International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

# Anti-Microbial and Anti-Fungal Activity of Methanol Extracts of Leaf and Root of Rauvolfia Micrantha Hook. F.

**Rajesham P[1], Kishore Kumar Godisela[2], A. Gangaprasad[3], Rama Devi B[4]**

[1]Department of Botany, Osmania University, Hyderabad

[2]Department of Biotechnology, Kakatiya Government College (A), Hanumakonda

[3]Professor, Department *of Biotechnology, University of Kerala, Kariavattam, Kerala.*

[4]Department of Botany, Osmania University, Hyderabad-50001
Corresponding Author Email: *rama81379[at]gmail.com*

**Abstract:** ***Phytomedicine and herbal remedies have long been a part of medical care. Medicinal plants have long been used by many different countries, ethnic groups, and cultures worldwide. Plant-based compounds are used in a number of industries, such as essential oils, agrochemicals, nutraceuticals, food additives, cosmetics, and pharmaceuticals. Rauvolfia micrantha Hook. f. has attracted a lot of interest in the biomedical sector because of its ability to lower blood pressure, which is mainly because of the reserpine found in the oleoresin that is collected from its roots. Numerous bioactive secondary metabolites with significant therapeutic value are produced by these medicinal plants. Numerous phytochemicals, especially indole alkaloids like reserpine, are abundant in the plant. Plant root extracts have been used for generations to treat neurological illnesses. Promising clinical trials have elucidated characteristics such as antihypertensive and antidiabetic effects. The plant's potential for anti-microbial and anti-fungal activities is estimated in this study. The study investigated the minimum drug concentration needed to effectively inhibit pathogenic bacteria and fungi in the methanol extract of Rauvolfia micranthe root and leaf. When compared to standard medications, we found that the root extract is more effective than the leaf extract.***

**Keywords:** *Rauvolfia micrantha Hook. F,* Reserpine, Medicinal plant, Alkaloids, Anti-microbial, Anti-fungal. Nutraceuticals.

## 1. Introduction

Many nations, ethnic groups and cultures have used medicinal plants since ancient times to treat various ailments. In addition to important minerals, vitamins and other nutrients, medicinal plants include a wide range chlorophyll, phytosterols, glycosides, phenols, flavonoids and diterpenes (Harish, (2024)). The strong pharmacological properties of medicinal plants and herbs make them very useful in the development of new drugs (Rajesham. P, 2023). Plant-based chemicals are used in a variety of industries, including pharmaceuticals, food additives, cosmetics, foodstuffs, agrochemicals and essential oils. Plants produce moderate amounts of secondary metabolites that are stored in specific regions such as roots and trichomes (Krochmal, 2021). These "small valuable" compounds are obtained mainly by chemical synthesis or direct extraction from plants specially cultivated for this purpose. Due to the complex structures and stereo- and regiospecific effects of these molecules, organic synthesis is usually an expensive and time-consuming process. Thus, the preferred technique is the direct extraction of these secondary metabolites from wild plants.

The genus Rauvolfia L. (Apocynaceae) is distributed throughout the tropical regions of the World (Koch, 2007). Currently, there are 110 species of Rauvolfia (R. B. Malabadi, et al. , 2024). The perennial arboreal shrub Rauvolfia micrantha Hook. f. (Apocynaceae) may be found at a height of 600 meters in the Tonnarelli and Travancore hills in southern India's Western Ghats. Being very different from the other species, R. micrantha is thought to be an endemic species of southwest India, specifically the Western Ghats. The roots of this plant contain notable alkaloids, such as ajmalicine, reserpine, sarpagine, reserpine, and serpentine, known for their antihypertensive, hypertensive, and tranquilizer properties (Anonymous., 1969). Notably, R. micrantha has been recognized as an alternative to Rauvolfia serpentina roots in business lots supplied to American buyers (HW.jr, 1954 ).

R. micrantha is used as a substitute of R. serpentina in traditional Indian medicine (Ayurveda) to treat a variety of nerve disorders, especially in the Kerala region (Jenipher, 2024; R. B. Malabadi, et al., 2024; Sahu, 1979). According to Sahu (1979), the plant is rare and exclusive to the southern forests of the Western Ghats. Regrettably, endemic habitats, restricted ranges, small populations in easily accessible areas, and human pressures on forestlands have contributed to R. micrantha's lessen in the wild (Rajesham P.1, 2023)

## 2. Materials and Methods

### Antibacterial activity

### 2.1Bacterial Strains:

The ATCC provided the bacterial strains used in the study, which included Gram positive strains of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC

33400), and Gram negative strains of *Pseudomonas aeruginosa* (ATCC 27853) and E. coli (ATCC 25922).

**2.2 Media Preparation for Anti-Bacterial Activity:**

**A) Nutrient Agar Media**
We purchased nutritional agar commercially (Himedia), weighed out 28.0 grams of powder, diluted it in 1000 milliliters of distilled water, and properly mixed it. In order to investigate the antibacterial activity, the dissolved nutrient agar was sterilized in an autoclave for 15 minutes at 1210 C. The media was then used to prepare plates.

**B) Nutrient Broth**
Nutrient broth was purchased commercially, and 1.3 grams of powder were weighed, dissolved, and well mixed with 100 milliliters of distilled water. The dissolved nutritional broth was used to prepare the inoculum after it was sterilized in an autoclave for 15 minutes at $121^0$ C.

**C) Preparation of stock solution**
Each validated test organism was subculture aseptically using two nutrient agar slants to create the stock culture of that organism. Two slants were maintained: a working set and a stock culture. As stock cultures, the bacterial cultures in the corresponding agar slants were kept at 4°C. Additionally, one counter-glycerol stock was kept at a temperature of 20°C.

**D) Inoculum preparation**
After the chosen bacterial pathogens were added to nutritional broth and cultured for 24 hours at 37°C, the suspensions were measured to ensure that they contained roughly 10-5 CFU/ml.

**2.3 Antibacterial Activity**

The Agar well-diffusion method was used to study chemical compounds with antibacterial properties. Using a range of bacterial pathogens such as E. coli, Pseudomonas aeruginosa, Streptococcus pneumonia, and Staphylococcus aureus, four concentrations (25, 50, 75, and 100 µl) were assessed. For 18 to 24 hours, the plates were incubated at 37°C. The activity index and the inhibitory zone's diameter (mm) were calculated at the end of the experiment. Readings were taken at three distinct fixed orientations, and the average results were recorded.

**2.4 Anti-Fungal Activity**

Candida albicans (MTCC 183), the fungus strain used in this investigation, was obtained via the Microbial Type Culture Collection (MTCC) of the Institute of Microbial Technology (IMTECH), Chandigarh.

**Sabouraud Dextrose Agar (SDA):** After weighing the commercially obtained Sabouraud dextrose agar and dissolving 32.5 grams of powder in 500 milliliters of distilled water, everything was well blended. After preparing the plates with the dissolved SDA, they were autoclaved for 15 minutes at $121^0$ C to test for antifungal activity.

**Antifungal activity:** The antifungal activity was evaluated using the well diffusion technique. The Candida albicans fungus was added to the prepared SDA culture plates using the spread plate method. The plates were incubated at 37+2ºC for 48 hours in order to detect fungal activity. After 48 hours, the plates were examined for zone development around the well, and the zone of inhibition (mm) was determined.

**2.5 Minimum Inhibitory Concentration (MIC):**

The lowest dose of an antimicrobial growth inhibitor (MIC) that, following an overnight incubation period, would prevent a bacterium from growing visibly is known as the minimum inhibitory concentration (MIC).

**Compound Preparation:**
Each compound was weighed once, at a concentration of one milligram per milliliter, and then dissolved in methanol. Standard amoxicillin was also made for the same sample.

**Culture Preparation:**
The culture loop was injected with 3 milliliters of nutrient broth and left in a shaking incubator at $37^0$ C for the whole night.

**Inoculum Preparation:**
After overnight growth, 20 µl of culture was removed and inoculated in 1.5 ml of nutrient broth. Different chemical concentrations were added and cultured at 37 0 c overnight in an incubator.

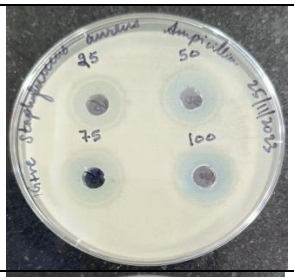
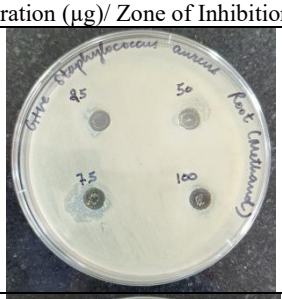
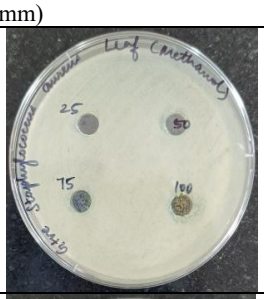
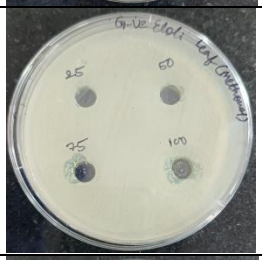
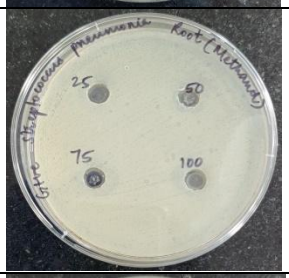
## 3. Result

**3.1 Anti-bacterial activity**

Using ampicillin as the standard antimicrobial agent, the well diffusion method was used to investigate the antibacterial characteristics of the methanolic extract from the root and leaf of the Rauvolfia micrantha plant. Pathogenic strains such as *E. Coli, Pseudomonas aeruginosa*, *Streptococcus pneumonia,* and *Staphylococcus aureus* were the focus of the investigation. In the evaluation, the effectiveness of ampicillin was contrasted with the methanolic extract of Rauvolfia micrantha Root, as shown in Table 1. The findings showed different patterns for various bacterial strains and concentrations. While the methanolic extract from Rauvolfia micrantha Root showed significant inhibition starting at lower concentrations, reaching a maximum zone of 12 mm at 75 µg, *Staphylococcus aureus* showed an increasing zone of inhibition in response to ampicillin, peaking at 21 mm with 100 µg concentration. *E. Coli* showed similar ranges of inhibition with ampicillin, ranging from 16 mm to 20 mm across doses, however the methanolic extract showed gradual increase inhibition registering a tiny 21 mm zone at 100 µg concentration. Increased ampicillin susceptibility was seen in *Streptococcus pneumonia*, where zones of inhibition increased from 17 mm to 23 mm at higher dosages. The Rauvolfia micrantha Root methanolic extract, on the other hand, showed only modest inhibitory effects, with a peak zone of 25 mm seen at 100 µg concentration. *Pseudomonas aeruginosa* showed a clear increase in susceptibility to ampicillin, with zones of

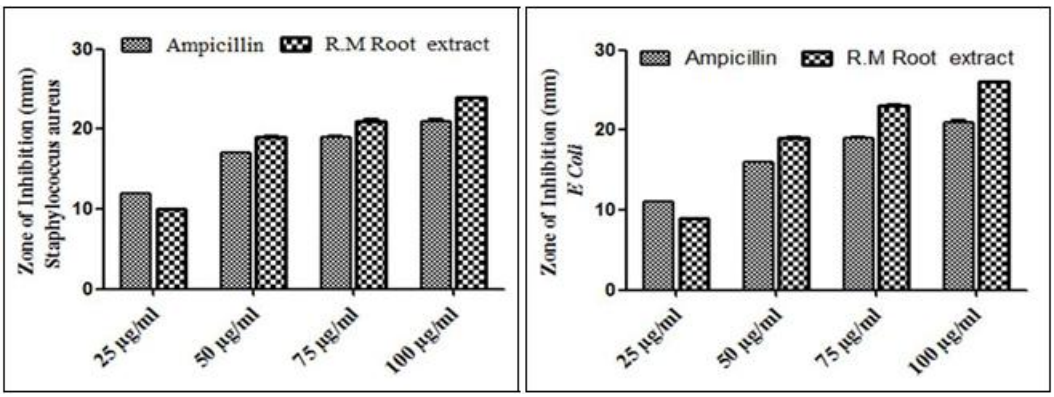
inhibition ranging from 17 mm to 21 mm. The zones measured 12 mm and 23 mm, respectively, however the methanolic extract showed very good inhibition, especially at dosages of 75 µg and 100 µg as shown in Figure 1.

**Table 1:** Anti-Bacterial Activity of Root and Leaf Methanolic extract of *Rauvolfia micrantha* with Standard Ampicillin against bacterial Strains- *Staphylococcus aureus, E coli, Streptococcus pneumonia* and *Pseudomonas aeruginosa*

| S. No | Bacterial Strain | Ampicillin | Methanolic extract of *Rauvolfia micrantha* Root | Methanolic extract of *Rauvolfia micrantha Leaf* |
|---|---|---|---|---|
| | | Concentration (µg)/ Zone of Inhibition (mm) | | |
| 1 | *Staphylococcus aureus* |  |  |  |
| 2 | *E coli* |  |  |  |
| 3 | *Streptococcus pneumonia* | |  |  |
| 4 | *Pseudomonas aeruginosa* | |  |  |

### 3.2 *Rauvolfia micrantha* Leaf Methonolic extract:

The table 1 represents the response of different bacterial strains to both Ampicillin and the methanolic extract sourced from *Rauvolfia micrantha* leaves, gauged by their respective zones of inhibition (mm).



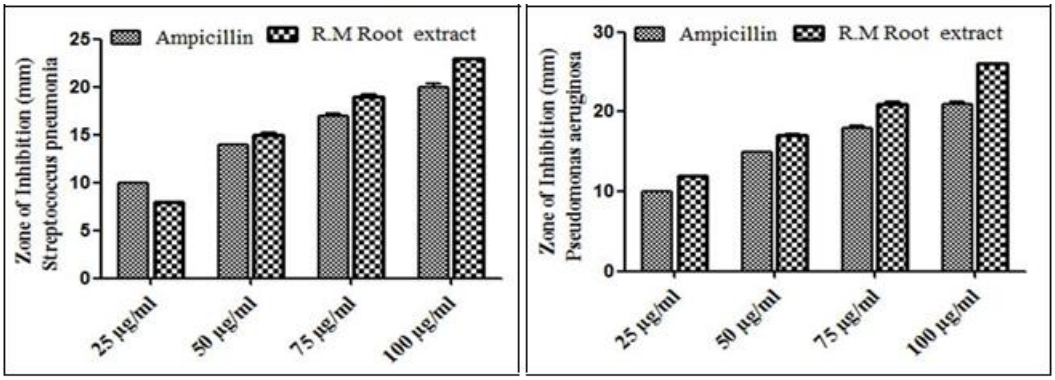



**Figure 1:** Anti-Bacterial Activity of methanolic root extract of *Rauvolfia micrantha* with Standard Ampicillin against bacterial Strains- *Staphylococcus aureus, E coli, Streptococcus pneumonia* and *Pseudomonas aeruginosa.*

When measuring the inhibition zones of *Staphylococcus aureus,* ampicillin causes a dose-dependent rise that ranges from 17 mm to 21 mm at increasing concentrations. On the other hand, at lower concentrations, the methanolic extract of Rauvolfia micrantha leaves shows little inhibition, with zones of 9 mm and 13 mm at 50 µg and 75 µg, respectively. On the other hand, a zone of 15 mm is registered at 100 µg, indicating a more marked inhibitory impact.

Similar tendencies in susceptibility are shown in *E. Coli*, where Ampicillin causes inhibitory zones that range in size from 16 mm to 21 mm at different concentrations. In contrast, the Rauvolfia micrantha leaf methanolic extract shows very modest inhibitory effects, with recording zones of 11 mm and 17 mm at 75 µg and 100 µg, respectively. Ampicillin exhibits considerable susceptibility to *Streptococcus pneumonia*, resulting in inhibitory zones that enlarge from 17 mm to 23 mm at increasing concentrations. *Pseudomonas aeruginosa* similarly displays increased Ampicillin susceptibility, with inhibitory zones measuring between 17 and 21 mm. The Rauvolfia micrantha leaf methanolic extract exhibits a moderate inhibition, particularly at 75 µg and 100 µg doses, with recording zones of 8 mm and 9 mm, respectively as shown in Figure 2.

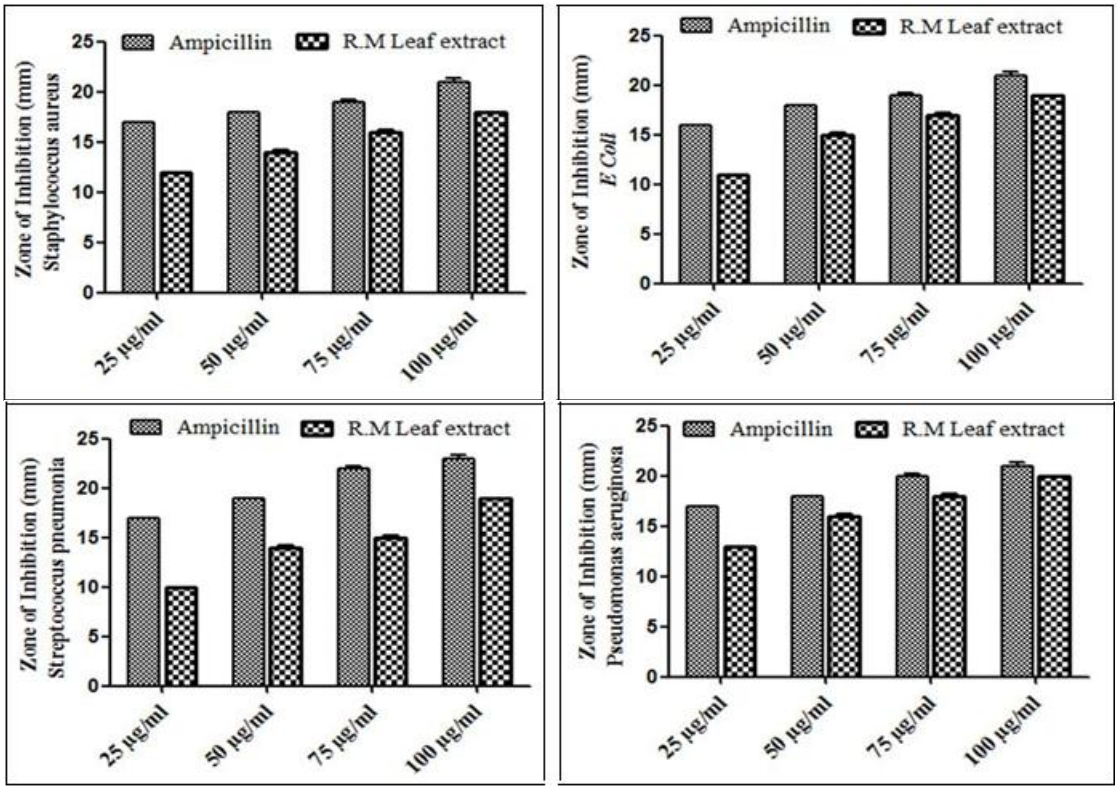



**Figure 2:** Anti-Bacterial Activity of Methanolic Leaf extract of *Rauvolfia micrantha* with Standard Ampicillin against bacterial Strains- *Staphylococcus aureus, E coli, Streptococcus pneumonia* and *Pseudomonas aeruginosa.*

Using the well diffusion method in SDA culture plates, the anti-fungal activity profile of plant extracts was conducted against fungal strains *Candida albicans* (MTCC 183). Fluconazole is used in this research as a common antifungal medication. The table 2 shows how different strains of Candida albicans react to fluconazole when combined with methanolic extracts from the root and leaf plant Rauvolfia micrantha. The corresponding zones of inhibition (mm) at various concentration levels (µg) were used to measure these responses.

Fluconazole is an antifungal drug that exhibits variable effectiveness against strains of *Candida albicans* at increasing doses. The zones of inhibition gradually extend

from 14 mm at 25 µg to 20 mm at 100 µg, confirming its potential to inhibit the growth of *Candida albicans* and showing a dose-dependent pattern.

| S. No | Fungal strain | *Fluconazole* | Methanolic extract of *Rauvolfia micrantha* Root | Methanolic extract of *Rauvolfi amicrantha Leaf* |
|---|---|---|---|---|
| | | Concentration (µg)/ Zone of Inhibition (mm) | | |
| 1 | *Canadia Albicans* |  |  |  |

**Table 2:** Anti-fungal Activity of Fluconozole and methanolic extracts of root and leaf of the Rauvolfia micrantha against fungal Strain- *Canadia Albicans*

In contrast, at lower dosages of 25 µg and 50 µg, the methanolic extract derived from the root of Rauvolfia micrantha does not show any appreciable inhibitory action on *Candida albicans*. But at higher dosages of 75 µg and 100 µg, a significant inhibitory zone appears, with dimensions of 11 mm and 14 mm, respectively. This suggests that the root extract might have antifungal activities, although they would only be noticeable at high concentrations.

Similarly, at lesser concentrations of 25 µg and 50 µg, the methanolic extract obtained from the leaf of Rauvolfia micrantha exhibits no inhibitory action. However, at 75 µg and 100 µg dosages, a sizable 9 mm and 11 mm zone of inhibition appears. This implies that, especially at higher doses, the root extract may have more potent antifungal properties than the leaf extract as shown in Figure 3.

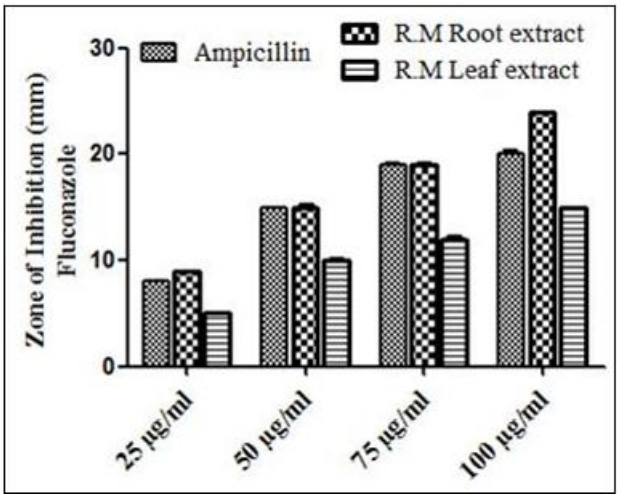



**Figure 3:** Anti-fungal Activity of Fluconozole and methanolic extracts of root and leaf of the Rauvolfia micrantha against fungal Strain- *Canadia Albicans*

### 3.3 Minimum inhibitory concentration

Following a 24-hour compound treatment, the tubes were examined, and the findings were recorded. Standard: Ampicillin (Concentrations 5, 10, 25, 50, 100 and 200µg/ml). Minimum inhibitory concentration of methanolic extract of the plant is carried out for bacterial species including *E coli* and *Staphylococcus aureus* using ampicillin as a standard antimicrobial. The study was conducted in nutrient broth using various concentrations of test compounds by incubating the samples and standard overnight at $37^0$ C temperatures.

The Figure 4 illustrates the susceptibility of *Staphylococcus aureus* and *Escherichia coli* bacterial strains to both Ampicillin and the methanolic extract derived from *Rauvolfia micrantha* Root at various concentrations (µg/ml). Concerning *Staphylococcus aureus*, the vulnerability to Ampicillin declines as the concentration escalates, witnessing a reduction in the minimum inhibitory concentration (MIC) from 0.267 µg/ml at 5 µg/ml to 0.348 µg/ml at 200 µg/ml. Conversely, the methanolic extract obtained from *Rauvolfia micrantha* Root exhibits diverse susceptibility levels, displaying MIC values ranging from 0.342 µg/ml at 5 µg/ml to 0.368 µg/ml at 200 µg/ml.

Likewise, for *Escherichia coli*, susceptibility to Ampicillin wanes as concentrations increase, with the MIC decreasing from 0.274 µg/ml at 5 µg/ml to 0.113 µg/ml at 200 µg/ml. In contrast, the methanolic extract sourced from *Rauvolfia micrantha* Root showcases a variable susceptibility pattern, with the MIC ranging from 0.285 µg/ml at 5 µg/ml to 0.146 µg/ml at 200 µg/ml.

In *Staphylococcus aureus*, the vulnerability to Ampicillin decreases as concentrations rise, with a decline in the minimum inhibitory concentration (MIC) from 0.299 µg/ml at 5 µg/ml to 0.140 µg/ml at 200 µg/ml. Conversely, the

methanolic extract from *Rauvolfia micrantha* leaf shows varying susceptibility, ranging from 0.344 µg/ml at 5 µg/ml to 0.162 µg/ml at 200 µg/ml.

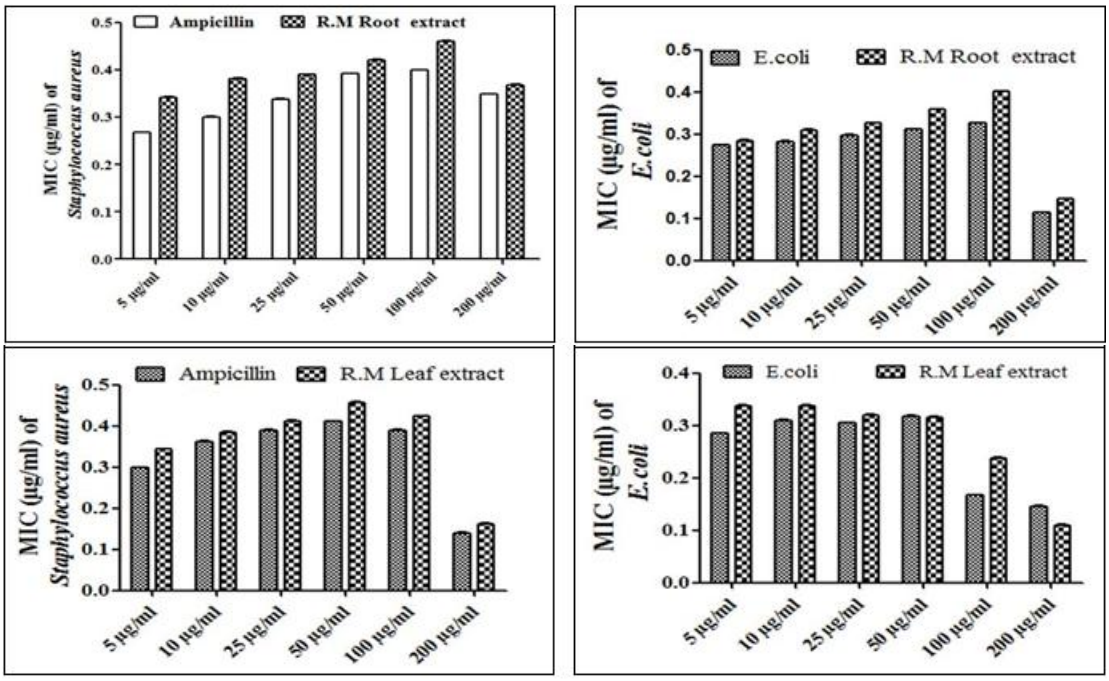



**Figure 4:** MIC studies for methanolic extract of root and leaf of Rauvolfia micrantha

Similarly, for *Escherichia coli*, susceptibility to Ampicillin declines with increasing concentrations, with MIC decreasing from 0.285 µg/ml at 5 µg/ml to 0.146 µg/ml at 200 µg/ml. Conversely, the methanolic extract sourced from *Rauvolfia micrantha* leaf exhibits diverse susceptibility patterns, with MIC values ranging from 0.338 µg/ml at 5 µg/ml to 0.110 µg/ml at 200 µg/ml.

## 4. Discussion

Numerous research have been conducted on the antioxidant activity and radical scavenging of various herbs and plants with medicinal benefits in an effort to identify medicinal plants as a natural source of antioxidants (AL, 2006; Dragland.S, 2003). Synthetic medications are not only costly and ineffective in underdeveloped nations for treating illnesses, but they also frequently include negative effects and adulterations. In order to control microbial infections, new infection-fighting techniques must be found. R. micrantha is used as a substitute for R. serpentina in traditional Indian medicine, specifically in the Kerala region, to treat various nerve ailments. Rauwolfia has a wide range of phytochemicals, including alcohols, steroids, glycosides, flavonoids, fatty acids, tannins, phytosterols, oleoresins, and alkaloids. More than fifty of the plant's indole alkaloids have been identified, making them the most significant alkaloids present in the plant (Verma KC, 2010). Indole alkaloids can be found in all sections of the plant, including the stem and leaves, although they are most concentrated in the root bark. Reserpine is a key alkaloid in the plant. The root contains the largest reserpine content, while the stems and leaves have lower levels (Ruyter CM, 1991).

The study examines the antimicrobial and anti-fungal properties of the root and leaf methanolic extraction of R. micrantha. In summary, the effectiveness of methanolic extract derived from Rauvolfia micrantha leaves varies, suggesting that it may be a viable source of antimicrobial drugs, whereas ampicillin consistently shows inhibitory effects across bacterial strains. At all tested concentrations shown in millimeters, the methanolic extract of Rauvolfia micrantha root did show a strong inhibitory effect. But none of the tested doses, shown in millimeters, show any discernible inhibitory effects from the methanolic extract of Rauvolfia micrantha leaves. We discovered that root extract exhibited a good inhibitory pattern when compared to the conventional medication Amphicilin. The same pattern was observed among both positive and negative microorganisms (R´ıos, 1988). In comparison to the leaf extract, the root extract demonstrated greater inhibition. We also investigated the anti-fungal activity of root and leaf extracts in comparison to the standard medication fluconazole. In root extraction, we discovered extremely good inhibition comparable to antifungal drugs. Leaf extraction exhibited only mild inhibition.

Due to the ineffectiveness of current medical treatments for treating bacterial infections in patients, it is necessary to carefully choose antibiotics based on a number of factors, including microbiological ones, in addition to aggressively searching for novel therapeutic approaches (Fukai, 2004). The term "minimum inhibitory concentration" refers to the in vitro thresholds at which particular bacterial strains become susceptible or resistant to an antibiotic (Krochmal, 2021). When compared to typical anti-microbial and anti-fungal medications, we found that root extract had significantly better inhibition than leaf extract.

## 5. Conclusion

Given the enormous number of therapeutic plants that have not yet been chosen and their phytochemical compositions

thoroughly studied, the future of medicinal plants is bright. The development of new diseases and pathogens that require alternative or complementary medicine will have an impact on medicinal plants' role in medicine in the future (A. S. Kutama, 2015). In this post genomic era, there is a need to develop safe, natural substances due to the growing concern in the medical industry regarding the spread of antibiotic resistance by microorganisms (M. M. A. El-Ghani, 2016). Given the enormous number of therapeutic plants that have not yet been chosen and their phytochemical compositions thoroughly studied, the future of medicinal plants is bright. Furthermore, creative research into novel natural bioactive compounds is required in order to create chemical libraries that will be helpful in the pipelines for drug development and discovery.

## References

[1] A. S. Kutama, I. I. D., W. Aisha, M. I. Auyo, U. Sharif et al., . (2015). “An overview of plant resources and their economic uses in Nigeria, *Global Advanced Research Journal of Agricultural Science, vol. 4, no. 2, pp. 042–067, .*

[2] AL, D. (2006). The antioxidant properties of alcoholic extracts from Sambucus nigra L. *LWT Food Sci. Technol. .*

[3] Anonymous. (1969). Pharmacognosy of Indigenous Drugs. . *Central Council for Research in Indian Medicine and Homoeopathy., (Vol. 1)*.

[4] Dragland.S. (2003). Several culinary medicinal herbs are important sources of dietary antioxidnats *Journal of Nutritation.*

[5] Fukai, T., Oku, Y., Hano, Y., Terada, S., (2004). Antimicrobial activities of hydrophobic 2-arylbenzofurans and an isoflavone against vancomycinresistant enterococci and methicillin-resistant Staphylococcus aureus. *Planta Medica 70,* (685–687.).

[6] Harish, K., Shanmugam Manoharan Sivasankaran, and Shanmugam Manoharan. ((2024)). "Indian Medicinal Plants with Multiple Pharmacological Efficacies: A Comprehensive Review." *Journal of Drug Delivery and Therapeutics 14. 1:*, 143-154.

[7] HW.jr, y. (1954). A pharmacognostical study of Rauwolfia. . *J Am Pharm Assoc Am Pharm Assoc.;, 43(2):70-8. doi: 10.1002/jps.3030430203.*

[8] Jenipher, C., and Muniappan Ayyanar. (2024). "Ethnobotanical Analysis of Medicinal Plants Used by Kani Tribals of Tirunelveli District (Tamil Nadu, India) in Treating Respiratory Diseases." *Journal of Herbal Medicine 43* (100826.).

[9] Koch, I., Kinsoshita, L.S. & Bittrich, R. (2007). Taxonomy novelties in Rauvolfia (Apocynaceae, Rauvolfiodeae) *from Brazil, Novon, 17: 462-417.*

[10] Krochmal, R. D.-W. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, . *Clinical Relevance.Pathogens 10, 165.*

[11] M. M. A. El-Ghani. (2016). “Traditional medicinal plants of Nigeria: an overview,” *Agriculture and Biology Journal of North America, vol. 7,* (no. 5, pp. 220–247,).

[12] Malabadi, R. B., et al. (2024). "Cannabis sativa: A therapeutic medicinal plant-global marketing updates. *World Journal of Biology Pharmacy and Health Sciences 17.2* (170-183.).

[13] Malabadi, R. B., et al. (2024). "Cannabis sativa: A therapeutic medicinal plant-global marketing updates." *World Journal of Biology Pharmacy and Health Sciences 17.2: 170-183.*

[14] R´ıos, J. L., Recio, M.C., Villar, A., (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of Ethnopharmacology 23,* (127–149.).

[15] Rajesham P.1, R. D. B. (2023). Optimising Plantlet Regeneration by Stem Node Explant Culture in Rauvolfia Micrantha Hook. F, A Vital and Endangered Medicinal Plant. *International Journal of Science and Research (IJSR)*

[16] Rajesham. P, R. D. B. (2023). Intense Plantlet Regeneration through shoot-tip explants of Rauvolfia micrantha Hook. F. An Important Medicinal Endangered Plant. *International Journal of Biotechnology Research Vol. 7(1), pp.* , 001-007, December,

[17] Ruyter CM, A. M., Illahi I, Stöckigt J. (1991). Investigation of the alkaloid content of Rauwolfia serpentina roots from regenerated plants. *Planta Med. 57(4):328–330.*

[18] Sahu, B. N. (1979). Taxonomy of Indian species-Rauvolfia serpentina. . *Today and Tomorrow’s Printers and Publishers, New Delhi, Vol II: pp. 70-71.*

[19] Verma KC, V. S. (2010). Alkaloids analysis in root and leaf fractions of sarpaghanda (Rauwolfia serpentina) *Agric Sci Dig., 30(2):133–135.*