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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY ASSESSMENT OF *WRIGHTIA TINCTORIA* (Roxb.) R.Br

C. S. Sailaja Lecturer in Botany, B T Government Degree College, Madanapalle -517325, Annamayya District, Andhra Pradesh, India
N. Nageseara Rao Lecturer in Botany, Government College (A), Rajamahendravaram- 533105, Andhra Pradesh., India
Y. T. Rajesh Babu Research Scholar, Department of Botany, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India baburajesh0999@gmail.com
J. Ramalakshmana Research Scholar, Department of Botany, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India
S. B. Padal Professor, Department of Botany, Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India

Abstract:
This study evaluates the inhibitory properties of successive extracts from the leaves and bark of *Wrightia tinctoria* for phytochemical constituents and antimicrobial efficacy against four bacteria. The leaves and bark were shade-dried and extracted using methanol, petroleum ether, and water. Qualitative phytochemical analysis revealed the presence of primary and secondary metabolites, including proteins, phenols, tannins, flavonoids, terpenoids, glycosides, saponins, and coumarins. The antimicrobial potential was assessed using the agar well-diffusion method against four pathogenic bacteria: *E. coli*, *Pseudomonas fluorescence*, *Bacillus subtilis*, and *Streptococcus mutans*. Methanol and petroleum ether extracts exhibited significant inhibitory activities, with the highest inhibition observed in *E. coli* from leaf petroleum ether extract (30 mm inhibition zone). These findings suggest the potential of *W. tinctoria* extracts in developing novel phytomedicines.

Keywords:
Antimicrobial activity, Phytochemical analysis, Ethnobotany, Phytomedicine, Bioactivities.

Introduction

Infectious diseases have long posed a significant threat to global health, with pathogens such as bacteria, viruses, fungi, and parasites causing a wide range of illnesses. These diseases, which can spread rapidly through populations, have historically led to widespread morbidity and mortality (Satapathy et al., 2023). The advent of antibiotics and vaccines has significantly reduced the impact of many infectious diseases; however, the emergence of multidrug-resistant (MDR) strains has become a major public health concern. MDR pathogens challenge the efficacy of existing antimicrobial agents, necessitating the exploration of alternative therapeutic options (Fauci & Morens, 2012). Traditional medicine, which encompasses a variety of health practices, knowledge, and beliefs incorporating plant, animal, and mineral-based medicines, has been used for centuries to treat a wide array of ailments, including infectious diseases. Traditional systems such as Ayurveda, Traditional Chinese Medicine (TCM), and indigenous practices across Africa and the Americas rely heavily on natural products derived from medicinal plants. These systems offer a rich repository of bioactive compounds that have evolved to address health conditions within specific cultural contexts (WHO, 2002). The World Health Organization (WHO) has recognized the value of traditional medicine and encourages its integration into modern healthcare systems to enhance primary healthcare delivery and promote holistic health (WHO, 2013).

Medicinal plants have been a cornerstone of traditional medicine and continue to provide a valuable source of new therapeutic agents. Phytochemicals, the bioactive compounds found in plants, exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties (Ramalakshmana et al., 2023; Rajesh et al., 2023). The exploration of plant-derived compounds has led to the discovery of many important drugs, such as quinine from *Cinchona* bark and

artemisinin from *Artemisia annua*, which are used to treat malaria (Newman & Cragg, 2012). The increasing interest in ethnopharmacology—the study of the medicinal use of plants by indigenous people—has further highlighted the potential of medicinal plants in developing novel treatments for infectious diseases, particularly those caused by MDR pathogens (Cowan, 1999). Due to the urgent demand for new antimicrobial drugs to combat multidrug-resistant diseases, scientifically investigating medicinal plants utilized in traditional medicine presents a promising pathway for drug development. These plants often contain complex mixtures of phytochemicals that can work synergistically to enhance therapeutic efficacy and reduce the likelihood of resistance development. By systematically studying these plants, researchers can identify and isolate bioactive compounds, evaluate their mechanisms of action, and assess their potential as lead compounds for new antimicrobial drugs (Atanasov et al., 2015; Sandhya et al., 2023).

Wrightia tinctoria (Roxb.) R.Br, commonly known as the Pala indigo plant or Dyer's oleander, is a small deciduous tree belonging to the family Apocynaceae. This plant is widely distributed across India, Southeast Asia, and Australia and has been traditionally used for its diverse medicinal properties. In various traditional medicine systems, *W. tinctoria* is valued for treating ailments such as psoriasis, jaundice, toothache, snake bites, and gastrointestinal disorders (Raghu et al., 2010). The phytochemical profile of *W. tinctoria* reveals a rich array of bioactive compounds, including alkaloids, flavonoids, terpenoids, phenolics, and glycosides. These phytochemicals are known to exhibit a wide range of pharmacological activities, contributing to the plant's therapeutic potential. Studies have shown that the leaves, bark, and seeds of *W. tinctoria* contain significant amounts of these compounds, which are responsible for the plant's medicinal properties (Jain et al., 2013). For instance, the presence of flavonoids and phenolic acids is associated with strong antioxidant activities, which can mitigate oxidative stress-related diseases (Singh et al., 2011).

Beyond its phytochemical richness, *W. tinctoria* has demonstrated various biological activities, making it a subject of increasing interest in pharmacological research. The plant exhibits potent antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities, among others. Its antimicrobial properties are particularly noteworthy, with studies indicating effectiveness against a range of pathogenic bacteria and fungi, including multidrug-resistant strains (Srivastava, 2014). This broad spectrum of biological activities underscores the potential of *W. tinctoria* as a source of novel therapeutic agents. Given the growing threat of antibiotic resistance, the exploration of traditional medicinal plants like *W. tinctoria* for new antimicrobial agents is timely and crucial. The plant's diverse phytochemical composition and proven biological activities provide a strong foundation for further research into its potential applications in modern medicine. By scientifically validating and harnessing these traditional knowledge systems, we can develop innovative treatments that address current and emerging health challenges. This study aims to provide an up-to-date phytochemical profile of *W. tinctoria* and evaluate its antimicrobial efficacy against MDR bacteria.

Materials and Methods

Plant Material Collection and Preparation

Wrightia tinctoria specimens were collected in December 2023 from the Botany Department garden at Andhra University. Fresh leaves were washed, air-dried in the shade, and ground into a fine powder, which was then stored in a refrigerator for further analysis.

Extraction

The powdered plant materials were subjected to Soxhlet extraction using three solvents: methanol, petroleum ether, and water. Each extract was concentrated using a rotary evaporator and stored at 4°C for subsequent analysis (Harborne 1984).

Phytochemical Analysis

Qualitative phytochemical screening of the extracts was conducted to identify the presence of primary and secondary metabolites, including proteins, phenols, tannins, flavonoids, terpenoids, glycosides, saponins, and coumarins (Stein 1990).

The following formula was used to determine the extractive values:
Yield (%) = W1/ W2 x 100
Where:
W1 = Weight of the extract following solvent evaporation
W2 = the plant sample's dry weight

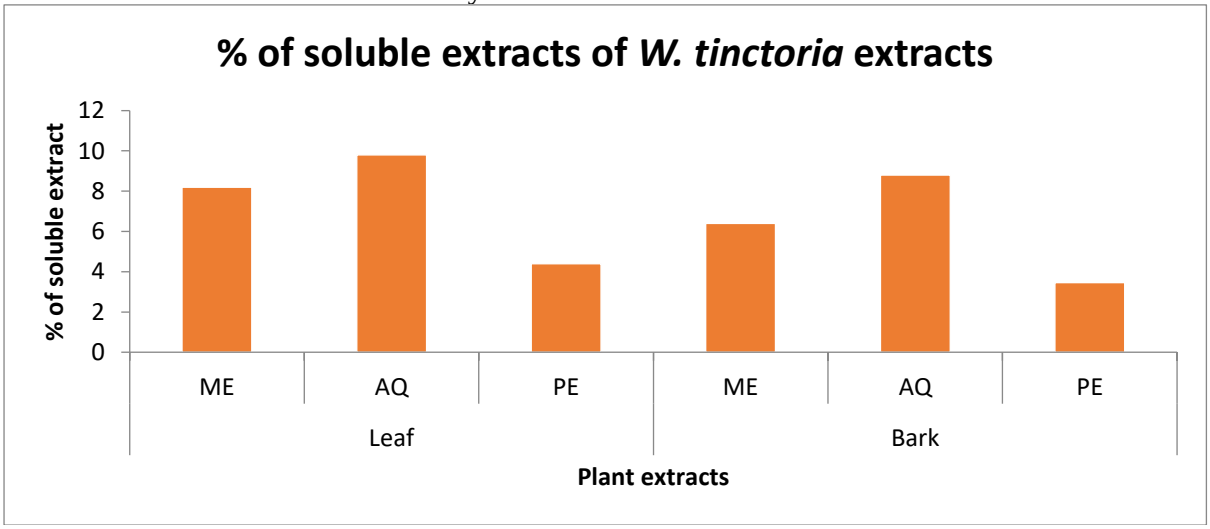
Antimicrobial Activity Screening

The antibacterial activity of the extracts was assessed by using the agar well diffusion method (Bolouiri et al., 2016). For Gram-positive bacteria, the strains include *Bacillus subtilis* (MTCC 28), and *Streptococcus aureus* (MTCC 497), and for Gram-negative bacteria, the strains include *E. coli* (MTCC 42), *Pseudomonas fluorescence* (MTCC 664), all of which were cultured in Muller-Hinton medium at 37 °C for 24 hours with Streptomycin serving as the positive control. The stock solutions of the test compound were prepared in concentrations of 500 mg/mL, 250 mg/mL, and 125 mg/mL with 10% DMSO. The 5mm diameter drilled wells on agar plates were supplied with 20 µL of plant extract. The plates were tested after a 24-hour incubation period at 37°C. To measure the antibacterial activity following incubation, the diameter of the inhibition zone was assessed. The findings of each test were averaged after being run three times. The positive and negative controls were streptomycin 100µg/ml and DMSO, respectively.

Results

Percentage of Soluble Extracts

The weight of the powdered leaf and bark materials of *W. tinctoria* used for extraction is consistent across all solvents at 30 grams. This consistency ensures that the comparison of the solvent's efficiency in extracting soluble material is fair and unbiased. The weight of the soluble extract varies depending on the solvent used. The highest percent yield from various extracts of the leaf was recorded in aqueous extract at 9.8% followed by methanol extract at 8.2% and the lowest percentage yield was recorded by petroleum extract with 4.4% yield. From bark extracts highest soluble compounds were reported from aqueous (8.8%) followed by methanol (6.4%) lowest reported from petroleum extract (3.45%). The choice of solvent can significantly impact the yield and quality of the extract. For instance, if one is interested in extracting a particular compound that is soluble in water, then distilled water might be the best choice due to its higher percentage yield. Aqueous extracts yielded the highest percentage of soluble components from both leaves and bark. Methanol and Petroleum ether extracts showed moderate to low extraction efficiency.



ME: Methanol extract, AQ: Aqueous extract, PE: Petroleum ether extract

Figure 1: Percentage of the soluble extracts of *Wrightia tinctoria* leaf and bark extracts

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of different solvent extracts of *W. tinctoria* leaf and bark samples revealed the presence of various bioactive compounds, as summarized in Table 1. In the leaf

extracts, methanol (ME) extract exhibited the presence of carbohydrates, coumarins, and saponins, whereas proteins and glycosides were detected in the petroleum ether (PE) extract. The aqueous (AQ) extract of leaves was positive for terpenoids and quinones. In contrast, bark extracts demonstrated a broader spectrum of phytoconstituents. Both methanol and aqueous extracts contained carbohydrates, with the petroleum ether extract also showing their presence. Coumarins and saponins were consistently found across all bark extracts, while terpenoids and glycosides were present in the aqueous and petroleum ether extracts. Additionally, phenols were detected exclusively in the methanol extract of the bark. Alkaloids and anthocyanins were absent in all tested extracts, indicating a specific profile of phytoconstituents based on the solvent used for extraction. The abundance and variety of these bioactive compounds suggest potential health-promoting properties and underline the antibacterial and antifungal activities previously observed in *W. tinctoria* extracts. However, some phytoconstituents were absent in certain extracts, likely due to the varying polarity of the solvents used for extraction (Okwe et al., 2016). The bioactive agents identified have health-promoting effects and may contribute to the observed antibacterial and antifungal activities.

Table 1: Preliminary phytochemical analysis of different solvent extracts of *W. tinctoria* leaf and bark extracts

Plant Constituents	LEAF			BARK		
	ME	AQ	PE	ME	AQ	PE
Carbohydrates	+	-	-	+	+	+
Proteins	-	-	+	-	-	-
Alkaloids	-	-	-	-	-	-
Phenols	-	-	-	+	-	-
Coumarins	+	-	+	+	+	-
Anthocyanins	-	-	-	-	-	-
Terpenoids	-	+	-	-	+	+
Glycosides	-	-	+	+	+	+
Saponins	++	++	++	+	+	+
Quinones	-	+	-	-	-	-

Antimicrobial Activity Screening

Leaf extracts:

The antimicrobial activities of *W. tinctoria* leaf extracts were evaluated against four bacterial strains: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Streptococcus mutans*, using the agar well-diffusion method. The results demonstrated that the methanol, petroleum ether, and aqueous extracts exhibited significant to moderate antibacterial activities against all tested strains (Table 2). The methanol extracts showed the highest inhibitory activity against *B. subtilis* and *P. fluorescens* at a concentration of 125µg/mL, with inhibition zone diameters of 11 ± 1 mm. At a concentration of 500 µg/mL, the petroleum ether extract of *E. coli* exhibited the widest inhibition zone diameter (11 ± 1 mm), followed closely by the methanol extract (10 ± 1 mm). The water extract demonstrated the lowest inhibition for *B. subtilis* at this concentration, with an inhibition zone diameter of 9 ± 0.57 mm.

For *Streptococcus mutans*, the petroleum ether extract at 250µg/mL showed the highest inhibitory activity with an inhibition zone of 11 ± 1 mm, followed by the water extract with a 10 ± 1 mm inhibition zone. The lowest inhibition was observed with the methanol extract for *E. coli* at 250µg/mL, with an inhibition zone of 9 ± 0.57 mm. Streptomycin was used as a positive control, producing inhibition zones of 30 mm and 33 mm, whereas DMSO, used as a negative control, showed no zone of inhibition. These results indicate that the methanol and petroleum ether extracts of *W. tinctoria* are potent antibacterial agents capable of exerting significant inhibitory effects on the tested pathogenic bacteria. Methanol and petroleum ether extracts demonstrated significant antimicrobial activity.

Table 2: Antimicrobial Activity of *Wrightia tinctoria* leaf extracts

Extract	Concentration (µg/mL)	*E. coli*	*B. subtilis*	*P. fluorescens*	*S. mutans*
Petroleum Ether	500 mg	11 ± 1	10 ± 1	9 ± 0.57	11 ± 1
	250 mg	10 ± 1	9 ± 0.57	9 ± 0.57	11 ± 1
	125 mg	9 ± 0.57	10 ± 1	10 ± 1	10 ± 1
Methanol	500 mg	10 ± 1	9 ± 0.57	11 ± 1	10 ± 1
	250 mg	9 ± 0.57	10 ± 1	10 ± 1	9 ± 0.57
	125 mg	10 ± 1	11 ± 1	11 ± 1	9 ± 0.57
Water	500 mg	9 ± 0.57	8 ± 0.57	10 ± 1	9 ± 0.57
	250 mg	8 ± 0.57	8 ± 0.57	9 ± 0.57	9 ± 0.57
	125 mg	8 ± 0.57	9 ± 0.57	10 ± 1	8 ± 0.57

Bark extracts:

The antimicrobial activity of *Wrightia tinctoria* bark extracts was evaluated against four bacterial strains: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Streptococcus mutans* using the agar well-diffusion method. The results indicated that methanol, petroleum ether, and aqueous extracts demonstrated significant to moderate antibacterial activities against all tested strains (Table 3). At a concentration of 500 µg/mL, the petroleum ether extract showed the highest inhibitory activity against *E. coli* with an inhibition zone diameter of 11 ± 1 mm. This was followed by the methanol extract with an inhibition zone of 10 ± 1 mm. The water extract exhibited the lowest inhibition zone for *E. coli* at 9 ± 0.57 mm. For *B. subtilis*, the methanol extract showed the least inhibition at 9 ± 0.57 mm, while the highest activity was also observed in the petroleum ether extract.

At a concentration of 250µg/mL, the petroleum ether extract displayed the highest inhibitory activity against *Streptococcus mutans* with an inhibition zone of 11 ± 1 mm. The aqueous extract showed a slightly lower inhibition zone of 10 ± 1 mm. For *E. coli*, the lowest inhibition was observed with the methanol extract at 9 ± 0.57 mm. At a concentration of 125 µg/mL, the methanol extract demonstrated the highest inhibitory activity against *Pseudomonas fluorescens* and *B. subtilis* with inhibition zones of 11 ± 1 mm. The aqueous extract also showed significant activity with inhibition zones of 10 ± 1 mm for both strains. The positive control, streptomycin, produced inhibition zones of 30 mm and 33 mm, indicating its high antibacterial efficacy. In contrast, DMSO, used as a negative control, showed no zone of inhibition. The highest inhibition zone was observed against *E. coli* with the leaf petroleum ether extract (30 mm). Other bacterial strains showed varying degrees of susceptibility to the different extracts.

Table 3: Antimicrobial Activity of *Wrightia tinctoria* bark extracts

Extract	Concentration (µg/mL)	*E. coli*	*B. subtilis*	*P. fluorescens*	*S. mutans*
Petroleum Ether	500 mg	11 ± 1	10 ± 1	9 ± 0.57	11 ± 1
	250 mg	10 ± 1	9 ± 0.57	9 ± 0.57	11 ± 1
	125 mg	9 ± 0.57	10 ± 1	10 ± 1	10 ± 1
Methanol	500 mg	10 ± 1	9 ± 0.57	11 ± 1	10 ± 1
	250 mg	9 ± 0.57	10 ± 1	10 ± 1	9 ± 0.57
	125 mg	10 ± 1	11 ± 1	11 ± 1	9 ± 0.57

Extract	Concentration (µg/mL)	*E. coli*	*B. subtilis*	*P. fluorescens*	*S. mutans*
Water	500 mg	9 ± 0.57	8 ± 0.57	10 ± 1	9 ± 0.57
	250 mg	8 ± 0.57	8 ± 0.57	9 ± 0.57	9 ± 0.57
	125 mg	8 ± 0.57	9 ± 0.57	10 ± 1	8 ± 0.57

The results of this study indicate that *Wrightia tinctoria* extracts possess significant antimicrobial properties, particularly against MDR bacteria. The presence of diverse phytochemicals in the extracts supports the traditional uses of the plant for treating various ailments. Previous studies have reported similar findings, highlighting the potential of *W. tinctoria* in developing novel antimicrobial agents. For instance, the study by Jose and Thomas, (2014) demonstrated the antibacterial activity of *W. tinctoria* leaf extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, corroborating our findings.

Similarly, Meenu et al. (2022) reported the presence of flavonoids and tannins in *W. tinctoria* extracts, which are known for their antimicrobial properties. The high inhibition zone observed in *E. coli* suggests that the plant's bioactive compounds could effectively target MDR strains, addressing the critical issue of antibiotic resistance. Further research should focus on isolating and characterizing these bioactive compounds to understand their mechanisms of action and potential therapeutic applications. This structured research article provides a comprehensive overview of the phytochemical profiling and antibacterial activities of *W. tinctoria*, contributing valuable insights into its potential as a source of novel antimicrobial agents.

Conclusion

The study titled "Preliminary Phytochemical Profiling and Evaluation of Antibacterial Activities of *Wrightia tinctoria* (Roxb.) R.Br" investigates the phytochemical composition and antimicrobial potential of *W. tinctoria*. The plant's leaves and bark were extracted using acetone, petroleum ether, and water, and then analyzed for phytochemicals like proteins, phenols, tannins, and flavonoids. Antibacterial tests against *E. coli*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Streptococcus mutans* showed significant activity, particularly with petroleum ether extracts against *E. coli* (30 mm inhibition zone). Methanol extracts also demonstrated substantial activity. Streptomycin and DMSO served as positive and negative controls, respectively. The study concludes that *Wrightia tinctoria*'s antimicrobial properties, supported by its diverse phytochemical profile, indicate its potential for developing new antimicrobial agents. Future research should focus on isolating specific bioactive compounds to validate their therapeutic potential.

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