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Phytochemical properties of Methanol Extract and Antimicrobial study of Less Polar Fractions of *Loranthus micranthus* (Linn.) parasitic on *Alstonia boonei*

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The leaves of *Loranthus micranthus* (Linn.) parasitic on *Alstonia boonei* were extracted with methanol by cold maceration method. The extract was purified further by column chromatographic technique with *n*-hexane and chloroform respectively. The extract was investigated for phytochemical constituents while the antimicrobial study of the *n*-hexane and chloroform fractions were evaluated using *Escherichia coli* and *Staphylococcus aureus*. The phytochemical study showed tannins, alkaloids, steroids and terpenoids to be moderately present while glycosides, flavonoids and saponins are fairly present. The antimicrobial study of the *n*-hexane and chloroform fractions showed activities on both bacteria. The minimum inhibitory concentration (MIC) of the *n*-hexane fraction was 12.42 and 0.49 mg/ml against *E. coli* and *Staph. aureus* respectively while the MIC of the chloroform fraction was 23.82 and 0.09 mg/ml against *E. coli* and *Staph. aureus* respectively.

Keyword: *Escherichia coli*, *Staphylococcus aureus, Loranthus micranthus*, Phytochemical analysis.

1. Introduction

In the past, almost all the medicines were from the plants, the plants being man's only chemist for ages. Herbs are staging a comeback, herbal 'renaissance' is happening all over the globe and more and more people are taking note of herbal therapies to treat various kinds of ailments in place of mainstream medicine. There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries [1]. The need for more potent, safe and affordable drugs has led to intensified research into herbal drugs, the result of which is the introduction of new herbal preparation for therapeutic uses [2]. The diversity of biological active substance of plant origin and their bioactivities are also of current interest among biomedical research [3]. Researches in natural alternative medicines exist [4, 5], as there are increasing evidence that many current drug therapies simple suppress symptoms and ignore the underlying disease process, in contrast many natural products appear to address the cause of many diseases and yield superior clinical results

Mistletoes which are hemi parasitic plants growing on different host trees and depend on their host plant for water and mineral nutrition, even though they produce their own carbohydrates through photosynthesis [6, 7]. It is an obligate semi-parasitic plant of *Kola acuminata, Baphia nitida, Citrus limon, Alstonia boone, and Pentaclethra macrophylla* [8]. The leaves of mistletoes are traditionally used in folkloric medicine of Nigeria for the treatment of diarrhea, epilepsy, hypertension and rheumatism [2] and the methanol extracts of mistletoes

parasitic on different host trees have been reported to have antidiarrhea [9], antidiabetic [10], antimicrobial [11] and immunomodulatory activities [12]. Studies have, however, shown that several factors play important role in the phytochemical composition and pharmacological activities of the mistletoe plant, such as: the host, specie of mistletoe used, season of harvest, etc. [13, 14, 10, 11, 15]. Earlier studies by these authors on the crude plant powder and some of its solvent fractions have established some significant antibacterial properties by *L. micranthus,* though with negligible anti-fungal activity [11, 15, 16].

Fig 1: Diagram of *Loranthus micranthus* parasitic on *Alstonia boonei*

Staphylococcus aureus is a Gram-positive eubacteria found on the surface of human skin and mucous membrane, other areas of human contact like air, soil, dust and food products. *S. aureus* is an opportunistic pathogen in human and animals and is one of the most frequent sources of hospital- and community-acquired infections [17]. Many isolates of *S. aureus* have evolved resistance to both synthetic and traditional antimicrobial agents posing potential epidemiological threat outside the hospital; these isolates can transfer resistant genes to other potential pathogens [18] and the accumulation of resistance factors has rendered the bacterium resistant to a variety of commonly used antibiotics [19].

This research was to explore the phytochemical properties and antimicrobial activity of *Loranthus micranthus* parasitic on *Alstonia boonei* on two different bacteria *E. coli* and *Staph. aureus.* This was because the host plant affects the phytochemical constituents and hence the bio-activity of the *Loranthus micranthus*. Owing to reported activities, the *n*-hexane and chloroform solvent fractions were evaluated for the antimicrobial activities on these organisms.

2. Materials and Methods

2.1 Materials

Methanol (Riedel de Hain, Germany), *n*-hexane (Riedel-de Haen, United Kingdom), chloroform (Sigma-Aldrich®, Germany), Thomas-Wiley Laboratory Mill, (Model 4) and distilled water (Lion Water, University of Nigeria, Nsukka)

2.1.1 Plant Material

The leaves of *L. micranthus* parasitic on *Alstonia boonei* were collected in January 2011 from Enugu-Ezike, Enugu State and were identified by Mr. A. O Ozioko, of Bio-resources Development and Conservation Programme (BDCP), Nsukka and a voucher specimen (LM1610) was deposited at the herbarium of the Institute. All other reagents were of analytical grade and were used without further purification.

2.2 Methods

2.2.1 Preparation of the plant material

The leaves of *L. micranthus* parasitic on *Alstonia boonei* were air-dried and extracted according to the procedure by Agbo *et al*., [7] with slight modification. Briefly the leaves were pulverized and the powder (500 g) extracted by cold maceration method in 3.0 L methanol at room temperature for 48 h with continuous agitation. The extract was concentrated in *vacuo* at 40 ºC and stored in the refrigerator till when used.

2.2.2 Purification of the methanol extracts using column chromatographic technique

Ten gram (10.0 g) of the dried methanol extract was purified further using column chromatographic method. The extract was mixed with silica gel 60 and dissolved in methanol. The mixture was dried and loaded unto glass column (150×1.5 cm, ID) which has been packed to two-third the length with 200 g slurry of silica gel (20-230 mesh). The column was eluted with 2.5 L of *n*-hexane and 2.5 L of chloroform. These fractions were concentrated *in vacuo* to obtain the dry solvent fractions.

2.2.3 Phytochemical screening of the methanol extract

Phytochemical analysis of the extract was performed using standard method [20, 21].

2.2.4 Determination of antimicrobial susceptibility

The antimicrobial susceptibility studies of the solvent fractions (*n*-hexane and chloroform) were carried out using the paper disc diffusion technique previously described by Cheebrough[22]. The stock solution of the n-hexane fraction was prepared as described below. Standard concentrations of the fraction was introduced into different 6 mm diameter paper discs with micropipette and preserved aseptically. The densities of the microorganisms were adjusted as per Mac Farland 0.5 standard and microorganisms were grown in nutrient broth for 18 h, and transferred to Muellar Agar (MHA).

The *n*-hexane fraction impregnated disc was introduced into the Muellar Hinton Agar already inoculated with the test microorganisms. The plates were incubated at 37 ºC for 24 h and zones of inhibition of the triplicate analyses were measured and the mean IZD determined. The same procedure was repeated for the chloroform fraction.

2.2.5 Determination of MIC of the different fractions

The MIC was determined using agar diffusion method. A stock was prepared by dissolving 100 mg of the fractions in 2 mL of Tween 80 or DMSO to get 50 mg/mL. A six two fold serial dilution of the stock (25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/mL were prepared. 24 h MacFarland standard of *E. coli* and *Staph. aureus* were prepared with sterile normal saline and 0.1 mL of each organism was smeared over the face of a sterile nutrient agar plate and 20 mL of the MacFarland standard was added and swirled for uniform distribution. Sterile cork borer was used to bore hole on the nutrient agar plate. This was allowed to stay on the bench for pre-diffusion time of 1 h and later incubated for 24 h. The zone of inhibition was measured and the mean inhibition zone diameter determined.

2.3 Statistical analysis

The results were analyzed using descriptive statistical method according to Woodson [23].

3. Results

The yield was assessed to know which of the solvent will be adopted in fractionating the L. *micranthus*. The yield of the fractions is shown in Table 1. The methanol extract had the highest yield while the chloroform extract had the lowest yield.

3.1 The Phytochemical study

Phytochemical screening of the methanol extract of the leafy twigs of *L. micranthus* parasitic on *Alstonia boonei* led to the identification of many bioactive constituents as shown on Table 2.

The use of herbal remedies in antimicrobial treatment is a common practice in many countries of the world including Nigeria. The potential inhibitory effects of *n*-hexane and chloroform

fractions of the methanol extract of *Loranthus micranthus* was evaluated on selected human pathogens- *E. coli* and *Staph. aureus.*

Table 1: Percentage Yield of Extract and Fractions.

Extract/Fractions	Colour	Mass (g)	Yield (%)
Methanol extract	Greenish black	10.00	2.00
n-hexane	Yellow	2.50	0.51
Chloroform	Greenish	0.60	0.12

Table 2: Phytochemical Studies of the Plant Extract

Secondary metabolites	Relative abundance
Alkaloids	++
Glycosides	+
Saponins	+
Steroids	++
Terpenoids	++
Tannins	++
Flavonoids	+

KEY: + Low in concentration, ++ Moderate in concentration

Table 3: Various concentrations (mg/ml) of *n*-hexane fraction of *Loranthus micranthus* leaves against *Staph. aureus* and *E. coli*

		Staph. aureus		***E. coli***	
Conc. (mg/ml)	**Log Conc.**	**Mean IZD (mm)**	**Mean IZD^2 (mm^2)**	**Mean IZD (mm)**	**Mean IZD^2 (mm^2)**
50.00	1.6990	6.02 ± 0.15	36.24	2.02 ± 0.02	4.08
25.00	1.3979	5.00 ± 0.00	25.00	2.00 ± 0.00	4.00
12.50	1.0969	4.15 ± 0.22	17.22	+	+
6.25	0.7959	4.12 ± 0.20	16.97	+	+
3.13	0.4949	4.00 ± 0.00	16.00	+	+
1.56	0.1938	3.00 ± 0.00	9.00	+	+
0.78	-0.1072	2.50 ±0.20	6.25	+	+

Key: + no activity or growth not inhibited.

Table 4: Various concentrations (mg/ml) of chloroform fraction of *Loranthus micranthus* leaves against *Staph. aureus* and *E. coli*

		Staph. aureus		*E. coli*	
Conc. (mg/ml)	Log Conc.	Mean IZD (mm)	Mean IZD^2 (mm^2)	Mean IZD (mm)	Mean IZD^2 (mm^2)
50.00	1.6990	4.00 ± 0.00	16.00	1.00 ± 0.00	1.00
25.00	1.3979	3.13 ± 0.13	9.80	3.00 ± 0.00	9.00
12.50	1.0969	3.10 ± 0.10	9.61	+	+
6.25	0.7959	3.07 ± 0.02	9.42	+	+
3.13	0.4949	3.02 ± 0.02	9.12	+	+
1.56	0.1938	3.00 ± 0.00	9.00	+	+
0.78	-0.1072	3.00 ± 0.00	9.00	+	+

Key: + no activity or growth not inhibited.

4. Discussion

The *in vitro* results of this study showed that the *n*-hexane and chloroform fractions of the plant were active against the test organisms.

Tables 1 and 2 showed that the fractions were more active against *Staph. aureus* being a Gram positive bacteria than *E. coli.* The MICs of the *n*-hexane and chloroform fraction were 0.49 mg/ml

and 0.09 mg/ml respectively against *Staph. aureus* and 12.42 and 23.82 mg/ml respectively against *E. coli.*

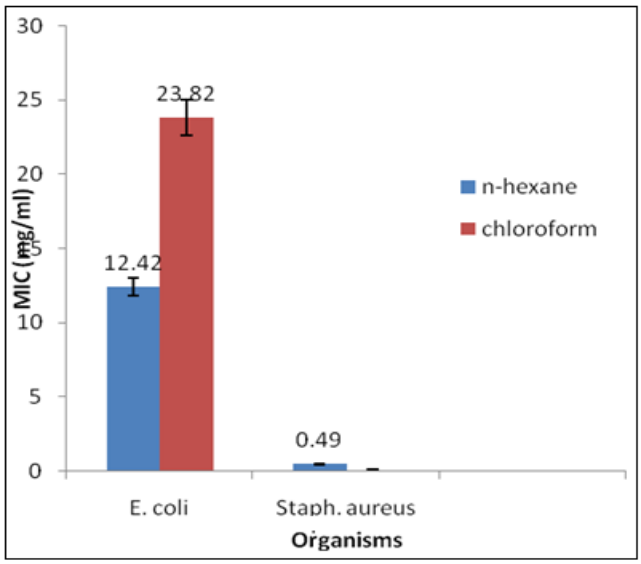

Fig 2: MICs of *n*-hexane and chloroform fractions of *L. micranthus* on *E. coli* and *Staph. aureus*

This showed that the fractions are more suitable for the treatment of any infection caused by *Staph. aureus* than *E. coli*. The reason for this can be drawn from the antimicrobial activity of amoxicillin used as a reference drug against both bacteria which has a MIC of 0.0000254 mg/ml and 0.0145 mg/ml respectively. Amoxicillin (penicillin) is more active against gram positive bacteria because they inhibit the growth of peptidoglycan crosslinks in the bacteria cell wall thereby weakening the cell wall; this imbalance is responsible for the rapid killing action of amoxicillin. This gives the mode of action thereby explaining the greater activity of the fractions against *S. aureus.*

The result of the phytochemical screening of the methanol extract of *Loranthus micranthus* showed the presence of different phytochemical constituents in several degrees of abundance. The presence of tannins, saponins and alkaloids enhanced the antimicrobial activity thereby increasing its broad spectrum of activity [7]. An analysis of the IZD's and phytochemical results suggested that the antibacterial activity observed in *L. micranthus* might have arisen as a result of a number of the phyto-constituents present in the plant. This followed from the results which clearly showed that no singular fraction/constituent could be said to be solely responsible for the antibacterial action of the plant. Among these constituents, however, tannins, flavonoids, terpenoids and alkaloids appear to have the greatest impact on the activity under study [10]. Metabolites like flavonoids and terpenoids are known to be synthesized by plants in response to microbial infection, and thus have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms) [24].

5. Conclusion

The solvent fractions have displayed antimicrobial activity and therefore justify the ethno medicinal uses of mistletoe in the treatment of bacteria infections. These solvent fractions are quite active but below those of standard antibiotics (Amoxicillin). Therefore, it is recommended that further investigations should be done on the chemical nature of the active components of the plant. This knowledge will help pharmaceutical chemists to alter the structure of these components in order to enhance their lethality on the test organisms.

6. Conflict of interest

The authors wish to declare no conflict of interest.

7. Acknowledgments

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