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Review

Genus *Decalepis*: Biology, Importance and Biotechnological Interventions

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Abstract: The steno-endemic species from the genus *Decalepis* are facing a high level of threat due to destructive wild harvesting. The genus claimed its paramount importance to mankind due to its unique tuberous root characteristics and exhibits a wide range of biological and medicinal properties, and is used in pharmaceutical and food industries. Plants of this genus are endemic to limited areas of peninsular India, such as the Eastern and Western Ghats, and according to the International Union for Conservation of Nature (IUCN), species from the genus *Decalepis* are considered globally critically endangered. The genus comprises of five species namely *Decalepis hamiltonii* Wight & Arn., *Decalepis arayalpathra* (J. Joseph & V. Chandras.) Venter, *Decalepis salicifolia* (Bedd. ex Hook. f.) Venter, *Decalepis nervosa* (Wight & Arn.) Venter, and *D. khasiana* (Kurz) Ionta ex Kambale. All the species of the genus *Decalepis* are being used by the tribal people and also in traditional Indian and Chinese medicine. International trade for this plant is also increasing, resulting in overharvesting. The traditional method of propagation, viz., seed germination and vegetative, are limited and jeopardizes the species population, whereas plant tissue culture provides the opportunity for extensive production of the plant in vitro without sacrificing their natural habitats. This review is aimed to systematize the up-to-date facts related to the Genus *Decalepis* with the exploration of their geographic distribution, chemical profile, pharmacology, biological activities, micropropagation, somatic embryogenesis, synthetic seed, and genetic transformation.

Keywords: medicinal plants; natural products; bioactive metabolites; tissue propagation

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1. Introduction

India, being a megadiverse country, possesses abundant biodiversity and it is home to more than 17,000 angiosperms [1,2], with four major hotspots (Himalaya, Western Ghats, Indo-Burma, and Sundaland) out of thirty-four hotspots [3]. The country represents 11% of the world's flora, and approximately 28% of the total Indian flora are endemic, with 33% of the angiosperms occurring in India [4]. Increasing human population and their intervention, deforestation, and agricultural land expansion in the forest area put these ecological regions at risk of extinction, requiring special attention [5,6]. The species from the genus *Decalepis* are confined to some forest area of Tamil Nadu, Kerala, and Andhra Pradesh in India [7]. Plants are found as patches on the exposed rocky slopes that faces high wind velocities and temperatures with moderate rainfall throughout the year [8]. Taxonomic distribution at family level recorded *D. hamiltonii* (Wight & Arn.), *D. arayalpathra* (J. Joseph and V. Chandras.) Venter, *D. salicifolia* (Bedd. Ex Hook.f.) Venter, *D. nervosa* (Wight & Arn.) Venter., and *D. khasiana* (kurz) Ionta ex Kambale as the accepted names of the plants under the family Apocynaceae ([9,10], http://www.theplantlist.org, accessed on

12 Feburary 2022). The species of *Decalepis* was assessed as Critically Endangered Globally and declared as a red listed medicinal plant by International Union of Conservation of Nature (IUCN) ([11,12], http://www.iucnredlist.org/details/50126587/0, accessed on 12 Feburary 2022). The National Biodiversity Authority of India (NBA) declared the species of high conservation concern [13]. The plants are in regularly use in traditional Indian and Chinese medicine to cure the disarray related to the lungs, digestive, and circulatory system, stomach ache, cancer-like affliction, peptic ulcer, and as rejuvenating tonic by the native Kani tribes of Southern Western Ghats [14,15].

The most important plant part is the root tuber, a rich center for different alkaloids and phenolics [16]. The tuberous root contains 2-hydroxy-4-methoxybenzaldehyde (2H4MB) (97%), which is a valuable source for the production of commercially important flavor compound vanillin [16–20]. The presence of aroma (2H4MB) in the tuberous root of these species places them as a potential substitute for *Hemidesmus indicus* on the international market [20,21]. The plant *H. indicus* is widely used in Ayurvedic and Unani systems of medicine because of its high medicinal value, viz., its anti-cancerous, anti-ulcer, anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-venom, and anti-microbial properties [22]. The tuberous root of *H. indicus* is very short and thin and rigidly attached to the soil, and in order to unearth the root tuber, extensive labor is needed, while *D. hamiltonii* tuberous roots are large and fleshy, loosely attached to the soil, and require less labor. Such availability of tuberous roots of the species from the genus *Decalepis* leads to the high demand for the plant in the global market as an alternative to *H. indicus* and thousands of tons are traded each year from uncultivated wild sources [12,23]. Overexploitation and haphazard collection put the genus into thrust for their survival. Thus, a convenient outline for the species from the genus *Decalepis* is needed for their sustained use, whose trade is regulated by Convention of International Trade of Endangered Species (CITES). Thus, the current communication detailing the facts about the species from the genus *Decalepis*, their geographical distribution, current status, chemical constituents, and pharmacological applications and biotechnological intervention for the advancement of *Decalepis* and their conservation through micropropagation.

2. Genus *Decalepis*: A Brief Outline

In a recent study, the recorded population of the genus *Decalepis* showed restricted distribution and occurrence due to destructive harvesting. Moreover, Niche specificity, low genetic diversity, restricted gene flow, damage caused by fruit wasp, and genetic differentiation has led to the reduction of the species in number [7]. Of the five species of *Decalepis*, *D. hamiltonii*, *D. nervosa*, and *D. khasiana* are climbing plants, while *D. salicifolia* and *D. arayalpathra* are erect shrubs found in poor soils on rocky hill slopes of evergreen forests [24,25]. The species is endemic to the Eastern and Western Ghats of peninsular India, except *D. khasiana*, which occupies the easternmost part of India [26].

D. hamiltonii, commonly known as Maredukommulu or Nannarikommulu in Telugu, Magalikizhangu in Tamil, and swallow root in English, is the most widely distributed species. The geographical distribution of this species is on the rocky slopes and crevices of dry and moist deciduous forests of Karnataka, Andhra Pradesh, and in Tamil Nadu [27]. However, despite its wide distribution, its population has gradually declined due to the destructive harvesting of the tuber [28]. *D. arayalpathra*, locally known as *Jankia arayalpathra*, is a perennial lactiferous shrub. Its distribution is limited to the Thiruvananthapuram district of Kerala and the Tirunelveli and Kanyakumari district of Tamil Nadu [28]. The forests of the Anamalai Hills (Kerala and Tamil Nadu), Nelliampathy (Kerala), and Marayoor (Kerala) have been documented to inhabit *D. salicifolia*. *D. nervosa*, is restricted to the Nilgiris mountains and the Kothgiri and Wellington region of Tamil Nadu [26].

3. Chemical Constituents and Metabolic Profiling

The demands of human beings are increasing continuously for phytomedicine due to its accuracy and consistency, leading to an emphasis on plants and their role in phy-

tomedicine bioactivity. A large number of plant species are slowly evolving due to many reactions, including environmental impact on habitat loss, destructive collection, etc. The overall effects endanger plant populations, and most of them are poorly explored and less studied. Therefore, such plant species need greater attention for their industrial use and effective conservation with the screening of the newer secondary metabolites. Various chromatographic techniques had provided us with a broad notion to explore the biochemical composition of plant and plant parts.

Considerable effort has been made to isolate and determine the metabolites available in the species from the genus *Decalepis*. The earliest intervention in this regard was available by Murti and Seshadri [29], who claimed the presence of Inositol, saponins, tannins, phenolics, alkaloids, flavonoids, etc. in root tuber of *D. hamiltonii*. Nagarajan et al. [30] have reported a few specific metabolites, i.e., 2-hydroxy-4-methoxy benzaldehyde (2H4MB), from volatile oils (96%), which is found to be an important constituent in *D. hamiltonii*. Further work has been done to investigate the metabolic content by George et al. [31] and confirmed the presence of 2H4MB in the tuberous root of *D. hamiltonii* as major metabolites. They have also found some other minor metabolites of pharmacological value, namely 2-hydoxybenzaldehyde (31.01%), 4-O-methylresorcylaldehyde (9.12%), benzyl alcohol (3.16%), etc. The GC-MS analysis of tuberous root extract of *D. hamiltonii* by Nagarajan et al. (2001) observed the presence of benzaldehyde (0.01%), salicylaldehyde (0.01%), methyl salicylate (0.04%), 2-phenylethyl alcohol (0.08%), ethyl salicylate (0.03%), and vanillin (0.45%) in minor amounts that are biologically significant. Nagarajan and Rao [32] developed a chromatographic technique in which they used gas as a mobile phase and found varying combination of the aromatic compound in *D. hamiltonii*. Other compounds, such as 4-hydroxyisopthalic acid, 14-aminiotetradecanoic acid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1,2-cylohexane diol, 2-(hydroxymethyl)-3-ethoxybenzaldehyde, bornerol, and ellagic acid have also been reported in *D. hamiltonii* root [33,34]. Moreover, α-atlantone (2.06% v/w of oil) and β-pinene (2.01%) have been isolated by Thangadurai et al. [35]. Table 1 represents the GCMS analysis of methanolic root extract of *D. hamiltonii*. This table shows the presence of bioactive constituents in methanolic root extract of *D. hamiltonii* with their retention time and area percentage.

Some studies on the determination and evaluation of phytocompounds in *D. arayalpathra* also revealed the presence of a compound similar to that reported in *D. hamiltonii*. Ahmad et al. [16] performed the screening of metabolites for methanolic extract of tuberous root of *D. arayalpathra* and its successive fractions that revealed the presence of the alkaloid, phenolics, tannins, carbohydrate, flavonoids, terpenoids, etc. Further GC-MS analysis enabled the identification of furaneol, 2H4MB, 4H3MB (4-hydroxy-3-methoxybenzaldehyde), nerol acetate, diazoacetone, benzoic acid, neryl acetate ester, hexacontane, mome inositol, inositol, tetrapentacontane, diethyl phthalate, 4-ethoxy-ethyl, hexacontane, methyl commate D, diazoacetone, squalene as a major metabolites with chromelin, anhydride, alcohol, myristate, Benzoic acid, Lup-20(29)-en-3-yl acetate, 2,6-Octadiene-1-ol, phthalic acid, and steraloids as minor metabolites [16]. The high-performance liquid chromatography (HPLC) studies of methanolic extract of *D. arayalpathra* confirmed the presence of 2H4MB as the major constituents with chlorogenic acid having potential biological activity [16–18]. George et al. [36] also noted the presence of 2H4MB in the root of *D. salicifolia* as major metabolites in their early work. A recent report by Das et al. [37] witnessed the occurrence of phenolic and flavonoids compounds on *D. nervosa* phytochemical screening.

Table 1. GC-MS analysis of methanolic root extract of *D. hamiltonii*.

Peak	R. Time	Area %	Name
1	8.13	1.86	2,3-Dihydro-3, 5-dihydroxy-6-methyl-4h-pyra
2	11.05	41.72	2-hydroxy-4-methoxy- Benzaldehyde
3	12.77	1.13	Vanillin
4	12.98	1.85	1,3,5,7-Tetravinyl-1,3,5,7-tetrabutoxycyclotetrasiloxane
5	13.53	0.62	Benzoic acid, 4-ethoxy-, ethyl ester
6	15.17	7.12	Ethyl. alpha.-d-glucopyranoside
7	16.29	4.87	Methyl. beta.-d-galactopyranoside
8	16.93	0.28	4,4-Dimethyl-2-adamantanol
9	17.89	0.94	Hexadecanoic acid, methyl ester
10	18.26	3.37	Pentadecanoic acid
11	18.78	6.88	Glucose
12	19.60	1.06	9-Octadecenoic acid (z)-, Methyl ester
13	19.97	3.55	cis-Vaccenic acid
14	20.16	0.52	Octadecanoic acid
15	23.81	0.22	Isosteviol methyl ester
16	24.48	0.21	Isosteviol methyl ester
17	24.62	1.58	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
18	25.33	0.80	1,2-Benzenedicarboxylic acid
19	27.50	0.20	Octadecanoic acid, 2,3-dihydroxypropyl ester
20	28.11	0.22	9-Octadecenamide
21	28.51	0.54	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23
22	34.70	1.37	Stigmast-5-en-3-ol, (3.beta.)-
23	36.30	0.81	Methyl Commate B
24	37.34	3.09	4,4,6A,6B,8A,11,11,14B-octamethyl-1,4,4A,5,6,6A,6
25	38.33	15.20	Methyl commate D

4. Pharmacology: Traditional and Modern Application

Species from the genus *Decalepis* have long been used by tribal people for the treatment of diseases related to the digestive system, lungs, circulatory system, and inflammation [38,39]. The species *D. hamiltonii* is widely used by the tribes of the Western Ghats; the roots of these plants are available in the local tribal markets in dried form and are used in the preparation of Ayurveda [39]. These dried roots are used to cure indigestion and also chewed according to their need [40]. It has been found to be effective and can be used to cure dysentery, cough, bronchitis, leucorrhea, uterine hemorrhage, skin disease, fever, indigestion, vomiting, chronic rheumatism, anemia, and blood diseases [40–42]. The people of Nannari, the forest regions of the Eastern and the Western Ghats, eat it in the form of pickles, which is used to treat bleeding ulcer, tuberculosis, asthma, and skin disease and also as a refreshing drink [41,42]. The root extract had also a great significance in the terms of industrial scale. It is widely used as food preservatives due to its anti-oxidant potential. In addition, it is in great demand for the nutraceuticals and pharmaceuticals industry [43].

5. Biological Activities

Several studies have been conducted regarding the pharmacological value of the species from the genus *Decalepis*. Compared to the other species, *D. hamiltonii* and *D. arayalpathra* have been extensively evaluated for their medicinal activities, as discussed below.

5.1. Anti-Oxidant Activity

The preservative role of anti-oxidants against the damage caused by the free radicals is well studied. Free radicals once formed are highly reactive and can start a chain reaction. Their main danger comes when they react with cellular components, viz., DNA or cell membrane. The reaction with these effects adversely affects the cell, viz., leading to DNA mutation and oxidation of protein or lipids. These adverse effects lead to the succession of different disease. Every living organism has a defense system to counter these adverse effects of free radicals; however, the natural anti-oxidant defense mechanism

is insufficient to prevent the damage and therefore calls for dietary intake of food rich in anti-oxidant compounds [44]. This demand attracts researchers to explore the anti-oxidant potential of the medicinal plant. An anti-oxidant prevents the damage of macromolecules and cells by interfering with free radicals. Srivastava et al. [33] reported six pure compounds from the methanolic extract of *D. hamiltonii* tuber, including a potent anti-oxidant compound bis-2,3,4,6-galloyl-α/β D glucopyranoside (aptly named decalepin). Furthermore, Murthy et al. [45] established that the potential anti-oxidant activity is due to the presence of 2H4MB in *D. hamiltonii*; they used different parts of the aromatic root, i.e., whole tuber, peel, tuber, medullary region, etc. Peel extract was found to have the highest anti-oxidant activity. Anti-typhoid activity of various root extracts has been examined by Kumuda et al. [46] in different solvent, i.e., petroleum and chloroform both show anti-bacterial activity. Ahmad et al. [16] performed anti-oxidant activity for *D. arayalpathra* root tuber through DPPH assay. They used methanolic extract (EM) and its successive fraction (EB; butanolic extract, EA; aqueous extract; EEA; ethyl acetate extract; EC; chloroform extract). EM (330 ± 1.56) μg L^{-1} was found to have the highest anti-oxidant efficiency.

5.2. Anti-Bacterial, Anti-Fungal, Anti-Tumor, and Ulcer-Preventive Activity

The extract of *D. hamiltonii* leaf in petroleum ether was used to investigate its anti-bacterial properties against different pathogens by using the agar well diffusion method [47]. The profound effect of the extract was most prone against the colony of *S. typhi* (11 mm) followed by *Proteus vulgaris* (6 mm) and *Klebsiella pneumoniae* (5 mm). The anti-bacterial activity of *D. hamiltonii* was also evaluated by Devi and Latha [48], who obtained an inhibitory effect against different bacterial strain, viz., *E. coli*, *K. pneumoniae*, *S. typhi*, *P. mirabilis*, *V. cholerae*, *S. sonnie*, *Serritias* species, *S. aureus*, and *B. subtilis* by the disc diffusion method. Fitzgerald et al. [49] identified the activity of 2H4MB against both Gram-positive and Gram-negative bacteria. Saradha and Bharti [50] evaluated the anti-bacterial efficiency of various solvents leaf and tuberous root of *D. nervosa* by using the agar well diffusion method against the five bacterial strains. Moreover, among the different solvents, methanol extract showed better inhibition against the tested bacteria.

Mohana and Raveesha [51] screened the aqueous root extract of *D. hamiltonii* for anti-fungal activity against the species of *Fusarium*, *Aspergillus*, *Penicillium*, *Drechslera*, and *Alternaria* through the use of the poisoned food technique. They found that the presence of the phenolic compound, 2H4MB, was the main constituent to show potent anti-fungal activities. In another study, the anti-fungal activity of the compound 2H4MB was isolated from the root tuber of *D. hamiltonii*, which showed an inhibitory effect for *F. proliferatum*, while being the least effective against *P. oryzae* [51].

The possible treatments for cancer are chemo- or radiotherapy, but this has serious side effects. Plants are an important resource for anti-cancer drugs such as Taxol, isolated from *Taxus brevifolia*, and vincristin and vinblastine, isolated from *Catharanthes roseus*. However, a great interest has developed among scientists to discover new resources. In this regard, *D. hamiltonii* root extract (DHA) was found to be effective against Ehrlich Ascites Tumor (EAT) cells in mice, which showed a significant reduction in ascites tumor volume and tumor cell counts and increased median survival time (MST) as compared to the anti-cancer drug cyclophosphamide (CP) [52]. The above observation demonstrates the anti-cancerous potential of DHA. A phenolic polysaccharide has been investigated in the extract of *D. hamiltonii* that contains in vivo anti-ulcer activities [34]. They found that phenolic plosachharide is involved in the up-regulation of mucin, anti-oxidant enzyme, the inhibition of H^+ and K^+-ATPase activity against ulcers in experimental animal models, and was also found to inhibit *Helicobacter pylori*.

6. Extinction and the Need for Conservation

Thus far, various reports have been published on the importance of the genus *Decalepis*, although the causes of extinction and conservation strategies have been less frequently studied. The root tuber, as the most important part of the plant, is used in various ways,

including on the international market to meet various industrial demands. In order to harvest the root, the entire plant must be cut from the soil, which leads to a destructive harvesting. The overall demand of the root tuber threatens the standing biomass of the plant in its natural habitat. According to the IUCN data for endangered plant, three peninusular Indian species of *Decalepis* have been placed in the endangered (*D. hamiltonii*) and critically endangered (*D. arayalpathra* and *D. salicifolia*) categories. Absence of seed dormancy, pollinator limitation, self-incompatibility, and lack of systematic cultivation are important factors restricting the distribution and conservation of the species [53,54], which act as a means to prevent plant spread and loss of its habitat, demonstrating the necessity of alternative means. Thus, of biotechnological intervention is needed for this genus to feed the pharmacological industries and other demands related to the genus *Decalepis*.

7. Biotechnological Interventions for the Conservation and Advancement of *Decalepis* Research

Biodiversity conservation is of primary concern worldwide, and many efforts have been made both domestically and globally. Conserving threatened and endangered plants is our primary concern in this wide field of plant sciences. There are many organizations in India that are engaged in the conservation and protection of such plants. Generally, in situ conservation strategies include preservation of germplasm in their natural environments. The Foundation for Revitalization of Local Health Traditions (FRLHT), a non-government organization based in southern India, is working and monitoring the species through in situ conservation (http://www.frlht.org/, accessed on 12 Feburary 2022). Figure 1 represents the different conservation strategies. This section presents a detailed study for the ex vitro conservation of species of *Decalepis* through micropropagation and tissue culture technology.

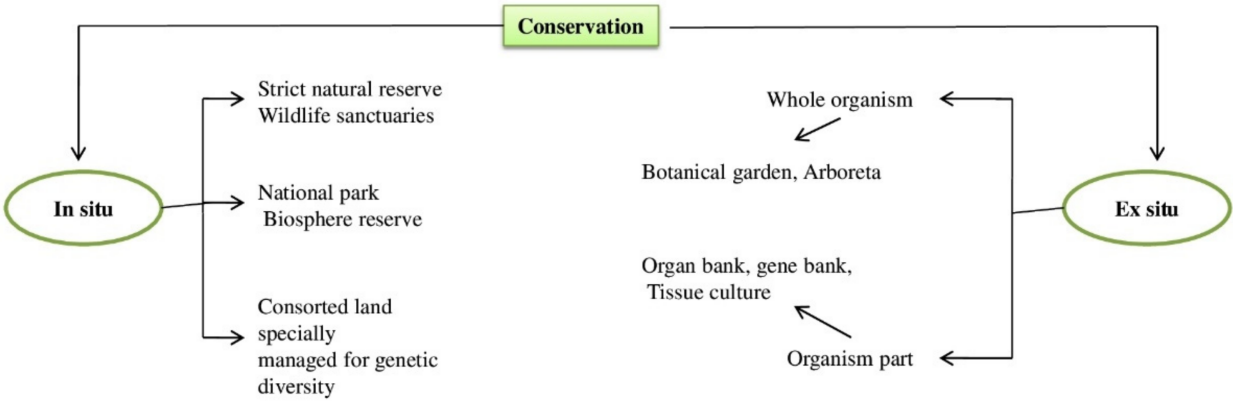

Figure 1. Plant tissue culture approaches in species from the genus *Decalepis*.

8. Micropropagation

Ex vitro conservation of important medicinal plants using in vitro propagation is generally carried out when the species count is low, hard to grow through the conventional breeding program, and overexploited with destructive harvesting. All these jeopardize the natural population of the plant and push plants into the categories of endangered or critically endangered. In vitro conservation of the biological material leads to preservation under aseptic conditions and can be done for short and longer periods [55]. The advanced technology of micropropagation gives us a new and improved perspectives over traditional propagation, which has been reported to have many advantages, such as the development of true-to-type plant, disease-free plants, and elite germplasm. In addition, several other applications, i.e., haploid production, somaclonal propagation, etc., are also associated with the modern techniques of biotechnology [55,56]. Figure 2 shows the possible strategies for the conservation and propagation of the genus *Decalepis*. Moreover, plant cell or tissue culture techniques has been found effective and more advantageous in studying plant

secondary metabolism, their mechanism of synthesis, their manipulation with effective elicitors, etc. [55,56]. Several studies have been conducted to develop viable methods for *D. hamiltonii*, *D. arayalpathra*, and *D. salicifolia* (Table 2) and details are discussed in this section. However, studies on *D. nervosa* and *D. khasiana* are lacking. Figure 3A–C show the mother plant of *D. hamiltonii*, *D. arayalpathra*, and *D. salicifolia*. George et al. [57] reported indirect shoot regeneration of *D. hamiltonii* through leaf-derived callus. Among various concentrations of cytokinin examined, treatment of 6-Benzyleaminopurine (BA) (1.9 mg L^{-1}) + 1-Naphthalene acetic acid (NAA) (0.05 mg L^{-1}) was found to be optimum for the differentiation of a maximum of 4.4 shoots per culture. To achieve normal growth and development in the regenerated shoot, application of 3% sucrose was optimum in the growth medium [57]. The technique of axillary bud differentiation was found to be more applicable to a higher number of shoot production [58]. They have found a treatment of BA (2.0 mg L^{-1}) + NAA (0.5 mg L^{-1}) to be the most effective, as a maximum of 12.8 shoots per explant could be produced in *D. hamiltonii.* However, Anitha and Pullaiah [59] increased the concentration of cytokinin treatment to maximize the regeneration efficiency of nodal explants, but could not be able to improve the response with protection of only four shoots per explant on MS medium containing BA (17.76 µM) + NAA (0.53 µM). The addition of phenylacetic acid (PAA) showed the influential response on shoot multiplication through nodal segment in *D. hamiltonii* [60]. A nutrient composition of MS + BA (31.08 µM) + PAA (14.68 µM) proved to be the best for maximum regeneration of the shoot, with 6.4 shoots per explant [60].

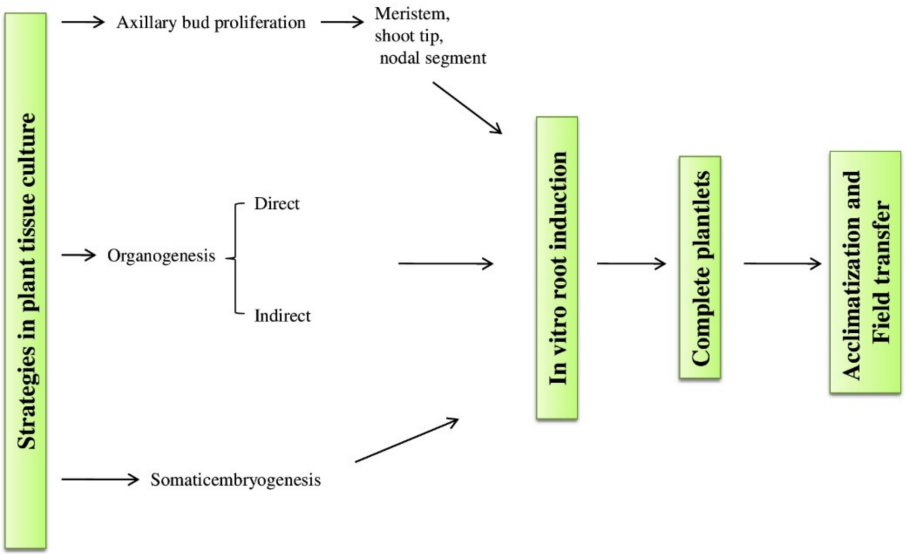

Figure 2. Strategies in the plant tissue culture for the in vitro propagation of the genus *Decalepis*.

Figure 3. Mother plant. (**A**) *D. hamiltonii*. (**B**) *D. arayalpathra*. (**C**) *D. salicifolia*.

Table 2. In vitro shoot regeneration using different explants in species from the genus *Decalepis*.

S. No	Species	Treatment	Regenerat-Ion	Mean No. of Shoots/Explants	Mean Shoot Length (cm)	Explants Used	Ref.
1	*D. salicifolia*	MS + BA (5.0 µM) + NAA (0.5 µM) + ADS (30.0 µM)	Direct	6.1	6.4	NS	[17]
2	*D. arayalpathra*	MS + BA (5.0 µM) + IAA (0.5 µM) + ADS (20.0 µM)	Direct	11.8	9.2	NS	[18]
3	*D. hamiltonii*	MS + BA (5.0 µM) + IAA (0.5 µM) + ADS (30.0 µM)	Indirect	8.2	6.5	NS	[61]
4	*D. arayalpathra*	MS + BA (12.96 µM) + 2-ip (2.48 µM) + NAA (2.68 µM)	Direct	1.0	16.0	NS	[62]
5	*D. hamiltonii*	MS + BA (1.9 mg L^{-1}) + NAA (0.05 mg L^{-1})	Indirect	4.4	7.2	Leaf	[58]
6	*D. hamiltonii*	MS + BA (2.0 mg L^{-1}) + NAA (0.5 mg L^{-1})	Direct	12.8	5.8	NS	[58]
7	*D. hamiltonii*	MS + BA (17.76 µM) + NAA (0.53 µM)	Direct	4.0	*	NS	[54]
8	*D. hamiltonii*	MS + TRIA (20 µg/L)	Direct	5.0	*	ST	[54]
9	*D. hamiltonii*	MS + BA (31.08 µM) + PAA (14.68 µM)	Direct	6.4	2.9	NS	[60]
10	*D. hamiltonii*	MS + BA (10.65 µM) + Zea (9.12 µM)	SE	8/SE	*	SE	[63]
11	*D. hamiltonii*	MS + BA (1.1 µM) + PG (800 µM/l) + GA_3 (5.8 µM)	Direct	4.0	3.5	Direct	[64]
12	*D. hamiltonii*	MS + 2-iP (4.9 µM)	Direct	6.5	*	Direct	[28]

* Information is not available in the published report. NS: nodal segment; ST: shoot tips; SE: somatic embryo. BA: 6-Benzyleaminopurine.; NAA: 1-Naphthalene acetic acid; ADS: Adenine sulphate; IAA: indole-3-acetic acid; 2-ip: 2-isopentanyladenine.; TRIA: Triacontanol; PAA: phenylacetic acid; Zea: Zeatin; GA: Gibberellic acid.

For improvement in the regeneration efficiency of various explants, the synergistic effect of growth additives, i.e., adenine sulphate (ADS), phloroglucinol (PG) with the cytokinin has been tested and effective results have been obtained. Gururaj et al. [64] found a maximum number of shoots when nodal explants were inoculated on MS medium augmented with BA (1.1 µM) + Gibberellic acid (GA3) (5.8 µM) + PG (800 µM) in *D. hamiltonii*. Subculture passes on media containing BA (5.6 µM) + PG (200 µM) + triacontanol (TRIA) (0.11 µM) gives elongated shoot with secondary shoot formation. Giridhar et al. [28] used shoot tips as explants for the regeneration and different concentrations of cytokinins were tried for the shoot organogenesis. The maximum number of shoots (6.5) was observed on MS + 2-isopentanyladenine (2-iP) (4.9 µM) and the subsequent multiplication was found to be significantly better on MS + 2-iP (2.5 µM) + GA3 (0.3 µM). Similarly, Sharma et al. [61] tried different combination of plant growth regulators (PGRs) and growth additives for the enhanced shoot regeneration and plantlets regeneration and found that MS medium supplemented with BA (5.0 µM) + indole-3-acetic acid (IAA) (0.5 µM) + ADS (30.0 µM) was more suitable for the production of a maximum of 8.20 shoots per explant with a mean shoot length of 6.54 cm in *D. hamiltonii*. They also noticed that the application of cytokinin alone was unable to give rise shoot regeneration in *D. hamiltonii*, but that it was able to induce nodulation (N1 and N2) at the base of the explants. N2 type nodule was able to give rise to a maximum number of 15.40 shoots per explant with a maximum shoot length of 4.56 cm when subcultured on BA (5.0 µM) + IAA (0.5 µM) + ADS (30.0 µM) + GA3 (1.0 µM) [61].

The micropropagation protocol is unsuccessful, unless effective rooting is programmed on the growing microshoot. A successful in vitro rooting protocol for *D. hamiltonii* was developed using silver nitrate ($AgNO_3$) [65]. They also tried ethaphone for rooting purposes. However, it brings baroque callusing at the base of microshoot. Furthermore, they used $AgNO_3$ in combination with ethylene which improved the rooting response. Reddy et al. [66] achieved rooting when the microshoots were transfer to a nutrient medium containing a combination of auxins indole-3-butyric acid (IBA) (8.8 µM) + IAA (1.43 µM). On the other hand, Giridhar et al. [61] found that the application of IBA (9.8 µM) was efficient for the root induction in the microshoots, while Gururaj et al. [64] obtained rooting response when microshoots are transferred to a media containing 5.38 µM NAA and 400 µM PG. Some other researchers have also advocated for the active participation of

PG and salicylic acid and their compatible interaction to induce root differentiation in in vitro-generated microshoots [60]. Table 3 represents the in vitro root induction using different phytohormones alone or in combination for *Decalepis* plants.

Table 3. In vitro root regeneration from the obtained shoot in species from the genus *Decalepis*.

S. No.	Species	Treatment	Rooting Response (%)	Mean No. of Root	Mean Root Length	Plantlet Survival (%)	Reference
1	*D. salicifolia*	$\frac{1}{2}$ MS + IBA (2.5 µM)	91.0	6.10	2.30	90	[17]
2	*D. arayalpathra*	$\frac{1}{2}$ MS + NAA (2.5 µM)	91.6	5.1	4.9	92.3	[18]
3	*D. hamiltonii*	$\frac{1}{2}$ MS + NAA (2.5 µM)	96.40	7.80	6.46	95.10	[61]
4	*D. hamiltonii*	MS + NAA (5.38 µM) + PG (400 µM)	*	*	*	80-90	[64]
4	*D. hamiltonii*	MS + IBA (9.8 µM)	100	3.0	2.4	60	[63]
5	*D. hamiltonii*	MS + TRIA (10 µg/L)	*	5.2	2.3	57	[54]
6	*D. hamiltonii*	MS + IBA (4.4 µM)	100	*	*	80	[66]
7	*D. hamiltonii*	MS + IBA (4.4 µM) + PG (69 µM) + activated charcoal (0.25%)	80	*	*	65	[66]

* Information is not available in the published report. IBA: indole-3-butyric acid; NAA: 1-Naphthalene acetic acid; PG: Phloroglucinol; TRIA: Triacontanol.

Limited reports are available on the micropropagation of *D. arayalpathra*. A detailed micropropagation protocol has been presented in Figure 4A–F for *D. arayalpathra*. Preliminary work has been done by Gangaprasad et al. [67] via single node culture. MS medium supplied with a distinct concentration of BA (0.1–5.0 mg L^{-1}) and all the concentration of BA was able to induce single shoot but the application of BA (0.1 mg L^{-1}) was found to be optimum for rapid growth in the shoots, as they attained a shoot length of 6.8 cm in 60 days. Then, after the nodal segment and shoot tip were taken from the in vitro-raised plant and inoculated on MS medium supplied with BA (0.5 mg L^{-1}), plants produced 8 cm long shoots in 30 days [67]. Sudha et al. [62] studied the action of cytokinin on a nodal segment of *D. arayalpathra* taken from the mother plant (12–16-week-old), seedling, and after being cultured on MS + BA (12.96 µM) + 2-iP (2.48 µM) + NAA (2.68 µM), which was capable of giving rise to single non-branching shoots with a length of 16–17 cm in 8 weeks. The cotyledonary node on the above nutrient composition initiated maxillary shoot induction, although the delicate shoot system is less suitable for mass propagation. Furthermore, they used a combination of cytokinin, i.e., BA and 2ip, and a concentration of BA (2.22 µM) + 2-ip (0.24 µM) was found to be the most suitable in producing a single thick shoot of 9.8 nodes from 18.0 cm long shoots within 5–6 weeks, and the basal nodes of shoots were used for subculturing purposes to enhance the stock of propagules [62]. A comparative study of node and shoot tip explants of *D. arayalpathra* has been carried out by Ahmad et al. [18]. They reported that the nodal segment was more responsive than the shoot tip explants and a combination of MS + 5.0 µM BA + 0.5 µM indole-3-acetic acid + 20.0 µM adenine sulphates produced 11.8 shoots per nodal segment and 5.5 shoots per shoot tip, with a mean shoot length of 9.2 and 4.8 cm, respectively. The induction of the root to the microshoot has been carried out with the application of auxin in a fresh medium. Among the different concentrations of auxin that have been evaluated, a concentration of 2.5 µM a-naphthalene acetic acid (NAA) in half-strength MS medium was found to be the best for the regeneration of 5.1 ± 0.1 roots per microshoot, with a root length of 4.9 cm [18]. However, the regeneration through leaf-derived callus is not reported yet, as callus formed through this method was found to be non-organogenic (Figure 5A,B).

Very few reports are available on the micropropagation of *D. salicifolia.* Ahmad et al. [17] developed a high frequency shoot regeneration system using a nodal segment. Different combinations of cytokinin, such as BA, Kn, and 2 iP, have been tried with different growth additives, such as ADS, Glu (glutamine), and PG. Between the tried concentration of cy-

tokinins and growth additives, the MS medium fortified with BA (5.0 µM) + NAA (0.5 µM) + ADS (30.0 µM) was proven to be optimal for the significant number of shoot proliferation, wherein the highest of 9.97 ± 0.01 shoots per explant with a maximum shoot length of 6.46 ± 0.1 cm was achieved. In vitro-raised microshoot was rooted on half strength MS + IBA (2.5 µM), which gave rise to 6.10 ± 0.07 roots per microshoot with an average root length of 2.30 ± 0.06 cm [17]. In a recent study by Rodrigues et al. [68], the combination of BA and IAA was found to be suitable for shoot tip proliferation, wherein a treatment of BA (2.2 µM) + IAA (5.7 µM) gave rise to an average shoot length of 5.8 cm in three weeks; moreover, a higher concentration of BA was found suitable for axillary bud proliferation. They obtained successful rooting in a modified MS medium with a low concentration of ammonium nitrate and potassium nitrate, along with 8% sucrose concentration, which is responsible for the production of 12.4 roots per shoot with a root length of 4.3 cm [68].

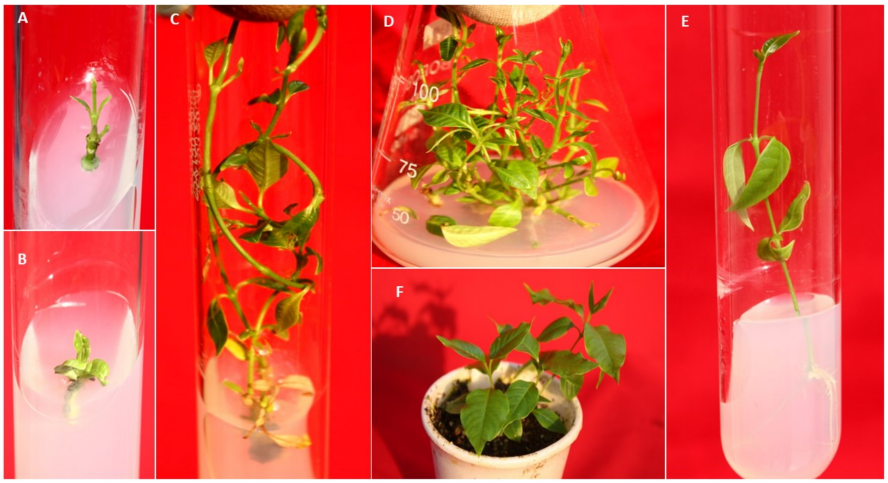

Figure 4. A micropropagation protocol for *D. arayalpathra*. (**A**,**B**) Different explants, viz., nodal segment and shoot tip explant inoculated on MS + 5.0 µM BA after 6 weeks of culture. (**C**) Proliferating NS-raised culture on MS + 5.0 µM BA after 6 weeks of culture. (**D**) NS-raised culture showing elongation on MS + 5.0 µM BA + 0.5 µM NAA + 20 µM ADS after 3 weeks of inoculation. (**E**) Development of fibrous roots on Half-strength MS + 5.0 IAA after 4 weeks of culture. (**F**) Successfully acclimatized in vitro rooted plantlets of sterile soilrite after 6 weeks of transfer.

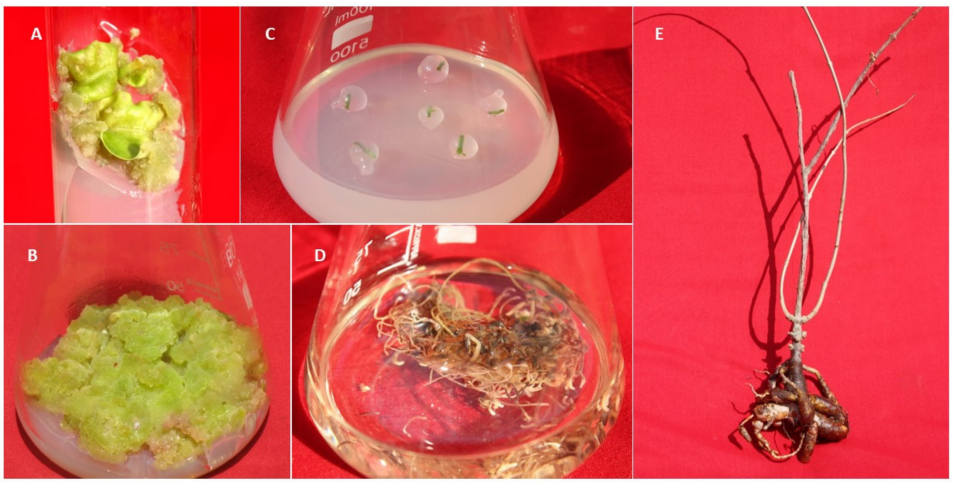

Figure 5. Callus formation derived from leaf explants of *D. arayalpathra* when cultured on MS medium supplemented with different PGRs. (**A**,**B**) *D. arayalpathra* leaf explant inoculated on MS + 1.0 µM BA + 1.0 µM NAA after 2 weeks and 6 weeks of culture, respectively. (**C**) Encapsulated NS on MS basal medium, 1-day-old culture. (**D**) Root culture. (**E**) Root tuber of *D. arayalpathra*.

9. Somatic Embryogenesis

Production of somatic embryos in *Decalepis* is a rare phenomenon, as there is only one preliminary report available from Giridhar et al. [63], who obtained somatic embryoids in the *D. hamiltonii* by using leaf explants. The callus was obtained from the leaf on MS + zeatin (13.68 µM) + BA (10.65 µM). Differentiation and maturation of embryos were obtained on the same nutrient medium. However, when an even lower concentration of zeatin was used, embryo differentiation was improved, and treatment of MS + Zea (4.56 µM) + BA (10.65 µM) was found to be more effective for the maturation of embryoids.

10. Synthetic Seed Production

Synthetic seed encapsulation has emerged as a new and advanced technology for the conservation of germplasm and exchange of plant material [69]. The somatic embryo, shoot tip, nodal segment, bulbs, etc. can be encapsulated in sodium gel, which are considered as a synthetic seed [70] (Figure 5C). Synseed technology has several advantages, such as the exchange of germplasm of this endangered and critically endangered plant, the possibility for longer-distance transport due to the small size of the capsules, and that these capsules could be stored up to 8 weeks at a low temperature (4 °C) [71]. However, fewer reports are available on synthetic seed technology used on the genus *Decalepis*. Germana et al. [71] developed a protocol for short-term storage and conservation of *D. hamitonii* using synthetic seed technology by encapsulating the juvenile nodal segment and concluded that 4% Na-alginate and 100 mM $CaCl_2 \cdot 2H_2O$ are optimal for the gelling matrix and ideal Ca-alginate beads synthesis. Furthermore, it was reported that the concentration of sodium alginate (Na-alginate) and calcium chloride ($CaCl_2 \cdot 2H_2O$) affects the encapsulation [60]. However, rooting was not possible in one step in this protocol, which calls for a separate rooting program. Therefore, the technique provides a novel way of storage and exchange of this endemic plant germplasm and tackles various problems related with long-distance transport of plant germplasm [61]. In a recent report by Rodrigues et al. [68] on encapsulation techniques in *D. salicifolia*, shoot tips and nodal explants were encapsulated in 3% sodium alginate and 100 mM $CaCl_2 \cdot 2H_2O$ and regeneration was achieved following storage at a low temperature up to 12 weeks.

11. Elicitation, Adventitious Root Culture, and Genetic Transformation

The tuberous root (Figure 5E) of the plant is a storehouse of several minor and major secondary metabolites, most importantly 2H4MB. The biological role of 2H4MB has continuously attracted researchers for many years, as this compound has been reported to be abundant in the genus *Decalepis*. Thus, the isolation of the compound becomes of major interest to many researchers. Giridhar et al. [72] reported an improved 2H4MB contents in the root system of *D. hamiltonii* when treated with TRIA. They tried TRIA soil drenching and showed a better yield of tuber with enhanced flavor compound, and achieved a significant increase (1.5 times more) as compared to the control when analyzed with GC-MS (Gas Chromatography and Mass Spectrometry). In a recent study, Ahmad et al. [73] developed the elicitation protocol for the enhancement of 2H4MB in root tuber-derived callus for the first time in this species. They used two elicitors, viz., chitosan and yeast extract, at various concentrations in a suspension culture, and chitosan was found to be more effective than yeast extract. A maximum biomass of 9.7 dry weight g/L and a maximum of 14.8 µg/g 2H4MB content at the optimum contact period of 72 h was obtained when the suspension culture was treated with 200 µM of chitosan [73]. Giridhar et al. [74] evaluated the 2H4MB content in root cultures of *D. hamiltonii*. They tried Dichloromethane as solvent with ethanol at defined ratio for qualitative and quantitative GCMS analysis. The maximum content (40 $\mu g g^{-1}$ dry weights) of 2H4MB in root was recorded after 45 days of culture when MS medium was augmented with α-naphthaleneacetic acid (NAA) (1.0 mg L^{-1}). Normal root culture for *D. arayalpathra* was also established by Sudha et al. [62] by using leaf and internodal explants of in vitro-raised shoot culture. To achieve this, they used MS medium augmented with BA (2.5 mg L^{-1}) + 2-iP (0.5 mg L^{-1}) + NAA (0.5 mgL^{-1}) and

subcultured at 5 weeks interval. The maximum 2H4MB (0.16%) content was recorded when the root was cultured on MS liquid medium supplemented with IBA (0.5 mg L^{-1}) + NAA (0.2 mg L^{-1}) and also favored maximum induction of roots (5.820 g). In a recent report of Rodrigues et al. [75] on adventitious root cultures of *D. salicifolia* for the production of 2H4MB, adventitious root biomass accumulation of 10.61 g fresh weight was obtained in woody plant liquid media containing 0.5 mg L^{-1} NAA + 0.3 mg L^{-1} IBA in 60 days of inoculation, and in comparison to the field-grown plant, the accumulation was 35-fold lower than the adventitious root culture. Figure 5D represents the adventitious root culture of *D. arayalpathra*.

Agrobacterium-mediated transformation of *D. arayalpathra* has been achieved by using different strains of *A. rhizogenes*, i.e., A4, MTCC 532, TR105, and LBA 5402 [75]. The juvenile hypocotyls explants were used for the infection and induction of hairy roots at higher frequency of (53.2 $\pm$ 0.3%) than cotyledons (32.1 $\pm$ 0.2%) when infected with TR105, which is the most virulent strain. They recorded that the formation of hairy roots took place in two ways, either directly from the wounds or followed by the formation of gall-like structures when cocultivated in half-strength MS basal medium. Irregular gall formation was observed during the culture, which was showing the active site for the induction of hairy root. They observed a maximum accumulation of 2H4MB (0.22% dry weight) recorded in the 6th week [75].

12. Concluding Remarks and Future Prospects

This review highlights the major advances of the genus *Decalepis* in terms of their distribution, biology, and importance. In addition, the review focused on the biotechnological interventions and their advancement in the genus *Decalepis*. The report clearly indicates the species from the genus *Decalepis* as a viable alternative of *H. indicus*. Of the five species (i.e., *D. hamiltonii*, *D. arayalpathra*, *D. salicifolia*, *D. nervosa*, and *D. khasiana*), *D. hamiltonii* is the most extensive and widely used species. However, a more biased approach is needed to focus on better conservation of these threatened species as well as improved production of 2H4MB. To date, effective protocols for direct shoot regeneration from various explant have been reported for *D. hamiltonii*, *D. arayalpathra*, and *D. salicifolia*, but they are lacking for *D. nervosa* and *D. khasiana*. Therefore, a clonal method should be developed for these two unexplored species from the genus *Decalepis*. Similarly, callus-mediated organogenesis, somatic embryogenesis, and synthetic seed production are less explored areas for all the species. Genetic transformation, identification, and manipulation of the 2H4MB biosynthetic pathway to enhance the production of 2H4MB are totally unexplored areas for the genus. The use of a molecular marker, germplasm preservation for long- and short-term duration, low-cost system of micropropagation, elicitation, adventitious root culture, and genetic manipulation of in vitro cultures for the production of 2H4MB are some of the areas of research that need special attention to advance the biotechnology of this economically and medicinally important genus *Decalepis*.

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