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A Review Study on the Taxonomic, Phytochemical, and Pharmacological Characteristics of "Small-Flowered Snakeroot" (*Rauwolfia micrantha*, Hook. F.)

Denna Susan Jacob[1*], Anjana E.S.[2], Avani Rajeev[3]

Department of Botany, CMS College Kottayam (Autonomous), Kerala-686001
*dennajacob92@gmail.com

ABSTRACT

India is one of the 12 mega-diversity countries in the World and has 47,000 plant and 89,000 animal species ranking the country sixth for harboring the largest number of threatened plant species.This unique land mass of India has one of the most varied tropical and subtropical climates, altitudinal ranges reaching up to 2,800 m, and many edaphic conditions. The mentioned factors confer a variety of micro-climatic/ecological niches that nurture one of the richest endemic flora of peninsular India. With its exceedingly environmentally heterogeneous biogeographic area, the Western Ghats as a whole is an abode of rich diverse flora. The present work has been conducted to review and assess the taxonomic characters, phytochemical properties, and pharmacological uses of *Rauwolfia micrantha*. *Rauwolfia micrantha* is an important evergreen, woody, shrub used in the modern medical system and also in Ayurveda, Unani, and folk medicine. It is also a source of pharmacologically important active chemicals and alkaloids. The data obtained from the present study will help in understanding the potential and importance of *Rauwolfia micrantha* and assert the significance of its conservation through micropropagation and other biotechnological tools.

Keywords: *Rauwolfia micrantha*, Apocyanaceae,Endemic, Alkaloids,Micropropagation

1. INTRODUCTION

Apocynaceae, the "Dogbane or Oleander family" of flowering plants is one of the prominent angiosperm families that comprises about 410 genera, 25 tribes, 49 subtribes, and 5500 species of trees, shrubs, herbs, stem succulents, woody vines, and lianas distributed in five subfamilies such as Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. Apocynaceae plants show a naturalized distribution and are widespread in the regions of Europe, Africa, Asia, Australia, and America, mostly in tropical and subtropical as well as temperate regions [47]. The main characteristics of the family include white latex or more rarely, watery juice, salvar-shaped corolla, and sagittate anthers [24]. The species of this family possess different compounds, such as alkaloids, cardenolides, triterpenoids, and iridoids which contribute to their overall biological activity or toxicity. Nearly all members of this family are poisonous[2] and many species are used medicinally because of the presence of cardiac glycosides and various alkaloids. Hence, they are included in different traditional medicinal systems such as Indian, Chinese, and Thai [8] while many species are cultivated as ornamentals for their attractive flowers and foliage.

Rauwolfia Plum. ex L. is a genus of evergreen trees and shrubs, commonly known as "Devil Peppers", in the family Apocyanaceae. It comprises about 80 species of tropical distribution. In India, the genus is represented by six species viz.; *R. hookeri* Srinivas et Chithra, *R. micrantha* Hook f., *R. serpentina* (L.) Benth. ex Kurz, *R. verticillata* (Lour.) Baill., R. tetraphylla L., and the introduced species *R. vomitoria* Afzel. Among the six species, *R. hookeri* and *R. micrantha* are endemic to the Western Ghats of south India [1].

Rauwolfia micrantha is a rare, endemic, annual shrub, reaching up to 1.5 to 2 m high. It contains alkaloids such as reserpine, reserpiline, and serpentine in its roots and is used to treat a variety of nervous disorders [24].

All *Rauwolfia* species are rich in alkaloids and other elite chemicals. The biological and therapeutic significance of the *Rauwolfia* species stimulated intensive research, resulting in the identification of several heterocyclic alkaloids with monoterpene indole skeletons [56]. According to Sahu (1983) and Gao (2012), reserpine, reserpiline, rescinnamine, ajmaline, ajamalacine, rauvolfinine, serpinine, serpentine, serpentinine, and yohimbine are some of the indole alkaloids with therapeutic and biological potential reported from various *Rauwolfia* species.

In the present review, an attempt has been made to collect information about the habitat, taxonomy, medicinal uses, phytochemistry, and pharmacology of a critically endangered species of the family Apocynaceae, the *Rauwolfia micrantha*, from various research conducted through the past years. The recent biotechnological advances to produce bioactive compounds of plant origin are also highlighted.

2. MATERIALS AND METHODS

2.1 STUDY AREA

Plants were collected from various locations of Kottayam during different seasons of 2021-2022 by using a vasculum and replanted in suitable locations. Kottayam is a district in the Indian state of Kerala. Bordered by the enchanting Western Ghats on the east and the Vembanad Lake and paddy fields of Kuttanad on the west, Kottayam is a scenic beauty located in Central Kerala. Kottayam is the first town in India acclaimed by the Ministry of Environment and Forests, Government of India, to be reformed as an Eco City. The city spreads over a total area of 2208 sq.km and lies between 9˚35' 29.1876" N latitudes and 76˚31' 19 .8156" E longitudes. Photographs of the plants were also taken by using Canon 12.1 Megapixel PowerShot Digital Camera.

2.2 METHODOLOGY

The study's methodology included a systematic review of the taxonomy, phytochemistry, pharmacology, and ecology of Rauwolfia micrantha using standard floras (Hooker 1872–1897, Gamble & Fischer 1915–1936) and available e-resources. The plants were enumerated according to APG IV (2016) and the nomenclature and citations follow IPNI (2011), verified with the online databases of WCSP (2017) and The Plant List Version 1.1 (2013). According to Ahmedullah & Nayar (1986) and Nayar (1997), endemism and the distribution of species were treated. RET (Rare, Endangered, and Threatened) status of the species was treated on a global scale based on IUCN (2011).

3. RESULTS AND DISCUSSION

3.1 SCIENTIFIC CLASSIFICATION

Kingdom: Plantae

Division: Angiosperms

Class: Dicotyledonae

Order: Gentianales

Family: Apocyanaceae

Genus: Rauwolfia

Species: R. micrantha

3.2 NOMENCLATURAL NOTES

The taxonomic confusion in the past has resulted in many misspelled and synonymous names.

- According to the Indian Biodiversity Portal, the plant has 2 accepted synonyms: Rauwolfia *membranifolia* Kerr and *Ophioxylon micranthum*.
- In India, it is locally known as Cheriyaveluthamalpori.
- The internationally accepted common names are Small-flowered snake root and Malabar Rauwolfia.

3.3 TAXONOMIC DESCRIPTION

Rauwolfia micrantha Hook. f. (Apocynaceae) is a rare, endemic, critically endangered, woody, medicinal shrub distributed at anelevation up to 600m in the Tirunelveli and Travancorehills of the Southern Western Ghats (South India). The medicinal property of the plant is mainly due to the wide spectrum of alkaloids such as reserpine, reserpiline, and serpentine in its roots [4].*R. micrantha* is used as a substitute for *R. serpentina*totreat a variety of nervous disorders in Ayurveda, especially in the state of Kerala, South India [56].

Rauwolfia micrantha is an annual shrub, reaching up to a height of about 1.5 to 2m with sparingly branched stems. Leaves are simple, alternate, elliptic-lanceolate or oblanceolate, 5-10 x 3-4cm, acuminate, peduncle terminal, erect, 4-6 flowered. The inflorescence is a corymbose cyme and consists of 20 ± 3.0 flowers. Flowers are small, white infundibuliform with a purple tinge inside, bisexual, actinomorphic, and hypogynous. Stamens 5, alternating with corolla lobes (alternipetalous), epipetalous, filamentous, very short, and sagittate. The pistil is elongated. Style long and slender with capitate, dumb-bell shaped, wetand papillate stigma. The ovary is bicarpellary syncarpous withone ovule in each carpel with axile placentation. Fruit is a smooth berry with 1 or 2 seeds. Seeds are rough, black, and with a reticulate surface [24,45].

(A) (B) (C)

Figure1: (A) Plant Bearing Fruit (B) Mature Flowering Plant (C) A Single Leaf

3.4 DISTRIBUTION, CLIMATE, AND PROPAGATION

The genus Rauwolfia L. (Apocynaceae) is distributed throughout the tropical regions of the World [42] in semi-evergreen and evergreen forests. The ideal climatic conditions prevailing in the Western Ghats region provide suitable habitats for *R. micrantha*. It is a woody shrub and may attain a height of 1.5-2m in dense Shola forests at an altitude of 550-650m[45]. The area receives both southwest and northwest monsoon with an average of 2000mm rainfall except in the Agasthyamalai region, where sometimes rainfall exceeds over 4000mm. The region harbors vegetation types such as moist deciduous, semi-evergreen, evergreen, Shola, montane grassland, etc., which form a natural home for *Rauwolfia micrantha*[44]. Under favorable climatic conditions,such as optimum light intensity, rainfall, relative humidity, temperature, and humus content of the soil,*Rauwolfia micrantha* starts flowering in the first week of March and reaches a peak during late May and June. The flower buds take 10-15 days from initiation to full bloom. The average lifespan of each flower is about 2-4 days after blooming. The flowers offer both nectar and pollen to visitors [44].

The floral analysis by Kulloli et al. (2009) indicated that each flower has 5 anthers and 2 ovules. A single anther contains 1200 pollen grains and the individual flower has around 6000 pollen grains. Hence P/O ratio was worked out as 3000 pollens per ovule (3000:1), which again substantiates entomophilous pollination[16]. The phenological studies prove useful in planning out conservation strategies and formulating measures for large-scale cultivation measures [74]. The flowers are visited and pollination occurs by honeybees (*Apis cerana*), pollen-collecting bees (*Trigona iridipennis*), ants (*Oecophylla smaragdina*), and butterflies such as *Pachliopta aristolochaea*, *Pachliopta hector*, *Euploea core*, and *Delias eucharis*[44].

Being protogynous and self-incompatible, the species is solely cross-pollinated and depends on external pollinating agents. The seed set is very low principally due to limited pollination and an isolated population. Predation, dormancy, and low seed viability lead to low seed germination. Furthermore, the flower offers very little rewards to the pollinators resulting in their visits to be on a lesser degree. The species exist in the isolated pockets of the southern Western Ghats and lacks its unique pollinator[44]. It was also reported that, at the time of the flowering of *Rauwolfia micrantha*, some entomophilous plants such as *Asystatia gangetica*, *Strobilanthus barbatus* (Acanthaceae), *Viola pilosa* (Violaceae),

Goniothalamus sp. (Annonaceae),*Sygyzium travancorica* (Myrtaceae), *Memecylon* sp. (Melastomaceae) and *Begonia integrifolia* (Begoniaceae) entered their peak flowering phase and secreted more nectar than *Rauwolfia micrantha*. Hence the pollinators that previously visited the flowers of *Rauwolfia micranthawere* attracted to visit the flowers with abundant nectar. Therefore, steady competition exists among the co-flowering plants for a particular pollinator(s) [44]. The views of Heinrich and Raven (1972) and Heinrich (1975,1979) show that it is the principal aim of insect pollinators to forage nectar as a source of energy and that they will select plants that secrete the most.

3.5 PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES

The roots of *Rauwolfia micrantha* are a potent source of valuable tranquilizer alkaloids [4]. In addition, it serves as a substitute for the roots of *Rauwolfia serpentina* in commercial lots offered in increasing amounts to American purchasers [75]. In the traditional system of medicine (Ayurveda), *R.micrantha* is also used, especially in the state of Kerala, as a substitute for *R. serpentina* to treat a variety of nervous disorders, such as insomnia and insanity [56].

Other nutrients and chemicals in the plant include phenolics, flavonoids, vitamins, and carotenoids. The extracts also had antioxidant properties in vitro.All parts of the plant, including the stem and leaves, contain indole alkaloids, but the root's highest concentration is seen in the bark[72].

3.5.1 PHENOLICCOMPOUNDS

The plant may be an antimicrobial agent because of the presence of phenolic compounds in it. Phenols are mainly responsible for the antioxidant properties of the plant as reported by Miller et al., 1996. The presence of phenolic compounds in the plant is an indication of antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory activities[51]. These compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates. Literature surveysreveal that most antioxidant activities from plant sources are correlated with phenolic compounds [12, 38, 35, 36].

3.5.2 FLAVONOIDS

Flavonoids are free radical scavengers and water-soluble antioxidants which have strong anticancer activity. Further, the flavonoids in the intestine lower the risk of heart disease. Flavonoids have anti-inflammatory activities and prevent oxidative stress to the cells in herbal medicine. Flavonoids have existed for over one billion years and also possess anti-ischemic, antiapoptotic, antihypertensive, and anti-thrombic activity. Flavonoid serves as an antioxidant by scavenging singlet oxygen, superoxide anion, and lipid-peroxy radicals [69]. Flavonoids are one of the most diverse and widespread groups of natural compounds and are probably the most important natural phenolics with radical scavenging properties [48]. Antioxidant activity directly depends upon the number and positions of hydroxyl groups, other substituents, and glycosylation of flavonoid molecules [10].

3.5.3 NUTRIENTS AND LIPOSOLUBLE PIGMENT COMPOSITION

Javanmardietal., (2003) [39] reported that the antioxidant activity of plant extracts may also come from other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins. Many

carotenoids are potent lipophilic antioxidants and are believed to prevent the onset of chronic diseases[13] and play essential roles in different plant processes.

Tocopherols were present in ample amounts in *Rauwolfia micrantha*. Nutritionally, the tocopherols (α-, β-, γ- and δ-tocopherol) and tocotrienols are important because they exhibit vitamin E activity and are traditionally regarded as potent physiological antioxidants [7].

Vitamin C has been proposed as a biological antioxidant, a chain-breaking scavenger for peroxy radicals, and also a synergist with vitamin E (one of the best quenchers for singlet oxygen), thus regenerating its activity [72].

Ascorbic acid was the most abundant vitamin found in *R. micrantha*. Carotenoids (carotene) and chlorophylls were also found in the studied plant species. Lycopene was found in very low amounts when compared to other nutrient compositions[72].

Singlet oxygen is powerfully quenched by carotenoids, especially β-carotene. The levels of vitamins C and E, and β-carotene found in the medicinal plants make them suitable antioxidant sources that might be used commercially to retard rancidity in fatty materials in food manufacturing, reduce the effects of aging, and help prevent oxidative stress-related diseases such as cancer, stroke, heart disease, several neurodegenerative diseases and cataractogenesis [19, 29]. Also, carotenoids have an important antioxidant role in cellular protection against lipid peroxidation, thus preventing the risk of illnesses such as cancer, cardiovascular disease, and macular degeneration. It also reduces the risk of cataracts and strengthens the immune system [53].

3.5.4 SUPEROXIDE ANION SCAVENGING ACTIVITY

Superoxide is biologically important because it can be degraded to form stronger oxidative species such as singlet oxygen and hydroxyl radicals [43]. The superoxide anion can be generated by illuminating a solution composed of riboflavin. *R. micrantha* also exhibits this property among other Rauwolfia species [72]. It was reported that the superoxide anion scavenging activity could be either due to the action of a free hydroxyl group of phenolic compounds or by a flavonoid molecule with polyhydroxylated substitution on ring A or B and a free 3-hydroxyl substitution [63].

3.5.5 REDUCING POWER

Reducing power is associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through dehydrogenation [21]. In this assay, the Fe^{3+}/ferricyanide complex is reduced to the ferrous form by antioxidants and can be monitored by measuring the intensity of navy-blue color at 700 nm [28]. *R. micrantha* exhibited a stronger reducing power compared to other Rauwolfiaspecies whileAscorbic acid has the strongest reducing power among all the chemical constituents in the species. At 100 g/mL, the reducing power of *R. micrantha* and ascorbic acid was analyzed to be 1.44 and 1.92, respectively by RagupathiG. (2012) [72]. The reducing power of Rauwolfia is most probably due to the presence of di- and mono-hydroxyl substitutions in the aromatic ring, which possesses potent hydrogen-donating abilities as described by Shimada et al. (1992) [61].

3.5.6 METAL CHELATING ACTIVITY

Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [22] and is effective as a secondary antioxidant because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [27].

Rauwolfia micrantha showed a concentration-dependent metal chelating activity which reached about 72 ± 3.59% when the concentration was 25µg/mL in comparison to other *Rauwolfia* species[72]. Furthermore, the chemical and biological diversity of aromatic and medicinal plants depend on such factors as growth habitat, climatic conditions, vegetation phase, and genetic modifications [48].

3.6 ALKALOIDS

Apocynaceae is a well-known alkaloid- rich family. Alkaloids and their derivatives are used extensively as medicinal agents because of their antispasmodic, analgesic, and bactericidal effects. The roots of *Rauwolfia micrantha* are a rich source of antihypertensive and tranquilizer alkaloids, such as ajmalicine, ajmaline, reserpiline, sarpagine, reserpine, and serpentine [4].

3.6.1 NEOSARPAGINE

In 1959, Rao and co-workers were isolated from *Rauwolfia micrantha,* a minor alkaloid, which they called neosarpagine, a vinyl isomer of sarpagine. There is no further mention of the occurrence of neosarpagine in the literature. As all of the physical data presented for neosarpagine are very similar to those of sarpagine, it seems to the writers, in agreement with Taylor, that neosarpagine probably was a more or less impure sample of sarpagine.

3.6.2 AJMALINE

Ajmaline (known by trade names Aritmina, Ritmos, and Gilurytmal) is an antiarrhythmic agent (class 1a) [40] named after the Unani medical practitioner, Hakim Ajmal Khan, from South Asia [54]. It is used to lower ST elevations in patients suffering from Brugada syndrome [34]. It is found in Catharanthus roseus [3] and most species of *the Rauwolfia* genus but the concentration is too low when extracted from the root part and also its bioavailability is insignificant, leading to the development ofa semisynthetic propyl derivative called prajmaline [33].

Ajmaline acts by altering the shape and threshold of the cardiac action potential. It blocks sodium channels, and a short half-life makes it a very suitable drug for acute intravenous treatments. Ajmaline acts by depression of intraventricular conduction. It leads to the prolongation of the P-Q interval, Q-T interval, QRS complex, and widening of the R wave. The drug is very famous in some countries for the treatment of Wolff-Parkinson-White syndrome [11]. It has also been used as a drug to challenge the heart's conduction system in cases of syncope and bundle branch block. It is used to diagnose Brugada disorder (genetic cardiac syndrome) and helps to distinguish the subtypes between patients with the syndrome [20]. Ajmaline group acts as a general depressant to the heart but has been reported to stimulate intestinal movements and respiration.

In a case study of 100 patients with essential hypertension, blood pressure was lowered significantly and the elevated serum cadmium levels (64.3%) in these individuals were also reported to be reduced on the administration of Ajmaline[64].

3.6.3 AJMALICINE

Ajmalicine has applications in the treatment of circulatory diseases by providing relief to normal cerebral blood flow, preventing strokes, lowering blood pressure, and affecting the function of smooth muscles [65]. Approximately, 3500 Kg of Ajmalicine is isolated from either Rauwolfia or Catharanthus spp. for the treatment of circulatory diseases [15].

3.6.4 SERPENTINE

Serpentine is a terpene indole alkaloid that is known as an inhibitor of topoisomerase (Type II) and has antipsychotic properties. The serpentine group causes paralysis of respiration, depression of the nerves, and stimulation of the heart [62]. The compound exhibit anticancerous, antineoplastic, and anti- hypertensive activitiesaccording to Van der Heijden, Jacobs, Snoeijer, Hallard, & Verpoorte, 2004.

3.6.5 RESERPILINE

It is a 10, 11 - dimethoxy stereo isomer of Ajmalicine. It has an amorphous base and is derived from *R. serpentina* following the extraction of ajmalicine. Isoreserpiline and reserpiline coexist. It is usefulfor the treatment of psychosis. It improves the affinity of dopaminergic-B2, muscarinic and serotonergic receptors toward binding reactions [65].

3.6.6 SARPAGINE

Sarpagine is a major class of structurally and biosynthetically related monoterpenoid indole alkaloids which possess useful biological activity ranging from anti-hypertensive to anticancer activity.

3.6.7 RESERPINE

Reserpine is the most prominent indole-alkaloid derived from *Rauwolfia* and has been regarded as an antidepressant, antihypertensive, antitumor, antimalarial, and tranquilizing agent. It was reported as a potent cytotoxic compound against drug- resistant tumor cells and it was concluded that it could be used in cancer therapy by Abdelfatah & Efferth (2015). It plays an important role in dysentery, ecbolic, fever, diarrhea, cornea opacity, and epilepsy. It is used to treat high blood pressure, arrhythmia, breast cancer, cardiovascular diseases, hypertension, mental disorders, and leukemia.

Physiologically, it binds with protein receptors, and vesicular monoamine transporters (VMATS) in membranes of specialized secretory vesicles of presynaptic neurons leading to pre-synaptic closure of calcium-gated ion channels and preventing intracellular neurotransmitters from binding to VMAT proteins therefore, stopping secretory vesicles from up taking neurotransmitters [9].

Reserpine has a higher affinity for VMAT2 and binds irreversibly to their receptors [58]. It is also used to treat symptoms of dyskinesia in patients suffering from Huntington's disease [46] by depleting catecholamine stores and is unable to trigger the release of catecholamine [55]. The reserpine-induced catecholamine release increases sensitivity to the effects of direct-acting sympathomimetics [23]. The tranquilizing effects of *R. serpentina* can result from the depletion of amino stores in the CNSby depressant action on the cerebral centers and it relaxes the general nervous system [59, 6]. Theeffect of reserpine in the vasomotor center causes diminished reflex in vasomotor responses leading to generalized vasodilatation, with a lowering of blood pressure [59].

3.7 CONCERNS ON CONSERVATION AND FUTURE PROSPECTS

In nature, *R. micrantha* is never over-exploited. Their populations are rapidly declining due to various ecological and biological reasons. Several factors, such as endemicity, restricted distribution, small populations in accessible areas, several anthropogenic pressures on forestlands, pollination limitation, poor seed viability, habitat destruction, fragmentation of populations, narrow environmental niche, poor seed set, lack of specific pollinating agent and their reproductive syndrome have contributed to the decline of *R. micrantha* in the wild[44]. All these causal factors either alone or in combination with others are responsible for its limited distribution in the wild. It is thus designated as a critically endangered plant. The plant has also been reported as rare and endemic to the forests of the Southern Western Ghats [56].

Conventional propagation is beset with problems of low germination, poor seed viability, and scanty and delayed rooting of seedlings and vegetative cuttings. Considering the urgent need for conservation, an in vitro propagation system can be achieved through shoot tip and nodal explant cultures [66]. There is an urgent need to apply non-conventional propagation methods for the conservation and future commercial delivery of *Rauwolfia* spp. [71]. The protocol for the plant regeneration from cultured root segments of *R. micrantha* through direct somatic embryogenesis and successful field establishment of the acclimatized plants has also been standardized by Sudha and Seeni in 2005.

Plant tissue culture is a well-known biotechnological tool for the rapid propagation of medicinal plants for commercialization [41], conservation [49], and cryopreservation [17,18]. Somatic embryogenesis and organogenesis have been the common pathways for the clonal propagation of superior medicinal plant species [26]. In vitro regeneration in *Rauwolfia* species had been implemented mainly by organogenesis [57,66,52] and rarely via somatic embryogenesis in *Rauwolfia vomitoria*[70] and *Rauwolfia caffra*[71]. In general, embryogenesis has been achieved using various plant tissues like cotyledon, hypocotyls, leaves, or internodes, but seldom root [73]. Organogenesis and somatic embryogenesis in root segment culture is particularly advantageous for genetic transformation studies using Agrobacterium rhizogenes. This also provides an ideal experimental system for investigating cell differentiation and understanding the regulatory mechanism of totipotency in plant cells. Moreover, plants regenerated from the root segment are suggested to be genetically uniform [60]. Chaturvedi (1981) [14] emphasized that root culture could be used for germplasm preservation of several plant species, including *R. serpentina*.

4. CONCLUSIONS

The present study has been done to review the taxonomic, ecological, and phytochemical characteristics and relevance of *Rauwolfia micrantha.* The study highlighted the ecological and biological threats to this plant species as it is declining in the wild due to overexploitation. Because of over-exploitation, the need for conservation, and low propagation rates, there are several reports of in vitro propagation and manipulation of this important medicinal plant. Several attempts have been made to conserve this threatened and endangered plant either by in-situ, ex-situ, or in-vitro conservation strategies. Exploration, characterization, evaluation, collection, domestication/cultivation, and ex-situ conservation in gene banks eventually support their sustained use by supplying quality planting material and certified raw drugs. Modern biotechnological tools, including in vitro and cryopreservation techniques, Gene banks, DNA fingerprinting, and bioreactor-mediated bioproduction of phytochemicals, are most relevant in this context. Micropropagation and eco-restoration support in-situ conservation activities, facilitating population enhancement in species where natural propagation is hindered because of destructive harvesting or reproductive barriers.

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6. REFERENCES

1. AhmedullahM., Nayar M.P,"Endemic Plants of the Indian Region – 1", Botanical Survey of India, Calcutta, India, 1986, 118, pp.
2. Aiyambo D, "Traditional uses of selected members of the Apocynaceae family in Namibia", Ministry of agriculture, water, and forestry, Windhock,2010.
3. AkhtarS., Ahmad J., Ahmad A, "In Stress Signalling in Plants: Genomics and Proteomics Perspective", Vol. 2, Spain: Springer, 2017, p. 37-67.
4. Anonymous, "The wealth of India: raw materials", Vol. V, New Delhi: Council of Scientific and Industrial Research, 1969, 376–391.
5. Anonymous, 1969b,"Colour identification chart", Flora of British fungi, Her Majesty's stationary office, Royal Botanic Garden, Edinburgh, London.
6. Avol M., Vogel P.J.,"Treatment of delirium tremens with reserpine (serpasil); a preliminary report",The Journal of the American Medical Association, 1955, 159:1516-20.
7. Azzi A,"Molecular mechanism of [alpha]-tocopherol action", Free Radical Biology and Medicine, 2007, 43:16–21.
8. Bhat P., Hegde G., Hegde G.R., "Ethnomedicinal practices in different communities of Uttara Kannada district of Karnataka for treatment of wounds", Journal of Ethnopharmacology, 2012, 143(2):501–514.
9. BlackmanJ.G., Campion D.S., Fastier F.N., "Mechanism of action of reserpine in producing gastric hemorrhage and erosion in the mouse", British Journal of Pharmacology and Chemotherapy, 1959, 14:112-6.

10. BouzizM., GrayerR.J., SimmondsM.S.J., DamakM., Sayad S.,"Identification and antioxidant potential of flavonoids and low molecular weight phenols in Olive cultivar Chemlali growing in Tunisia", Journal of Agricultural and Food Chemistry, 2005, 53: 236– 241.
11. BrugadaJ., BrugadaP., BrugadaR., "The ajmaline challenge in Brugada syndrome: A useful tool or misleading information?", European Heart Journal, 2003, 24:1085-6.
12. Cai Y.Z., Luo Q., Sun M., Corke H., "Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer", Life Sciences, 2004, 74: 2157–2184.
13. Carrapeiro M.M., Donato J., GoncalvesR.C., Saron M.L.G., Godoy H.T., Castro I.A., "Effect of lycopene on biomarkers of oxidative stress in rats supplemented with [omega]-3 polyunsaturated fatty acids", Food Research International, 2007, 40, 939–946.
14. Chaturvedi H. C., Sharma M., Prasad R. N., "Micropropagation and germplasm preservation of some economic medicinal plants. "Rao, A. N., ed. Tissue culture of economically important plants", Proceedings of COSTED-ANBS Symposium, Singapore, 1981, 301–302.
15. Chaudhary R., Singh B., Chhillar A.K, "Ethnomedicinal importances of Rauwolfia serpentine L. Benth. Ex Kurz", Prev Treat Dis, 2011, 5:144-50.
16. Cruden R.W.,"Pollen-ovule ratios- A conservation indicator of breeding system in flowering plants", Evolution, 1977, 31: 32-46.
17. Decruse S.W., Seeni S., Pushpangadan P.,"Cryopreservation of alginate-coated shoot tips of in vitro-grown Holostemma annulare (Roxb.) K. Schum, an endangered medicinal plant: influence of preculture and DMSO treatment on survival and regeneration", 1999.
18. Decruse S. W., SeeniS., Pushpangadan P., "Effect of cryopreservation on seed germination of selected rare medicinal plants of peninsular India", Seed Science and Technology, 1999, 27: 501–505.
19. Dewick P.M.,"Medicinal Natural Products", John Wiley and Sons, Lda, 2008.
20. DobbelsB., De Cleen D., Ector J., "Ventricular arrhythmia during ajmaline challenge for the Brugada syndrome",EP Europace, 2016, 18:1501-6.
21. Duan X., Wu G., Jiang Y., "Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention", Molecules (12), 2007, 759–771.
22. Duh P.D., Tu Y.Y., Yen G.C., 1999. "Anti-oxidant activity of water extract of Harng Jyur (Chrysanthemum morifoliun Ramat)", Lebensmittel - Wissenschaft &Technologie, 1999, 32, 269–277.
23. Eger E.I. 2nd, Hamilton W.K., "The effect of reserpine on the action of various vasopressors", Anesthesiology, 1959, 20:641-5.
24. Gamble, Flora of Presidency of Madras, Vol. II, Adland & Sons, 1921,808, pp.
25. Gamble, Flora of Presidency of Madras, Vol. II, Adlord and Son Ltd, WC, London, 1953.
26. Gary A. T., Brent H. M.,"Establishing a micropropagation system for American ginseng (Panax quinquefolium)", HortScience, 1986, 21:232–236.
27. Gordon M.H., "The mechanism of the antioxidant action in vitro. In Hudson, B.J.F. (Ed.), Food Antioxidants", Elsevier, London/New York, 1990, pp. 1–18.
28. Gupta S., Prakash J., 2009. "Studies on Indian green leafy vegetables for their antioxidant activity", Plant Foods for Human Nutrition- Springer, 2009, 64, 39–45.
29. Halliwell B.,"Antioxidants in human health and disease", Annual Review of Nutrition, 1996, 16, 33–50.

30. Heinrich B.,"Energetics of pollination", Annual Review of Ecology and Systematics, 1975, 6: 139-170.
31. Heinrich B.,"Resource heterogeneity and patterns in foraging bumblebees", Oecologia, 1979, 40: 235-245.
32. Heinrich B., Raven P.,"Energetics and pollination ecology", Science, 1972, 176: 597-602.
33. HinseC.,StöckigtJ., "The structure of the ring-opened N beta-propyl-ajmaline (Neo-Gilurytmal) at physiological pH is obviously responsible for its better absorption and bioavailability when compared with ajmaline (Gilurytmal)", Pharmazie, 2000, 55:531-2.
34. HongK., Brugada J., OlivaA., Berruezo-Sanchez A., Potenza D., Pollevick G.D., et al., "Value of electrocardiographic parameters and ajmaline test in the diagnosis of Brugada syndrome caused by SCN5A mutations", Circulation, 2004, 110:3023-7.
35. Huang B., Ban X., He J.S., Tong J., Tian J., Wang Y.W., "Heteroprotrective and antioxidant activity of ethanolic extracts of edible lotus (Nelumbo nucifera Gaern.) leaves", Food Chemistry, 2010a, 120, 873–878.
36. HuangB., Ban X., He J.S., Zeng H., ZhangP., WangY.W., "Heteroprotective and antioxidant effects of the methanolic extract from Halenia elliptica", Journal of Ethnopharmacology, 2010b, 131, 276–281.
37. HussainS.R., GillardJ., Gillard P., "Hydroxyl radical scavenging activity of flavonoids", Phytochemistry, 1987, 26(9), 2489-2491.
38. Jain A., Soni M., Deb L., Jain A., Rout S.P., Gupta V.B., Krishna K.L., "Antioxidant and heteroprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. Leaves", Journal of Ethnopharmacology, 2008, 115, 61–66.
39. Javanmardi J., Stushnoff C., Locke E., Vivanco J.M.,"Antioxidant activity and total phenolic content of Iranian Ocimum accessions", Food Chemistry, 2003, 83, 547–550.
40. KieseckerC., Zitron E., Lück S., Bloehs R., Scholz E.P., Kathöfer S., et al., "Class Ia anti-arrhythmic drug ajmaline blocks HERG potassium channels: Mode of action", Naunyn - Schmiedebergs Archives of Pharmacology- Springer, 2004, 370:423-35.
41. Kitto S. L., "Commercial micropropagation", HortScience, 1997, 32:1012–1014.
42. Koch I., Kinoshita L.S., Bittrich R., "Taxonomy novelties in Rauwolfia (Apocynaceae, Rauvolfiodeae) from Brazil, Novon", 2007, 17: 462-417.
43. Korycka Dahl M., RichardsonM., "Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids", Journal of Dairy Science, 1978, 61, 400–407.
44. Kulloli S.K., Sreekala A.K., "Pollination Ecology of Rauwolfia micrantha Hook.F. (Apocynaceae): A Critically Endangered Medicinal Plant from the Southern Western Ghats", Phytomorphology, 2009, 59 (3 & 4): 96–101.
45. Kulloli S.K., SreekalaA.K., Rama SubbuR., "Floral biology of Rauwolfia micrantha Hook.F.: a rare and endemic Medicinal plant of Western Ghats, India", Scientific transactions in environment and technovation., 2008, 1(4): 174-177.
46. Lehman E., Haber J., Lesser S.R., "The use of reserpine in autistic children", The Journal of Nervous and Mental Disease, 1957, 125:351-6.

47. Mahmood T., Meer F., Munir F., Nazar N.,Naveed I., "Genetic diversity of selected Apocynaceae species based on chloroplast gene rps11", Journal of Medicinal Plant Research, 2011, 5(17), 4382–4387.
48. Miliauskas G., Venskutonis P.R., Van Beek T.A., "Screening of radical scavenging activity of some medicinal and aromatic plant extracts", Food Chemistry, 2004, 85, 231–237.
49. Nadeem M., Palni L. M. S., Purohit A. N., Pandey H., Nandi S. K.,"Propagation and conservation of Podophyllum hexandrum Royle: an important medicinal herb", Biological Conservation, 2000, 92:121–129.
50. Nayar M.P., Biodiversity challenges in Kerala and science of conservation biology. In: Pushpangadan, P. & Nair, K.S.S. (eds.), Biodiversity of Tropical Forest - The Kerala Scenario, The State Committee on Science, Technology, and Environment (STEC), Kerala, India, 1997,40, pp.
51. Pan Y., Wang K., Huang S., WangH., Mu X., He C., "Antioxidant activity of microwave-assisted extract of longan (Dimocarpus longan Lour.) peel", Food Chemistry, 2008, 106, 1264–1270.
52. Patil V. M., Jayanthi M.,1997. "Micropropagation of two species of Rauwolfia (Apocynaceae)", Current Science, 1997, 72:961–965.
53. Rao A.V., Rao L.G., "Carotenoids in human health", Pharmacological Research, 2007, 55, 207-216.
54. Ravishankar B., Shukla V.J., "Indian systems of medicine: A brief profile", African Journal of Traditional, Complementary and Alternative Medicines, 2007, 4: 319-37.
55. Sagi S., Avula B., Wang Y., Khan I.,"Analysis of Alkaloids from the roots of Rauwolfia serpentina using UHPLC- MS", Planta Medica, 2016, 82: PA17.
56. Sahu B.N., "Taxonomy of Indian species: Rauwolfia serpentina, vol. II", New Delhi: Today and Tomorrow's Publishers, 1979, 70- 71.S.
57. Sarker K.P., Islam A., Islam R., HoqueA.,Joarder O,"In vitro propagation of Rauwolfia serpentina through tissue culture", Planta Medica, 1996, 62: 358 – 359.
58. Schuldiner S., Lucy, Edwards R.H.,"Reserpine binding to a vesicular amine transporter expressed in Chinese hamster ovary fibroblasts", Journal of BiologicalChemistry, 1993, 268:29-34.
59. ShamonS.D., Perez M.I., "Blood pressure lowering efficacy of reserpine for primary hypertension", Cochrane Database of Systematic Reviews, 2009, 4:CD007655.
60. Sharma K., Yeung E.C., Thorpe T.A., "Histology of shoot bud ontogeny from seedling root segments of Brassica napus L", Annals of Botany, 1993, 71:461–466.
61. Shimada K., Fujikawa K., Yahara K., Nakamura T.,"Antioxidative properties of xanthan on the auto-oxidation of soybean oil in cyclodextrin emulsion", Journal of Agricultural Food Chemistry, 1992, 40, 945–948.
62. Siddiqui S., Siddiqui R., "Chemical examination of the roots of Rauwolfia serpentina Benth.", Journal of the Indian Chemical Society, 1931, 8:667-80.
63. Sidduraju P., Mohan P.S., Becker K.,"Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers, and fruit pulp", Food Chemistry, 2002, 79, 61–69.
64. Smith W. M., "Treatment of mild hypertension: Results of a ten-year intervention trial", Circulation Research, 1977, 40 (5) Suppl 1: I98-105.
65. Srivastava S.K., Agrawal A.K., Singh S.C., KhannaV.K., Singh J., Nath C., et al.,"Antipsychotic Agents and Standardized Antipsychotic Fractions from Rauwolfia tetraphylla and Process of their Isolation", US Patent No. 20120184576 A1, 2010.

66. SudhaG.G., SeeniS., "In Vitro Propagation of Rauwolfia micrantha, a Rare Medicinal Plant", Plant Cell, Tissue and Organ Culture, 1996, 44 (3): 243–248.
67. Sudha C. G., Obul Reddy B., Ravishankar G. A., Seeni S., "Production of Ajmalicine and Ajmaline in Hairy Root Cultures of Rauwolfia micrantha Hook F., a Rare and Endemic Medicinal Plant", Biotechnology Letters, 2003, 25 (8): 631–636.
68. Sudha C.G., Seeni, S., "Spontaneous Somatic Embryogenesis on in Vitro Root Segment Cultures of Rauwolfia micrantha Hook, F.- A Rare Medicinal Plant", In Vitro Cellular & Developmental Biology – Plant, 2006, 42 (2): 119-123.
69. TorelJ., Cillard J., Cillard P., "Antioxidant activity of flavonoids and reactivity with peroxy radicals", Phytochemistry, 1986, 25, 383-385.
70. Tremouillaux-Guiller J., Chenieux J.C., "Somatic embryogenesis from leaf protoplasts of Rauwolfia vomitoria shoots cultures", Plant Cell Reports, 1991, 10:102–105.
71. Upadhyay N., Makoveychuk A. Yu., Nikolaeva L. A., Batygina T. B., "Organogenesis and somatic embryogenesis in leaf callus culture of Rauwolfia caffra Sond", Journal of Plant Physiology,1992, 140:218–222.
72. Vadakkemuriyil D. N., Rajaram P., Ragupathi G., "Studies on methanolic extract of Rauwolfia species from Southern Western Ghats of India- In vitro antioxidant properties, Characterisation of nutrients and phytochemicals", Industrial Crops and Products, 2012, 39: 17-25.
73. Vuorela P., Caldentey K.M.O., Lipponen J., Hiltunen R., "Spontaneous somatic embryogenesis and plant regeneration from root cultures of Peucedanum palustre", Plant Cell Reports, 1993, 12:453–456.
74. Wafai B.A., Siddique M.A.A., BeighS.Y., Dar N.A., "Reproductive Biology of Patris (Aconitum hetorophyllum Wall. Ex Royle; Rannuculaceae) an important rare and threatened Himalayan Drug plant", HUF-IUFRO InternationalWorkshop on Prospects of Medicinal Plants, Y.S. Parmar, University of Agricultural Sciences, Solan, H.P., India, 1996.
75. Youngken, Heber W., "Malabar Rauwolfia, Rauwolfia Micrantha Hook.F.", Journal of the American Pharmaceutical Association, 1954, 43 (3): 141–143.