



REVIEW ARTICLE

Botany, phytochemistry, pharmacology and ecological status of *Rauvolfia* Plum. ex. L. (Apocynaceae) from the Southern Western Ghats: A review

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Abstract

Rauvolfia species of the family Apocynaceae (Dogbane family) are a rich source of pharmaceutical compounds. This genus includes shrubs and evergreen trees distributed across tropical and subtropical forests. Five *Rauvolfia* species are found in the Southern Western Ghats of India namely, *R. serpentina*, *R. tetraphylla*, *R. densiflora*, *R. micrantha* and *R. beddomei*. Due to their phytochemical composition, include alkaloids, flavonoids and terpenes. *Rauvolfia* species have been traditionally used to treat various ailments, including epilepsy, hypertension, snake bites among other ailments. Amongst these, *R. serpentina* is widely used in traditional and modern medicine as a primary source of reserpine alkaloid. However, the escalating demand for their medicinal properties has led to adulteration practices with compromised plant quality. This review compiles information on the biogeography, taxonomy, morphology, ethnopharmacology, phytochemistry, pharmacology, economic significance, cytogenetics, propagation and adulteration issues of *Rauvolfia* in the Southern Western Ghats of India. These facets of the review address the challenges associated with exploiting the plant species and provide insights into their current research status in the context of the region's biodiversity and medicinal heritage.

Keywords: adulteration; biogeography; ethnopharmacology; phytochemistry; *Rauvolfia*; Southern Western Ghats

Introduction

Among the 36 global biodiversity hotspots, the Western Ghats are among the most significant (1). The Western Ghats are one of the four biodiversity hotspots located in India (2). In the terms of floral diversity, the Western Ghats are the second-richest biogeographic region after the Himalayas. They extend from Kanyakumari to Tapi river and span the states of Gujarat, Maharashtra, Kerala, Tamil Nadu, Karnataka and Goa. The Western Ghats are a treasure trove of valuable medicinal plants, contributing to their status as a complex ecosystem (2). They harbor a wide variety of plant species, many of which are used in traditional healthcare and are also threatened. The Southern Western Ghats exhibit abundant plant diversity, with both annuals and perennials spanning a wide range of flora, from lower to higher plants (3). The Southern Western Ghats are the most prolific area in terms of indigenous species density and vegetation composition, particularly regarding medicinal plants and the presence of unique taxa within Peninsular India. A multitude of plant species in this region are now experiencing a severe population decline (4-6) (Fig. 1).

Rauvolfia is a prominent genus in the Apocynaceae family. It is widespread in tropical regions, inhabiting diverse

environments such as open grasslands, savannas, forests and limestone hills (7). In 1753, Linnaeus established the genus based on the species *R. tetraphylla* (8). The genus was first discovered in the sixteenth century and now includes about 110 species (9). Using the Latin form of Rauwolf's name, French botanist Charles Plumier originally named the genus *Rauvolfia* in 1703 to honour German botanist and physician Leonhard Rauwolf (1535-1596). Later, Swedish botanist Carl Linnaeus adopted the nomenclature *Rauvolfia* in his book *Species Plantarum* (10). The genus is also known as Devil's pepper and includes both shrubs and evergreen trees. Members of this genus commonly found in the tropical and subtropical forests of India, Central and South America, Sri Lanka, Africa, China, Japan, Burma and Java. Five species of *Rauvolfia* are found in the Southern Western Ghats of India, including *Rauvolfia serpentina* L. Benth. ex Kurz.; *Rauvolfia tetraphylla* L.; *Rauvolfia micrantha* Hook. f.; *Rauvolfia beddomei* Hook.; *Rauvolfia densiflora* Benth ex Hook. f. (11) (Fig. 2).

Rauvolfia species occurs in various parts of the Western Ghats in Southern India, thriving in diverse ecosystems of this mountainous region that spans multiple states such as Karnataka, Kerala, Tamil Nadu, Goa and Maharashtra.

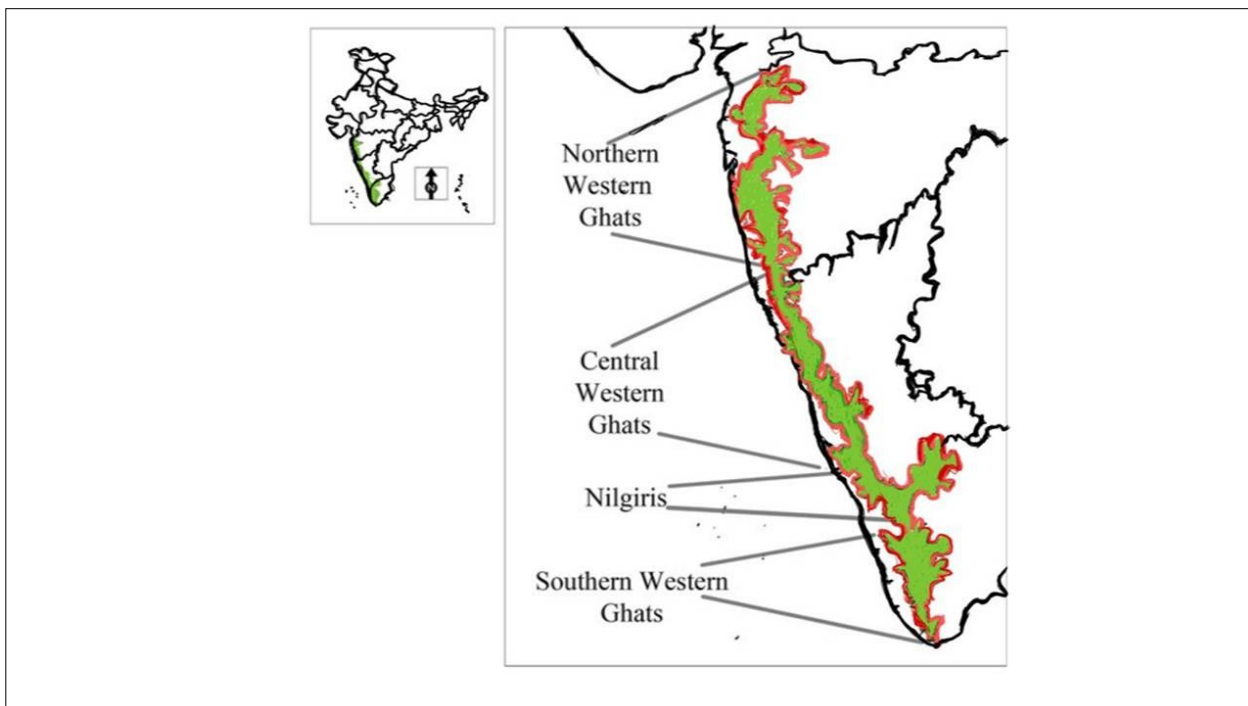


Fig. 1. Map of study area.



Fig. 2. *Rauvolfia* species from Southern Western Ghats of India.

According to literature, six *Rauvolfia* species occur in India, including *R. vomitoria* Afzel (12). *Rauvolfia* genus hold significance in traditional and contemporary medicine (13-15). In this genus, most species are trees or shrubs featuring whorled leaves, terminal inflorescences, flowers with tubular shapes and colours of petals ranging from white to purplish. Their ovaries range from apocarpous to completely syncarpous, surrounded at the base by a nectar-bearing disc (8). Moreover, they produce drupaceous fruits containing one or two visible seeds enclosed within a stony endocarp. Genetic reservoirs are presently encountering formidable challenges due to heightened anthropogenic endeavours, including

deforestation, habitat modifications and unsustainable extraction for commercial purposes (16).

According to the International Union for Conservation of Nature (IUCN), diverse members of the *Rauvolfia* genus, including *R. serpentina*, have been classified as endangered. For centuries, the plants from the *Rauvolfia* genus have been employed in Indian folk medicine to treat a variety of ailments such as epilepsy, hypertension, intestinal disorders, insanity, insomnia, schizophrenia, hysteria, constipation and cardiac and liver diseases (12). Additionally, these plants have been utilized as tranquilizers, anthelmintics and antidotes for snake and venomous reptile bites (17). Phytochemicals with bioactive



Fig. 4. Field trips for collection of *Rauvolfia* species from different locations of study area.

the genus *Rauvolfia* from the Southern Western Ghats of India. There are over 100 species of *Rauvolfia* worldwide. Despite being indigenous to India, *R. serpentina*, also known as Sarpagandha, exhibits a global distribution. The plant thrives in regions characterized by a warm and humid climate, particularly where the soil ranges from sandy alluvial loam to red laterite loam (23). It is prevalent across tropical regions, inhabiting diverse environments such as open grasslands, savannas, forested areas and limestone hills (7). These specific environmental conditions, along with temperatures ranging from 10-35 °C and precipitation levels of 250-500 cm, create an ideal habitat for the plant's optimal growth. *R. serpentina* is predominantly found in the Asian subcontinent, spanning countries such as China, Bangladesh, Pakistan, Nepal, Myanmar, Bhutan, Laos, Sri Lanka, Vietnam, Thailand and Indonesia. In India, the plant is distributed across various regions, including Punjab, Jammu and Kashmir, Delhi, Himachal Pradesh, Uttar Pradesh, Assam, Sikkim, Bihar, West Bengal, Arunachal Pradesh, Goa, Kerala, Tamil Nadu and the Andaman-Nicobar Islands (24).

R. tetraphylla, another species is identified by various names, including wild snake root and 'be still' tree in English; Papataku in Telugu; Pampukaalaachchedi in Tamil; Barachandrika in Hindi; Patalagarudi in Odia and Vanasarpagandha in Sanskrit is facing critical endangerment. Indigenous to the West Indies, *R. tetraphylla* has become naturalized in several countries, including India, Myanmar, Bangladesh, Pakistan, Nepal and Sri Lanka and is often cultivated in gardens. In India, it is found in various states, including Odisha, Madhya Pradesh, Karnataka, Andhra Pradesh, Kerala, West Bengal, Bihar and Tamil Nadu (25). Both species, *R. serpentina* and *R. tetraphylla*, are found throughout the Southern Western Ghats. *R. tetraphylla* exhibits a more abundant distribution compared to *R. serpentina*, which is relatively rare.

Additionally, *R. densiflora* is another species in the genus, native to India and Sri Lanka. In India, *R. densiflora* is found in various regions, including Uttara Kannada district in Karnataka, Kannur, Wayanad, Malappuram, Palakkad, Thrissur, Idukki, Kottayam and Thiruvananthapuram districts of Kerala and the Megamalai wildlife sanctuary in Theni district, Tamil Nadu (26). *R. micrantha* an endangered and endemic woody medicinal shrub, is found at elevations up to 600 m above sea level in the Southernmost part of the Western Ghats (11, 27-31). Other names for this species include Malabar *Rauvolfia* and small-flowered snakeroot (12). In an unpublished study, Robert Wight first reported *R. micrantha* as *Ophioxylon micranthum*. Based on Robert Wight's collection from Malabar, a princely territory in the former Madras Presidency, Hooker considered it an invalid name and designated it as a "new species" (32). It was reclassified as an endangered species of Peninsular India, while it is critically endangered in the Western Ghats (28, 33). Researchers described this species as "a slender shrub, apparently very scarce" and included it in the Flora of the Presidency of Madras, based on collections by Rama Rao and Wight from Travancore and Malabar, respectively (34). The rare species *R. beddomei* is endemic in its distribution to the evergreen forests of the Southernmost parts of Western Ghats (11).

Botany and systematic of the *Rauvolfia* grown in Southern Western Ghats of India

The genus *Rauvolfia* belongs to the family Apocynaceae. It is classified as follows: Kingdom - Plantae; Phylum - Angiospermae; Subphylum - Eudicotidae; Class - Asterids; Order - Gentianales and Family - Apocynaceae, Subfamily - Rauvolfioideae; Tribe - Vinceae and Subtribe - Rauvolfiinae Benth. & Hook. f. *Rauvolfia* is the largest genus within the tribe Vinceae and is widely distributed within the Apocynaceae family (8). The pantropical genus *Rauvolfia* is categorized into two primary lineages, one consisting entirely of paleotropical species and the other composed of neotropical species (8).

Most of sections, series and subseries previously identified in classifications of *Rauvolfia* are paraphyletic. Some researchers suggest two out of the fourteen sections as monophyletic (8). Other researchers suggests that one out of eleven sections are monophyletic (8).

Most species in this genus are trees or shrubs with whorled leaves, terminal inflorescences, tubular flowers with tubular shapes and petal colours ranging from white to purplish. The ovaries range from apocarpous to completely syncarpous and are surrounded at the base by a nectar-bearing disc (8). Additionally, they produce drupaceous fruits containing one or two visible seeds enclosed within a stony endocarp. Genetic reservoirs of these species face significant threats from deforestation, habitat changes and overharvesting (16). Several members of the genus *Rauvolfia*, including *R. serpentina*, have been listed as endangered by the IUCN. Discriminating character between *R. micrantha* and *R. beddomei* is the feature of the peduncle (34). The former possesses a comparatively short and smooth peduncle. In contrast *R. beddomei* has a long peduncle with node-like articulations (35) (Fig. 5). Based on a comparative analysis, scientists reported that fruit and seed characteristics can be used to distinguish between species and provided an artificial key (also called taxonomic key or identification key) to support this finding (36). Literature reports exist regarding the comparative carpology of *Rauvolfia* species from South India (37). In this study, it was noted that all species exhibited a sigmoidal pattern in the morphological and physiological characteristics of fruit/seed development. The study also observed in terms of fruit and seed dry mass accumulation, *R. hookeri* and *R. micrantha* exhibited a single-sigmoidal pattern, whereas other species showed a double-sigmoid pattern.

Researchers recognized flavonoids as chemotaxonomic markers in endemic and endangered species of *Rauvolfia* in the Southern Western Ghats of India (11). Flavonoid composition in all five species documented in the Southern Western Ghats of India and findings indicated that, within the closely related species *R. beddomei* and *R. micrantha*, the flavonol kaempferol is exclusively found in *R. beddomei*, serving as a distinctive chemotaxonomic marker between these two species (11).

Additionally, luteolin, a flavone, can be proposed as a chemotaxonomic marker for *R. densiflora*, as it is the only species among the five studied *Rauvolfia* species displaying the presence of luteolin. The cluster analysis results for *R. densiflora* also highlighted the species' distinctiveness from others in the study. The dendrogram produced through the unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis in certain research depicted the connections and variations among the five species (11). The cluster analysis distinctly organized the five species into three clusters. *R. serpentina* and *R. tetraphylla* were placed in the first cluster, *R. beddomei* and *R. micrantha* in the second (these two have morphological similarities, moreover only distinguishable feature is peduncle morphology and length) and *R. densiflora* constituted the third group.

ITS2 serves not only as a conventional phylogenetic marker but also as an excellent candidate for DNA barcoding due to its inclusion of highly variable sites, facilitating the identification and differentiation of the five *Rauvolfia* species found in the Southern Western Ghats of India (38). The phylogeny studies on members of the Rauvolfioideae subfamily and genus *Rauvolfia* has not kept up, most likely partly because of multiple genera or species with small, inconspicuous, white, salverform flowers that look identical but lack charisma. Phylogenetic analysis based on Indian species or members from Western Ghats has never been conducted till date.

Tribes within Rauvolfioideae were formerly divided by systematists using fruit features (39). The sinistrorse aestivation of the corolla lobes in buds and the anthers' non-attachment to the style-head are characteristics of the Rauvolfioideae (40). Though fruit characteristics have been shown to exhibit oscillatory evolution in Rauvolfioideae, many researchers encouraged the utilization of fruit features in conjunction with other morphological traits for systematics studies of Rauvolfioideae members (39-45). Because of their comparable habits, flower colours and other characteristics, *R. micrantha* and *R. hookeri* (*R. beddomei*), another unique species of the Western Ghats, are closely related.



Fig. 5. Morphological characters of *R. micrantha* and *R. beddomei*.

Our field observations showed a conspicuous difference in the fruit morphology of *R. micrantha* and *R. beddomei*, though both species are fleshy fruit-bearing. The former has partially syncarpous fruits with seeds embedded in fleshy pulp, while *R. beddomei* has apocarpous fruit with numerous small seeds. The abortive tendency was palpable in one of two syncarpous fruits of *R. micrantha*. The main difficulty is finding characters that accurately represent and articulate these two species in a formal categorization. The development of DNA-based molecular markers can resolve the issue and suggest more information regarding the phylogeny of these related species. *R. micrantha* with *R. membranifolia* in their reports and relinquished the position of *R. micrantha* as an "endemic" to the Western Ghats (46). A broader circumscription to define the species of *R. micrantha*, *R. vietnamensis*, *R. littoralis* and *R. indosinensis* in addition to *R. membranifolia* conspecific (46, 47). The plant list was adopted these observations and based on these studies, *R. membranifolia* from *R. micrantha* as a separate species *R. membranifolia* integrated as a heterotypic synonym with *R. littoralis*, *R. indosinensis* and *R. vietnamensis* (48-50).

Several molecular phylogenetic investigations have found that the majority of Rauvolfioideae tribes are non-monophyletic since the categorization of Endress and Bruyns (40, 51-54). Sporadic reports are available on the phylogenetic studies on the genus *Rauvolfia*. The phylogenetic position of *R. verticillata* using the plastid genome with twenty other published plastid genomes of the Apocynaceae family, a phylogenetic tree was constructed with *Gentiana officinalis* and *Gelsemium sempervirens* as the outgroups (55). Based on their study, they concluded that *R. verticillata* (*R. densiflora*) is more closely related to *Catharanthus*. Morphology and sequences from five DNA sections of the chloroplast genome (*matK*, *rbcl*, *rpl16* intron, *rps16* intron and 39 *trnK* intron) showed the relationships within Rauvolfioideae (Apocynaceae) (39, 40, 56).

Cytogenetics of genus *Rauvolfia*

Based on chromosome number data, *Rauvolfia* is characterized by a fundamental chromosome count of $x = 11$ (35). Polyploidization and subsequent intraspecific hybridization have led to the emergence of diploid, tetraploid, hexaploid and octaploid species or cytotypes within the *Rauvolfia* genus. These mechanisms have significantly contributed to the evolutionary development of diverse chromosome numbers, thereby fostering species diversification within the genus (57). Chromosome data for *R. serpentina* and *R. tetraphylla*, with the following results: the cumulative length of somatic chromosome complements in *R. serpentina* measured 79.91 μm , while in *R. tetraphylla*, it amounted to 153.94 μm (57). *R. serpentina* possesses 22 chromosomes ($2n = 22$), whereas *R. tetraphylla* has 66 chromosomes ($2n = 66$) (57). The range of chromosome lengths in *R. serpentina* was 2.76-4.33 μm , while in *R. tetraphylla* it was 1.68-2.88 μm . All four chromosomes in *R. serpentina* and three in *R. tetraphylla* displayed full fluorescence when treated with chromomycin A3 (CMA). The CMA-bands were distributed throughout the entire chromosomal structure rather than being confined to terminal or centromeric regions, indicating the presence of accumulated GC-rich sequences. Similarly, following 4'-6-diamidino-2-phenylindole (DAPI) staining, six chromosomes in *R. tetraphylla* exhibited complete fluorescence, suggesting a prevalence of AT-rich sequences throughout these chromosomes.

Morphology and ethnopharmacology of various species of *Rauvolfia* from Southern Western Ghats

R. serpentina

General morphology: *R. serpentina*, also known as *Ophioxylon septentium* L., is globally distributed in tropical Indian regions (58-61). The most popular vernacular name for this plant is Sarpagandha (59). The antihypertensive properties of *R. serpentina* root led to the plant's identification as either *Rauvolfia* root or serpentine root was studied in Western medicines (62). It is a woody, perennial, evergreen, glabrous undershrub that grows upright. The roots are aromatic, fleshy and long, with infrequent branching and display greyish-yellow to pale brown cork.

The stem is cylindrical and approximately one meter in length. The leaves, arranged in 3-4 whorls or occasionally opposite, are slender with a tapering base, showing a dark green upper surface and a pale green lower surface. The flowers are arranged in irregular corymbose cymes, forming clusters that range in colour from white to pink to red. The inflorescence comprises red pedicels and calyces, along with white petals. Flowering typically occurs from March to May in the Indian climate. The fruits are drupes, found singly or in pairs (didymous), initially green and turning into a shiny purplish-black to black when ripe, each containing ovoid seeds (63).

Ethnopharmacology: Historically, indigenous communities employed therapeutic flora to alleviate various health conditions (64-67). *R. serpentina* is one of the ethnomedicinally significant plants employed by tribal communities for drug acquisition for hypertension, sleeping disorders and anxiety. The plant mentioned by Sushruta, approximately 600 BC, has been traditionally employed in ayurvedic formulations (68). The use of this plant for treating hypertension in India is notable in rural regions (69, 70). It has a historical association with addressing issues such as insomnia and mental disorders. Older women or village physicians used to soak the roots of this plant in rose water for administration (71).

This indigenous plant is dispersed in diverse floristic zones across India, highlighting substantial natural variability. Since the Vedic period, diverse ethnic groups have utilized *R. serpentina* to treat a variety of ailments such as neurological disorders, infectious diseases and gastrointestinal disorders (60, 72, 73). Formulations derived from the roots of *R. serpentina* have been used for centuries in India to treat intestinal issues, disorders of the central nervous system and snake bites. Moreover, it has been used to trigger uterine contractions and as an antihelmintic (74). The people of Jhapa District, located in eastern Nepal, employ the juvenile shoot essence to treat pneumonia during the initial phases of infection (75). In the Unani medical tradition, the *Pitkriya* concoction features the extract derived from this plant, exhibiting hypnotic, diuretic, anesthetic and nervine sedative properties (76). The populace of Jaunsari in Garhwal Himalaya, Uttaranchal, has historically employed for alleviating nervous ailments, anxiety and epilepsy (77). The inhabitants of Madhupur, Tangail, Bangladesh, have observed that applying newly extracted leaf juices from *R. serpentina* can effectively ward off eye inflammation (78).

R. tetraphylla

General morphology: *R. tetraphylla* is considered an endangered plant species originated in West Indies but adapted to the Indian environment about a century ago (12). It is a noteworthy botanical member of the Apocynaceae family, cultivated for its substantial medicinal worth and therapeutic attributes (79). The common name of this species is 'still tree' (80). It is reported a range of synonyms linked to *R. tetraphylla* (81). The morphological features of the species are as follows: perennial, hairy shrub with an upright stem that reaches a height of 4-6 feet. The leaves are asymmetric and uneven, measuring 5-9 × 3-4 cm, elliptical-ovate in shape and pointed at the apex. They are typically arranged in groups of four. Terminal corymbose cymes showcase cream-coloured flowers, approximately 5 mm wide, with slender and rounded calyx lobes bearing cilia. The petals are white and about 3 mm long, with short lobes and tubes. The ovoid drupes are smooth, remain in pairs at the top, are 5-10 mm in diameter and turn purple when mature (79). Blooms occur consistently throughout the year.

Ethnopharmacology: *R. tetraphylla* is also called wild snakeroot (82). It is used as a calming treatment for conditions relating to sleep, hypochondria, blood pressure problems, mental illnesses and central nervous system disorders (83). This plant has many uses for native tribes in South India and has a wide ethnobotanical significance. A plethora of alkaloids derived from this plant, demonstrating noteworthy pharmacological attributes, including anticancer, antipsychotic, antihypertensive, antimicrobial, antioxidant, anti-inflammatory and antidiarrheal properties (84). Moreover, the therapeutic application of root extracts extends to treating ailments such as cholera, diarrhoea, fever, colic and dysentery (81, 85-89). Additionally, these extracts demonstrate efficacy in managing intestinal problems (90). The alkaloids obtained from this plant are used in therapies linked to cardiovascular and mental interventions (91). The qualities of these alkaloids have been shown to combat hypertension (88, 92, 93). *R. tetraphylla* root and leaf extracts inhibit the growth of the *Staphylococcus aureus* bacteria and the fungus *Fusarium oxysporum*, respectively (79).

The fruits produce a black dye utilized for treating skin diseases (12). The Kaattu Naika tribe in Wayanad district, Kerala, utilizes *R. tetraphylla* roots to induce uterine contractions in challenging childbirth situations (84). Concurrently, the Kurichya tribe in Wayanad district eases stomach aches by consuming a decoction of shoots thrice daily. The roots are used to treat several ailments, including syphilis, have calming effects, reduce swelling, function as a diuretic, ease stomatitis, have anti-inflammatory qualities, control gingivitis, act as an expectorant, ease toothache, sore throat, treat ulcers and alleviate anxiety (94). Labdane diterpene obtained from the stem of *R. tetraphylla* shows anticancer activities (95). The Irular tribe in Tamil Nadu employs plants to treat snakes and animal bites and insect stings (84).

R. micrantha

General morphology: The morphological features of *R. micrantha* are a seasonal shrub 1.5 to 2 meters tall and features sparsely branched stems with simple and alternate leaves (28). The leaves, ranging in shape from elliptical to lanceolate, measure 5-10 × 3-4 cm and acuminate. The peduncle is erect and terminal and bears 4-6 flowers. The diminutive flowers

exhibit a white, funnel-shaped structure with a purple-tinged interior, forming extended corymbose cymes. They feature five epipetalous, filamentous stamens, notably short and sagitate. The elongated pistil includes a lengthy style and a dumbbell-shaped capitate stigma. The syncarpous and bicarpellary ovary has one ovule in each locule. The smooth berries, bearing 1 or 2 seeds, are black with a textured surface displaying a reticulate pattern.

Ethnopharmacology: *R. micrantha* treats diverse health issues, including peptic ulcers, diabetes, coughs, mouth infections, colds, stomach aches and rheumatism (84). It is also recorded in the traditional Chinese medicine (TCM) systems and in India's Ayurvedic system of medicine (96). Crushed green fruit is blended with coconut oil and applied to sores, boils, cuts and carbuncles. In the Wayanad district of Kerala, the Kurumba tribe employs leaf juice to address pain in the affected ear. Moreover, they apply latex from the leaves topically to relieve earaches. In the Idukki district of Kerala, the Malapandaram tribe consumes fresh leaves for placenta expulsion, employs leaf galls to ease childbirth and eats a mixture of ground bark and coconut to induce sterility and contraception (84). Crushed root or bark from the stem is administered to alleviate urinary tract infections and heated leaves covered with oil are a poultice for injuries such as sprains and fractures. It is also used in Brugada syndrome, a disorder affecting cardiac electrical signalling those results in manifestations like heart palpitations, loss of consciousness, dizziness and associated with abrupt demise. The leaf sap combined with water is ingested to manage low blood pressure or anaemia. Various plant sections are employed to address cough, tuberculosis and injuries caused by stingrays. *R. micrantha* contains a higher amount of reserpine, a monoterpene indole alkaloid (50). Therefore, *R. micrantha* is employed as a replacement for *R. serpentina* on a commercial basis, as per the existing documents. The roots are a significant repository of tranquilizer alkaloids with antihypertensive properties, serving as a substitute for *R. serpentina* in treating nervous disorders in Kerala region (27). The plant extracts contain apigenin, emerging as an antitumor and anticancer phytochemical (97, 98).

R. beddomei

General morphology: *R. hookeri*, alternatively known as *R. beddomei*, is a closely related species found exclusively in the evergreen Southern area of the Western Ghats (12, 27, 30). It exhibits dichotomous branching, reaching a 1.5-2 m height and distributed at elevations up to 700 m. It is an endangered species according to IUCN status and endemic to the Southern Western Ghats of India (99, 100). The plants are shrubs with leaves typically arranged in threes, measuring 4-10 × 2-3.5 cm, shaped like elliptic-lanceolate, pointed at the top and tapering at the base. Clusters of flowers arise from the leaf axils, exhibiting dichotomous branching. Slender peduncles extend 3-8 cm in length. The small flowers have a pinkish-white corolla, with a 0.4-0.5 cm long tube and swollen beneath the throat where the stamens attach. The throat is hairy, with five lobes measuring 0.1-0.15 cm each. The five stamens are included and a cup-shaped disc is present. The carpels are partially fused halfway up, with two ovules, a united style (except at the base) and a capitate stigma bifurcated at the tip. The drupes are partially fused, each measuring 0.5-0.6 cm in length and 0.3-0.5

cm in width, ovoid with a beak and turn purple when ripe. The seeds, typically 1-2 in number and wrinkled.

Ethnopharmacology: Local people use this plant to treat indigestion, diabetes, piles, headache, toothache, cough, cold, urinary troubles, body temperature and white discharge in women. *R. hookeri* (*R. beddomei*) serves as an alternative to *R. serpentina* and *R. tetraphylla*, displaying noteworthy properties and is extensively utilized by tribes in South India for easing stomach pain and muscle aches, promoting uterine contraction, addressing coughs and colds, managing skin diseases and treating mental disorders and hypertension (84).

R. densiflora

General morphology: Plants are shrubs with milky latex up to 1.5 m height, leaves are 3-4, at a node 14 × 6 cm, obovate abruptly acuminate, base acute to alternate subcoriaceous with prominent lateral nerves. Petiole is 2 cm long and cymes are terminal or axillary. The peduncle is 5-8 cm long and flowers are white, pedicellate and pedicels are 1 cm long. Calyx lobes acuminate glabrous and reddish. Corolla is 1 cm long lobes ovate acute. Stamens are 5 and anther sagitate. Carpels are 2 and 2 ovules in each carpel. The style is filiform, with two clefts at the base and a stigma calyptriform. Capsules are 10 × 8 cm in size, oblong and smooth.

Ethnopharmacology: It is consumed as a contraceptive after crushing bark with coconut. Bark juice is used to ease childbirth. Fruits are ground into a paste in coconut oil and applied externally to heal cuts and wounds. The paste prepared from unripe fruits is used to treat psoriasis. The fruit sap is used to treat ringworm. Leaves are powdered with salt and used to treat cough. Root sap is a remedy for cold, cough, peptic ulcer and mouth infections. Leaf extract in water is used to treat fever, tuberculosis, sough and sting of sting rays. Root or bark powder is used to treat urinary tract infections. After warming with oil, leaves are used to massage sprains and broken bones. Crushed leaf sap is used to treat rheumatism. The paste prepared from leaves is used to cure insect bites, scorpion stings, snake bites and hypertension.

Phytochemistry of *Rauvolfia* species reported from Southern Western Ghats of India

The classification of genus *Rauvolfia* is marked by the existence of substantial quantities of monoterpenoid indole alkaloids (MIAs) like serpentine, ajmaline, reserpine, deserpidine, rescinnamine and yohimbine (12). These compounds demonstrate a varied range of structures and biological effects. The alkaloids belong to three categories: indole alkaloids with weak basic properties, those with intermediate basicity and potent anhydronium bases (12, 101-103). 224 MIAs, two additional alkaloids and fourteen non-alkaloidal compounds have been enumerated from *Rauvolfia* (104). The constituents present in *Rauvolfia* encompass alkaloids, flavonoids, fatty acids, terpenes, iridoids, sterols and sugars. The predominant category of potent phytochemicals identified is indole alkaloids, primarily localized in the roots.

Every part of the *R. serpentina* plant harbours alkaloids, with the root containing the highest proportion, approximately 85-90 % of the overall alkaloid content (19). The total alkaloid yield in *R. serpentina* varies from roughly 0.8 % to 1.3 % of the dry weight (105-107). The primary alkaloid in *R. serpentina* is reserpine, initially identified in 1952 (108). A comprehensive

collection of compounds documented from *R. serpentina* has been assembled (19). A thorough repository of molecules from the *R. serpentina* plant has been created as a database (109). Among these, 86 phytomolecules were sourced from roots, stems, leaves or root bark, predominantly from the roots of *R. serpentina*. Fifty-five molecules were exclusively from cell culture, including hairy root culture and three were common to both the plant and cell culture. The specific plant part was unspecified for six molecules (19). Alcohol-based extracts of *R. serpentina* showed the presence of quercetin, rutin and kaempferol (110-112). Five new indole alkaloids were discovered: N(b)-methylisoajmaline, 3-hydroxysarpagine, yohimbic acid, N(b)-methylajmaline and isorauhimbinic acid in the dried roots of *R. serpentina*. Another alkaloid, 21-O-methylisoajmaline, of the ajmaline type, was found in the roots of *R. serpentina*, along with twenty-one known compounds. These compounds included β -sitosterol, loganic acid, tetrahydroalstonine, stigmaterol, reserpine, reserpine, yohimbine, 6'-O-(3,4,5-trimethoxybenzoyl) glomeratose A, venoterpine, isoajmaline, rescidine, methyl 3,4,5-trimethoxy-trans-cinnamate, suaveoline, β -sitosterol 3-O- β -D-glucopyranoside, 3-epi- α -yohimbine, stigmaterol 3-O- β -D-glucopyranoside, 7-deoxyloganic acid, ajmaline, (+)-tetraphyllicine, 3-hydroxysarpagine and sarpagine (113). The water extract of *R. serpentina* seeds contained polysaccharides, yielding three methylated derivatives each of glucose and mannose after methylation and sulfuric acid hydrolysis (114).

R. tetraphylla is revealed to harbour a diverse array of secondary metabolites, notably alkaloids, with reserpine standing out as a significant alkaloid (25, 115, 116). *R. tetraphylla* contains twenty-two known indole alkaloids (84, 117). *R. canescens*, a synonym for *R. tetraphylla*, was found to produce ten indole alkaloids in Southern Srilanka namely corynanthine, ajmaline, α -yohimbine, deserpidine, isoreserpine, yohimbine, reserpine, arcine, isoreserpine and lankanescine (118-120). Reserpine, verticillatine, ajmaline, spagatine, dispegatine and vellosimine constitute the primary alkaloids extracted from the root of *R. densiflora* (syn. *R. verticillata*) (121). Additionally, rescinnamine, reserpine, reserpine, sarpagine, isoreserpine and densiflorine have been documented as alternative alkaloids present in *R. densiflora* (84). The chloroform extract of *R. verticillata* yielded three indole alkaloids such as 7-hydroxynoracronycine, raunescine and sandwicine and one acridone alkaloid, identified as ajmalicine B (13). Notably, the acridone alkaloid represents a novel compound extracted from the *Rauvolfia* for the first time (14). Additionally, five newly discovered hexacyclic MIAs, namely, 17-epi-rauvovertine A, 17-epi-rauvovertine B, rauvovertine A, rauvovertine B and rauvovertine C, have been isolated from *R. verticillata* (*R. densiflora*) (122).

Various principal bioactive compounds reported in the five *Rauvolfia* species are listed in Table 1 and 2 contains the uses of significant alkaloids reported from these five species of *Rauvolfia*. The chemical structure of major phytochemicals is given in Fig. 6.

Propagation of *Rauvolfia* species

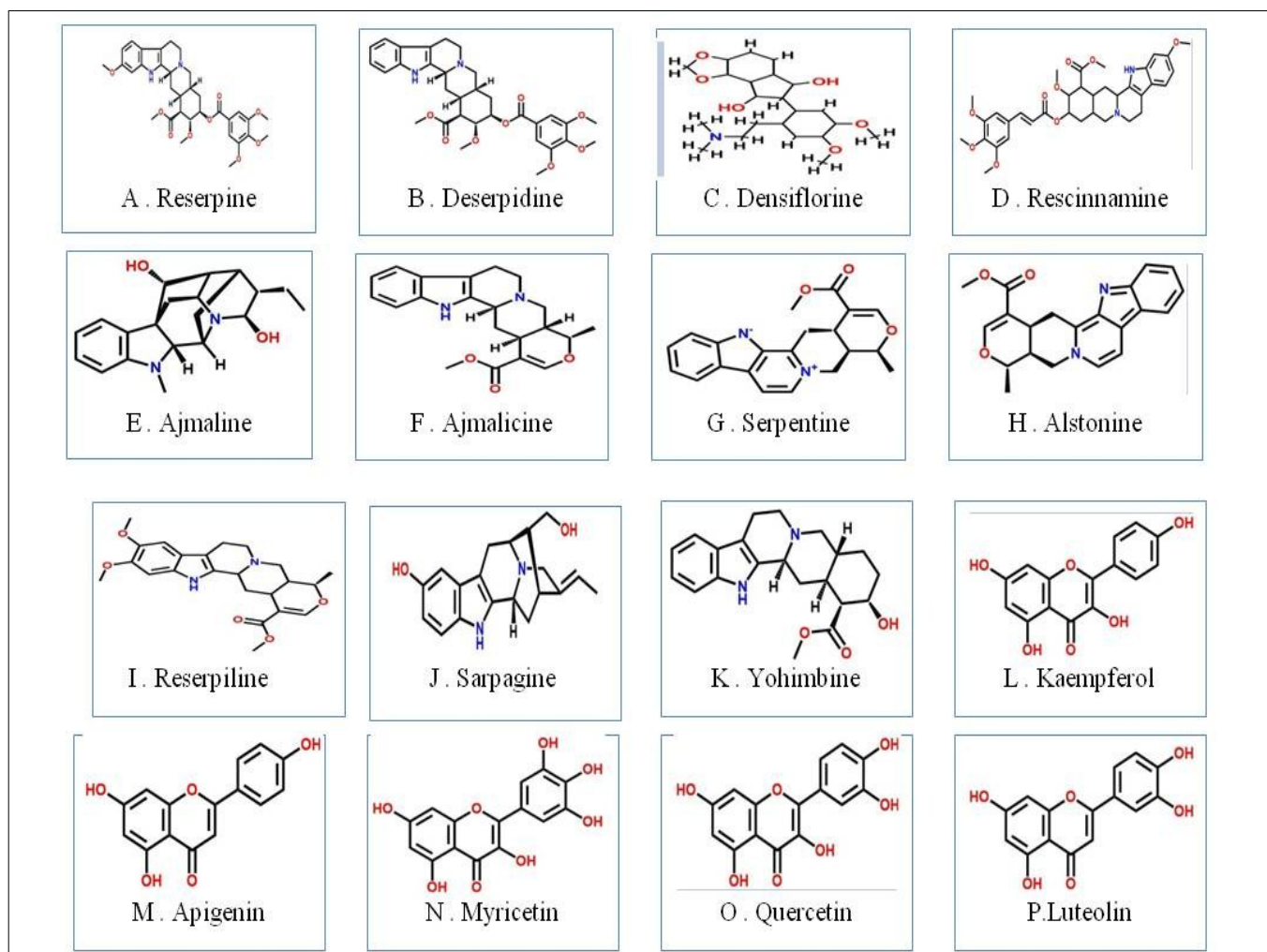
The relentless depletion of sarpagandha from forests, coupled with neglect of its regeneration, has led to a decline in wild populations, pushing it into a precarious and endangered

Table 1. List of bioactive phytochemicals reported from five species of *Rauvolfia* from Southern Western Ghats of India

Species name	Principal phytochemicals reported	References
<i>R. serpentina</i>	Reserpine, Raubasine, Ajmalicine, Isoraubine, Raugalline, Rauwolfine, Serpine, Rescinamine, Rescinnamine, Rescinnamidin,	(9)
	Rescinnaminol, Isoraubimic acid, Yohimbic acid, Arbutin, Rescidine, deserpidine, Seredine, Reserpig acid	(64)
	Myricetin, Quercetin, Cyanidin, Delphinidin	(66)
		(103)
<i>R. tetraphylla</i>	Reserpine, 8-octadecenoic acid, Pentadecanoic acid, Ajmaline, Yohimbine, Reserpiline, Iso reserpine, Deserpidine, Isoreserpiline, Lankanescine, Corynanthine, Aricine, Seredine, Rescidine, 18-hydroxy yohimbine, Reserpig acid, Labdane diterpene, Alstonine, Ajmalicine, Phenanthrenone, β carotene, Myricetin, Quercetin, Cyanidin	(29)
		(64)
		(116)
		(123)
<i>R. micrantha</i>	Reserpine, Quercetin, Apigenin, Ajmaline, Ajmalicine, Yohimbic acid, Seredine, Deserpidine, Rescidine, Reserpig acid, Yohimbine	(124)
<i>R. densiflora</i>	Luteolin, Quercetin, Sarpagine, Reserpine, Ajmaline, Densiflorine, Vitamin C	(64, 125)
<i>R. beddomei</i>	Reserpine, Quercetin, kaempferol, Apigenin, Kaempferol, kaempferol-3-O-D-glucoside, Apigenin-3-O-D-glucoside, Apigenin-7-O-neohesperidoside, Lycopene	(12, 64, 84)
		(18, 29, 64)

Table 2. List of major alkaloids from 5 species of *Rauvolfia* reported from Southern Western Ghats of India and their uses

Name of the phytochemical	Uses	Reference
Reserpine (indole alkaloid)	Antihypertensive, Antidepressant, Antipsychotic, Tranquilizer, Inhibits monoamine transporter, Anti-cancerous	(126, 127)
Alstonine (indole alkaloid)	Antitumor, Antipsychotic	(128-130)
Serpentine (alkaline anhydronium alkaloid)	Antioxidant, Tranquilizer, Antihypertensive, Anti-cancerous, DNA intercalating agent	(85, 102, 130)
Ajmaline (terpene indole alkaloid)	Blocks Na^+/K^+ channel, Treats abnormal heart beats (antiarrhythmic)	(101, 126)
Deserpidine (indole alkaloid)	Antihypertensive, Tranquilizer, Treats schizophrenia, Inhibits melanoma, brain metastasis (MBM)	(131-133)
Reserpiline (indoline alkaloids having intermediate alkalinity)	Antipsychotic, Antihypertensive	(89)
Yohimbine (indole alkaloid)	Blocks alpha adrenoreceptor, Aphrodisiac chemical	(93)
Rescinamine (indole alkaloid weakly alkaline)	Helps in sleep, Antihypertensive	(12, 66)
Ajmalicine (indole alkaloid)	Antagonist activity to androgen receptor, Antihypertensive, Tranquilizer	(126)

**Fig. 6.** Chemical structure of commonly found phytochemicals from the various species of *Rauvolfia* reported from Southern Western Ghats of India. (A-K: alkaloids) and (L-P: flavonoids) drawn in Chemdraw (<https://chemdrawdirect.perkinelmer.cloud/js/sample/index.html>) by taking Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>) database as reference.

state, particularly in India (133). Uncontrolled harvesting, habitat loss, human and biological interference and over-exploitation pose additional significant threats to the wild resources of *Rauvolfia*. There is an imperative need to develop advanced agro-technology for its systematic domestication and cultivation. The availability of high-quality planting material is indispensable for profitable cultivation. Although the crop propagated by seeds is renowned for providing superior quality material and increased yield, the germination of sarpagandha seeds is notably deficient. Soaking of *R. serpentina* seeds overnight with gibberellic acid solution (GA₃) enhances seed germination (134). Mechanical and chemical scarification of *R. serpentina* seeds did not enhance germination, it suggests that germination inhibitors may exist within the seeds (135). The germination process of *R. serpentina* seeds seems intricate, necessitating additional research to comprehend the underlying mechanism. Seed-mediated propagation is ineffective due to dormancy and a low germination percentage. GA₃ and potassium nitrate (KNO₃) for *R. tetraphylla* seeds and GA₃ and hydrogen peroxide (H₂O₂) for *R. serpentina* seeds to address dormancy and expedite germination (136). Their findings revealed that *R. serpentina* germination percentage increased to 48.65 % (GA₃) and 34.94 % (H₂O₂) from the original 11.27 % for untreated seeds. Conversely, the initial germination percentage for *R. tetraphylla*, at 31.26 %, elevated to 56.66 % (GA₃) and 52.70 % (KNO₃). *R. serpentina* is a paramount medicinal plant in India, but prolonged intense exploitation has rapidly diminished its natural reserves. Consequently, it is classified as endangered, featuring on the convention on international trade in endangered species of wild fauna and flora (CITES) list due to substantial commercial depletion. The issue is compounded by low seed germination rate, hampering natural propagation and cultivation (137).

Historically, seed propagation has been employed, but this approach has numerous drawbacks, including low seed setting, subpar seed germination and a heightened likelihood of genetic variation. These issues not only diminish the quality and quantity of secondary metabolites but also impede the swift propagation of this plant. Therefore, using synthetic seed production technology, specifically through alginate encapsulation, proves to be an efficient method for the short-to mid-term storage and exchange of germplasm, concurrently with *in vitro* propagation for *Rauvolfia* spp. (121, 138-141). A technique for effective *in vitro* conservation of *R. serpentina* genetic material by enclosing or encapsulating shoot tips (137). The resultant synseeds showcase peak storage potential lasting up to 14 weeks and a heightened regrowth frequency. This method provides a remedy for the intrinsic issue of limited seed germination, potentially facilitating a simple pathway for the widespread cultivation of this vital medicinal plant. The *in vitro* clonal multiplication of *R. serpentina*, involving direct regeneration from shoot tip explants (142). The cuttings from 1.5-2 years old *R. serpentina* plants collected from various parts to facilitate propagule development (143). Root formation and successful propagule development from cuttings taken from root, stem and root-stem junctions were 62 %, 42 % and 78 %, respectively. Lower concentrations of naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-butyric acid (IBA) stimulated root formation and propagule

development from stem cuttings. Notably, 2,4-D at 5 ppm demonstrated the most substantial positive influence on both root formation and propagule development (100 %), followed by IBA (83 % at 50 ppm) and NAA (66 % at 10 ppm). The combination of IBA and NAA had a modest positive effect on rooting (143, 144). *R. serpentina* exhibited increased growth under double or triple doses of nitrogen, phosphorus and potassium (NPK), especially with higher nitrogen (N) supply. The crude alkaloid contents of the roots experienced a noteworthy increase only under elevated nitrogen levels.

R. serpentina can also be propagated from shoot or nodal shoot explants by clonal propagation procedure using Murashige and Skoog (MS) medium with the addition of IAA and 6-benzylaminopurine (BAP). MS medium procedure with BAP and IAA can be used to multiply axillary buds of *R. micrantha* and *R. tetraphylla* (145). The primary impediment to the natural seed propagation of *R. tetraphylla* lies in inadequate seed germination (146). Despite the seeds demonstrating high viability (83.1 %) in their natural surroundings and containing viable embryos, it is evident that viability alone does not explain the suboptimal seed germination of this valuable medicinal plant. To address this issue, an efficient protocol has been devised for *R. tetraphylla* (146). Among the various treatments, the highest *ex vitro* seed germination rate (13.33 %) was attained when the seeds underwent treatment with 100 ppm of GA₃. The response of *in vitro* germination varied under different conditions, with the rate significantly surpassing that of *ex vitro* germination. In MS medium without plant growth regulators supplements, the rate reached 78 %, while it was 70 % and 78 % in cotton beds under light and dark conditions, respectively. Additionally, an incubator's temperature of 37 °C yielded an 80 % germination rate.

The hard seed coat in *R. tetraphylla* hinders germination and its removal facilitates germination. Plantlets cultured *in vitro* were effectively acclimatized in a natural environment, producing flowers and generating seeds. Optimal results for *in vitro* shoot regeneration, reaching a success rate of 90.7 %, were achieved using nodal segments cultured on MS medium supplemented with 2.2 mg/LBA and 0.1 mg/L NAA. *R. micrantha* can also be propagated by somatic embryogenesis of root segments (147). Alkaloids like ajmaline and ajmalicine may be produced from *R. micrantha* using hairy root cultures (148). Vegetative propagation of hardwood and softwood of *R. densiflora* is facilitated by using plant growth regulators such as IAA and IBA. At the same time, axillary buds and leaf explants showcase better results during callus induction when the MS medium is used in addition to 2,4-D in micropropagation (149). Culturing shoot tip explants of *R. beddomei* on a medium containing half-strength MS medium, supplemented with various cytokinins (6-benzylaminopurine, thidiazuron and 6-furfurylaminopurine), either individually or in combinations, resulted in the production of a lot of shoots (150). Currently, *in vitro* biotechnological aspects, including direct regeneration, indirect regeneration through callus formation, synthetic seed production, somatic embryogenesis, hairy root culture and polyploidy induction, contribute significantly to the successful propagation of different *Rauvolfia* species (151).

Economic importance of *Rauvolfia* species and demand of *R. serpentina* in global herbal market

For the Western medicine on antihypertensive and nootropic attributes of the *R. serpentina* root resulted in its recognition as either serpentine root or *Rauvolfia* root (62). *R. serpentina* stands out among the 178 medicinal plant species engaging in substantial trade in India, with an annual domestic demand of 588.7 tonnes in 2004–2005 (18). Notably, India is a prominent source for a noteworthy share of the drug's commercial supply to the USA and European countries (152). The essential ayurvedic products derived from sarpagandha, include sarpagandha ghan vati, sarpagandha yoga, sarpagandha churna and maheshwari vati. Recognizing its agro-economic significance, some farmers in Prayagraj have taken up sarpagandha cultivation (153). The crop typically matures over a three-year period, during which the subaerial parts dry and the primary roots extend to a depth of 0.9 meters. The average yield is approximately 3000 kg of dried roots per hectare and 8–10 kg of seeds (153). The plant's roots and leaves are valuable components. Due to the lucrative nature of its cultivation, farmers have ventured into commercial production, discovering that the average cultivation cost is around Rs. 110000.00 per hectare. At the same time, the gross return amounts of about Rs. 420000.00 per hectare, which results in a favourable benefit-cost ratio of 1:2.81, surpassing that of traditional crops (153). The price of *Rauvolfia* supplements in various countries is very high, ranging around Rs. 250.00–Rs. 8000.00 per 100 g due to the presence of highly crucial medicinal bioactive compounds such as reserpine, ajmaline, sarpagine and apigenin. Almost every part of these plants has medicinal value, though the numbers of bioactive compounds differ from species to species (12).

According to the data acquired from Indian trade (Import/Export) statistics (2016), India exports 113.0 Kg (3,283.8 USD/Kg), 1.01 kg (4,745.0 USD/Kg), 0.20 Kg (724.5 USD/ Kg) and 0.55 Kgs (9,483.1 USD/Kg) of reserpine API (active pharmaceutical ingredient) to China, Thailand, Russia and Canada respectively.

Adulteration of *Rauvolfia* species in the market samples

The fraudulent inclusion of adulterants is a prevalent deceit in the trade of herbal raw materials. An adulterant plant may share similarities with a genuine plant but is substantially distinct, inferior, less potent or contains a lower percentage of active constituents (154). India needs 650 tons of dry *Rauvolfia* annually (155). The adulteration process differs depending on the resources available. *R. heterophylla*, *R. canescens*, *R. caffra* and *R. vomitoria* were recognized for having varying levels of indole alkaloids and were employed as suitable alternatives to *R. serpentina* (156, 157). *R. serpentina* roots could be tampered with *R. hookeri*, *R. tetraphylla*, *R. micrantha*, *R. vomitoria* and *R. verticillata*. These species function as stand-ins because, although there are some variations in the quantity and quality of phytochemicals, they resemble real herbal medication. *R. serpentina* experiences two types of adulteration. Due to the easy availability of similar species like *Clerodendrum paniculatum*, *Ophiorrhiza mungos* and invasive species *R. tetraphylla*, these species are sold in the market instead of *R. serpentina* and this is regarded as intentional adulteration. As we observed during field visits, the habitat overlapping of *R.*

serpentina with morphologically similar species like *Chassalia curviflora* leads to using the later as an unintentional adulterant. *Ophiorrhiza mungos*, white and red flower varieties of *Clerodendrum* spp. (*Clerodendrum paniculatum*), *Chassalia curviflora* and roots of other less effective *Rauvolfia* spp., such as *R. perakensis*, *R. beddomei*, *R. densiflora* and *R. tetraphylla*, are found as adulterants in market samples of *Rauvolfia* root due to its high industrial demand (80) (Fig. 7a & b). *R. micrantha* and *R. beddomei* display notable morphological resemblances, posing a challenge in distinguishing them, even when they are in the flowering stage (97). Though the medicinal worth of *R. beddomei* is inferior to that of *R. micrantha*, mainly due to the less reserpine, *R. beddomei* is used as an adulterant for *R. micrantha*.

Ecological threats of the genus from the study area

The genetic wealth of this plant faces substantial risks due to heightened human activities such as deforestation, habitat modifications and unsustainable harvesting for commercial purposes (158). The concept of a forest gene bank entails intentionally establishing and tailoring a diverse collection of genetic resources to protect the genetic diversity of the *R. serpentina* plant. The species was classified as "endangered" in the Southern regions of India, encompassing states like Karnataka, Kerala and Tamil Nadu, as well as in central India. Based on data from workshops hosted by the conservation assessment and management plan (CAMP), several researchers have documented a gradual population decline of over 50 % between the 1985 and 1995 censuses. These researchers attribute this decline to habitat loss resulting from human consumption and use for medicinal purposes (159).

The indigenous populations of *Rauvolfia* species in the Western Ghats have undergone a notable decrease in recent decades, predominantly due to widespread root harvesting and the removal of plants from their native environments (160). The rapid decline of this species in India, characterized by low alkaloid content, can be attributed to genetic erosion (161). In 1998, another CAMP workshop in Bhopal categorized the same species as "critically endangered" in Maharashtra, with an observed decline rate exceeding 80 % from 1988–1998 (162). Most populations were at risk primarily due to the collection for medicinal use and trade (163). Subsequent research findings led various researchers to determine that the species was categorized as "vulnerable" in Southern India, encompassing Kerala, Odisha and Tamil Nadu (164). Researchers indicated that fragmentation could significantly affect the pronounced genetic differentiation observed in *R. serpentina*. This finding suggests that the primary factors jeopardizing this species encompass human disruptions like extensive deforestation, forest fires and agricultural activities. The challenges lie in sustaining seed viability across extended intervals between seed years, protecting seeds from animal damage and addressing difficulties in germination and dispersal. While losing individual plants in specific locations may not promptly reduce genetic diversity, such losses pose perilous long-term repercussions. Preserving endangered species hinges on sustaining a sizable population.

The degradation of its habitat and excessive harvesting for medicinal purposes pose significant threats to this plant (146). Its small stature, slow growth, limited regeneration and



Fig. 7 (A, B). *Rauvolfia* species using as adulterants.

limited distribution render it highly susceptible to human exploitation. Additionally, pharmaceutical companies heavily rely on materials obtained from naturally occurring plants, contributing to the rapid depletion of this endangered plant species. The combination of indiscriminate collection and limited cultivation has led to its scarcity, resulting in its classification as an endangered plant in India (165). The extensive use of this plant for its diverse medicinal applications is a major threat (136). In another report, the plant is described as critically endangered due to widespread and unregulated wild collection, poor seed germination and insufficient commercial plantations (166).

Despite the favorable climatic conditions in the Western Ghats region, creating a suitable habitat, the populations of these shrubs are dwindling rapidly due to various ecological and biological factors. Factors such as endemism, restricted distribution, small populations in accessible areas, significant anthropogenic pressure on forest land, constraints in

pollination and inadequate seed viability have collectively contributed to its global decline, designating it as rare and endangered (27). The Western Ghats stands out as the richest in biodiversity in terms of the diversity of plant species and the prevalence of unique taxa within Peninsular India. A multitude of plants in this region, *R. beddomei* included, are progressively becoming scarcer due to excessive commercial exploitation (4).

Regrettably, these plants confront imminent peril from poachers due to inappropriate harvesting techniques and ineffective preservation approaches. *R. serpentina*, renowned for yielding the coveted reserpine drug with calming and antihypertensive attributes, is especially susceptible. Its inclusion in the endangered plant roster is ascribed to unregulated use and insufficient traditional propagation and conservation methodologies (20). Considering their economic value and the precarious state of the ecosystem resulting from unsustainable extraction, species like *R. serpentina* demand particular attention. Because reserpine and other

pharmacologically active alkaloids have been found in allied species of *Rauvolfia*, such as *R. tetraphylla* and *R. micrantha*, a recent study from India has shown that these roots are heavily exploited. Habitat overlapping with invasive species like *Chasalia curviflora* or *Osbeckia stellata* and invasive allied species like *R. tetraphylla* are the primary threat to *R. serpentina* and *R. micrantha*. Throughout our field survey, we have noticed that habitat overtaking by these invasive alien species is key to the biodiversity loss of medicinally significant species like *R. serpentina* and *R. micrantha* (Fig. 8a-c).

The species *R. micrantha* is sparsely found in natural habitats due to significant threats like habitat degradation, population fragmentation and a limited ecological niche (28). Factors such as endemism, restricted small populations in accessible areas, significant anthropogenic pressure on forest land, constraints in pollination and inadequate seed viability have collectively contributed to its global decline, designating it as both rare and endangered (27, 31). While conducting our field excursions, we witnessed stone mining quarries and stone crusher units in Travancore (specifically in Palai, Poovarani and Kangazha), significantly impacting the natural environments of *R. micrantha*. These units' airborne dust and rock fragments are disrupting the population hindering flower pollination. Concurrently, the areas surrounding these quarries are being cleared, further contributing to the loss of habitat for the species.

Our extensive field work revealed the following reasons: significant threats to the *R. densiflora* species in the Wagamon area of the Southern Western Ghats of India. In the development of tourism-related facilities like hotels, resorts and golf courses in the natural habitat has resulted in habitat loss of *R. densiflora*. Another major threat to *R. densiflora* in this region is the installation of 11 KW electricity power lines. These power lines necessitate the clearance of vegetation and subsequent maintenance beneath them. The routine removal of flora by electricity board officials for the upkeep of these power lines also negatively influences the natural environment of the *R. densiflora* species. There are limited opportunities to mitigate these impacts on *R. densiflora* by adjusting the routes of the power lines. We have observed that removing the *R. densiflora* population for agricultural use and eliminating weeds along national/state highways under the national rural employment guarantee scheme initiative is also leading to the degradation of the natural habitat of *R. densiflora* (Fig. 9). We have noticed massive devastation of *R. densiflora* populations in Periya (Nempoyil-Mananthavady highway) study area regions as part of land clearing for agricultural purposes.

Locals and officials in Thenmala ecotourism project have observed the main issues concerning domestic and foreign tourists are wildlife disturbances, non-biodegradable waste disposal, destruction of natural habitats, destruction of cultural heritage and conflicting attitudes (167). This habitat destruction and anthropogenic activities can be considered a major threat to the species. *R. beddomei* population is progressively becoming scarcer due to excessive commercial exploitation (4). During our visits, we observed two notable alterations in land utilization within the research area. The transition from forested areas to agricultural land and the shift from agricultural activities to non-agricultural uses. This signifies that adverse human-induced pressures, managerial

shortcomings and natural elements have transformed the predominant natural vegetation in the Shenduruny area (the native habitat of *R. beddomei*) into grasslands. Forest pressure continues to escalate driven by expanding agricultural activities, encroachment and population migration. Several factors contribute to forest degradation in the study zone, with population increase due to migration particularly noteworthy. The local community comprises individuals of diverse backgrounds, including settlers, migrants and cultivators residing on the fringes of the forest alongside tribal communities in and around the study area. The individuals who arrived during the construction of dams at Neyyar and Peppara have primarily contributed to the land pressure in the Shenduruny area. Other factors include encroachment, the transformation of forest land for agriculture and plantation activities. Presently, with the intervention of the Rubber Board, the main beneficiaries appear to have gained significantly. Recently, the increasing emphasis on promoting recreation has had extensive implications for their lives. There is a lack of control over the shifting cultivation of Kani tribals and encroachment by individuals from the plains.

Current status of research

Rauvolfia, a significant genus relevant in pharmaceutical and agricultural contexts, has been extensively examined by diverse researchers for different aspects, Such as phytochemistry, ethnopharmacology, biodiversity conservation biology and population biology. In the current context, researchers are particularly exploring the genus for its diverse medicinal attributes, including but not limited to anticancer, antioxidant, psychotic and antimicrobial properties. The anticancer effects of methanolic extracts from *R. serpentina* leaves on HepG2 and Hells cells (168). The suitability of habitats for a valuable non-timber forest product, focusing on *R. serpentina* (169). The antihypertensive and anti-hyperlipidemic effects of *Allium sativum* and *R. serpentina* in a rat model induced with hypertension and high-fat conditions (70). The impact of *in-vitro* culture age on the morphology, antioxidant activities, reserpine production and genetic fidelity of Indian snakeroot (*R. serpentina*) (170). The impact of varying concentrations and combinations of plant growth regulators on the regeneration of plantlets in *R. serpentina* from cell suspension culture (171). There are reports about multi-omics approaches for breeding in medicinal plants, focusing specifically on *Rauvolfia* species (172). Analytical techniques for measuring natural indole alkaloids found in *Catharanthus* and *Rauvolfia* (173).

Chemometric approach based on nuclear magnetic resonance (NMR) to identify agents with chemo-sensitizing properties for colorectal cancer within *R. vomitoria* (174). The anti-fouling capabilities and conducted an *in-silico* analysis of carotenoids and fatty acids derived from *R. tetraphylla* (175). The precise distribution of monoterpenoid indole alkaloids within *R. tetraphylla*, employing advanced high-resolution mass spectrometry imaging techniques (176). *Pseudocercospora rauvolficola*, a new species responsible for foliar disease in India's medicinal plant *R. serpentina*, was discovered and studied (177). The growth of published literature for five species of *Rauvolfia* reported from the Southern Western Ghats of India is given in the graph (Fig. 10).





Fig. 8 (A-C). Habitat overlapping of *Rauvolfia* species with allied or invasive species.



Fig. 9. Devastation of *R. densiflora* natural habitat for agricultural land clearing.

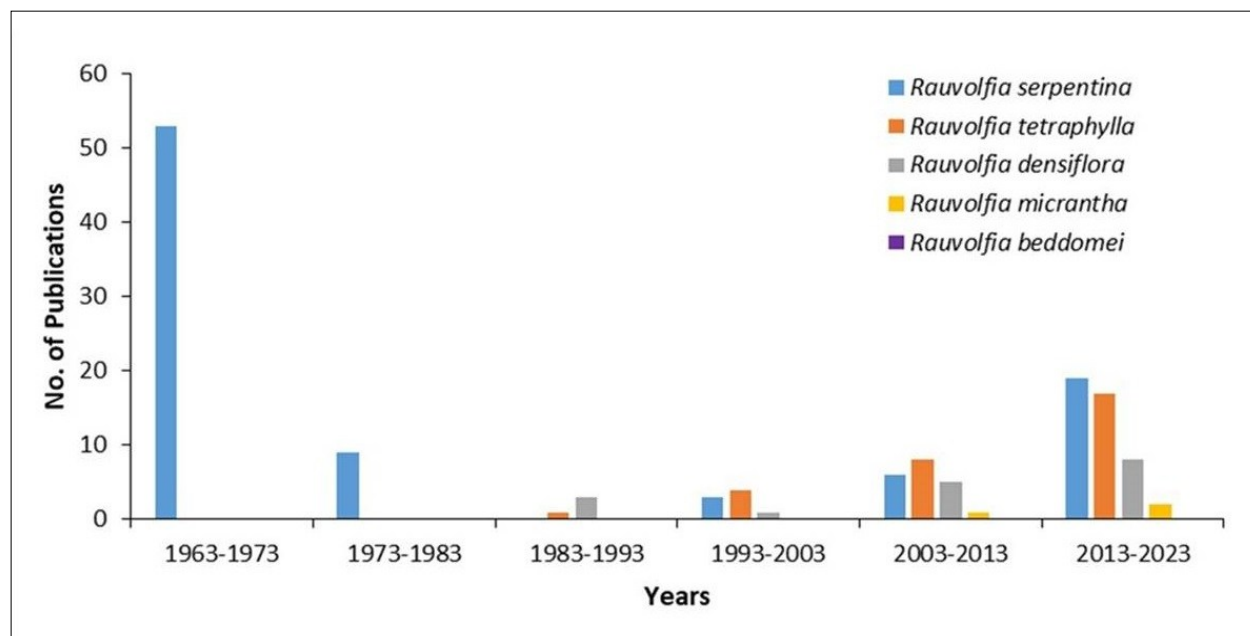


Fig. 10. Number of published papers from 1963 to 2023 for five species of *Rauvolfia* reported from Southern Western Ghats of India on the basis of Pubmed database.

Conclusions and future perspectives

The present review furnishes about five *Rauvolfia* species reported from the Southern Western Ghats of India. This is the first study that provides comprehensive information about the five reported species of genus *Rauvolfia* from the Southern Western Ghats of India. The study encompasses information on the biogeography of five *Rauvolfia* species from Southern Western Ghats of India, taxonomy and chemotaxonomic markers for the species, general morphology, ethnopharmacology, phytochemistry, principal phytochemicals and their structures, the use of phytochemicals in treating various diseases, cytogenetics, plant propagation, adulterations, threats to the plants, conservation status, economic importance, current research status and the growth of published literature associated with the five *Rauvolfia* species such as *R. serpentina*, *R. tetraphylla*, *R. micrantha*, *R. densiflora* and *R. beddomei*. While prior literature predominantly focuses on two species, *R. serpentina* and *R. tetraphylla*, there is limited existing data on the other three species. Given their medicinal significance and the presence of bioactive compounds crucial to the pharmaceutical industry, these plants face anthropogenic exploitations, resulting in their critical endangerment and susceptibility to extinction. Extensive exploitation has pushed these species to extinction in their natural habitat. Phytochemical analysis of threatened *Rauvolfia* species such as *R. beddomei* in the Western Ghats, has identified as alternative sources of reserpine. This could help to reduce the risk associated with *R. serpentina* decline. Furthermore, integrating analytical techniques in investigating phytochemicals from the endangered *Rauvolfia* species significantly aids in dereplication.

Despite biotechnological intervention studies for drug extraction, it is imperative to reinforce cytogenetics, bioprospecting, ecology and molecular authentication using DNA barcoding and conservation biology studies. DNA-based molecular authentication of *Rauvolfia* plants is crucial for ensuring the quality and safety of herbal pharmaceuticals and nutraceuticals. This approach has the potential to substantially enhance the medical efficacy and economic viability of herbal

products. Addressing somaclonal variations associated with culture systems is pivotal in any *in vitro* conservation effort. Strong partnerships are needed among institutions and centers of excellence, specifically focused on researching diverse facets of genetic conservation for these endangered medicinal plants. Recently, gene cloning technology has experienced substantial advancements across various ecological realms. The application of cloned segments of chromosomal DNA as genetic markers, often denoted as 'RFLPs' (restriction fragment length polymorphism), has garnered noteworthy importance. It is recommended that phylogenetic studies be strengthened, as they play a crucial role in guiding conservation policies when conservation biologists face challenging decisions regarding preserving species that are at risk of extinction. Phylogenetics reveals the historical development of genetic sequences and provides overarching principles that allow us to anticipate future changes. This holds fundamental significance and proves highly valuable for various practical applications. This review will help future researchers develop strategies to protect these species from extinction, ensuring judicious utilization of these plants and their products for human welfare. Such measures contribute to species preservation and benefit the country's economy, as these plants serve as major sources of precious chemicals for treating various diseases.

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Authors' contributions

VDN conceived, designed and edited the manuscript. VDN has conducted the field study and collection of various species from the study area. PA has compiled the first draft of the manuscript. GA critically reviewed the manuscript. KA has provided laboratory facilities during fieldwork. SA assisted in formatting the manuscript as per journal's guidelines. NA and TA have assisted in locating and collecting species from various places in the study area. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest : The authors declare that they do not have any conflict of interest.

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