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**RESEARCH ARTICLE**

# Evaluation of preliminary Phytochmical, Antidandruff and Antioxidant activity of medicinally and economically important plant, *Wrightia Tinctoria* (Apocynaceae)

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## ABSTRACT

The plant samples (leaf and bark) of *Wrightia tinctoria* were collected from the nearby areas of Coimbatore, Tamil Nadu. Preliminary phytochemical analysis, *in-vitro* antioxidant activity and antidandruff activity studies were done using ethanolic and acetone extracts from the leaf and bark of *Wrightia tinctoria.* Phytochemical analysis revealed the presence of Alkaloids, Phenols, Glycosides, Steroids, Terpenoids and Tannins. Antioxidant activity evaluated by the Phosphomoybdenum assay and Metal chelating assay showed that the bark samples exhibited the most antioxidant activity compared to leaf samples in both assays. The antifungal activity of leaf and bark (acetone and ethanol) extracts of *W.tinctoria* on the growth of dandruff causing fungi *Malassezia fufur* done by disc diffusion method, revealed that the extracts of *Wrightia tinctoria* are actively involved in controlling the dandruff causing fungi.

**Keywords:** *Wrightia tinctoria,* Antifungal activity, Antioxidant activity, *Malassezia furfur*

## Introduction

Medicinal plants, rich in bioactive compounds like alkaloids and flavonoids, play a crucial role in traditional and modern medicine. They offer therapeutic benefits for physical, biological, and mental health, and are increasingly popular for their potential to develop new medications. Medicinal plants with antifungal properties provide a natural and effective alternative to synthetic antifungal drugs, reducing health risks and the likelihood of resistance[1],[2],[3].

Phytochemicals are bioactive substances found in plants, offering health benefits beyond basic nutrition. These compounds, such as tannins, alkaloids, terpenoids, and flavonoids, protect plants and enhance their color, aroma, and flavor. Over 4,000 phytochemicals have been identified, each with unique properties. Plants are also rich sources of natural antioxidants, including vitamins C and E, polyphenols, and carotenoids. These antioxidants neutralize free radicals, reducing oxidative stress and the risk of chronic diseases like cancer, cardiovascular, and neurodegenerative disorders. They play a crucial role in the body's defense against free radical-induced damage.

## Dandruff

Dandruff, characterized by the shedding of small white flakes from the scalp, affects about 5% of the population, with higher prevalence in males and those aged 20-30[4]. It is commonly caused by the lipophilic fungus *Malassezia*, which thrives on sebum [5],[6],[7]. The condition may be influenced by genetic, environmental factors, and worsens in winter [8],[9]. Treatments include antifungal agents like amphotericin B, clotrimazole, ketoconazole, and other compounds such as salicylic acid and zinc pyrithione.

### *Wrightia tinctoria*

*Wrightia tinctoria*, a small evergreen tree from the Apocynaceae family, is native to tropical Africa and Asia and known in Tamil as "Paalai" (Vedhanaryanan *et al*., 2013). This plant is rich in secondary metabolites and is used in traditional medicine for various ailments, including skin diseases and liver issues. It exhibits diverse pharmacological properties, such as anti-anticancer activities [10], [11]. Its leaves, seeds,



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and fruits are consumed as vegetables and used in hair oil formulations for their antidandruff properties, with the leaves also producing a blue dye and the bark and seeds treating psoriasis [6].

## Materials and Methods

### Collection of Plant Material

*Wrightia tinctoria* leaves and bark were collected in and around Coimbatore, Tamil Nadu, India. The leaves and the bark of *W. tinctoria* were collected from natural and undisturbed areas.

### Preparation of Plant Extracts

Twenty-five grams of powdered bark and leaves of *Wrightia tinctoria* were successively extracted with 300 mL of ethanol and acetone for 48 hours using a Soxhlet apparatus. The extracts were then stored in an airtight container at 4°C in a refrigerator until further examination.

### Preliminary phytochemical screening of leaf and bark (acetone and ethanol) extracts of *Wrightia tinctoria*

The extracts were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant materials [12]

### Test for Alkaloids

To 1mL of each extract in two separate test tubes, 2-3 drops of Dragendroff's and Meyer's reagents were separately added. An orange red precipitate/turbidity with Dragendroff's reagent or a white precipitate with Meyer's reagent would indicate the presence of alkaloids.

### Test for Flavonoids

To 4 mL of each of the extracts, a piece of magnesium ribbon was added, followed by concentrated HCl dropwise. A colour ranging from crimson to magenta indicates the presence of flavonoids.

### Test for Glycosides

Keller Kiliani test: To the 2 mL of extracts, 1 mL of glacial acetic acid with ferric chloride and concentrated sulphuric acid were added. The appearance of a blue colour indicates the presence of glycosides.

### Test for Saponins

One mL of extract was added to a test tube and 5 mL of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponin.

### Test for Tannins

Two mL of the extracts were diluted with distilled water in separate test tubes and 2-3 drops of a 5% ferric chloride ($Fecl_3$) solution were added. A green-black or blue-black colouration indicated the presence of tannin.

### Test for Terpenoids

Five mL of extracts were mixed with 2 mL of chloroform and concentrated $H_2SO_4$ to form a layer. A reddish-brown colouration of the interface showed the presence of terpenoids.

### Test for Steroids

Two ml of the extracts were taken in separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydride, followed by the addition of chloroform. Concentrated sulphuric acid was added to the side of the test tube. The formation of a brown ring at the interphase of the two liquids and the appearance of a violet colour in the supernatant layer indicated the presence of steroids.

### Test for phenols

Five mL of the concentrated extracts were taken and 2 mL of a neutral ferric chloride solution was added. The appearance of violet colour indicates the presence of phenols.

### Antioxidant Activity

### Phosphomolybdenum Assay

The antioxidant activity of the bark and leaf extracts (ethanol and acetone) was assessed using the green phosphomolybdenum complex method[13]. Samples (40 µL) or ascorbic acid (standard) in 1 mM DMSO, or distilled water (blank), were mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes, then cooled to room temperature. Absorbance was measured at 695 nm, and results were expressed as mg AAE/g extract.

### Metal Chelating Activity

The ferrous ion chelating activity of bark and leaf extracts (acetone and ethanol) was measured using the method[14]. Extract samples (100 µL) were mixed with 50 µL of 2 mM FeCl2 and 200 µL of 5 mM ferrozine. After standing at room temperature for 10 minutes, absorbance was measured at 562 nm, with EDTA as the standard. Results were expressed as mg EDTA equivalents/g extract.

**Anti-dandruff activity by antifungal method**

**Sample Collection**

Dandruff samples were collected by scraping visible flakes from the scalp using a sterile scalpel. The samples were observed under a compound microscope, treated with 10% KOH, and stained with methylene blue.

**Preparation of Saboraud dextrose Agar**

The collected dandruff was inoculated onto sterile Sabouraud dextrose agar (SDA) using the spread plate method. SDA was prepared with peptone, dextrose, and agar. The plates were incubated at 32°C to 37°C for 3-5 days. Characteristic white growths around the flakes indicated the presence of organisms causing dandruff.

**Growth and Identification**

The organism was identified using cultural, microscopic, and biochemical methods. *Malassezia furfur* was isolated in pure culture on Sabouraud dextrose agar with added chloramphenicol. This medium supports the growth of medically significant fungi, while chloramphenicol inhibits unwanted bacterial flora

**Inoculum Preparation**

The pure culture was inoculated onto Sabouraud Dextrose agar using buds and incubated at 37°C for two days. Discs containing varying concentrations of ethanolic and acetonic extracts of bark and leaf were prepared to determine the minimum inhibitory concentration (MIC) of each extract. A 2% fluconazole disc served as a control. The antifungal activity of the extracts was assessed by the disc diffusion method. After incubation for 48 hours, the plates were removed, and the zone of inhibition around each disc was measured in millimeters. Each concentration was tested in triplicate.

**Fresh Leaf and Bark Sample of *Wrightia tinctoria* collected from in and around Coimbatore locality**

**Powdered sample of leaf and bark of *Wrightia tinctoria***

**Culture and Growth pattern of *Malassezia furfur* on Sabouraud's Dextrose Agar Media and Microscopic observation of *Malassezia furfur***

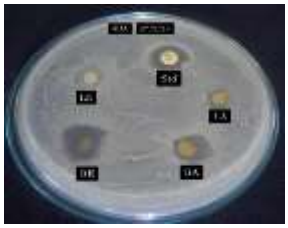
**Dandruff**

**Prepared**

**isolation of**

**Microscopic**

**Antidandruff activity of leaf and bark extracts of ethanolic and acetonic extracts of *Wrightia tinctoria* against *Malassezia furfur* at 5 and 10 mg/mL concentration respectively**



**Results And Discussion**

**Table 1. Phytochemical Screening of leaf and bark (acetone & ethanol) extracts of *Wrightia tinctoria***

| Sl. No | Phytochemical Constituents | Ethanol Extract | | | Acetone Extract | | Test Name |
|---|---|---|---|---|---|---|---|
| | | Leaf | Bark | | Leaf | Bark | |
| 1 | Alkaloid | +++ | ++ | | - | - | Dragendroff's test |
| 2 | Flavonoids | - | - | | - | - | Shinoda's test |
| 3 | Phenol | - | - | | +++ | - | Ferric Chloride |
| 4 | Glycosides | - | - | | - | + | Keller Kiliani test |
| 5 | Steroids | - | + | | - | - | Liebermann-Burchard's test |
| 6 | Terpenoids | + | - | | ++ | - | Salkowski test |
| 7 | Saponin | - | - | | | - | Foam test |
| 8 | Tannin | ++ | - | | +++ | - | Acetic anhydride |

**+; moderately present, ++; highly present,** -; **absence**

Phytochemical evaluation of *Wrightia tinctoria* extracts (ethanol and acetone) revealed a range of secondary metabolites. Alkaloids were prominent in both the leaf and bark ethanol extracts, showing strong reactions in Dragendroff's test. Flavonoids were absent in all extracts, while phenols were abundant in the acetone leaf extract. Glycosides were detected solely in the acetone bark extract, and steroids were found only in the ethanol bark extract. Terpenoids were present in both ethanol and acetone extracts, and tannins were notably abundant, especially in the acetone leaf extract. Saponins were not detected in any of the extracts.

These findings align with previous studies on *Wrightia tinctoria*. [2] reported the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins, although our study did not detect flavonoids or saponins. [15] identified alkaloids and flavones in methanolic extracts, supporting the presence of alkaloids observed in our study. [16] noted various secondary metabolites contributing to the plant's pharmacological activity, which is consistent with our findings of diverse bioactive compounds. Similarly, [17] also identified several secondary metabolites, reinforcing the medicinal potential of *Wrightia tinctoria*.

***In vitro* Antioxidant Activity**

**Phosphomolybdenum Assay**

The phosphomolybdenum assay revealed significant differences in antioxidant activity among extracts. Ethanol extracts of leaves (249.58 ± SD) showed higher activity than acetone extracts (234.27 ± SD), while acetone extracts of bark (321.9 ± SD) were more effective than ethanol extracts (103.4 ± SD), with all differences being statistically significant ($p < 0.05$). These findings align with previous research by [18] which also demonstrated strong antioxidant activity in *Wrightia tinctoria*.

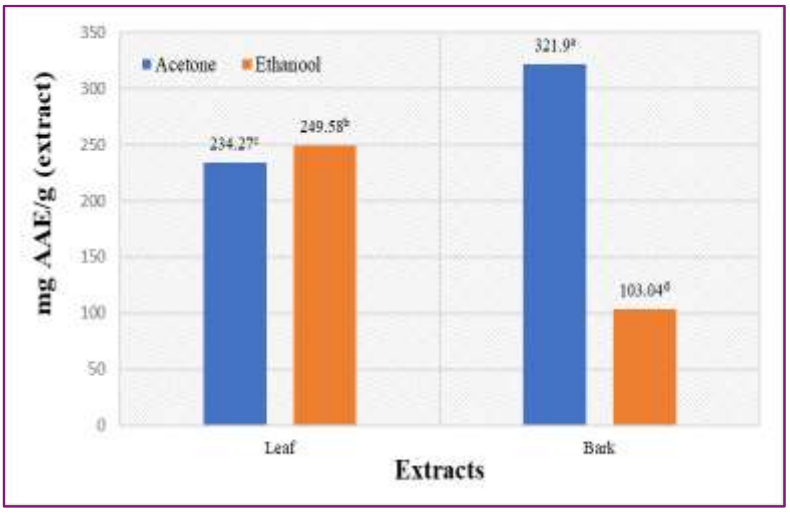



**Figure 1: Assay Phosphomolybdenum assay in the leaf and bark (acetonic and ethanolic) extract of *Wrightia tinctoria*. Statistically significant at p < 0.05, where a > b > c. AAE – Ascorbic Acid Equivalents Values are mean of triplicate determination (n=3) ± standard deviation.**

**Metal Chelating Activity**

The metal chelating assay revealed that ethanol extracts from both leaf (63.86 ± SD) and bark (141.54 ± SD) were more effective than acetone extracts, with statistically significant differences ($p < 0.05$). The positive control, BHT (210), exhibited the highest activity. These results are consistent with Dhanabal et al. (2009), who reported high antioxidant activity in *Wrightia tinctoria* leaves with an IC50 of 14.12 ± 0.71 µg/mL, and [17] , who confirmed antioxidant potential using DPPH. [19] also noted significant antioxidant activity (IC50 of 53.64 µg/mL) in TiO2 nanoparticles from *Wrightia tinctoria*. These findings highlight the plant's diverse antioxidant properties and the impact of extraction methods.

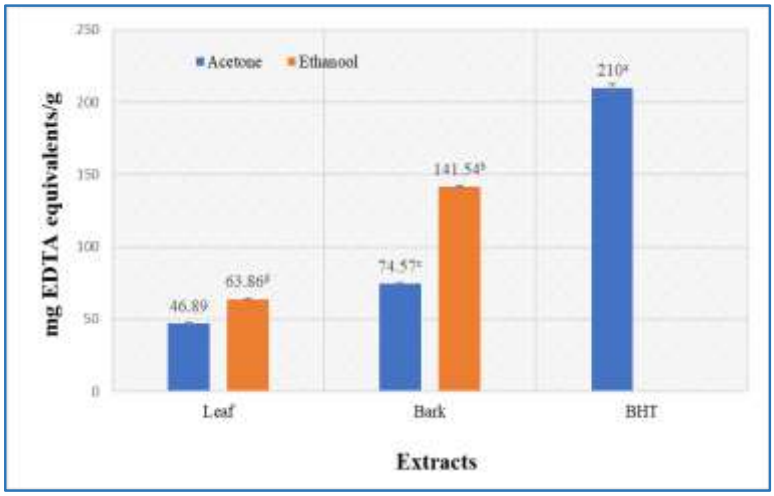



**Figure 2: Metal chelating Activity in the leaf and bark (acetonic and ethanolic) extract of *Wrightia tinctoria.* Statistically significant at p < 0.05 where a > b > c. Values are mean of triplicate determination (n=3) ± standard deviation.**

**Table 2: Mean radius of zone of inhibition of leaf and bark ethanol and acetone extracts of *Wrightia tinctoria* against *Malassezia furfur.***

| Zone of inhibition (mm) | | |
|---|---|---|
| **Plant Extract** | **5 mg/ml** | **10 mg/ml** |
| Leaf Acetone | 1.3 ±0.11 | 5 ± 0.11 |
| Leaf Ethanol | 0.5±1.5 | 7 ± 0.17 |
| Bark Acetone | 1.5±0.34 | 10.1 ± 0.38 |
| Bark Ethanol | 0.9± 1.0 | 10.7 ± 0.14 |
| Standard (Flucanazole) | 6.0±0.36 | 11 ± 0.51 |

The antidandruff activity of *Wrightia tinctoria* extracts varied by solvent and concentration. Ethanol extracts of bark showed the highest inhibition (10.7 ± 0.14 mm) at 10 mg/mL, followed by acetone extracts of bark (10.1 ± 0.38 mm). Leaf extracts, both ethanol and acetone, showed lower inhibition (7 ± 0.17 mm and 5 ± 0.11 mm, respectively). Fluconazole, the standard antifungal, showed consistent inhibition. These results highlight the potential of *Wrightia tinctoria*, especially the bark ethanol extract, for further therapeutic exploration.

**Conclusion**

The study found that the leaves and bark extracts (acetonic and ethanolic) of *Wrightia tinctoria* contain various phytochemicals with potential to control dandruff-causing fungi and exhibit antioxidant activity.

**Acknowledgement**

**Conflict of Interest**

Author has no conflict of interest.

**References**

1. Moorthy, K., Aravind, A., Punitha, T., Vinodhini, R., Suresh, M., & Thajuddin, N. (2012). In vitro screening of antimicrobial activity of *Wrightia tinctoria* (Roxb.) R. Br. *Asian Journal of Pharmaceutical and Clinical Research, 5*(5), 4.
2. Vedhanaryanan, P., Unnikannan, P., & Sundaramoorthy, P. (2013). Antimicrobial activity and phytochemical screening of *Wrightia tinctoria* (Roxb.) R. Br. *Journal of Pharmacognosy and Phytochemistry, 2*(4), 123-125.
3. Nirat, B. K., & Agnihotri, S. K. (2023). Preliminary phytochemical screening of *Wrightia tinctoria*.
4. Deviha, M. S., & Pavithram, K. S. (2015). Antifungal activity by ethanolic extracts of medicinal plants against Malasseziafurfur: A potential application in the treatment of Dandruff. Int J Pharmtech Res, 8(3), 440-444.
5. Amutha, E., Sivakavinesan, M., Rajaduraipandian, S., & Annadurai, G. (2023). Identification of phytochemicals capping the biosynthesized silver nanoparticles by Wrightia tinctoria and evaluation of their in vitro antioxidant, antibacterial, antilarvicidal, and catalytic activities. Emergent Materials, 6(2), 525-534.
6. Karmakar, E., Das, P., Yatham, P., Kumar, D., Mukhopadhyay, S., & Roy, S. S. (2023). Seedpod extracts of Wrightia tinctoria shows significant anti-inflammatory effects in HepG2 and RAW-264.7 cell lines. Natural Product Research, 37(18), 3158-3162.
7. Khyade, M. S., & Vaikos, N. P. (2014). Wrightia tinctoria R. Br.-a review on its ethnobotany, pharmacognosy and pharmacological profile. Journal of Coastal Life Medicine, 2(10), 826-840.
8. Daulat, C. A., & Pradip, D. T. (2023). A review on commercial cultivation and collection aspects of Wrightia tinctoria. Journal of Pharmacognosy and Phytochemistry, 12(6), 321-324.
9. Srinroch, C., Sahakitpichan, P., Techasakul, S., Chimnoi, N., Ruchirawat, S., & Kanchanapoom, T. (2019). 2-Aminobenzoyl and megastigmane glycosides from Wrightia antidysenterica. Phytochemistry Letters, 29, 61-64.
10. Nagalakshmi, M. A. H., & Murthy, K. S. R. (2015). Phytochemical profile of crude seed

oil of Wrightiatinctoria R. BR. and Wrightiaarborea (Dennst.) Mabb. by GC-MS. Int. J. Pharm. Sci. Rev. Res, 31(2), 46-51.
11. Ragunath, C., Kousalya, L., Venkatachalam, R., & Anitha, S. (2022). Green synthesis of hydroxyapatite nanoparticles from wrightia tinctoria and its antibacterial activity. BioNanoScience, 12(3), 723-730.
12. Harborne, J.B. Phytochemical methods, London. Chapman and Hall, Ltd. 1973; 49-188.
13. Zahan, R., Nahar, L., Mosaddik, A., Rashid, M. A., Hassan, A., & Ahmed, M. (2013). Evaluation of antioxidant and antitumor activities of Wrightia arborea. Journal of Basic & Applied Sciences, 9, 625.
14. Donato, R., Sacco, C., Pini, G., & Bilia, A. R. (2020). Antifungal activity of different essential oils against Malassezia pathogenic species. Journal of ethnopharmacology, 249, 112376.
15. Ragunath, C., Kousalya, L., Venkatachalam, R., & Anitha, S. (2022). Green synthesis of hydroxyapatite nanoparticles from wrightia tinctoria and its antibacterial activity. BioNanoScience, 12(3), 723-730.
16. Dhanabal, S. P., Raj, B. A., Muruganantham, N., Praveen, T. K., & Raghu, P. S. (2012). Screening of *Wrightia tinctoria* leaves for anti-psoriatic activity. *Hygeia - Journal for Drugs and Medicine, 4*(1), 73-78
17. Khan, N., Ali, A., Qadir, A., Ali, A., Warsi, M. H., Tahir, A., & Ali, A. (2021). GC-MS analysis and antioxidant activity of *Wrightia tinctoria* R. Br. leaf extract. *Journal of AOAC International, 104*(5), 1415-1419.
18. Muthu, S., Gopal, V. B., Soundararajan, S., Nattarayan, K., Narayan, K. S., Lakshmikanthan, M., ... & Perumal, P. (2017). Antibacterial serine protease from Wrightia tinctoria: Purification and characterization. Plant Physiology and Biochemistry, 112, 161-165.
19. Giordani, C., Simonetti, G., Natsagdorj, D., Choijamts, G., Ghirga, F., Calcaterra, A., ... & Pasqua, G. (2020). Antifungal activity of Mongolian medicinal plant extracts. Natural product research, 34(4), 449-455.

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