



## Review

# *Decalepis salicifolia* (Bedd. ex Hook. f.) Venter: A steno-endemic and critically endangered medicinal and aromatic plant from Western Ghats, India

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*Decalepis salicifolia* (Bedd. ex Hook. f.) Venter is a potential medicinal and highly aromatic plant species confined to the southernmost part of the Western Ghats of India. The plant is well known for its traditional uses among the various tribal communities of south India. The tubers of the plant possess characteristic vanillin-like aroma due to the presence of the compound 2-hydroxy-4-methoxybenzaldehyde. The tubers are used to substitute *Hemidesmus indicus* in various herbal formulations. The plants in the wild are continuously uprooted for their roots, leading to the irreversible destruction of the whole plant. The resulting tremendous loss of populations in the wild led to the species being declared as critically endangered by IUCN. Our group is working on the various aspects of this species including population status, distribution mapping, prospection, and conservation management. In the present review, we have brought out the available information till date on *D. salicifolia*, including taxonomy, ethno-medicinal uses, phytochemistry, pharmacology, population status, and conservation efforts along with research gap and lacunae to provide direction for further research into this less explored medicinal and aromatic plant.

**Keywords.** 2-Hydroxy-4-methoxybenzaldehyde; conservation; endangered; *Uleria salicifolia*; vanillin; Western Ghats

## 1. Introduction

*Decalepis salicifolia* (Bedd. ex Hook. f.) Venter (syn. *Uleria salicifolia* Bedd. ex Hook. f.), known as willow-leaved swallow-root, belongs to the family Apocynaceae (earlier in Asclepiadaceae). The genus *Decalepis* Wight

and Arn. is represented with *D. salicifolia* (Bedd. ex Hook. f.) Venter, *D. arayalpathra* (J. Joseph and V. Chandras.) Venter (syn. *Janakia arayalpathra* J. Joseph and V. Chandras.), *D. hamiltonii* Wight and Arn., *D. khasiana* (Kurz) Ionta ex Kambale, and *D. nervosa* (Decne. ex Moq.) Venter (Kambale *et al.* 2016). *D. salicifolia* is endemic to

the south Western Ghats of India, and has been declared as critically endangered (CR) by International Union for Conservation of Nature (IUCN) because of population loss by overexploitation, poor seed establishment, habitat degradation, and forest fires (Ved *et al.* 2015). Habitat loss is due to the construction of roads, agricultural expansion, and human settlements. The present article encompasses a complete review of all known aspects of *D. salicifolia* covering population distribution, medicinal properties, scientific authentication of traditional uses, propagation, and conservation status of the plant. The study thus helps in providing a single platform to conservation biologists, environmentalists, biotechnologists, and policy makers for concerned interventions.

## 2. Nomenclature

The species was described in 1876 by Beddome and was placed in the genus *Uteria* as an unnamed species. The name was validly published later in 1883 by Hooker in *Flora of British India* as *Uteria salicifolia* Bedd. ex Hook. f. and was considered as a monotypic genus. Later in 2001, Venter merged the genus into *Decalepis* and named it as *D. salicifolia*, based on the floral characters being similar to the characteristic features of the genus, viz. ovate-like corona lobes, an obconical style-head with flat apex containing translator hollows, and pollen in pollinia (Venter and Verhoveen 2001). Further, Kambale *et al.* (2016) validated *D. salicifolia* with typifications along with the other species of the genus *Decalepis*. *Decalepis salicifolia* is reported as an accepted name with the synonym *Uteria salicifolia* (<http://www.theplantlist.org/tpl1.1/record/kew-2758604>). Although the species relationships in *Decalepis* group are not well resolved, *D. arayalpathra* and *D. salicifolia* are strongly supported as sister species in both morphological and molecular analysis based on their ITS sequence data (Ionta 2009).

## 3. Etymology and vernacular names

The name *Decalepis* is derived from the Greek word *deca* meaning ten, *lepis* meaning scale, with reference to coronal scales and staminal filaments (Patil 2017). The species epithet '*salicifolia*' comes from its leaves resembling those of *Salix* (a genus of Salicaceae commonly known as the willow). The name *Uteria* originates from the word 'Uttallari' meaning 'jungle beauty' in Tamil (Quattrocchi 2012). The Malasar, Kadar, and Muthuvan tribal communities of Kerala in south India

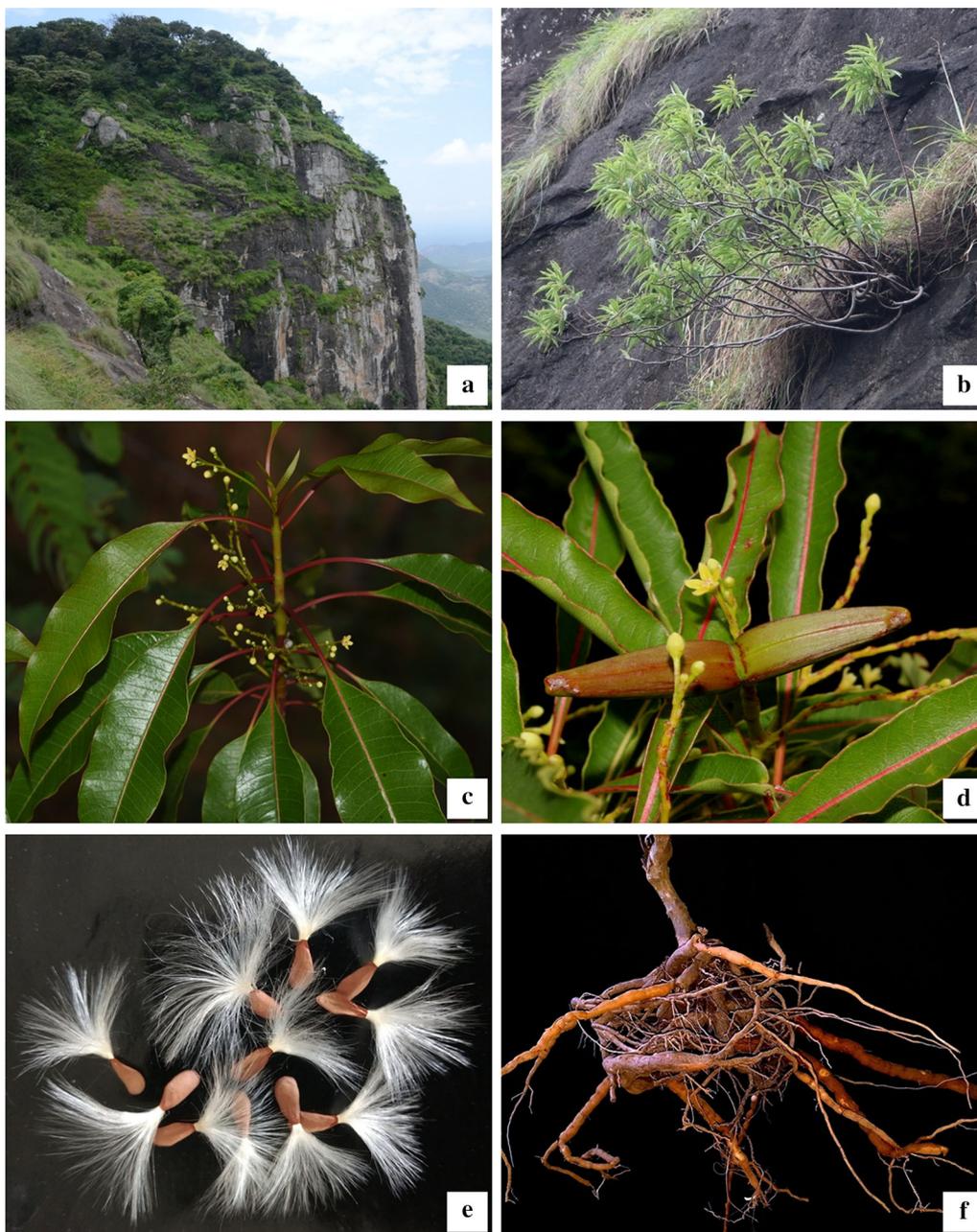
refer the plant as 'Mahali kizhangu': 'Mahali' refers to 'Mahalakshmi', the Hindu Goddess of wealth, and 'kizhangu' means root tubers (Radhakrishnan *et al.* 1998). The plant is also locally known as 'Marappala' in Kerala (Menon 2003). The Kadar tribe of Anamalai in Tamil Nadu knows the plant by the name 'Utleer' (Ravikumar and Ved 2000). The plant is also known as 'Shedi Mahali' and 'Para Mahali', 'shedi' meaning shrub and 'para' meaning rock in Tamil.

## 4. Taxonomic description

The plant is taxonomically characterized as perennial, deciduous, erect shrub (figure 1b), glabrous, branchlets with prominent and persistent leaf scars. The whole plant secretes abundant milky, sticky, and thick latex. Leaves are opposite, whorled or scattered, usually clustered at the ends of the branchlets. Leaves are narrowly lanceolate in shape, generally 7–10 cm, sometimes up to 23 cm long. Leaf stalk is up to 3 cm long, leaf base wedge-shaped, apex acuminate, margin wavy or sometimes toothed with minute rounded teeth, hairless, lateral nerves about 35 pairs, closely parallel. Flowers are in dichotomously branched cymes, bisexual; about 5 mm across, greenish yellow, shortly stalked, and peduncles up to 8 cm long. Calyx is very small, 5-lobed, each with two-minute glands. Corolla rotate, 5-lobed, slightly overlapping and twisted to the right, corona scales 5, small, rounded. Stamens inserted on the base of the corolla-tube, filaments short, anthers ovate, connivent and adherent to the style-apex, pollen-masses in pairs in each cell, granular. The ovary is of two carpels, many ovules, style apex convex. Fruit follicles are 2 in number, smooth, lanceolate, divaricate, about 5 cm long, and 1 cm wide (figure 1d). Each follicle contains several seeds which are broadly ovate, thin, flat, with a cluster of white hair at the tip (figure 1e). Roots are in clusters of numerous tubers, fleshy, moniliform in shape (figure 1f) (Ravikumar and Ved 2000; Gamble 1921).

### 4.1 Pollinium structure

Pollen tetrads are grouped to form a pollinium of 138–218 × 80–131 μm in size. Distal exine wall is smooth and consists of a compact stratum (tectum), 0.15–0.22 μm thick, subtended by granular stratum, 0.26–0.37 μm thick, with larger granules towards inside. The proximal wall possess pores bearing the same exine stratification as the distal wall with tectum 0.14–0.16 μm thick and granular stratum 0.27–0.33 μm thick. Tectum



**Figure 1.** (a) Habitat of *D. salicifolia* in rocky slopes and (b) in shrubby habit. (c) Flowering twig, (d) fruit, (e) mature seeds with hair or coma, and (f) tuberous roots.

and granular stium of adjoining tetrads may be fused when pores of adjacent tetrads are opposite to each other. Inner walls consist of the tectum and granular stratum and contain wall bridges (Rudolf *et al.* 1998).

#### 4.2 Morphological distinction from other species of the genus

*D. salicifolia* greatly resembles *D. aryalpathra* as both are erect shrubs, while the others are scandent to

climbing twiners. Both the species possess tuberous moniliform roots whereas those of *D. hamiltonii* are cylindrical and non-tuberous in case of *D. khasiana*. The stems of *D. salicifolia* are succulent, wrinkled when dry, grayish wax covered when old, while rest of the species are woody, reddish when young and purplish when old. The floral sepals of *D. salicifolia* are more broadly obovate and thinner, than those of other species, lacking an obvious midrib. The most evident distinguishing feature from *D. aryalpathra* is the shape of leaves. *D. salicifolia* possesses short-petioled,

linear lanceolate long leaves, in contrast to the long petioled, elliptic to ovate leaves of *D. arayalpathra*. Another important distinction is the minutely erose leaf margins and weakly brochidodromous venation of *D. arayalpathra* in contrast to strongly brochidodromous venation and an intramarginal vein in *D. salicifolia* (Ionta 2009; Sharma and Shahzad 2014). Longer inflorescence and absence of disc between stamens is also one of the major differences from its closely allied species *D. arayalpathra* (Radhakrishnan *et al.* 1998).

## 5. Habit and habitat

*D. salicifolia* is a profusely branching shrub and grows up to 2.5 m height. The plant flourishes in deciduous and evergreen forests at an altitude range of 600 to 1750 m above mean sea level. It prefers to grow horizontally on 90-degree steep rocky slopes, open big rock boulders, and rocky crevices of windswept vertical cliffs. The root stock is tuberous, cylindrical, moniliform, up to 50 cm long, approximately 4 cm thick, and pleasantly aromatic (Ravikumar *et al.* 2001). The plants shed their leaves from December to February, and new leaf initiation occurs in mid-February. The flowering starts in April extending up to October and fruit set begin from June and prolong up to November. The plant is generally found associated with *Chrysopogon* sps. *Ensete superbum* (Roxb.) Cheesman, *Linum mysurense* B. Heyne ex Benth. *Phoenix loureiroi* Kunth, *Strobilanthus* sps. (Ravikumar *et al.* 2001). In the natural habitat, *D. salicifolia* is often misidentified with young plants of *Alstonia venenata* R. Br. due to similarities in their leaf morphology.

## 6. Distribution

The species is endemic to the south Western Ghats of Tamil Nadu and Kerala, India (Ved *et al.* 2015). It was first located by Beddome in 1876 from Poonachi, Tunakkadavu Valley of Anamalai Hills, Coimbatore district, Tamil Nadu. The species was later located in another range of Coimbatore hills by Fischer in 1921. In 1999, Matthew recorded the species in Pambar Shola, Kodaikkanal hills, Tamil Nadu (Ravikumar *et al.* 2001). The species was relocated in its type locality of Annamalai hills, in the Top Slip reserve forest after about 115 years by Ravikumar *et al.* (2001). It was also located along Thekkadi Valley in Kurudi malai Reserve forests (Sankar *et al.* 2007). In Kerala,

the species was recorded near Irumbupalam in Thiruvananthapuram, Nelliampathi Hills of Palakkad district and Marayoor forests of Idukki district (Ravikumar *et al.* 2001). Recently, our group has located new populations in the mountain ranges of Chinnar in Kerala, Attakati, Kadampari, Udu-malappettai, and Valparai in Tamil Nadu (Gokul *et al.* 2020).

## 7. Ethnomedicinal uses

Knowledge on the traditional uses of the plant comes from Malasar, and Kadar tribes inhabiting in the Nelliampathy forests as well as from the Muthuvan tribes of Parambikulam forests (Anamalai hills), Palakkad district, Kerala (Radhakrishnan *et al.* 1998). The plant is considered divine by these tribes as it is found to grow only in inaccessible rocky crevices of mountains. The rhizome is usually tied by the tribal communities on their huts with the belief that it will invite good fortune. Tribal communities use the powdered dried tuber in hot water which is administered orally, twice a day for fifteen days, for treating asthma. The Malasar tribal community uses the decoction of the rhizomes to treat debility due to tuberculosis. The decoction is consumed four times a day continuously for 40 days. The chopped and water boiled rhizomes of *D. salicifolia* are also used for making pickles, which are considered to be beneficial in intestinal ailments and bleeding due to ulcers. The crushed rhizomes are also consumed orally by the Muthuvan tribe for treating skin diseases (Radhakrishnan *et al.* 1998). The plant is used in intestinal discomforts and is used as a galactagogue with *Asparagus racemosus* Willd. *Foeniculum vulgare* Mill. and *Ficus glomerata* Roxb. (Quattrocchi 2012). The fresh tubers are crushed and boiled with tea powder (*Camellia sinensis*) and consumed as a refreshing drink.

## 8. Phytochemistry

Preliminary phytochemical screening of the ethanolic extract of *D. salicifolia* tubers through high-performance thin-layer chromatography (HPTLC) revealed the presence of steroids, alkaloids, terpenoids, saponins, and tannins (Rao *et al.* 2004). The root tuber volatile oil also contains  $\alpha$ -pinene, cineol, limonene, rutin, among others (George 2009). The chemical basis of the vanillin-like aroma present in the tuberous roots was provided by George *et al.* (2011). The volatile oil

was characterized by infrared spectroscopy, mass spectroscopy,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR. The pure compound isolated from volatile oil fraction was identified as 2-hydroxy-4-methoxybenzaldehyde ( $\text{C}_8\text{H}_8\text{O}_3$ ) (figure 2a), an isomer of the popular and widely used flavoring compound vanillin (4-hydroxy-3-methoxybenzaldehyde) (figure 2b).

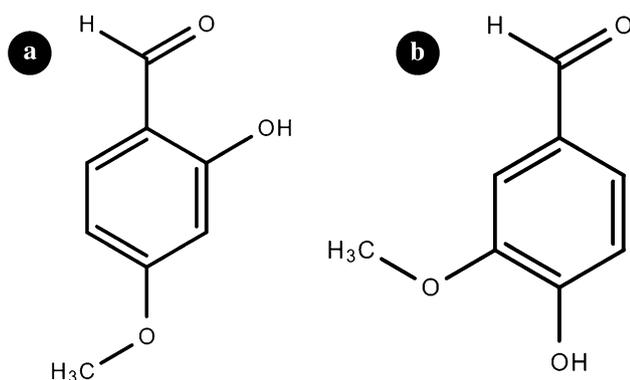
### 8.1 2-Hydroxy-4-methoxybenzaldehyde: An isomer of vanillin

2-Hydroxy-4-methoxybenzaldehyde (2H4MB) is also known by other chemical names, viz. 4-methoxysalicylaldehyde, 2-hydroxy-p-anisaldehyde, and 2-formyl-5-methoxyphenol (PubChem). 2H4MB (mol. weight 152.149 g/mol) is a fragrant phenolic compound, known to be found in the roots of the plants belonging to the family Apocynaceae (Kundu and Mitra, 2016). Very few plants are reported that contain 2H4MB, viz. *Hemidesmus indicus* (L.) R. Br. Ex Schult (Nagarajan *et al.* 2001a), *D. hamiltonii* (Nagarajan *et al.* 2001b), *D. arayalpathra* (Verma *et al.* 2014), *Periploca sepium* Bunge (Wang *et al.* 2010), *Mondia whitei* (Hook. f.) Skeels of Apocynaceae family, *Rhus vulgaris* Meikle, and *Sclerocarya caffra* Sond. of Anacardiaceae (Kundu and Mitra 2016). Recent studies from our group reported that the volatile oil from the tubers of *D. salicifolia* contain upto 95% of 2H4MB (Gokul *et al.* 2020). The compound is reported to act as an antimicrobial agent (Phadke *et al.* 1994), bioinsecticide (George *et al.* 1999), contact toxicity against fruit flies (*Drosophila melanogaster*) and maize weevil (*Sitophilus zeamais*) (Chu *et al.* 2012), taste modifier (Mukonyi and Ndiege 2001), larvicide (Mahanga *et al.* 2005), antifungal agent (Mohana *et al.* 2009), anti-

*Helicobacter pylori* activity (Srikanta *et al.* 2011), anti-bio-deteriorative, antiaflatoxicogenic (Mohana and Raveesha 2010), and anti-oxidant (Murthy *et al.* 2006; Wang *et al.* 2010). Schiff bases of the compound exhibit anti-aflatoxicogenic activity (*Aspergillus flavus*) and anti-microbial activity against *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* (Harohally *et al.* 2017).

The tyrosinase inhibitory potential of 2H4MB has been proven against mushroom tyrosinase (Kubo and Kinoshita 1999; Kundu and Mitra 2014). Further investigations can reveal the underlying action against enzymatic melanogenesis thus establish potent use in anti-browning of foods and treating skin hyperpigmentation. The compound is reported to inhibit acetyl cholinesterase (AChE) which is an important property for the treatment of Alzheimer's disease and other neurological disorders (Kundu and Mitra 2013). It is also found to inhibit neuro-degeneration in *Caenorhabditis elegans* signifying its potential as a nutraceutical with neuro-protective properties (Kamireddy *et al.* 2018). The anti-fumonisin activity of the compound renders it potential for use against fusarial growth and mycotoxin contamination on food and feed stuffs (Thippeswamy *et al.* 2015). The compound is also reported to exhibit anticancer properties in terms of generation of reactive oxygen species (ROS), cell cycle arrest, and induction of apoptosis in breast cancer cells (Thangam *et al.* 2019). Pottie *et al.* (2011) reported that urolithin M7 can be synthesized from 2H4MB by inverse electron demand Diels-Alder (IEDDA) reaction.

Due to its vanillin-like aroma, 2H4MB is used as a flavoring agent in bakery products and as a coolant and blood purifier in local beverages (Wang *et al.* 2010; Verma *et al.* 2014). The compound is also approved by the U.S. Food and Drug Administration (FDA) in the category of generally recognized as safe (GRAS) flavoring substances. The Flavor and Extract Manufacturers Association (FEMA) recommends the standard average maximum use level in the range of 25–1000 ppm in beverages, baked goods, breakfast cereals, candies, milk products, seasonings, and flavors among others (Smith *et al.* 2009). *Decalepis* roots are illegally smuggled in the international market due to the demand for its extract. The market for *Decalepis* extract is mostly driven by *D. hamiltonii*, however the use of *D. arayalpathra* and *D. salicifolia* is known and the harvested roots in fresh or dry form are not distinguishable. The market demand is predicted to increase steadily during 2018–2028 and the prominent global market players are Alpspure Lifesciences, Herbo Nutra,



**Figure 2.** (a) Chemical structure of 2-hydroxy-4-methoxybenzaldehyde. (b) Chemical structure of 4-hydroxy-3-methoxybenzaldehyde (Vanillin).

John Aromas, Nature and Nurture Healthcare (PMR 2020).

## 8.2 Production of 2H4MB by plant tissue culture techniques

Owing to the diverse properties of the compound and the scarcity of the plants in the wild, attempts have been made to produce the compound in *in vitro* cultures. 2H4MB is produced in suspension cultures obtained from root tuber callus of *D. salicifolia* at a concentration of 14.8 µg/g with chitosan elicitation at 200 µM for a period of 72 hrs (Ahmad *et al.* 2019). It is also produced in cell suspension cultures of *D. hamiltonii* with maximum accumulation of 0.44±0.001 mg/100 g dry weight on ferulic acid feeding (Matam *et al.* 2017). Production of 2H4MB by harnessing the potential of hairy root culture has been successfully achieved in *D. arayalpathra* (Sudha *et al.* 2013), and *D. hamiltonii* (Samydurai *et al.* 2013). In addition, *in vivo* enhancement of 2H4MB is also obtained in *D. hamiltonii* by using triacontanol (Giridhar *et al.* 2005) and by the application of arbuscular mycorrhizal fungi (Matam and Parvatam 2017).

## 9. Pharmacological studies

The wide utilization of the plants in traditional medicine and the reverence given to the plant by the tribal communities has necessitated the pharmacological investigations. Very few studies are reported on the most well applied ethnopharmaceutical uses of the *D. salicifolia* rhizome. These studies are mainly based on crude solvent extracts, thereby suggesting the need for further detailed critical assays to identify the active chemical constituents.

### 9.1 Antiulcer activity

The traditional claims of the antiulcer activity of the rhizome were scientifically authenticated by investigating its phytochemical and pharmacological activity. The effect of ethanolic extracts of *D. salicifolia* rhizome on physical (cold stress, pylorus ligation) and chemical factor (ethanol, aspirin, acetic acid, cysteamine) induced acute and chronic gastric ulcer in rat models are reported. The extract exhibited dose-dependent ulcer protective effect, and also significantly reduces ulcer incidence and severity of duodenal ulcer. The extract offers protection against ethanol-induced

depletion of gastric wall mucus by enhancing gastric wall mucus secretion thus demonstrating cytoprotective nature of *D. salicifolia* rhizome (Rao *et al.* 2004).

### 9.2 Hepatoprotective activity

Remya *et al.* (2010) investigated the anti-hepatotoxic activity of ethanolic extract of *D. salicifolia* rhizome. Significant hepatoprotective activity was recorded against paracetamol or carbon tetrachloride-induced hepatic damage. Serum marker enzymes like glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (SAKP), and bilirubin (SB) restored to normal in rat models pretreated with *D. salicifolia* rhizome extract before hepato-toxin administration.

### 9.3 Anticancer activity

George *et al.* (2016) evaluated the antiproliferative potential of the solvent extracts of *D. salicifolia* rhizome against human chronic myelogenous leukemia cell line (K562). The methanol extract showed higher inhibition of cell proliferation (IC<sub>50</sub>: 0.18 mg/ml) as compared to petroleum ether and chloroform extract (IC<sub>50</sub> 0.25 mg/ml).

## 10. Patented product

A herbal formulation ointment for the treatment of cuts, burns, and wounds comprising an aqueous alcoholic extract of *D. salicifolia* rhizome along with *Jatropha curcas* L. *Clerodendrum infortunatum* L. and *Centella asiatica* (L.) Urb. has been patented (Patent no.: US7344737 B2) (Pushpangadan *et al.* 2008). The formulation has antimicrobial and antifungal activity, assisting in the regeneration of dead cells and connective tissues, angiogenesis, and re-epithelialization.

## 11. Conservation status

*D. salicifolia* is listed as critically endangered (at the global level) in the wild under the criteria B2ab (ii, iii) (Ved *et al.* 2015). The fleshy tubers being the medicinally potential part, the plants are uprooted destructively leading to the irreversible damage of the plant population. Moreover, anthropogenic forces are also one of the main reasons for the habitat loss. A population in the adjacent area of Annamalai hills in Kerala

has vanished as a consequence of the construction of two dams (Ravikumar *et al.* 2001). Construction of roads, agricultural expansion, and human settlements are also the reasons for habitat loss causing a sub-population in Palani hills likely to be extinct (Ved *et al.* 2015). The plant has very narrow restricted distribution, and the current population is depleting with the incessant decline of extent, area, and quality of habitat (Ved *et al.* 2015). To conserve the existing populations of *D. salicifolia* in their natural habitats, medicinal plants conservation area (MPCA) has been established in Anapady, Palakkad district of Kerala (Begum *et al.* 2016). The MPCA with *D. salicifolia* as a flagship species is estimated to consist of more than 500 clumps of the plant (Sankar *et al.* 2007). It is expected that the MPCA program will promote *in situ* conservation of the species (Ved *et al.* 2015).

### 11.1 Anthropogenic pressure and trade of tubers

The field explorations made by our group reveals that the extant populations of *D. salicifolia* species are fragmented due to the construction of ghat roads from Pollachi (Tamil Nadu) to Chalakudy, Marayoor, and Munnar (Kerala) and to 53 private estates of plantation crops (tea and coffee), hydroelectric dams (Kadamparai, Alayar, Solayar, and Tirumoorhi in Tamil Nadu as well as in Tunakadavu and Parambikulam in Kerala), and power stations (Attakati). The high biotic pressure due to land use changes by tea and coffee estates, spices, and lemongrass cultivation by tribals has adversely impacted the *D. salicifolia* population.

The root of sariva or sarsaparilla plant, *Hemidesmus indicus*, is the component of the ‘Sariva bheda’ Ayurvedic formulations which include Amritamalaka taila, Drakshadi churna, Shatavari rasayana, and Yeshtimadhu taila (Nayar *et al.* 1978). The major chemical constituents, pharmacognostic, and therapeutic properties of *H. indicus* and the species of *Decalepis* viz. *D. arayalpathra*, *D. hamiltonii*, and *D. salicifolia* are significantly similar (Nayar *et al.* 1978; Nagarajan *et al.* 2001a; Nagarajan *et al.* 2001b; Verma *et al.* 2014). Hence, the vanilla-fragranced roots of *D. salicifolia* along with those of *D. arayalpathra* and *D. hamiltonii* are used as a substitute to meet the herbal raw drug market demand for *H. indicus* (Nayar *et al.* 1978; Mishra *et al.* 2017; Nandy *et al.* 2020). *H. indicus* grows firmly rooted, bearing thin roots, whereas the *Decalepis* species have large fleshy roots loosely attached to the soil, making it less laborious for collection as compared to the former. The tribals and

people inhabiting estates are engaged in the collection of *D. salicifolia* tubers for the traders of Pollachi and Marayoor raw drug markets (mandis). The fresh tubers are sold at the price of ₹40–60 per kg whereas dried tubers are sold at ₹140–180 per kg. All the accessible areas, where populations of *D. salicifolia* were once abundant are completely harvested for the tuberous roots. The plants that still exist are those that are dangling along the inaccessible rocky slopes and crevices, which were evident in our surveys. The wild populations are under tremendous pressure due to the indiscriminate uprooting of plants for the tubers.

As a means of accurate identification and to discriminate the species of *Decalepis* and *H. indicus*, a DNA barcode is developed by our group. The barcode comprises of rbcL+ matK + ITS which will aid in detection of adulteration or substitution as well as prevent illegal trade of this endangered species (Mishra *et al.* 2017).

### 11.2 Population genetic diversity

The survival of the population both in short and long term is determined by the genetic variation within and among populations, which necessitates the study of plant genetic diversity in *D. salicifolia* to develop suitable conservation programme. Investigation of population genetic diversity using various molecular markers is of great importance for the genetic resource characterization. The genetic diversity in the wild populations of *D. salicifolia* was assessed recently by our group using inter-simple sequence repeats (ISSR) markers showing low genetic diversity, low gene flow, and high genetic differentiation (Gokul *et al.* 2020). Majority of the genetic variance was found to reside among the populations. Further, the diversity parameters indicated greater risk of genetic drift due to the added affects of small population size, fragmentation, and geographic isolation. The study revealed the significance of immediate interventions for the conservation of the species. The percentage content of 2H4MB was also found to vary across the populations. Based on the study, genetically diverse populations were prioritized for conservation.

## 12. Propagation endeavors

*D. salicifolia* is mainly propagated through seeds in the wild. The seeds are flattened, elliptic to obovate with a coma of white to yellowish hair at the micropylar end. The coma aids in anemochory or seed dispersal by wind, however, the narrow habitat preference could be



**Figure 3.** Germinated seed with persistent seed coat hindering the efficient development of root and shoot.

impeding its survivability. The plant propagation is constrained by poor fruit set and seed germination (Gangaprasad *et al.* 2003). However, detailed seed biology studies are lacking in *D. salicifolia*. Studies in *D. hamiltonii* indicate that the seeds are sensitive to drying, with low seed viability (Sharma and Shahzad 2014), and the seed coat is thick inhibiting efficient water imbibitions and thus germination (Anandalakshmi and Prakash 2010). Our preliminary seed germination experiments in *D. salicifolia* also reveal similar results. We have also observed that the thick seed coat hinders the emergence of root and shoot for several days after germination (figure 3) which could be the reason for poor seed establishment in the wild. The rapidly depleting wild population of *D. salicifolia* has necessitated interventions in plant propagation.

### 12.1 *Ex vitro* propagation

Saradha and Samyudurai (2015) studied the rooting ability of leafy stem cuttings treated with different concentrations of rooting hormones and rooting media (coir pith, vermiculite, and forest soil). The highest number of roots with 92% rooting was obtained in stem cuttings treated with 2000 ppm indole-3-butyric acid (IBA) planted in coir pith rooting medium. The rooted cuttings showed 96% survivability.

### 12.2 *In vitro* propagation

*D. salicifolia* is reported to have poor *in-vitro* regeneration potential (Gangaprasad *et al.* 2003). A few efforts for propagation through tissue culture have been successful

despite the impediment. Gangaprasad *et al.* (2003) reported axillary bud proliferation in nodal explants cultured in Murashige and Skoog (MS) medium fortified with 0.5 mg/L 6-benzylaminopurine (BAP) and 0.01 mg/L naphthaleneacetic acid (NAA). *In vitro* shoots were rooted in quarter strength MS medium containing 1.0 mg/L IBA, hardened in the mixture of sand, soil, farmyard manure (1:1:1) exhibiting 63% field survival rate. Later, George *et al.* (2007) obtained multiple shoots ( $4.2 \pm 0.9$ ) using plumule as explants in MS medium supplemented with 1.0 mg/L BAP and 0.1 mg/L kinetin (KIN), which were rooted in quarter strength MS medium. Field establishment of rooted plants was successful post hardening for two weeks in a mixture of sand and soil (1:1). Ahmad *et al.* (2018) reported multiple shoot ( $9.9 \pm 0.01$ ) induction using nodal explants in MS media comprising of 5  $\mu$ M BAP, 0.5  $\mu$ M NAA, and 30  $\mu$ M adenine sulfate, with successful rooting in 2.5  $\mu$ M IBA and 90% survival rate in soilrite. Recently, our group for the first time established micropropagation using shoot tips explants from *in vitro* germinated seedling (Rodrigues *et al.* 2020). Shoot tip proliferation was achieved in MS medium supplemented with 2.2  $\mu$ M BAP and 5.7  $\mu$ M IAA. Multiple shoot ( $3.5 \pm 0.06$ ) induction was obtained with nodal explants in MS medium supplemented with 11.1  $\mu$ M BAP. Rooting was achieved in quarter strength MS medium with reduced nitrate concentration and 8% sucrose followed by 92.8% survival during hardening. Gas chromatography coupled mass spectroscopy analysis of essential oil from micropropagated plants showed 2H4MB production in quantities similar to the field grown plants. Further, sodium alginate encapsulated shoot tip and nodal explants of *D. salicifolia* can be stored up to 12 weeks at 4°C without the loss of regeneration ability demonstrating the possibility of short term storage (Rodrigues *et al.* 2020).

A recent record for the first time reports *de novo* regeneration of shoots from leaf callus with  $7.0 \pm 0.46$  shoots per explant in MS media supplemented with 1.0 mg/L BAP and 0.1 mg/L IAA. The shoots were rooted in 0.6 mg/L IBA and showed 97.5% survival during acclimatization (Saradha and Samyudurai 2018).

## 13. Future prospects

In view of the rapidly depleting population in the wild, propagation studies by conventional and biotechnological methods have been undertaken by few researchers to aid conservation of the species. Our group has established the genetic diversity profile as well as a micropropagation protocol providing an *in vitro* gene bank of *D. salicifolia*. In an effort for *ex*

*situ* conservation, a gene bank of about 500 live plants has been established at CSIR–CIMAP Research Centre, Bengaluru. Efficient conservation of endangered species can be achieved by a combined approach of *in situ*, *ex situ* and *in vitro* conservation. Introduction into cultivation or domestication of such species largely alleviates the risk of extinction. The *ex situ* gene bank established in our campus proves the possibility of growing the plants in plain fields without the niche specific to the species. Further, our efforts are continued towards development of agro-technology and also the production of 2H4MB in *in vitro* cultures.

Compared to *D. hamiltonii* and *D. arayalpathra*, the species *D. salicifolia* is scientifically less explored. *D. salicifolia* is well known among the local tribal communities for its medicinal properties and is recently being explored for its pharmaceutical use. A few of its traditionally claimed activities are validated scientifically. Therapeutic potential of plants of the same genus as well as those with similar chemical constituents are reported plentiful. It paves ways for possible explorations in *D. salicifolia* which still holds immense prospects for bioprospection. So far no scientific and methodological investigation is reported in the literature regarding the identification and chemistry of the active principles or the mechanism of action. Pharmacological activities that are reported are based on crude solvent extracts. Thus, extensive exploration is required for the identification of the active principles that renders the claimed healing properties of the plants and thereby to elucidate the mode of action. This plant is a promising subject for further investigations especially for the three preliminarily proven anti-ulcer, hepato-protective, and anti-cancer properties, being the most prevalent ailments of the modern society.

The major compound present in its tubers, 2-hydroxy-4-methoxybenzaldehyde is an isomer of industrially important flavor and aroma compound vanillin. The compound has the characteristic flavor and fragrance of vanillin that is naturally obtained from the pods of *Vanilla planifolia* Jacks. ex Andrews. Vanillin is the most popularly used flavoring compound worldwide. The demand for vanillin far exceeds its supply, and thus the cheaply available synthetic vanillin is extensively used despite the associated ill effects. There exists an incessant quest for natural sources of the high-value vanillin and compounds with similar properties. The compound 2H4MB offers a promising natural alternative to synthetic vanillin.

Considering the critically endangered status of the species, it is irrational to use the plant as such whereas biotechnological interventions can be used for manipulation and effective utilization of the natural

compound along with due conservation efforts. Adventitious and hairy root cultures, along with cell suspension cultures can serve as an alternative source for the compound (2H4MB) to reduce the pressure of overexploitation. Despite being an important group of plant secondary metabolites, enzymatic route of benzoic acid and its derivatives is not well defined (Qualley *et al.* 2012). Investigations aiming regulation of these compounds at the upstream of their amino acid precursors are extensive. Plant hydroxybenzoates are known to originate from phenylalanine; however, they are also reported to originate directly from shikimate via chorismic acid (Chakraborty *et al.* 2008). The compound 2H4MB is reported to be formed via the phenylpropanoid pathway (Chakraborty *et al.* 2008), modulated further upstream by the shikimate pathway (Kundu *et al.* 2012). Although these claims are based on elicitor stimulated accumulation of the compound 2H4MB in *H. indicus*, biotechnological manipulations in *D. salicifolia* can be executed with backing from such studies. A recent study in root cultures of *D. hamiltonii*, pointed out 4-CL (4-coumarate CoA ligase) enzyme as the branch point of phenylpropanoid pathway in channeling ferulic acid into intermediates that further convert into 2H4MB (Kamireddy *et al.* 2017). Elicitation and subsequent examinations aid the trace of preceding enzymes involved in the response which further provide important insights into the biosynthetic pathways of 2H4MB. On the other hand, inhibition studies and precursor feeding are also important in elucidating the metabolic pathway. These approaches coupled with cell or organ culture systems offers a promising means for both secondary metabolite production and elucidation of the biosynthetic pathway in *D. salicifolia*.

Life history and ecology of the plant, reproductive and seed biology, along with the threats and biological constraints in propagation and survivability requires to be essentially investigated. Species management can be possible by habitat protection and restricted harvest. *Ex situ* conservation could serve as a safe refuge in case of unexpected and untimely species depletion in the natural habitat. The plant being endemic and critically endangered needs to be effectively conserved by application of both conventional and biotechnological methods. Tissue culture technique may be ideal for the mass multiplication of *D. salicifolia* which has the added advantage of minimal source plant requirement for the establishment of *in vitro* cultures. However, when resources are limiting, multiplication using stem cuttings may be cost effective. The availability of sufficient source

plant material may still be a limitation with this approach. The species is not under cultivation and the only known distribution of the species is in the wild, which is rapidly depleting. Systematic efforts towards mass multiplication and introduction into cultivation are needed to reduce exploitation of wild populations as well as to meet the market requirements.

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