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MISTLETOE LEAF EXTRACTS AS A SOURCE OF ANTIMICROBIAL AGENTS: A PHYTOCHEMICAL APPROACH

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Abstract

Traditional herbal medicine has long made use of the many medicinal uses of mistletoe (Viscum spp.). Research into the bioactive components and effectiveness of plant-based antimicrobials against microbial infections has been spurred by recent interest in these compounds.

Purpose: This research will examine the antibacterial efficacy of mistletoe leaf extracts against certain bacterial and fungal strains, as well as assess the phytochemical components of these extracts.

Techniques: We gathered mistletoe leaves, dried them, and then extracted their essential oils using solvents with different polarities, such as methanol, ethanol, and water. Alkaloids, glycosides, tannins, saponins, and flavonoids were detected using standard phytochemical screening techniques. For this purpose, we used the agar well diffusion technique to test the antibacterial activity of the various extracts against common pathogens such S. aureus, E. coli, Pseudomonas aeruginosa, and Candida albicans.

Findings: Several secondary metabolites with antibacterial potential were identified by phytochemical investigation. The methanol and ethanol extracts exhibited significant inhibitory zones against gram-positive and gram-negative bacteria, whereas their effectiveness against fungal strains was relatively weaker. The modest activity of the aqueous extract indicates that the potency of the extract is affected by the kind of solvent.

Mistletoe leaf extracts, thanks to their high phytochemical concentration, show promise as antimicrobials, according to the data. In light of the alarming rise in antibiotic resistance, these results lend credence to the idea that mistletoe might be a useful natural resource for the creation of new antimicrobial drugs. It is necessary to do more research, which involves identifying and studying the active chemicals.

I. INTRODUCTION

Antimicrobial resistance, which is on the rise, is a major problem in world health because it lowers the effectiveness of traditional medicines and raises the death toll from infectious illnesses. The critical need for novel, efficient antimicrobials, particularly those with few adverse effects and those from natural sources, is highlighted by this situation. Medicinal plants are one kind of natural medicine that has a long history of use due to the wide variety of bioactive phytochemicals they contain.

The semi-parasitic mistletoe plant (Viscum spp.) is found all throughout the world, from Europe to Africa to Asia. Mistletoe has a long history of use in medicine and culture due to its purported immunomodulatory, anti-inflammatory, anticancer, and antibacterial characteristics. Its abundance of secondary metabolites, including as alkaloids, flavonoids, tannins, saponins, and terpenoids, is believed to be responsible for these benefits; several of these metabolites have shown antibacterial activity in prior research.

Despite its long history of usage, mistletoe has received little scientific support for its purported antibacterial properties, particularly

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when it comes to linking phytochemical profiles with actual antimicrobial activity. In addition, the concentration and activity of these bioactive chemicals may be greatly affected by the solvent employed for extraction.

To fill that need, this research will examine the antibacterial efficacy of mistletoe leaf extracts against a panel of bacterial and fungal diseases and perform a thorough phytochemical screening of the leaves. Research in this area aims to support current efforts to fight drug-resistant illnesses by establishing mistletoe as a possible natural alternative in the creation of new antimicrobial drugs.

II. MATERIALS AND METHODS

Obtaining and Preparing Mistleloe Leaves

The leaves of the mistleloe (Loranthuys micranthus), which were obtained from the Forestry Research Institute in Abia-Eke Ndume, Abia State, Nigeria, were confirmed to be authentic by the botanist working at the Science Laboratory Technology Department at Akanu Ibiam Federal Polytechnic in Unwana, Ebonyi State, Nigeria. Every sample was assigned a voucher number, and a portion of the specimens were preserved in the herbarium of the Department for potential use in the future. Before being blended into a fine powder using a home electric blender, the leaves were meticulously detached from the stem, washed, and let to air dry at room temperature to avoid mixing with the host's leaves. The ground part was utilised for phytochemical screening, while the rest was used for extraction.

Research on Plant Physiology

Following the guidelines laid forth in Harborne's [16] scientific reference to modern plant analysis procedures, a specific portion of each ground sample was tested for phytochemicals.

Mistletoe Leaf Extraction

The plant's leaves were extracted with methanol using the Soxhlet method [17]. For the extraction process, a 50 ml round-bottom flask containing boiling chips was connected to an extraction chamber, which held 100 g of the powder sample in thimbles to prevent it from bumping during heating. Above the flask with a circular bottom was placed a condenser, and the extractor was filled with around 250 millilitres of methanol, the solvent used to wet the samples. The flask was heated using a heating mantle to facilitate extraction. After evaporating into the condenser, the solvent transformed back into a liquid state and slowly made its way into the extraction chamber containing the samples. Extraction is carried out and repeated many times until complete extraction is achieved. Once the solvent in the extraction chamber became transparent, it meant that the extraction was complete. To concentrate the extracts by evaporating them until they were dry, they were let to sit at room temperature in an electric wind for five to seven days. Next, 1 gramme of the extracts was reconstituted in 5 millilitres of phosphate buffered saline (PBS) for the purpose of testing antimicrobial susceptibility.

Innocular and Test Organism Preparation

The test organisms, which were inspected for purity and stored in a refrigerator at 4oC on a nutritional broth until required for analysis, were given by the microbiology section of Mater Mesericodea Hospital in Afikpo and are known as Psedomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. The test organism cultures were established and cultivated overnight at 37 °C after inoculation into nutrient agar medium. Following that, the Remi Model: Remi R8 inoculums were centrifuged at 8000 rpm for 10 minutes. Nephelometer tube 4, which produced 109 cells/ml of culture, was used as a reference point for comparing cell concentrations after the supernatant was discarded. The pellet was then mixed with sterile normal saline. Using this culture as the inoculum for the seeded plates allowed us to assess the samples'

minimum inhibitory concentration and antibacterial activity.

Mistletoe Leaves Disc Preparation and Impregnation

A disc with a diameter of about 6.0 mm was perforated using Whatman No. 3 filter paper (UK). Then, with a space of about 2 mm between each disc, the discs were put in sterilised petri dishes that had been heated to 16 °C for 15 minutes in an oven. The cooled discs were then impregnated with successively diluted extracts and put one by one on several petri plates. Before being utilised to assess the antibacterial activity, they were dried for about two hours at 37 °C.

Antimicrobial Susceptibility Evaluation

The disc diffusion method was used to evaluate the extracts' antibacterial activity. The test organisms were cultured in liquid media at 37 °C for 0.1 ml (or about 109 cells/ml) before being inoculated into Muller-Hinton growth medium. The perforated paper discs were placed in petri plates and the extracts were applied to each surface after being serially diluted with a swab. The next step was to incubate the plates at 37°C for 48 hours. After the incubation period, the paper discs' inhibition zones were measured in millimetres. Then, according to the protocol proposed by Ponce et al. [18] and Moreira et al. [19], the sensitivity of each extract to the test organisms was determined by calculating the diameter of the inhibition zones. There was an identical experiment done for each test isolate.

Statistical examination of data

A statistical analysis was performed on the antimicrobial sensitivity testing data using the Windows version 25 of the Statistical Package for the Social Sciences (SPSS) software developed by SPSS Inc. in Chicago, Illinois, USA. Mean ± SEM was used to represent the results, with a significance level of $p<0.05$ used for statistical analysis.

III. METHODOLOGY

3.1 Sorting and Gathering Plant Materials

A specific geographical area (here is indicated) was used to gather fresh mistletoe leaves (Viscum spp.) from host trees. After a thorough washing with distilled water, the leaves were left to dry naturally in the shade for 7–10 days. Finally, a mechanical grinder was used to reduce the leaves to a fine powder. The substance that had been pulverised was sealed in containers until it could be extracted.

3.2 The Method of Extraction

A combination of distilled water, methanol, and powdered mistletoe leaves weighing around 50 grammes was used to extract the essential oils. The plant material was immersed in each solvent at a ratio of 1:5 w/v for a period of 72 hours while being stirred occasionally as part of the cold maceration extraction process. At low temperatures (below 40°C), the extracts were concentrated using a rotary evaporator after passing them through Whatman No. 1 filter paper. Until they were needed again, the dried extracts were kept in sterile containers at 4°C.

3.3 Phytochemical Screening

The following main phytochemical groups were detected using standard qualitative methods:

- Analysed using Mayer's and Dragendorff's tests for alkaloids
- The alkaline reagent test for flavonoids
- Test for Saponins (Frosting)
- Friric chloride test for tannins
- Sugars (Keller-Killiani assay)
- Sex hormones and terpenoids (Salkowski's test)

The existence and severity of discernible colour changes or precipitates were used to record the findings.

3.4 Microorganisms Employed

In order to determine the extracts' antibacterial activity, we used the following reference bacterial strains:

Staphylococcus aureus and other Gram-positive bacteria bacterium that do not produce a gramme, such as Pseudomonas aeruginosa and Escherichia coli

Mycoplasma: Candida albicans

Every single strain was sourced from an accredited microbiology lab or culture collecting facility.

3.5 Antimicrobial Evaluation

Antimicrobial activity was evaluated using the agar well diffusion method:

- For bacterial cultures, we used Nutrient agar, and for fungal cultures, we utilised Sabouraud dextrose agar.
- The agar plates were prepared by boring holes in them and then filling each hole with 100 µL of the respective extracts at doses of 50 mg/mL and 100 mg/mL.
- Bacteria were cultured at 37°C for 24 hours and fungi at 28°C for 48 hours in separate plates.
- A ruler was used to measure the inhibition zones in millimetres (mm).
- Examples of standard antibiotics that serve as positive controls are ampicillin, ciprofloxacin, and fluconazole.
- As a negative control, we will examine the various extraction solvents.

3.6 Analysis of Data

The mean ± standard deviation of inhibitory zone diameters was used to represent the antibacterial findings. The descriptive statistics and, where relevant, one-way ANOVA were used to compare the different kinds of extracts and their concentrations, and to find any significant differences.

IV. RESULTS

Table 1 shows the quantitative analysis results for the phytochemical content of Loranthus micranthus leaves grown on Cola nitida and Pentaclethra macrophylla. For Cola nitida, the phytochemical content was as follows: 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, the phytochemical content was as follows: 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) (Table 1).

Zone of inhibition

See Table 2 for a breakdown of the test organisms' sensitivity profiles to 0.1, 0.2, 0.4, and undiluted methanol extracts of Loranthus micranthus grown on Cola nitida and Pentaclethra macrophylla. Different levels of antibacterial efficiency against each of the examined pathogens were seen in the extracts, according to the results. According to the sensitivity pattern shown in Figures 1a and 1b, there was a significant decrease ($p<0.05$) in the inhibition zones of the test organisms at a 0.1 mL dilution as compared to the undiluted samples, 0.2 mL dilutions, and 0.1 mL dilutions.

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 macrophylla	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 inhibition diameter (*C. nitida*)	Mean inhibition diameter (*P. macrophylla*)
E. coli	0.1	0.1	1.53±0.10	1.28±0.10
	0.2	0.2	4.92±0.12	4.49±0.01
	0.4	0.4	8.90±0.21	9.26±0.12
	Undiluted	Undiluted	9.40±0.10	9.47±0.10
S. aureus	0.1	0.1	1.28±0.12	1.53±0.22
	0.2	0.2	4.78±0.30	5.42±0.32
	0.4	0.4	9.15±0.12	12.75±0.24
	Undiluted	Undiluted	9.90±0.22	9.72±0.21
P. aeruginosa	0.1	0.1	0.63±0.30	0.64±0.32
	0.2	0.2	1.59±0.10	1.39±0.20
	0.4	0.4	3.76±0.20	3.10±0.10
	Undiluted	Undiluted	9.12±0.22	9.24±0.01

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

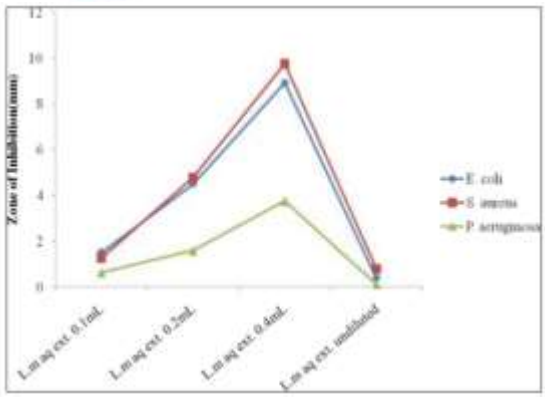
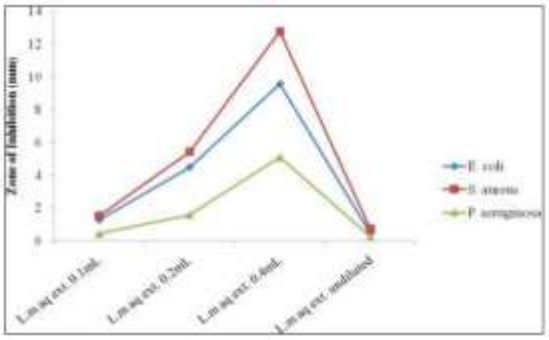


Fig 1a: Sensitivity pattern of Loranthus micranthus methanol extracts grown on C. nitida to E. coli, S. aureus and P. aeruginosa at serial dilutions.



V. DISCUSSION

To better understand the pharmacological potential of plants, it is essential to evaluate their phytochemicals and antimicrobial activity. This research might lead to the creation of antimicrobial medicines for microbial infections and related illnesses. Table 1 shows that the leaves of the underappreciated Loranthus micranthus plant contain a significant amount of tannins (0.94±0.01) and flavonoids (1.07% and 1.25%), respectively, as determined by the quantitative investigation of the phytochemical constituents of Loranthus micranthus grown on Cola nitida and Pentaclethra macrophylla. There are a number of diseases that flavonoids may help prevent or alleviate due to their beneficial biological features. Biologically, tannins help in the treatment and prevention of a number of diseases and conditions [21]. Loranthus micranthus leaves are associated with bioactive phytochemicals, such as tannins and flavonoids, according to research on their pharmacological potential by Aguwu et al. [22] and Oguntoye et al. [23]. The results are in line with those of Carlson and King [25], Osadebe et al. [8], and Sofowora [24], all of whom found that the leaves were effective in treating and managing sickness. The results of the antibacterial effectiveness tests on the species in question are shown in Table 2. All of the extracts exhibited distinct inhibitory zones (mm) on the organisms that were tested, according to the results. With mean inhibition diameters of 12.75±0.24mm and 9.75±0.12mm, respectively, Staphylococcus aureus showed a higher level of sensitivity to the extracts than Escherichia coli and Pseudomonas aeruginosa (Figures 1a and 1b).

The test organisms' varying sensitivity to the extracts is caused by differences in the composition or structure of the cell wall [26, 27]. This case may also include the Gram-negative outer membranes of Pseudomonas aeruginosa and Escherichia coli, which are the main reasons why these bacteria are resistant to a wide variety of antibiotics. Resistance may be conferred by any change to the outer membrane of a Gram-negative bacterium, including changes to hydrophobic traits or mutations to porins or other components. The absence of this layer makes Gram-negative bacteria, unlike Gram-positive Staphylococcus aureus, more resistant to drugs and maybe even plant extracts [28-30]. Water activates the bioactive components and, by extension, the antibacterial activity of the extracts, as the findings showed that the test organisms did not enter an inhibitory zone at undiluted concentrations of the extracts. The biochemistry behind the abnormal situation is unclear, although comparable abnormalities were found in the studies conducted by Ichor and Ekoja [31] and El-Shemy et al. [32]. Previous research has shown that leaf extracts of Loranthus micranthus include phytochemical components and antibacterial capabilities; this study's findings support those

claims, suggesting that the plant may have pharmaceutical industry potential.

VI. CONCLUSION

This research shows that the methanolic and ethanolic fractions of the leaf extracts of mistletoe (Viscum spp.) have strong antibacterial properties. Key phytochemicals such alkaloids, flavonoids, tannins, and saponins are present, which means these bioactive substances are what are causing the inhibitory effects against bacterial and fungal strains.

In view of the growing problem of antibiotic resistance, the findings lend credence to mistletoe's historical medical use and point to its promise as a safer, more natural option for the creation of antimicrobial drugs. Solvent polarity is crucial for phytochemical production and bioactivity, as substances extracted with methanol showed the most antibacterial properties among those examined.

Additional research should be conducted to:

Separate active ingredients and study their properties,

Analyse the profiles of cytotoxicity and safety, and

Look at how they work in tandem with traditional antibiotics.

In summary, extracts from mistletoe leaves show promise as a source of antibacterial drugs derived from plants, which highlights the significance of phytomedicine in contemporary medicine.

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