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Phytochemical and Antibacterial Studies of Eastern Nigerian Mistletoe (*Loranthus Micranthus*) Parasitic on *Pentacletra Macrophylla* and *Parkia Biglobosa*

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Abstract : The comparative studies of methanol extracts of the leaves of *Loranthus micranthus* parasitic on *Parkia biglobosa* and *Pentacletra macrophylla* on the basis of their phytochemical and antimicrobial properties was carried out. The leaves of *Loranthus micranthus* parasitic on *P. biglobosa* and *P. macrophylla* were extracted with 2.5 L methanol by cold maceration at room temperature. Preliminary phytochemical screening showed that both extracts had similar phytochemical constituents namely: terpenoids, carbohydrates, reducing sugars, saponins, glycosides, steroids and resins. The antimicrobial assays of the extracts were studied on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris, Klebsiella pneumoniae* and *Salmonela typhii*. The minimum inhibitory concentrations (MIC) of the extract are 14.81, 14.34 mg/mL for *L. micranthus* parasitic on *P. macrophylla* and 16.27, 19.60 mg/mL for *P. biglobosa* respectively against *E. coli* and *P. vulgaris*. Also, the minimum bactericidal concentrations (MBC) of the extracts are 25.87, 14.81 mg/mL and 18.78, 18.78 mg/mL for *L. micranthus* parasitic on *P. macrophylla* and *P. biglobosa* against *E. coli* and *P. vulgaris*.
Keywords: *Loranthus micranthus*; *Parkia biglobosa*; *Pentacletra macrophylla*; *Escherichia coli*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Proteus vulgari; Salmonela typhii;*.

1.0. Introduction

Human kind has been subjected by wide varieties of micro-organisms since the dawn of recorded history, and therefore novel antimicrobial agents are needed to address this problem[1]. As a result of this quest, plants remain the most common source of antimicrobial agents. Plants are the richest bio resources of drugs of traditional medicinal systems, modern medicines, folk medicines, and pharmaceuticals, food supplements, intermediate and chemical entitled for synthetic drugs[2]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound as antibacterial agents[3]. Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antibacterial activity of medicinal plants[3]. Ethno-pharmacologist, botanist, microbiologist etc, are searching for phytochemicals which could be developed for treatment of infectious diseases[4]. African mistletoe is used folkloric medicine in the treatment of epilepsy, hypertension, headache, infertility, cancer, menopausal syndrome and rheumatism[5].

Loranthus micranthus Linn (loranthaceae) is the eastern Nigerian species of the African mistletoe, which has been used widely in ethnomedicines as anti-diabetic, anticancer, antihypertensive, antimicrobial[6].

They belong to the plant family *viscaceae* of order *santalaies* parasitic on *Pentacletra macrophylla* and *Parkia biglobosa*. In Ibo, *P. macrophylla* is referred to as "Ukpaka" and P. *biglobosa* is known as "Ugba". The leaf of *P. biglobosa* is known to provide ingredient that is used in treating leprosy and hypertension[7]**.** The antidiabetic and antimicrobial activities of *L. micranthus* have also been reported [8]. It is equally used in many parts of West Africa as an antimicrobial and antispasmodic agent[9]**.** The leave together with the root is used in Gambia for the treatment of sore eyes. The leaves of P. *macrophylla* are used in Ghana in treating of diarrhea and some other industrial uses, such as fuel wood and charcoal[10].

This study is aimed at investigating the variation in the *Phytochemicals* and antimicrobial[13-21] constituents of *Loranthus micranthus* leaves sourced from *Parkia biglobosa* and *Pentacletra macrophylla*.

2.0. Materials and Method

Fresh leave of *Loranthus micranthus* parasitic on *Pentacletra macrophylla* and *Parkia biglobosa* were collected from Enugu-Ezike, Enugu State of Nigeria; between the month of January and February and was identified and authenticated at Centre for Ethno-medicine and Drug Development Program, University of Nigeria, Nsukka. Fehling's solutions A and B, Dragendorff's reagent, Wagner's reagent, 5 % Iron (III) chloride, 1 % Aluminum (III) chloride, Bromine water, Aqueous ammonia, Ethanol, Methanol, Nutrient agar, α-naphthol, Concentrated tetraoxosulphate (VI) acid, Concentrated hydrochloric acid, Lead acetate solution, Mayer's reagent were of analytical.

2.1. Preparation of Plant Material

The leaves of *Loranthus micranthus* parasitic on *Pentacletra macrophylla* and *Parkia biglobosa* were air dried and pulverized using Thomas–Wiley Laboratory Mill, Model 4. Seven hundred gram (700 g) of the powered leaves was extracted with 2.5 L of methanol by cold maceration at room temperature for seven days. The extracts were filtered with chess cloth and then with Whatman filter paper to obtain the clear filtrate. The clear filter was concentrated with in vacuo using rotary evaporator. The extract was stored in the refrigerator throughout the experiments.

2.2. Phytochemical Analysis of the Methanol Extracts

The Phytochemical analysis was done using standard method and carried out in the Department of Pharmaceutical Chemistry, University of Nigeria, Nsukka.

2.3. Antibiotic Assay of the Methanol Extracts

The minimum inhibitory concentration (MIC) was determined using the agar dilution method. Two fold serial dilution of the extract (6.25, 12.5, 25, 50, 100, 200 mg/mL) were prepared using sterile distilled water and poured into separate sterilized Petri dishes. 20 mL of molten nutrients agar were poured into Petri dish, swirled slowly and allowed to set and dry. Agar plate containing 20 mg/mL of Ampicillin (positive control) were also streaked with the micro-organisms. The agar plates were incubated at 37 ^{o}C for 24 hours. MIC was determined as the lowest concentration of the extract or ampicillin that did not permit visible growth as compared to the negative control[11]**.**

The minimum bactericidal concentration (MBC) was determined by the same experimental procedure as that of MIC. The agar plates for the determination of MBC were incubated for 72 hours at 37 ^{o}C.

3.0. Results:

Table 3.1: Results of the Phytochemical Analysis of the methanol Extracts of *Loranthus micranthus* parasitic on *Parkia biglobosa* (MPB) and *Pentacletra macrophylla* (MPM)

	Relative abundance	
Secondary metabolite	LPB	LPM
Alkaloids	++	-
Flavonoids	+++	-
Glycosides	+++	+
Steroids	++	++
Saponins	+++	+
Tannins	+++	-
Carbohydrates	++	++
Proteins	-	-
Resins	+++	-
Acidic compound	-	-
Oil	-	-
Reducing Sugar	+++	+
Terpenoids	++	+++

Key (-) = Absent (+) = Low in abundance, (++) = Moderate in abundance
(+++) =High in abundance, LPM = Mistletoe parasitic on Parkia biglobosa
LMP = Mistletoe parasitic on Pentacletra macrophylla

Table 3.2 below shows the results of the percentage yield of the extracts.

Extract	Colour	Mass(g)	%Yield
LPB	Dark-green	12.22	1.75%
LPM	Rusty-brown	10.08	1.44%

Key:
LPB: *Loranthus micranthus* parasitic on *Parkia biglobosa*
LPM: *Loranthus micranthus* parasitic on *Pentacletra macrophylla*

Table 3.3: Results of the Antibiotic Assay (MIC) of methanol extract of

***Parkia biglobosa* against some bacteria**

Extract concentration (mg/mL) and IZD (mm).						
Bacteria	200	100	50	25	12.5	6.25
S. aureus	+	+	+	+	+	+
E. coli	16	12	9	6	3	+
P. aeruginosa	+	+	+	+	+	+
P. Vulgaris	15	10	7	5	3	+
K. pneumonia	+	+	+	+	+	+
S. typhii	+	+	+	+	+	+

Key: +: No inhibition

Table 3.4: Result of the Antibiotic Assay (MIC) of Methanol Extract of *Pentacletra macrophylla* against some bacteria

Extract concentration (mg/ml) and IZD (mm).						
Bacteria	200	100	50	25	12.5	6.25
S. aureus	+	+	+	+	+	+
E. coli	16	12	9	6	3	+
P. aeruginosa	+	+	+	+	+	+
P. Vulgaris	15	10	7	5	3	+
K. pneumonia	+	+	+	+	+	+
S. typhii	+	+	+	+	+	+

KEY: +=No Inhibition

Table 3.5: Results of the Log of concentration of extract and IZD^2 $(mm)^2$ of *Parkia biglobosa*

Bacteria	**2.301**	**2.000**	**1.699**	**1.398**	**1.097**
E. coli	256.0	144.0	81.0	36.0	9.0
P. vulgaris	225.0	100.0	49.0	25.0	9.0

Table 3.6: Result of the log of concentration of extract and IZD^2 $(mm)^2$ of Pentacletra macrophylla.

Bacteria	**2.301**	**2.000**	**1.699**	**1.398**	**1.097**
E. coli	100.0	64.0	36.0	16.0	4.0
P. vulgaris	144.0	100.0	49.0	25.0	9.0

Table 3.7 Results of the Antibiotic Assay (MBC) of the methanol extract of *Pentacletra macrophylla*

Concentration of Extract (mg/ml) and IZU (mm)						
Bacteria	200	100	50	25	12.5	6.25
S. aureus	+	+	+	+	+	+
E. coli	8.20	6.40	4.00	2.00	+	+
P. aeruginosa	+	+	+	+	+	+
P. Vulgaris	10.00	8.00	6.00	4.00	2.00	+
K. pneumonia	+	+	+	+	+	+
S. typhii	+	+	+	+	+	+

Key: + = No inhibition/activity

Table 3.8: Log of concentration MBC and IZD^2 (mm^2) of methanol extract of Pentacletra macrophylla

Organisms	**2.301**	**2.000**	**1.699**	**1.398**	**1.097**
E. coli	67.20	41.00	16.00	4.00	+
P. vulgaris	100.00	64.00	36.00	16.00	4.00

Table 3.9: Results of the Antibiotic Assay (MBC) of the methanol Extract *Parkia biglobosa*

Concentration of Extracts (mg/ml) and IZD (mm)					
Organisms	200	100	50	25	12.5
S. aureus	+	+	+	+	+
E. coli	15	10	8	5	2
P. aeruginosa	+	+	+	+	+
P. Vulgaris	14	9	6	4	2
K. pneumonia	+	+	+	+	+
S. typhii	+	+	+	+	+

Table 3.10: Results of the log of concentration and IZD 2 (mm) 2 of Parkia biglobosa.

Bacteria	2.301	2.000	1.699	1.398	1.097
E. coli	225.0	100.0	64.0	25.0	4.0
P. vulgaris	196.0	81.0	36.0	16.0	4.0

Table 3.11: Result of the Antibiotic Assay (MIC) of Ampicillin at a concentration of 20 mg/mL

Bacteria	Inhibition Zone Diameter (mm)
S. aureus	6.0
E. coli	9.0
P. aeruginosa	6.0
P. vulgaris	8.0
K. pneumonia	16.0
S. typhii	10.0

Discussion:

The phytochemical screening of the methanol extracts revealed the presence of alkaloids, glycosides, flavonoids, reducing sugars, saponins, tannins, terpenoids and steroids. These classes of compounds are known to show curative activities against several pathogens and therefore explain its use traditionally for the treatment of wide arrays of illness[12]. The methanol extracts of *Loranthus micranthus* leaves showed varying degree of antibacterial activities against test organisms (table 3.3). The extracts had a broad activity on bacteria at concentrations of 200 mg/mL. The broadest spectrum observed is with *E. coli* IZD of 16 mm at a dose level of 200 mg/mL and the least spectrum activity is observed against P. vulgaris with an inhibition zone diameter of 15 mm. it is observed that this extract showed no activity against S. *aureus*, P. *aeuriginosa*, K. *pneumonae* and S. *typhii* respectively. This may be attributed to the presence of soluble phenolic and polyphenolic compounds. Table 3.11 shows the result of the MIC of the control drug ampicillin and it is observed that it showed a very good broad spectrum activity against all the bacteria and this can be attributed to the pure form of the drug as compared to the crude methanol extract of *Loranthus micranthus*.

The minimum inhibitory concentrations (MIC) of the methanol extract for different organisms ranged between 2.00-16.00 mg/mL. For ampicillin it ranged between 6.00-16.00 mg/mL. The minimum bactericidal concentration (MBC) of the extracts for different bacteria ranged between 2.00-15.00 mg/mL (table 3.9). The inhibitory effect of methanol extracts of Loranthus micranthus against pathogenic bacterial strains suggests that the plant is a potential candidate for drug development for the treatment of ailments caused by these pathogens.

Conclusion

The plant materials sourced from *Pentacletra macrophylla* and *Parkia biglobosa* was found to show relatively better spectrum of antibacterial activity when compared with that sourced from other host trees. The claim of the use of *Loranthus micranthus* as antibacterial agent is scientifically justified by the fact that it contains important therapeutical phytochemicals and show significant inhibition against bacteria.

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