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Phytochemical and Antibiotic Evaluation of the Methanol Extract of *Loranthus micranthus* Linn Parasitic on Kola Accuminate

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Abstract: The methanol extracts of Loranthus micranthus Linn on Kola acuminata leaves were investigated for phytochemical studies and antibiotic assay, using Staphylococcus aureus and Escherichia coli. The leaves were extracted with methanol by cold maceration method. The extracts were further purified by column chromatography method. The phytochemical analysis showed flavonoids, resin, carbohydrates and tannins to be present in abundance, while alkaloids, saponins, reducing sugars, glycosides, terpenoids, steroids and acid compounds are moderately present. Fats, proteins and oils are absent. The sensitivity of the extracts could not inhibit the growth of Escherichia coli, but could inhibit the growth Staphylococcus aureus. The minimum inhibitory concentration of the extract against Staphylococcus aureus is 2.91 mg/ml.
Keywords: Loranthus micranthus, Staphylococcus aureus, Escherichia coli, phytochemical analysis.

Introduction

The use of plant parts by various human traditions in the preparation of herbal remedies is as old as human history[1]. These medicinal plants are used for the treatment of kinds of ailments such as skin infections, sores, intestinal and respiratory conditions[2-3]. Literature revealed that ancient people and old cultures over ages knew the use of several medicinal plants that help in curing disease states[4]. In primitive medical history based on human efforts, trials and errors, some natural toxic products were also recorded[5]. It is interesting to notice that worldwide opinion has not yet fully changed and people with low resource and living in remote areas, still make use of those herbal remedies as part of their traditional healing systems[6]. Needless to say that medicinal plants use has persisted as a long standing tradition in all cultures of the world. Moreover the activities of different medicinal plant extracts against microorganisms supported the scientists in developing pharmaceutical industry. Several medicinal plants used in Chinese, Africa, Arab, German and local Asia systems of medicine are part of herbal pharmacopoeias[7]. Screening of medicinal herbs and drug products have become a potential source of biodynamic compounds of therapeutic value[8]. Ethnopharmaceutical studies have become increasingly valuable in the recent past and medicinal plants, are now part of health care system. The medicinal plants conservation programs and their sustained supply are part of global health strategy [9, 10].

Furthermore, recent reports suggested that in some cases, synthetic antibiotics are no more effective to treat some infections disease because of bacterial resistance. Some drug products were found to cause health hazards if used in higher dose range. The observed bacterial resistance and undesirable side effect of certain

antibiotics, led scientific community to find new antibacterial compounds from medicinal plants or to prepare synthetic and semi-synthetic antibacterial drug products with low toxicity[11]. Several medicinal plant extracts, plant products and isolated phytochemical constituents showed highly significant antimicrobial activity which added more to encourage research on such potential natural products/drugs[12]. *Loranthus micranthus* Linn is the Eastern Nigeria species of the Africa mistletoe which has been used widely in ethnomedicines as anticidiabetics[13, 14], anticancer, antihypertensive, antimicrobial e.t.c. Different research teams have worked on various species of the plant and demonstrated some pharmacological activities, which supported the ethnomedicinal uses[15]. This research aims at extracting and evaluating the antimicrobial and phytochemical properties of the methanolic extract of *Loranthus micranthus Linn* parasitic on *Kola acuminate*

Leave and Seed of *Kola acuminata*

Materials and Methods

Plant materials:

The leaves of *Loranthus micranthus Linn* were collected from Owerre Eze Orba Nsukka in Enugu state of Nigeria between the months of February and March. The plant was identified and authenticated by Mr. A.O. Ozioko of Bioresources Development Conservation Programme (BDCP) Nsukka.

Reagents:

Fehling's solution A and B, Dragendorff's reagent, Wagner's reagent, Mayer's reagents, 5 % Iron (III) Chloride, 1 % aluminum (III) Chloride, Ethanol, Concentrated tetraoxosulphate (VI) acid, Concentrated Hydrochloric acid, α- naphthol, Ethanol, Methanol were sourced commercially from Sigma-Aldrich and Riedel de Ham, Germany and were used without further purification.

Phytochemical screening:

Phytochemical analysis of the methanol extracts was performed using standard method [17, 18].

3.3 Antibiotic Assay

100 mg of extract was dissolved with 2 cm^3 of DMSO or tween 80 to get 50 mg/mL concentration (stock solution). 1 cm^3 of the stock was taken and serially diluted in double dilution up to 6 tubes. The minimum inhibitory circulation (MIC) was determined using agar diffusion method. Two fold serial dilution of the exhaust (50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 (mg/mL) were prepared in sterile distilled water and poured into separate sterilized Petri dishes. 20 mL of molten nutrient agar were poured into the Petri-dishes, swirled slowly and allowed to set and dry**.**

Each set of plate were streaked with broth culture of *Staphylococcus* aureus, *Escherichia coli*, The agar plates containing no extract (negative control) and the plates containing 4, 0.4,0.2, 0.05 and 0.025 mg/mL of amoxicillin. MIC amoxicillin (positive control) were also streaked with micro-organisms. The agar plates were incubated at 37 ^{o}C for 24 hours. The MIC of amoxicillin did not permit visible growth as compared to the negative control. The zone of inhibition was measured and the inhibition zone diameter was recorded**.**

3.3.1 Statistical Analysis

The results are expressed as "Mean IZD ± SEM". The significance of difference between means was determined by Student's *t-test* values of $P < 0.05$ were considered significant and $P < 0.05$ as highly significant**.** All statistical procedure was performed according to the method of Woodson

4.0. Results and Discussion

Result of the phytochemical assay of the methanol extract of *Loranthus micranthus Linn* parasitic on *Kola acuminate* showed the presence of different phytochemical constituents in several degrees of abundance **(table 4.1).** The extracts showed that flavonoids, resins, carbohydrates and tannins are in high abundance, while alkaloids, saponins, reducing sugars, glycosides, terpenoids, steroids and acid compounds are moderately present, while protein, fats and oils are absent.

Table 4.1: Results of Phytochemical Assay of the Methanol Extract of Kola Acuminata

S/N	Phytochemical Constituents	Relative Abundance
1	Alkaloids	++
2	Flavonoids	+++
3	Saponins	++
4	Tannins	+++
5	Reducing Sugars	++
6	Glycosides	++
7	Terpenoids	++
8	Steroid	++
9	Resins	+++
10	Carbohydrates	+++
11	Proteins	_
12	Fats and oils	_
13	Acidic Compounds	++

Keys:
+++ = Abundantly present
++ = Moderately present
+ = present
- = Absent.

Table 4.2 : Antibiotic assay of methanol extract of *Micranthus loranthus Linn* leaves against some bacteria at various concentrations (mg/ml)

Mean IZD(mm) ± SEM							
Organism	50±0.33	25±0.00	12.5±0.10	6.25±0.25	3.125±0.15	1.5625±0.33	0.78125±0.35
Staphylococcus **aureus**	3± 0.22	2± 0.44	2.5± 0.00	+	+	+	+
Escherichia **coli**	+	+	+	+	+	+	+

Key: + = No activity or growth not inhibited.

The *Loranthus micranthus Linn* parasitic on *Kola acuminate* methanol fraction which was dissolved with dimethyl sulphur oxide (DMSO) showed that the result has no activity against *Escherichia coli*, but has activity against *Staphylococcus aureus* (**table 4.2**) meaning it can be used to cure *Staphylococcus aureus* caused

diseases. The minimum inhibitory concentration (MIC) of the methanol extract against *Staphylococcus aureus* is 2.91 mg/ml. The different phytochemical constituents with different relative abundance found in *Staphylococcus aureus* have so many curative impacts on animals and human beings. For example: *Flavonoids* are highly abundant in *Loranthus micranthus Linn* and have potential to be biological response modifier such as anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities**.**

They are antioxidants and have medicinal properties, especially their purgative role in prevention of cancers and cardiovascular diseases**.** Researches have shown that epicatechin, quercetin and luteolin can inhibit the development of fluids that result in diarrhea**.** Human studies show that flavonoids may reduce the risk of cardiovascular disease and stroke**.** Flavonoids have many medicinal uses in connection with the following conditions and health concerns: Chronic venous insufficiency (rutin), oedema (water retention) coumarin, (hydroxyethylrutosides), hepatitis (catechin), bruising, cold sores, diabetes (bilberry). Others are dysmenorrhea (rutin plus vitamin B3 [niacin] and vitamin C), Gingivitis (periodontal disease) (in combination with vitamin C), haemorrhoids (hydroxyethylrutosides derived from rutin), retinopathy (bilberry), skin ulcers (diosmin, hesperidins), allergies, atherosclerosis (quercetin, bilberry), cancer (naringenin), capillary fragility glary fragility (hesperidins, quercetin rutin). Also included are cataracts (quercetin, bilberry), diabetes (quercetin), glaucoma (rutin), hay fever (quercetin, hesperidins, rutin), macular degeneration (bilberry), measles, menopause (hesperidins), menorrhagia (heavy menstruation), night blindness (bilberry) and peptic ulcer (quercetin)**.**

Tannins are found very abundant in concentration in the methanol extract of *Loranthus micranthus Linn* and have a lot of pharmacological applications;

- ❖ antioxidant example, geramin and corilagin
- ❖ anti tumor example; agrimoniin and genothein B
- ❖ anti HIV example; gemin D and trapanin B
- ❖ anti mutagenicity example; ellagic acid**.**

Alkaloids which are moderately present are very important in pharmaceutical industry. These alkaloids display so many activities, like antimalarial (quinine) antihypertensive (reserpine), antitumor (comptothecin) and smooth muscle relaxant (theophylline). Alkaloids are used to stop haemorrhage, it is an antiprotozoal agent. They have sedative effect on nervous system**.**

Terpenoids which are moderately present has so many medicinal and pharmacological importance. Citral a type of terpenoids is used as mosquito repellant, taxol and cucurbitacins a type of terpenoids are anti tumour. Citral is also used as the starting material for the synthesis of vitamin A.

Artemisinin also; a type of terpenoids is sesquiterpene peroxide with potent antimalarial activity. It is a disinfectant and deodorant. It can be used as local anaesthetic, which is utilized in dentistry, although large dose can result to hallucination. It leads to excitatory sensation of taste and smell**.**

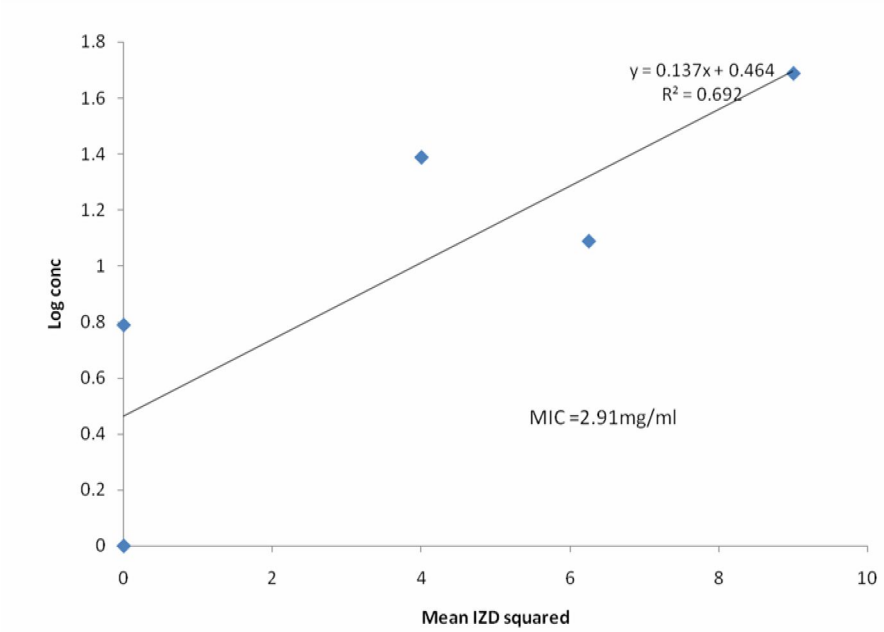
Steroids found moderate in *Loranthus micranthus Linn* has a lot of medicinal application. They are used in the treatment of various bone degenerative diseases, example bisphosphonates. It is also used as the anti inflammatory drugs, example dexamethasone.

Saponins found moderate in *Loranthus micranthus Linn* is used in agriculture as wetting agent, nematicide, and insect repellant, for increasing crop production and for fungal control. They are used in cosmetics**.** The excellent surfactant properties of saponins are made use of in pharmaceutical formulations as emulsifying agents and solubilizing agents. Oil soluble vitamins can be solubilized with saponins for easy incorporation into aqueous mixtures. Saponins are used as vaccine adjuvant for the production of vaccines used in the treatment of rabies, foot-and-mouth diseases and feline leukaemia**.** A range of pharmacological effects have been reported for saponins among which are anti-inflammatory effects; irregularities in heart action; cholesterol lowering effects, changes in the microstructure of cell antimicrobial, antifungal, antiviral and mullusidal effects have also been reported for saponins**.** They irritate the membranes of respiratory and digestive tract. Saponins are used for the treatment of rheumatoid arthritis, Addison's disease and various inflammatory conditions. Saponins cause a depletion of body cholesterol by preventing its re-absorption, thus increasing its excretion**.**

Table: 4.3. Logarithm of concentration of the methanol extracts of *Micranthus loranthus Linn* and means IZD squared.

Mean IZD^2 (mm)							
Organism	1.69	1.39	1.09	0.79	0.49	0.19	-0.11
Staphylococcus aureus	9	4	6.25	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+

IZD=inhibition zone diameter



4.4 Conclusion

Due to the extensive research done on the extract of *Loranthus micranthus Linn,* it has been found that *Loranthus micranthus Linn* has so many medicinal and pharmacological uses. The outcome of this research showed that it had growth inhibition on *Staphylococcus aureus,* meaning it could be used to cure *Staphylococcus aureus* caused diseases and infections, but had no growth inhibition on *Escherichia coli* and may not be used to cure *Escherichia coli* caused diseases and infections.

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