

Full Length Research Paper

Phytochemical and antimicrobial activity of petroleum ether extract of the African Mistletoe (*Loranthus micranthus* Linn) leaves

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The leaf of the African Mistletoe (*Loranthus micranthus* Linn) parasitic on *Kola acuminata* in Nsukka, Eastern Nigeria was studied. The crude petroleum ether extract (CPE) and ethanol extract of the CPE residue (EER) of *Loranthus micranthus* Linn were subjected to phytochemical and antimicrobial evaluation. Results of the phytochemical screening showed that EER had proteins, carbohydrates and reducing sugars while CPE had none of these metabolites. Flavonoids was absent, while tannins, steroids, saponins and terpenes were present in CPE and EER. Results of the antimicrobial activity revealed that CPE, EER and even standard antibiotic, gentamycin did not elicit any activity against *A. niger* and *C. albicans*. CPE and EER were active against *S. aureus*, *S. paratyphi*, *B. subtilis* and *K. spp*. Furthermore, the highest inhibition was noted in CPE (25.00) against *S. aureus* while the least was observed in EER (14.00) against *P. aeruginosa*. Altogether, the study suggests that CPE and EER extracts of leaf of *Loranthus micranthus* Linn parasitic on *Kola acuminata* in Nsukka area of Eastern Nigeria may serve as source for therapeutic compounds, thereby justifying its use in alternative medicine and potential for development into orthodox drugs.

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

INTRODUCTION

Following the emergence of hitherto unknown disease causing microbes that require renewed strategies on their treatment and prevention, further drug research and development from any viable source is imperative. In this regard, the investigation of indigenous plants may yield positive results (Obasi et al., 2011). Ever before the advent of orthodox medication, plants extracts have served as dependable sources of medicine in traditional African societies and beyond (Obasi et al., 2011).

However, the practice of herbal medicine in the developing parts of the globe lacked scientific basis or standards, thereby limiting their possible benefits. To remedy this and ensure their maximal use and efficacy the quality and consistency of these herbal medications should be ascertained and maintained (Ukoha et al.,

2011).

Loranthus micranthus is a semi parasitic shrub. It grows on host trees where it obtains nutrients and support. In Nigeria, Western Africa, host trees on which *Loranthus micranthus* could grow include, *Kola acuminata*, *K. nitida*, *Mangifera indica*, *Azadirachta indica*, *Jatropha curcas*, and *Persia* sp. (Osadebe and Ukweze, 2004). In Nigeria and other parts of Africa, the plant is used in ethno medication against diabetes and hypertension, schizophrenia and an immune booster (Osadebe and Omeje, 2009; Osadebe et al., 2004). In particular, (Obatomi et al., 1996; Osadebe and Omeje, 2009; Ameer et al., 2010) reported the possibility of *Loranthus micranthus* acting as antihypertensive, which according to Egbuonu (2010) is a class of drugs that should induce a reduction in blood pressure and prevent the complications of hypertension or high blood pressure with minimal induction of adverse response.

Research report indicated that the composition and activities of mistletoe are dependent on host-tree and

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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 activities of mistletoe may therefore be dependent on the type of solvent used for its extraction. The present study therefore, attempts to determine the phytochemical contents of crude petroleum ether (CPE) extract and ethanol extract of the CPE residue (EER) in relation to their antimicrobial activity on some common pathogens. The aim is to contribute to scientific knowledge on the development of potential human uses for *L. micranthus* Linn leaf parasitic on *K. acuminata* in alternative as well as orthodox drugs and diets.

EXPERIMENTALS

Solvent materials

The solvents used (petroleum ether, ethanol) 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 *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Candida albicans* (*C. albicans*), *Klebsiella spp* (*K. spp*), *Salmonella paratyphi* (*S. paratyphi*) and *Aspergillus niger* (*A. niger*)

Collection and preparation of plant material

Fresh leaves of *L. micranthus* were collected from *Kola acuminata* in Nsukka, Enugu State, South Eastern Nigeria. The specimen was then identified by Mr. Ozioko of Bioresources Conservation and Development Programme (BCDP) Nsukka, air dried for about eight days and pulverized using electric 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 residue was macerated with ethanol to obtain the ethanol extract of the residue (EER). The extracts were filtered separately with Whatman filter

paper. The respective filtrate was concentrated with rotary evaporator and then preserved in a refrigerator as concentrated extract until subsequently used for the research study (phytochemical and antimicrobial screening).

Phytochemical screening

The phytochemical analysis of the CPE and EER extracts of *L. micranthus* Linn from *Kola acuminata* was by standard methods as described in Evans (2000). Specifically, the extract and extract of the residue 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 extracts (2.5 mg/ml) 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 (Cleudson *et al.*, 2007; Esimone *et al.*, 2003). Essentially, nutrient agar powder (28g) was weighed out and dispersed in 1 litre of deionised water. The mixture was allowed to stand for 10 minutes before swirling to mix properly. It was sterilized by autoclaving for about 15 minutes at 121°C, 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. paratyphi*, *A. niger*, *K. spp* and *P. aeruginosa*) and a fungus (*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, 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 calculated from the intercept on the

TABLE I. Phytochemical Analysis of the Extracts

| | CPE | EER |
|------------------------|-----|-----|
| Proteins | - | ++ |
| Carbohydrates | - | + |
| Reducing Sugars | - | + |

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

TABLE II. Phytochemical Analysis of the Extracts

| | CPE | EER |
|-------------------|-----|-----|
| Alkaloids | - | ++ |
| Glycosides | - | ++ |
| Flavonoids | - | - |
| Resins | ++ | - |
| Tannins | +++ | +++ |
| Steroids | ++ | ++ |
| Saponins | + | ++ |
| Terpenes | +++ | ++ |

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

Table III. Antimicrobial Screening Test of the Extracts (IZD in mm) at 2.5 mg/ml for extracts and 25 µg/ml for standard antibiotic.

| | CPE | EER | Gentamycin |
|-------------------------------|-------|-------|------------|
| <i>Escherichia coli</i> | 20.00 | nz | 40 |
| <i>Pseudomonas aeruginosa</i> | Nz | 14.00 | 33 |
| <i>Aspergillus niger</i> | Nz | nz | nz |
| <i>Candida albicans</i> | Nz | nz | nz |

Note: Results are expressed as simple mean of two tests nz = no inhibition zone

Table IV. Antimicrobial Screening Test of the Extracts (IZD in mm) at 2.5 mg/ml for extracts and

| 25 µg/ml) for standard antibiotic. | CPE | EER | Gentamycin |
|---|-------|-------|------------|
| <i>Staphylococcus aureus</i> | 25.00 | 19.00 | 55 |
| <i>Salmonella paratyphi</i> | 21.00 | 15.00 | 40 |
| <i>Bacillus subtilis</i> | 18.00 | 20.00 | 40 |
| <i>Klebsiella spp</i> | 20.00 | 17.00 | 50 |

Note: Results are expressed as simple mean of two tests

log concentration axis.

RESULTS

Phytochemical screening

Results of the phytochemical screening showed that EER had proteins, carbohydrates and reducing sugars

while CPE had none of these metabolites (Table I). Comparison between CPE and EER revealed the absence of flavonoids in both extracts. However, with the exception of resins that were present in CPE as well as alkaloids and glycosides that were present in EER, tannins, steroids, saponins and terpenes were present in CPE and EER (Table II).

Antimicrobial activity

CPE, EER and even standard antibiotic, gentamycin did not elicit any activity against *A. niger* and *C. albicans*. CPE showed no activity against *P. aeruginosa* while EER had no activity against *E. coli*. The least inhibition was in EER (14.00) against *P. aeruginosa* (Table III).

However, CPE and EER were active against *S. aureus*, *S. paratyphi*, *B. subtilis* and *K. spp*. Furthermore, the highest inhibition was in CPE (25.00) against *S. aureus* (Table IV).

DISCUSSION

Tannins, steroids, saponins and terpenes were present in CPE and EER. These secondary metabolites may confer *L. micranthus* with therapeutic activities (Rabe, 2000). For instance, saponin is an antioxidant phytochemical (Gupta, 2002) whereas, tannins have biological activities that may favor the prevention and management of many ailments (James et al., 2007).

Alkaloids and glycosides were present only in EER, suggesting more activity in the residue extract. Alkaloids were the most efficient, therapeutically significant plant substance (Njoku and Akumefula, 2007) hence, EER may be more effective in ethnomedicinal and orthodox therapy.

Flavonoids have biological activities that are of benefit in the prevention and management of many ailments (James et al., 2007). However, flavonoids was absent in CPE and EER. Earlier, (Erah et al., 1996) had associated antimicrobial activity with the presence of tannins and flavonoids. Therefore, the absence of flavonoids in the extracts might limit their potentials and uses. Flavonoids inhibit platelets aggregation (Formica and Regelson, 1995), due to the possible efficient detoxification and antioxidant activities (Iweala, 2009).

Previously, aqueous and methanolic leaf extracts of *L. micranthus* also contain tannins, terpenoids, flavonoids, and alkaloids phytoconstituents, which have been implicated for the various pharmacological activities of the plant including antibacterial and antidiabetic properties (Osadebe and Ukwueze, 2004; Osadebe and Omeje, 2009; Osadebe et al., 2004; Uzoichukwu and Osadebe, 2007; Obatomi et al., 1994).

Results of the phytochemical screening showed that EER had proteins, carbohydrates and reducing sugars while CPE had none of these metabolites, apparently

indicating that the EER could also be a good source of dietary energy and nutrients. EER might be beneficial as source for nutrients. However, its use as nutrient may be limited by the presence of high tannins that may for instance, decrease the digestibility of proteins via its formation of insoluble complexes with proteins (Nzewi and Egbuonu, 2011)

Results of the antimicrobial activity revealed that CPE, EER and even standard antibiotic, gentamycin did not elicit any activity against *A. niger* and *C. albicans*. In particular, *C. albicans* is a fungus and the present result is a possible pointer that the extracts do not possess antifungal potential, hence may not be useful as broad spectrum antibiotic.

CPE showed no activity against *P. aeruginosa*. This may be because *P. aeruginosa* is a gram negative bacterium, hence more complex and less susceptible (Gould and Booker, 2000). It probably resisted the CPE and EER of *L. micranthus* leaf, due to its cell permeability (James et al., 2007) or inherent point mutation (Obasi et al., 2011).

The varying degree of anti bacterial activities on most of the tested pathogens suggests that these extracts of mistletoe could be of benefit in the development of potent drugs against diseases caused by these pathogens. In particular, EER was active against *P. aeruginosa*. *P. aeruginosa* is a gram negative bacterium, hence more complex and less susceptible (Gould and Booker, 2000). Thus, this is an important observation, suggesting the potential of harnessing EER of *L. micranthus* Linn leaf parasitic on *K. acuminata* in the development of drug against *P. aeruginosa*, hence warrants further investigation.

Taken together, the study suggests that CPE and EER of *L. micranthus* Linn leaf parasitic on *K. acuminata* may serve as source for compounds with therapeutic potentials, dietary energy and nutrients.

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