

Multifaceted analysis of *Wrightia tinctoria*: Extraction, physicochemical profiling, and phytochemical exploration

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Abstract

Wrightia tinctoria, also known as pala indigo plant or dyer's oleander plant, is a valuable medicinal tree found across India and neighboring regions. This study aims to comprehensively analyze *Wrightia tinctoria* through Soxhlet extraction, physicochemical profiling, and phytochemical exploration. The introduction provides a background on the botanical significance of *Wrightia tinctoria*, its traditional medicinal uses, and its chemical composition, emphasizing its importance in various traditional healing practices across different regions. The materials and methods section outlines the selection of *Wrightia tinctoria* leaves, seeds and stem for the study, their collection, and the preparation of leaf powder. Soxhlet extraction using different solvents was conducted to isolate active compounds. Physicochemical analysis involved determining moisture content, ash content, pH, and solubility. Additionally, qualitative phytochemical screening was performed to identify various phytoconstituents. Results reveal the extraction yields of *Wrightia tinctoria* seed, leaf, and stem using ethanol as the solvent for Soxhlet extraction. Physicochemical properties, including moisture content, ash content, pH, and solubility, varied across different plant parts, highlighting their diverse chemical compositions and potential applications. The phytochemical analysis of crude and hydroalcoholic extracts demonstrates the presence and concentration of alkaloids, flavonoids, tannins, saponins, and glycosides in *Wrightia tinctoria*. Differences in phytochemical composition among plant parts and extraction methods underscore the plant's pharmacological diversity and therapeutic potential.

Keywords: *Wrightia tinctoria*; Soxhlet extraction; Phytochemical analysis; Medicinal tree; Pharmacological diversity

1. Introduction

Wrightia tinctoria, commonly known as pala indigo plant or dyer's oleander plant, is a small deciduous tree belonging to the family *Apocyanaceae*. It has two subspecies: *Wrightia tinctoria* sub species *W. tinctoria* and *W. tinctoria rothii*, distributed across India, Myanmar, Nepal, Timor, and Vietnam. In India, it is prevalent in most of peninsular and central regions, excluding the northern and north-eastern states. Traditionally, *Wrightia tinctoria* is utilized to treat various ailments such as diarrhoea, toothache, headache, jaundice, gynaecological disorders, piles, ringworm, and other skin diseases. In Chhattisgarh (India), the germinated seed of *W. tinctoria* is used to traditionally cure jaundice. In South India, it is known as "jaundice alkaloids," indicating its medicinal usage (Battu et.al, 2018).

Wrightia tinctoria, a small deciduous tree prevalent across various regions of India, holds significant medicinal value. In Ayurvedic medicine, its leaves are utilized to address toothaches and hypertension, while the bark and seeds are employed to alleviate diverse digestive and skin issues. Notably, the leaves are particularly effective in treating skin ailments and are integrated into Siddha medicine for psoriasis management. Key compounds such as indigotin, indirubin, tryptanthrin, isatin, anthranilate, and rutin are extracted from *W. tinctoria* leaves, alongside β -amyryn, lupeol, β -

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sitosterol, and ursolic acid. The leaves possess an acrid taste, whereas the bark and seeds taste bitter. Additionally, the leaves exhibit thermogenic and hypotensive properties, while the bark and seeds are noted for their thermogenic, carminative, anthelmintic, depurative, and aphrodisiac attributes (Meenu et.al, 2022).

Chemical analysis of *W. tinctoria* reveals the presence of alkaloids, triterpenoids, lipids, carbohydrates, steroids, and flavonoids. Its wide array of pharmacological properties includes hepatoprotective, anti-helminthic, anti-diarrhoeal, anti-psoriatic, diuretic, anti-cancer, anti-ulcer, analgesic, and antioxidant effects. In Ayurvedic medicine, *W. tinctoria* is described as having titka, kashaya, rooksha, sita, and katu properties. Traditional remedies involving *W. tinctoria* include using oil obtained from soaking its leaves in coconut oil to treat psoriasis. Chewing its leaves alone or with salt alleviates toothache, while applying leaf and stem bark pastes on the forehead or administering them orally provides relief from headaches. Stem bark paste mixed with water is consumed twice a day for abdominal pain relief. *W. tinctoria*, including its leaves, bark, and seeds, is also reported to have aphrodisiac potential and antipyretic activity, along with traditional usage for curing breast cancer (Ganesan, 2008, Rao et.al, 2011, Singh et.al, 2010 and Nagarani et.al 2011).

1.1. Traditional medicinal applications

The bark of this plant is traditionally utilized to boost lactation, alleviate abdominal discomfort, address skin issues and injuries, act as a fever reducer, combat dysentery, diarrhea, and hemorrhaging, and serve as an antidote for snake venom. Its seeds are also employed as a remedy for enhancing libido. Concerning the potential risks associated with estrogen, including heightened chances of endometrial hyperplasia, carcinoma, breast cancer, and thromboembolic ailments, several natural substances exhibiting promising contraceptive effects in initial investigations couldn't be further explored due to their estrogen-like properties. Furthermore, the leaves are used topically as compresses for mumps and herpes, and sometimes chewed to relieve toothaches. In traditional medicine, dried and powdered roots of the *Wrightia tinctoria* plant, in conjunction with *Phyllanthus amarus* (keezhanelli) and *Vitex negundo* (nochi), are mixed with milk and ingested orally by women to enhance fertility. The bark and seeds are efficacious against psoriasis and non-specific dermatitis, possessing anti-inflammatory and anti-dandruff attributes, and are thus incorporated into hair oil formulations (Bigoniya et.al, 2011, Srivastava 2014).

The present research focused on documenting the pharmacological investigations of the traditional medicinal plant *W. tinctoria*. It involved the preparation of *W. tinctoria* Seed, Leaf, and Stem Crude using various solvents, followed by the identification of chemical components and assessment of antibacterial properties. The objective was to evaluate its potential for use in treating skin ailments.

2. Materials and Methods

2.1. Plant Selection

Wrightia tinctoria was chosen for its medicinal attributes, widespread availability, and ease of access. The plant is recognized for its efficacy in treating skin ailments and possesses various pharmacological properties conducive to wound healing. Among its different aerial components such as stems, leaves, petioles, flowers, fruits, seeds, and barks, leaves etc., stem, leaves and seeds were specifically chosen for the investigation.

2.2. Collection and Identification of Leaves

During the flowering season, fresh leaves of *Wrightia tinctoria*, along with stems and seeds, were gathered from agricultural land near Vidisha, Madhya Pradesh.

2.3. Preparation of sample

The freshly collected parts of *Wrightia tinctoria* underwent thorough washing with running water and double distilled water to eliminate any impurities. Subsequently, they were air-dried in shade for 20 days to reduce excess moisture and prevent the degradation of active compounds. Following the drying process, the leaves, seeds and stems were ground into a fine powder and stored for further use.

2.4. Extraction

To prepare the plant extracts, the gathered leaves of *W. tinctoria* underwent a washing process followed by sun-drying for a duration of 6 days. Subsequently, the dried leaves were ground into a fine powder, which was then sieved to ensure a smooth texture. The powdered material was stored in a cool and dry environment until the extraction process. Extraction was carried out utilizing ethanol (90% v/v) in a Soxhlet apparatus maintained at 60°C. After extraction, the

solvent was completely evaporated using a hot air oven. The resulting extract was preserved in an airtight container at 4°C until required (Khan, Z., & Ansari, 2018).

2.5. Physicochemical Analysis

To assess the purity and quality of the *Wrightia tinctoria* leaf, stem and seed powder, the following ash and extractive values were determined following procedures outlined in previous studies.

2.6. Total Ash

Approximately 2 grams of *Wrightia tinctoria* leaf powder was heated in a silica dish, not exceeding 450°C, until the powder turned white, indicating the absence of carbon. The remaining residue was cooled and weighed.

Total Ash Value (% w/w) = (Weight of residual ash / Weight of original sample) × 100

2.7. Water Soluble Extractive

Around 5 grams of *Wrightia tinctoria* leaf, stem and seed powder was placed in a sealed flask with 100 mL of chloroform. The mixture was vigorously shaken for six hours, left undisturbed for the subsequent 18 hours, and then filtered. Approximately 25 mL of the filtrate was evaporated and dried at 105°C to determine the percentage of water-soluble extractive.

2.8. Alcohol Soluble Extractive

Similarly, 5 grams of *Wrightia tinctoria* leaf, stem and seed powder was placed in a closed flask with 100 mL of 95% ethanol separately. The flask was shaken periodically for six hours, allowed to settle for 18 hours, and then filtered. Approximately 25 mL of the filtrate was evaporated and dried at 105°C to determine the percentage of alcohol soluble extractive.

2.9. Moisture Content

The *Wrightia tinctoria* leaf, stem and powder was dried at 105°C for 5 hours, cooled, and weighed. This process was repeated hourly, and the difference in weights was calculated to determine the percentage of moisture content.

2.10. Solvent Selection

Petroleum ether, ethyl acetate, ethanol, and aqueous solvents were chosen to facilitate tissue penetration and dissolution of active constituents within the leaves, stems and seeds.

2.11. Qualitative phytochemical

Screening involves examining the compounds derived from plants. This screening process helps in identifying the profile and chemical makeup of the extracts. Various chemical tests were conducted on the obtained extracts to detect different phytoconstituents (Harborne, 2012).

3. Result and Discussion

The Soxhlet Extraction of *Wrightia tinctoria* seed, Leaf, and Stem using Ethanol as the solvent for a duration of 6 hours reveals the following extraction yields:

Table 1 Extraction yield of Soxhlet Extraction of *Wrightia tinctoria* seed, Leaf, and Stem.

Plant Part	Extraction Yield (%)
Seed	2.5
Leaf	1.8
Stem	2.2

From the results, it is evident that the highest extraction yield was observed in the seeds (2.5%), followed by the stem (2.2%), and then the leaves (1.8%). This indicates that the seeds contain a higher concentration of soluble compounds compared to the leaves and stems. The variation in extraction yields could be attributed to differences in the composition of phytochemicals present in each plant part, as well as variations in the solubility of these compounds in ethanol. Further analysis of the extracted compounds and their potential pharmacological properties could provide valuable insights into the therapeutic potential of *Wrightia tinctoria* across different plant parts.

3.1. Physicochemical Properties of *Wrightia tinctoria* Seed, Leaf, and Stem

Table 2 Physicochemical Properties of *Wrightia tinctoria* Seed, Leaf, and Stem

Property	Seed	Leaf	Stem
Moisture Content (%)	5.3	3.8	4.2
Ash Content (%)	1.1	2.5	2.0
pH	5.8	6.2	5.5
Solubility	Insoluble	Partially soluble	Soluble

Based on the provided data for the Physicochemical Properties of *Wrightia tinctoria* Seed, Leaf, and Stem, the following conclusions can be drawn:

3.1.1. Moisture Content (%)

The moisture content is highest in the seed, followed by the stem and then the leaf. This indicates that the seeds retain more moisture compared to the other plant parts.

3.1.2. Ash Content (%)

The ash content is lowest in the seed, followed by the stem and then the leaf. This suggests that the seed contains fewer inorganic residues compared to the leaf and stem.

3.1.3. pH

The pH levels vary slightly across the different plant parts, with the leaf having the highest pH, followed by the seed and then the stem.

3.1.4. Solubility

The solubility properties vary significantly among the plant parts, with the seed being insoluble, the leaf being partially soluble, and the stem being soluble in water. This indicates differences in the composition of soluble compounds present in each plant part.

Overall, the physicochemical properties of *Wrightia tinctoria* vary across its different plant parts, suggesting diverse chemical compositions and potential applications in various fields such as pharmacology, cosmetology, and agriculture.

3.2. The Phytochemical Analysis of *Wrightia tinctoria* Seed, Leaf, and Stem Crude Extracts and Hydroalcoholic Extracts

Table 3 Phytochemical Analysis of *Wrightia tinctoria* Seed, Leaf, and Stem Crude Extracts and Hydroalcoholic Extracts.

Phytochemical	Crude Extract	Hydroalcoholic Extract
Alkaloids	Positive for all parts	Increased in stem
Flavonoids	Present in seed and leaf	Enhanced in leaf
Tannins	Notable in stem	Higher levels in seed
Saponins	Predominant in seed	Comparable levels in all parts
Glycosides	Detected in all parts	Higher concentration in stem

Based on the results, the following conclusions can be drawn:

3.2.1. Alkaloid

- Crude Extract: Alkaloids are present in all parts of the plant.
- Hydroalcoholic Extract: The alkaloid content is increased in the stem compared to other parts.

3.2.2. Flavonoids

- Crude Extract: Flavonoids are detected in the seed and leaf.
- Hydroalcoholic Extract: Flavonoid concentration is enhanced in the leaf extract.

3.2.3. Tannins

- Crude Extract: Tannins are notable in the stem.
- Hydroalcoholic Extract: The seed extract shows higher levels of tannins compared to other parts.

3.2.4. Saponins

- Crude Extract: Saponins are predominant in the seed.
- Hydroalcoholic Extract: Comparable levels of saponins are found in all plant parts.

3.2.5. Glycosides

- Crude Extract: Glycosides are detected in all parts of the plant.
- Hydroalcoholic Extract: The stem extract exhibits a higher concentration of glycosides compared to other parts.

Overall, the phytochemical analysis reveals variations in the presence and concentration of bioactive compounds across different plant parts and extraction methods. These differences may indicate diverse pharmacological activities and potential applications of *Wrightia tinctoria* in traditional medicine, pharmaceuticals, and other industries. Further research is warranted to explore the specific properties and therapeutic potential of each phytochemical constituent.

In conclusion, this multifaceted analysis sheds light on the pharmacological properties and potential applications of *Wrightia tinctoria* in traditional medicine and pharmaceutical industries. Further research is warranted to explore the specific bioactive compounds and their mechanisms of action, paving the way for the development of novel therapeutic interventions derived from *Wrightia tinctoria*.

4. Conclusion

The comprehensive analysis of *Wrightia tinctoria* through Soxhlet extraction, physicochemical profiling, and phytochemical exploration underscores its pharmacological diversity and therapeutic potential. The study revealed that the extraction yields varied across different plant parts, with seeds having the highest yield (2.5%), followed by stems (2.2%), and leaves (1.8%). This suggests a higher concentration of soluble compounds in the seeds. Physicochemical properties also differed, with seeds exhibiting the highest moisture content and lowest ash content, indicating fewer inorganic residues compared to leaves and stems. pH levels were relatively similar, while solubility varied significantly, highlighting the diverse chemical compositions of each plant part.

Phytochemical analysis demonstrated the presence of alkaloids, flavonoids, tannins, saponins, and glycosides, with variations across crude and hydroalcoholic extracts. Notably, alkaloid content was highest in the stem for hydroalcoholic extracts, flavonoids were enhanced in the leaf, tannins were most notable in the seed, and saponins were predominant in the seed. Glycosides showed higher concentrations in the stem.

These findings highlight the rich and varied chemical profile of *Wrightia tinctoria*, reinforcing its traditional medicinal uses and potential applications in pharmacology and other fields. The differences in phytochemical composition among plant parts and extraction methods suggest the need for further research to isolate specific bioactive compounds and elucidate their mechanisms of action. This could lead to the development of novel therapeutic interventions, leveraging the diverse pharmacological properties of *Wrightia tinctoria*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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