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REVIEW ARTICLE **OPEN ACCESS**

Indigofera tinctoria: traditional dye or a modern medicine?

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ABSTRACT
Indigofera tinctoria is traditionally used for extraction of fabric staining dye- indigo, also called 'neel 'in local Indian languages. In ancient cultures, it was also used as manure to ensure soil fertility before planting crops. Through the years, diverse uses of the plant have been studied, many of which are in different forms of medicine. The extracts from different parts of tinctoria plant are reported to have been used for treatment of various ailments ranging from epilepsy to ulcers. Recently, many groups have studied the various bioactive compounds present in extracts of tinctoria. This has opened up a window of opportunities to explore these extracts for purposes of medicine and for use in modern forms of drug delivery. This review focuses on the traditional use of I. tincoria in alternate forms of medicine and newer therapeutic mechanisms like nanoparticles.
Keywords: *Indigofera tinctoria,* indigo, alternate medicine, drug delivery

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INTRODUCTION

I*ndigofera tinctoria* a full sun-loving plant that grows best in medium moisture and well-drained soil. It requires moist soil during the growing phase but can withstand a little drought when the plant is full-grown. It is a deciduous tropical shrub that grows to 203 feet tall and wide. It produces light green leaves and pink or violet flowers which are followed by seed pods that grow to 2 inches long. The classification of *Indigofera tinctoria* is as follows:

Kingdom- Plantae. Subkingdom- Tracheobionta
Superdivision- Spermatophyta. Division- Magnoliophyta
Class- Magnoliopsida. Subclass- Rosidae.
Order- Fabales. Family- Fabaceae.
Genus- Indigofera L Species- Indigofera tinctoria L
Chromosome number- 2n=16

This plant has numbers of uses like medicine, herbal dye, homeopathic treatment and pharmacological properties.

*I.tinctoria*is believed to be originated from India, and native plant to India, China, parts of central Asia, and also parts of Arica. Before naturalization, it has greatly been cultivated for Indigo (Neel). In Australia, South-East Asia, many countries of South Africa and Arabia it occurs mainly in wild or through the process of naturalization. Because of its historical exploitation and massive cultivation, its distribution is found pan tropically [1].

There is a history of usage of Indigo that was extracted from *I.tinctoria* [2]. The oldest Sanskrit texts from India have mentionedthe use of *Indigofera* and the dye extracted from the same. According to Egyptian scriptures and texts, the blue dye from *Indigofera* was used for textiles of Egyptian mummies. One of the oldest archeological textiles dating back to the 11th or 12th century from West Africa has different textile patterns such as stripes, checks, etc. done with Indigo. In 1566, Andre Alvares de Almada, a Portuguese explorer mentioned that Indigo was extracted from the *Indigofera* in Guinea.

Since ancient times for dyeing textiles blue, Indigo was used. This Indigo was derived from the leafy twigs of the plant. Indigo has been referred to as 'the king of dyes' because of its mesmerizing blue color and its property of mixing with other natural dyes to produce a wide range of colors. There is no other such plant in human civilization that has gathered so much acceptance and importance as a dye plant as *Indigofera*.

Indigo is considered the most important dye of plant origin in western Africa also. Western Africa is one of the major centers known for textile decoration techniques [3]. These techniques are based upon two concepts of 'resistdyeing' and linked with 'Indigo dyeing' in which parts of clothes are covered with wax or starch and then the cloth piece is soaked in Indigo. It produces a blue background on the untreated part of the cloth piece. Despite the historical use of Indigo, extracted from *Indigofera*, there has been a rapid decline in the use of *Indigofera* as it has been replaced by synthetic indigo, not only at industrial use but also at the craft level.

Apart from the traditional use of extraction of natural dye, *Indigofera tinctoria* is also used green manure [4]. In India, it is especially used in coffee plantations or before sowing rice, maize, sugarcane, etc. Although *Indigofera tinctoria* is hardly used for fodder, it is extensively used as manure because it is a good nitrogen catch crop, thereby reduces the use of fertilizers. In the Philippines, it is a great choice as manure in rice fields because it reduces the use of Nitrogen fertilizer to about half. Indigofera twigs have used a toothbrush in Cameroon.

TRADITIONAL USES

In traditional ways of treatment, *Indigofera* leaf extracts are often used to treat nervous disorders, epilepsy, asthma, fever, and stomach, liver spleen disorders [5]. It is also used as rabies prophylactic and at times the lotion is used for sore skin, ulcers, hemorrhoids, etc. [6]. In India, a tincture prepared from the seeds of *Indigofera* is used to kill lice. There are various references from across the globe of usage of *Indigofera* for medicinal purposes such as root extract for toothache in Cameroon, medicine against syphilis and gonorrhea in Tanzania, as an antidote for snakebite or insect bite in India, etc. *Indigofera* has high LD50 thus put it low on toxicity level. *Indigofera* has antitoxic effects and has certain sedative properties as well (7).

Use in Homeopathy and naturopathy:

Indigofera tinctoria has been used in Homeopathy. It was introduced into Homeopathy as a remedy for epilepsy. Clinical use of *Indigofera tinctoria* comprises of the following – Acne, Amenorrhoea, prolapse of anus, diarrhea, epilepsy, toothache, sciatica, strictures of Urethra, worm infestation, etc. *Indigofera tinctoria* has extensively been proved and used by homeopaths especially with the patients who had been receiving large doses of medicines, as per the prescriptions from the old school of treatment.

Marked action of I*ndigofera* is on the nervous system, as a result, it is considered very useful in the treatment of epilepsy, especially in those cases where patients experience great sadness. The patient has a happy mood and always intends to be busy. Neurasthenia and hysteria are some of the keynote symptoms. *Indigofera* has also been used for snakebite and when pure powdered Indigo was placed on the affected areas, it cured snake and spider poison. *Indigofera* has also been found useful in patients with stricture of the esophagus. *Indigofera* has acted upon almost all the organs of the body.

It has been found very useful in the treatment of patients having vertigo with nausea and convulsions. Patient experiences the sensation of a tight band around the forehead. A patient has an undulating sensation through the whole head and feels as if the brain were frozen. There is a sensation as if the head were larger than its natural size. The patient feels as if it had occupied more space. There is the sensation of heat and bubbling in the occiput as if produced by boiling water. There is excessive pain in the occiput and the patient feels as if a bunch of hair was torn out from the crown of the head.All the above-mentioned sensations make the patient gloomy and he tends to cry at night. *Indigofera's* action on the nervous system has been of tremendous use. It has also been used in patients with hysterical symptoms where pain predominates along with excessive nervous irritation. Epilepsy appears in the patient with concomitant symptoms such as flashes of heat from the abdomen to the head. The fit of epilepsy begins with dizziness. Reflex spasms from worms.

Indigofera has marked action upon the respiratory system as well. It has been used to cure excessive sneezing and bleeding from the nose. The patient also complains of pressure and roaring in the ears. Apart from the respiratory system, it has been used to treat ailments of GIT and urinary systems too. The patient has a metallic taste along with eructations, bloating, and anorexia. The patient may also experience flushes of heat rising from stomach to head. There is a marked feeling as if the rectum would fall. The patient gets up at night with horrible itching at the anus. There is a constant desire to urinate. Urine appears turbid during physical examination.

It has also been used in the treatment of sciatica where pain travels from the middle of the thigh to the knee. The patient experiences boring pain in the knee-joint which gets better by walking. Sciatica pain gets worse after every meal. There have been found general modalities of *Indigofera* while dug proving. Most of the symptoms get worse during rest and sitting and get better by pressure, rubbing, and continuous motion.

Indigofera has marked action upon female sexual organs as well and has been used to treat ailments related to it. It acts as an emmenagogue and is used in patients where menstruation occurs too early. The patient complains of a stinging sensation in mammae that gets relieved momentarily by rubbing. There is a complaint of burning in mammae during the menses. *Indigofera* is advised for prescription in Third to thirtieth potency.

It is an anti-toxicant and has hemostatic and sedative properties [8]. The plant extracts are used in cancer, piles, chronic bronchitis, asthma, healing of ulcers, and dropsy. Its leaves, stems, and roots have been reported to promote hair growth [9].

Bioactive compounds:

Traditionally, indigo, a blue-colored compound obtained from *I. tinctoria*is used in the textile industry to dye fabric. Sarangi et al. elucidated the biosynthesis pathway of indigo using whole transcriptome analysis of tinctoria leaf extract [10]. More such studies are required to understand and adopt similar approaches for manufacturing synthetic dyes. Indican, a colorless compound is converted into blue-colored indigo through multistep glycosylation by UDP-glucosyltransferase [11]. It catalyzes the addition of UDP-glucose to indoxyl, an unstable compound that dimerizes and oxidizes into indigo. These reactions could be up-scaled for industrial production of indigo or similar fabric dyes. Indirubin and indigtone obtained from the leaves of *I. tinctoria*have been used to treat hydrophobia [12].

*I. tinctoria*is known to have a protective effect against a variety of conditions. These effects stem from various bioactive compounds present in extracts obtained from its roots, stem, or leaves. Andreotti et al used pyrolysis followed by mass spectrometric analysis [13]. The first significant peak corresponding to 2-methylpyrrole was seen at the retention time of 10.42 minutes followed by several other small compounds. Indigotinederivative1,2-Dihydro-3H-indol-3-one and 1,3-Dihydro-2H-indol-2-one, cleavage product of indirubin were seen at 16.72 and 22.00 minutes, respectively.

PHARMACOLOGICAL PROPERTIES

*I. tinctoria*has been traditionally used for several ailments including liver issues, constipation, palpitations in the heart [14]. More recently it has been reported to be beneficial as an anti-cancer agent and an antioxidant [15, 16]. *I. tinctoria*has been known to play a role in strengthening innate immune response through immunomodulatory effects on macrophages and lymphocytes [17]. In a separate study, it was demonstrated that*I. tinctoria*could enhance immune responses in rats through increased antibody titer, TNFα secretion, macrophage response and NK cell cytotoxicity (18). The group used noise-immunosuppressed Wistar rats and orally fed *I. tinctoria* aqueous extracts for 48 days.Indirubin also regulates T-cell homeostasis in immune thrombocytopenia (ITP) [19]. They used PBMCs from ITP patients and showed that indirubin could modulate the number of T_{reg} cells in these cultures. Additionally, a murine model of ITP was used to demonstrate that platelet counts in these animals could be manipulated by indirubin administration. The group elucidated that indirubin acts through PD1/PTEN/AKT signaling pathway to exert its effect in ITP and thus could be used as a plausible therapeutic agent to treat ITP.

I tincoria aqueous extract is also reported to have a neuro-protective role in rats [20]. Its methanolic extract is shown to have an anti-seizure effect in Wistar rats [21]. Rats were orally fed methanolic extract of *I. tincoria*. On the 14th day, seizures were induced in rats using an electro-convulsiometer. The rats that had previously been fed *I. tincoria* had a marked reduction in tonic hindleg extension by almost 6 folds. Also, reduction in clonic convulsion was comparable to diazepam, a standard medication for epilepsy.

The antioxidant properties of *I. tinctoria*have been investigated by multiple groups. In one of the studies, the free radical scavenging potential of *I. tinctoria*was evaluated using 2,2-diphenyl-1-picrylhydrazy (DPPH), nitric oxide (NO), and superoxide anion (O^{-2}) scavenging [22]. The free radical scavenging abilities of I tinctoria extract were significant however were much less than ascorbic acid. Singh et al also looked at the free radical scavenging abilities of *I. tinctoria*by investigating DPPH, sulfur dioxide, nitric dioxide, hydroxyl ion scavenging, and metal chelation [23]. They used ethanol and 80% ethanol to obtain ethanolic and hydroethanolic extracts of *I tinctoria*. This study again proved that *I. tinctoria*has noteworthy free radical scavenging abilities but is less potent than traditional antioxidants like ascorbic acid and tocopherol.

*I. tinctoria*has been reported to have specific hepato-protective effects. A study conducted by Singh et al elucidated that trans-tetracos-15-enoic acid (TCA) present in indigotin could reverse signs of liver damage in albino rats [24]. They induced hepatic injury and evaluated various parameters like serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase, and triglycerides associated with liver damage. The rats treated with TCA showed significant dose-dependent alleviation of hepatic damage. Another study used D-galactosamine and carbon tetrachloride to induce

liver toxicity [25]. Treatment with *I. tinctoria* resulted in reduced LDH leakage and decreased urine urea levels, typical indicators of hepatic toxicity.

USE IN NANOPARTICLE SYNTHESIS

Traditionally, nanoparticles for therapeutic purposes are synthesized using a series of complex chemical reactions that often use toxic chemicals and also result in the formation of a pollutant side product. To overcome this, bio-inspired synthesis or green synthesis is used. It is environment-friendly, clean, straightforward, and cheaper than other synthetic processes. *I. tinctoria*leaf extract has been used for the microwave-assisted synthesis of nanoparticles [8]. The leaf extract was mixed with silver nitrate and chloroauric acid to obtained silver and gold nanoparticles respectively using a domestic microwave. The presence of tannins, saponins, alkaloids, etc. reduced the metal ions thereby forming nanoparticles. A visible change of color and sharp absorption peaks of the nanoparticles confirmed their synthesis. The authors reported potent anti-microbial, antioxidant, and anti-cancer effects of the Itinctoria functionalized nanoparticles. Prashanth *et al.* [26] synthesized zinc oxide nanoparticles using leaf extract of *I. tinctoria*. The chemotherapeutic potential of these nanoparticles was evaluated using DU-145 and Calu-6 cell lines. The nanoparticles showed marked cytotoxicity against these cells and had an IC_{50} value of about 2µg/ml. Also, these ZnOnanoparticles did not cause hemolysis up to 2mg/ml enabling these particles to be systemically injected without plausible toxic effects.

I.tinctoria L is a plant with multiple uses in old traditional medicines and industry. More advanced techniques might be used to manufacture various dyes, synthesis of nano particles, and for various medicines preparation without any side effects. The herbal properties of the plant need to be studied in more detail for better uses.

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