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Endangered Liana of the Western Ghats, *Coscinium fenestratum* (Gaertn.) Colebr: An Overview

Naincy Sagar and R. Vasudeva[1]

Department of Forest Biology and Tree Improvement, College of Forestry, Sirsi-581 401, India
[1]University of Agricultural Sciences, Dharwad-580 005, India
E-mail: nancysagar2018@gmail.com

Abstract**:** *Coscinium fenestratum* (Gaertn.) Colebr. is a rare and endangered liana present in the Western Ghats, which is recently gaining greater importance in the pharmaceutical sector due to its medicinal properties. Globally, this species is distributed in India, Sri Lanka, Taiwan, Cambodia, Vietnam, Peninsular Malaysia, Western Java, and Borneo. Medicinal plants are important components of the biodiversity of the Western Ghats. The high anthropogenic pressures and associated fragmentation of natural forests have resulted in the loss of habitat and species. Increasing public interest in phytochemical-based medicine along with rapid expansion in pharmaceutical industries has resulted in the increase in demand for medicinal plants like *Coscinium fenestratum* resulting in a major threat due to over-exploitation from natural habitats in the absence of cultivation. This review article discusses the background of this endangered species of Western Ghats to create awareness of the conservation and research of such an important natural resource in the country.

Keywords**:** Rare, Endangered, Fragmentation, Anthropogenic

Since time immemorial, different classical medicinal systems such as Ayurveda, Siddha and Unani are being practiced in the country along with innumerable local folk medicinal traditions. The rich medicinal plants used in such systems are mainly distributed in two hot spots of diversity - Northeastern region and the Western Ghats. The Western Ghats cover a mere 5 per cent of the country's total land area in the country, it is believed to be more than 27 percent of the country's plant species which is a remarkable high level of endemism ranging from 25 to 60 per cent of recorded species. *Coscinium fenestratum* (Gaertn.) Colebr is a woody climber which grows wild in the natural rainforest reserves in Sri Lanka and India. This species is listed as endangered in the IUCN Red Data Book and in the Convention on International Trade in Endangered species of wild Fauna and Flora (CITES) listing because of its large-scale harvesting for medicinal use. *Coscinium fenestratum* is a slow growing liana which takes 15 years to reach its reproductive stage. But due to its huge demand for industrial consumption, it gets chopped down before it is fit to regenerate, and also the traders directly engage tribes and other collectors for the supply of the raw drug. The combination of rampant destruction of the forests along with over-exploitation of the species for the raw drug market and very slow rate of regeneration has seriously depleted its population (Tushar and Udayan, 2005).This belongs to the order Ranunculales, and family Menispermaceae.

Geographical distribution and status of *Coscinium fenestrstum*: *C. fenestratum* is a dioecious and a large woody climber of the family Menispermaceae, indigenous to the Indo-Malayan region. In India, it is restricted to the Western Ghats, mostly in the high rainfall wet evergreen forests, moist evergreen, semi-evergreen and semi-deciduous forests at 500-750 m altitude (Mohanan and Sivadasan 2002). In India, *C. fenestratum* is found in Kerala, Tamil Nadu, and some regions of Karnataka. In Kerala, natural populations have been identified in Thiruvananthapuram, Thrisuur, Malappuram, Palakkad, Kollam, Idukki, Waynad Kannur and Kozhikodi districts (Udayan et al 2004). Only a tiny band of high rainfall hilly slopes of the central Western Ghats in Karnataka was predicted to be highly suitable for the species, suggesting a high habitat-specificity and restricted distribution of *Coscinium fenestratum*. A total of 163 adult individuals and 975 regenerating individuals were enumerated in all the eight natural populations studied in three populations both adult and regeneration structures were healthy; large deficiencies of higher size class of adults and of individuals in class I (< 40 cm height) regeneration were observed in other five populations (Thriveni et al 2015). Due to a lack of information on the population size, trend, and threats in Cambodia, Vietnam, and West Malaysia, as well as information on whether this species is wild or introduced in these countries, this species is categorized as Data Deficient. Further

research is needed on the native distribution of this species and on the population size, trends, and threats in Cambodia, Viet Nam and West Malaysia (Ved et al 2015). The threat status of this species has been assessed as Critically Endangered for Karnataka, Kerala and Tamil Nadu in India, due to a more than 80% decline in the wild populations over the last 30 years (Ravikumar and Ved 2000).

Morphology and anatomy of liana: Malamanonmani and Mehalingam (2019) studied the transverse section of the leaf of *Coscinium fenestratum* and reported the presence of a circular hyaline hypodermal layer with three-layered parenchymatous cells. The presence of thick-walled parenchyma and small isolated calcium oxalate crystals were also observed in the cortical region. The anatomy of the stem revealed the occurrence of 8 to 10 parenchymatous layers in the cortical zone and thick-walled sclerenchymatous zone with lignification.

Germination and dormancy of seeds: Senerath (1991) provided best method of fruit collection and seed germination in *C. fenestratum* in populations of Sri Lanka and ripe fruits must be depulped and exposed to direct sunlight for six hours to increase germination. Ramasubbu et al (2011) conducted an experiment to analyze the biological characters and germination rate of seeds of *C. fenestratum* in the natural as well as in the laboratory condition. The fresh seeds showed 65% viability and 28% germinability. However, the seeds pretreated with GA3 (1000-4000 ppm) showed 55 to 70% germinability and 79% of the seed germination was observed after 6 months. The temperatures above 30°C and dark conditions facilitate germination of the mature seeds after split opening the hard seed coats by exposing them to direct sunlight for 6 hours followed by dipping the seeds in 2250mg/L GA_3 solution for 24 hours to reduce the time taken for germination by removing inhibitory chemicals, facilitating embryo growth and reducing inherent ABA/GA_3 ratio (Warakagoda et al 2014). Bhat (2015) investigated the seed germination of five medicinal plants of Western Ghats. In case of *C.fenestratum*, a total of 91.25% germination was recorded with the highest of 42.5% in the 4th week. Anilkumar et al (2010) observed that the germination of seeds of the liana was enhanced to 93% as the initial seed moisture content was reduced to 10% upon exposure to open laboratory conditions. When fresh seeds were pre-treated with 2 to 10% KNO_3 or GA_3 3000 ppm, the germination was enhanced to 95% respectively. Seeds kept exposed for two months at laboratory conditions lost their viability, while the seeds stored for four to six months inside the polycarbonate bottles expressed 90% germination within a month of sowing.

Medicinal uses of the liana: The stem of *C.fenestratum* has long been used in South India and Sri Lanka as a yellow dye and bitter tonic and in Europe under the name False Calumba or Tree Turmeric. The roots and stem contain alkaloids berlambine, dihydroberlambine, noroxyhydrastine, berberine, etc. The medicinally active compound of *C. fenestratum* is berberine, an isoquinoline alkaloid with numerous bioactivities (Birdsall and Kelly1997). Ashalatha and Gopinath (2019) analysed the leaf and stem samples of *C. fenestratum* by High-performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) and the compounds identified were berberine, jatrorrhizine, palmatine, tetrahydropalmatine, tetrahydroberberine, magnoflorine, isocorydine, glaucine and ecdysterone which is a plant sterol compound. Phytochemical investigation on *C.fenestratum* (Gaertn.) Collebr, revealed the presence of significant amounts of ecdysterone in the stem (0.22%) and leaves (0.12%), in addition to berberine. Ecdysterone was characterized using High-Performance Liquid Chromatography (HPLC), Infrared Spectroscopy (FT-IR), and Liquid Chromatography-Mass Spectroscopy (LC-MS). Isolation of this multi- functional bioactive compound will throw light on the chemical basis for the various pharmacological effects of *Coscinium* plant extract. Boberok

Source: Tushar et al (2008)

Fig. 1. *Coscinium fenestratum*

et al (2010) mentioned that Berberine had a weak activity against Gram-negative bacteria and is more potent against Gram-positive bacteria, including *Mycobacterium tuberculosis* and MRSA (Methicillin-Resistant *Staphylococcus aureus*) by the MDR pump NorA inhibition**.** Wang et al (2019) observed the impact of berberine on human hepatocarcinoma cell survival, demonstrating that its anti-tumor properties could be mediated both by apoptosis and autophagy. Pongkittipha et al (2015) reported that berberine showed a better direct-antioxidant activity of the derivatives containing phenolic groups than berberine in a cell-free system. For cell-based system, berberine was able to exert better cytotoxic activity than its derivatives. Berberine derivatives containing a single and four phenolic groups showed improved up-regulation of SOD gene expression. Tran and Stefan (2001) reported that the old parts or roots of the medicinal *liana C. fenestratum* was crushed and boiled for drinking in case of stomachache in the buffer zone of Bach Ma National Park, Vietnam. Shirwaikar et al (2005) concluded that alcoholic stem extract of *C. fenestratum* effect carbohydrate metabolism and observed the antioxidant status in streptozotocin–nicotinamide induced type 2 diabetic rats. Oral administration of *C. fenestratum* stem extract in graded doses caused a significant increase in enzymatic antioxidants such as catalase, superoxide dismutase, glutathione synthetase, peroxidase, and glutathione peroxidase and in the nonenzymatic antioxidants ascorbic acid, ceruloplasmin and tocopherol. The medicinal plants used by Kaadar tribe in Thrissur, Kerala were studied by Udyan et al. (2005) which revealed that the mature stem cuttings of *C. fenestratum* a liana of the family Menispermeaceae were boiled in water and taken internally against jaundice and joint pain. Among the different extracts of *C.fenestratum*, methanolic stem extract showed moderate activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Betula subtilis* . Methanol leaf extract had maximum activity against *Staphylococcus aureus*) and lowest against *B. subtilis* respectively (Goveas et al 2013). Preliminary phytochemical screening of *C.fenestratum* fruit extracts revealed the presence of alkaloids phenols, flavonoids, tannins, steroids, and resins, which are responsible for biological properties. The combined aqueous and methanol extract resulted in significant anthelmintic and antioxidant properties in a dose-dependent manner. The anthelmintic activity test was carried out against *Pheretima posthuma* and *Taenia solium* with the extract at varying concentrations of 25, 50, 100 and 150 mg/mL and compared with standard albendazole (25 and 50 mg/mL) and saline (0.9%) as a control. All the extracts exhibited concentration-dependent paralytic effect and a significant activity was observed with the combined methanol and aqueous extract (Das et al 2018).

Variation in berberine content: Yields of berberine content in crude extracts of *C. fenestratu* between 9.87-16.38% dry weight while Berberine content in the dried powder and in the crude extract was in the ranges of 1.71-2.89% w/w and 11.84-18.45% dry weight, respectively in a study. (Rojsanga and Gritsnapan., 2005). The recovery of standard berberine was 97.58-98.71% (%RSD = 3.85), and the limit of detection and quantitation were 25 and 50 ng/ spot, respectively. Eighty percent ethanol gave a higher content of berberine than 50% ethanol. Berberine contents from maceration, percolation and Soxhlet extraction with 80% ethanol were 3.37, 3.08 and 2.67 % w/w, respectively (Rojsanga et al., 2006). Khan et al. (2008) reported for the first time the role of phytohormones on the production of berberine in *C. fenestratum* from in vitro calli cultures. Berberine with the retention time of 8.49 min and enhanced dry weight (1.788%) from the petiole explant has been reported for the first time in this study. The presence of berberine was first checked by preparative thin layer chromatography (TLC) and then confirmed by High-Pressure Liquid chromatography (HPLC) and mass spectrometry. The root and stem bark samples of *Berberis asiatica* from lower altitudes, having a larger size class contained more Berberine. The Berberine concentration was highest in summer and lowest in the rainy season. Low level of soil moisture and higher level of soil potassium produced higher Berberine concentrations (Harish et al 2011). Method of extraction and method of drying significantly influenced the Berberine yield in *C. fenestratum*. Methanol cold extraction is the best and efficient method for the highest product recovery. Highest yield of Berberine (4.06 % w/w) was obtained in the shade dried samples followed by the sundried samples (3.21% w/w) extracted with cold methanol (Babu et al.,., 2012). The average berberine content irrespective of age, sex and tissues ranged between 0.64 to 3.01 %. Out of 45 adult individuals, 18 individuals yielded more than 5 % of berberine in the root samples. Further, the herbivore attack resulted in a significant increase in the berberine content of leaves. (Thriveni et al 2017).

Improvement of Berberine through selection and clonal multiplication: Nair et al (1992) conducted an experiment to study Berberine synthesis in callus and cell suspension cultures of *Coscinium fenestratum* which were established from sterile petiole segments on MS medium, supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D) and benzyl amino purine (BAP). The cells in the culture produced Berberine as the major compound. NAA stimulated the product synthesis over 2,4-D and the presence of light enhanced the Berberine synthesis. The root samples

collected from Sarhan provenance had the highest Berberine content. Practically all the sources of *Berberis aristata* suppressed more than 50% fungal growth of *Rhizoctonia solani* and less than 50% in the cases of *Colletotrichum* and *Fusarium* (Rashmi et al 2009).

Genetic diversity: Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic diversity of 14 individuals belonging to seven populations of *C. fenestratum* (Gaertn.) Colebr. (Menispermaceae). 18 decamer primers used for the analysis generated 99 scorable bands of which 79 were polymorphic. Coefficient of similarity ranged from 0.6604 to 0.9809. Variation within population was slightly higher than between populations. The similarity between individuals within and between populations was found (Narasimhan et al 2006). Thriveni et al (2013) observed that the species, *Coscinium fenestratum* exhibited a moderate to low level of intra population genetic diversity among seven populations of Central Western Ghats. The population had weak structure (K = 2) with one single widespread gene pool indicated that gene flow and inbreeding have been likely the major driving force in shaping the current population genetic structure of *C. fenestratum*.

Recent advancement in sexual methods of reproduction: *In vitro* propagation study was undertaken to produce the multiple shoots in *Coscnium fenestratum.* Murashige and Skoog (MS) medium supplemented with 1.0 µM kinetin (Kin) and 0.25 µM 2,4-Dichlorophenoxy acetic acid (2,4-D) was used for the epicotyl explants. From each explant, maximum five shoots were obtained. It was reported that repeated subculture favoured the increase in shoot length and the number of shoots per explant in the media containing Kin and 2,4-D.The higher concentrations of either cytokinin used: butyric acid (BA) or Kin caused stunting of multiple shoots with small and narrow leaves (Senarath 2010). Warakagoda et al (2017) reported that in case of *Coscinium fenestratum*, mature double nodal cuttings resulted in the highest shoot proliferation rate (3.90 shoots/explant) when cultured on WPM medium supplemented with 2.0 mgL^{-1} 6-benzylaminopurine,1.0 mgL^{-1} thidiazuron and 0.4 mgL^{-1} 2,4-dichlorophenoxyacetic acid. Shoots were separated and transferred to WPM medium devoid of plant growth regulators for regeneration into plantlets and gave over 60 % survival rate. Karthika et al (2019) in a study *Coscinium fenestratum* reported that the nodal explants when inoculated on MS medium and supplemented with BAP and TDZ at 2.0 and 1.0mg/L respectively produced high frequency of shoots (91.01%) and the same shoots subcultured on half strength MS medium supplemented with IBA alone at 0.6mg/L produced high frequency of roots (97.42%). Renuka et al (2019) revealed that for direct regeneration of liana, the ideal explant was shoot tip with 20µM/l Kinetin + 0.25µM/l 2,4-D & 20µM/1 BAP + 0.25 µM/1 2,4-D and for indirect regeneration, petiole with 6µM//l 2,4-D+. Petiole and leaf explants of *Coscinium fenestratum* were induced to form callus when cultured on vermicompost extract media along with coelomic fluid. Vermicompost medium and vermicompost extract medium with coelomic fluid showed the maximum (100 per cent) callus induction. Light induced the darkening of the callus tissue. The suspension media turned deep yellow because of the release of alkaloid, which was extracted with 80 per cent ethanol and subjected to Dragendrof's reagent which showed fluorescent colour under UV which confirmed positive test for berberine (Kashyap et al 2016).

CONCLUSION

Herbal remedies have become more popular in the treatment of variety of ailments. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity. Reserves of herbs and stocks of medicinal plants in developing countries are diminishing and in danger of extinction as a result of growing trade demands for new plant-based therapeutic markets. As the demand for medicinal plant is growing, some of them are increasingly being threatened in the natural habitat. *C. fenestratum* is one among the families having several medicinal values from root to fruit. Because of its medicinal uses in Ayurveda, Unani and Siddha systems of medicine, collection of wild plants from natural habitat has made the species endangered. Therefore, the present review attempts to create awareness for the protection of such genetically rich source in the country.

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