

A review on phytochemical, pharmacological, and pharmacognostical profile of *Wrightia tinctoria*: Adulterant of kurchi

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ABSTRACT

Wrightia tinctoria R. Br. belongs to family Apocynaceae commonly called as Sweet Indrajao, Pala Indigo Plant, Dyer's Oleander. "Jaundice curative tree" in south India. Sweet Indrajao is a small, deciduous tree with a light gray, scaly smooth bark. Native to India and Burma, *Wrightia* is named after a Scottish physician and botanist William Wright (1740-1827). Sweet Indrajao is called dhudi (Hindi) because of its preservative nature. The juice of the tender leaves is used efficaciously in jaundice. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases. Oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory, and anti-pyretic activities and to be effective in the treatment of psoriasis. The plant is reported to contain presence of flavanoid, glycoflavones-iso-orientin, and phenolic acids. The various chemical constituents isolated from various parts of the plant are reported as 3,4-Seco-lup-20 (29)-en-3-oic acid, lupeol, stigmasterol and campesterol, Indigotin, indirubin, tryptanthrin, isatin, anthranillate and rutin Triacontanol, Wrightial, cycloartenone, cycloeucalenol, β -amyrin, Alpha-Amyrin, and β -sitosterol, 14 α -methylzymosterol. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol, were isolated and identified in addition to several more common phytosterols. The Triterpinoids components of the leaves and pods of *Wrightia tinctoria* also isolated. This article intends to provide an overview of the chemical constituents present in various parts of the plants and their pharmacological actions and pharmacognostical evaluation.

Key words: Pharmacology, phytochemicals, therapeutic uses, *wrightia tinctoria*

INTRODUCTION

Wrightia tinctoria R.Br. (Family: Apocynaceae) commonly called "Indrajau" is distributed throughout the world and occurs abundantly in India. It is a deciduous tree with white fragrant flowers. The seeds and bark of this plant are used in Indian traditional medicine as anti-diarrheal and anti-dysenteric.^[1] Sweet Indrajao is a small, deciduous tree with a light gray,

scaly smooth bark. Native to India and Burma, *Wrightia* is named after a Scottish physician and botanist William Wright (1740-1827). From a distance, the white flowers may appear like snowflakes on a tree. The fruits pendulous, long-paired follicles joined at their tips. The hairy seeds are released as the fruit dehisces. The leaves of this tree yield a blue dye called Pala Indigo. Sweet Indrajao is called dhudi (Hindi) because of its preservative nature. Supposedly, a few drops of its sap in milk prevent curdling and enhance its shelf life, without the need to refrigerate. The wood of Sweet Indrajao is extensively used for all classes of turnery. It is made into cups, plates, combs, pen holders, pencils, and bedstead legs. It is commonly used for making Chennapatna toys.^[2]

Medicinal uses: Ethnomedically, the bark of this plant is used as a galactagogue to treat abdominal pain, skin diseases and wounds,^[3] as an anti-pyretic,^[4] anti-dysenteric, anti-diarrheal- and anti-hemorrhagic^[5] agents, and as an antidote for snake poison.^[6] Seeds of this plant are also used as an aphrodisiac.^[7] In view of the reported severe health hazards of estrogen, such as increased risk of endometrial hyperplasia and carcinoma,^[8,9] breast cancer,^[10] and thromboembolic diseases.^[11] A large number

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of natural products showing promising anti-fertility activity in preliminary studies could not be pursued due to their associated estrogen-agonistic activity.^[12]

The leaves are applied as a poultice for mumps and herpes. Sometimes, they are also munched to relieve toothache. In folk medicine, the dried and powdered roots of *Wrightia* along with *Phyllanthus amarus* (keezhanelli) and *Vitex negundo* (nochi) are mixed with milk and orally administered to women for improving fertility. The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and hence is used in hair oil preparations.

Pharmacognostical evaluation

A preliminary pharmacognostical study on the leaves of *Wrightia tinctoria* (Roxb) R.Br. studied to determine various parameters of pharmacognostical standards such as ash values, extractive values, phytochemical tests, and microscopical characters of leaf powder. The shade-dried powder and various solvent extracts (*viz.*, methanol, 70% ethanol, aqueous, dichloromethane, chloroform, ethyl acetate, and petroleum ether) have been analyzed for their phytoconstituents and fluorescence characters. The methanolic extract was found to contain presence of triterpenes. The data generated for the pharmacognostical evaluation on *Wrightia tinctoria* leaves may be useful for establishing the standardization protocols. The HPTLC analysis data indicated that the collected *Wrightia tinctoria* leaves contain 47.6 mg of lupeol/g of the total methanolic extract.^[13]

Pharmacognostical and physicochemical standardization of ethnopharmacologically important seeds of *Lepidium sativum* Linn. and *Wrightia tinctoria* R. Br. Performed. The morphological, microscopical, and physicochemical standards developed in this study will provide referential information for identification of these crude drugs and standardization. Quality control standardizations of the various medicinal plants used in traditional medicine is becoming more important today in view of the commercialization of formulations based on these plants. *Lepidium sativum* Linn. and *Wrightia tinctoria* R. Br. seeds are evaluated as per WHO recommendation, various physicochemical and phytochemical evaluation parameters for quality control of medicinal plants are performed. In view of their medicinal importance and taxonomic confusion, morphology and microscopy, physico-chemical parameters, fluorescence analysis, preliminary phytochemical screening, and quantitative estimation were performed to establish the salient diagnostic characters.^[14]

The present paper deals with the pharmacognostical study of leaf of *Wrightia tinctoria* for its identification and to distinguish it from the co-existing weeds and adulterants. It has been used mainly for psoriasis and some other disorders. Since there is no proper information regarding this plant, efforts were devoted to study the pharmacognostical properties of this plant.^[15]

Wrightia tinctoria (Indrajao), belonging to the family of Apocynaceae, is distributed in Rajasthan, Madhya Pradesh, and in Tamil Nadu. The plant is used in Siddha system for treating psoriasis, snake bites, and various inflammations. The present study is carried out to determine the pharmacognostical parameters and anti-microbial properties. It includes the transverse section, powder microscopy, physicochemical parameters, and anti-microbial studies.^[16]

The bark of *W. tinctoria* is used as an adulterant for the well-known drug, *Holarrhena antidysenterica*. The pharmacognostic characters of *W. tinctoria* (collected from India) are presented. Such characters can be used to enable to identification of this herbal drug.^[17]

H. antidysenterica and *H. pubescens*, an ingredient of the formulation Kurchi Bismuth Iodide, has often been confused and adulterated with another member of the same family, *W. tinctoria*. A comparative study was carried out on seeds of both medicinal plant species. Ash values, extractive values, and results of elemental analysis are reported, and physical characteristics of the seeds are described. The chloroform and methanolic extracts of both seeds showed anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The bitter value was determined as 11 000 for *H. antidysenterica*; *W. tinctoria* seeds are tasteless.^[18]

Seeds of *H. antidysenterica* and *H. pubescens* are used in India for treating dysentery and a wide range of other digestive disorders. In appearance, they resemble those of *W. tinctoria*, which do not have the same medicinal properties. Data are presented on seed characteristics with distinguishing features.^[19]

Pharmacological evaluation

Anti-psoriatic activity

The hydro-alcoholic extract of *Wrightia tinctoria* leaves was evaluated for anti-psoriatic activity by mouse tail test. Anti-psoriatic activity was performed at a dose 200 mg/kg body weight in mice (25-30 g). Isoretinoic acid (0.5 mg/kg) was used as the standard. Degree of orthokeratosis, drug activity, and the relative epidermal thicknesses were calculated and statistically analyzed. The extract was also evaluated for its antioxidant potential by DPPH, nitric oxide, and hydrogen peroxide radical scavenging assays. The extract produced significant ($P < 0.01$) degree of orthokeratosis compared to control, and the drug activity was found to be 70.18%, which is more potent than the standard (57.43%). The extract showed prominent antioxidant activity in all the assays. The present study concludes that the selected plant has anti-psoriatic activity and can be used for psoriasis treatment.^[20]

Anti-diabetic activity

In the present study, investigation has been carried out to evaluate the effect of the different extracts of the leaves of *Wrightia tinctoria* on alloxan-induced diabetic rats of wistar strain. The experiment was carried out using six groups of albino rats.

Chloroform extract showed a significant anti-diabetic activity when compared to the standard drug glibenclamide.^[21]

Anti-diabetic activity

The present work was undertaken to investigate various extracts of fruit of *Wrightia tinctoria* (Family- Apocynaceae) for anti-diabetic activity in alloxan-induced diabetic rats. A comparison was made between the action of extracts and known anti-diabetic drug glibenclamide (10 mg/kg body weight). Oral administration of methanolic extract at a dose of 300 mg/kg/b. wt and ethyl acetate extract at a dose of 200 mg/kg/b. wt exhibited a significant ($P < 0.001$, $P < 0.001$) hypoglycemic activity in normal rats and significant ($P < 0.001$, $P < 0.001$) anti-hyperglycemic activity in alloxan-induced diabetic rats, respectively. The maximum reduction in blood glucose level was observed after 4 hours in case of methanolic and ethyl acetate extracts with a percentage protection of 37% and 42%, respectively. In long-term treatment of alloxan-induced diabetic rats, the degree of protection was determined by measuring blood glucose on 0, 1, 2, 4, 7, 14th day. Both the extracts showed a significant anti-diabetic activity comparable with that of glibenclamide. These results indicate that the *W. tinctoria* fruit extracts possess significant anti-diabetic activity.^[22]

Anti-microbial properties

The present investigation focuses on *in vitro* anti-microbial properties and phytochemical analysis of aqueous and methanolic extracts of two different colored mature seed varieties of *Wrightia tinctoria*. The phytochemical screening revealed the presence of carbohydrates, reducing sugars, alkaloids, sterols, glycosides, phenolics, tannins, flavonoids, and amino acids. Greater effectivity was observed against gram-positive bacterial pathogens such as *Staphylococcus aureus* ATCC 25923, *S. aureus*, *S. citreus*, and *B. cereus* than the gram-negative strains. The methanolic seed extracts were largely inhibitory against pathogenic yeasts like *Trichophyton rubrum*, *Candida albicans*, *C. parapsilosis*, and *Cryptococcus*. The results indicated that the methanolic extract of the brown variety seeds is pharmacologically more active than that of the beige variety seeds. The aqueous extracts of both the seed varieties were moderately effective against *S. aureus* ATCC 25923 and *S. citreus* with no effect against the fungal strains.^[23]

Anti-diabetic activity

In the present investigation, the methanolic extract of leaves of *Dodonaea viscosa* (*D. viscosa*) and pods of *Wrightia tinctoria* (*W. tinctoria*) were evaluated for anti-diabetic activity. The anti-diabetic activity was studied using the glucose uptake by isolated rat hemi-diaphragm *in vitro* model. The value of glucose uptake by rat hemi-diaphragm for *D. viscosa* was 13.80 ± 0.1697 and for *W. tinctoria* was 9.384 ± 0.3944 as compared to control (5.34 ± 0.12) and insulin 15.45 ± 0.12 in mg/g/min. The results strongly suggest that *D. viscosa* will be alternative choice for the treatment of diabetes mellitus caused in the consequences of resistance to stimulatory effect of insulin on Glut-4 protein.^[24]

Anti-ulcer activity

The purpose of the present study was aimed at evaluating the anti-ulcer activity on leaves of *Wrightia tinctoria* on albino rat. The anti-ulcer activity of the *Wrightia tinctoria* methanolic extract (TM) and *Wrightia tinctoria* 70% ethanolic extract (T70E) were compared with carboxy methyl cellulose (CMC), pylorus control, aspirin, and standard famotidine, which was evaluated by employing aspirin plus pylorus ligation-induced ulcer model. The biochemical parameters like volume of gastric juice secretion, pH, free acidity, total acidity, and ulcer index and percentage inhibition were studied at the concentration of 200 mg/kg body weight. The plant methanolic extract showed significant gastro-protective activity of 65.89% when compared with the standard drug famotidine (20 mg/kg), which showed 75.34%. The result suggested that the methanolic extract of *Wrightia tinctoria* leaves possesses anti-ulcer effect. The observed effect may be due to the presence of bioactive constituents.^[25]

Free radical scavenging activity

Attempt has been made to evaluate reducing power and free radical scavenging activity of ethanolic extract of *Wrightia tinctoria* Roxb bark and *Schrebera swietenoides* Roxb bark individually. *In vitro* antioxidant evaluation was done by measuring the reducing power and inhibition of superoxide production. The results suggest that the ethanolic bark extract of the *Wrightia tinctoria* Roxb and *Schrebera swietenoides* Roxb has the ability to suppress the oxidation, and it was also found that *Schrebera swietenoides* Roxb extract has more activity than *Wrightia tinctoria* Roxb extract.^[26]

Toxicological profiles

The research work was conducted with the leaf solvent extracts of *Wrightia arborea* and *Wrightia tinctoria* to make toxicological profiles by employing Brine Shrimp Assay method (BSA) (*Artemia Salina* LEACH). The LC₅₀ values were determined for both the plant solvent extracts respectively in mg/ml of active compounds and extracts. It was found that the leaf ethanolic and methanolic extracts were toxic for the Brine Shrimp Naupli. The results indicated that *Wrightia tinctoria* leaf ethanol (70%) extract and methanolic extract showed LC₅₀ values of 471.604 and 517.038 mg/ml, respectively. While the *Wrightia arborea* leaf ethanol (70%) extract and methanolic extracts showed LC₅₀ values of 498.213 and 531.082 mg/ml, respectively. The remaining solvent extracts showed no toxicity (as found more than 1000 mg/ml) in BSA method.^[27]

Acute oral toxicity investigation

Abelmoschus manibot and *Wrightia tinctoria*, belonging to the botanical family Malvaceae and Apocynaceae, have been traditionally used by the locals in India for treatment of various ailments. The current study reports the outcome of acute oral toxicity investigation of *Abelmoschus manibot* and *Wrightia tinctoria* on ICR mice. No mortalities or evidence of adverse effects have been observed in ICR mice following acute oral administration at the highest dose of 2500 mg/kg crude extracts of *Abelmoschus manibot* and *Wrightia tinctoria*. This is the first report on the acute oral toxicity of *Abelmoschus manibot* and *Wrightia tinctoria*, and the findings of this study are in agreement with those of *in vitro*

experiments and thus provide scientific validation on the use of the leaves of *Abelmoschus manibot* and *Wrightia tinctoria*.^[28]

Anthelmintic potential

The present communication deals with the comparative studies on anthelmintic potential of methanolic and aqueous extracts of *Cymbopogon citratus* and *Wrightia tinctoria* against *Pheritima posthuma*. Methanolic and aqueous extracts of both were used as test solutions. Piperazine citrate was used as standard drug and normal saline as a control. Study involved the determination of time of paralysis as well as time of death of worms. The results revealed that methanolic extract of *Cymbopogon citratus* leaves have better anthelmintic activity than that of *Wrightia tinctoria* extracts. Further, it will be interesting to isolate the active chemical constituents from both the plants.^[29]

Anti-diabetic activity

The aim of this study is to evaluate the anti-diabetic activity of two Indian Ayurvedic herbs using an oral glucose tolerance test and blood insulin levels to understand the mechanism of action using the Zucker diabetic rat model. Herbal extracts of *Wrightia tinctoria* and *Parthenocissus quinquefolia* at a dose of (250 mg/kg body weight) were used throughout the study. Following a glucose challenge of 2 gm/kg using oral gavage, a timed glucose tolerance test was used to determine the ability of these extracts to alter glucose levels in diabetic animal model. The glucose-lowering activities of these extracts were then compared to the controls. Both tested herbal extracts have shown to exhibit significant ($P < 0.05$) hypoglycemic activity compared to the control. *W. tinctoria* and *P. quinquefolia* have an anti-diabetic activity, which reduced the blood glucose level in oral glucose tolerance test significantly compared with the control. To further understand their mechanism of action, blood insulin levels were also studied using an insulin Elisa assay. These studies revealed that the herbal extract of *P. quinquefolia* has direct correlation between glucose and insulin levels. However, *W. tinctoria* significantly lowered blood glucose levels ($P < 0.05$), while it did not show any correlation between blood glucose and insulin levels. Based on these findings, it can be concluded that hypoglycemic effects of *Wrightia tinctoria* are more complicated than *P. quinquefolia* and may involve other possible mechanism.^[30]

Cytotoxic activity

The cytotoxic activity of the alcoholic extracts of some traditional plants of Chhattisgarh state, India used to treat cancer. *In-vitro* cytotoxic activity of alcoholic extracts of five plants i.e. *Artocarpus heterophyllus*, *Alangium salvifolium*, *Buchanania lanzan*, *Sesbania grandiflora*, and *Wrightia tinctoria* was studied against human breast cancer (MCF-7) and human leukemia (HL-60) tumor cell lines using the thiazolyl blue test (MTT) assay. From the result, it can be found that the *Sesbania grandiflora* extract has potent *in vitro* cytotoxic activity.^[31]

Wound healing

In recent years, oxidative stress and free radicals have been implicated in impaired wound healing. *Abelmoschu manibot* (L.)

Medik, Malvaceae and *Wrightia tinctoria* R.Br, Apocynaceae plants, widely used in Ayurveda, possesses anti-inflammatory and anti-microbial properties. The present study was undertaken to assess the potential of petroleum ether and methanolic extracts in wound healing in Wistar albino rats. The rats were divided into six groups of six animals each. Group 1 is normal wounded control, group 2 received standard drug, and the other 4 groups were treated with two different doses each of petroleum ether and methanolic extract of *A. manibot* and *W. tinctoria*. The wound healing parameters were evaluated by using incision wounds in extract-treated rats, standard, and controls. Both the doses of petroleum ether and methanolic extract significantly increased wound breaking strength when compared with the control group.^[32]

Anti-inflammatory activity

In the present study, the bark of *Wrightia tinctoria* was investigated for anti-inflammatory activity by carrageenan-induced rat paw edema and cotton pellet-induced granuloma method. The various extracts showed inhibition of rat paw edema and percent granuloma changes at dose of 200 mg/kg when compared to control group. The activity was compared with that of standard drug diclofenac sodium (13.5 mg/kg/bw, p.o).^[33]

Anthelmintic activity

The aim of the present study was to determine the anthelmintic activity of crude petroleum ether and chloroform extracts of leaves of *Wrightia tinctoria* using *Pheretima posthuma*. Three concentrations (2.5, 5.0, 7.5 mg/ml) of each extracts were studied in the activity, which involved the determination of time of paralysis and time of death of the worms. Piperazine citrate is used as standard reference and normal saline as control. The present study proves the potential usefulness of leaves of *Wrightia tinctoria* as comparable anthelmintic agent.^[34]

Anti-fungal activity

Present study was designed to investigate the *in vitro* anti-fungal activity of certain medicinal plants and the pure compound indirubin isolated from *Wrightia tinctoria*. The hexane, chloroform, methanol, and ethanol extracts of six different plants were investigated against dermatophytes, non-dermatophytes, and yeasts. Chloroform extract of *Wrightia tinctoria* leaf was fractionated using column chromatography, and the major compound was identified using spectroscopic techniques. Anti-fungal activity was studied by spore germination test using agar dilution method. The minimum inhibitory concentration (MIC) was determined using broth micro dilution method. *Wrightia tinctoria* showed promising activity against dermatophytic and non-dermatophytic fungi. Leaf chloroform extract showed activity at 0.5 mg/ml against *Trichophyton rubrum*, *Epidermophyton floccosum*, *Aspergillus niger*, and *Scopulariopsis brevicaulis*. The major compound, identified as indirubin, exhibited activity against dermatophytes such as *Epidermophyton floccosum* (MIC = 6.25 µg/ml); *Trichophyton rubrum* and *Trichophyton tonsurans* (MIC = 25 µg/ml); *Trichophyton mentagrophytes* and *Trichophyton simii* (MIC = 50 µg/ml). It was also active against non-dermatophytes (*Aspergillus niger*, *Candida*

albicans, and *Cryptococcus* sp.) within a MIC range of 0.75-25 µg/ml. The indole compound indirubin from *Wrightia tinctoria* showed anti-fungal activity and may be useful in the treatment of dermatophytosis.^[35]

Wrightia tinctoria was investigated for the preliminary phytochemical analysis and characterization by various instrumental techniques. Indole derivatives such as isatin, indirubine, tryphanthrine, and fatty acids were identified. Methanolic extract of leaf parts of *Wrightia tinctoria* (WT) have been studied against replication of HCV in Huh 5.2 cells. The 50% effective concentration for inhibition of HCV in RNA sub-genomic replicon replication in huh 5-2 cells (luciferase assay) by CWT was found to be 15 µg/mL. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells by 50% was greater than 50 µg/mL.^[36]

Hematological, biochemical, histological, and antioxidant enzyme status

The effect of sub-acute administration of *W. tinctoria* bark extract on some hematological, biochemical, histological, and antioxidant enzyme status of rat liver and kidney investigated, following 21 and 45 days treatment. The animals were observed for gross physiological and behavioral responses, food and water intake, and body weight changes. Free radical scavenging activity and histopathology was done on liver and kidney samples. *W. tinctoria* showed significant hemopoiesis with increase in body weight signifying anabolic effect. It significantly reduced serum SGOT level and increased glucose levels. *W. tinctoria* caused increased SOD activity of liver along with catalase of both liver and kidney and decreased liver peroxidase ($P < 0.001$). These features indicate that *W. tinctoria* upto 1000 mg/kg daily dose is safe and has potential to be consumed for long time in management of various diseases.^[37]

Anti-nociceptive activity

The pharmacological profile of hydro-alcoholic extract of *Wrightia tinctoria* (Roxb) R. Br. investigated in mice and rats using various models. The effects of the extract were observed in three different dose levels 300, 500, and 1000 mg/kg as extract does not show any sign of toxicity up to 3000 mg/kg dose. Investigations were carried out against thermal, chemical, and mechanical noxious stimuli to study anti-nociceptive activity and on pentobarbitone-induced hypnosis. Carrageenan-induced paw edema and cotton pellet-induced granuloma model were employed to test anti-inflammatory activity. The parameters taken for diuretic activity were urine volume and renal excretion of Na⁺, Cl⁻, and K⁺ ions. Study revealed moderate analgesic effect against thermal ($P < 0.001$ to 0.01) and chemical ($P < 0.05$) noxious stimuli and anti-inflammatory activity ($P < 0.001$ to 0.01) at the 1000 mg/kg dose. Extract is devoid of any sedative activity. *Wrightia tinctoria* extract considerably increases urine volume, acting as strong kaliuretic.^[38]

Anti-bacterial activity

The anti-bacterial activity of petroleum ether (60-80°), 95% alcohol, and 40% aqueous alcohol extracts of bark of *W. tinctoria*

was evaluated against Gram-positive and Gram-negative organisms by cup plate diffusion method. The 95% alcohol and 40% aqueous alcohol bark extracts exhibited anti-bacterial activity against the tested organisms.^[39]

Pregnancy-interceptive activity

The pregnancy-interceptive activity of the stem bark of *Wrightia tinctoria* R.Br. (Family Apocynaceae) investigated during the pre implantation, peri-implantation, and early post-implantation periods by oral route in adult female Sprague-Dawley rats. The ethanolic extract of the stem bark and its serial fractions were administered to female rats on days 1-7 or 1-5 post-coitum (Day 1: Day of sperm-positive vaginal smear) by the oral route. At autopsy on day 10 post-coitum, the number and status of corpora lutea and implantations were recorded. For estrogen-agonistic activity, immature rats ovariectomized 7 days earlier received the test extract or the vehicle once daily for 3 days and, at autopsy on day 4, uterine weight, status of vaginal opening, and extent of vaginal cornification were recorded. The ethanolic extract of the stem bark of *W. tinctoria* R.Br. inhibited pregnancy in 100% of rats when administered orally at a 250 mg/kg dose on days 1-7 or 1-5 post-coitum. On fractionation, the hexane-soluble, chloroform-soluble, water-soluble, and water-insoluble fractions showed 100% anti-implantation effect, while *n*-butanol-soluble fraction intercepted pregnancy in 75% of animals when administered in the days 1-5 post-coitum schedule. In immature rat bioassay, the active ethanolic extract and its fractions exhibited moderate to potent estrogen-agonistic activity, which might be responsible for their contraceptive action in this species. Findings demonstrate the anti-fertility activity of the ethanolic extract of the stem bark of *W. tinctoria* and its hexane-soluble, chloroform-soluble, water-soluble, and water-insoluble fractions. Studies that pursue promising natural products (to identify contraceptive agents from natural sources lacking potent estrogenic activity) towards a fruitful conclusion for development/lead generation should continue.^[40]

Anti-ulcer activity

Evaluation of the anti-ulcer activity of *Wrightia tinctoria* bark extract investigated in induced acute gastric ulcers in rat.^[41]

Wound-healing activity

The wound-healing activity of ethanol extract of *W. tinctoria* bark screened by using incision, excision, and dead space wound models and evaluated histopathological and biochemical changes of granuloma tissue. The bark powder of *W. tinctoria* was extracted with 95% ethanol by continuous heat extraction and was subjected to phytochemical investigation and screened for wound-healing activity in the incision, excision, and dead space wound models in rats. A supportive study made on granuloma tissue to estimate the hydroxyproline content and histopathological examination to determine the pattern of lay-down for collagen using Masson Trichrome stain. Triterpenoids, steroids, and saponins were present in ethanol extracts of barks of *W. tinctoria*. In the re-sutured incision wound model, the ethanol extract showed significant

breaking strength ($P < 0.01$) compared to the control. The ethanol extract promotes better wound-healing by increasing the percentage wound closure and decreasing epithelization time ($P < 0.001$) compared to the control. Statistically significant increase ($P < 0.001$) was observed in breaking strength and hydroxyproline content of ten-day-old granuloma of drug-treated animals compared to control animals in the dead space wound model. The results of the present study reveal that ethanol extract of bark of *W. tinctoria* have significant wound-healing activity. The pro healing action seems to be due to the increased synthesis of collagen, its cross-linking as well as better alignment and maturation. This may be attributed to the presence of triterpenoids in the title plant.^[42]

Phytochemical evaluation

The present paper deals with HPTLC finger printing studies on two ethnomedicinally important wrightia species, viz., *Wrightia tinctoria* and *Wrightia arborea*. The high performance thin layer chromatographic finger print parameters have been developed for methanolic leaf extracts to fix standards. At shorter (254 nm) and longer (366 nm) wavelength, the resolution was better for these extracts and hence, these wavelengths can be taken for obtaining optimum HPTLC finger printing for this medicinal plant.^[43]

777 Oil is a topically applied Ayurvedic formulation used for the effective treatment of psoriasis. The formulation is composed of leaf extract of *Wrightia tinctoria* and *Oleum Cocos nucifera*. A selective, sensitive, and reproducible HPLC method was developed for analyzing marker compound of *Wrightia tinctoria* (Rutin) in 777 Oil for routine standardization purpose. The chromatography was performed on Phenomenex C₁₈ (250 × 4.6 mm, 5.0 μm particle) column using methanol-water (60:40, v/v) as mobile phase; adjusted to pH 3.0 by orthophosphoric acid. The flow rate was 1.0 mL/min. with detection at 360 nm. The values of retention times and capacity factor were 3.88 and 0.40, respectively. The calibration plot showed a good linear relationship between response curve and concentration in the range of 1.0-1000.0 μg mL⁻¹ with regression coefficient 0.9998. The detection (LOD) and quantification (LOQ) limits were found 27.0 and 95.0 ng mL⁻¹, respectively. The statistical analysis proved that the method was precise, reproducible, selective, and accurate for the analysis of rutin in 777 Oil. The developed HPLC method is useful for the qualitative and quantitative estimation of rutin in 777 Oil and other products of traditional systems of medicine.^[44]

Aim of this study was to identify and characterize the bioactive principles from the woody stem of *Wrightia tinctoria*. For isolation of the compounds, the powder of dried woody stem of *Wrightia tinctoria* was subjected to hot extraction with petroleum ether and subjected to chromatography. Three compounds (PEW-1, PEW-2, and PEW-3) were isolated and purified by chloroform. Mass spectrum of PEW-1, PEW-2, and PEW-3 showed a parent molecular ion [M⁺] peak at m/z 426, which corresponds to the molecular formula C₃₀H₅₀O, 412 corresponds to C₂₉H₄₈O, and 400 corresponds to C₂₈H₄₆O. In the ¹H-NMR spectrum of PEW-1, H-3 proton appeared as a triplet of a double doublet (tdd) at δ

3.21, H-29 proton gives two multiplets at δ 4.71 and δ 4.56, in ¹H-NMR spectrum of PEW-2, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.62, and H-6 olefinic proton showed a multiplet at δ 5.14. Two olefinic protons appeared downfield at δ 4.16 (m) and δ 4.14 (m). Six methyl proton appeared at δ 1.27, δ 1.19, δ 1.07, δ 1.00, δ 0.98, and δ 0.91 for methyl group and in the ¹H-NMR data of PEW-3, H-3 proton appeared at δ 3.21 as a triplet of a double doublet H-6 olefinic proton showed a multiplet at δ 5.10 and six methyl proton appeared at δ 1.27, δ 1.14, δ 1.09, δ 1.00, δ 0.98, and δ 0.95 for methyl group. From the physical, chemical, and spectral characteristics, PEW-1, PEW-2, and PEW-3 were concluded as lupeol, Figure 1 stigmasterol, and campesterol.^[45]

3,4-Seco-lup-20 (29)-en-3-oic acid, Figure 2 a rare triterpene, was isolated from *Wrightia tinctoria* R.Br (Family: Apocynaceae). It appeared promising to study the structural chemistry of this compound because of its unique bioactivity. X-ray diffraction analysis demonstrated that this compound consists of three six-membered rings and one five-membered ring. The first six-membered ring (A) is in the twist boat form while the remaining two six-membered rings (B, C) are in the chair form. The crystal of this compound belongs to the monoclinic crystal system and space group P21. Lattice constituents are as follows: a = 13.074 (6) Å, b = 11.972 (5) Å, c = 17.394 (7) Å; a = 90.00, b = 98.20 (13), c = 90.00; V = 2695 (2) Å³, d = 1.094 Mg/m³, Z = 4. The optimized geometry of the reported molecule has been calculated with the DFT/B3LYP theory using 6-31G (d, p) basis set.^[46]

Chemical constituents were isolated from leaves of *W. tinctoria*, *W. tomentosa* [*W. arborea*], and *W. coccinea* (collected from India). Indigotin, indirubin, Figure 3 tryptanthrin, isatin, anthranillate, and rutin [rutoside] were isolated and identified as major constituents of *W. tinctoria* and *W. tomentosa*. Anthranillate and rutin were the major constituents of *W. coccinea*. Indigotin was found in fresh plant material, and indirubin was an artifact formed during the drying process after harvesting. Seasonal variation in the chemical constituents of leaves was studied using HPTLC and HPLC analyses; similar variation patterns in the 3 species were observed. The concentration of indigotin-indirubin combination steadily increased from August to November. In contrast, concentration of isatin and anthranillate increased in December and January, at the expense of indigotin-indirubin. Isatin was produced by the autoxidation of indigotin. Tryptanthrin concentration also

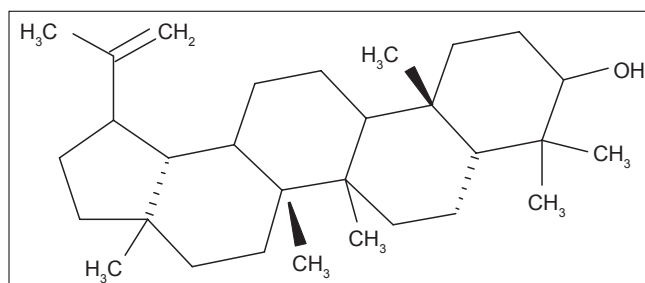


Figure 1: Lupeol

increased, periodically, in May (at the expense of isatin) and in January. Plausible pathways for the formation of these indole metabolites are appraised on the basis of circumstantial and synthetic evidence.^[47]

Triacontanol and tryptanthrin Figure were newly isolated from *Wrightia tinctoria* leaves, collected from Pacha-Palode, Kerala, India, in July 1994.^[48]

W. tinctoria is used in Indian traditional medicine to treat psoriasis, stomach pains, toothache, and as an anti-dysenteric. Wrightial, Figure 5 and 4 known compounds (cycloartenone, cycloeucalenol, β -amyrin, and β -sitosterol), were isolated from the MeOH extract of the immature seed pods of *W. tinctoria* (collected from the Mannanoor forest, Andhra Pradesh, India). The structure of wrightial was established from spectral analysis and by chemical correlation.^[49]

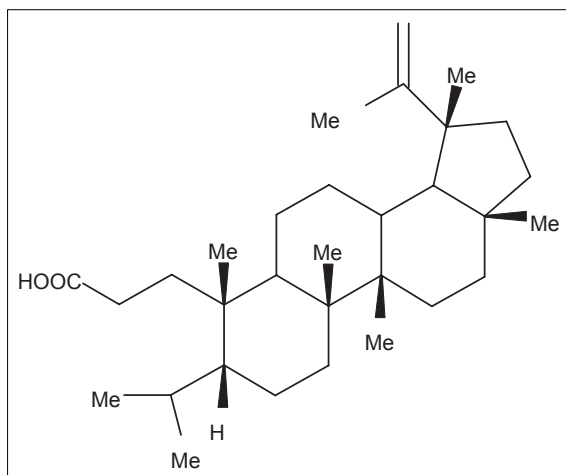


Figure 2: 3, 4-Seco-lup-20 (29)-en-3-oic acid

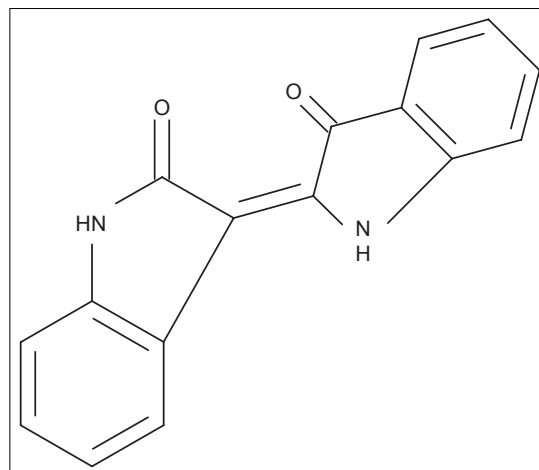


Figure 3: Indirubin

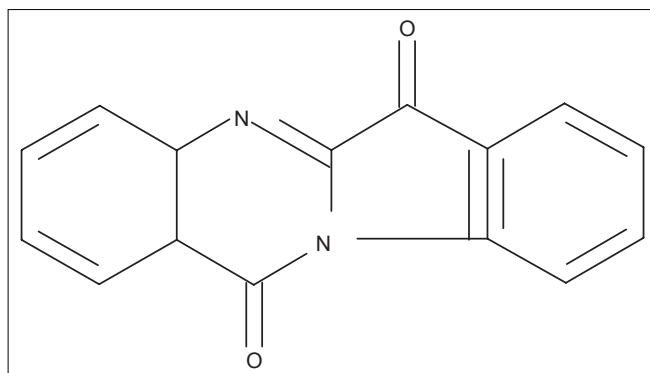


Figure 4: Tryptanthrin

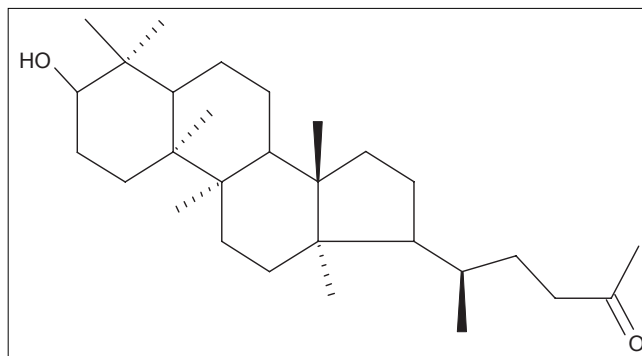


Figure 5: Wrightial

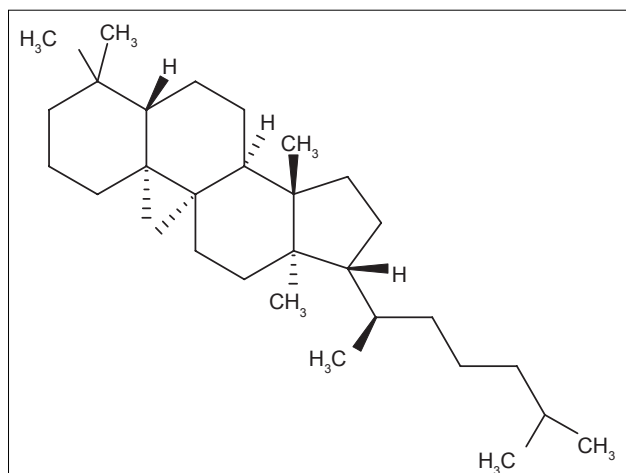


Figure 6: Cycloartenone

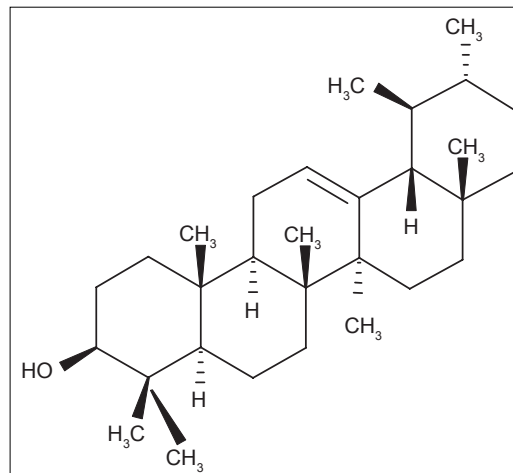


Figure 7: Alpha-Amyrin

W. tinctoria is used in traditional medicine to treat psoriasis. Cycloartenone Figure 6 and cycloeucalenol were isolated from the immature pods of this species, collected from Mannanoor Forest, Andhra Pradesh, India.^[50]

The structure of a new sterol isolated from the unsaponifiable lipid fraction of the seed lipid was shown to be 14 α -methylzymosterol by comparison with a synthetic authentic compound. Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol, were isolated and identified in addition to several more common phytosterols.^[51]

The Triterpenoids components of the leaves and pods of *Wrightia tinctoria* isolated.^[52]

Alpha-Amyrin Figure 7 was isolated from bark extracts of both *W. tomentosa* and *W. tinctoria*.^[53]

CONCLUSION

This review shows that *Wrightia tinctoria* is an important medicinal plant with diverse pharmacological spectrum. Few novel chemical constituent isolated from the *Wrightia tinctoria* showed anti-cancer, anti-HIV, and anti-diabetic (type 2 diabetic) properties too. Further evaluation need to be carried out on *Wrightia tinctoria* in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind port in carrying out this study at the laboratory

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