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Full Length Research Paper

Phytochemical properties of some solvent fractions of petroleum ether extract of the African mistletoe (*Loranthus micranthus* Linn) leaves and their antimicrobial activity

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The leaves of the African Mistletoe (*Loranthus micranthus* Linn) on *Kola acuminata* in Nsukka, Eastern Nigeria were studied. The chloroform (A), ethyl acetate (B) and ethyl acetate residue (C) fractions of crude petroleum ether extract of *L. micranthus* Linn were subjected to phytochemical and antimicrobial evaluation. Results reveal the presence of protein in fraction C, whereas flavonoids, steroids and terpenes were present in all the fractions, but in a low quantity. Fractions A and B had high, while fraction C had moderate quantity of tannins. However, high quantity of resins was present in fraction C followed by fraction A. Fraction B showed the highest susceptibility (25) to *Bacillus subtilis*, and was active against the fungus, *Candida albicans*, suggesting its higher potency and fungistatic potential. Apart from *Klebsiella* spp., the other organisms were not susceptible to fraction A, apparently owing to high resins but no protein content. Fraction C was active against the highest number of organisms (*Pseudomonas aeruginosa, B. subtilis and Klebsiella* spp.). In all, this study suggests that A, B and C fractions of crude petroleum ether leaf extract of *L. micranthus* Linn is parasitic on *K. acuminate*, may serve as source for compounds with therapeutic potentials. However, the fractionating solvents used in this study may not be the preferred choice for fractionating the active principles of *L. micranthus* Linn. Interestingly, the possible fungistatic potential of *L. micranthus* Linn parasitic on *K. acuminata* as observed in fraction B may be exploited in the design and development of fungistatic drug.

Key words: Phytochemical, antimicrobial, fungistatic, *Loranthus micranthus* Linn, *Kola acuminata*, mistletoe.

INTRODUCTION

Extracts of plants and plant parts are good sources of medicine in traditional African societies and beyond (Obasi et al., 2011). The dependence on plant-sourced medicine apparently diminished with the advent of orthodox medication. However, the emergence of hitherto unknown disease causing microbes necessitates further drug research and development from any viable source including indigenous plants (Obasi et al., 2011).

Furthermore, the possible benefits from alternative medical practice, especially in the developing parts of the globe, were limited by lack of scientific basis, warranting that the quality and consistency of the alternative medicines be ascertained and maintained for their maximal use and efficacy (Ukoha et al., 2011).

Loranthus micranthus is a semi parasitic shrub that grows on host trees. In Ojoto and other Igbo speaking parts of Eastern Nigeria, it is called '*nbu nnunu*' literary meaning 'carried by bird' in apparent recognition of the role played by birds in dispersing the shrub. The host trees on which *L. micranthus* could grow include, *Kola acuminata, Kola nitida, Mangifera indica, Azadirachta*

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indica, Jatropha curcas and *Persia* sp. (Osadebe and Ukwueze, 2004). In Nigeria and other parts of Africa, the plant is used in ethno medication against diabetes and hypertension, schizophrenia and as an immune booster (Osadebe et al., 2004; Osadebe and Omeje, 2009). Furthermore, reports of possible hypotensive potential of *L. micranthus* abound (Obatomi et al., 1996; Osadebe and Omeje, 2009; Ameer et al., 2010).

Tannins, terpenoids, flavonoids and alkaloids phyto-constituents have been implicated for the various pharmacological activities, including antibacterial and antidiabetic properties of the plant (Obatomi et al., 1994; Uzochukwu and Osadebe, 2007; Osadebe and Omeje, 2009). Furthermore, the aqueous extract of mistletoe (*Tapinanthus bangwensis*) evoked hypoglycaemic and hypocholesterolaemic activity in normoglycaemic rats (Iheanacho et al., 2008)

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 (*Loranthus micranthus* Linn).

The distribution of phytochemicals (anti nutrients) in a plant may affect the quality (Nzewi and Egbuonu, 2011) and possibly content of other phytochemicals. For instance, tannins may form insoluble complexes with proteins, thereby decreasing protein digestibility and quality (Uzoechina, 2007).

Therefore, the activities of mistletoe may also be dependent on the type of solvent used for its extraction or fractionation.

The present study therefore, attempts to determine the phytochemical contents of some solvent fractions of crude petroleum ether (CPE) extract in relation to their antimicrobial activity on some common pathogens. The aim was to contribute to scientific knowledge on the development of potential human uses of *L. micranthus* Linn in alternative as well as orthodox medicine and diet.

MATERIALS AND METHODS

Solvent and chromatographic materials

The solvents used (petroleum ether, chloroform are ethyl acetate) are Sigma-Aldrich® grade. Other chemicals and standard grades of silica gel (70 to 230 mesh) and reagents used were of certified grade and quality and were used without further purification.

Test organisms

The test organisms were clinical isolates of patients attending University of Nigeria Medical Centre, Nsukka donated to the Department of Microbiology, University of Nigeria Nsukka, Nigeria and include bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Salmonella paratyphi*) *and* fungi *(Aspergillus niger* and *Candida albicans*).

Collection and preparation of plant material

Fresh leaves of *L. micranthus* were collected from *K. acuminata* in Nsukka, Enugu State, South Eastern Nigeria. The specimen was then identified by a taxonomist in Bioresources Conservation and Development Programme (BCDP) unit, University of Nigeria, Nsukka, air dried for about eight days and pulverized using grinding machine. A known amount (1000 g) of the resultant powdery specimen, was extracted with 4.0 L of petroleum ether by Soxhlet method to obtain the crude petroleum ether extract (CPE). The extract was filtered separately with Whatman filter paper. The filtrate was concentrated with rotary evaporator. Ten grams of the dried CPE was fractionated sequentially with chloroform and ethylacetate to obtain fractions A and B, respectively while the ethylacetate residue became fraction C. The fractions were then stored in a refrigerator until used for the study (phytochemical and antimicrobial screening).

Phytochemical screening

The phytochemical analysis of the CPE extract fractions of *L. micranthus* Linn from *K. acuminata* was done using standard methods as described in Evans (2000). Specifically, the fractions were screened for saponins, glycosides, proteins, steroids, reducing sugars, alkaloids, flavonoids, tannins, terpenes, carbohydrates and resins.

Antimicrobial activity screening

The minimum inhibitory concentrations (MIC) agar dilution assay

The minimum inhibitory concentration was determined by agar dilution method, described by Adeniyi et al. (1996) and Harbone (1998). Essentially, one two-fold serial dilution of the fractions were prepared in sterile distilled water and poured into separate sterilized Petri dishes. The concentration was selected based on the preliminary sensitivity tests on the microorganisms as has been used and reported by many authors (Cleidson et al., 2007; Esimone et al., 2003). Essentially, nutrient agar powder (28 g) was weighed out and dispersed in 1 L of deionized water. The mixture was allowed to stand for 10 min before swirling to mix properly. It was sterilized by autoclaving for about 15 min at 121°C, and cooled to 40°C. Then, 20 ml of molten nutrient agar Sabouraud dextrose agar (SDA) (OXOID, UK) (for fungi) was poured into the Petri dishes, swirled slowly and then allowed to set and dry. Each set of agar plate was streaked with the broth culture of bacteria (*B. subtilis*, *S. aureus*, *E. coli, S. paratyphyi, A. niger, Klebsiella* spp. and *P. aeruginosa*) and fungi (*A. niger* and *C. albicans*). The agar plates without extract or standard antibiotic (the negative control) and the plates containing 2.5 µg/ml of a standard antibiotic, gentamicin sulphate (GS) (the positive control) were also streaked with the micro organisms. The agar plates were incubated at 37°C for 24 h (for the bacteria) and at 25°C for 48 h (for the fungus). The inhibition zone diameter (IZD), the measure of activity, was consequently determined by plotting the square of the inhibition zone diameter (IZD^2) against the log concentration of the extract and the MIC was calculated from the intercept on the log concentration axis.

RESULTS

Phytochemical screening

Results of the phytochemical screening showed that

Table 1. Phytochemical analysis of the extracts and the solvent fractions.

Parameter	A	B	C
Proteins	—	—	+
Carbohydrates	—	—	—
Reducing Sugars	—	—	—

+ = Present; — = absent.

Table 2. Phytochemical analysis of the extracts and the solvent fractions.

Parameter	A	B	C
Alkaloids	—	—	—
Glycosides	—	—	—
Flavonoids	+	+	+
Resins	+	—	+++
Tannins	+++	+++	++
Steroids	+	+	+
Saponins	—	—	—
Terpenes	+	+	+

+ = Present; - = absent; multiple pluses indicate the degree of abundance.

fraction C had only protein, but no carbohydrates and reducing sugars. However, fractions A and B had none of these metabolites (Table 1).

None of the fractions (A, B and C) had alkaloids, glycosides and saponins. On the other hand, the fractions had flavonoids, steroids and terpenes in low quantity, but had tannins in high quantity. However, resins was present in fraction C in high quantity and in fraction A in low quantity but was not present in fraction B (Table 2).

Antimicrobial activity

In comparison with standard antibiotic, gentamycin, none of the fractions had activity against *E. coli, S. aureus and S. paratyphyi*, whereas none including gentamycin showed any activity against *A. niger*. Furthermore, only fraction B showed activity against the fungus, *C. albican*, whereas the other fractions and even gentamycin did not show any activity against the fungus (Table 3).

As shown in Table 4, fraction C inhibited the highest number of microorganisms (*P. aeruginosa, B. subtilis and Klebsiella* spp.), whereas fraction B inhibited only *B. subtilis,* while fraction A inhibited only *Klebsiella* spp.

DISCUSSION

Medicinal value of plants lies in the bioactive phyto-components of the plants (Veermuthu et al., 2006). Thus, the phytochemical constituents of the leaves of African mistletoe (*L. micranthus* Linn) parasitic on *K. acuminata* in Nsukka, Eastern Nigeria were studied. The chloroform (A), ethyl acetate (B) and ethyl acetate residue (C) fractions of crude petroleum ether extract of *L. micranthus* Linn were subjected to phytochemical and antimicrobial evaluation.

Phytochemical screening results revealed the presence of protein in fraction C, but not in A or B, suggesting that fraction B probably preserved the protein integrity of the petroleum ether extract. The fractions (A, B and C) had low quantity of flavonoids, steroids and terpenes, suggesting low therapeutic activity (Rabe, 2000) which could limit the choice of these solvent for the fractionation of active medicinal ingredients from *L. micranthus* Linn leaves on *K. acuminata*. Flavonoids have antioxidant potentials that could enhance the body defense against pathology induced free radical generation (Al-Humaid et al., 2010). Steroids ensure hormonal balance by serving as potent starting material in the synthesis of sex hormones (Okwu, 2001).

Tannins have biological activities that may favor the prevention and management of many ailments (James et al., 2007). In this study, fractions A and B had high, whereas fraction C had moderate quantity of tannins. This may indicate higher therapeutic benefits in fractions A and B than in C. However, excess tannins could be toxic because tannins, as metal ion chelator, could decrease the bioavailability of iron leading to anemia (Ukoha et al., 2011).

Furthermore, therapeutic activity was associated with alkaloids (Renu, 2005; Njoku and Akumefula, 2007) and saponins (Rabe, 2000). Hence, the absence of alkaloids, glycosides and saponins in fractions A, B and C, appears to negate the efficacy of the use of *L. micranthus* Linn leaves in ethno-medicinal practice. Therefore, the solvents may not be effective in fractionating the active principles of *L. micranthus* Linn leaves. Results of the phytochemical screening showed the absence of carbohydrates and reducing sugars in the fractions. This may indicate that the *L. micranthus* Linn leaves fractionate of these solvents may not offer good source of dietary energy. Previously, aqueous and methanolic leaf extracts of *L. micranthus* contained alkaloids phyto-constituent (Obatomi et al., 1994; Osadebe and Ukwueze, 2004; Osadebe et al., 2004; Uzochukwu and Osadebe, 2007; Osadebe and Omeje, 2009), suggesting that the absence of these phytoconsistuents as observed in this study may be attributed to the fractionating solvents used.

The antimicrobial screening results revealed that fraction B showed the highest susceptibility (25) to *B. subtilis*, and was active against the fungus, *C. albicans*, suggesting its higher potency and fungistatic potential, perhaps due to the absence of resins. Concentration is an important factor that influences antimicrobial activity (Min et al., 2008). Thus, fraction B from the leaves of *L. micranthus* Linn probably posses adequate concentration

Table 3. Antimicrobial screening test of the extracts and fractions (IZD in mm) at 2.5 mg/ml for extracts and 25 µg/ml for standard antibiotic.

Parameter	A	B	C	Gentamycin
Escherichia coli	–	–	–	40
Staphylococcus aureus	–	–	–	55
Salmonella paratyphi	–	–	–	40
Aspergillus niger	–	–	–	–
Candida albicans	–	14	–	–

Results are expressed as simple mean of two tests.
– = No inhibition zone; IZD = Inhibition zone diameter.

Table 4. Antimicrobial screening test of the extracts and fractions (IZD in mm) at 2.5 mg/ml for extracts and 25 µg/ml for standard antibiotic.

Parameter	A	B	C	Gentamycin
Pseudomonas aeruginosa	–	–	16	33
Bacillus subtilis	–	25	15	40
Klebsiella spp.	19	–	19	50

Results are expressed as simple mean of two tests.
– = no inhibition zone; IZD = Inhibition zone diameter.

and lethality against *C. albicans* hence, could be considered as an active compound for use in the formulation of potent fungistatic agent/drug. Furthermore, high quantity of resins was present in fraction C followed by fraction A, but not in fraction B.

Generally, other workers attributed antimicrobial effects of plant extracts to the presence of phytochemicals (Nweze et al., 2004; Ogueke et al., 2007), notably tannins and flavonoids which have been shown to posses antibacterial properties (Draughon, 2004). The fractions in this study failed to inhibit some of the tested pathogens, including *E. coli*, *S. aureus, S. paratyphi* and *A. niger*, probably because these pathogens possess a mechanism for detoxifying the active principles in the fractions (Ogueke et al., 2007).

In all, the study suggests that A, B and C fractions of crude petroleum ether leaf extract of *L. micranthus* Linn parasitic on *K. acuminata* may serve as source for compounds with therapeutic potentials, thereby justifying the use of *L. micranthus* Linn leaves in alternative medicine. However, the fractionating solvents used in this study may not be preferred choice for fractionating the active principles of *L. micranthus* Linn. Interestingly, the possible fungistatic potential of *L. micranthus* Linn parasitic on *K. acuminata* as observed in fraction B may be exploited in the design and development of fungistatic drug.

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