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INDIGOFERA ASPALATHOIDES VAHL EX. DC. (SIVANAR VEMBU): A PHYTO PHARMACOLOGICAL REVIEW

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ABSTRACT: Since ancient times, herbal drugs are used in traditional system of medicine such as Siddha, Ayurveda and Unani. The allopathic system of medicine adopted many medicinal plants to derive newer molecules for challenging diseases. The plant *Indigofera aspalathoides* which is well known as ‘Sivanar vembu’ is widely used for skin diseases. Phytochemical and Pharmacological researchers have screened it for its anti-cancerous and antioxidant property. This review focuses all updated information about its authentication, phytochemistry and pharmacology. The phytochemical evaluation of the plant showed phenolic compounds, tannin, alkaloids, triterpenes, flavones, saponin and steroids. This drug is widely screened for its anti-cancerous and anti-oxidant activity. Anti-microbial, hypoglycemic, hepatoprotective, anti-inflammatory and antiviral activity were also reported. A thailam (oil prepared by traditional extraction procedure) prepared from this plant is widely used by the Siddha practitioners for various ailments. This review focuses the lot of medicinal properties of the plant through the scientific validation and strongly supports the traditional claim.

INTRODUCTION: Plants are the major source for the discovery of new products of medicinal values for drug development. Many of the drugs sold today are derived from plants and simple synthetic modifications were done to obtain newer molecules. India has a rich flora of medicinal plants. They are used in both herbal and conventional medicine.

They not only help to treat diseases but also helpful to regain good health in which the pharmaceutical drugs are lacking. Therefore, in recent years medicinal plants have received renewed attention of scientists and there are spurt of publications on their chemistry, pharmacology and clinical investigations.

Indigofera aspalathoides widely used in traditional medicines has tremendous medicinal potential owing to its biological functions. Based on this, the present review focuses the phytochemical and pharmacological activities of Sivanar vembu (*Indigofera aspalathoides*).

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The plant *Indigofera aspalathoides* belongs to the family Fabaceae (Papilionaceae) which is popularly known as Sivanar vembu or sivan vembu in Tamil, is a low under shrub with copiously terete spreading branches. It is found in South India and Srilanka and is traditionally used for treating various skin disorders and tumours¹.

Classification:

Kingdom	: Plantae
Order	: Fabales
Family	: Fabaceae
Sub family	: Faboideae
Tribe	: Indigofereae
Genus	: Indigofera. L
Species	: <i>Indigofera aspalathoides</i> Vahl

Vernacular names²

Sanskrit	: Ratakohomba, sivanimba
Latin Name	: K-Nila
Kannada	: Sivamballi
Malayalam	: Manali
Tamil	: Iraivan vembu, Sivanarvembu
Trade name	: Wiry Indigo

The leaves, flowers and tender shoots are cooling and demulcent. The leaves are used for leprosy, cancerous affections, abscesses, dandruff and also for edematous tumours².

This plant is one of the main ingredients of a Siddha preparation (thailam) used for chronic eczema (Saraswathy *et al.*, 1998). The roots soak in coconut oil and they used for chronic eczema, acute tumor and psoriasis. The root is chewed in toothache and aphthae³.

Botanical description⁴: It is an erect herb. Some are small trees up to 5-6m (16-20 ft) tall. Stem is dark brown when young, grayish white, branched 0.7cm to 1.5cm width. Roots are brown colored,

woody, lateral roots present 0.5 to 3.0 cm width. The leaf is trifoliate, pale green, obanceolate, digitate, sessile and crowded on the young branches, stipules minute.

Pharmacognostic features⁵:

- Leaflets:** Leaflets folded adaxially with the lamina being vertical and the other being laterally deflexed. The unique feature of adaxially folded leaflets is the presence of wide, circular, canals in the midrib and leaf margins. The distribution of the canals is also variable. The canals are 50µm in diameter.
- Lamina:** The lamina is distinctly bifacial. It consists of square or rectangular, wide, thin walled epidermal cells which are 15-20µm wide. The mesophyll is differentiated in to adaxial zone of palisade cells, median level of circular cells and adaxial zone of spongy parenchyma cells. The palisade cells are in two rows; they are narrow and cylindrical and compactly arranged. The spongy parenchyma cells are three layered; they are lobed and loosely arranged. The lamina is 250 µm thick.
- Midrib:** The midrib possesses a small circular collateral vascular bundle which is situated in median part. In the midrib there is no canal. The vascular bundle consists of three or four shoot, parallel lines of xylem elements and small group of phloem elements. The abaxial epidermis consists of prominent papillae cells.
- Venation:** The lamina possesses reticulate venation system with wide, irregularly shaped vein islets and well defined vein terminations which are either simple or forked terminally. The vein terminations possess cylindrical cluster of tracheids. These tracheids are short and cylindrical with pitted thickenings on their walls. The tracheids are 30 µm long and 10 µm wide.
- Stem:** The thickness of the stem is 900 µm and consists of intact epidermis wide heterogeneous cortex, secondary phloem, secondary xylem and wide pith. Epidermis consists of small spindle shaped thick walled cells. The inner epidermis consist of one or two layers of chlorenchyma cells followed by a

few layers of parenchyma cells next to the parenchyma zone occurs a discontinuous cylinder of gelatinous fibers of 3 or 4 cells wide.

6. **Root:** Young root is 800 μm thick. It consists of fairly wide, superficial, periderm with wide shallow fissures. Periderm is followed by parenchymatous & thick continuous sclerenchyma cells. Secondary phloem is wide and continuous. Secondary xylem is dense and compact, compressing wide, circular thick walled diffuse mass of vessels and sclerenchymous ground tissue. The root measures 2-5 mm thick. It has wider and deeply fissured periderm and discontinuous, radial segments of fibers with cortical region. The xylem cylinder is 1.5 mm thick and consists of about nine, fan-shaped radial bands of vessels and fibers and wide dilated rays. The radial level xylems are further cleaved into smaller units by dilated rays; within each xylem bands wide circular thick walled radial chain of vessels occur.
7. **Crystals:** Calcium oxalate crystals of prismatic type are fairly abundant in the leaf mesophyll, cortical cells of the stem and phloem parenchyma of the root. They are diffuse in distribution. In the stem and root, the crystals also occur in the ray parenchyma cells.

8. **Powder microscopy:** The leaf powder consists of abundance of characteristic 'T' shaped epidermal trichomes. They have a short, unicellular central stalk with two laterals and opposite arms, spreading parallel to the surface. The walls are thick with minute cuticular spires. The trichome is 350 μm long and 30 μm thick in the middle.
9. **Phytochemistry:** The phytochemical analysis shows steroids, triterpenes, alkaloids, phenolic groups, flavones, saponin, tannin, sugar, catechin, amino acid and reducing sugar^{4, 6}. The total content of phenols and tannins of *Indigofera aspalathoides* were studied by using spectrophotometric methods. The plant is rich in phenols (47.38 \pm 1.532) than tannins (34.59 \pm 1.788)⁶.

A new compound indigocarpan and the known compound mucronulatol were isolated from chloroform extracts of *Indigofera aspalathoides*. Spectroscopic methods including single x-ray analysis were used to elucidate their structure⁷. The GC-MS analysis of methanolic extract of *Indigofera aspalathoides* showed ten major peaks which indicate the phytoconstituents in it. Among them Dodecanoic acid, tetradecanoic acid and n-Hexadecanoic acid have the property of anti-oxidant and antimicrobial activities⁸.

TABLE 1: GC – MS ANALYSIS OF *INDIGOFERA ASPALATHOIDES*⁸

S. No.	RT	Name of the compound	Molecular formula	MW	Peak Area
1.	10.99	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	1.61
2.	13.47	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	39.70
3.	16.34	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	11.14
4.	18.96	9,12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	280	5.45
5.	19.37	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	6.46
6.	19.03	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	1.31
7.	20.95	Kaur-16-ene3	C ₂₀ H ₃₂ O ₂	272	1.98
8.	21.26	Pregnanetrial	C ₂₁ H ₃₆ O ₃	336	4.83
9.	22.35	5-(1-Lsopropenyl-4,5dimethylbicyclo(4.3.0)nonan-5-yl)-3-methyl-2-pentenol acetate	C ₂₂ H ₃₆ O ₂	332	3.15
10.	0.02	2-Methoxy-4 α -methylandroster-2-en-17ol-1-one 5 β	C ₂₁ H ₃₂ O ₃	332	24.38

RT - Retention time, MW- Molecular weight (MW)

In-vitro studies: Various extracts of *Indigofera aspalathoides* was investigated for its antifungal activity by using disc diffusion method. Among the extracts, methanol extract showed maximum inhibitory activity against (*Candida albicans*; *Candida parapsilosis*; *Candida tropicalis*) with

zone of inhibition of 13 mm, 14 mm and 16 mm, respectively followed by ethyl acetate (zone of inhibition of 13 mm, 15 mm and 16 mm respectively) and hexane (zone of inhibition of 15 mm, 16mm and 18mm respectively). The

methanol extract effectively inhibits all the test pathogen⁹.

Various extracts of leaves and roots of *Indigofera aspalathoides* were tested against 13 microbial species including 8 bacteria and 5 molds as test organisms by disc agar method. The petroleum ether, chloroform and acetone leaf and root extracts of this plant showed antibacterial activity against *B. cereus*, *E. aerogens*, *S. typhi*, *P. vulgaris* and *S. aureus*, while the methanol extract of *Indigofera aspalathoides* shows both antibacterial and antifungal activity. However, the methanol root extract showed maximum inhibition zone against *P. vulgaris* (22mm). Root extracts showed superior antibacterial and antifungal activity¹⁰.

The antimicrobial property of naturally prepared kuzhi thailam (extract prepared by using traditional method) from *Indigofera aspalathoides* with commercially available product of *Indigofera aspalathoides* kuzhithailam was compared by using agar gel diffusion method.

Result showed superior activity of naturally prepared oil than commercially available oil¹¹.

Oral administration of the methanol extract of *Indigofera aspalathoides* (MEIA) and *Wedelia calendulaceae* (MEWC) at the dose of 250 and 500 mg/kg body weight increased the life span and non-viable cell count, decreased tumor volume and viable cell count of the tumor bearing mice, when compared to that of EAC control mice. Both MEIA and MEWC seemed to increase the RBC count, Hb % and lymphocytes. There was also decreased level of neutrophils¹².

Pharmacological studies: The plant was investigated for different pharmacological activities but still more has to be explored. It was widely investigated for anti tumour activity and antioxidant activity.

Anti-cancer activity: The antitumor activity of ethanol extract of *Indigofera aspalathoides* (EIA) was evaluated against the Ehrlich ascites carcinoma (EAC) tumor model. It increased the survival time and normal peritoneal cell count. Hematological parameters and protein which were altered by tumor inoculation, were restored¹³.

The anticancer and antioxidant effects of the aqueous extract of *Indigofera aspalathoides* on fibrosarcoma were investigated in male albino rats. The activities of antioxidant enzymes like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in blood serum, liver, and kidney of control and experimental animals, respectively, have been studied. The levels of antioxidant enzymes CAT, SOD and GPx, were corrected to near normalcy in fibrosarcoma-bearing animals by treating with aqueous extract of *Indigofera aspalathoides*. The observation of *Indigofera* on treatment of fibrosarcoma in male albino rats enhanced the recovery from 20-MCA-induced fibrosarcoma due to its antioxidant property¹⁴.

The chemo preventive effects of *Indigofera aspalathoides* was studied on chemically induced carcinogenesis in rats. The activity levels of nucleic acids such as total DNA and RNA and hexose, hexosamine, and sialic acid in liver and kidney of treated rats were used to monitor the chemopreventive role of the plant extract. The observed increase in the levels of DNA, RNA, hexose, hexosamine, and sialic acid in liver and kidney tissues of fibrosarcoma-bearing animals reached near normal state after the treatment with aqueous extracts of *Indigofera aspalathoides*^{15, 16}.

In the evaluation of therapeutic efficacy of an aqueous extract of *Indigofera aspalathoides* against growth of transplanted experimental fibro sarcomas in wistar male albino rats, reduced tumour size was noted in drug treated group of animals. Hepatic microsomal Phase II enzymes such as UDPGT and GST were reduced significantly¹⁷.

Indigofera aspalathoides has protective effect in Dalton's ascitic lymphoma which is assessed by the intraperitoneal injection of 400mg/kg of ethanolic extract of *Indigofera aspalathoides* (EEIA)¹⁸.

Influence on Hepatic Xenobiotic enzymes and chemo preventive effect of ethanolic extract of *Indigofera aspalathoides* (EIA) was tested on DMBA induced hamster buccal pouch carcinoma model. It has been observed that hamster treated with EIA showed a significantly low level of hepatic phase I enzyme and high level of Phase II enzymes, which might be the reason for its chemopreventive effect¹⁹.

Oral administration of ethanol extract of *Indigofera aspalathoides* (250 mg/kg) was investigated for chemo preventive effect in male wistar rats. It effectively suppressed liver tumor induced with DEN as revealed by decrease in the levels of extend of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO), glutathione peroxidase (Gpx) and glutathione S-transferase (GST) with a concomitant increase in enzymatic antioxidant (superoxide dismutase and catalase) levels when compared to those in liver tumor bearing rats.²⁰

Anti-oxidant activity: The free radical scavenging activity of two fractions from the leaves of *Indigofera aspalathoides* was investigated. To analyze the activity four in vitro models namely-DPPH radical, ABTS radical, Nitric oxide radical and hydroxyl radical scavenging assays were used. Both fractions showed significant antioxidant activity when compared to standard antioxidants. Even though chloroform fraction contains lesser amount of polyphenolic compounds, it exhibits more radical scavenging activity than the ethanol fraction which indicates the role of structural features of polyphenolic compounds with respect to their antioxidant potential²¹.

Anti-inflammatory activity^{22, 23}: The indigo-carpen and mucronulatol were evaluated for cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitory activities and antioxidant properties. The compound indigocarpan showed significant COX-1 inhibition (IC₅₀ 30.5 µM) and its in vivo anti-inflammatory activity was found to be comparable to the standard drug ibuprofen. Molecular docking studies revealed the binding orientations of indigocarpan in the active sites of COX-1 and COX-2⁷. Anti-inflammatory activity of alcoholic extract of *Indigofera aspalathoides* was reported by Amala Bhaskar et al., Rajkapoor et al also reported anti-inflammatory activity of dried stem of *Indigofera aspalathoides* in rats.

Anti-arthritic activity: The effect of ethanol extract of stems of *Indigofera aspalathoides* Vahl (EIA) was evaluated for anti-arthritic activity on complete Freund's adjuvant-induced (CFA-induced) arthritis in rats.

The extract was administered orally at doses of 250 and 500 mg/kg daily for 30 days. On days 7, 14, 21 and 30, the paw volumes were measured. There was a significant alteration in biochemical parameters in EIA administered arthritic rats along with significant alteration in SOD, GPx, LPO and catalase levels, which proves an eminent anti-arthritic effect of EIA against CFA, induced arthritis in rats²⁴.

Hepatoprotective activity: The alcoholic extract of stem of *Indigofera aspalathoides* was evaluated for its antihepatotoxic activity against CCl₄-induced hepatic damage in rats. The activity was evaluated by using biochemical parameters and histopathology. The histopathological changes of liver sample were compared with respective control. The extract showed remarkable hepatoprotective effect²⁵.

Ethanol extracts of *Indigofera aspalathoides* and *Bauhinia variegata* were studied for hepatoprotective effect against hepatotoxicant paracetamol. The hepatoprotective effect of these extracts is evident from the near normal level of the biochemical parameters such as SGOT, SGPT, ALP, and GGPT which are affected by paracetamol - induced hepatotoxicity. In antioxidant studies the alcohol extract of *Indigofera aspalathoides* and *Bauhinia variegata* was found to have antioxidant effect against free radicals, LPO, SOD, catalase and GPx generated during paracetamol-induced hepatotoxicity when compared to control group animals.

Histopathological studies of liver section showed regenerative changes in hepatocytes after the treatment with alcohol extracts of *Indigofera aspalathoides* and *Bauhinia variegata*. The alcohol extract of *Indigofera aspalathoides* and *Bauhinia variegata* produced hepatoprotective effect in a dose dependent manner²⁶.

Anti-diabetic activity: Preliminary investigation was carried out to evaluate the antidiabetic effect of the alcoholic extract by oral glucose tolerance test (OGTT), normoglycaemic and anti hyperglycaemic activity in streptozotocin (STZ) – nicotinamide induced non-insulin dependent diabetes mellitus rats. Graded dose (250 and 500mg/kg) of the alcoholic extract suspended in gum acacia were administered to normal and experimental diabetic

rats. Effect on glucose tolerance and hyperglycemic studies showed only less remarkable decrease in blood glucose level at both dose levels as compared to glibenclimide. Normoglycaemic study revealed significant percentage decrease in blood glucose level from the initial value in normal rats 21.20% and 25.20% (250 and 500mg/kg respectively) as compared to the control group 1.85%. Result showed that alcoholic extract of *Indigofera aspalathoides* is a source of compounds with antidiabetic activity²⁷.

Nephro protective activity: The methanol extract of *Indigofera aspalathoides* was studied against gentamicin induced nephrotoxicity in wistar male albino rats. Various biochemical markers such as blood urea, serum creatinine, serum uric acid, serum electrolytes and antioxidant parameters such as Renal SOD, catalase, LPO and GPx were analysed. The result showed significant reduction in elevated serum marker levels and significant increase in renal SOD, catalase level. Histopathological study revealed the protective effect at the dose level of 500mg/kg while the 250mg/kg showed only partial protection²⁸.

Wound healing activity: The wound healing property of chloroform extract of *Indigofera aspalathoides* vahl. Ex DC. was evaluated in two different dose levels employing excision wound model. The wound treated with plant drug showed higher rate of wound contraction, increased level of Hydroxy proline, Hexosamine, SOD, Ascorbic acid and decreased levels of Lipid peroxides as well as histopathological studies also showed progressive collagenation and few macrophages compared to the control rats²⁹.

Anti-viral activity: The anti-viral activity (Respiratory viral infection RSV) of a poly herbal formulation which contains *Indigofera aspalathoides* was investigated in Juvenile chinchillas and BALB/c mice. A dose-effect was clearly detectable in chinchillas inoculated with increasing doses of RSV.

At the lower dosages assayed, no signs of illness were noted at any time of post challenge. Conversely, animals that received higher dosages of the virus without poly herbal formulation showed signs of acute respiratory tract infection.

Nasal lavage fluids recovered from the RSV-infected chinchillas (control) had an abnormal yellowish-green tint and was notably turbid. There was no such change observed in poly herbal formulation treated group of animals. Preliminary evidence in support of restriction of viral replication to the uppermost airway following the challenge was supported by the absence of viral plaques when tracheal mucosa and lung tissue recovered 4 days after challenge in the treatment group even after receiving highest dose of RSV.

This clearly indicates that the poly herbal formulation pretreatment prevent viral multiplication within 4 days of challenge³⁰.

CONCLUSION: The complete literature and resources available on this plant shows the medicinal value of *Indigofera aspalathoides* to possess potent anti-neoplastic and antioxidant property. Thus traditional system of medicine provides biologically active molecules and lead to new drug discovery.

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