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An Overview on Commercial Cultivation and Collection Aspects of *Wrightia tinctoria*

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Abstract: *Since ancient times human utilized the nature to cure various ailments. The knowledge of medicinal plants resulted in the development of various indigenous systems of medicine worldwide. Serendipitous discovery as well as scientific approach on the reason for medicinal properties of plants gave the knowledge of chemical constituents such as secondary metabolites in plants. Wrightia tinctoria which is commonly known as 'Danthapala' is a known potential medicinal plant, the leaves of which is traditionally used in the treatment of psoriasis and non-specific dermatitis in Siddha and Ayurvedic systems of medicine and distributed in tropical region belongs to the family Apocynaceae. This plant is beneficial for the treatment of dandruff, various scalp and skin disorders. Phytochemical and pharmacological investigation on the various parts of the plant showed anti-ulcer, anti-inflammatory, analgesic, anthelmintic, anti-cancer, anti-dandruff, wound healing and anti-anxiety activity. The current review focus on providing an update on the recent pharmacological and phytochemical investigations on the plant by researchers around the globe with special emphasis on Antisporiatic, Antifungal, Antibacterial, Antiviral, Cytotoxic, Anti-inflammatory, Anti-diabetic, Analgesic, Hepatoprotective, Anthelmintic, and Wound healing activities.*

Keywords: *Wrightia tinctoria; pharmacological; phytochemical; antisporiatic; wrightial; indurubin.*

I. INTRODUCTION

Wrightia tinctoria (Roxb) R.Br is comparatively a small deciduous tree, bark is scaly and smooth, young parts are glabrous or puberulous. Leaves variable, 7.5-15 cm in length and 2.5-5.7cm in width, shape is elliptic, lanceolate, apex is acuminate, surface glabrous or the young leaves puberulous, base acute or rounded, main nerves 6-12 pairs, petioles are 3- 4mm in length. Flowers are white and fragrant, arranged in lax terminal cymes about 12.5cm diameter with slender dichotomous branches; minute ovate bracts, glabrous calyx, glandular inside; segments 2.5mm in length, oblong, apex rounded and with membranous Since December 2019, a novel coronavirus disease (COVID-19) has rapidly spread across China, triggering a global outbreak and raising significant public health concerns. On January 30, 2020, the World. margins. Corolla is short, tube 3mm long; obtuse; corona of numerous linear scales, some inserts with the filaments & some on the corolla lobes. Fruits, 25-50cm (l) 6- 8mm(w), cylindrical, tapering to both ends, glabrous striate, cohering at the tip. Seeds 1.3- 2cm in length pointed at apex. From a Scottish physician and botanist William Wright (1740 - 1827) *Wrightia* is named. The intention of the current review is to explore different parameters of plant like general description, distribution, chemical constituents, traditional uses and to highlight pharmacological activities studied in recent literatures. The search strategy adopted for this purpose focused on the databases like pubmed, scopus and web of science from inception to 2021.

A. Plant Profile



Fig.1. *W. tinctoria* flowers and pods

- 1) Synonym: Sweet Indrajao, Pala indigo plant, Dyers's oleander, Dantappala, Vetpala
- 2) Kingdom: Plantae
- 3) Order: Gentianales
- 4) Family: Apocyanaceae
- 5) Genus: Wrightia
- 6) Species: tinctoria
- 7) Origin: India, Burma

B. Geographical Distribution

Rajputana, Central provinces, Deccan, Konkan, S.M Country, Circars, W. Ghats of Madras presidency, Ceylon, Burma-Tunor. Seasons Leaves fall in December or January, renewed in April- may. Flowering happens after the leaves, mid May to late June. Fruit conspicuous in November, ripens by the following summer.

C. Uses

The bark and the seeds of *Wrightia tinctoria* used in bilious troubles & flatulence. Seeds are anthelmintic and aphrodisiac. Arthritic fevers are cured by both from the leaves and bark. Decoction prepared from the leaves and bark is stomachic. The dried and ground bark is rubbed over the body in dropsy. The fresh leaves are very pungent and are chewed for relief from tooth ache. The latex produced from the plant is cream in colour and its coagulum is used in code wire insulation, floor furnishings and adhesives. Fresh latex is proteolytic and curdles milk. Bark is especially useful in piles, skin diseases and bilious troubles. Bark is used as tonic.

II. PHARMACOLOGICAL ACTIVITY

A. Antimicrobial Activity

Kannan et al studied *Wrightia tinctoria* leaf extracts screened against skin bacteria and dermatophytes by in vitro. The hexane, methanol and ethanol extracts were tested using agar dilution method and broth micro dilution method. The Methanol and ethanol extracts showed antibacterial activity. The minimum inhibitory concentration of methanol and ethanol extracts were found to be 0.5 mg/ml for *Bacillus subtilis* and *Staphylococcus epidermidis* and 0.25 mg/ml for *Staphylococcus aureus*. The hexane extract was active against *Trichophyton rubrum* and *Trichophyton tonsurans* at 2 mg/ml.

N Al Zaqri et al synthesised zirconium oxide nanoparticles using *Wrightia tinctoria* leaf extract. Green synthesis method was used for the synthesis of ZrO₂- NPs. ZrO₂-NPs formation was confirmed by XRD spectra analysis and DLS. Zeta potential revealed well stabilized ZrO₂-NPs and it exhibited 94% degradation for RY 160 dye. ZrO₂-NPs using *Wrightia tinctoria* leaf extract showed remarkable antibacterial activity.

B. Antiulcer Activity

Wrightia tinctoria methanolic extract (TM) and *Wrightia tinctoria* 70% ethanolic extract (T70E) were studied for antiulcer activity and was compared with carboxy methyl cellulose, pylorus control, Aspirin and standard famotidine. Aspirin plus pylorus ligation induced ulcer model was used for the study. *Wrightia tinctoria* crude extract exhibited excellent antiulcer activity against experimentally induced acute gastric ulcer model

C. Anticancer Activity

S Ramalakshmi et al studied the anticancer property of the leaves of *Wrightia tinctoria* on HeLa Cells. The methanolic extract was evaluated by in-vitro method for cytotoxic effect by employing MTT assay. The potency of each concentration was calculated in terms of percent decrease in viable HeLa cells and compared to the control value. At 76.1 µg/ml crude extract showed antiproliferative activity (IC₅₀). The extract showed dose dependent anticancer effect.

D. Antiinflammatory Activity

PR Tharkar et al investigated the anti- inflammatory activity of bark of *Wrightia tinctoria* by carrageenan- induced rat paw oedema and cotton pellet induced granuloma method The various extracts showed inhibition of rat paw oedema at dose of 200mg/kg and also showed granuloma changes when compared to control group. Diclofenac sodium (13.5 mg/kg /b w, p.o) was used as the standard for comparison.

NA Aleykutty et al studied the dried leaves of *Wrightia tinctoria* for anti-inflammatory and analgesic effects. Antiinflammatory activity was studied by using HRBC membrane stabilization method and carrageenan induced rat paw oedema model. Ethyl acetate fraction exhibited 67.21% protection in rat paw oedema model at a concentration of 400mg/kg. Ethyl acetate fraction also showed remarkable analgesic potential when studied using hot plate method and acetic acid induced writhing in mice.

E. Antidiabetic Activity

AK Shukla and Papiya Bigoniya studied the effect of total flavonoid isolated from *W. tinctoria* seed on alloxan induced diabetic model by assessing body weight change, relative organ weight, BG level, and serum lipid parameters. The effect of *W. tinctoria* seed flavonoid fraction was not significant on hyperglycemia and other disturbed biochemical parameter induced by alloxan, but it has significant effect on normalization of serum creatinine level and lowering of TG and relative weight of liver indicating possible presence of kidney and liver protective property.

R Asok Raj et al evaluated petroleum ether extract of *Wrightia tinctoria* for hypoglycaemic activity in Alloxan-induced diabetic rats. The extract exhibited reduction of serum glucose levels (74.39%) at the dose of 400 mg/kg.

F. Antifungal Activity

K Ponnusamy et al investigated the in vitro antifungal activity of leaf extracts and indirubin, an important constituent of *Wrightia tinctoria*. Leaf extracts showed promising activity against dermatophytic and non-dermatophytic fungi. At dose of 0.5 mg/ml leaf extract was active against *Trichophyton rubrum*, *Epidermophyton floccosum*, *Aspergillus niger* and *Scopulariopsis brevicaulis*. Whereas Indirubin, exhibited activity against dermatophytes such as, *Trichophyton rubrum*, *Trichophyton simii*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Trichophyton tonsurans*. Indirubin also exhibited activity against *Cryptococcus sp.*, *Aspergillus niger*, and *Candida albican*.

G. Wound Healing Activity

M Yariswamy et al evaluated the wound healing potential of *Wrightia tinctoria* latex protease. Excision wound model in mice was used to evaluate the healing potential. Neosporin was used as the standard drug for comparison. The progression of healing was observed using histological examinations, wound contraction, collagen content, catalase and MMP activity. Re- establishment of skin structure, complete epithelialisation and accelerated wound healing were observed by histological examination on day 9 which confirmed the wound healing effect.

H. Antipsoriatic Activity

Antipsoriatic activity of *Wrightia tinctoria* extract was evaluated by mouse tail test. Longitudinal sections of tail skin were prepared and it was stained with hematoxylineosin. Histometrical analysis of specimens showed potent activity of extract (63.94%) than standard isoretinoic acid (48.52%). Both standard and sample increased the epidermal thickness when compared to control.

9. Post Coital Interceptive Activity

G Keshri et al worked on post coital interceptive activity in *Wrightia tinctoria*. 250-mg/kg dose of ethanolic extract of the stem bark inhibited pregnancy in 100% of rats on Days 1– 7 or 1–5 postcoitum. The hexane, chloroform fractions and water soluble and water- insoluble fractions showed 100% anti-implantation effect. The n- butanol fraction intercepted pregnancy only in 75% of animals. They concluded that estrogen- agonistic activity of the active ethanolic extract and its fractions might be responsible for their contraceptive action.

I. Antioxidant Activity

H Jamshed et al screened *Wrightia tinctoria* leaves and seeds for antioxidant potential. The extracts were evaluated using free radical scavenging assays like DPPH and ABTS. Reducing power abilities of extracts were also noted by fluorescence recovery after photobleaching [FRAP] and TAC. In DPPH method IC₅₀ was 45.4 µg/ml and TAC₅₀ mg GAE/g. In ABTS *Wrightia tinctoria* showed IC₅₀ 31.7 µg/ml and FRAP 2.5 mMol Fe+2/g. The results confirmed significant antioxidant potential of *Wrightia tinctoria* compared to other antioxidant-rich medicinal plants.

III. PHYTOCHEMISTRY

The phytochemical constituents of pods without seeds are cycloartenone, cycloartanes, cycloeucaleanol besides alpha and beta amyirin, terpene wrightial, oleanolic acid, ursolic acid and the betasitosterol. Beta amyirin is also present in leaves and stem bark. Stem bark also contains lupeol and beta sitosterol.

A. Phytochemical Studies

Preliminary phytochemical analysis of *Wrightia tinctoria* methanolic extract showed that it contains alkaloids and flavones. The analysis of *Wrightia tinctoria* methanolic extract was conducted using different analytical instruments like UV, HPLC, TLC and GC revealed that indole derivatives like indurubine and isatin were present. The outcome of Gas Chromatographic analysis showed the presence of myristic acid, behenic acid and palmitoleic acid.

The phytochemical investigation of the bark of *Wrightia tinctoria* showed the existence of alkaloids, phenolics, saponins, tannins terpenoids, steroids, triterpenoids, flavonoids and carbohydrates.

Similarly S Sridhar et al found out that carbohydrates, steroids, phenols, saponins, flavonoids, tannins and proteins were present in the leaves of *Wrightia tinctoria*.

A study investigated by SR Sankar et al indicated that alkaloids, terpenoids, glycosides, flavanoids, saponins and phlobatannins were present on the leaves.

Table 1. Pharmacological activities of various parts of *W.tinctoria*

Activity studied	Plant Parts Used
Antisporiatic activity	leaves
Antifungal	leaf
Antifungal	leaf
Antibacterial and antifungal	leaf
Antibacterial	leaf
Antibacterial	bark
Wound healing	latex
Anthelmintic	leaf
Analgesic	leaf
Cytotoxic	Stem bark
Cytotoxic	Flowers
Cytotoxic	Leaves
Anti-inflammatory	Stem bark
Anti-inflammatory	Stem bark
Anti-inflammatory	Leaf
Anti-inflammatory	Leaf

Indigotin, isatin, and anthranillate as vital constituents of *Wrightia tinctoria* by HPTLC, HPLC, UV-VIS, IR and EI-MS. Indigotin is present in fresh leaves of live plants and indirubin is formed during drying after collecting of the leaves. This conversion is due to hydrolytic reaction and auto-oxidation. The variation of chemical constituents of leaves due to seasonal changes was investigated using HPTLC and HPLC techniques, and showed a steady rise in indigotin-indirubin concentration from August to November, whereas isatin and anthranillate concentration rose during December and January.

Autoxidation of indigotin resulted in the production of isatin.

Along with the phytochemicals, *W. tinctoria* is also found to contain important enzyme. Proteases are commercially important class of enzymes and the hydrolytic property of the enzyme is exploited in various biotechnological processes. The researchers isolated Wrightin, serine protease, from the sap of *Wrightia tinctoria*, which is an economical source of protease for commercial use. The plant also has Wrightial, a triterpenoid, besides, Cycloartenone, Cycloeucaenol, β amyrin, and β -sitosterol as phytochemicals.

By comparing a synthetic genuine molecule to a novel sterol isolated from the unsaponifiable lipid of *Wrightia tinctoria* seed, it was determined to be 14- methylzymosterol.

Desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24- dehydropollinastanol, four rare sterols, were extracted and identified.

To concentrate the milk clotting proteases, a study conducted by Rajagopalan et al isolated proteases from *Wrightia tinctoria* bark and partially purified them using a non- chromatographic approach called three phase partitioning (TPP). The interfacial phase (IP) with 60 percent ammonium sulphate and 1:1 crude enzyme to t-butanol yielded the highest recovery and purification fold of protease activity. The enzyme fraction's optimal pH and temperature were found to be 7.5 and 50 degrees Celsius, respectively. Inhibition studies revealed its serine nature. Non-denaturing PAGE, Zymography, and 2D PAGE of IP revealed the existence of three caseinolytic proteases with molecular weights of 95.62 kDa, 91.11 kDa, and 83.23 kDa, respectively, and pI values of 3.89, 5.45, and 5.43. IP in both aqueous and lyophilized form was exceptionally stable, retaining full activity for 3 weeks at 4 °C.

IV. CONCLUSION

Use of *Wrightiatinctoria* in ayurvedi and siddha system of medicine for its effects against psoriasis and epidermal thickening and drying problems. It is added in hair oil preparations as it effectively minimises dandruff. Th pharmacological studies proves its pharmacological significance such as antiviral, anti-inflammatory, cytotoxic, hepatoprotective, wound healing, post coital interceptive, anthelmintic, antinociceptive, antioxidant, antiviral, antifungal, antibacterial, antidandruff and antipsoriatic activity. Total flavonoid isolated from *W. tinctoria* seed lack hypoglycemic effect.

Alkaloids, saponins, terpenoids, flavones, triterpenoids, tannins, steroids, carbohydrates, glycosides, Indole derivatives like isatin and indurubine, and fixed oils like myristic acid, palmitoleic acid, behenic acid, acid indigoid compounds reflects its phytochemical abundance. So the present study suggests that the proved phytochemical and biological characteristics makes *Wrightia tinctoria* a promising drug to the pharmaceutical industries and a good candidate for more exploration to the future.

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