricensis* – Root), Dt-L (*Dennettia tripetela* - leaf) and Ad-R (*Anthocleista djalonensis* - Root);

MIC (mg/ ml) = Minimum Inhibitory Concentration

TA (ml/g) = Total Activity (Quantity of material extracted from 1 g of plant material in mg, divided by MIC in mg/ml [21].

Values were expressed as mean ± SEM; n=3; Analysis was processed with (SPSS-version 20.0) software and a one way (ANOVA) at P≤0.05.

CONCLUSION

The over all report of this study had scientifically justified their uses in Traditional medicine. The findings also were consistent with the earlier reports on the plant samples. *Calliandra portoricensis* that exhibited good antibacterial and antifungal activities was a good candidate for further investigation. *Croton zambasi*cus ranked second as an equally good antibacterial agent but not as effective an antifungal agent.

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