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Antimicrobial activity of *Mallotus oppositifolium* extractives

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Summary
A bioactivity monitored phytochemical examination of the morphological parts of *Mallotus oppositifolium* utilizing the hole-in-plate bioassay procedure against Gram-positive, Gram-negative bacteria and fungal isolates resulted in the location of significant antimicrobial activity in the acidic fraction (HAF) of the hexane extract of the powdered leaves.Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were 32.5 µg/ml and 65 µg/ml against *Pseudomonas aeruginosa* NCTC 6750 and 25 µg/ml and 50 µg/ml against *Staphylococcus aureus* NCTC 6571 respectively. These activities were found comparable with standard drugs.

Keywords: *Mallotus oppositifolium, euphorbiaceae, antimicrobial.*

Résumé
Une biactivite controlee de l'examination phytochimique des parties morphologiques de *mallotus oppositifolium,* utilisant les plonques internes trouces de la procedure qualiitative et quantitative contre Gram - positif, Gram - negatif des bacteries et des isoles de moisissures ont aboutit a la localisation significative de l'activite antimicrobienne dans la fraction acidique de l'hexane (HAF) extrait de la pondre des femitles. Les valeurs minimales de concentration ihibitive (MIC) et de concentration minimales bactericioles etaient de 32, 5 µg/ml et 65 µg/ml contre *pseudomonas aeruginosa* NCTC 6750 et 25 µg/ml et 50 µg/ml contre *staphylococcus aureus* NCTC 6571, respectivement. Ces activites ont ete tronve comparable avec les medicaments standards.

Introduction

Mallotus oppositofolium is a shrub of up to 12ft high, which inhabits old farms in the secondary forest. The leaves are broad ovate in shape, the flowers are rose-scented, creamy white to yellow in colour while the fruits are smooth and 3-celled. It is commonly referred to as 'oju-eja' in the South-Western region of Nigeria (1,2). The leaves are locally employed in anti-infective preparations used in the treatment of dysentary, sores, pneumonia, wounds, amongst others (2,3). The seed is poisonous, while it is employed with the leaves of *Piliostigma thonningii* is used as an antidote(4). Rottlerin (an anthraquinone-like compound) has been extracted from the leaves of *M. oppositofolium*. The antimicrobial activity of this plant species is yet to be reported in literature.

Materials and methods

Plant material

Mallotus oppositifolium (Geisel mull-Arg) leaves and roots were collected from the botanical garden of the University of Ibadan. Voucher specimen PGUI 110 was deposited in the Department of Pharmacognosy herbarium.

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The leaves were air dried and communited in the Wood Chemistry Department, University of Ibadan.

Phytochemical screening

Standard phytochemical screening procedures (5) revealed the presence of alkaloids, tannins, cardiac glycosides and anthraquinones.

Preparation of extracts.

Forty grams of powdered leaves and 20g of powdered roots were exhaustively and successively extracted with n-hexane, methanol and water. Extracts were concentrated in vacuo to low volumes . The hexane leaf extract (HL 1.2g) was extracted with 10% HCl solution to give the basic fraction (HBF) and the remaining hexane portion contained the neutral and the acidic fractions. Subsequent extraction with 10% NaOH produced the acidic fraction (HAF) while the remaining was the neutral fraction (HNF).

Aqueous salt solutions were converted into free forms by treating with acid or base as appropriate and extracting into an organic solvent as indicated in Figure 1.

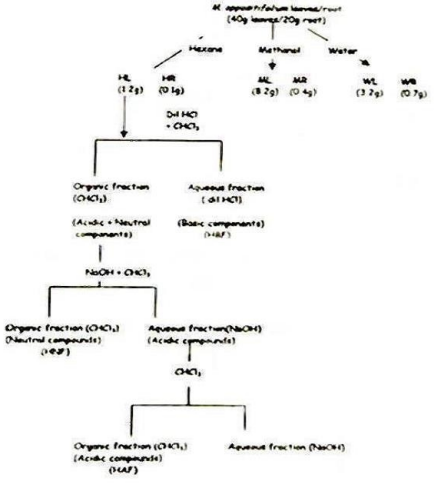

Fig 1: Schematic diagram of the fractionation procedure of *M. oppositifolium* extractives

Thin layer chromatography:

Analytical: Silica gel GF_{254} (Merck) 0.25mm thick, activated at 100°C before use.

Solvent system (SSI): Ethylacetate : methanol : water (10:1.3:0.7).

Visualisation: $FeCl_3$, 5% KOH.

Antimicrobial assay

Test organisms:Escherichia coli NCTC 7001, *Pseudomonas aeruginosa* NCTC 6750, *Staphylococcus aureus* NCTC 6571,

5. Trease GE and Evans WC. Pharmacognosy, 14th Edition, London W.B. Saunders. 1996; 268-269.

6. Hugo WB and Russel AD. Pharmaceutical Microbiology. 3rd Edition Blackwell Scientific Publication. 1983; 167.

7. Onawunmi GO. Evaluation of the antimicrobial activity of citral. Letters in Applied Microbiology. 1986; 9; 105-108.

Bacillus cereus LSCV and *Candida albicans* LSCV.

Antimicrobial agents: Ampicillin hydrochloride (Lab Oftalmiso, Spain), Gentamicin sulphate (Lek, Yugoslavia) and Tioconazole (Pfizer, Nigeria).

Antimicrobial evaluation :The hole-in-plate bioassay method (6) was used. One hundred millilitre molten sterile nutrient broth was cooled to 50 °C and inoculated with 0.5 ml of overnight culture of test organism. Twenty millilitre quantities of the inoculated medium was each poured into a 9 cm agar plate and allowed to set.

Equidistant wells of 6mm were bored into the agar using a sterile cork borer and the wells were filled with the particular extract under test.

All extracts were reconstituted into 50% v/v methanol in water which was used as a control. Eighty microlitres of crude extracts (25 µg/ml), purified fractions (10 µg/ml) and drug controls gentamicin (2.5 µg/ml), ampicillin (2.5 µg/ml) and tioconazole (1mg/ml) were poured into the wells. The plates were left at room temperature for 45 minutes and incubated at 37 °C for 24 hours for bacterial isolates and 25 °C for 48 hours for fungal isolates.

The diameter of the zones of inhibition were measured after the incubation period. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) measurements were determined by the broth dilution method (7).

Results and discussion

The results obtained suggest that the crude extracts and purified fractions have considerable antimicrobial activity (Table 1). The hexane leaf extract produced preferred activity against all test micro-organisms when compared with the methanol and aqueous fractions of both the powdered leaves and root. Subsequent fractionation of the hexane leaf extract into the acidic, basic and neutral fractions showed that activity resided in the acidic fraction (HAF). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the acidic fraction hexane leaf extract (HAF) were found comparable with those of the drug controls used (Table 2).

Thin layer chromatographic(TLC) analysis of the HAF revealed the presence of three phenolic compounds with Rf 0.13, 0.22 and 0.67 using ethylacetate:methanol:water (10:1.3:0.7) as solvent system. These compounds differed from rottlerin which had earlier been isolated from *M.oppositifolium* in both Rf values and colour reactions.

The above results however indicate that *M.oppositifolium* possess antimicrobial activity which justifies its use locally in antiinfective preparations . Further work is being carried out in our laboratory to isolate the bioactive constituents.

Table 1: Results of the antimicrobial assay of the crude extracts and purified fractions of *M. oppositifolium*.

	Zone of Inhibition (mm)											
Organisms	HL	ML	WL	HR	MR	WR	HAF	HBF	HNF	Ampicillin	Gentamicin	Tioconazole
E.coli	10.0±0.2	12.0±0.4	11.0±0.2	12.0±0.2	12.0±0.0	-	17.0±0.2	-	-	14.0±0.0	7.5±0.2	NT
B.cereus	10.5±0.8	12.0±0.2	-	9.0±0.6	12.0±0.4	-	17.0±0.0	12.0±0.0	18.0±0.2		11.5±0.4	NT
S.aureus	11.5±0.0	-	9.5±0.8	11.0±0.6	-	10.5±0.2	21.0±0.2	14.0±0.4	13.0±0.2	20.5±0.0	15.0±0.4	NT
P.aeruginosa	15.0±0.2	-	12.0±0.4	12.0±0.4	12.0±0.4	-	16.5±0.4	-		11.5±0.8		NT
C.albicans	15.0±0.0	-	-	11.5±0.4	-	-	10.0±0.4	-		NT	NT	12.0± 2

Key:

HL	= *Hexane Leaf*	*HAF*	= *Hexane Leaf, Acidic Fraction*
ML	= *Methanol Leaf*	*HBF*	= *Hexane Leaf, Basic Fraction*
WL	= *Water Leaf*	*HNF*	= *Hexane Leaf, Neutral Fraction*
HR	= *Hexane Root*	-	= *No activity*
MR	= *Methanol Root*	*NT*	= *Not tested*
WR	= *Water Root*		

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of acidic fraction hexane leaf extract (HAF)

Organism	HAF		Ampicillin		Gentamicin	
	MIC(ug/ml)	MBC(ug/ml)	MIC(ug/ml)	MBC(ug/ml)	MIC(ug/ml)	MBC(ug/ml)
Ps. Aeruginosa	32.5	65	NT	NT	1.25	2.5
S.aureus	25	50	1.52	3.13	1.25	1.25

References

1. Keay RWJ, Onochie CFA and Stanfield DF. Nigerian trees, Vol. II. Fed. Govt. Printers, Lagos, Nigeria. 1964; 152-153.
2. Iwu MM. Handbook of African medicinal plants. CRC press. Florida. 1993; 125-128.
3. Irvine FR. Woody plants of Ghana. Oxford University Press. London. 1961; 68-69.
4. Burkill HM. The Useful Plants of West Tropical Africa. Edition 2, Vol. 2, Families E-I. Royal Botanic Gardens, Kew. 1994; 144-150.