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A SYSTEMATIC REVIEW ON PHARMACOLOGY, PHARMACOGNOSY AND PHYTOCHEMISTRY OF *BAPTISIA TINCTORIA*

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ABSTRACT

Baptisia tinctoria (Wild indigo; Fabaceae) has long tradition of use in Indian systems of medicine for the treatment of much disease. Roots of *B. tinctoria* were successively extracted to obtain n-hexane, chloroform, methanol and water extracts. Preliminary phytochemical screening of ME showed presence of alkaloids, steroids, flavonoids, triterpenoids, coumarins and tannins as major classes of phytoconstituents. This review attempts to encompass the available literature on *Baptisia tinctoria* with respect to its pharmacognostic characters, chemical constituents and summary of its various pharmacological activities.

Key words: *Baptisia tinctoria*, Fabaceae, Pharmacology, Phytochemistry, Pharmacognosy.

INTRODUCTION

This is commonly known as Wild Indigo, a plant that grows abundantly throughout the United States. The tincture is prepared from the fresh root and the bark of this plant. Wild indigo (*Baptisia tinctoria*), also called *Sophora tinctoria* is a herbaceous plant belonging to the legume family (Fabaceae) and is widely used in traditional medici lane (Bone, 2003). Its roots are used in traditional South American Indian medicine to treat wounds, ulcers, and inflammation. Currently, it is considered an immunomodulatory drug and has been introduced into homeopathy since the middle of the 19th century.

Cultivated *B. tinctoria* plants have been used to treat pneumonia, influenza, and tuberculosis. Tea made from this herb was used to wash smallpox lesions internally and externally. An infusion of *B. tinctoria* and *Juniperus utahensis* has been used as a drug for kidney disease. Moreover, *B. tinctoria* poultices have been used to treat snake bite injuries. Well-known anticancer agents include vitamin C, vitamin E, carotenoids, and flavonoids. Recently, peptides with antioxidant properties have been identified in various plant and creature sources.

Synonym: *Sophora tinctoria L*, Baptisia *gibbesii* small, wild indigo, yellow false indigo

Taxonomy:

- Kingdom : Plantae
- Clade : Trancheophytes
- Clade : Angiosperms
- Clade : Eudicots
- Clade : Rosie's
- Order : Fabales
- Family : Fabaceae
- Sub-Family :Faboideae
- Genus : Baptisia
- Species :B.tinctoria

Geographical source:

Baptisia tinctoria is found throughout the eastern United States, West to Minnesota and South to Florida. The multiple bushy stems of *Baptisia tinctoria* reach 2-3 feet tall. The leaves are silver green each is divided into three leaflets about 1/2 inch long. The flowers are yellow and grow in spikes 1/2 to 3 inches long.

Macroscopical:

Fleshy up to 4 cm in thickness, usually cut into the elongated cylindrical segments, the crown form 5 to 8

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cm in thickness, more or less warty and marked by stem scars; outer surface dark brown; usually longitudinally wrinkled, transversely warty or the thicker pieces covered with soft corky layer, fracture though and surface whitish.

Microscopical:

Powder: Light greyish to greyish-brown, starch grains numerous, simple or 2 to 4 compound, the individual grains spheroidal, plane-convex up to 16 µ diameter, the larger grains occasionally showing a central cleft, fragments of parenchyma the cells of which are filled with starch, relatively few fragments of slightly lignified cork tissue with cells being yellowish-brown walls, tracheae with slit like or bordered pores associated with fragments of sclerenchymafibres that are long, thickwalled, fragments of medullary rays with cells having thick, lignified,porous walls and containing starch.

Pharmacognostic studies:

The roots of the plant contain flavonoids, baptigenin, pseudobaptigenin, coumarins, tannins, triterpenesaponins, glycoproteins, polysaccharides, and quinolizidine alkaloids.

Major chemical compounds

Bioactive compounds identified in the plant include biochanin A, cytisin, daidzin, formonetin, fustin, garbanzol, ononin, crobol, pseudobapligenin, scopoline, sparteine, tectoridin, and tectorigenin.[Phytochemical dictionary. A handbook of bloactive compounds from plants, Harbome, J.B. et al., 1999, p. 976). The roots also contain arabinogalactans (heteroglycans with a molecular weight of 25000-500000), glycoproteins, flavones such as apigenins, apigenins 7-0 rhamnoglucoside, luteoline, luteoline 7-0 glucoside, lutéoline 7-0 rhamnoglucoside, isoflavones such as genisteine, genisteine 7-0 - rhamnoglucosides,

Phytochemical Screening and Acute Toxicity Studies:

The percentage yield (w/w) of ME of *B. tinctoria* roots was found to be 16.98% w/w. The ME was further fractionated with ethyl acetate. Yield of EAF was found to be 26.58% w/w in relation to the ME. The HE, ME, EAF and RME of *B. tinctoria* roots were screened for different classes of phytoconstituents using specific standard reagents. Phytochemical screening showed presence of fixed oils in HE; proteins, carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, tannins and coumarins in ME; alkaloids, flavonoids, steroids, triterpenoids, tannins and coumarins in EAF and proteins, carbohydrates in RME. Preliminary phytochemical screening showed the presence of only fixed oils in HE of *B. tinctoria* roots. HE was not subjected to acute toxicity studies as it was used only for defatting of plant material. No mortality and lethality was observed in mice treated with 2 g/kg dose of the ME of *B. tinctoria* roots. CNS studies of ME of *B. tinctoria* roots were performed at 200 and 400 mg/kg, i.e., 1/10th and 1/5th dose employed in acute toxicity studies.

Obtaining the extract from *Baptisia tinctoria*:

The extracts to be used according to the invention are produced by conventional methods of extracting plants or parts of plants. Regarding the appropriate conventional extraction methods such as maceration, remaceration, digestion, agitation maceration, ultrasonic extraction, countercurrent extraction, percolation, repercolation, evacuation (extraction under reduced pressure), diacolation and solid-liquid extraction under continuous reflux, the extraction is carried out in a Soxhlet extractor.

An Acidic Arabinogalactan-Protein from the Roots of Baptisiatinctoria

An Acidic Arabinogalactan protein (AGP) isolated from an aqueous extract of roots of wild indigo [baptisiatinctoria (L).R.Br.]

Glycoproteins from *Baptisia tinctoria* Roots by Affinity Chromatography and Iso electric Focusing

Polysaccharide-containing fractions from *Baptisia tinctoria* roots have been described as being immunologically active. For further purification of these active glycoproteins, affinity chromatography and isoelectricfocussing were applied. As starting material, we used a retentate from Baptisiasabetinctoria roots obtained by ultrafiltration (cut-off point: 10000 poly daltons).

For affinity chromatography, antibodies were raised in rabbits against the glycoprotein fractions with the highest activity. The IgG fraction of the antiserum was purified on Pro tein-G Sepharose Fast Flow (Pharmacia), and then coupled to char CNBr-activated Sepharose 4 B (Pharmacia) that was packed in a C 10/10 column (Pharmacia). After application of the sample, dete washing was performed until no protein could be detected any longer. Then we started elution under acidic conditions. The obtained fractions were analyzed by means of SDS-PAGE and gala the amount of active glycoproteins was determined using a specific ELISA (4)

Preparative iso electric focussing was performed in the Rotofor cell (Bio-Rad). The sample was initially fractionated in a pH range between 3 and 10. After determination of the pH value, the single fractions were analyzed by means of SDS PAGE. Positive protein-containing fractions were pooled and re-applied to the Rotofor cell. During refractionation, a nar rower pH gradient was used, which resulted in an improved fo cussing. The fractions were again analyzed using SDS-PAGE HIB and ELISA. Positive glycoproteins revealed pH values between 4 and 6

Lyophilisates of the positive fractions from immune affinity chromatography as well as those from preparative isoelectricfocussing exerted enhanced

immunomodulating ac tivity in vitro. It is concluded that both methods are suitable for the purification of immunologically active glycoproteins from the roots of Baptisiatinctoria.

Determination of total phenolic composition with HPLC analysis

HPLC analysis was performed as described previously by Park et al. (2018). Quantitative analysis of phenolic compounds was performed using an Agilent 1100 series HPLC system (Agilent Technologies Inc., Waldbronn, Germany) equipped with a diode array detector (DAD) and autosampler and column compartment. The system was equipped with a ZORBAX Eclipse XDB-C18 column (4.6 × 250 mm, 5 μm) (Agilent Technologies Inc., Santa Clara, CA, USA), and the column temperature was set at 30°C. Elution was performed using 70% mobile phase A (0.1% acetic acid in HPLC water), 17% B (methanol), and 13% C (acetonitrile), the flow rate being 1.0 mL/min, and detection being performed at 278 nm under ultraviolet light for phenolics and flavanols. The total running time was 45 min. The water and ethanol extracts of *B. tinctoria* were filtered through a 0.20 μm SM13P020NL filter (Hyundai Micro, Seoul, South Korea) and injected into the HPLC system (injection volume, 10 μL).

Determination of the total phenol content (TPC)

The TPC was determined using a mixture of 1 mL of 95% ethanol and 1 mL of the extract. First, 5 mL of DW was added to 1 mL of sample extract solution, followed by 0.5 mL 1 N Folin-Ciocalteu reagent (Junsei chemical Co. Ltd., Tokyo, Japan), and the solution was mixed well in a vortex mixer KMC-1,300V (Vision Scientific Inc., Daejeon, Korea). The mixture was allowed to stand for 5 min, after which 1 mL of Na2CO3 was added. Subsequently, a UV spectrophotometer (Optizen 3220, Merasys Co. Ltd, and Seoul, Korea) was used to measure the optical density (OD) at 725 nm. TPC was estimated using a standard curve with gallic acid as the standard (mg/g) (Folin and Denis, 1912).

Pharmacological activites:

Antioxidant and skin health-enhancing activities

The antioxidant and enzyme inhibitory activities of *B. tinctoria* roots had not been investigated. Therefore, *B. tinctoria* extract could exhibit antioxidant activities, such as DPPH and ABTS radical cation scavenging activities, as well as the activities of HAase, tyrosinase, elastase, and collagenase, as a natural anti-inflammatory substance.

Antidepressant activity:

Laca mice (either sex, body weight 20-25 g) were used for pharmacological activites. Animals were acclimatized to laboratory conditions daily for 1 h for seven days before starting the experiment. Groups of six animals were used in all sets of experiments. The test drugs were administered orally with the help of an oral cannula fitted on a tuberculin syringe. The antidepressant and locomotor activity studies were investigated using well established models such as forced swim test (FST) and open field test, respectively, as per standard procedures. Distilled water+Tween 80 (2%) were used as a vehicle for preparing various test doses of crude extracts, fractions and the standard drug. Imipramine (Triko Pharmaceuticals, Rohtak, Haryana, India; 15 mg/kg, p.o.), was used as standard antidepressant drug.Hesperitin is being reported for the first time in this plant. A perusal of literature revealed that hesperitin and its glycoside hesperidin exhibited strong antidepressant activity.

Central Nervous System Activity

Anti-stress Activity

The ME and EAF of *B. tinctoria* roots were evaluated for anti-stress activity in mice using cold swim test. The ME (400 mg/kg) and EAF (106 mg/kg) significantly reduced duration of immobility in mice with respect to control and statistically equivalent to the standard drug

Antianxiety Activity

The ME and EAF of *B. tinctoria* roots were screened for antianxiety activity in mice using EPM model. The ME (200 or 400 mg/kg) and EAF (106 mg/kg) significantly increased number of entries and average time spent by mice in open arms of EPM with respect to control, but therapeutic level equivalent to the standard drug was not achieved at all tested doses The results suggest that ME and EAF of *B. tinctoria* roots exhibit mild antianxiety activity.

Sedative Activity

Potentiation of thiopentone sodium induced-sleeping time assay was employed to assess sedative activity of the ME and EAF of *B. tinctoria* roots. The mean latency time and duration of sleep after acute oral administration of methanol extract (200 or 400 mg/kg, p.o.), EAF (106 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) and the control (vehicle) have been shown in table 1. In this assay, thiopentone sodium (40 mg/kg, i.p.) was administered to all groups of mice to induce sleep. The standard drug, diazepam at the dose of 1 mg/kg significantly potentiated thiopentone sodium induced sleeps in mice with respect to the control. It is clearly evident from the table 1 that mean duration of sleep observed in mice treated with vehicle was 49.66 min, whereas standard drug potentiated sleep in mice to 86.33 min. The mean duration of sleep observed in groups of mice treated with ME and EAF was statistically equivalent to the vehicle treated group. These observations infer that

ME and EAF were devoid of sedative activity.

Anticonvulsant Activity

All extracts of *B. tinctoria* roots were subjected to anticonvulsant activity in mice using maximal electro-shock test. It is clearly evident from table 1 that ME and EAF were devoid of anticonvulsant activity as mean duration of extensor phase in mice treated with ME and EAF were found to be statistically equivalent to control group of mice treated with vehicle. On the other hand, phenytoin (a standard anticonvulsant drug) significantly reduced duration of extensor phase in mice and protected all the mice from mortality due to MES- induced convulsions.

Treatment of typhoid

Typhoid is one of the most serious infectious bacterial diseases in third world countries. It is usually treated by traditional antibiotics but due to the appearance of antibiotic resistant strains physicians opt for phyto products and other alternative medicines for the treatment of typhoid. Baptisia, an extract from indigo plant root, has been proved to be highly effective ultradilute medicine for the treatment of typhoid; however, the mode of action of the ultradilute extract is uncertain. Due to the antigenic variations of Salmonella it seems to induce immuno system by activating both T and B cells by the formation of antibodies. This principle seems to be highly effective for the development of typhoid vaccine. The present studies found that Baptisia administration possibly caused a salmonella-like reaction in the body as this extract produces an endogenous antibody similar to salmonella reaction. Thus, this study suggests that *Baptisia tinctoria* extract can be used for the prevention and treatment of typhoid.

Anti-inflammatory Effect:

The anti-inflammatory effect of wild indigo (*Baptisia tinctoria*) root in Raw 264.7 cells. We prepared two extracts of *B. tinctoria*: one in water and the other in 50% ethanol. Then we evaluated the toxicities of the *B. tinctoria* root extracts at 10 to 100 mg mL-1 concentrations in Raw 264.7 cells and observed 80% cell viability. An anti-inflammatory effect of *B. tinctoria* root extract in lipopolysaccharide (LPS)-stimulated Raw 264.7 cells was observed with concentrations of 10, 30, and 50 μg·mL-1. The results showed that 77.27–66.82% of nitric oxide (NO) production was inhibited by 50 μg·mL-1 *B. tinctoria* root extract. The protein expression of inducible NO synthase (iNOS) expression dramatically decreased by 93.14% and 52.65% in Raw 264.7 cells treated with water and ethanol extracts of *B. tinctoria* root, respectively. Moreover, cyclooxygenase-2 (COX-2) protein expression decreased by 42.85% and 69.70% in Raw 264.7 cells treated with water and ethanol extracts of *B. tinctoria* root, respectively. Furthermore, the mRNA expression of pro-inflammatory markers, such as tumor necrosis factor alpha, interleukin-1β, interleukin-6, monocytechemoattractant protein-1, and prostaglandin E synthase 2, was significantly suppressed in a concentration-dependent manner. Additionally, the *B. tinctoria* root extracts effectively inhibited enzymes involved in physiological activities. The *B. tinctoria* root extracts showed excellent anti-inflammatory effects and can be used as a functional material for biological activities.

Antimicrobial

These indications are reflective of a good antimicrobial phytomedicine. Moderate in vitro activity has been reported for extracts against Staphylococcus aureus(6), and yet surprisingly few other studies into antimicrobial activity or clinical studies appear to have been published on the use of Wild Indigo alone. Clinical trials involving combinations of Baptisiatinctoria root, Echinacea purpurea root, Echinacea pallida root and Thujaoccidentalis reported an improvement in cold symptoms earlier than placebo(7, 8, 9). Enhanced phagocytic activity by leukocytes was also reported for a combination of Baptisiatinctoria, Eupatorium cannabinum and Arnica with Echinacea angustifolia, than that measured for Echinacea alone(10).The fact that large doses can be emetic, may account for some of this relative paucity of scientific studies into the Wild Indigo's antimicrobial potential.

Table.no:1 Desorption of isoflavones according to the indicated rate:

Eluent fraction number ( )	Eluted substance	Yield of the dried fraction(weight fraction/weight raw extract)
Water	Polar Alkaloids	41.49%
Methanol 20%	Polar Alkaloids	1.12%
Methanol 40%	Polar Alkaloids	3.58%
Methanol 40% and 60%(B)	Polar Alkaloids, Daidzein glycoside, Genistein glycoside	3.51%
Methanol 60%(C)	Polar Alkaloids,Daidzein glycoside,Genisteine glycoside,Formonetine glycoside	4.03%

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Methanol 60% and 80%(D)	Non-Polar Alkaloids,Daidzeine glycoside,Genistein glycoside,Formononetin,Biochanin,glycoside	22.76%
Methanol 80%(E)	Non-Polar Alkaloids, Dadzein, Genistein glycoside Formononetin glycoside, Biochanin	3.20%
Methanol 80%(F)	Non-polar alkaloids, dadzein, genistein, formononetin glycoside, Biochanin glycoside	1.48%
Methanol 80% and Methanol 100%(G)	Non-Alkaloids, Genistein, Formononetin	3.26%

Fig.1 Aerial view of *Baptisia tinctoria*

Fig.2 *Baptisia tinctoria* root

Fig.3 - Genistein.

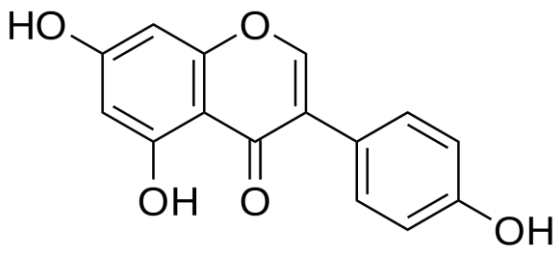
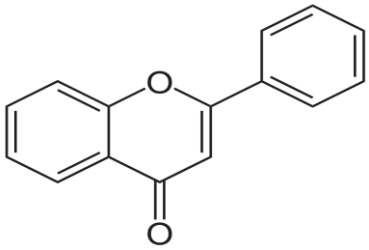


Fig.4- Flavonoids



DISCUSSION & CONCLUSION:

More than 100 phytochemicals compounds have been characterized and isolated from various parts of the plant *baptisia tinctoria* including phenol content, proteins, carbohydrates, alkaloids, Flavonoids, steroids, triterpenoids, Tannins and coumarin phenol content has been analyzed by using HPLC analysis All the reports pharmacological activites like. Anti-Oxidant, Antidepressants, anti-stress, anti-anxiety, sedative activity anti convulsant. Skin health enhancing activities and treatment of thyroid has been observed due to the phytochemicals present in plants.

Extensive research into the Phytochemistry& Pharmacological Properties of *Baptisia tinctoria* from its use as a Remedy for a various medical Conditions Almost a hundred Secondary metabolites have been identified from the aerial Parts. Several researches have suggested that *Baptisia tinctoria* extracts and isolated compounds could have a wide therapeutic potency range. More research is needed to uncover key features of *Baptisia tinctoria* in medicinal Practice.

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