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Phytochemical evaluation and antimicrobial potential of methanol extracts of mistletoe (*Loranthus micranthus*) leaves grown on cola tree (*Cola nitida*) and oil bean tree (*Pentaclethra macrophylla*)

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Abstract
This study evaluated the phytochemical and antimicrobial potential of methanol extracts of *Loranthus micranthus* (Mistletoe) plant grown on *Cola nitida* (Cola tree) and *Pentaclethra macrophylla* (oil bean tree) on three bacterial pathogens; *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* using standard methods. Phytochemical analysis results showed the following (%); for *Cola nitida*: alkaloids (0.49±0.01), flavonoids (1.07 ±0.01), saponins (0.71±0.01), steroids (0.45±0.01), tannins (0.94±0.01) and for *Pentaclethra macrophylla*, alkaloids (0.39±0.01), flavonoids (1.25±0.01), saponins (0.43±0.03), steroids (0.77±0.01) and tannins (0.84±0.02) respectively. Antimicrobial evaluation revealed that the extracts produced antimicrobial activity on the test microorganisms. Zone of inhibitions at 0.4ml dilution of the extracts were significantly higher ($p<0.05$) than those at 0.1mL, 0.2mL dilutions and undiluted. *Staphylococcus aureus* was most sensitive to the extracts with mean diameter of (12.75±0.24mm)**.** From these findings, *Loranthus micranthus* leaves contain pharmacological ingredients and therefore could be useful in antimicrobial regimen formulation for microbial infection diseases treatment.

Keywords: Antimicrobial Potential, Aqueous Extracts, *Loranthus micranthus*

Introduction
Herbs with healing ability used for infectious diseases treatment mostly in rural areas and in most culture has been an ancient practice [1].
These herbs apart from serving as vitally necessary constituent of human diet could also be useful in different disease treatment [2, 3].
Over 80% of the populations in third world countries like Nigeria, Togo, Ghana and Cameroon are highly dependent as their health care delivery on the use of traditional and herbal medicines [4]. It has been reported that different extracts of plant origin have been found to show antimicrobial potentials towards some common clinical bacterial pathogens growth [5, 6].
Some plants have been shown to have wide range of pharmaceutical effects making them potent pharmaceutical agents used in clinical intervention in disease treatment and management and such plants include *Loranthus micranthus* [7, 8] and *Moringa oleifera* [9].
Loranthus micranthus is especially interesting botanically because it is a partial parasite (a hemiparasite) that grows on the branches or trunk of trees and actually sends out haustorium that penetrates into the tree xylem and takes up nutrients [10]. *Loranthus micranthus* as a hemi-parasite is capable of growing on their own, like other plants as it can produce their own food by photosynthesis [10].
Loranthus micranthus is locally known as kauchi in Hausa, Ofomo in Yoruba, Nenor in Tivi and Nbu-nnunu in Igbo speaking parts of Nigeria [11]. The leaves and young twigs are used by herbalist and it is popular in Europe especially in Germany for treatment of circulatory and respiratory system problem [12, 13].
Loranthus micranthus teas and infusions are recommended in traditional medicine because of its usefulness in preventing and management of the incidence of stroke and improvement of heart functions in some parts of Nigeria [14].

The constituents of *Loranthus micranthus* largely depend to some extent on the host tree on which the plant has grown [15]. The resistance of standard antibiotic medication among clinically important pathogens has been in the increase in recent times which have provoked research investigation, seeking for healing ability of some extracts of herbal/plant origin with the view of containing the threat. Many rural populace have resorted into the use of herbals such as mistletoe leaves (*Loranthus miranthus*) for some years past for clinical intervention in disease treatment and management with little or no psychological result of perception and learning and reasoning underlying their use in ethno-medicine. The aim of the study therefore is to carry out phytochemical and assessment of the antimicrobial potential of aqueous extracts of mistletoe leaves (*Loranthus micranthus*) grown on cola tree (*Cola nitida)* and oil bean tree (*Pentaclethra macrophylla*)

Materials and Methods

Mistleloe Leaves Procurement and Preparation

The leaves of the mistleloe (*Loranthuys micranthus*) were obtained from the Forestry Research Institute, Abia-Eke Ndume, Abia State, Nigeria which were authenticated by the Botanist in the Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria. The samples were assigned with a voucher number and some specimens were deposited in herbarium of the Department for reference purposes. The leaves were carefully detached from the stalk to avoid mixing up with the leaves of the host, washed with clean water, rinsed and then air dried at room temperature and then blended to fine powder forms using a household electric blender. Pulverized part was used for phytochemical screening while the remaining part was used for the extraction.

Phytochemical Studies

Phytochemical analysis was carried out on some quantity of each of the ground samples following the scientific guide to modern technique of plant analysis as recommended by Harborne [16].

Extraction of Mistleloe Leaves

Soxhlet extraction method [17] was used to obtain the methanol extracts of the plant (leaves). A 100g portion of the powder samples was wrapped in thimbles and the thimbles containing the plant material to be extracted was placed in an extraction chamber which was fitted to a 50ml capacity round bottom flask containing boiling chips to prevent bumping during heating. Above the round bottom flask was fitted a condenser and about 250ml of the solvent (methanol) was poured into the extractor to wet the samples. The flask was then heated using a heating mantle and the solvent evaporated into the condenser where it condensed and was converted into the liquid form that trickled into the extraction chamber containing the samples, thereby promoting extraction. The extraction process is continued and repeated several times until complete extraction was achieved. A clear solvent in the extraction chamber indicated a complete extraction. The extracts were then concentrated by evaporation to dryness by standing under room temperature in the breeze of an electric for five to seven days. Then the extracts were reconstituted 1g in 5ml of phosphate buffered saline (PBS) which was used for the antimicrobial susceptibility testing.

Preparation of Innocular/Test Organisms

The test organisms, *Psuedomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* used in this study were obtained from the Microbiology Unit of Mater Mesericodea Hospital, Afikpo and were checked for purity and were maintained on a nutrient broth at 4^{o}C in the refrigerator until it was required for analysis. Then the test organisms' cultures were prepared by inoculating them into nutrient agar medium and incubated at 37^{o}C overnight. The inoculums were then centrifuged with Remi Model: Remi R8 at 8000rpm for 10 mins. The supernatant was discarded and the pellet was mixed in sterile normal saline and the concentration of the cell was matched to the Nephelometer tube no 4 which gave a cell concentration of 10^9 cells/ml of culture. This culture was used as inoculum for the seeded plates in determining the antimicrobial activity and minimum inhibitory concentration of the samples.

Preparation and Impregnation of Mistletoe Leaves Disc

About 6.0mm diameter discs were perforated using Whatman No. 3, filter paper (UK) and arranged allowing a distance of about 2mm between them in petri dishes which were sterilized at 16^{o}C for 15mins in an oven. After cooling, the discs were impregnated with serial dilutions of the extracts and arranged separately on different petri dishes and further dried at 37^{o}C for about 2 hrs and then used for the determination of the antimicrobial activity.

Antimicrobial Sensitivity Test

The disc diffusion method was used to determine the antimicrobial activity of the extracts. About 0.1ml (approximately 10^9cells/ml) of the test organisms grown in liquid media at 37^{o}C was inoculated on Muller-Hinton growth media and then spread on the entire surface of the petri dishes containing the perforated papers discs with serial dilutions of the extracts using a swab. Then the plates were incubated at 37^{o}C 48hrs. After the incubation period, the inhibition zones around the paper discs were measured in millimeters and the sensitivity of each extracts on the test organisms were classified by the diameter of the inhibition zones according to the procedure as recommended by Ponce *et al.* [18] and Moreira *et al.* [19]. The experiment was repeated in duplicate for all the test isolates.

Statistical data analysis

The data obtained from the antimicrobial sensitivity testing were analyzed statistically using the Statistical Package for Social Sciences (SPSS) software for windows version 25 (SPSS Inc., Chicago, Illinois, USA) and the results were presented as mean ± standard mean of error (SEM) and the level of statistical significance was taken at $p<0.05$ confidence level.

Results

Quantitative analysis results as presented in Table 1 showed the phytochemical content of the leaves of *Loranthus micranthus* grown on *Cola nitida* and *Pentaclethra macrophylla* as the following (%); for *Cola nitida*: alkaloids (0.49±0.01), flavonoids (1.07 ±0.01), saponins (0.71±0.01), steroid (0.45±0.01), tannins (0.94±0.01) and for *Pentaclethra macrophylla*, alkaloids (0.39±0.01), flavonoid (1.25±0.01), saponins (0.43±0.03), steroids (0.77±0.01) and tannins (0.84±0.02) respectively (Table 1).

Zone of inhibition

The sensitivity pattern of the test organisms on the methanol extracts of *Loranthus micranthus* grown on *Cola nitida* and *Pentaclethra macrophylla* at different dilution, 0.1, 0.2, 0.4 and undiluted is shown on Table 2. The results revealed that

the extracts had varying levels of antimicrobial activity against all the test microorganisms. The sensitivity pattern as shown in Figures 1a and 1b revealed that the inhibition zones of the test organisms at 0.1ml dilution showed significant decrease (p<0.05) than those at 0.2ml dilution for methanol extracts of *Loranthus micranthus* grown on *Cola nitida* and *Pentaclethra macrophylla* an. For the respective organisms, the extracts at 0.4mL dilution showed significant increase (p<0.05) in zone of inhibition compared to those at 0.1mL, 0.2mL dilutions and undiluted respectively.

Table 1: Phytochemical composition of the methanol extract of *Loranthus micranthus* (%)

Sample	Alkaloids	Flavonoids	Saponins	Steroids	Tannins
Cola nitida	0.49±0.01	1.07 ±0.01	0.71±0.01	0.45±0.01	0.94±0.01
Pentaclethra maxrophylla	0.39±0.01	1.25±0.01	0.43±0.03	0.77±0.01	0.84±0.02

Presented as mean ± standard mean of error (SEM) of duplicate determinations.

Table 2: Serial dilutions of extracts (ml) and diameter of the inhibition zones (mm)

Test organism	*C. nitida* of *L.micranthus*	*P. macrophylla* of *L. micranthus*	Mean inhibitor diameter *(C. nitida)*	Mean inhibitor diameter *(P. macrophylla)*
E. coli	0.1	0.1	1.53±0.10	1.28±0.10
	0.2	0.2	4.52±0.12	4.49±0.01
	0.4	0.4	8.93±0.21	9.56±0.12
	Undiluted	Undiluted	0.40±0.10	0.45±0.10
S. aureus	0.1	0.1	1.28±0.12	1.53±0.22
	0.2	0.2	4.78±0.20	5.42±0.32
	0.4	0.4	9.75±0.12	12.75±0.24
	Undiluted	Undiluted	0.80±0.22	0.72±0.21
P. aeruginosa	0.1	0.1	0.63±0.30	0.44±0.32
	0.2	0.2	1.59±0.10	1.59±0.20
	0.4	0.4	3.76±0.20	5.10±0.10
	Undiluted	Undiluted	0.12±0.22	0.24±0.01

Presented as mean ± standard mean of error (SEM) of duplicate determinations.

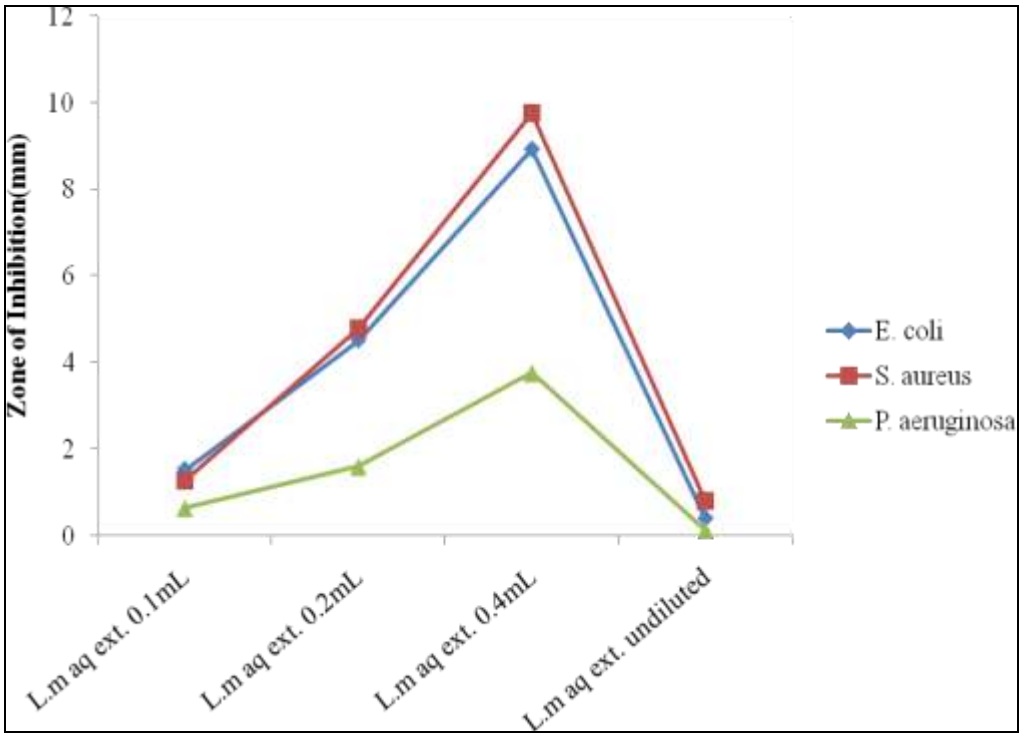

Fig 1a: Sensitivity pattern of *Loranthus micranthus* methanol extracts grown on *C. nitida to E. coli, S. aureus* and *P. aeruginosa* at serial dilutions.

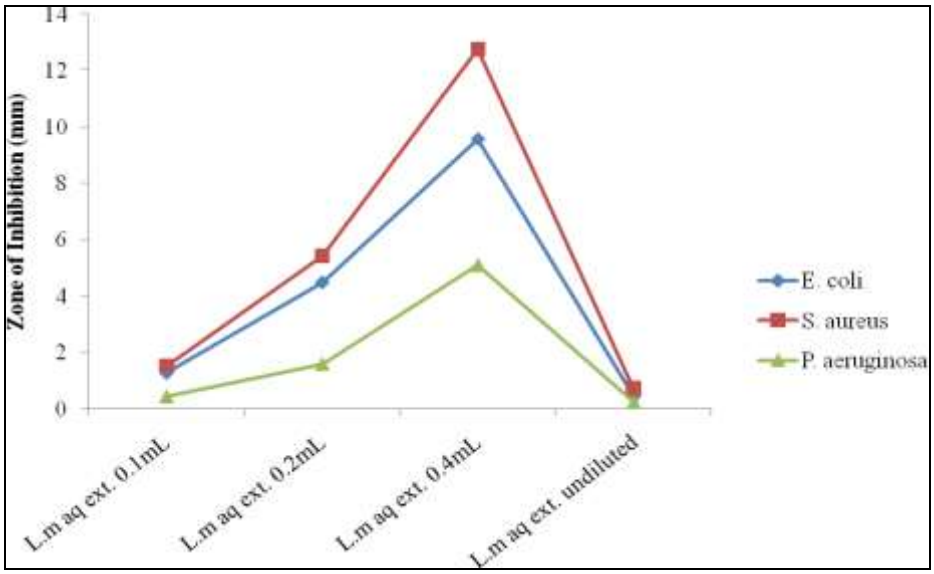

Fig 1b: Sensitivity pattern of *Loranthus micranthus* methanol extracts grown on *P. macrophylla to E. coli, S. aureus* and *P. aeruginosa* at serial dilutions.

Discussion

The evaluation of phytochemicals and antimicrobial activity of leaves and other plants parts is a veritable tool in discovering the pharmacological potentials of such plants which could be useful in antimicrobial regimen formulation for microbial infection and other related diseases treatment.

Quantitative analysis of the phytochemicals results of *Loranthus micranthus* grown on *Cola nitida* and *Pentaclethra macrophylla* (Table 1), indicated that this non valued plant leaves con tain appreciable amount of flavonoids (1.07%) and (1.25%) and tannins (0.94±0.01) and (0.84±0.02) respectively. Flavonoids have biological activities that are of benefit in the prevention and management of many ailments [20]. Tannins have biological activities that favour the prevention and management of many ailments [21].

The reports of Aguwu *et al.* [22] and Oguntoye *et al.* [23] on the pharmacological potential of *Loranthus micranthus* leaves suggests that it must be linked with the bioactive phytochemicals such as flavonoids and tannins. The reports are in agreement with the findings of Osadebe *et al.* [8], Sofowora [24] and Carlson and King [25], who also reported the usefulness of the leaves in disease treatment and management.

Table 2 presents the results of the antimicrobial potential of this leaves on the test organisms. The results revealed the various inhibition zones (mm) exhibited by the extracts on the test organisms. Among the clinical bacterial pathogens, *Staphylococcus aureus* was observed to be more sensitive to the extracts than *Escharichisa coli* and *Pseudomonas aeruginosa* with mean inhibition diameter of 12.75±0.24mm and 9.75±0.12mm respectively (Figures 1a and 1b).

The difference in susceptibility of the test organisms to the extracts is due to the composition or morphology of the cell wall [26, 27]. The outer membranes of *Escherichia coli* and *Pseudomonas aeruginosa* being Gram-negative bacteria is the main reason for resistance to a wide range of antibiotics which may be applicable in this case also. Any alteration in the other membrane by Gram-negative bacteria like changing the hydrophobic properties or mutations in porins and other factors can create resistance. *Staphylococcus aureus* being a Gram-positive bacterium lack this important layer which makes Gram-negative bacteria more resistant to antibiotics and possibly plant extracts than Gram-positive bacteria [28-30].

The result recorded no inhibition zone on the test organisms in undiluted concentration of the extracts which is an indication that water activates the bioactive components and hence antimicrobial activity of the extracts. Although the biochemistry underlying the abnormal situation might not be clear, however, Ichor and Ekoja [31]. and El-Shemy *et al.* [32]. in their respective research findings reported similar anomaly. Our findings in this study are supportive and in line with other researchers in the related area of interest that *Loranthus micranthus* leaves extracts contain phytochemical constituents and antimicrobial potential and therefore hold promise in pharmaceutical industry.

Conclusion

Loranthus micranthus leaves contain appreciable amount of flavonoids (1.07%) and (1.25%), tannins (0.94±0.01) and (0.84±0.02) respectively from *Cola nitida* and *Pentaclethra macrophylla* and other bioactive components (alkaloids, saponnins, steroids, tannins). The acclaimed invaluable pharmaceutical potential and antimicrobial properties exhibited by the extracts towards the microorganisms could be linked to the bioactive constituents of the extracts. The difference in susceptibility of the test organisms to the extracts is due to the composition or morphology of the cell wall.

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