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Evaluation of the antioxidant and antibacterial activity of Ruellia simplex extract

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Abstract
The increasing fascination in potent nutraceuticals has prompted scientists and breeders to investigate the possibility of decorative plants as surrogate reservoirs of valuable biological compounds. Ornamental plants, such as flowers, leaves, and fruits, are not only architecturally pleasing and employed for landscaping, but also provide a diverse array of phytochemicals that can be harnessed for several practical uses. Ruellia simplex L, sometimes referred to as the Mexican petunia plant, is found to southern India. In current study, an evaluation was conducted to examine the antibacterial effectiveness of crude methanolic extracts of R. simplex L. leaves by assessing their antioxidant activities. A maceration process was used to extract the dried leaves of R. simplex L. The phytochemical analysis detected the existence of flavonoids, triterpenoids, carbohydrates, alkaloids, and phenolic bioactive substances. These findings were further corroborated by FTIR spectroscopy, which revealed the specific functional groups present in the characteristic phenols and flavonoids. An antioxidant investigation conducted in vitro shown that the crude methanolic extracts exhibited significant free radical scavenging activity, with an IC50 value of 2.08 µg/mL. This antibacterial investigation shown significant efficacy against Staphylococcus aureus (MTCC-96; strain-6571), Klebsiella pneumoniae (MTCC-39; strain-418), and Escherichia coli (MTCC 119; strain-9483). Significantly, there have been no previous reports of such a study for R.simplex. Hence, it might be regarded as a treatment for certain pathological conditions.

Keywords: Antioxidant, IC50, Ornamental plants, R. simplex L.

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1. INTRODUCTION

Acanthaceae includes several dicotyledonous herbs, shrubs, and vines (Hughes and Lev-Yadun, 2023). These plants are epiphytes that only produce blooms. This family has 4300 species in 346 genera, making it one of the top 12 most diversified flowering plant families (Ibrahim et al., 2023). Its principal distribution facilities are in numerous countries, including Indonesia, Malaysia, Africa, Brazil, Central America, and Asia. However, this family's systematic position and circumscription have been debated. This is due to its wide ecological range and genetic and morphological diversity. Acanthaceae have been categorized at the familial, sub-familial, tribe, and sub-tribal levels by many scholars (Ajuru et al., 2022). These classifications use pollen morphology. The Acanthaceae family is useful for curing several deadly diseases. These chemicals contain secondary metabolites such alkaloids, phenols, terpenoids, tannins, quinones, cardiac glycosides, saponins, carbohydrates, flavonoids, and proteins. These secondary metabolites have several medical uses. These compounds have anti-inflammatory, antiviral, antioxidant, and anti-diabetic properties. Acanthaceae family members have been used to treat respiratory problems, as well as antiviral, antipyretic, and anti-asthmatic qualities (Khan et al., 2017).

Numerous flowering plant species in the Ruellia genus are beautiful and therapeutic. Many Ruellia L species have been studied for their antibacterial properties. Bioactive substances such alkaloids, flavonoids, and phenolics give Ruellia extracts antibacterial properties. Bacteria, fungi, and viruses are inhibited by these chemicals. Ruellia species are very effective against Staphylococcus aureus and Escherichia coli. Ruellia extracts may also fight fungal infections due to their antifungal characteristics. These bioactive chemicals may damage microbial cell membranes, disrupt critical biological processes, or improve antimicrobial activity synergistically, according to studies. Ruellia's antibacterial potential is important for traditional medicine and new therapeutics. Finally, Ruellia L species have antibacterial capabilities against a variety of microbes. Ruellia's bioactive chemicals and mechanisms may help create novel antibiotics or herbal therapies for infectious disorders. Due to their potential traditional uses, Ruellia, an Acanthaceae plant, has been extensively studied. Pharmacological activities in these three taxa have also been thoroughly described.

The genus Ruellia L holds considerable therapeutic significance (Wangia, 2021). The collection comprises 250 distinct and widely recognized species of decorative plants, often referred to as Ruellias. These plants are found in tropical and temperate climates worldwide. The genus has numerous species that possess significant ingredients such as glycosides, flavonoids, alkaloids, and triterpenoids. These constituents have valuable use in the synthesis of diverse medicines. Plant extracts from the Ruellia genus have a range of beneficial qualities, including anti-hypertensive, antinociceptive, analgesic, antispasmolytic, antioxidant, antiulcer, antidiabetic, antipyretic, and anti-inflammatory effects.

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Based on the survey described before, we have put forth the current study to assess the antioxidant and antibacterial properties of Ruellia simplex extract.

2. METHODOLOGY

2.1 Material

2.1.1 Plant material collection and validation: Plant material was collected from MDU in Rohtak. The plant sample was verified by the Department of Botany, MDU, Rohtak.

Figure: *Ruellia simplex image* from botanical garden MDU, Rohtak

2.1.2 Microbes sample collection

Staphylococcus aureus (MTCC-96; strain-6571); Klebsiella pneumoniae (MTCC-39; strain-418), and Escherichia coli (MTCC 119; strain-9483) were collected from Microbial Type Culture Collection, housed at the Institute of Microbial Technology (IMTECH), Chandigarh.

2.2 Phytological Screening

Organoleptic Studies: Evaluation of the the leaf's border, apex, base surface, venation, and inflorescence were done according to Kakkar et al. (2023).

Macroscopic and microscopic assessment: It was done similar to method described by Pal et al. (2022).

Powder Microscopy: It was done similar to method described by Kakkar et al. (2023), by utilising plant powder.

Total Ash: The 2-4g sample was heated in a silica crucible to a thin layer. The temperature was slowly raised to 500-600°C until the material was white and carbon-free. The crucible was cooled and the material's weight was recorded. It was then wetted with 2ml of water or saturated ammonium nitrate (if the ash contains carbon). Drying in a water bath and hot plate and igniting

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again stabilized the sample's weight. The treated material was cooled in a desiccator for 30 minutes, weighed correctly, and ash content was measured accordingly. (Kokate et al. 1991)

$$\% \text{ Ash} = \left(\frac{\text{Mash}}{\text{Mdry}}\right) \times 100$$

Mash= mass of ash sample; Mdry= mass of dry sample

Foreign matter determination*:* Contamination, foreign debris, and discolouration that could damage plant components was checked. (Chase and Pratt,1949 and Kokate et al., 1991)

% Foreign Organic Matter = (Organic matter weight/Total weight) ×100

Measurement of moisture*:* Plant samples were properly dried to assess water content (%). The plant sample were weighed and dried at 239º F (115ºC) to a consistent state. Dry weight of plant sample was recorded again after cooling. Final moisture content was estimated using this numerical equation (Chase and Pratt, 1949).

% W=((A-B)/B)×100

Where W is the sample's moisture weight in %.

Wet sample weight: g; dry sample weight: g

2.3 Phytochemical study of plant extracts

Plant extraction preparation: The crude methanol extract was made by soaking 10 g of dried plant powder (leaves) in 100 ml of methanol on a rotary shaker for 24 hours. After filtering and centrifuging at 5000 g for 15 minutes, the extract was dried under reduced pressure. The crude methanol extract yields 20.93% of the dry material. Extract was stored in sealed bottles at 4ºC. (Parekh and Chanda, 2007)

Phytochemical screening

Carbohydrate Test: The Molisch test: Filtrate was treated with α-naphthol solution and a few drops of concentrated H2SO4 added to the test tube. At the intersection of two layers, a reddish violet ring indicates carbohydrate.

Protein test: Ninhydrin test: Mixing Ninhydrin reagent with filtrate and heating it was the process. Blue or purple indicates amino acids.

Test for alkaloids: Wagner's test: Potassium iodide and iodine were added to the filtrate. Alkaloids were confirmed by brown precipitate.

The Hager test: Hager's reagent was added to the filtrate. Alkaloids were confirmed by yellow precipitate.

Saponin detection Foam test: In the experiment, extract was mixed with 1 ml of alcohol and diluted with distilled water. The liquid was vigorously agitated in a graduated cylinder for 15 minutes. Saponins are indicated by surface foam.

Test for tannins: The filtrate was mixed with a little 0.1% ferric chloride. A brownish-green or blue-black color indicates tannins.

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Test for phenols: Filtrate was be treated with 10% ethanolic ferric chloride. Phenols were confirmed by the hue change to deep blue or blue-green (Sapunyo et al. 2023).
Flavonoids test: After treating the filtrates with 5ml of 1:1 diluted ammonia solution, concentrated H2SO4 was added. Yellow tints indicated flavonoids and fade over time
Terpenoids test: The test tubes received 5 ml of filtrates and 3 ml of concentrated H2SO4. Reddish-brown hues indicated terpenoids.
Detection of fixed oils and fats Spot test: The experiment consisted of putting modest amounts of petroleum ether and benzene extracts onto two filter sheets. The presence of solidified oil is indicated by the formation of oil stains above the filter paper.

2.4 Quantitative analysis of plant extract: FTIR was utilized for quantitative analysis of plant extract **(Abraham et al., 2018; Gore et al 2023).**

2.5 Evaluation of antimicrobial activity

According to Nortjie et al. (2022), antimicrobial activity was assessed. Three pathogens were inoculated on Mueller-Hinton agar (MHA) medium for a disc diffusion test to determine antibacterial activity. After centrifuging fresh isolate cultures at 8000 rpm for 15 minutes, the supernatants were removed. Each isolate was placed on blank discs with 40 µl of supernatant. *Staphylococcus aureus* (MTCC-6538), *Klebsiella pneumoniae* (K36), E. coli (MTCC 29181) and -inoculated MHA medium. After incubating all agar medium at 37°C for 24 hours, pathogen growth inhibition zones and isolated microbe inhibitory activities was assessed.

2.6 Evaluation of antioxidant activity (Rodríguez-Yoldi., 2021)

DPPH Radical Scavenging: The experiment eliminated DPPH radicals with 1,1-diphenyl-2-picrylhydrazyl (DPPH). Carefully poured one millilitre of plant extract in a sterile test tube and added 0.5 millilitres of 0.3 millimolar DPPH in methanol. After stirring, the solution was left at room temperature for 15 minutes in a lightless environment. The negative control was 2.5 ml DPPH and 1 ml ethanol. The positive control used L-ascorbic acid comparable to the extracts. After incubation without light, a spectrophotometer was measured absorbance at 517 nm. Repeated the experiments.

% radical scavenging activity as ((Ac – As))/Ac)×100,

where As is sample absorbance and Ac is control absorbance.

3. RESULT & DISCUSSION

Organoleptic Studies

The organoleptic characteristics, including the leaf structure such as margin, apex, base, surface, venation, and inflorescence, were assessed. The leaves were dark green in color, opposite in arrangement, and lanceolate in shape, measuring approximately 15-30 cm in length and 1-2 cm in width. The veins were prominently visible on the abaxial (lower) surface, and the leaf margins were either undulating.

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Microscopic and macroscopic evaluation: The epidermal cells were rectangular to polygonal in shape, with a thickened outer wall. Stomata were predominantly of the anomocytic type, scattered across the leaf surface, particularly on the abaxial side. The vascular bundles in the midrib are collateral, with xylem vessels arranged in a radial pattern. Additionally, the mesophyll is differentiated into a palisade and spongy parenchyma, reflecting the typical dicotyledonous leaf structure. Trichomes were sparse but present, primarily on the leaf margins and stem, contributing to the plant's slightly rough texture. These microscopic characteristics, combined with the macroscopic observations, aid in the accurate identification and understanding of *Ruellia simplex*.
Powder Microscopy The powdered sample shows polygonal to rectangular epidermal cell fragments with thicker walls. Anomocytic stomata, where guard cells were surrounded by a variety of subsidiary cells without a clear pattern, are distinctive. Powder containsed trichomes, typically non-glandular, unbranched, and vary in length, giving the plant a slightly gritty appearance. Xylem vessels—elongated, thick-walled cells with a lignified structure—indicated vascular tissue. Scalariform or spiral thickening patterns were common in these vessels, depending on magnification. Mesophyll tissue segments highlighted the difference between dicotyledonous plants' palisade and spongy parenchyma. Numerous calcium oxalate crystals in needle-like raphides or prismatic crystals, were seen which help identify Ruellia simplex.

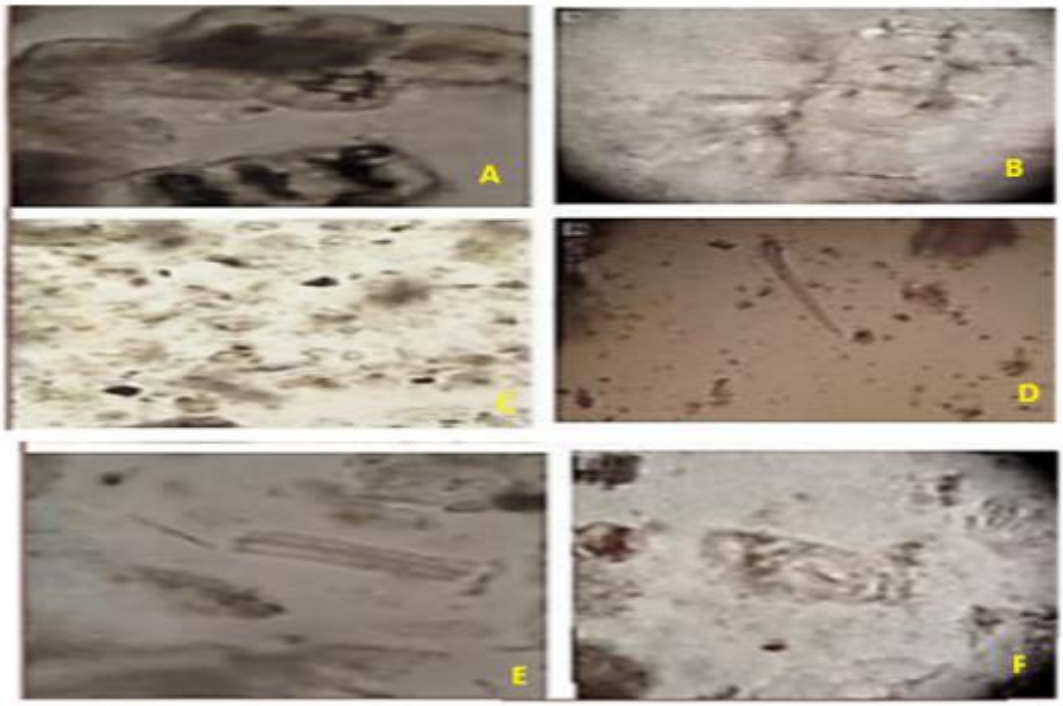

Figure: Powder Microscopy depicting A) Vascular tissue B) Epidermal cells C) Calcium oxalate crystals D) Unicellular trichome E) Conducting elements F) Stomata

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Ash Content: The result of the ash content measurement in *Ruellia simplex* provides quantitative data on the inorganic residue present in the plant material. Typically, the total ash content is expressed as a percentage of the dry weight of the sample. This percentage helps assess the quality and purity of the plant material. In the current study the ash content of *Ruellia simplex* typically found to be 1.35 %.

Foreign matter: Medicinal plant components must be free of mold, bug, and animal waste contamination. No odd scent, color change, sliminess, or decomposition should be present. In this investigation, foreign matter was measured at 1.15 ± 0.10.

Moisture Content: The ethnomedicinal plant samples were carefully dried to evaluate their water content in percentage. High-quality plants have minimal moisture. The study indicated that plant has a moisture content of 0.02 ± 0.01, demonstrating its high quality.

Phytochemical Screening

The present investigation involved an initial analysis of the chemical components in the leaf extract. The results indicated the presence of alkaloids, tannins, saponins, flavonoids, triterpene.

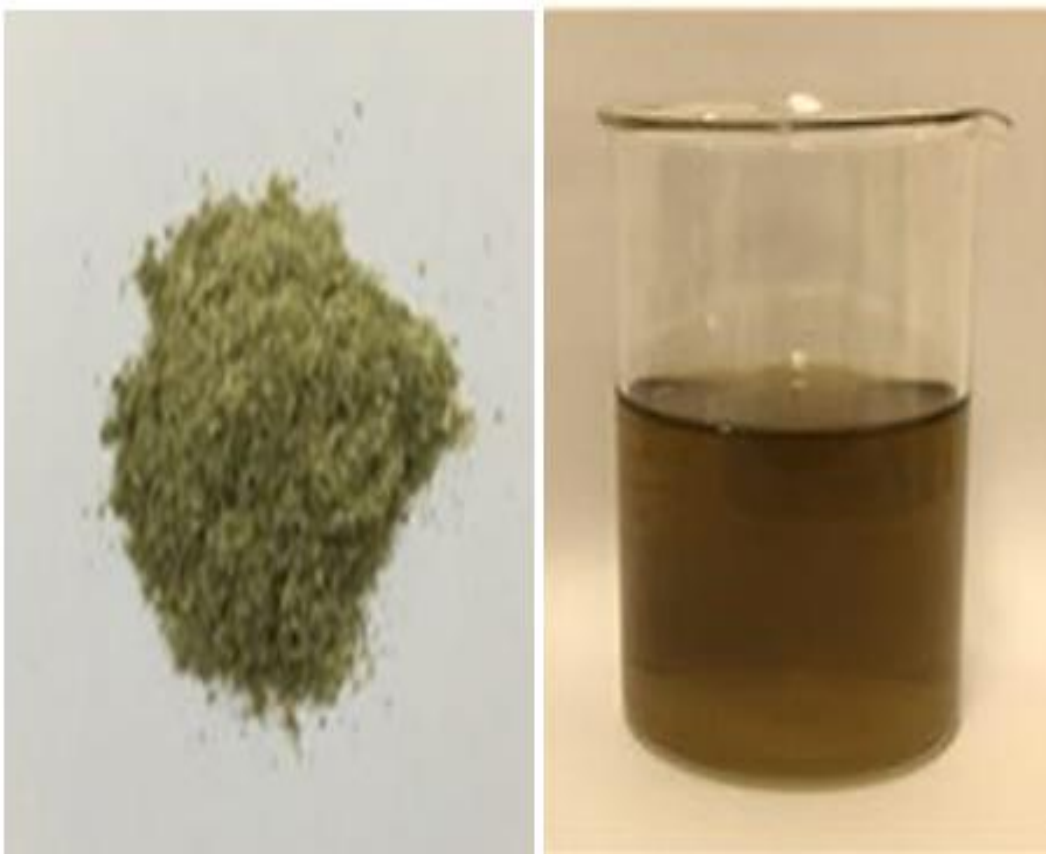

Figure: Powder of *Ruellia simplex* and its Leaf Extract

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Table: Preliminary phytochemical analysis of *R.simplex*. extract

S.No.	Constituents	Test	Methanol Extract
1.	Carbohydrate	Molisch Test	+
2.	Protein	Ninhydrin test	--
3.	Alkaloids	Wagner's test	++
4.	Saponins	Foam Test	+
5.	Tannins	Ferric Chloride	+
6.	Phenols	Ethanolic Ferric Chloride	+
7.	Flavonoids	Conc. H_2SO_4	++
8.	Terpenoids	Conc. H_2SO_4	+
9.	Fats and oils	Fat Spot Test	++

$^{+}$ shows traces present; $^{++}$ shows abundance; - shows absent

The qualitative examination of the extracts showed the presence of carbohydrates, proteins, oils, and lipids. Additionally, active secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, and total phenols were detected using established methods. Phytochemical screening serves the purpose of identifying the components present in plant extracts, determining the most abundant one, and also exploring for biologically active substances that can be utilized in the production of beneficial medications. They constitute an integral component of the plant's immune system, safeguarding it against viral, bacterial, fungal, and parasitic threats. Phytochemicals can provide humans with similar protective benefits. Phytochemical screening methods are used to identify and authenticate raw and final herbal medicines. These approaches help detect the specific components and properties that are unique to each species. Methanolic extracts used for the study exhibited remarkable favorable phytochemical outcomes, which were verified by a considerable alteration in color. Previous studies of Ruellia species have exhibited highest phenolic content as well as highest flavonoid content (57.8%) Ukwubile et al., 2023

FTIR Analysis

The FTIR (Fourier Transform Infrared Spectroscopy) analysis of *Ruellia simplex* primarily focuses on identifying the functional groups present in its chemical constituents. This analysis is significant

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for understanding the plant's potential medicinal properties and chemical composition. In the current study, the FTIR spectrum of *Ruellia simplex* revealed several key absorption peaks corresponding to different functional groups. **O-H Stretching (around 3400 cm^{-1})**-This broad peak indicates the presence of hydroxyl groups, likely due to the presence of phenolic compounds, which are common in plant extracts. **C-H Stretching (around 2900 cm^{-1})-** These peaks are associated with the aliphatic C-H bonds, indicating the presence of fatty acids and other hydrocarbon chains. **C=O Stretching (around 1700 cm^{-1})**-This sharp peak is indicative of carbonyl groups, which suggests the presence of esters, aldehydes, or carboxylic acids—common in fatty acids and flavonoids. **C=C Stretching (around 1600 cm^{-1})-**This region corresponds to the aromatic C=C bonds, which are indicative of the aromatic rings found in many polyphenols and flavonoids. **C-O Stretching (around 1200-1300 cm^{-1})-** The presence of these peaks suggests the existence of alcohols, ethers, or esters, further supporting the identification of complex organic molecules like glycosides or polysaccharides. Previous studies conducted by Ukwubile et al., 2023, also exhibited the similar wavelengths.

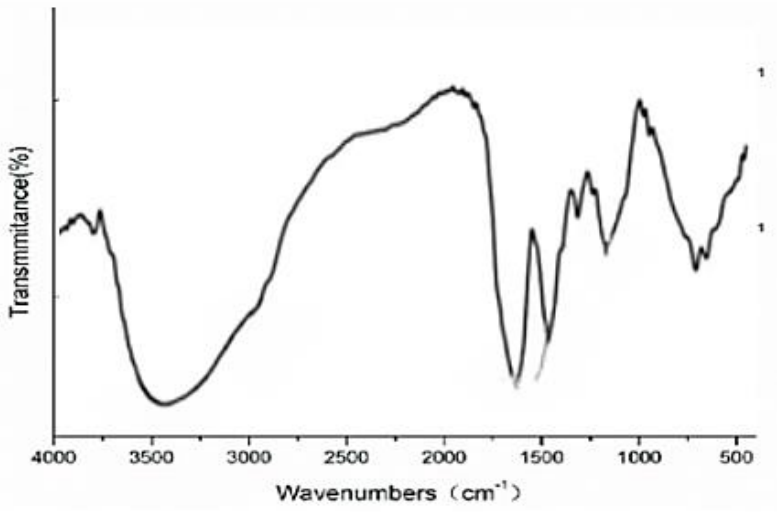

Figure: FTIR Spectra of Plant Extract

Antioxidant activity

In vitro DPPH scavenging activities

The DPPH radical scavenging technique is frequently employed to assess the antioxidant effectiveness of different compounds over a period of time. This approach entails evaluating the capacity of molecules to scavenge and diminish DPPH free radicals in a controlled laboratory setting. The outcome of DPPH radical scavenging is a transition in hue from blue to yellow, which can be quantified at a wavelength of 517 nm. The current investigation found that the methanolic

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extract of leaves from R.simplex L. f. demonstrated significant in vitro DPPH radical scavenging action, which increased with higher doses. Nevertheless, the DPPH radical scavenging activity of the conventional ascorbic acid was markedly superior to that of the R.simplex L. f. leaves extract.

Table: *In vitro* DPPH scavenging activities of *R.simplex L. f.*

Conc (µg/ml)	**Ascorbic Acid**	***R.simplex* Leaf extract**
100	57.09 ±0.02	42.61 ±0.03
200	68.80±0.02	51.54±0.01
300	75.07 ±0.03	60.01 ±0.02
400	81.01 ±0.01	68.99 ±0.03
500	82.43 ±0.03	76.05 ±0.03
600	87.02 ±0.01	79.00 ±0.02
IC_{50}	2.65	2.08

The values are expressed as mean ± SEM. (P > 0.05, one-way ANOVA)

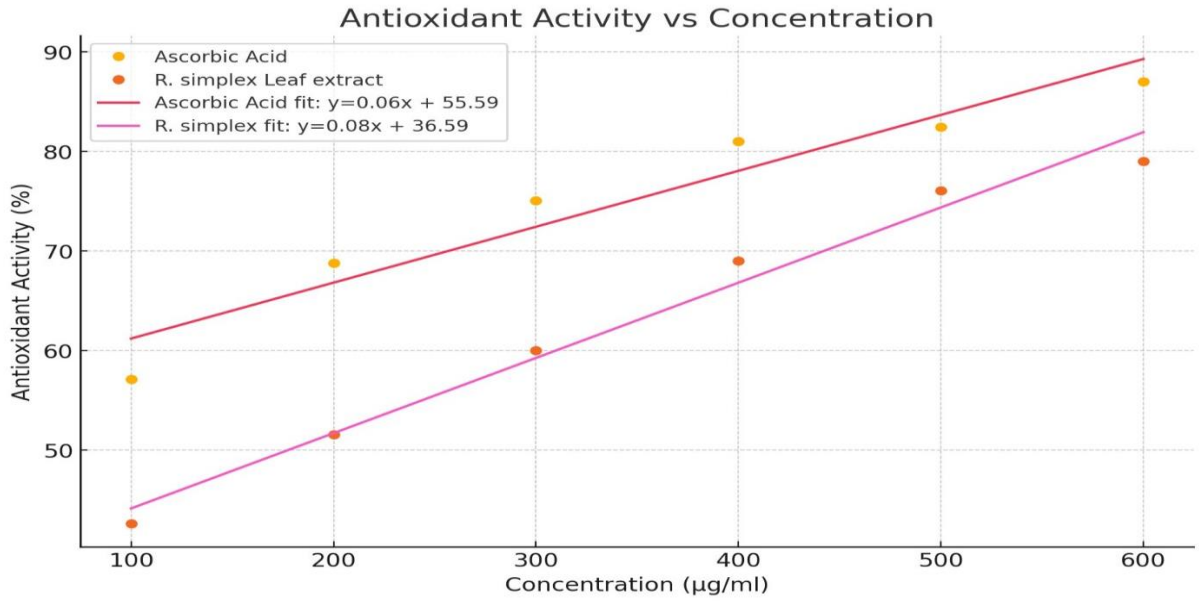

Figure: Graph showing the antioxidant activity of Ascorbic Acid and *R. simplex* Leaf extract at different concentrations, along with their corresponding linear regression lines.

***In-vitro* antimicrobial activity**

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The frequent utilization of these drugs by humans has resulted in the emergence of drug resistance in human infections, posing significant challenges for researchers in devising secure and effective treatments for infectious diseases. Multiple research teams have conducted studies on the antibacterial and radical-scavenging characteristics of plant extracts, revealing their strong effectiveness in combating bacteria and scavenging radicals, without any adverse side effects. The prior research indicated that the leaves. The present study found that the plant formulations had remarkable efficacy against *Staphylococcus aureus* (MTCC-96), *Klebsiella pneumoniae* (MTCC-39), and *E. coli* (MTCC-119), with an increase in inhibitory zones that was dependent on the dosage. The methanolic extract exhibited a dose-dependent increase in the inhibitory zone, ranging from 9.09 to 16.05 mm for E. Coli and 7.88 to 17 mm for Staphylococcus aureus. Klebsiella pneumoniae showed the highest level of inhibition within the range of 8.65 to 17.5 mm.

Table: Growth inhibition zone diameter (mm) of pathogens at various concentrations of leaf extract of *R.simplex*

	Zone of Inhibition in (mm)at Various Concentrations of R.simplex (ug/ml)			
Bacterial Species	**300**	**600**	**900**	**Standard**
Staphylococcus aureus (MTCC-96)	9.98	14.07	14.10	19.24
Klebsiella pneumoniae (MTCC-39)	9.23	15.60	16.12	20.77
E.coli (MTCC-119)	11.17	13	13.3	18

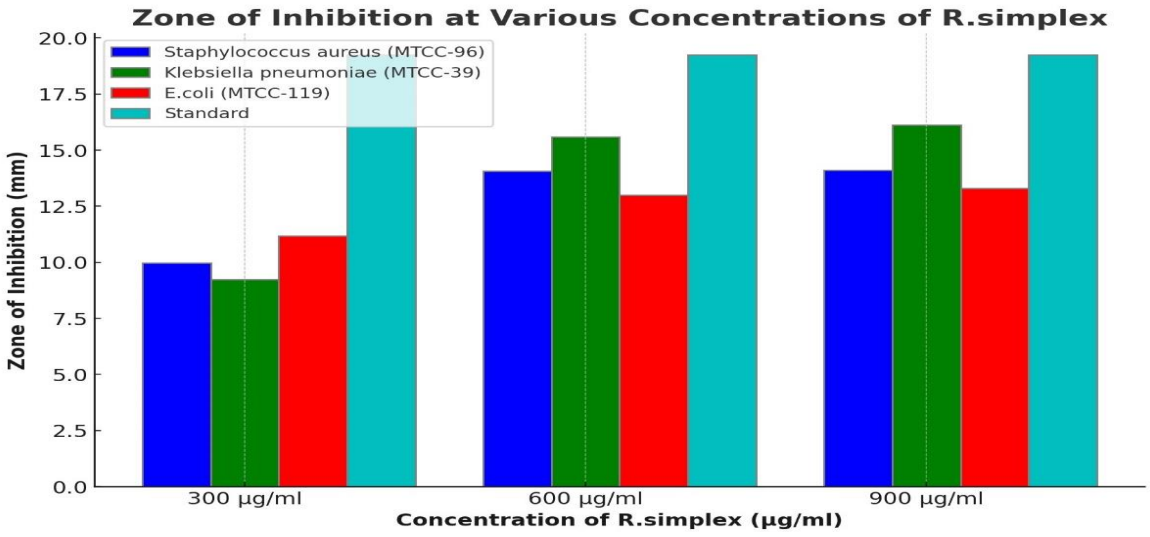

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Figure: Bar graph representing the zone of inhibition (in mm) for different bacterial species (with their respective strain numbers) at various concentrations of *R.simplex* (in µg/ml), compared to the standard. This visualization should help you analyze how each bacterial species responds to the different concentrations.

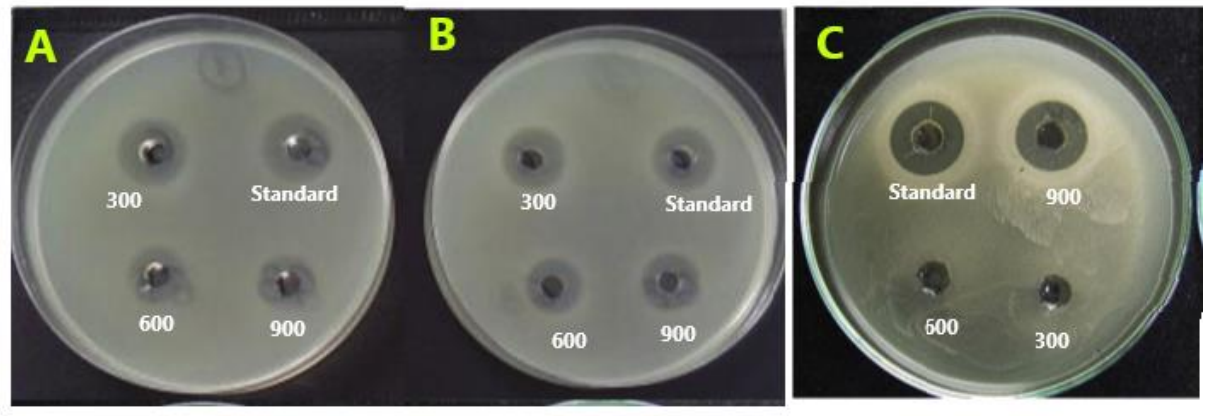

Figure: Figure representing the zone of inhibition (in mm) for different bacterial species (A- *E.coli*; B: *K.pneumoniae*, C: *S.aureus*) at various concentrations of *R.simplex* (in µg/ml), compared to the standard.

CONCLUSION

Science and breeders are studying beautiful plants as surrogate reservoirs of vital biological substances due to the increased interest in potent nutraceuticals. Flowers, leaves, and fruits are lovely and utilized for landscaping, but they also contain phytochemicals with many uses. Mexican petunia Ruellia simplex L grows in southern India. This study examined crude methanolic R. simplex L. leaf extracts for antibacterial efficacy using antioxidant activity. Dry R. simplex L. leaves were macerated. Flavonoids, triterpenoids, sugars, alkaloids, and phenolics were phytochemically identified. These findings were supported by FTIR spectroscopy showing phenol and flavonoids' functional groups. In an in vitro antioxidant research, the crude methanolic extracts demonstrated significant free radical scavenging activity (IC50 = 2.08 µg/mL). Staphylococcus aureus, Klebsiella pneumoniae, and E. coli were stopped. Interestingly, no R.simplex study has been recorded. Therefore, present ornamental plant have significant relevance in medicine, as it contains bioactive compounds that might be utilized in traditional and modern medicine.

REFERENCES

Abraham, A., Samuel, S., & Mathew, L. (2018). Pharmacognostic evaluation of *Curcuma longa* L. rhizome and standardization of its formulation by HPLC using curcumin as marker. *Int. J. Pharmacogn. Phytochem. Res*, *10*, 38-42.

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open access journal
Impact Factor: 8.3 www.ijesh.com ISSN: 2250 3552

Chase Jr, C. R., & Pratt, R. (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *Journal of the American Pharmaceutical Association*, *38*(6), 324-331.

Gore, D. D., Ahmad, F., Tikoo, K., Bansal, A. K., Kumar, D., & Singh, I. P. (2023). Comparative quantitative analysis of fruit oil from *Hippophae rhamnoides* (seabuckthorn) by qNMR, FTIR and GC–MS. *Chinese Herbal Medicines*, *15*(4), 607-613.

Hughes, N. M., & Lev-Yadun, S. (2023). Why do some plants have leaves with red or purple undersides. *Environmental and Experimental Botany*, *205*, 105126

Ibrahim, N. D., Seow, L. J., Sekar, M., Rani, N. N. I. M., & Lum, P. T. (2022). Ten commonly available medicinal plants in Malaysia with potential sun protection factor and antioxidant properties–A review. *Pharmacognosy Journal*, *14*(2).

Kakkar, R. A., Haneen, M. A., Parida, A. C., & Sharma, G. (2023). The known, unknown, and the intriguing about members of a critically endangered traditional medicinal plant genus Aconitum. *Frontiers in Plant Science*, *14*, 1139215.

Khan, I., Jan, S. A., Shinwari, Z. K., Ali, M., Khan, Y., & Kumar, T. (2017). Ethnobotany and medicinal uses of folklore medicinal plants belonging to family Acanthaceae: An updated review. *MOJ Biol Med*, *1*(2), 34-38.

Kokate, C. K. (1991). Practical Pharmacognosy. 3^< rd> ed. New Delhi. *VPBN*, *3*, 107-111.

Melk, M. M., & El-Sayed, A. F. (2024). Phytochemical profiling, antiviral activities, molecular docking, and dynamic simulations of selected Ruellia species extracts. *Scientific Reports*, *14*(1), 15381.

Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. *Plants*, *11*(15), 2011.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, *10*(2).

Rodríguez-Yoldi, M. J. (2021). Anti-inflammatory and antioxidant properties of plant extracts. *Antioxidants*, *10*(6), 921.

Samy, M. N., Sugimoto, S., Matsunami, K., Otsuka, H., & Kamel, M. S. (2015). Chemical constituents and biological activities of genus Ruellia. *International Journal of Pharmacognosy*, *2*(6), 270-279.

Sapunyo, W. L., Mbaria, J. M., Kanja, L. W., Omolo, M. J., & Onyancha, J. M. (2023). Phytochemical screening, toxic effects, and antimicrobial activity studies of Digitaria abyssinica (hochst. Ex A. Rich.) Stapf (Poaceae) Rhizome extracts against selected Uropathogenic microorganisms. *Evidence-Based Complementary and Alternative Medicine*, *2023*(1), 4552095.

Ukwubile, C. A., Nettey, H., Malgwi, T. S., & Menkiti, N. D. (2023). Ruellia simplex C. Wright (Acanthaceae): Antinociceptive, anti-inflammatory, and antidiabetic activities of a novel fatty acid isolated from its leaf extract. *International Journal of Plant Based Pharmaceuticals*, *3*(1), 32-40.

Wangia, C. O. A. (2021). *Pharmacological, Chemical, Chromatographic and Spectroscopic Profiles of Extracts and Bioactive Compounds from Selected Kenyan Ruellia Species* (Doctoral dissertation, JKUAT-COHES).