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Chemical characterization and antimicrobial activity of *Loranthus micranthus* Linn leaves

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The leaves of *Loranthus micranthus* Linn on shear butter tree was harvested in Suleja Nigeria for phytochemical screening, antioxidant activities, antimicrobial properties and proximate analyses. The crude ethanol extract of *L. micranthus* was observed for its antimicrobial activities at various concentrations (60, 30 and 15 µg/disc) using agar diffusion method, and was tested on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyhi-1*, *Klebbsiella pneumoniae,* and *Proteus mirabilis* and the results documented. Gradient extractions of ethanol (A), petroleum-spirit (B) and ethyl acetate (C) extracts were screened for phytochemical analysis and the result for ethanol extract showed positive results for tannins, glycosides, saponins, phenols, cardiac glycosides, phlobatanins, volatile oil, steroids and balsams; petroleum spirit extract indicated positive result for tannin, phenols, alkaloids, cardenolides, terpenoids, flavonoids, carbohydrates, resins, and volatile oil. While ethyl acetate extract indicated positive results for tannins, phenols, alkaloids, cardenolides, terpenoids, carbohydrates, cardiac glycosides, resins, and balsams. The anti-oxidant activities of the crude ethanolic extract of the leaves also showed positive results when compared with the ascorbic acid standard as the percentage inhibition of the extract increase as concentrations of the extract decreases relative to the standard. Proximate analysis results indicated that moisture content contain 6.497%, ash content 11.67%, crude fibre 19.54%, crude fibre 19.54%, crude protein 1.487%, crude fat 11.04 and carbohydrate 49.76%.

Key words: *Loranthus micranthus* Linn, phytochemical, antimicrobial, anti-oxidant properties, proximate.

INTRODUCTION

African mistletoe (*Loranthus micranthus* Linn) belongs to the family Loranthaceae, popularly called Kauchi and ewe afomon in Fulani and Yoruba tribes of Nigeria, respectively; it is an obligate semi-parasitic evergreen tropical plant, normally found growing on a variety of trees, including palm fruit, mahogany and other tropical plants. Bird feeds on the fruit from the plant and the undigested seeds from the bird droppings spread on trees branches and thus germinate on the host plant. It is among the plants whose leaves are used for pharmaceutical practices (Krenzelok et al., 1997). There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicine especially in developing countries (Murray, 1998). In Nigeria, *L. micranthus* Linn is used as remedy for several human and animal ailments that include stomach ache, diarrhoea, dysentery, wound and cancer. *L. micranthus* has been analyzed and observed to contain lecithin, viscotoxin, polysaccharides and many phytochemicals as an active ingredient (Leoper, 1999). It has been reported to have hypoglycaemic properties since it decreases the blood glucose level and has effects of controlling the loss of body weight which occurs during diabetes mellitus (Obatomi et al., 1994; Akinjobi et al., 2004).

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Mistletoe preparations are commonly used in complementary medicine as anticancer, antidiabetic, bacteriostatic and antihypertensive agents (Grossarth et al., 2007; Osadebe et al., 1998). *L. micranthus* Linn has been used in the treatment of atherosclerosis, AIDS, skin problems, such as acceleration of skin ageing, epilepsy, infertility in men and women, menopausal syndrome and rheumatism (Bonnia et al., 1998; Nweze et al., 2004). The mistletoes plant has also shown broad spectrum of antimicrobial properties against certain drug resistant bacterial and fungal organisms of farm animals (Deen et al., 2002; Okwueze, 2004). The composition and activities of mistletoe are dependent on host-tree and harvesting period (Obatomi et al*.,* 1994). In particular, Osadebe et al. (2004) showed the host-tree variation of anti-diabetic activities of Eastern Nigerian species of African mistletoe (*L. micranthus* Linn). The activities of mistletoe may also be dependent on the type of solvent used for its extraction or fractionation (Egbuonu et al., 2011). This present study therefore, attempt to determine phytochemical screening, antimicrobial activity, anti-oxidant properties and proximate of leave of *L. micranthus* Linn.

MATERIALS AND METHODS

Plants

The leaves of *L. micranthus* Linn on shear butter tree was collected from Suleja and dully identified at Sheda Science and Technology Complex Abuja. The leaves were separated from the whole plant and air-dried at room temperature (about 26°C) for four weeks and it was pulverized at point of brittle into uniform powder and was stored in an air-tight container in dark.

Phyto-chemical screening

Gradient extraction

The extraction was performed sequentially by extracting 0.102 kg of the leaves powder first with 300 ml of petroleum spirit (40 to 60°C) followed by the same volume of ethyl acetate and finally ethanol using soxhlet apparatus, each of the extracts were collected into 250 ml beaker and air-dried at room temperature. Using the method previously described by Harbome (1973), Trace and Evans (1989) and Sofowora (1993), phytochemical screening of the leaves fractions was carried out qualitatively.

Determination of antioxidant activity

The radical scavenging activities of the crude ethanolic leave extract against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrch-Sigma) were determined by UV-Visible Spectrophotometry at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described (Brand-Williams et al., 1995; Ayoola et al., 2008). The following concentrations of the extracts were prepared, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol (Analar grade). Vitamin C (Ascorbic acid) was used as the antioxidant standard at concentrations of 0.500, 0.250, 0.125, 0.063 and 0.03125 mg/ml. One milliliter of the extract was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol and thereafter the decrease in absorption was measured on a UV-Visible double beam Spectrophotometer 10 min later. A blank solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against ascorbic acid standard and the percentage inhibition was calculated. All test and analyses were run in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation (Ayoola et al., 2008):

Inhibition (%) = {[Ab-Aa]/Ab} × 100

where Ab is the absorption of the blank sample (without the extract) and Aa is the absorption of the extract.

Proximate analysis

Moisture, protein, fat, ash, crude fibre and carbohydrates were determined according to AOAC (2000). All the samples analyzed were done in triplicates. Moisture content was determined by accurately weighing few grams of the sample and dried in an oven set at 105°C to a constant weight, the percentage weight loss was determined. Fat was assayed by extracting the sample for 24 h with petroleum ether (boiling point range 40 to 60°C) in a soxhlet extractor. Ash content was determined by incinerating few grams of the sample in a furnace (Carbolite-RHF 1600) for 4 h at 550°C, the percentage ash content was determined. The protein content of the sample was determined using micro kjeldah method.

Preliminary antimicrobial screening of the extract

Preparation of turbidity standard

A 0.5 McFarland standard was prepared as described by CLSI/NCCLS (2006). 1% v/v solution of sulfuric acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water and mixed well. A 1.175% w/v solution of barium chloride was prepared by dissolving 2.35 g of dehydrated barium chloride ($BaCl_2.H_2O$) in 200 ml of distilled water. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 1% sulfuric acid solution and mixed well. A small volume of those turbid solutions was transferred to a storage bottle and stored in the dark at room temperature until required for use.

Standardization of Inoculums

Using inoculating loop, enough material from an overnight culture of the test organisms were transferred into a tube containing about 2.0 ml normal saline, until the turbidity of the suspension matched the turbidity standard 0.5 McFarland (CLSI/NCCLS, 2006).

Disc preparation

Whatman No.1 filter paper discs of (6 mm in diameter) were punched out with aid of paper punch and placed in Bijour bottles, which were sterilized by autoclaving at 121°C for 15 min and keep in a refrigerator until required for use.

Disc antimicrobial activity testing

Agar diffusion method as modified and adopted from European

Table 1. Phytochemical screening result of *L. micranthus* extracts.

Test	Petroleum spirit (A)	Ethyl acetate (B)	Ethanol (C)
Carbohydrate	++	+	-
Alkaloids	-	++	+
Tannins	+	+	+
Glycosides	+	-	+
Saponins	-	-	++
Steroids	-	-	-
Flavonoids	+	-	-
Resins	+	+	-
Phenols	+	++	+
Terpenoids	+	-	-
Cardiac glycosides	-	++	++
Balsam	_	+	++
Volatile oil	+	-	+
Phlobatannins	+	-	+
Cadenolides	+	++	+
Triterpenoids	-	-	-

Committee on Antimicrobial Susceptibility Testing (EUCAST, 2012) was employed. The freshly prepared Mueller-Hinton agar plates were dried in a dryer for about 15 min to remove surface moisture at 40 to 45°C using (Genlab Widness England, Model DC 125) glassware dryer.

The discs were prepared using serial double dilution by dissolving 0.06 g of the extract in 1 ml of the solvent (DMSO). Half (0.5 ml) of the extract was introduce into 50 sterile discs in Bijour bottle to make 60 µg/disc concentration. 0.5 ml of the solvent was added into the remaining stock solution making it 1 ml, 0.5 ml was taken and placed into another Bijour bottle containing 50 sterile discs and labeled 30 µg/discs. Another 0.5 ml was taken and placed into another Bijour bottle containing 50 sterile discs and labeled 15 µg/discs. With each disc was capable of adsorbing 0.01 ml of the solution, the procedure was employed to prepare 15, 30 and 60 µg/disc concentrations. The plates were aseptically inoculated uniformly with test organism by streaking methods. With the aid of a sterile forceps, impregnated paper discs (Whatman No. 1 filter paper) containing the extract at different concentrations (60, 30 and 15 µg/disc) were arranged in three directions and pressed firmly onto the inoculated agar surface to ensure even contact including positive control at the center of the plate and negative control on the other side. Each disc was sufficiently spaced out and kept at least 15 mm from the edge of the plate and 25 mm from disc to disc to prevent overlapping of zones. The plates are incubated at 37±2°C for 24 h. The zone diameters of the semi-confluent growths were measured with the aid of a meter rule to the nearest millimeter.

RESULTS AND DISCUSSION

The results of the phytochemical screening, antioxidant activity, and proximate antimicrobial screenings of *L. micranthus* Linn are presented in Tables 1 to 4.

The result from Table 1 shows that the ethanol extract (C) of *L. micranthus* Linn contains tannins, glycoside, saponins, phenols, alkaloids, carenolides, cardiacglycosides, phlobatannins, volatile oil, steroids and balsam. While the petroleum spirit extract (A) contains tannins, phenols, alkaloids, cardenolides, terpenoids, flavonoids, carbohydrate, resins, and volatile oil. The ethyl acetate extract (B) contains tannin, phenols, alkaloids, terpenoids, flavonoids, carbohydrates, cardiac glycosides, resins and balsams. These findings are in agreement with those of Egbuonu et al. (2011) and Orji et al. (2012) that indicated occurrence of tannin, terpenoids, phenols, flavonoids and resins. Although, Egbouo et al. (2011) recorded the absence of alkaloids, glycosides and saponins as against the results obtained in this study, this variation might be due to difference in host plant and geographical location as suggested by Osadebe et al. (2004). It was also observed that tannins, phenols and cardenolides were present in all the extracts. The petroleum spirit and ethyl acetate extracts showed positive result for alkaloids, while terpenoids are present in petroleum spirit and ethyl acetate extracts. Resins and carbohydrate also showed positive results for petroleum spirit and ethyl acetate extracts. It was observed that all the fractions contain phenols that have antioxidant potential that could enhance the body defense against pathology induced free radical generation (Al-Humaid et al., 2010). It was observed that the solubility and reactions of the phytochemicals with the reagents are solvent dependent (Natural product isolation, goggle book). The result from Table 3 shows that saponins, glycosides, phenols and phlobatanins are very polar compounds and hence soluble in ethanol layer (polar solvent). Alkaloids and flavonoids are considered as compounds of intermediate polarity hence soluble in ethyl acetate. While resins, triterpenoids and terpenoids are soluble in non polar solvents (ethyl acetate and petroleum spirit). The phenolic compounds are known to possess antioxidant properties (Gomez–Alonzo et al.,

Table 2. Percentage inhibition of *L. micranthus* ethanol crude extract at different concentrations compared with vitamin C.

Concentration (mg/ml)	0.500	0.250	0.125	0.0625	0.03125
L. micranthus percent inhibition	61.49	70.21	74.26	77.02	76.39
Vitamin C (Standard) percent inhibition	90.19	90.47	91.32	91.50	85.00

Table 3. Proximate analysis of *L. micranthus.*

Parameter	Moisture	Ash	Crude fibre	Crude protein	Crude fat	Carbohydrate
Results (%)	6.400	11.67	19.54	1.487	11.04	49.76

Table 4. Extract minimum zone of inhibitions against test organisms.

Test organism	Disc potency (µg/disc)			Positive control CLP. (60 µg/disc)	Negative (DMSO)
	15	30	60		
S. aureus	NA	8	9	25	NA
E. coli	NA	7	8	22	NA
S. paratyhi-1	NA	7	7	22	NA
K. pneumonia	7	8	12	22	NA
P. mirabilis	NA	7	10	24	NA

CLP: Chloramphenicol; µg: microgram; DMSO: dimethyl sulphoxide; NA: No activity.

2003). Steroids ensure hormonal balance by serving as potent starting materials in the synthesis of sex hormones (Okwu, 2001). Therapeutic characteristics were associated with alkaloids (Renu, 2005; Njoku and Akemefula, 2007) and saponins (Rabe, 2000). Flavonoids have both antifungal and antibacterial activity and anti-inflammatory properties (Iwu, 1999).

DPPH gives a strong absorption band at 517 nm in visible region. When odd electron becomes paired off in the presence of free radical of free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the degree of radiation in absorbance measurement is an indication of the radical scavenging power of the extract. The percentage of inhibition of the tested extract increases as the concentration of the extract decreases. However, the potent (77.02 at 0.0625 mg/ml) when compared with vitamin C (91.50 at 0.0625 mg/ml). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are suspected to be tumourigenic (Ito et al., 1985). Therefore, there is a need to search for potential antioxidant compounds especially from herbs that can replace their synthetic counterparts. The highly antioxidant activity of the plant at low concentration shows that the plant could be very useful for the treatment of ailments resulting from oxidative stress such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections (Onocha et al., 2011).

Proximate analysis of leaves of the plant was also examined, as shown in the Table 3, moisture content 6.497%, ash content 11.67%, crude fibre 19.54%, crude protein 1.487% and carbohydrate. The high ash content present is an indication of high minerals content which promote good body metabolism. The petroleum ether extract was shown to have a fixed oil that looks stick and gummy, the fixed oil can be used as adhesive in paper industry or as a binder in pharmaceutical industries. The result of antimicrobial screening using disk diffusion method from Table 4, the extract shows a promising result of 12 and 10 mm at 60 µg/disc on both *Klebsiella pneumonia* and *Proteus mirabilis*, respectively. The zone of 9 and 8 mm also shows 60 µg/disc on *Staphylococcus aureus* and *Escherichia coli* while the least zone shows *Salmonella paratyphi-1* at the highest concentration (60 µg/disc). Despite the variation in concentrations, the results agree with that of Orji et al. (2012) which show the activity of *L. micranthus* extract on the same clinical isolate such as *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. In comparism with the standard chloramphenicol, the extract posses low antimicrobial activity as indicated in Table 4. Furthermore, the extract is of concentration dependent which shows that increase in concentration will results in winder zone of inhibition. However, previous work (Egbuonu et al., 2011) shows that ethyl acetate and petroleum extracts from the plant did not have activity on *E. coli* at any concentration as against the result obtained

in this study. This variation might be due to different in host plant and different geographical location as suggested by Osadebe et al. (2004) and hence their phyto-constituents and pharmacological properties. Besides, increase in concentrations as shown in Table 4 did not have any further effect on the zone of inhibition of *S. paratyphi-1* suggesting that the organism developed a resistance against the plant extract even at higher concentration. It might be that the *S. typhi* strains under study have acquired genes for resistance of *L. micranthus.*

Conclusion

The results of the analyses shown earlier have demonstrated that the leaves of *L. micranthus* Linn could be a good therapeutic preparation in treatment of various infections, serves as an anti-oxidant and source of minerals in the body. And it might be exploited in design and development fungistatic and bactericidal drugs.

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