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Antimicrobial Studies of Aqueous Extract of the Leaves of *Lophira Lanceolata*

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ABSTRACT

The antimicrobial activity of aqueous extract of the leaves of *Lophira lanceolata* was tested on *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Selaginella selaginoides*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Streptococcus pyogens*. The micro organisms were chosen because they are associated with dysentery, diarrhoea , skin infection, especially burn sites, wounds, pressure, sores and ulcer, The In vitro- antimicrobial assay recorded the zones of inhibition of bacterial and fungal growths. Extracts with inhibition zones greater than (>10mm), were considered active The (MBC) of the aqueous extract showed considerable inhibition of the micro-organisms at higher doses. While the extract inhibited *Escherichia coli* and *Streptococcus pyogens*, at 800 mg/kg, the extracts are active at 1000 mg/kg. From the result of the MIC it was observed that the leaves aqueous extract shows comparable minimum inhibitory concentration on all the micro organisms with growth of *Escherichia coli, Streptococcus aureus, Streptococcus* Pyogens and *Selaginella selaginoides Pseudomonas aeruginosa* at 6.25mg/ml while for, *Bacillus subtilis, Aspergillus niger, Shigella dysenteriae* and *Candida albicans* showing at 12.5 mg/ml The leaves aqueoues extract shows comparable MBC to Tetracycline and Ciprofloxacin, for organisms with 3.125 mg/ml for *Shigella dysenteriae*, *Bacillus subtilis*, 6.25mg/ml for *Candida albicans*, *Aspergillus niger* while showing 12.5 mg/ml for *Escherichia coli, Staphylococcus, Pseudomonas aeruginosa*, *Streptococcus pyogens* and *Selaginella selaginoides.* This is being reported in this plant for the first time.

Keywords: Antimicrobial activity; Minimum Inhibitory Concentration; *Lophira lanceolata;*

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INTRODUCTION

Lophira lanceolata is a multipurpose tree. Its seeds are eaten, but more commonly in the past than at present; now they are mainly used to extract edible oils. The oil also has cosmetic and medicinal uses and is suitable for making soap. The wood is hard and heavy and is locally used e.g. for mortars, railway sleepers and in bridge construction. It is also used in house construction and to make agricultural and household tools. It is excellent firewood producing hot flames and little smoke and is also a good source of charcoal. Edible caterpillars are grown on the tree; in northern Cameroon, where they are called 'dessi', 'sankadang' or 'sélénibétéyo' in the Gbaya language, they are collected, traded and consumed by several tribes. The flowers are fragrant and an important source of honey, e.g. in Nigeria. The bark of the plant is used as a colorant in Nigeria and other part of West Africa to prevent cooked yam from becoming dark. During the dry season, the foliage is browsed by cattle.

In traditional medicine the oil is used to treat dermatosis, toothache and muscular tiredness. Rubbing the skin with the oil prevents dryness. The oil is mixed with porridge and given to children as a tonic. The sap of the tree is used to treat tiredness by the Dii, Fulbe and Gbaya people in Cameroon. In Mali pounded roots, mixed with flour are used to treat constipation, while its concoction is used to cure chronic wounds. A concoction prepared from the roots is drunk by women against menstrual pain, intestinal troubles and malaria. The bark of the roots and trunk is used against pulmonary diseases. The bark is also used to treat fevers and gastro-intestinal problems, and in southern Nigeria the root bark is a remedy for yellow fever.

The young stems and sometimes the roots are commonly used as chew-sticks, and an infusion of the bark is used as a mouthwash against toothache in Guinea, Mali and Nigeria. An infusion of the young twigs is used to treat fever, respiratory tract infections and dysentery. Concoctions of young fresh or dried leaves taken in the form of a drink are given to treat pain caused by intestinal worms, dysentery and diarrhoea in children, while as a steam bath it is said to cure general tiredness and rheumatism. Pain caused by worms can also be treated by eating young fresh leaves. Decoctions of the young red leaves are also employed in the treatment of headache, hypertension and syphilis. Culturally, the leaves and wood of *Lophira lanceolata* are very important for the Dii people. The leaves are used for traditional dances and masks are made from the wood. The medicinal uses are probably inseparable from the ceremonial uses of the leaves [5].

While some flavonoids may help control growth in some plant other class of flavonoids can protect this plant from infection by viral, fungal and bacterial.

Flavonoids of many types have antiviral effects in animals. In human cell lines (Hela cells) Herpesvirus hominis is inhibited by quercetin at levels of 300-µg ml^{-1} [8]. Most of the flavonoids investigated have an inhibitory activity toward one or more bacterial or fungal studied. Antihelmintic activities have also been reported for flavonoids [4].

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MATERIALS AND METHODS

Plant material and extraction

The plant under screening is a spermatophyte collected from, Sakaru village along Jos road Zaria, in December 2005 and identified at the Herbarium section, Biological Science Department Ahmadu Bello University Zaria as *Lophira lanceolata* a voucher coded 4002B was deposited in the Herbarium.

An unknown weight of *Lophira lanceolata* leaves was collected around Sakaru village, Jos road Zaria in December 2005, identified by A.B.U Herbarium unit Department of biological science and was dried and grounded to powder. 200g of the powdered leaves was extracted to exhaustion using water by cold process (maceration) the combined aqueous extract was concentrated. These was use for the screening

Bacterial and fungal Strains used

The Gram – positive organisms used in this study are: *Bacillus subtilis, Selaginella selaginoides*, *Shigella dysenterae* and *Staphylococcus auraus*, while Gram – negative organisms are: *Escherichia coli Streptococcus* pyogene, and *Pseudomonas aeruginosa*. The fungal strains are *Candida albicans* and *Aspergillus niger* – which are laboratory isolates.

Antimicrobial Susceptibility Studies

Disc diffusion technique as described by Chung *et al*., 1990 [1] was used. Each extract and standard antibiotics were independently tested in duplicate. Diameters of zones of inhibition ≥10 mm were considered active [12].

Minimum inhibitory concentration (MIC)

MIC was determined using the broth dilution technique [10, 11].

Minimum Bacterial concentration (MBC)

MBC were determined by using the broth dilution technique previously described by Vollekova *et al*., (2001)

Micro organism used

Clinical isolates of *Escherichia coli, Staphylococcus aureus, Shigella dysenterae*, *Streptococcus pyrogenes, Selaginella selaginoides*, *Bacillus subtilis Candida albicans, Aspergillus niger and Pseudomonas aeruginosa* were used.

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Testing Microbial Susceptibility

The methods of inoculation were carried out according to (Monica 2000). Tetracycline and Ciprofloxacin were used as standard drug. Stock solution was prepared in sterile distilled water in a concentration of 1mg/ml. Solutions of different concentrations were made from ethanol and aqueous extract of the leaves of *Lophira lanceolata* and from tetracycline/ Ciprofloxacin (standard) drug.

Concentrations used

Extract are prepared using the general fomular to get the required concentration of the extract in mg/mls. The highest concentration is 6000mg/mls, 1000mg/ml, 500mg/ml and 10mg/mls.

MIC Procedure

Equal volume of nutrient broth was dispensed into bottles where known concentrations raging from higher to the lowest of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.563mg/ml, 0.780mg/ml 0.390mg/ml 0.195mg/ml 0.098mg/ml were prepared. Also equal volume of known concentration and microbial isolates (*Staphylococcus aureus*, *Pseudomonas* aeruginosa, *Candida albicans*, *Streptococcus* pyogene, *Escherichia* coli, *Shigella dysenterae*, *Selaginella selaginoides*, *Bacillus subtilis* and *Aspergillus niger*)

RESULTS AND DISCUSSION

Phytochemical screening of the leaves extracts of *Lophira lanceolata* showed that the extract contains carbohydrates, tannins, saponins, cardiac glycosides, terpenoids, steroids, anthraquinone, and flavonoid. Alkaloid was absent in this species.

Flavonoids have certain nutritive effects though they are non-nutritive compounds [2]. A considerable research has been directed towards their activity as anti-oxidant and radical scavenging properties [3] as well as anti-mutagenic and anti-carcinogenic properties and also their potential in the prevention of coronary heart disease [6].

The use of flavonoids has been epidemiologically studied. The use of flavonoids in the treatment of disease is to large extent much older than the science of chemistry. In view of the safe and effective treatment, a study of the role of Silymarin in the management of non-B acute viral hepatitis was investigated, which showed significantly earlier recovery from hepatomegaly and enlarged spleen in patients receiving Silymarin. Also, treatment with hydroxyl ethyl rutosides significance improved the sensation of limb swelling bursting pain, heaviness, tension and mobility [9].

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Table 1: Microorganisms susceptibility to aqueous extract of the leaves of *Lophira lanceolata*

Extract Conc.(mg/ml)	Microorganism /zone of inhibition								
	E.c	S.a	P.e	C.a	S.p	B.s	A.n	S.d	S.s
400	R	16	18	12.	R	14	12	16	18
600	R	18	20	18	R	18	18	18	26
800	R	22	20	26	R	22	24	22	32
1000	32	22	22	36	28	40	32	38	36
Tetracycline 5µg/ml	50	35	50	50	40	50	40	50	50
Ciprofloxacin, 5µg/ml	50	50	50	50	50	50	50	50	50

Key: S.a = *Staphylococcus aureus*, S.d = *Shigella dysenteriae*, S.s = *Selaginella selaginoides* , B.s = *Bacillus subtilis*, E.c = *Escherichia coli*, P.e = *Pseudomonas* aeruginosa, C.a = *Candida albicans*, An = *Aspergillus niger*, *S.p = Streptococcus pyogens*.

Table 2: Minimum inhibitory concentration of some micro-organisms produced by leaves aqueous extract of *Lophira lanceolata*.

Extract Drug conc. (mg/ml)	Micro- organisms to the aqueous extract of *Lophira lanceolata*								
	E.c	B.s	A.n	S.a	P.e	C.a	S.p	S.d	S.s
100	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-
6.25	+	-	-	+	+	-	+	-	+
3.125	+	+	+	+	+	+	+	+	+
1.563	+	+	+	+	+	+	+	+	+
0.781	+	+	+	+	+	+	+	+	+
0.390	+	+	+	+	+	+	+	+	+
0.195	+	+	+	+	+	+	+	+	+
0.098	+	+	+	+	+	+	+	+	+
Tetra cycline 5µg/ml	50	50	50	50	50	50	50	50	50
Ciprofloxacin5µg/ml	50	50	50	50	50	50	50	50	50
MIC	12.5	6.25	6.25	12.5	12.5	6.25	12.5	6.25	12.5

Key: S.a = *Staphylococcus aureus*, S.d = *Shigella dysenteriae*, S.s = *Selaginella selaginoides* , B.s = *Bacillus subtilis*, E.c = *Escherichia coli*, P.e = *Pseudomonas aeruginosa*, C.a = *Candida albicans*, An = *Aspergillus niger*, *S.p = Streptococcus pyogens* + = growth, - = no growth.

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Table 3: Minimum Bactericidal Concentration of Micro-Organisms produced by Leaves Aqueous Extract Of *Lophira Lanceolata*.

Extract conc. (mg/ml)	Micro- organisms to the aqueous extract of *Lophira lanceolata*								
	E.c	P.e	S.a	C.a	S.p	B.s	A.n	S.d	S.s
0.098	+	+	+	+	+	+	+	+	+
0.195	+	+	+	+	+	+	+	+	+
0.390	+	+	+	+	+	+	+	+	+
0.781	+	+	+	+	+	+	+	+	+
1.563	+	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	-	+	-	+
6.25	+	+	+	-	+	-	-	-	+
12.5	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-
Tetracycline (5µg/ml)	50	50	50	50	50	50	50	50	50
Ciprofloxacin (5µg/ml)	50	50	50	50	50	50	50	50	50
MBC	12.5	12.5	12.5	6.25	12.5	3.125	6.25	3.125	12.5

Key: S.a = *Staphylococcus aureus*, S.d = *Shigella dysenteriae*, S.s = *Selaginella selaginoides* , B.s = *Bacillus subtilis*, E.c = *Escherichia coli*, P.e = *Pseudomonas aeruginosa*, C.a = *Candida albicans*, An = *Aspergillus niger*, *S.p = Streptococcus pyogens* + = growth, - = no growth.

The antimicrobial activity of the plant was tested on *Candida* albican, *Shigella dysenteriae*, *Bacillus subtilis*, *Aspergillus niger*, *Escherichia coli*, *Staphylococcus*, *Pseudomonas aeruginosa*, *Streptococcus pyogens*, and *Selaginella selaginoides*.

The micro organisms were chosen because they are associated with dysentery, diarrhoea , skin infection, especially burn sites, wounds, pressure, sores and ulcer, (Monica, 2000). The In vitro- antimicrobial assay recorded the zones of inhibition of bacterial and fungal growths. Extracts with inhibition zones greater than (>10mm), were considered active [12].

The aqueous and ethanol extract showed considerable inhibition of the micro-organisms at higher doses. While the extract inhibited *Escherichia coli* and *Streptococcus pyogens*, at 800mg/kg, the extracts are active at 1000mg/kg.

From the result of the MIC it was observed that the leaves ethanolic extract shows comparable minimum inhibitory concentration on all the micro organisms with growth of *Candida*, *Shigella dysenteriae*, *Bacillus subtilis* and *Aspergillus niger* at 6.25mg/ml while for *Escherichia coli*, *Staphylococcus*, *Pseudomonas aeruginosa, Streptococcus*. Pyrogens and *Selaginella selaginoides* showing at 12.5 mg / ml.

The leaves ethanolic extract shows comparable MBC to Tetracycline and ciprofloxacin, for organisms with 3.125mg/ml for *Candida*, *Shigella dysenteriae*, *Bacillus subtilis*, *Aspergillus*

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niger while showing 12.5 mg / ml for *Escherichia coli*, *Staphylococcus*, *Pseudomonas* and *Streptococcus pyogens*, *Selaginella selaginoides.*

The leaves aqueous extract shows comparable MBC to Tetracycline and Ciprofloxacin, for organisms with 3.125mg/ml for *Shigella dysenteriae*, *Bacillus subtilis*, 6.25mg/ml for *Candida* albican, *Aspergillus niger* while showing 12.5 mg / ml for *Escherichia coli*, *Staphylococcus*, *Pseudomonas aeruginosa* and *Streptococcus pyogens*, *Selaginella selaginoides.*

CONCLUSION

The in-vitro antimicrobial activity of the leaves extract were assayed using the agar plate diffusion and broth technique with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* euroginosa, Streptococcus pyogens, Selaginella selaginoides, at (MIC) of 12.5 mg/ml and *Bacillus subtilis*, *Shigella dysenteriae*, *Candida albicans*, *Aspergillus niger*, at (MIC) of 6.25 mg/ml as test organism.

The extract was active on *Shigella dysenteriae*, and *Bacillus subtilis*, at (MBC) of 3.125 mg/ml confirming the traditional use. Also active on two fungi, *Candida albicans*, and *Aspergillus niger*, at (MBC) 6.25 mg/ml this is been reported for the first time

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