

Indigofera tinctoria Linn - A Phytopharmacological Review

Saraswathi Motamarri N^{1*}, Karthikeyan¹ M, Rajasekar S¹ and Gopal V²

¹Faculty of Pharmacy, PRIST University, Thanjavur, Tamilnadu, India.

²College of pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry-6, India.

ABSTRACT

A Large proportion of the world population especially the developing countries rely on the traditional system of medicines. The use of herbs in medicine is getting popularized because of its natural origin with no or lesser side effects. *Indigofera tinctoria* is a branching shrub used in traditional medicines, Ayurveda, sidha and unani. A galactomannan, composed of galactose and mannose in molar ratio of 1:1.52, Glycoside (Indian), Coloring matter (Indigotin), Flavonoids, terpenoids, alkaloids and glycosides, Indigotine, Indirubin, rotenoids are phytochemical constituents of *Indigofera tinctoria* are mainly responsible for its wide therapeutic actions. Studies conducted on *Indigofera tinctoria* showed that it possesses Anti hyperglycemic activity, Anti bacterial, Anti oxidant, Cytotoxicity Effect, Anti-inflammatory activity, Anti hepatoprotective activity, Antidiabetic activity, Anticonvulsive agent. In Traditional system of medicine it has medicinal properties as it is used in constipation, liver disease, heart palpitation, gout, bitter, thermogenic, laxative, trichogenous and expectorant. The present review attempts to provide comprehensive information on phytopharmacological properties of *Indigofera tinctoria* Linn for further research.

Key Words: *Indigofera tinctoria* Linn, phytoconstituents, pharmacological activities, Therapeutic uses.

INTRODUCTION

India is a country with rich natural resources with variety of medicinal plants. In contrast to synthetic drugs, Herbal drugs enjoy the advantages of comparatively less toxic than synthetic drugs, more harmony with the biological system and affordable to all classes of people⁵. In the last few decades, Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system. Medicinal plants play an important role in traditional health care systems as well as in international herbal and pharmaceutical markets. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds that produce a definite physiological action on the human body¹. The world health organization has reported that nearly 65-80% of the world's population in developing countries depends on the traditional medicine for their primary health care and treatment. This is because that the herbal medicines are cheap, and have natural origin^{2,3}. The plant *Indigofera tinctoria* belongs to the family Fabaceae which popularly known as Neeli in Tamil and found throughout India. The roots, stems and leaves of *Indigofera tinctoria* are bitter, thermogenic, laxative, trichogenous, expectorant, anthelmintic, gastropathy, splenomegaly, cephalgia, cardiopathy, hepatoprotective anticancer, epilepsy, neuropathy, chronic

bronchitis, asthma, ulcers, skin diseases, diuretic and are useful for promoting the growth of hair⁴. The plant was found to contain indirubin and indigotone where the juice extracted from the leaves is useful in the treatment of hydrophobia⁵.

Biological Source

Nili (leaf) consists of Dried leaf of *Indigofera tinctoria* Linn belongs to the Family Fabaceae. *Indigofera tinctoria* is a shrub, 1.2-1.8 m high, found throughout and widely cultivated in many parts of the country⁷.

Toxonomy of Plant

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Subfamily : Faboideae
Tribe : Indofereae
Genus : *Indigofera*
Species : *L.tinctoria*
Binomialname : *Indigofera tinctoria*

Botanical Description

Throughout India, Mainly as an escape from cultivation⁸.

Synonyms^{7,8}

Eng : Indian indigo

Hin : Nili

Kan : karunili

Mal : Neelamar Amar

San : Nililini

Tam : Avuri

Tel: Nili Chettu, Nili

Parts Used: Whole Plant**Description of Plant**

A branching shrub upto 2m high with 7-13 leaflets. Leaves are green when fresh and greyish black on drying, tender branches bluish red in color, flowers many in nearly sessile lax spicate racemes which are much shorter than the leaves with red or pinkish colour; fruits cylindrical pods, pale greenish grey when young and dark brown on ripening with 10-12 seeds[8].

Macroscopic

Plant occurs mostly in the form of leaflets and broken pieces of rachis; leaflet 1-2.5 cm long and 0.3-1.2 cm wide, oblong or oblanceolate with very short mucronate tip; pale green to greenish-black; no characteristic odour and taste.

Microscopic**Leaf-**

Petiole- appears nearly circular in outline having two lateral wings; epidermis single layered covered externally with thin cuticle and followed internally by single layered collenchymatous cells; pericycle present in the form of continuous or discontinuous ring, vascular bundles collateral and three in number, large one present in central and two smaller in lateral wings; pit composed of round to oval, thin-walled parenchymatous cells a few prismatic crystals of calcium oxalate present in phloem and pith region.

Midrib -Shows epidermis, cuticle and hair, similar as in petiole; beneath epidermis on lower side single or 2-3 layers of collenchyma on upper side present, both followed by 2-3 layers of thin-walled parenchyma; vascular bundle single collateral and crescent shaped

Lamina- Show dorsiventral structure; epidermis, cuticle and hair, similar as in petiole and midrib; palisade 2-3 layers; spongy parenchyma 2-4 layers, a few patches of veins scattered between palisade and spongy parenchyma, prismatic crystals of calcium oxalate rarely present in mesophyll cells; paracytic stomata and hair present on both surfaces but abundant in lower surface⁷.

Phytochemical Constituents

A galactomannan, composed of galactose and mannose in molar ratio of 1:1.52, isolated from

seeds and partially characterized Flavonoids, terpenoids, alkaloids and glycosides^{1,9,7} Indigotine, Indirubin, rotenoids¹¹

Traditional Uses

Indigofera tinctoria is used in constipation, liver disease, heart palpitation and gout¹⁰

The roots, stems and leaves are bitter, thermogenic, laxative, trichogenous, expectorant, anthelmintic, tonic, naturopathy, splenomegaly, echolalia, cardiopathy, chronic bronchitis, asthma, ulcers, skin diseases, diuretic and are useful for promoting growth of hair. The juice expressed from the leaves is useful in the treatment of hydrophobia. An extract of the plant is good for epilepsy and neuropathy. The plant possesses anti-toxic property⁸. The plant is stimulant, alternative, deobstruent and purgative. Indigo is antiseptic and astringent. The Juice of the leaves and indigo in powder are used mixed with honey in enlargement of liver and spleen, epilepsy and other nervous affections. In hydrophobia two ounces of fresh juice with an equal quantity of milk is given in the morning for 3 days as a prophylactic; Juice is also given in asthma, whooping cough, palpitation of heart, in some lung diseases and kidney complaints as in dropsy. Decoction of the root is given in calculus; Juice of the young branches mixed with honey is a used application for aphthae of the mouth in children. An Infusion of root is given as an antidote in cases of poisoning by arsenic. Externally, leaves crushed are used as stimulant, Poultice or plaster in various skin affections, to haemorrhoids etc., and to cleanse and heal wounds and ulcers. Powdered indigo also is used for sprinkling on ulcers. It is applied mixed with castor oil to the navel of children to promote the action of the bowels and mixed with warm to the pubes and hypogastria as it stimulates bladder and therefore useful in cases of retention of urine¹²

Pharmacological Actions**Anti hyperglycemic activity**

Amarnath V Bangar *et al.*, (2011) were postulated that investigate anti-diabetic and nephroprotective activity of *Indigofera tinctoria* leaves, using STZ-induced diabetic rats as model for clinical type-1 and type-2 diabetes. At a regular interval of an experimental protocol blood glucose, urinary creatinine, total proteins and organs to body weight ratio were studied. The histo-pathological study was carried out by STZ-induced diabetic and anti-diabetic rat's pancreas. Statistical analysis of the results shown that in STZ-induced diabetic rats chloroform and alcohol extracts of *I. tinctoria* leaves at 40, 80, 160 and 200 mg/kg doses. Significant effect of alcoholic extract from 4th day to 16th day of the study. *I. tinctoria* leaves extract improved renal creatinine clearance and reduce renal total protein loss demonstrating

nephroprotective properties. The organ to body weight ratio studies carried out on last day, shown pancreas and liver specific effects of *I.tinctoria* leaves. These results were also supported by histopathological studies. The present study conclude that alcoholic extract of *I.tinctoria* leaves with long-term treatment may be beneficial in the management of type-1 and type-2 diabetes¹³.

Anti bacterial, Anti oxidant and Cyto toxicity Effect

Renukadevi K.P *et al.*, (2011) study has been under taken with an objective to determine the antibacterial, anti oxidant and cytotoxic activity of the leaf extract of *indigofera tinctoria*. Anti bacterial activity was carried out on *in vitro* lung cancer cell line. The extract screened for phyto chemical analysis was found to contain bioactive compounds like flavonoid, saponins, tannins, steroidal terpenes, phenols and anthraquinone were identified by GC-MS analysis. The leaf extract *I.tinctoria* having the ability to inhibit the growth of gram positive bacteria namely *Staphylococcus aureus*, *Bacillus pumilus* and *Streptococcus pyogenes* and zone of inhibition was observed 16 and 17 mm, respectively but not shown growth of inhibition on gram negative bacteria *Escherichia* and *Pseudomonas aeruginosa*. Strong antioxidant activity was observed both qualitatively and quantitatively. The strong antioxidant was observed at 250 µg/ml-1 with an IC₅₀ value of 51.66 which is higher than that of standard ascorbic acid. The cytotoxic effect of *I.tinctoria* leaf extract on lung cancer cell line NCI-H69 was studied. The percentage cell viability of cells was found to decrease at increasing concentration. GC-MS analysis of the leaf extract shows 6 compounds. This study suggests that ethanol extract of *Indigofera tinctoria* have profound antibacterial, antioxidant and cytotoxic effect¹⁴.

Anti-Inflammatory effect

Pramodm K.Tyagi *et al.*, (2010) elucidate the anti-inflammatory activity of Ethanol extract of *I.tinctoria* leaves (500 & 1000 mg/kg). when compare to control as well as positive control Ibuprofen (Standard drug) group values are expressed as mean and SD. Statistical significance was determined using the student's t-test. Values with $p < 0.01$ were considered significant. The present study indicated that oral administration of ethanol extract of *I.tinctoria* linn dose dependently improve the potent anti-inflammatory activity. The extract lowers the carrageenan induced rat paw oedema. Further pharmacological and biochemical investigations are essential to elucidate the mechanism of action¹⁵.

Anti hepatoprotective activity

Muthulingam .M *et al.*, (2010) were investigated

the antihepatotoxic efficacy of aqueous extract of *I.tinctoria* (250, 500 mg/kg) and silymarin (25 mg/kg) against paracetamol induced liver damage in rats. Paracetamol at the dose of 3 g/kg body weight orally one day only produced liver damage in rats as manifested by the significant rise in serum levels of aspartate aminotransferase (AST), alanine-γaminotransferase (ALT), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), bilirubin, cholesterol and decrease the protein level compared to control. Treatment of rats with *I.tinctoria* and silymarin once daily for twenty eight days to paracetamol treated will increase in protein level. Furthermore, liver tissues were processed for histopathological observation. The extract alone treated rats did not adversely affect the serum biochemical and histopathological observation. The antihepatotoxic efficacies of the *Indigofera tinctoria* extracts were reported to be significant⁵.

Anti diabetic activity

Verma S.M *et al.*, (2010) estimate the total percent reduction in blood glucose level of the diabetes induced rabbits at different time intervals after administration of *I.tinctoria* leaf extract were considered for statistical analysis. Upon statistical evaluation (two-way analysis of variance), a significant ($p < 0.01$) difference was observed in total % reduction in blood glucose between different groups of rabbits while same was not observed within the group ($p > 0.05$) which indicating differ in concentration and nature of standard drug and methanolic leaf extract. Student's t-test was conducted for blood glucose level of different rabbits after administration of the leaf extract. The results have shown that there is a significant ($p < 0.001$) difference in blood glucose level between control versus standard drug and the test (Table-1). The further substantiate extracts that *I.tinctoria* Linn methanolic leaf extract possess significant antidiabetic activity¹.

Anti-Epileptic property

Asuntha G *et al.*, (2010) investigate the anti-Epileptic property of *I.tinctoria* induced in male albino Wistar rats by injection of lithium chloride (3 mEq/kg, i.p.) follow after 24 h administration of pilocarpine (30 mg/kg, i.p.). After 1h injection of pilocarpine different doses of the ethanol extract of *I.tinctoria* were administered orally. The severity of status epilepticus was observed and recorded using Racine scoring system for every 15 min for 90 min and thereafter every 30 min for another 90 min. In terms of the thiobarbiturate-reactive substances (TBARS) in-vivo lipid peroxidation of rat brain tissue was measured. Determined both in-vitro free radical nitric oxide (NO) and 2-diphenyl-2-picryl hydrazyl (DPPH) scavenging activities of the extract. The status epilepticus was significantly

reduced following oral administration of the extract at a dose of 500 and 1000 mg/kg. No test animal group exhibited stage 4 seizure. The extract also exhibited antioxidant activities in both in-vivo and in-vitro studies. The ethanol extract of *I.tinctoria* was found to be useful in controlling lithium / pilocarpine induced status epilepticus in albino rats⁴.

Phytochemical Study and Physiological Evaluation

Sauabh Jain *et al.*, (2010) evaluate the phytochemical studies on *I.tinctoria* were it is East Asia originated shrub belonging to the Fabacea Family. The leaves of *Indigofera tinctoria* leaves were collected locally, shade dried and extracted with methanol and petroleum ether by using Soxhlet apparatus. The yield of methanolic and petroleum ether extracts of leaves were 21% and 7.85% , respectively. For methanolic extracts of *I.tinctoria* (leaves) the preliminary phytochemical screening was carried out for the presence of alkaloids, flavonoids, carbohydrate glycosides, tannins, terpenoids, phenol and absence of steroids and saponins. The physical evaluation was carried out for the determination of water-soluble extractive value, Hexane-soluble extractive value, methanol-soluble extractive value, ash value includes total ash, acid insoluble ash and water-soluble ash, and moisture content for leaves of *I.tinctoria*¹⁶.

Neurotransmitters concentrations

Madhan Mohan.E, *et al.*,(2010) evaluate the effect of methanolic extract of *Indigofera tinctoria* (MEIT) on neurotransmitters concentrations in rat brain after induction of seizures by MES and PTZ. The leaves of *Indigofera tinctoria* Linn. (Family: Fabaceae) is traditionally used in the epilepsy and other nervous disorders, bronchitis and liver ailments. The relationship between seizure activities and altered the neurotransmitters such as noradrenaline, dopamine and serotonin in forebrain of rats in MES and PTZ seizure models is estimated. In MES model, MEIT(200 & 400 mg/kg) showed significantly restored the decreased levels of brain monoamines such as noradrenaline, dopamine and serotonin. Similarly in PTZ model, MEIT showed significantly increased the neurotransmitters in forebrain of rats. Thus, this study conclude that methanol extract of *Indigofera tinctoria* increased the neurotransmitters on rat brain, which may be decreased the susceptibility to MES and PTZ induced seizure in rats¹⁷.

Antinociceptive property

Saravanakumar *et al.*,(2009) investigated the analgesic activity of ethanol extract of *Indigofera tinctoria* Linn (Fabaceae) leaves (EEIT) in chemical models of nociception in mice. EEIT at

doses of 100, 200 and 400mg/kg p.o produced an inhibition of 21.71%, 42.62% and 72.38%, of the abdominal writhes by acetic acid induced in mice. In the formalin test, the administration of 100,200 and 400mg/kg p.o had no effects in the first phase (0–5 min) but produced a dose-dependent analgesic effect on the second phase (15–40 min) with inhibitions of the licking time of 26.62%, 46.5% and 60.07%, respectively. In hot plate method, EEIT at the dose of 400mg/kg showed the mild analgesic and EEIT 100 and 200 mg/kg showed non significant analgesic effect due to peripheral analgesic activity. Based on the results of this study, we suggest that the peripheral analgesic effect of *Indigofera tinctoria* may be attributed to inhibition of prostaglandin release and other mediators involved. Further studies are needed to evaluate the mechanism of action of the analgesic activity of the *Indigofera tinctoria*¹¹.

Anthelmintic activity

Gunasekaran balamurugan *et al.*,(2009) investigated the anthelmintic activity of *Indigofera tinctoria* Linn (whole plant) against *Pheretima posthuma*. Various concentrations (50 and 100 mg/ml) of each extract were tested in the assay, which involved the determination of paralysis time and death time of the earth worms. The methanol extract exhibited a maximum anthelmintic activity comparable to standard drug Piperazine citrate (10 mg/ml). The petroleum ether and chloroform extracts exhibited a modest activity. The Preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts¹⁸.

Protective Effect of Flavonoidal Fraction

Kameswaran Ravichandran *et al.*,(2008) studied the chemopreventive effect of the flavonoidal fraction of *Indigofera tinctoria* in swiss albino mice using 16 week medium term model of benzo(a)pyrene(BP)-induced lung tumor. lung tumor was induced biweekly through oral incubation of BP for 4 weeks. The oral administration of flavonoidal fraction of *I.tinctoria* (100 mg/kg) showed a significant increase in anti oxidant enzymes like super oxide dismutase (SOD), glutathione reductase(GR), catalase(CAT) and non enzymatic antioxidants like reduced glutathione(GSH), vitamine C and vitamine E were significantly increase when compare to BP treated groups. The effect is much more pronounced in pretreatment regime than in post treatment regime. The levels of lung marker enzymes were significantly decreased in both the treatment regimes when compared to cancer induced group. The levels of lipid peroxidation were significantly decreased in the flavonoidal fraction of *I.tinctoria* treated regimes. The elevated levels of glycoproteins were normalized in the animals

subjected to flavonoidal fraction of *I.tinctoria* treatment. The histopathological studies conformed the protective effect of the extract by showing the reappearance of alveolar spaces in the treatment regimes, where the effect is pronounced much in the pre treatment regime than the post treatment regime. From the results obtained it is concluded that flavonoidal fraction of *I.tinctoria* had protective effect on BP induced lung cancer¹⁹.

Anti proliferate activity

Thiruvznmioor Ravichandran *et al.*, (2008) was investigated the antiproliferative activity of aerial parts of the plants in human non Small Cell lung cancer cells A-549. The results showed that the flavonoidal fraction of methanolic extract of the aerial parts of the plant inhibited the proliferation of A-549 cells as measured by MTT assay. Flow cytometric analysis showed that flavonoidal fraction of methanolic extract of *I.tinctoria* blocked cell cycle progression in G0/G1 phase. In addition flavonoidal fraction of methanolic extract of *I.tinctoria* induced A-549 cell apoptosis as determined by propidium iodide staining. We suggest that its overall chemopreventive effect of flavonoidal fraction of *I.tinctoria* against lung cancer and can possibly be considered as for future therapeutic application²⁰.

Antidyslipidemic activity

Anju Puri *et al.*, (2007) investigated the antidyslipidemic activity of the alcoholic extract from *Indigofera tinctoria* as well as its three other components, that is chloroform, butanol and aqueous fractions in dyslipidemic hamsters that were fed a high fat diet. The chloroform fraction showed a significant decrease in the plasma triglycerides (TG, 52%) ($P < 0.001$), total cholesterol (TC, 29%) ($P < 0.05$), glycerol (Gly, 24%) and free fatty acids (FFA, 14%). This decrease was also accompanied by an increase in high density lipoproteins (HDL) by 9% and an increased HDL-C/TC ratio of 52% at the dose of 250 mg/kg of body weight²¹.

CONCLUSION

The vast study done on the plant proved that the plant has many important phytochemical compounds like galactomannan, composed of galactose and mannose in molar ratio of 1:1.52, Glycoside (Indian), Coloring matter (Indigotin), Flavonoids, terpenoids, alkaloids and glycosides, Indigotine, Indirubin, rotenoids and other related compounds. These compounds were found to be responsible for many pharmacological activities such as Cyto toxicity Effect, Anti hyperglycemic activity, Anti oxidant, Anti-inflammatory activity, Anti bacterial, Anti hepatoprotective activity, Anti diabetic activity, Anticonvulsive agent. Plant has therapeutic uses as medicinal properties as it is

used in constipation, liver disease, heart palpitation and gout, bitter, thermogenic, laxative, trichogenous, expectorant, anthelmintic, tonic and diuretic and are useful for promoting growth of hair and in naturopathy, splenomegaly, echolalia, cardiopathy, chronic bronchitis, asthma, ulcers, and skin diseases. Hence, this plant provides a significant role in the prevention and treatment of a various disease and in the protection of the system from damage. The therapeutic potential of *I.tinctoria* is effective and versatility is such that further detailed research appears crucial.

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