

Review: Propagation and Production of Secondary Metabolites and its after Study on Lung Cancer Cell Line Evaluation of Antibacterial, Antioxidant effects of *Indigofera tinctoria* Linn.

Raj Kumar Ahirwar¹, Hitesh Raj Wankhede², Shradha Choudhary³, Dr. Smt Rakesh Mehta⁴.

¹Research Scholar

⁴Head of Department in Botany and Biotechnology, M.G.M. P. G. College, Itarsi (M. P.)

Abstract

There is study of basic objective to review of the antimicrobial, antioxidant and cytotoxic activity of the different methanolic solvents screened for phytochemical analysis was found to contain bioactive compounds like steroids, saponins, flavonoids, terpenoids, phenolic compound, anthraquinones, tannins and reducing sugars. Antibacterial activity was carried out against gram positive and gram negative bacteria. Antioxidant property was determined quantitatively. Study of cytotoxic activity of the methanolic root extract was carried out on human lung cancer cell line. Among the different solvent extracts used in the study, the methanolic root extract showed highest antimicrobial and cytotoxic activity against microbes and human cancer cell line respectively. The aerial part of the plant was investigated for its anti proliferative activity in human Non Small Cell Lung Cancer.

Indigofera Tinctoria

The plant *Indigofera tinctoria* Linn. (fabaceae) popularly known as “true indigo” is a common remedy for various ailments. It has been cultivated from worldwide centuries. The Indigo dye is shrub one to two meter height. It may be annual, biennial or perennial. Roots and leaves are used for epilepsy and hydrophobia. The phytoconstituents are responsible for the pharmacological screening in the presence

of phytochemical constituents. Dry powder is used in the treatment of asthma (Savithramma., et al., 2007). The aerial parts of the plants were used in treatment of antiproliferative activity in human non small cell lungs cancer cell A-549 (Kameswaran., et al., 2008). The hepatoprotective treatment is given by aerial parts of *Indigofera tinctoria* Linn.(Singh et al., 2001). The antidyslipidemic activities are given in aerial

parts of *Indigofera tinctoria* Linn. (Puri et al., 2007).

Indigofera is one of the oldest coloring agents known to man and is among the most widely used naturally dye in the world.³ Medicinally the chines use *Indigofera tinctoria* to clear the liver, detoxify the blood, alleviate pains and reduce fever. Sap from the whole plant of *I. Hirsute* is used in the case of injury to the eyeball and inflammation of the eyelids.

Cancer Cell

Cancer cells are growing and divide at an unregulated, quick pace. Although cancer cells can be quite common in a person, they are only malignant when the other cells (particularly natural killer cells) fail to recognize and destroy them. In the past a common belief was that cancer cells failed to be recognized and destroyed because of a weakness in the immune system. However more recent research has shown that the failure to recognize cancer cells is caused by the lack of particular co-stimulated molecules that aid in the way antigens react with lymphocytes.

Review

Secondary metabolites

Plant have developed a complex biochemical defense system that including carotenoids and flavanoids. Flavonoid compounds, as secondary metabolites are considered to play a major role in protecting plants from UV-B damage (Liang et al., 2006). These flavonoids generally absorb the light in the region of 280~320 nm and thus are capable of acting as a UV filter, thereby protecting the photosynthetic tissues from damage (Siefermann, 1987). Flavaonoids stabilize and protect the lipid phase of the thylakoid membrane, and are quenchers of the excited triplet state of chlorophyll and singlet oxygen (Agawal and Rathore, 2007). Apart from the flavonoids, carotenoids also have antioxidant properties which act as an internal filter against UV-B radiation. Plants scavenge reactive oxygen species by detoxification mechanism produced by enzymatic antioxidant such as catalase, peroxidase, superoxide dismutase and phenylalanine ammonia-lyase etc (Moran and Porath, 1980).

- 1. Mann R. A. et al., (1990)**, there are studies a range of 4-9.5 t/ha dry matter yield of indigo with N addition of 107-257 kg N/ha was obtained under lowland conditions; under upland, the respective figures were 3-6 t/ha with 87-180 kg N/ha.
- 2. Meenakshisundaram sreepriya et al., (2001)** the aerial part of the plant was investigated for its antiproliferative activity in human Non Small Cell Lung Cancer A-549. The results showed that the flavanoidal fraction of methanolic extract of the aerial parts of the plant inhibited the proliferation of A-549 cells as measured by MTT assay.
- 3. B. Singh et al., (2001)** obtained by fractionation of a petroleum ether extract of the aerial parts of *Indigofera tinctoria*, showed significant dose related hepatoprotective activity against ccl_4 induced liver injury in rats and mice. Hexobarbitone induced 'sleeptime', zoxazolamine induced 'paralysis time', and levels of transaminases, bilirubin and total protein in serum were employed as indices of liver injury.
- 4. Jowita Orska-Gawrys et al., (2003)** As the main individual chemical components of natural dyes, anthraquinone, indigoid and flavonoid dyes including alizarin, purpurin, luteolin, apigenin, carminic acid, ellagic acid, gallic acid, laccaic acids A and B and indigotin were found.
- 5. M. Chitra et al., (2003)** two different dose volumes of *Indigofera tinctoria* (5ml/kg of the body weight and 10ml/kg of body weight) were given to determine the hepatoprotective efficacy. The effect of the extract was found to be dose dependent and the altered levels of AST, ALT, ALP and other serum parameters such as total protein, total bilirubin are showing normal values.
- 6. B. Singh et al., (2006)** TCA was found to reverse the altered hepatic parameters in experimental liver damage. In the safety evaluation study the oral LD_{50} was found to be more than 2000 mg/kg, with no signs of abnormalities or any mortality for the 15 day period of observation after administration of a single dose of drug in mice.
- 7. Chonyu Chen et al., (2008)** in the ultraviolet resistance test, the sodium hydrosulfite with polygonum tinctorium showed the best result with T% 0.19. The dyed cotton fabric also revealed the characteristic functional peak of indigo blue at 1624 cm^{-1} via FT-IR test.

8. Saravana Kumar A *et al.*, (2009). The study reported the significant delay in clonic seizure induced by PTZ and dose dependent decrease in duration of hindleg extensor phase in MES model. In MES model, MEIT showed significant reduction in duration of hindleg extension with 200 mg/kg dose and effect was dramatically reduced with 400mg/kg.

9. Neelawan P ongsilp and Achara Nuntagij., (2009) a total of 215 root-nodule bacteria were isolated from 3 medicinal legumes including *Indigofera tinctoria*, *Pueraria mirifica* and *Derris elliptica* Benth. Naturally grown in 16 provinces of Thailand. The isolates were evaluated for DNA polymorphism using randomly amplified polymorphic DNA (RAPD) analysis. Isolates generated 92 identical RAPD profiles, indicating highly significant genetic diversity among isolates from distinct geographic areas.

10. K. C. Ravindran *et al.*, (2010) the impact of UV-B radiation (UV-B, 280~320nm) on growth, biochemical and antioxidant enzyme activity was studied in *Indigofera tinctoria* (L.) Seedling, commonly used as a green manure. The supplementary UV-B radiation significantly decreased the growth, development and changes in UV-B absorbing compounds such as anthocyanin and flavonoids.

11. Chonchanok Leelahawonge *et al.*, (2010) fourteen root-nodule bacteria isolated from the medicinal legume *Indigofera tinctoria* were characterized for their phenotypic features including growth curves, utilization of carbon and nitrogen sources, antibiotic resistance, vitamin requirement and growth under different conditions.

12. S. Selvakumar and C.M.Karunakaran., (2010) Different parts of medicinal plants have been used to cure specific ailments. Today, there is wide spread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacy-therapeutic agents in medicinal plants.

13. G Asuntha *et al.*, (2010) the present study was designed to verify this claim. The severity of status epilepticus was significantly ($p < 0.01$) reduced following oral administration of the extract at 500 and 1000 mg/kg doses.

14. Saurabh Jain et al., (2010) the yield of petroleum ether and methanolic extracts of leaves were 7.85% and 21%. Respectively. The preliminary phytochemical screening was carried out for the presence of alkaloids, flavonoids, carbohydrate glycosides, tannins, terpenoids, phenol and absence of steroids and saponins for methanolic extracts of *Indigofera tinctoria* (leaves).

15. G. Balamurugan and P. Muralidharan., (2010) the oxidative stress reducing effect of methanol extract of *Indigofera tinctoria* leaves (250 and 500 mg/kg) was investigated on β -amyloid (25-35) peptide-induced Alzheimer's disease in mice.

16. B. R. Sarkar et al., (2011) the ethanol extract of *Indigofera tinctoria* (250, 500 & 1000 mg/kg bw, p.o.) Reduced acute paw oedema volume induced by sub-planter injection of carrageenan (0.1ml of 1% solution) in Wistar Albino rats using plethysmometer. Ibuprofen (100 mg/kg bw, p.o.) Were used as a standard drug (positive controls). Ethanolic extracts of leaves of *Indigofera tinctoria* Linn (500 & 1000 mg/kg bw) showed potent anti-inflammatory activity when compared to control as well as positive control Ibuprofen (standard drug) group.

17. K. P. Renukadevi and S. Suhani Sultana., (2011) Strong antioxidant activity was observed both qualitatively and quantitatively. The strong antioxidant was observed at 250 μ g ml⁻¹ with an IC₅₀ value of 51.66 which is higher than that of standard ascorbic acid. The cytotoxic effect of 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.

18. Saraswathi Motamarri N et al., (2012) *Indigofera tinctoria* is a branching shrub used in traditional medicines, Ayurveda, siddha 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.

19. G. Priyadarsini et al., (2012) Nephrotoxicity were induced in wistar albino rats by intraperitoneal administration of Cisplatin 5mg/kg. Effect of concurrent administration of AKL and AKRL *Avuri kudineer* at a dose of 500 mg/kg and 1000mg/kg were given for respective animal

groups by oral route was determined using serum creatinine and blood urea and change in body weight as indicators of kidney damage.

20. Magesh vijayan et al., (2012) *Indigofera tinctoria* Linn was traditionally been used in the ancient herbal medicine in India/China for epilepsy, nervous disorders, bronchitis, and liver ailments; and also used as an anti cardiovascular, but there was no literature evidence substantiating its antibacterial activity.

21. S. Rajaperumal et al., (2013) The present study has been under taken with an objective to determine the antimicrobial, anti oxidant and cytotoxic activity of the methanolic root extract of *Indiofera aspalathoides* (Vahl ex Dc) by using different solvents(methanol, ethanol, aqueous). Determination of cytotoxic activity of the methanolic root extract was carried out on human lung cancer cell line NCL H460. Among the different solvent extracts used in the study, the methanolic root extract showed highest antimicrobial and cytotoxic activity against microbes and human cancer cell line respectively.

22. Nagarajan anusuya and sellamuthu manian., (2013) *Indigofera tinctoria* L. (Fabaceae) is traditionally used in Indian and Chinese medicinal systems for various ailments including cancer, liver disorder, inflammation, ulcers and nervous disorders

Summary

The main objective of this study is Secondary metabolites production and evaluation of anti-bacterial and antioxidant activity of *Indigofera tinctoria* Linn. *In vivo* testing of the anti-bacterial and antioxidant activity of ethanol extract of leaves of *Indigofera tinctoria*. The Study of Flow cytometric that flavanoidal of methanolic

extract of *I. tinctoria* blocked cell cycle progression. In addition flavanoidal fraction of methanolic extract of *I. tinctoria* induced cancer cell apoptosis as *I. tinctoria* activity might be potentially contribute to its overall chemo preventive effects against lung cancer and can possibly be considered for future therapeutic application.

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