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Wrightia tinctoria R. Br.-a review on its ethnobotany, pharmacognosy and pharmacological profile

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PEER REVIEW

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Comments

The present review provides comprehensive information on the traditional uses, ethnopharmacology, pharmacognosy, phytochemistry, biological activities, pharmacological activities and toxicology of *W. tinctoria*. The data provided in the present manuscript is worthy and written in good format of the journal.
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ABSTRACT

Different parts of *Wrightia tinctoria* R. Br. (Apocynaceae) (*W. tinctoria*), have been extensively used in Indian systems of medicine such as Ayurveda, Siddha and Unani for the treatment of jaundice, malaria, psoriasis and many other ailments. The present review has been primed to describe the existing data on the information on the traditional uses, botany, pharmacognosy, phytochemical constituents, pharmacological activities and toxicology of *W. tinctoria*. The information was gathered via electronic search (using Google Scholar, NOPR, Pubmed, Elsevier, Medline Plus and Web of Science) and library search for the books on traditional medicine as well as the articles published in peer-reviewed journals. The plant is rich in compounds containing alkaloids, saponins, indoxy yielding O-glycoside(s), phenolics, flavonoids, isatin tryptanthrin, anthranillate, rutin, β-isatin, tryptophan, indigotin, indirubin, wrightial and sterols. The vast number of literature found in database revealed that the extracts of different parts of *W. tinctoria* showed significant pharmacological actions. Clinical studies indicated a broad range of applications in the treatment of psoriasis and other skin diseases. We suggest that there is a need for further investigations to isolate active principles that confer pharmacological action. Therefore, identification of such active compound is useful for producing safer drugs in the treatments of various ailments.

KEYWORDS

Wrightia tinctoria, Ethnobotany, Traditional uses, Pharmacognosy, Pharmacological profile

1. Introduction

Wrightia tinctoria R. Br., (Apocynaceae) (*W. tinctoria*), a small deciduous tree is known by various names, *e.g.* Ivory tree, Easter tree, Sweet indrajao and Pala indigo-plant (English). In India, it is locally recognized by its different vernacular names, the most commonly used ones are Indrajava, Svetkutaja, Krsnkutaja (Sanskrit), Kalakuada (Marathi) and Mitha indrajau (Hindi)[1]. The whole plant or its specific parts (bark, leaf, seed and root) are known to have medicinal properties and have a long history of use by indigenous communities in India[2]. The medicinal value of this plant for the treatment of a large number of human ailments is mentioned in Ayurveda, Siddha, Unani and folk medicine[3-6]. In the last more than three decades, several studies have been carried out on this medicinal plant species to facilitate evidence in favor of its conventional uses. The rationale of this review is to provide comprehensive information on the traditional uses, ethnopharmacology, phytochemistry, pharmacological

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research and toxicology of *W. tinctoria* to explore its therapeutic potential, highlight the lacunae in our present knowledge and explore future research possibilities.

1.2. Geographical distribution and ecology

The plant of *W. tinctoria* is widely distributed in Asia, Africa and Australia and are known to be the native of Australia, India, Myanmar, Nepal and Vietnam[1]. The plant mostly occurs in the Western, Central and Peninsular India[7]. The plant grows well in arid, semi-arid and moist regions with a wide range of soil types. This plant is especially common along hillsides and valleys and is often found as undergrowth in deciduous forests. The timber *i.e.* white wood is of high quality and value for turnery, carving, toy making, matchboxes, small boxes and furniture. Leaves, flowers, fruits and roots constitute the source of an indigo-yielding glucoside, which produces a blue dye or an indigo dye[8].

1.3. Botanical description

W. tinctoria is a small deciduous tree, 5-8 m tall and has a scaly, smooth bark. Leaves are variable, 6-15 cm×3-6 cm, elliptic-lanceolate or oblong-lanceolate, acuminate, glabrous or the young leaves puberous beneath, base acute or rounded; main nerves 6-12 pairs and petioles 3-4 mm long. Flowers are white, fragrant, in lax terminal cymes, bracts minute, ovate. Calyx is glabrous, glandular inside, segments or teeth 2.2-5.0 mm long, oblong, rounded at the apex and with membranous margins. Corolla tube is short, 2.5-3.0 mm long, white, lobes 6-8 mm long, oblong and obtuse. Corona consists of numerous linear scales, some inserted with the filaments and some on the corolla-lobes (Figure 1).

Figure 1. Flowering twig of *W. tinctoria*.

Stamens are at the top of the corolla tube. Filaments are short, dialated, anthers exserted, sagittate conniving around and adhering to the stigma. The ovary is of 2 carpels, free or connate; ovules many in each carpel. Fruits are made of 2-distinct pendulous, follicles 20-40 cm long, cylindrical, slightly tapering to both ends, glabrous, striate, cohering at first at the tip only. Seeds are 1.2-2.0 cm long, pointed at the apex with deciduous coma often more than 3.0-3.7 cm long at the base[9,10].

2. Traditional uses

Numerous traditional uses have been recorded for *W. tinctoria*[2]. In Karnataka and Tamilnadu (states of India), the tree named as "jaundice curative tree", since the juice of its tender leaves is used as an effective drug for treatment of jaundice[11]. Fresh leaves are pungent and when crushed fresh leaf filled in the cavity of decayed tooth or chewed under tooth relieves toothache. Juice of leaves is also employed against serpent bite and considered febrifuge, stomachic and tonic[2,3,11-13].

In Siddha medicine, the plant is widely used in the treatment of psoriasis and other skin diseases[4,7,14]. Leaves soaked in coconut oil are kept in hot sunlight for a day and then this oil is used for the treatment of psoriasis[15-17]. Oil 777 prepared from fresh leaves of plant with coconut oil has been assigned to be analgesic, anti-inflammatory and antipyretic activities[18], to be effective in the treatment of psoriasis[19].

Bark is used as tonic, anthelmintic, antidiarrheal, febrifuge and aphrodisiac; it is used in treatment of seminal weakness, flatulence, colic diarrhea, leprosy, psoriasis, haemorrhoids dipsia, helminthiasis fever, digestive, stomachic, constipating, depurative, febrifuge, burning sensation and dropsy[4,11,12,20,21].

Seeds are useful as a tonic, carminative, anthelmintic, astringent, aphrodisiac and febrifuge and for treatment of stomach disorder[20-22]. In Unani medicine, the seeds of *Wrightia* are differently known as "Lisanul-e-Asafir", Inderjao Shireen, and Meetha Inderjao and have been reported to have used for cure disorders of central nervous system and claimed to have analgesic, aphrodisiac, tonic and emmenagogue actions[3,5,6,21]. *W. tinctoria* have been recommended for the treatment of infections of the chest (in asthma), colic and as diuretic[3]. Extracts of the root and leaves possess hypotensive activity[1].

According to an ethnobotanical information, the plant bark is of considerable medicinal value for bronchitis, piles

and is used in scorpion sting and snakebite[23]. In Nasik district of Maharashtra state of India, infusion of bark is administered to mothers for a week to increase lactation[24]. People inhabiting in Seshachalam hills of Andhra Pradesh, India apply a paste of crushed stem bark along with bark of *Ailanthus excelsa* on wounds once in a day for 3 d[25]. In tribal villages of Chitheri Hills of Dharmapuri district in Tamilnadu, the bark decoction is given to cure piles, whereas bark and seeds are used together to treat various ailments[26]. A crushed fresh leaf when filled in the cavity of decayed tooth relieves toothache[27]. The native practitioners in and around Chitoor district, India, have claimed that the leaves of *W. tinctoria* are used for treating diabetes[28]. Tribes of Southern Rajasthan use the latex of plant externally on vagina for easy delivery[29]. These claims of medicinal properties are to be substantiated by clinical trials in humans. Table 1 summarizes ethnomedicinal uses reported for *W. tinctoria*[30-57].

3. Pharmacognostical studies

3.1. Leaves

The leaf is dorsiventral and amphistomatic. The stomata are paracytic. The trichomes are 3-7 celled, thick walled and uniseriate. Adaxial epidermis has striations. Mesophyll is differentiating into 1-layered palisade and spongy tissue of loosely arranged cells. Midrib shows an arc shaped vascular bundle. In quantitative microscopy, the stomatal indexes have been found to be 21.0. Vein islet number and vein-let termination number are 21.0 and 21.8 respectively[58-60]. The histochemical color reactions were carried out in transverse section of the fresh leaf. The results indicated the presence of lignin, starch, fats, alkaloids, saponins, tannins, flavonoids and calcium oxalate crystals. Histochemical localization of certain important compounds enables to get a preliminary idea of type of compounds and their accumulation in the plant tissues[60].

Table 1
Ethnomedicinal uses of *W. tinctoria*.

Plant part	Method of preparation	Use	References
Leaf	Paste	Skin diseases; external and internal application	[30]
	Paste	Toothache and swelling gums	[31,32]
	Pounded leaves mixed with coconut oil	Eczema, psoriasis and other skin diseases	[33-39]
	Unspecified	Skin disease, wound healing	[40]
	Juice mixed with lime and turmeric powder	Swellings	[25,41]
	Infusion from coconut oil	Chronic wounds (veterinary medicine)	[31]
	Paste with *Santalum album* and *Allium cepa*	Chronic wounds (veterinary medicine)	[31]
	Juice	Jaundice	[42]
	Oil obtained from the paste of leaves of *W. tinctoria* and *Azadirachta indica*	Eczema	[33,43]
	Half a teaspoon of dried leaf powder	Respiratory ailments	[44]
Bark	Stem bark crushed with those of *Ailanthus excelsa*, paste applied daily once for 3 d	Boils, wounds	[25]
	Powdered and mixed with coconut oil	Wounds	[45]
	Unspecified	Galactogogue, abdominal pain	[46]
	Unspecified	Antipyretic	[47]
	Unspecified	Cure skin diseases, wounds	[40,48]
	Unspecified	Antidote for snake poison	[49]
	Decoction	Antidysenteric, antidiarrhoeal and antihaemorrhagic agent	[50,51]
	Paste	Skin diseases, ringworm and in leprosy	[52]
	Infusion	Stomach disorder	[53]
Stem	Unspecified	Toothbrush	[31]
	Decoction	Cure stomach disorder	[44]
Latex	Unspecified	Skin disease, wound healing	[40]
Root	Decoction	Epilepsy	[54]
	Unspecified	Laxative	[40]
	Decoction	Cure stomach disorder	[44]
Whole plant	Unspecified	Antioxidant, Antinociceptive	[55]
Seed	Powdered and mixed with coconut oil	Wounds	[45]
	Unspecified	Piles, worm infestation and pain	[51]
	Juice	Indigestion	[56,57]
Unspecified	Decoction	Wounds	[45]

3.2. Bark

Transverse section of bark consists of tangentially elongated thick walled, suberised 6-8 layers of cork cells, arranged in radial alignment or rows followed by phellogen composed of tangentially elongated parenchyma cells. Phelloderm is a wide, parenchymatous and interspaced with phloem fibers and stone cells. The ground tissue has stone cells in old bark. Starch grains and prisms of calcium oxalate crystals are present in parenchyma cells. Phloem fibers and medullary rays are mostly uniseriate and few are biseriate[59,61,62].

3.3. Seed

Seeds of *Holarrhena antidysenterica* are well known to treat dysentery and are confounded with those of *W. tinctoria*. A comparative pharmacognostical study was carried out on seeds of both the species. The seeds of the former taxon have been characterized by having hairs on the micropylar whereas in *W. tinctoria* they are developed at the chalazal end. The seeds of these two taxa have different patterns of folding of cotyledons and spermodermal ornamentation as well. Heavy tanniferous deposition in the outer epidermal cells of the seed coat in *H. antidysenterica* and its scanty deposition in *W. tinctoria* is also an additional character to be used as a distinguishing feature[63-65].

3.4. Root

Root epidermis is composed of compactly arranged smaller cells. Periderm formation has been observed. Many layered cork is made up of radially aligned rectangular cells. Below it lies many layered cortex and phloem. Phloem cells show the presence of starch and oxalate crystals. Secondary growth is more. Xylem occupies a considerable part. Medullary rays are made up of 1-2 rows of cells. Vessel elements in the roots are variable in length and diameter with simple perforations. Pits are simple, alternate and thickly arranged. The tail is short or long with mostly pointed end[59].

4. Phytochemistry

Most of the health promoting and disease curing potential of plants and their plant products are associated to their phytoconstituents. Accumulating evidence showed the presence of bioactive phytoconstituents in leaf, bark, root and seed of *W. tinctoria*. Preliminary phytochemical screening of the leaves showed the presence of alkaloids, cardiac glycosides, flavonoids, tannins, terpenoids[60,66-68]. Further detailed phytochemical characterization studies reveal presence of triacontanol and tryptanthrin[69] along with indigotin, indirubin, tryptanthrin, isatin, indoxyl-yielding O-glycosides, rutin and anthranillate[70]. However, indirubin has been reported only in the dried leaves and not in freshly collected ones[70]. Furthermore, content of indigotin and indirubin depends on seasonal variation.

Interestingly, taxol, an anticancer drug, which exhibited strong cytotoxic activity in the *in vitro* apoptosis assay; isolated from a leaf spot fungus, *Phyllostica tabernaemontanae*, of leaves of *W. tinctoria*[71]. Similarly, a new protease named "Wrightia" purified from the latex of the plant *W. tinctoria* is thought to be a potential candidate for various applications in food and biotechnological industries[72]. The "777 oil", a coded drug of Siddha system of medicine, has been derived from the leaves of *W. tinctoria* by insulation with coconut oil as a base. This process of preparation in the Siddha medicine is called "Sooriya Pudam"[15]. Alam *et al*. suggests modifications in "777 oil" by employing the bark of *W. tinctoria* instead of leaves[73]. Thin layer chromatography characterization of both the products reveals that they are chemically identical.

The stem bark of *W. tinctoria* reported to contain β-amyrin, lupeol, β-sitosterol, stigmasterol, campesterol and a triterpenoid, flavonoid, steroids, alkaloids and phenolics[59,62,74-76].

The mature powdered pod showed the presence of co-occurrence of β-amyrin, ursolic acid and oleanolic acid along with β-sitosterol[77]. The wrightial, a new terpene and other phyto constituets such as cycloartenone, cycloeucalenol, β-amyrin and β-sitosterol were isolated from methanol extract of the immature seed pods[78]. In addition to this, a new sterol 14 α-methylzymosterol along with four rare plant sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol have also been obtained from seeds[79]. The hexane extract of seed pods contains oleonolic acid[77]; whereas ursolic acid and isoricinolic acid have been further separated from the seedpods and seed oils[80]. Thus far, root of *W. tinctoria* received less research interest pertaining to phytochemical constituents. However, our preliminary phytochemical studies of root reported the presence of cardiac glycosides, saponins, pseudotanins and terpenoids[59].

5. Biological and pharmacological activity

So far, *W. tinctoria* has been reported to possess an array of biological and pharmacological properties that include antidiabetic, antimicrobial, antimalarial, anthelmintic, *etc.*

5.1. Antidiabetic activity

Diabetes is one of the prevalent diseases of carbohydrate metabolism, resulted into hypoglycemic condition. It has been observed that hyperglycemic condition leads to various complications such as retinopathy, nephropathy, *etc*. Zito *et al*. studied ocular aldose reductase inhibitory activity to *W. tinctoria* at 1 mg/mL as aldose reductase and sorbitol dehydrogenase to accelerate the formation of sorbitol from glucose in insulin-sensitive tissue leading to the development of cataracts in lens *i.e*. one of the common nephropathic condition in diabetes[81]. The results of this study showed that *W. tinctoria* strongly inhibits aldose reductase (92.92%) with IC_{50} 5 μg/mL[81]. Similarly, Kumar *et al*. have reported alpha glucosidase inhibitory activity of *W. tinctoria*[82]. Results showed that the bark ethanol extracts have significant *in vitro* intestinal alpha glucosidase inhibitory activity at 1 500 mcg/mL[82].

Raj *et al*. investigated the hypoglycemic and hypolipidemic activity of petroleum ether extract of *W. tinctoria* in alloxan induced diabetes in albino Wistar rats[83]. Oral administration of petroleum ether leaves extracts at doses 200 and 400 mg/kg exhibited a significant reduction in elevated levels of serum glucose, total cholesterol, and triglycerides. The results of this work confirmed *W. tinctoria* have significant application in diabetes mellitus illness and its related complications[83]. Later, these investigators also reported the antidiabetic effect of petroleum ether extract of *W. tinctoria* leaves in streptozotocin induced diabetic rats[84].

5.2. Antimicrobial activity

Antimicrobial effect investigations provide rationale information for the traditional uses of *W. tinctoria* in treating microbial infections such as the dental diseases, dysentery, respiratory ailments, psoriasis and other skin diseases. The leaves hold potent antimicrobial properties against dermatophytic microbes. Kannan *et al*. screened different solvent extracts against skin bacteria and dermatophytes[85]. The methanol and ethanol extracts were found to be more active against studied bacteria (at MIC 0.5 mg/mL for *Bacillus subtilis, Staphylococcus epidermidis* and 0.25 mg/mL for *Staphylococcus aureus*) whereas, hexane extract found to be potent against dermatophytic fungi such as *Trichophyton rubrum* and *Trichophyton tonsurans* at 2 mg/mL[85]. Similarly, in another study, *in vitro* antibacterial activities of the petroleum ether, chloroform, methanol and water extracts of leaves of *W. tinctoria* were studied against *Staphylococcus aureus* (*S. aureus*) (Gram-positive) *Escherichia coli* (*E. coli*) (Gram-negative) as test organisms[86]. The chloroform extract of *W. tinctoria* registered the highest zone (24 mm) against *E. coli*, followed by aqueous extract with *S. aureus* (12 mm) and *E. coli* (14 mm). The antibacterial study of different solvent extracts such as petroleum ether, chloroform, acetone and methanol of *W. tinctoria* leaf has been carried out in our laboratory. It was a chloroform extract that exhibited the highest antibacterial activity against *S. aureus* (24 mm), *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* (*P. aeruginosa*)[87], followed by acetone and methanol extracts. Interestingly, the observed antibacterial activity is almost equal, and/or more when compared to standard ampicillin[87]. Indirubin isolated from the chloroform extract of leaves of *W. tinctoria* tested for its antibacterial activity on *S. aureus*, *P. aeruginosa etc.* and efflux pump inhibitory (EPI) activity. Indirubin showed antibacterial activity against both Gram-positive and Gram-negative strains; and drug resistant to *S. aureus*; the observed MIC was 12.5 mg/mL for *S. aureus* and 25 mg/mL for *Streptococcus epidermidis*. *W. tinctoria* had significant EPI property and further clearly showed the synergistic effect along with ciprofloxacin. This indicated that indirubin has the capacity to block the Nor A efflux pump, which leads to cell susceptibility, due to increasing concentration of ciprofloxacin within the cell[88]. *Pityrosporum ovale* (*P. ovale*) is a yeast causing infection of skin and scalp leading to dandruff. Krishnamoorthy and Ranganathan studied *in vitro* antifungal activity of the oil made from leaf extracts of *W. tinctoria* and *Hibiscus rosasinensis* against *P. ovale*[89]. The results of this study indicated MIC ranges between 500-1 000 μg/mL indicating fungicidal action of the drug.

Anbuganpathi *et al*. also studied the antibacterial and antifungal effect of chloroform and aqueous extracts of *W. tinctoria*[90]. Interestingly, the authors reported no antifungal activity of all the studied extracts, but good bactericidal activity was reported to all the studied extracts[90]. Similarly, Krishnamoorthy *et al*. studied *in vitro* and *in vivo* antifungal effect of the Dano, a poly-herbal hair oil, containing extracts of *W. tinctoria*, *Cassia alata* and *Azadirachta indica*[91]. The results of this study also showed that Dano inhibited the growth of *P. ovale* and *Candida*

albicans with MIC at 30 mg/mL and 50 mg/mL respectively. Further clinical studies on 10 volunteers with severe dandruff in the age group of 18-22, showed mild traces to nil scaling with successive days of treatment[91]. Vijaykumar *et al*.[92] carried out an investigation of *W. tinctoria* leaf extracts along with other species for the antimycotic activity against *P. oval* and showed the antifungal property as they progressively inhibited the growth of *P. ovale* on saboured dextrose agar medium[92]. It was reported that the methanol and chloroform extract prepared from the woody stem bark of *W. tinctoria* indicated the broad spectrum and significant antimicrobial activity against various microbes used[74]. In addition, we have demonstrated potent antibacterial activity of chloroform extract followed by acetone and methanol extracts, while petroleum ether indicated negative inhibition[93]. Jayechandran *et al*. screened the antibacterial potential of *W. tinctoria* using various solvents *viz*., hexane, petroleum ether, ethyl acetate, chloroform, acetone and methanol on bacterial cultures of *E. coli* (MTCC 1195), *Klebsiella pneumoniae* (MTCC 2405), *Enterobacter aerogens* (MTCC 2823), *S. typhi* (MTCC 733), *Proteus vulgaris* (MTCC 1771), *P. aeruginosa* (MTCC 2642), *S. aureus* (MTCC 1430), *Bacillus cereus* (MTCC 1272)[94]. Petroleum ether, chloroform, acetone and hexane extracts showed the highest inhibition zone between 19-22 mm against *E. coli, Proteus mirabilis* and *Klebsiella pneumoniae*, respectively, while the moderate antibacterial activity was attributed to hexane, acetone and chloroform extracts[94]. In another study, hexane, methanol, chloroform, ethyl acetate and water extracts of leaves of *W. tinctoria* were subjected to *in vitro* antibacterial screening against plant pathogenic bacteria using disc diffusion method[95]. Results showed that ethyl acetate and methanol extracts have significant antibacterial activity against Gram-negative bacteria with MIC 50 μg/mL for *Xanthomonas campestris* and *Erwina* sp.

5.3. Antimalarial activity

Tryptanthrin, an indolquinazolin isolated from *W. tinctoria* leaves[70], was tested against *Plasmodium falciparum*. Further, tryptanthrin and several of its analogues were also tested for *Plasmodium falciparum* and showed very low IC_{50} values: 69 ng/mL for tryptanthrin and 0.43-10 ng/mL for analogues. These compounds were particularly active against atovaquone-, chloroquine- or mefloquine-resistant strains[96]. Valanite reported that the observed antimalarial activity of tryptanthrin could be due to its immunostimulating potential[97]. In addition to this, leaves of *W. tinctoria* are known to possess larvicidal effects against *Culex quniquefasciatus*[98].

5.4. Anthelmintic activity

Anthelmintic activity of crude petroleum ether and chloroform extracts of leaves of *W. tinctoria* using *Pheretima posthuma* as a model organism were studied at three concentrations (2.5, 5.0, 7.5 mg/mL) of each extract. Petroleum ether and chloroform extract of *W. tinctoria* caused significant paralysis (125.83 and 94.5 seconds) and death (162.33 and 140.28 seconds) of *Pheretima posthuma* respectively[99]. Minimum time taken by the methanol extract of *W. tinctoria* leaves (100 mg/mL) were 13.97 and 23.3 min to cause paralysis and death of the worms respectively[100].

5.5. Analgesic and anti-inflammatory activity

Reddy *et al*. evaluated the antinociceptive activity of petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of *W. tinctoria* bark on acetic acid-induced writhing test in mice[101]. The ethyl acetate, acetone and methanol extracts showed significant antinociceptive activity and were comparable with acetylsalicylic acid[101]. In the study of Bigonia *et al*. in 2008, the ethanol (70%) extract of *W. tinctoria* bark showed significant analgesic activity in Eddy's hot plate reaction and tail flick method. The authors claim the observed analgesic activity could be due to the presence of steroids in the extract and concluded that *W. tinctoria* bark extract is effective against acute phasic pain[102].

In another experiment, Tharkar *et al*. investigated for anti-inflammatory activity of bark in carrageenan-induced rat paw oedema and cotton pellet induced granuloma animal models[103]. The results of this study exhibited that the aqueous and chloroform extract treated group showed significant ($P<0.05$) reduction in paw oedema from 4-5 h, while methanolic extract and diclofenac sodium treated group showed a significant reduction ($P<0.05$) in paw oedema from 2-5 h when compared to control group[103]. Further, the observed anti-inflammatory activity of chloroform extract of *W. tinctoria* bark was attributed to the presence of the flavonoid[104,105]. Jain and Bari investigated anti-inflammatory activity of petroleum ether and methanol extracts of *W. tinctoria* bark using carrageenan and histamine-induced paw edema animal models[106]. The results of this study exhibited that the extract possessed significant dose dependent anti-inflammatory activity. Aleykutty *et al*. studied analgesic and anti-inflammatory

effects of *W. tinctoria* leaf extract in rats and mice[107]. The anti-inflammatory effect was investigated by human red blood cell membrane stabilization method and carrageenan induced hind paw edema method. Ethyl acetate fraction showed 67.21% protection at concentration 400 mg/kg. The ethyl acetate fraction was studied for its analgesic effect on acetic acid-induced writhing test and hot plate method in mice and was found to be effective. Above findings justify the traditional use of *W. tinctoria* leaves against inflammation and pain in psoriasis, hemorrhoids and swelling gums[107]. The leaves revealed different constituents such as flavonoids, steroids, glycosides, saponins, sterols, tannins and phenolics. Phenolics possess various biological properties such as antioxidant, anti-inflammatory and estrogenic activities[108]. Phenolics and flavanoids were used for the prevention and cure of various diseases, and mainly associated with free radicals. Flavanoids are known to possess anti-inflammatory activity by inhibiting the cycloxygenase responsible for the synthesis of inflammatory prostaglandins[109]. In addition, flowers of *W. tinctoria* depicted anti-inflammatory activity as revealed by Sethuraman[110].

5.6. Antiulcer activity

Bigonia *et al.* reported antiulcer activity to bark ethanol extract against experimental induced acute gastric ulcer model[111]. The extract significantly reduced the ulcer index induced by both pyloric ligation and ethanol at 100 mg/kg dose; similarly, extract treatment significantly reduced the secretary parameters such as volume and pH of gastric juice, free and total acidity. The extract has a significant effect on gastric mucous substances. By increasing total carbohydrate content and decreasing total protein content in pyloric ligated ulceration, all these effects are comparable to ranitidine (20 mg/kg). The results indicated that *W. tinctoria* bark extract protects gastric mucous membrane by improving microcirculation, increased capillary resistance or by precipitating microproteins. In another study, Divakar and Devi evaluated antiulcer activity of leaves of *W. tinctoria* evaluated against experimentally induced acute gastric ulcer model in albino rat[112]. The antiulcer activity of methanol was evaluated by comparing with carboxy methyl cellulose, pylorus control, aspirin and standard famotidine, by employing aspirin plus pylorus ligation induced ulcer model. The biochemical parameters such as volume of gastric juice secretion, pH, free acidity, total acidity, 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 (65.89%), when compared with the standard drug famotidine (20 mg/kg). The results suggested that the methanolic extract of *W. tinctoria* leaves possess anti-ulcer effect[112].

5.7. Immunomodulatory activity

Bigonia *et al.* reported immunomodulatory activity of *W. tinctoria* bark alcohol extract on nonspecific and specific immune responses, studying the parameters such as survival study, carbon clearance test, delayed type hypersensitivity and hemagglutinating antibody titer[113]. *W. tinctoria* extract at 400 mg/kg body weight increased the survival rate of rats against *E. coli* induced abdominal sepsis up to 15 d post infection. The extract showed significant homeopathic activity and raised neutrophils count significantly, which was further confirmed by increased phagocytic response against inert particles. *W. tinctoria* exhibited decreases in delayed type hypersensitivity response. Further, extract enhanced both primary and secondary humoral responses in rats sensitized with bovine serum albumin. The results of this study substantiate that *W. tinctoria* bark extracts have moderate non-specific immunostimulant[113]. In another study, Thabah *et al.* investigated the immunomodulatory activity of the bark extracts such as petroleum ether, ethanol and aqueous alcohol of *W. tinctoria* by using delayed type hypersensitivity reaction and carbon clearance assay[114]. Petroleum ether and aqueous alcohol extracts (200 and 400 mg/kg, *p.o.*) produced a significant increase in delayed type hypersensitivity in response to sheep red blood cells. Petroleum ether extract showed better activity than aqueous alcohol in delayed type hypersensitivity response. Aqueous alcohol extracts at dose 200 and 400 mg/kg, *p.o.*, showed a significant dose dependent increase in the phagocytic activity. The results revealed that aqueous alcohol possesses immunostimulant activity in carbon clearance assays whereas the petroleum ether extract and aqueous alcohol showed immunomodulatory activity in the delayed type hypersensitivity model[114].

5.8. Wound healing activity

The aqueous and alcoholic extracts of the stem bark of *W. tinctoria* were investigated for their antipyretic and wound healing potential in albino rats using excision model. Wound healing activity was assessed by the percentage of wound contraction and the period of epithelization; there was wound contraction activity by using extracts from the

14th day of topical application. Further, yeast induced pyrexia model was used for evaluation of antipyretic activity in albino rats and fall in body temperature of febrile rats was taken as an indication of antipyretic action. The drug extracts had significant antipyretic activity[115]. Jain and Bari reported the potential effect of petroleum ether and methanolic extracts of bark in wound healing in Wistar albino rats[116]. The wound healing parameters were evaluated by using incision wounds in extract-treated rats, standard and controls. Both 5% and 10% doses of petroleum ether and methanol extract significantly increased wound breaking strength when compared with the control group and the ethanol extract indicated, wound healing effect in the rats[117]. The observed significant wound healing activity was attributed to the presence of antioxidant compounds such as flavonoids and saponins. The enhanced wound healing may thus be due to the free-radical scavenging action of the plant, enhanced level of antioxidant enzymes in the tissues, antimicrobial potential which control microbial colonization and subsequent proliferation.

5.9. Effect against psoriasis and other skin diseases

Psoriasis is a chronic recurrent problem affecting the skin, nails, and joints and it is one of the papulo-squamous disorders. The current treatments available for psoriasis include local application of emollients, moisturizers, tars, anthralins, topical corticosteroids, vitamin A and D analogs and systematic treatment in the form of corticosteroids, methotrexate, cyclosporine, etretinate and other immunomodulators as well as hydroxyurea. Photo-chemotherapy and alternative medicine have been also extensively used for health care and are supporting to be a new era of medication. There are increasing research efforts to develop herbal formulations to treat psoriasis. Clinic and histopathlogical evaluation of ointment formulation prepared from *W. tinctoria* and *Cocos nucifera* (RegSoR) suggests superior efficacy of herbal formulation with a best result of formation of the granular layer and marked disappearance of the spongiform pustules, dermal vessel tortuosity and normalization. The reduction of the dermal infiltrates observed with herbal treatment. The formulation is found to be safe and non-toxic to liver, kidney, and haemopoietic system[118]. Emulsion of *W. tinctoria* showed reversal of parakeratosis to orthokeratosis in mouse-tail test with enhanced hyperplasia and diminished parakeratotic scales with tendency for separation forming healthy layer beneath the response that matches with standard method of treatment of retinoids[15,119,120]. United States Patent (No. 5858372), obtained from the pharmaceutical preparation for topical treatment of skin disorders, particularly psoriasis, comprises the ingredients of latex extracted from the leaves of *W. tinctoria*, urea and polyethylene glycol[121].

In the case of allergies of externally manifesting as urticaria, eczema and psoriasis, a specific plant drug store cottage (*W. tinctoria*) has been tried in the form of an oily extract for the treatment of psoriasis with encouraging results. Fresh leaves of the plant are crushed and kept embedded in coconut oil under the sun for 5-7 d and the oil is subsequently filtered out. This oil is used for local application and for oral administration in doses of 5 mL twice a day for 3 months. Trials made in a series of 40 patients showed significant relief in symptoms, reduction in scaling and shrinkage of the affected surface area. The drug does not show any side effects[16].

5.10. Other activities

W. tinctoria was reported to have hepatoprotective activity[122]. Further, Bigoniya and Rana investigated the hepatoprotective effect of triterpene fraction isolated from the stem bark of *W. tinctoria* (containing lupeol, β-amyrin and β-sitosterol) against CCl_4-induced hepatotoxicity in comparison with known standard sylmarin in the rat[123]. Pretreatment with triterpene fraction 125, 250 and 400 mg/kg, *p.o.* once a day for 4 d before CCl_4 and continued further 3 d, attenuated the CCl_4-induced acute increase in serum serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase and alkaline phosphatase activities and considerably reduced the histopathological alterations. Further, triterpene fraction reduced thiopentone-induced sleeping time, suggesting the protection of liver metabolizing enzymes. The authors claim triterpenes administration changed the tissue redox system by scavenging the free radicals and by improving the antioxidant status of the liver replenished the depleted hepatic glutathione and superoxide dismutase. Triterpene pretreatment improves bromsulphalein clearance of the CCl_4-intoxicated liver and increases the cellular viability. These effects substantiate protection of cellular phospholipid from peroxidative damage induced by highly reactive toxic intermediate radicals formed during biotransformation of CCl_4. Triterpene fraction afforded protection against the hepatic abnormalities due to the presence of lupeol and β-amyrin. The study supports the traditional use of *W. tinctoria* bark in treatment of liver diseases. Sathianarayanan *et al.* investigated diuretic activity on male albino rats using water and alcoholic

extract of leaves of *W. tinctoria*[124]. The extracts showed a potent diuretic effect with increase in electrolyte concentration in urine, when compared to standard drug (frusemide) in albino rats[124].

Antidiarrheal potential of ethanol bark extract and isolated steroidal alkaloid fraction of *W. tinctoria* was investigated on different experimentally induced diarrhea models of rats, isolated rat ileum, and on enteric bacterium. The extract at 500 and 1 000 mg/kg dose, and steroidal alkaloid fraction at dose 50 and 100 mg/kg, significantly inhibited the frequency and wetness of faecal droppings in castor oil-induced diarrhea. Further, extract and steroidal alkaloid fraction decreased propulsion of charcoal meal and reduced prostaglandin E2-induced enteropooling. Steroidal alkaloid fraction reduced amplitude, frequency, and tone of spontaneous gut movement; also inhibited acetylcholine-induced contraction of rat ileum. The results of this study confirmed good antidiarrheal activity of *W. tinctoria* against secretory, osmotic, motility related, and inflammatory diarrhea[125]. The bark extract plays an important role in infertility. The ethanol extract and its hexane-soluble, chloroform-soluble, water-soluble and water-insoluble fractions of the bark of *W. tinctoria* when administered orally during pre-implantation, peri-implantation and early post implanation in adult female of Sprague-Dawley rats showed anti-implantation effect with moderate to potent estrogen-agonistic activity at 250 mg/kg dose; indicating antifertlity activity of extract[126]. However, authors suggested that further studies on clinical and isolation of active natural products for development of newer agent from natural origin are required to identify contraceptive agents from natural sources lacking potent estrogenic activity. Kumar *et al*. evaluated the reducing power and free radical scavenging activity of ethanol extract of *W. tinctoria* bark[127]. *In vitro* antioxidant evaluation was done by measuring the reducing power and inhibition of superoxide production. The study demonstrates that the ethanol bark extract has significant antioxidant and free radical scavenging activity[127].

Moreover, neuropharmacological potential of seeds of *W. tinctoria* has also been studied[128]. 50% alcoholic extract when studied for central action on mice, the drug diminished the alertness, grooming and spontaneous locomotory activity and pain response in gross behavioral studies. Analgesiometer test using hot wire for providing thermal stimulus in the rats showed significant analgesia in all the doses studied (150, 200, and 300 mg/kg, body weight). The higher dose of 200 and 300 mg/kg showed significant supramaximal electroshock seizure indicating anticonvulsant activity of test drug with LD_{50} determined to 891.25 mg/kg in the mice. These results indicate that the drug has central depressant, muscular relaxant and anticonvulsant activity[128]. Murugnandan *et al*. also studied anxiolytic and antidepressant effect of *W. tinctoria* leaves on brain monoamines and metabolites in rats[129]. In another interesting study, Selvam *et al*. have attempted antiviral activity and cytotoxicity of *W. tinctoria*[130]. Different extracts of leaves selected against replication of HIV-1 (IIIB) in MT-4 cells and hepatitis C virus in Hue 5.2 cells. The cytotoxicity of leaves also tested against mock-infected MT-4 cells and Hue 5.2 cells. These extracts of the leaves have been evaluated for anti HIV-activity and cytotoxicity against HIV-1 (IIIB) replication in acutely infected MT-4 cells. None of the extracts exhibited anti-HIV activity in acutely infected MT-4 cells. However, the chloroform extract exhibited a maximum protection at 48% of the MT-4 cells against the cytopathic effect of HIV-1 (IIIB) at subtoxic concentration. The anti-hepatitis activity revealed that 50% effective concentration for the inhibition of hepatitis C virus subgenomic replicon replication in Huh 5.2 cells (luciferase assay) by chloroform extract was 10 μg/mL, whereas ether and ethanol extracts inhibits hepatitis C virus RNA synthesis at the concentration (EC_{50}) of 23 and 29 μg/mL, respectively. The concentration of extracts reduced the growth of exponentially proliferating Huh 5.2 cells by 50% was greater than 50 μg/mL[130].

6. Toxicological assessment

Lakshmi and Divakar screened the toxicological profiles of leaves of *W. tinctoria* by employing brine shrimp assay method (*Artemia salina* Leach) using seven different leaves to extract, namely ethanol (70%) methanol, water, petroleum ether, dichloromethane, ethyl acetate and chloroform[131]. Among the seven different solvent leaf extracts of *W. tinctoria*, the ethanolic (70%) and methanolic leaf extracts showed an LC_{50} of 471.604 μg/mL and 517.038 μg/mL. The other remaining solvent extracts taken from the leaves showed no toxicity in brine shrimp assay method. The percentage mortality LC_{50} was also calculated by constructing graphs considering log concentration versus percentage mortality. However, after 24 h, all the samples showed a significant lethality but these were not accepted for safer and more accurate result. Because after 24 h, some nauplii may die normally as their life span is 24-48 h. The toxicity that was found in the other vials of different concentrations might be due to the toxic property of the

plant extracts. *W. tinctoria* may contain some cytotoxic constituents that may be soluble in methanol and (70%) ethanol than other solvents used. *In vivo* safety and efficacy of bark extracts of *W. tinctoria* at dose 1 000 mg/kg have also been studied on some haematological, histological, biochemical and antioxidant enzyme status of rat liver and kidney[132].

7. Conclusion

Biologically active compounds from the natural sources have attracted the scientists working on the health problems. *W. tinctoria* is one of the plants, with a great medicinal potential claimed to contain the varieties of phytochemicals, which play an important role in one or other ways in different biological activities. The traditional application of leaves in human beings for the treatment of jaundice and recent study performed on human cell line (Huh 5-2) evidenced the antihepatitis viral property of leaf extracts[130] validates its traditional claim against jaundice. However, the findings obtained on concentrating the activity of *W. tinctoria* extract requires further detailed investigation. Various poly-herbal formulations containing *W. tinctoria* are available in the markets for psoriasis, diarrhea, dysentery, dandruff and for rejuvenation of joint function. It is the leaf extract of *W. tinctoria* which forms the major constituent in the Siddha preparation for the treatment of psoriasis. The experimental evidence of antimicrobial property has been produced in the laboratory against the skin microorganism supports the claims of the plants used in the cure of psoriasis in the Siddha system[85]. *In vitro* studies on the extracts from this species have shown a range of antimicrobial activities. However, in some studies the type of inhibitory activity, the concentrations of inhibition, the method used for screening and positive controls are not well defined. This suggests that there is a need for some of these to be re-evaluated using accepted research methods.

There is an increased interest in the search of plant compounds that may inhibit the bacterial efflux pump, since efflux is a common resistance mechanism employed by bacteria. An effective EPI could have significant benefits, including restoration of antibiotic sensitivity in a resistant strain bacteria and a reduction in the dose of antibiotic required for inhibition, possibly reducing adverse drug effects[133,134]. Another significant role of EPI is that when used with an antibiotic it delays the emergence of resistance to that antibiotic[135]. There are prospects of isolating and using EPIs clinically from leaves of *W. tinctoria*, since a compound indirubin from the leaves of this species has shown such activities against *S. aureus*[88]. The extracts obtained from different organs in above mentioned pharmacological studies using various solvents have been evaluated for multi-effective property of the plant *W. tinctoria*. It is worth to isolate some pure phytopharmaceuticals which in turn can be used as lead molecules for synthesizing novel agent having good therapeutic value.

A critical analysis of the present study indicates the fact that although the number of diseases, for which *W. tinctoria* acts as a medicine in one or other way are large, yet, in view of the wide range of medicinal uses of *W. tinctoria* mentioned in ethnobotanical survey, Ayurveda, Siddha, Unani system and otherwise, it is mandatory that more clinical and pharmacological studies should be conducted to investigate the unexploited potential of the plant. Furthermore, with regard to the development of quality herbal medicine, the standardization of extracts, phytopharmacology of different extracts, isolation and characterization of active phytopharmaceuticals, elucidation of mechanism of action of the isolated compound as well as clinical trials of the compounds are much needed.

W. tinctoria represents a most valuable medicinal plant sheltering a variety of the important chemoconstituents which confer most of the characteristics of the plants. Based on the facts, it is concluded that this plant species may form a good potential source in the drug development.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

W. tinctoria belonging to family Apocynaceae, is an important medicinal plant used by indigenous communities in India. Different parts of this plant such as stem bark, leaf, seed and root are known to have medicinal properties. The medicinal value of this plant for the treatment of a large number of human ailments is mentioned in Ayurveda, Siddha, Unani (codified drug system) and folk medicine (non codified drug system). In the last more than three decades, several studies have been carried out on this medicinal plant to facilitate evidence in favor of its traditional uses. The authors wanted to discuss future research opportunities through the present review in the form of its comprehensive information on the traditional uses, ethnopharmacology, phytochemistry, pharmacological research and toxicology.

Research frontiers

The voluminous data on traditional uses, ethnopharmacology, phytochemistry, pharmacological research and toxicology can be a foundation for future research opportunities. The root of this plant is less studied organ and consists of many phytochemical constituents. Moreover, many traditional uses of this plant are still not substantiated or validated by studying *in vivo* and *in vitro* methods. So further study in this regard can be available for future studies.

Related reports

The present review provides comprehensive information on the traditional uses, ethnopharmacology, pharmacognosy, phytochemistry, biological activities, pharmacological activities and toxicology of *W. tinctoria* to explore its therapeutic potential for future biprospecting. The data on *W. tinctoria* was gathered via electronic search (using Google Scholar, NOPR, Pubmed, Elsevier, Medline Plus and Web of Science) and library search for articles published in peer-reviewed journals also the contribution of authors in the form of their own research. Furthermore, information was also included from referred books on traditional medicine and ethnopharmacology.

The results exhibit that a wide range of chemical compounds such as alkaloids, saponins, indoxy yielding O-glycoside(s), phenolics, flavonoids, isatin tryptanthrin, anthranillate, rutin, β-isatin, tryptophan, indigotin, indirubin, wrightial and sterols have been found in the species. Many pharmacological studies revealed that plant has antimalarial, antimicrobial, antidiabetic, antioxidant, immunomodulator, anti-inflammatory, analgesic and anthelmintic activities together with the wound healing property, besides its traditional uses. A clinical study further suggests that the plant has broad range of applications in the treatment of psoriasis and other skin diseases.

Innovations and breakthroughs

The authors collected the pertinent data from the entire world, where the plant species is distributed; moreover the authors shared their own contribution in the form of research on pharmacognosy and antimicrobial activity. The lacunae left in the research can be a future perspective in the field of phytochemistry and pharmacology.

Applications

Such type of review provided comprehensive information to industry, research organization and institutes as well as beginners in the field of phyto pharmacology research.

Peer review

The present review provides comprehensive information on the traditional uses, ethnopharmacology, pharmacognosy, phytochemistry, biological activities, pharmacological activities and toxicology of *W. tinctoria*. The data provided in the present manuscript is worthy and written in good format of the journal.

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