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Review

A review on *Decalepis hamiltonii* Wight & Arn.

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Decalepis hamiltonii **is a climbing shrub with aromatic tuberous roots distributed in Southern parts of Peninsular India. Its tuberous roots are widely used as a health drink and are well known for its medicinal properties. *D. hamiltonii* is one of the important plants in Ayurvedic system of medicine in India and are used in curing various diseases like stomach disorders, gastric ulcers and to stimulate appetite. This plant has been used in the preparation of several herbal drugs like Amrutamataka taila, Drakshadi churna, Shatavari rasayana and Yeshtimadhu taila. In the present review, traditional uses, uses as food and health drinks, phytochemistry, pharmacology and conservation of this endangered species are highlighted. A number of phytochemical compounds have been isolated from this plant; of these, 2-hydroxy-4-methoxy benzaldehyde (HMB) is an abundant aromatic bioactive compound with greater biological significance. The tubers have antimicrobial, antipyretic, antiulcer, antidiabetic, antioxidant, anti-inflammatory, chemoprotective, cytoprotective, insecticidal, neuroprotective and hepatoprotective activities. Natural seed germination is very low in this species, that is, 6% because of hard seed coat, less seed dormancy period and due to self-incompatibility. *In vivo* and *in vitro* conservation methods have been standardized to this endangered taxon by studying seed germination, field cultivation methods and by developing rapid micropropagation techniques. Among different plant growth regulators tested, 6-benzylaminopurine (BAP) played a significant role in shoot multiplication, whereas, indole-3- butyric acid (IBA) and silver nitrate ($AgNO_3$) influence rooting efficiently. The review aims to provide sufficient baseline information for further works and commercial exploration.**

Key words: *Decalepis hamiltonii*, phytochemistry pharmacology, conservation.

INTRODUCTION

Decalepis hamiltonii Wight & Arn. is a monotypic, glabrous, climbing shrub belonging to the family Periplocaceae (Kostel.) Schltr (earlier under Asclepiadaceae) (Figure 1 A and B). This is an endemic and endangered medicinal plant and grows largely in moist as well as dry deciduous forests, scrub jungles of southern parts of Deccan Peninsula and the Western Ghats of India (Gamble and Fischer, 1957). This plant known by its names of Maredu Kommulu, Nannari Kommulu, Madina Kommulu, Barre Sugandhi and Maredu Gaddalu in local language, whereas in English it is named as swallow root. It prefers to grow along rocky slopes, big rock boulders and rocky crevices and small mounds where there is thick vegetation at an altitude from 300 to 1200 m. It has good medicinal importance and used in wide drug preparations. Pharmacognostical study of roots of *D. hamiltonii* was investigated by Nayar et al. (1978) for proper identification during drug preparation. Shefali et al. (2009) reviewed the pharmacognosy, phytochemistry and pharmacology of *D.*

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Figure 1. *Decalepis hamiltoii* Wight & Arn. root collection by tribal peoples and habit.

hamiltonii. The present review assesses the potential of *D. hamiltonii* in relation to its traditional uses and in terms of findings based on modern biochemical research. The link between conventional remedies and resent research in various areas has been well established in other plants. This paper aims to provide the up to dated information regarding biochemical, pharmacological and conservation studies of economically important medicinal and aromatic plant *D. hamiltonii* which will provide a path for further research in pharmaceutics and beverages for human welfare and to establish high frequency regeneration protocols for effective protection of this endangered taxon.

PLANT DESCRIPTION

D. hamiltonii Wight & Arn. is a perennial, woody climber, the stem and branches are articulated, angled and reaches a girth of 5 cm. Branchlets are terete with swollen, winged nodes. The leaves are opposite, simple, elliptic, ovate, 2.5 × 1.5 to 5 cm, subcoriaceous, longitudinally folded, base attenuate to truncate, margin entire to undulate, apex subacute to obtuse; petiole to 1 cm. Milky latex is present in whole plant. The roots are aromatic and highly valued for medicinal properties. The swollen roots possess strong vanillin like odour, which have fleshy outer layer and woody inner core. The roots are pale brown in colour and elongated to grow up to 150 cm in length and 3.5 cm width. In each plant 4 to 10 roots arise from the rootstock. Cymes trichotomous; peduncle to 2 cm; bracts and bracteoles lanceolate, 1.5 mm; pedicel to 1 cm; calyx lobes oblong, tinged with brown, 2 mm, chartaceous, valvate, acute. Corolla cream, 7 mm across, campanulate; lobes spreading, 3.5 mm, valvate, villous within, acute to recurved. Stamens connivent; pollinia horizontal; pollinial bags closely adherent, flat, 0.6 mm; caudicle indistinct; receptacle minute. Corona double, staminal; outer scale-like, truncate, 1 mm; inner flat, adhering to gynostegium, 2 mm. Glands 5 at the base of corolla, alternating with stamens, 1 mm, 2 fid. Ovaries subglobose, 1 mm; style 0.7 mm; stigma obtuse or flat. Follicle oblong or lanceolate, cylindric 5.5 × 3 cm; epicarp thick, crinkled; seed ovate 6 × 4 mm; teats angled, chartaceous, tipped with long, white, silky coma. During summer the plant appears deciduous, vegetative structures become dry whereas the fruit follicles and tubers persist.

Ecology

Dry deciduous, leaf fall, January; new foliage, February to April; flowering, May to August; fruit, January onwards,

with horny pericarp that gets shrunk and persist till next season.

Traditional uses

The roots are being used in Ayurveda, the ancient Indian system of medicine, to stimulate appetite, relieve flatulence and as a general tonic (Vadavathy, 2004). The roots are also used in folk medicine as general vitaliser and blood purifier. *D. hamiltonii* roots are used as a substitute for *Hemidesmus indicus* in Ayurvedic preparations because of the similar aromatic properties (Nayar et al., 1978). It is also used as demulcent, diaphoretic, diuretic and tonic. It is useful in the loss of appetite, fever, skin disease, diarrhea and in nutrition disorders (Wealth of India, 1990). It is also used in treatment of epileprisy and central nervous system disorders (Murti and Seshadri, 1942). People procure and habitually carry the roots with them and chew the same whenever the digestion may seek relief. This root extract is taken orally to rejuvenate the body (Reddy et al., 2006). The roots of these taxa are considered as Sariva Bheda in Ayurvada and are used in the preparation of several herbal drugs like Amrutamataka Taila, Drakshadi Churna, Shatavari Rasayana and Yeshtimadhu Taila (George et al., 1999a). The root of Sariva is also used for treating fever, intrinsic haemorrhage, kushtha, erysipelas, poisoning, paediatric rejuvinative/rasayana and during pregnancy (Venugopal, 2002). Recently, the anticonvulsant activity was reported in this species (Ambavade et al., 2003). These roots are encouraged to use in the form of powder and infusion to treat wounds and bronchial asthma (Manivannan, 2010). The root extract of *D. hamiltonii* has been shown to contain significant antidiabatic, antiatheroscrerotic and hepatoprotective properties (Naveen and Khanum, 2010). Recently it has been reported that roots of *D. hamiltonii* possess diuretic property (Arutla et al., 2012).

Phytochemistry

The root of *D. hamiltonii* has shown to contain a number of compounds (Table 1) like aldehydes, alcohols, ketones, sterols and triterpenes such as amyrin and lupeols derivatives (Murti and Seshadri, 1941a, b, c, d), resinol, saponins, tannins, inositol, fatty acids (Murthi and Seshadri, 1942). *D. hamiltonii* root has a strong aromatic odour and its volatile oil (0.68%) which contains 2-hydroxy-4-methoxy benzaldehyde (HMB, 96%) as a major compound. Further gas chromatography mass spectroscopy (GC-MS) analysis of this oil showed, along with the major component, the presence of benzaldehyde (0.017%), salicylaldehyde (0.018%), methyl salicylate (0.044%), benzyl alcohol (0.016%), 2-phenylethyl alcohol (0.081%), ethyl salicylate (0.038%), *p*-anisaldehyde (0.01%), and vanillin (0.45%) in minor quantities which are biologically significant (Nagarajan et al., 2001). Whereas the hydrodistillation of *D. hamiltonii* roots yielded an essential oil (0.33% v/w) that contained HMB (37.45%), 2-hydroxybenzaldehyde (31.01%), 4-*O*-methylresorcylaldehyde (9.12%), benzyl alcohol (3.16%), and α-atlantone (2.06%) as major constituents, along with aromatic aldehydes (Thangadurai et al., 2002). HMB was determined in roots of *D. hamiltonii* and *H. indicus* by using GC analysis and found that the quantity of this aromatic compound varied from 0.03 to 0.54% (Nagarajan and Rao, 2003). Production of HMB in roots of tissue culture raised and acclimatized plants were evaluated with that to naturally growing plants. Similar profile in production of HMB as that of wild plants was observed. A maximum of 0.14 and 0.12% of HMB production was noticed in roots of tissue culture raised plants and acclimatized plants, respectively (Giridhar et al., 2004b). Phytochemical investigation of the methanolic extract fractionated on a silica gel column showed three major fractions. When the active fractions were further subjected to preparative thin layer chromatography (TLC) and silica gel column chromatography yielded six pure compounds. The purified compounds were further characterized by MS, ^{1}H NMR, ^{13}C NMR and two-dimensional NMR spectroscopic techniques and identified as HMB, *p*-anisaldehyde, vanillin, borneol, salicylaldehyde, and bis-2,3,4,6-galloyl-α/β-D-glucopyranoside and the latter compound was named as decalepin (Harish et al., 2005).

Production of root specific flavour compound HMB from normal roots of swallow root raised *in vitro* was examined for qualitative by TLC and quantitative by GC-MS analysis and found that maximum root biomass and the maximum content of flavour compound (40 ± 2.1 µg/L dry weight) was produced after 45 days of growth on MS medium containing 1.0 mg/L NAA (Giridhar et al., 2005c). Enhancement of root specific flavor compound 2-hydroxey-4-methoxy benzaldehyde in micropropagated plants of *D. hamiltonii* under triacontanol treatment was reported by Giridhar et al. (2005b). They found administration of triacontanol (0.228 µM/L) twice to the soil by giving second treatment with a gap of 60 days after initial treatment enhanced the yield of flavor compound. Five novel antioxidant compounds were isolated and characterized by NMR and MS from the aqueous extract of the roots of *D. hamiltonii* by Srivastava et al. (2006a). It was shown that the aqueous extract of the roots of *D. hamiltonii* is a cocktail of antioxidants namely, 4-hydroxyisophthalic acid, ellagic acid, 14-aminotetradecanoic acid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1, 2-cyclohexane diol, 2-hydroxymethyl-3-methoxybenzaldehyde, 2,4,8 trihydroxybicyclo [3.2.1]octan-3-one; bis-2,3,4,6-galloyl-a/b-D-glucopyranoside and borneol (Srivastava et al.,

Table 1. Phytoconstituents reported in *Decalepis hamiltonii*.

S/N	Type of extract	Technique used	Phytochemical compounds estimated and isolated	References
1	Various solvent fractionation	Gel permeation and gas chromatography	Pectic polysaccharide	Srikanta et al. (2007)
2	Petroleum ether	TLC, ^{1}H NMR, ^{13}C NMR and mass spectral analysis	2-Hydroxy-4-methoxybenzaldehyde	Mohana et al. (2009)
3	Aqueous	UV visible spectroscopy and colorimetric method	Total phenols and flavonoids	Samydurai and Thangapandian (2012a)
4	Dichloromethane	TLC and GC	2-Hydroxy-4-methoxybenzaldehyde	Murthy et al. (2006)
5	Various solvent fractionation	UV visible spectroscopy and colorimetric method	Free, conjugated and insoluble-bound phenolic acids	Nayaka et al. (2010)
6	Volatile oils	GC	2-Hydroxy-4-methoxybenzaldehyde	Nagarajan and Rao (2003)
7	Petroleum ether	TLC, ^{1}H NMR, ^{13}C NMR and mass spectral analysis	2-Hydroxy-4-methoxybenzaldehyde	Mohana et al. (2009)
8	Aqueous	TLC, RP HPLC, UV, IR, Lc-MS, ^{1}H NMR, ^{13}C NMR	Ellagic acid	Srivastava et al. (2007)
9	steam distillation	TLC and GC-MS	2-Hydroxy-4-methoxybenzaldehyde	Giridhar et al. (2005b,c)
10	Ethanol	UV, IR, LC-MS, and 2D HMQC NMR	Decalpoline	Naveen et al. (2012)
11	Aqueous	UV, IR, LC–MS, and NMR	2-(Hydroxymethyl)-3-methoxybenzaldehyde	Srivastava and Shivanandappa (2011b)
12	Aqueous	UV, IR, LC–MS, and NMR	4-Hydroxyisophthalic acid	Srivastava et al. (2012b)
13	Methanol	Colourimetric method and LC–MS	Phenolics and flavonoids	Surveswaran et al. (2010)
14	Different	-	Aldehydes, alcohols, ketones, sterols and triterpenes such as amyrin and lupeols derivatives	Murti and Seshadri (1941a,b,c,d)
15	Different	-	Resinol, saponins, tannins, inositol, fatty acids	Murthi and Seshadri (1942)
16	Volatile oil	GC-MS	HMB (96%), benzaldehyde (0.017%), salicylaldehyde (0.018%), methyl salicylate (0.044%), benzyl alcohol (0.016%), 2-phenylethyl alcohol (0.081%), ethyl salicylate (0.038%), *p*-anisaldehyde (0.01%), and vanillin (0.45%)	Nagarajan et al. (2001)
17	Essential oils	GC-MS	HMB (37.45%), 2-hydroxybenzaldehyde (31.01%), 4-*O*-methylresorcylaldehyde (9.12%), benzyl alcohol (3.16%), *α*-atlantone (2.06%) and aromatic aldehydes	Thangadurai et al. (2002)
18	Methanolic	MS, ^{1}H NMR, ^{13}C NMR, and two-dimensional NMR	HMB, *p*-anisaldehyde, vanillin, borneol, salicylaldehyde, and bis-2,3,4,6-galloyl-α/β-D-glucopyranoside	Harish et al. (2005)

Table 1. Contd.

19	Aqueous	NMR and MS	4-Hydroxyisophthalic acid, ellagic acid, 14-aminotetradecanoic acid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1, 2-cyclohexane diol, 2-hydroxymethyl-3-methoxybenzaldehyde, 2,4,8 trihydroxybicyclo [3.2.1]octan-3-one; bis-2,3,4,6-galloyl-a/b-D-glucopyranoside and borneol	Srivastava et al. (2006a)
20	methanol–acetone–water (7:7:6, v/v/v)	HPLC	Hydroxybenzoate and cinnamate derivatives. gallic, gentisic, protocatechuic and p-coumaric acids	Nayaka et al. (2010)
21	Various solvent fractionation	Flash and HPTLC chromatography	Vanillin	Tiwari et al. (2012)

2006a; Srivastava et al., 2007). A convenient high performance liquid chromatography (HPLC) method for separation of triterpenoids from swallow roots was developed by Nagarajan and Rao (2007). Semi synthetic chalcones, 4-hydroxy-3-(3-(4-methoxy-phenyl)-acryloyl) benzoic acid (a^1) and 3-(3-(4-ethoxy-phenyl)-acryloyl)-4-hydroxy-benzoic acid (b^1) were synthesized by an aldol condensation between 3-acetyl-4-hydroxy benzoic acid (iv) and isolated aromatic aldehydes from tuberous roots of *D. hamiltonii* in the presence of potassium hydroxide as a base (Reddy et al., 2009). HPLC analysis of phenolic acid extracts showed the presence of hydroxybenzoate and cinnamate derivatives. Among the phenolics identified gallic, gentisic, protocatechuic and p-coumaric acids were the major compounds (Nayaka et al., 2010). Surveswaran et al. (2010) investigated the presence of phenolic phytochemicals in Indian medicinal plants of Asclepiadoideae and Periplocoideae and found higher levels of total phenolics and flavonoids in leaves of *D. hamiltonii*. The presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar in the crude extracts of *D. hamiltonii* roots was confirmed by Samydurai and Thangapandian (2012c). Recently, vanilin was isolated from the roots of *D. hamiltonii* by using flash chromatography and the purified compound was quantified and validated through high performance thin layer chromatography (HPTLC) (Tiwari et al., 2012).

Foods and beverages

Dried tubers of *D. hamiltonii* are available in the country drug stores and local tribal markets. Due to the presence of aroma and its health promoting properties, these root tubers are consumed as pickles and beverages (Harish et al., 2005; Reddy et al., 2007). The rhizome is largely used for pickling along with curd or lime juice and it tastes like a ginger pickle (Jacob, 1937; Anonymous, 1952). Recently, a process for the debittered beverage and pickles has been developed by Central Food Technological Research Institute (CFTRI) (Chauhan et al., 2000).

The roots are characterized by sarsaparilla like taste accomplished by a tingling sensation on the tongue as described in the wealth of India (Wealth of India, 1990). These are also used as flavoring agents and used as a substrate for vanillin obtained from an orchid (*Vanilla planifolia*) and used in preparation of ice-creams, chucklers and in other foods and soft drinks. Even though vanillin has been synthesized since from 1874, natural source of this flavor is still in demand and now the roots of *Decalepis* are being used as substitute to it (Vedavathy, 2004). Approximate nutritional composition of edible root tubers of *D. hamiltonii* was evaluated and found that the tubers contain 1,650 kj of energy, 84.05% of moisture, 1.24 g lipids, 2.39 g of carbohydrates, 2.37 g proteins and 10.51 g of fiber for 100 g of roots (Samydurai and Thangapandian, 2012b).

The dried roots are used in preparing popular cool drink locally known as nannari, which has a cooling effect in summer without any toxic side effects in human beings (Vijayakumar and Pullaiah, 1998). Latter the traditional preparation of health drinks nannari, sharbat was explained by Raju and Ramana (2011). The fresh roots of *D. hamiltonii* were first washed under running tap water and dried under open sunlight. Then these roots are cut into small pieces and further dried in sunlight for about 3 days and then powdered and stored. During the preparation, the required quantity of powder (100 g/L) is added to water and allowed to stand for about 12 h and then boiled for 2 to 3 h until the water turns into wine red colour. Then the liquid is filtered and to the filtrate sugar and water are added and again boiled to obtain highly viscous concentrate. This liquid concentrate is then cooled to room temperature and again filtered and stored in bottles. It can be stored without spoil for about 5 months and is used to prepare health drink.

PHARMACOLOGICAL STUDIES

Antibacterial activity

The essential oil of *D. hamiltonii* was investigated for its antimicrobial activity. The oil inhibited the growth of *Escherichia coli*, *Salmonella typhi* and *Saccharomyces warum* at minimal concentration of 30 mg in case of 10 super (8) numbers of microorganisms/5 ml of broth. It prevented microbial growth in khoa and tomato puree for 7 and 21 days, at refrigerated temperature, when used at concentrations of 0.16 g/100 g and 0.08 g/100 ml, respectively. The major antimicrobial component of essential oil responsible for the inhibition of microbial growth was identified as HMB (Phadke et al., 1994). The antimicrobial activity of essential oil was tested against food borne pathogens responsible for food spoilage and human pathologies. It exhibited strong antimicrobial activity against *Bacillus cereus*, *Bacillus megaterium*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Micrococcus roseus* and *Staphylococcus aureus* at a concentration range of 1:0 with inhibitory activities of 27, 23, 16, 19, 22, 19, and 23 mm, respectively. The roots of *D. hamiltonii*, therefore, may be considered as an inexpensive source of an essential oil rich in antimicrobial compounds against food borne pathogens (Thangadurai et al., 2002).

The antimicrobial activity of different solvent (petroleum ether, benzene, chloroform, ethyl acetate and methanol) extracts of *D. hamiltonii* roots was tested against 15 different food related microorganisms. Among the solvent extracts, methanolic and petroleum ether extracts are the most active exhibiting growth inhibition of 11 and 9 of the 15 organisms, respectively (Thangadurai et al., 2004). Elizabeth et al. (2005) also demonstrated that the *D. hamiltonii* possesses strong antimicrobial property. Mohana et al. (2008b) investigated the antimicrobial potential of *D. hamiltonii* with some other medicinal plants against human pathogenic bacteria. Antibacterial potential of crude petroleum ether extract obtained from leaf callus tissue of *D. hamiltonii* was evaluated against five bacterial pathogens (*S. typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E. coli*) using agar well diffusion method. Among the tested bacterial strains, inhibitory activity of the callus extract was minimum against *K. pneumoniae* (5 mm) and *P. vulgaris* (6 mm), whereas maximum inhibitory activity was observed against *S. typhi* (11 mm) (Thangavel et al., 2011). The petroleum ether, chloroform, and ethyl acetate extracts were examined against typhoid causing organisms. Among these extracts, petroleum ether and chloroform showed a significant activity against *S. typhi, Salmonella paratyphi* A and *S. paratyphi* B, respectively in comparison with ciprofloxacin (Kumuda et al., 2011). Different solvent extracts were tested against four gastrointestinal pathogenic which causes skin disease.

The bacteria include two Gram positive, *S. aureus*, *Bacillus subtilis* and two Gram negative bacteria, *E. coli, K. pneumoniae*. 1 ml of petroleum ether containing 0.098 and 0.049 µg of extracts were recorded as minimal inhibitory concentration (MIC) and minimal inhibitory dose (MID), respectively, which exhibited greater activity against tested pathogens (Samydurai and Thangapandian, 2012c). The *in vitro* antibacterial activity of the various extracts of *D. hamiltonii* was studied against different bacterial species *E. coli, Klebsiella pneumoniae, S. typhi*, *Proteus mirabilis, Vibrio cholerae, Shigella sonnie, Serritias* species, *S. aureus* and *B. subtilis* by disc diffusion method. All the extracts were found to possess different degrees of antibacterial activity except aqueous extract. (Devi and Latha, 2012a)

Antifungal activity

The antifungal activity of aqueous extract of *D. hamiltonii* was evaluated at different concentrations by poisoned food technique against eight species of *Fusarium*, ten species of *Aspergillus*, three species of *Penicillium*, two species of *Drechslera* and *Alternaria alternata*. It was observed that aqueous extract showed significant antifungal activity against all the tested pathogens. Species of *P. chrysogenum* was completely inhibited at 10% concentration. *D. halodes* and *Aspergillus fumigatus* were inhibited at 20% concentration, whereas *Fusarium lateritium* and *Fusarium moniliforme* were inhibited at a higher concentration of 50%. *D. hamiltonii* was further subjected to different solvent extraction using petroleum ether, benzene, chloroform, methanol and ethanol to identify the solvent extract having high activity. It was observed that petroleum ether extract showed highly significant antifungal activity followed by benzene and chloroform extracts (Mohana and Raveesha, 2007). Mohana et al. (2008a) found that the antifungal active principle is a phenolic compound. TLC separation of the phenolic fraction using chloroform as an eluting solvent revealed the presence of seven bands, but the antifungal activity was observed only in band five with resolution or retardation factor value 0.77. The antifungal active compound is identified as HMB based on NMR and MS analysis. The MIC varied between 200 and 700 mg/ml depending on the fungal species. Seed treatment of the active principle significantly increased seed germination and seed vigour with a corresponding decrease in seed mycoflora. The antifungal active compound was effective against all the 24 fungal species tested suggesting broad-spectrum antifungal activity. This plant being an edible one can be exploited in the management of seed-borne pathogenic fungi and the prevention of biodegradation of grains and mycotoxin elaboration during storage.

In vitro antifungal activity assay of different concentrations of HMB isolated from *D. hamiltonii* against

six important seed-borne fungal pathogens, namely, *A. alternata*, *Drechslera tetramera*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Pyricularia oryzae* and *Trichoconis padwickii* isolated from paddy seeds revealed that, the compound HMB showed significant antifungal activity. Among the fungi tested, *F. proliferatum* showed the highest inhibitory activity, whereas *P. oryzae* showed least inhibitory activity. The MIC varied between 350 and 650 µg/ml depending on the fungal species. Comparative evaluation of the active compound with the synthetic fungicide thiram at recommended dosage revealed that, the antifungal activity of the active compound obtained from the plant was almost equivalent (Mohana et al., 2009). The maximum inhibitory activity of 1 ml of petroleum ether containing 0.098 and 0.049 µg of extracts were recorded as MIC and MID against *Candida albicans* which causes skin disease was reported by Samydurai and Thangapandian (2012a). Thippeswamy et al. (2012) reported significant antifungal activity against *C. albicans* and *C. neoformans*.

Insecticidal property

Research work at CFTRI has shown that the roots of *D. hamiltonii* protect the stored grains from insect infections and formulated a novel biopesticide (George et al., 1998). They explained the detailed procedure for the formulation of biopesticide from clean dried roots of *D. hamiltonii* in a step wise manner. They are making the clean dried roots into powder, extracting powdered roots using supercritical carbondioxide/carbon-dioxide and polar and non-polar solvents such as herein at a temperature in the range of 40 to 80°C and at a pressure in range of 100 to 160 bars for a period of 24 to 72 h and getting a creamish white, waxy material from the extract useful as biopesticide. The insecticidal activity of *D. hamiltonii* roots was tested against the grain pests *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. Wheat grains were mixed with 5 and 10% air-dried root powder and infested with *S. oryzae*. The treatments achieved 96 and 100% mortality, respectively, and no progeny emerged from either treatment. Against *R. dominica*, the 5 and 10% treatments achieved 86 and 100% mortality. The progeny emerged per adult insect was 0.004 and 0.000, respectively. The volatile fraction was mainly composed of HMB. In terms of contact toxicity, *T. castaneum* was highly susceptible to HMB, with ED_{50} and ED_{95} values of 1.64 and 6.24 µg/cm², respectively. *S. oryzae* was least susceptible of the 3 pests. Whole grains treated with powdered root and extracts retained their germination capacity up to 3 years (George et al., 1999b).

Rajashekar and Shivanandappa (2010) have isolated a novel bioactive molecule from the roots of *D. hamiltonii* that shows insecticidal properties against the stored-product insects *S. oryzae*, *R. dominica* and *Callosobruchus chinensis* by contact bioassay with lethal concentration (LC_{50}: 0.033 to 0.044 mg/cm^2). This compound proved effective as a grain protectant against stored product insects of wheat and green gram. Root extracts of *D. hamiltonii* were tested for insecticidal activity against the stored products pests, *R. domonica, S. oryzae, Stigobium pancieum, T. castaneum* and *C. chinensis*, by using residual and contact toxicity bioassays. Methanolic extract showed LC_{50} value of 0.14 mg/cm^2 for all the test species in a filter paper residual bioassay. Reduction of F1 progeny was observed in treated grain stored for 3 to 4 months. The extract did not affect the germination of the treated grains and served as a natural grain protectant (Rajashekar et al., 2010).

Antiulcer property

Gastric ulcer is a multi step disease caused due to imbalance between mucosal defense and aggressive factors. Swim stress-induced ulcers with an ulcer index (UI) of 6.0 ± 0.01 was protected up to 43 and 72% at 100 and 200 mg/kg body weight by aqueous extract of swallow root (SRAE), respectively. Rats fed with SRAE showed normal levels of antioxidant enzymes and thiobarbituric acid reactive substances (TBARS). SRAE also normalized ~3.1 and 2.4 folds of increased H^+–K^+–ATPase and gastric mucin, respectively in ulcerous animals. In addition, SRAE also possessed reducing power, free radical scavenging ability with an IC_{50} of 0.17 µg/ml gallic acid equivalent (GAE) and DNA protection properties. Antioxidant, proton pump inhibition as well as boosting of gastric mucin have been implicated to be responsible for antiulcer property of SRAE (Naik et al., 2007). Pectic polysaccharide from swallow root (SRPP), containing phenolics at 0.12 g GAE/g, prevented stress-induced gastric ulcers by 80 to 85%. Normal regulation of gastric mucin, antioxidant/antioxidant enzymes and H+, K+-ATPase in ulcerous animals upon treatment with SRPP was noticed. Histopathological analysis revealed protection to the disrupted gastric mucosal layer and epithelial glands (Srikanta et al., 2007).

Latter alteration of matrix metalloproteinase's, gastric mucin and prostaglandin E_2 levels during polysaccharide mediated ulcer healing was determined in acetic acid induced gastric ulcer models (Wistar albino rats). The potential ulcer healing effect of SRPP was evidenced by ~ 90% reduction in ulcer index; improvement in the antioxidant defense such as increase of glutathione levels together with significant reduction in lipid and protein oxidation and protection to damaged gastric mucin. Histological studies revealed the recovery of mucin by rejuvenation of mucosal epithelium and enhancement of high molecular mass mucin. Matrix Metallo Proteinases (MMPs) that are involved in tissue

injury was found to be modulated by SRPP treatment in addition to increased cytoproductivity due to enhanced synthesis of PGE_2 that necessitates the active proliferation of gastric mucin cells. Further, reduction in ~3 folds of galectin-3, an inflammatory marker suggests gastro protection against acid induced inflammation and gastric wall damages. This indicates the effectiveness of SRPP in ulcer healing (Srikanta et al., 2010). *Helicobacter pylori* mediated gastric ulcers in cancer patients is one of the common global problems since it was found to colonize ~50% of gastric ulcer/cancer patients. *D. hamiltonii* extracts have been depicted with medicinal properties. Attempted to explore antimicrobial property particularly anti-*H. pylori* activity by using HMB was studied. HMB was isolated from the roots of *D. hamiltonii* by hydrodistillation and cold crystallization method; identified by HPLC and characterized using electrospray ionization mass spectrometry (ESI-MS) and confirmed by NMR studies as a compound of molecular mass 152 Da. Isolated HMB was found to inhibit the growth of *H. pylori*, a potential ulcerogen in a dose dependent manner with MIC of ~39 µg/ml; further, it was studied by the lysis of *H. pylori* by electron microscopy and electrophoretic studies. The mechanism of action indicated the counteracting effect of vacuolating toxin (VacA) of *H. pylori* which otherwise would lead to host cell cytotoxicity. Further, the increased binding ability of HMB to DNA and protein offered an impact on DNA protectively and bioavailability (Srikanta et al., 2011).

Antioxidant and cytoprotective activates

Currently, there is a great deal of interest in the study of natural compounds with free radical scavenging activity, because of their potential role in maintaining human health and preventing diseases. In *D. hamiltonii*, different parts of the root, mainly the whole tuber, peal, tuber without peal and medullary portion were subjected to find out the antioxidant rich fraction and its activity (Murthy et al., 2006). The extract was found to contain HMB, medullary portion was found to contain high amount of HMB followed by peal which also showed significant antioxidant activity, but low when compared with the standard pure HMB. In *D. hamiltonii* among all the solvent extracts tested the methanolic and aqueous extracts showed high antioxidant activity measured as scavenging of DPPH (Srivastava et al., 2006b). Isolation of new anti oxidant compound ellagic acid from the aqueous extract of roots of *D. hamiltonii* and its antioxidant and cytoprotective effect was reported by Srivastava et al. (2007). Srivastava and Shivanandappa (2009) reported that the aqueous extract of *D. hamiltonii* has boosted the antioxidant status in rat brain and liver. The cytoprotective and antioxidant activity of free, conjugated and insoluble-bound phenolic acids of *D. hamiltonii* was investigated. A total phenol content of 20.72, 7.97 and 11.52 mg gallic acid equivalents (GAE)/g for free, conjugated and insoluble bound phenolic acid extracts, respectively were identified. At 0.12 lg/ml concentration, conjugated phenolic acids showed 87% cytoprotection (on NIH 3T3 cells) compared to free phenolic acids (47%) and insoluble bound phenolic acids (65%). DPPH radical scavenging activity indicated an IC_{50} of 0.046, 0.06 and 0.128 lg/ml for SRCP, SRIBP and SRFP, respectively. SRCP also showed higher reducing power and DNA protection property (Nayaka et al., 2010). Superior antioxidant activity of *Decalepis* rhizome of all the selected medicinal plants was reported by Naveen et al. (2011).

Antioxidant and cytoprotective properties of several bioactive compounds isolated from *D. hamiltonii* were investigated by several biologists (Srivastava and Shivanandappa, 2011b; Srivastava et al., 2012a, b, c, d; Naveen et al., 2012; Samydurai and Thangapandian, 2012a). They are 2-(hydroxymethyl)-3-methoxybenzaldehyde (HMMB) with IC_{50} values in the nanomolar (5 to 214) range (Srivastava and Shivanandappa, 2011b), 4-hydroxyisophthalic acid (4-HIPA) with IC_{50} values in the nanomolar (2 to 187) range (Srivastava et al., 2012a), 4-(2-hydroxypropan-2-yl)-1-methylcyclohexane-1,2-diol (HPMCD) with IC_{50} values in the nanomolar (56 to 582) range (Srivastava et al., 2012 b), 2,4,8-trihydroxybicyclo [3.2.1]octan-3-one (TBO) with IC_{50} values in the nanomolar (42-281) range (Srivastava et al., 2012c) and 14-aminotetradecanoic acid (Srivastava et al., 2012d). All these bioactive molecules are potent scavengers of superoxide (O_2 -), hydroxyl (OH), nitric oxide (NO), and lipid peroxide (LOO) physiologically relevant free radicals. They also exhibited concentration dependent secondary antioxidant activities, such as reducing power, metal chelating activity, and inhibition of protein carbonylation. Further, at nanomolar concentration prevented $CuSO_4$-induced human LDL oxidation. Apart from the *in vitro* free radical scavenging activity, they demonstrated cytoprotective activity in primary hepatocytes and Ehrlich Ascites Tumour (EAT) cells against oxidative stress inducing xenobiotics. The mechanism of cytoprotective action involved maintaining the intracellular glutathione (GSH), scavenging of reactive oxygen species (ROS), and inhibition of lipid peroxidation (LPO). The ethanol extract of *D. hamiltonii* roots was subjected to antioxidant activity-guided fractionation by repeated silica gel column chromatography to get a pure antioxidant compound and further subjected to extensive analysis of UV, IR, LC-MS, and 2D HMQC NMR by Naveen et al. (2012) to predict the structure. Its structure was elucidated as decalpoline. Decalpoline exhibited multiple antioxidant properties like DPPH scavenging activity, superoxide radical scavenging activity, lipid peroxidation inhibitory activity, metal chelating and total reducing activity (Naveen et al., 2012).

Anti-inflammatory and antipyretic activity

The methanol extract of roots of *D. hamiltonii* at 250 and 500 mg/kg was examined in the carrageenan-induced rat paw edema and cotton pellet induced chronic inflammatory models. A significant dose-dependent anti-inflammatory activity in both models was noticed. The extract exhibited considerable antipyretic activity at 250 and 500 mg/kg, p.o., in brewer's yeast induced pyrexia (Lakshman et al., 2006). The *in vitro* anti inflammatory activity of the pure compounds isolated from *D. hamiltonii* was studied in mitogen induced peripheral blood mononuclear cells (PBMCs) employing [^{3}H] thymidine uptake assay and their effect on cytokine expression. The inhibition of nuclear factor B (NF-B) activity in the presence of pure compounds was determined in J774 A.1 cells. The cytotoxicity of the compounds was tested using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit. Lupeol acetate (compound 1) and (2S)-5,7,4-trihydroxy flavanone 4-O-dglucoside (compound 2) isolated from *D. hamiltonii* roots showed antiinflammatory activity by showing non cytotoxic properties, down regulating TNF-and IL-2 specific mRNA, besides up regulating the synthesis of mRNA of IL-10 and by inhibiting the NF-B activation in J774 A.1 cells (Ashalatha et al., 2010).

The effect of ethanolic extract of roots of *D. hamiltonii* on the acute and chronic anti-inflammatory activities in Wistar rats was evaluated by Ghosh et al. (2011). Ethanolic extract of powdered root of *D. hamiltonii* was prepared and acute inflammatory profile was studied on the basis of paw edema induced by the phlogistic agents (carrageenan, serotonin and histamine, respectively). The chronic anti-inflammatory activity was studied after inducing inflammation with formaldehyde. There was a maximum inhibition of 34.48% in carrageenan induced, 21.71% in serotonin induced and 25.35% in histamine induced acute inflammatory models with ethanolic extract of *D. hamiltonii.* In the chronic inflammatory model, a progressive inhibition of 25.26% (3rd day), 31.25% (5th day), 33.90% (7th day), 44.82% (9th day), 50.40% (11th day) and 46.01% (13th day) was observed indicating that *D. hamiltonii* is a strong anti-inflammatory and anti-arthritic agent that blocks histamine and serotonin path ways (Ghosh et al., 2011).

Chemo protective activity

Chemo protective effect of methanolic extract of *D. hamiltonii* against cyclophosphamide (CTX) induced toxicity was investigated. Intraperitoneal administration of the extract significantly increased the total WBC count (3166±202 cells/cm^2), bone marrow cellularity (680±70.1 cells/femur), alpha-esterase positive cells (641±26.2 cells/4000 cells), weights of organs such as a spleen and lungs, in CTX treated animals. *D. hamiltonii* administration significantly decreased the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), creatinine and urea in serum and increased their levels in liver and kidney. Histopathological analysis of small intestine also suggests that extract could reduce the CTX induced intestinal damage. Similarly, *in vivo* studies using *D. hamiltonii* showed that the extract could significantly decrease the level of super oxide dismutase (SOD) in serum of the treated animal (Shathish et al., 2012).

Anxiolytic activity

The petroleum ether, alcoholic and water extracts of *D. hamiltonii* root were examined for anxiolytic activity by using elevated plus maze model (EPM) and open field test (OFT) in mice. Alcoholic extract exhibited potent anxiolytic effects at 10, 30 and 100 mg/kg, i.p. in both methods of all the solvent extracts tested, whereas petroleum ether and water extract were less effective (Ambavade et al., 2008).

Neuroprotective property

The neuroprotective potential of the *D. hamiltonii* root aqueous extract (DHRAE) was studied against ethanol induced oxidative stress in the rat brain. Ethanol, single dose (5 g/kg body weight), induced oxidative stress in the rat brain which was evident from the increased lipid peroxidation and protein carbonylation, reduced glutathione (GSH), and suppressed activities of antioxidant enzymes such as SOD, CAT, glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione-S-transferase (GST). Pretreatment of rats with multiple doses of DHRAE, 50 and 100 mg/kg body weight, for 7 consecutive days significantly prevented the ethanol-induced oxidative stress. The neuroprotective potential of DHRAE can be attributed to the known antioxidant constituents or its interaction with antioxidant response elements (AREs) which needs to be ascertained (Srivastava and Shivanandappa, 2010b). Differential inhibition of ChE (acetylcholinesterase + butyrylcholinesterase) in various rat brain regions by a single dose of DDVP (1/3 LD_{50}) after 16 h of treatment and protective potential of the aqueous extract of the roots of *D. hamiltonii* (DHA), against DDVP-induced ChE inhibition was investigated. Pretreatment of rats with multiple doses of DHA, 50 and 100 mg/kg body weight, for 7 consecutive days did not produce any significant change in ChE activity. Pretreatment of rats with DHA, at high dose, significantly protected against DDVP-induced ChE inhibition in all the brain regions except cerebellum, whereas pretreatment

of rats with DHA, at low dose, showed significant protection in striatum, cortex, and pons against DDVP-induced ChE inhibition. The protective activity of DHA can be attributed to the characterized potent antioxidant constituents who could have an important role in preventing ChE inhibition by inducing the DDVP detoxifying enzymes (Srivastava and Shivanandappa, 2011a).

Antidiabetic activity

Ethanolic extract of the roots of *D. hamiltonii* was evaluated for its antidiabetic and hypolipidemi activities in experimentally induced diabetic rats. Administration of ethanolic extracts (100, 200 and 400 mg/kg, oral) once daily up to 28 days to diabetic rats reduced blood glucose and glycosylated hemoglobin, and increased insulin levels significantly. Triglycerides (TG), total cholesterol (TC), VLDL and LDL levels were significantly decreased, whereas HDL levels were increased. The extract also significantly reduced reactive TBARS, SOD and increased the levels of reduced GSH and CAT. It is evident that alcoholic extract of the roots of *D. hamiltonii* can be effectively used as antidiabetic and hypolipidemic (Ragini et al., 2010).

Hepatoprotective effect

The hepatoprotective activity of the aqueous extract of the roots of *D. hamiltonii* with known antioxidant constituents was studied against ethanol and carbon tetrachloride (CCl_4) induced liver injury in rats. Pretreatment of rats with aqueous extract of the roots of *D. hamiltonii*, single (50, 100 and 200 mg/kg body weight) and multiple doses (50 and 100 mg/kg body weight for 7 days) significantly prevented the ethanol and CCl_4 (1 ml/kg body weight) induced hepatic damage as indicated by the serum marker enzymes (AST, ALT, ALP, and LDH). Parallel to these changes, the root extract also prevented ethanol and CCl_4 induced oxidative stress in the rat liver by inhibiting lipid peroxidation and protein carbonylation, and restoring the levels of antioxidant enzymes (SOD, CAT, GPX, GR, and GST) and GSH. The biochemical changes were consistent with histopathological observations suggesting marked hepatoprotective effect of the root extract in a dose dependent manner (Srivastava and Shivanandappa, 2006, 2010a).

The hepatoprotective activity of the methanolic extract of the roots of *D. hamiltonii* was also investigated. The hepatotoxicity produced by acute CCl_4 administration was found to be inhibited by pretreating the rats with crude methanolic extract of the roots of *D. hamiltonii* prior to CCl_4 induction. Hepatotoxic inhibition was measured with the decreased levels of hepatic serum marker enzymes (glutamate-pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and lipid peroxide formation. Imbalance level of GSH and antioxidant enzymes such as CAT, GPX and GR were normalized in rats pretreated with *D. hamiltonii* extract. Pathological changes of hepatic lesions caused by CCl_4 were also improved by pretreatment with the *D. hamiltonii* root extract (Harish and Shivanandappa, 2010). Hepatotoxicity was induced by administering a oral dose of acetaminophen (2 g/kg body weight) to rats for 10 days. When rats with hepatotoxicity were treated with the extract of *D. hamiltonii*, the serum ALT, AST, ALP, bilirubin and total protein levels reverted to near normal, while the hepatic concentration of CAT, SOD, GSH and ascorbic acid were significantly increased and that of LPO significantly lowered, this indicating that the extract of *D. hamiltonii* has strong hepatoprotective property (Devi and Latha, 2012b). Recently, it has reported that treatment with aqueous (50 and 100 mg/kg body weight p.o.) extract of *D. hamiltonii* mitigated the CycloPhosphamide induced oxidative stress and protected the liver from toxicity (Zarei and Shivanandappa, 2013).

Effect on reproduction

The effect of crude extract from *D. hamiltonii* on reproductive potential of male rats was investigated by Shereen (2005). The rats were fed with diets containing *D. hamiltonii* root powder and it was found that there was no significant effect on the fertility or fecundity and the survival of the pubs born to those rats.

Conservation studies

D. hamiltonii is an endangered, endemic, non timber forest resource of Southern peninsular India which requires attention to develop simple propagation techniques (Jacob, 1937). The aromatic roots of *D. hamiltonii* are highly prized for their role in preparation of natural cool drinks and Ayurvedic drugs. Even though it has great economic importance, the plant has restricted distribution, that is, endemic to southern states of India. The tribal people collect the roots regularly to sell in the market. It was observed that there is a large demand for the roots of *D. hamiltonii.* Many tribal families have selected the root collection activity as a livelihood throughout the year. Natural population of *D. hamiltonii* is declining due to over exploitation and habitat destruction and now it was considered as endangered. Regeneration of this species is severely affected, since most of the plants were harvested in a reproductively immature

stage. So, it is important to investigate the seed storage behavior and germination strategies. Development of *in vitro* and *in vivo* cultivation methods are the pre-requisites steps for conservation and commercialization of this endangered and economically important aromatic plant.

Seed viability and germination studies

Conservation of *D. hamiltonii* is very difficult due to low percentage of seed germination for *in vitro* and *ex vitro* cultivation of this species. Raju and Ramana (2009) conducted experiments on *ex vitro* seed germination and seedling establishment in *D. hamiltonii* and found that only 18% of seed germination and 6% seedling establishment to the total seeds sown. Anandalakshmi and Prakash (2009) have investigated on viability and germination of *D. hamiltonii* seeds by conducting several experiments *in vitro*. They observed that moist filter papers are found to be better substrata for germination than sand. Pretreatment studies revealed that soaking in hot water (60°C) for 24 h significantly improved the germination percentage from 83 to 98% on moist filter paper, and from 15 to 28% on sand. Pretreatment study coupled with microscopic studies indicated that about 14% of seeds were found to be hard seeded. Seeds could tolerate desiccation up to 10% moisture content (65% germination), while nil germination was recorded at 5% seed moisture content. The seeds were found to be chilling sensitive showing total mortality at 10°C. Seed deterioration studies showed rapid depletion of seed metabolites indicative of short viability. Based on storage behaviour, *D. hamiltonii* seeds could be classified as intermediate seeds. From seed perspective, the hard seed coat, the need for a well aerated substrate and the short viability could be the likely impeding factors for inadequate regeneration of the species. Saini and Giridhar (2012) found that exposing seeds to 0.3% H_2O_2 resulted in effective (94%) seed germination in *D. hamiltonii*. Similarly, 100% of immature zygotic embryos germination on MS medium supplemented with GA_3 (0.05 ppm), 6-benzylaminopurine (BAP, 1.0 ppm) and TDZ (1.0 ppm) was also reported.

Cultivation

Development of *in vivo* cultivation methods is an important issue for its commercial development as food and health drink. Vedavathy (2004) standardized the cultivation of *D. hamiltonii*. Loamy soils are well suited for the growth of this plant. However, soils inter mixed with rocks will show adverse effect on growth; the length and thickness of the roots mostly depend on the soil rather than the environment. The plant is disease free and needs no special care. It can be propagated by seeds and stem cuttings. Seed cultures are preferred for commercial propagation. The seeds have short viability; hence fresh seeds are to be used to get healthy seedlings. Direct sowing of the seeds in fields is not an appropriate method. Development of seedlings in nursery beds or in polythene bags and then transplantation in to the field is recommended. Germination starts within 2 weeks and continue for another week. Plantation of 3 months old seedlings with 3 to 4 leafs in main field at 60 × 60 cm will give good yield. The field should be irrigated with water twice in summer and once in winter. It can also be cultivated under rain fed conditions.

In vitro conservation

A number of biologists tried to standardize the *in vitro* propagation systems for conservation of this endangered species (Table 2). Optimization of three quality factors (the number of multiple shoots, shoot length, and number of leaves) pertaining to regeneration of plantlets from leaf calli of *D hamiltonii* were investigated (George et al., 2000). The variables evaluated were the levels of sucrose, BAP and NAA. The increase in shoot number was evident in clusters of shoots obtained when the NAA concentration was least (0. 26 µM/L) and the BAP was maximum (8.43 µM/L), while the sucrose has no effect, whereas maximum shoot length was obtained when sucrose concentration was 3%, NAA concentration minimum (0.26 µM/L) and BAP concentration maximum (8.43 µM/L). Similar type of report, that is, the influence of BAP at higher concentration (8.88 µM/L) and lower concentration of NAA (0.5 µM/L) on effective shoot regeneration from axillary buds of *D. hamiltonii* was reported by Bais et al. (2000a). A maximum of 4 shoots per explant were formed when nodal explants were cultured on medium fortified with 17.76 µM/L BAP and 0.53 µM/L NAA (Anitha and Pullaiah, 2002). Reddy et al. (2002) studied the effect of triacontanol (TRIA) on shoot multiplication from *in vitro* derived shoot tips of *D. hamiltonii*. TRIA was administered at 2 to 20 µg/L. TRIA resulted in the highest promotion of axillary shoot proliferation at maximum concentration of 20 µg/L. An efficient two-stage protocol was developed for induction of multiple shoots from single node *in vitro* shoot tip explants of *D. hamiltonii* (Giridhar et al., 2003). It was found that phenyl acetic acid (PAA) had a synergistic effect on shoot multiplication when treated with BAP. They suggested that PAA can be helpful for multiple shoot induction from nodal explants, elongation of primary shoots and initiation of adventitious shoots from primary shoots. MS medium containing BAP (2.22 to 31.08 mM/L) and NAA (0.27 to 10.74 mM/L) or PAA (7.34 to 36.71 mM/L) was used to initiate shoot formation from nodal explants. Maximum number of shoots per culture was produced on a medium containing 31.08 mM/L BAP

Table 2. Tissue culture students in *Decalepis hamiltonii*.

S/N	Medium composition	Explant	Response	References
1	MS + BAP (8.88 µM/L) + NAA (0.5 µM/l)	Nodes	Shoot proliferation	Bais et al. (2000a)
2	MS + $AgNO_3$ (40 µM/L)	*In vitro* derived shoots	Rooting	Bais et al. (2000b)
3	MS + NAA (0. 26 µM/L) + BAP (8.43 µM/L)/ 3% sucrose	Leaf callus	Multiple shoots	George et al. (2000)
4	MS + IBA (8.8 mM/L) + IAA (1.43 mM/L)	Micro shoots	Rooting	Reddy et al. (2001)
5	MS + BAP (17.76 µM/L) + NAA (0.53 µM/L)	Seedling explants	Multiple shoots	Anitha and Pullaiah, (2002)
6	MS + TRIA (20 µg/L)	*In vitro* derived shoot tips	Shoot proliferation	Reddy et al. (2002)
7	MS + BAP (31.08 mM/L) + PAA (14.68 mM/L)	Single node *in vitro* shoot tips	Shoot proliferation	Giridhar et al. (2003)
8	MS + BAP (1.1 µM/L) + GA_3 (5.8 µM/L) + PG (800 µM/L)	Nodes	Multiple shoots	Gururaj et al. (2004)
9	MS + zeatin (13.68 mM/L) + BAP (10.65 mM/L)	Leaf	Somatic embryogenesis	Giridhar et al. (2004a)
10	MS + 2iP (4.9 mM/L)	Shoot tips	Multiple shoots	Giridhar et al. (2005a)
11	MS + BAP (5.0 µM/L) + IAA (0.5 µM/L) + Ads (30.0 µM/L)	Nodes	Shoot regeneration from synthetic seeds	Sharma and Shahzad (2012)

and 14.68 mM/L PAA, whereas maximum shoot length and nodes were obtained on medium containing 22.2 mM/L BAP and 14.68 mM/L PAA. Shoots subcultured on MS medium containing 22.2 mM/L BAP and 14.68 mM/L PAA induced elongated shoots along with secondary shoot formation. Similar type of report by using phloroglucinol (PG) along with BAP, GA_3 and TRIA was reported by Gururaj et al. (2004). They found that 1.1 µM/L BAP, 5.8 µM/L GA_3, and 800 µM/L PG induced maximum number of shoots. Sub culturing of the shoots onto MS medium containing 5.6 µM/L BAP, 200 µM/L PG, 0.011 µM/L TRIA produced elongated shoots along with secondary shoot formation.

The influence of 2.5 to 7.5 mM/L 2iP, 4.4 to 17.7 mM/L BAP, 2.3 to 4.7 mM/L KN, 2.8 to 6.8 mM/L TDZ and 2.3 to 11.4 mM/L zeatin alone and in combination with 0.3 to 0.9 mM/L (IAA) on *in vitro* multiple shoot production was investigated by Giridhar et al. (2005a). The maximum number of multiple shoots (6.5±0.4) was induced from shoot tips cultured on MS medium containing 4.9 mM/L 2iP. But, both zeatin (9.1 mM/L) and KN (4.7 mM/L) in combination with IAA (0.6 mM/L) were able to produce a maximum of 5.0±0.4 and 5.1±0.4 multiple shoots, respectively. Further elongation of shoots and adventitious shoot formation was obtained on medium containing 2.5 mM/L 2iP and 0.3 mM/L GA_3.

A novel protocol has been developed for inducing plantlets through somatic embryogenesis from leaf cultures of *D. hamiltonii* within 12 to 14 weeks (Giridhar et al., 2004a). Callus was obtained from leaf sections on MS medium supplemented with a NAA, BAP and 2,4-D. Nodular embryogenic callus was developed from the cut end of explants on media containing 2,4-D and BAP, whereas compact callus developed on media containing NAA and BA. Upon subsequent transfer of explants with primary callus onto MS media containing zeatin and/or GA_3 and BAP, treatment with zeatin (13.68 mM/L) and BAP (10.65 mM/L) resulted in the induction of the highest number of somatic embryos directly from nodular tissue. The maturation of embryos took place along with the induction on the same medium. Embryogenic calluses with somatic embryos were sub cultured onto MS basal medium supplemented with 4.56 mM/L zeatin and 10.65 mM/L BAP. After 4 weeks, more extensive differentiation of somatic embryos was formed. The mature embryos developed into complete plantlets on growth regulator-free MS medium.

Development of plantlets that survive effectively in the field conditions is an important issue to be remembered in developing any successful micropropagation system. *In vitro* rooting plays an important role in successful field establishment of plantlets. Bais et al. (2000b) for the first time investigated on *in vitro* rooting of *D. hamiltonii* by using silver nitrate ($AgNO_3$). They found that medium comprising of MS salts with IAA (2.88 µM/L), induced poor rooting and root emergence did not occur until after 25 days. The resultant roots were stunted. However, the addition of 40 µM/L $AgNO_3$ improved root initiation and

elongation. The promotive effects of $AgNO_3$ on rooting may result from inhibition of ethylene action. Upon addition of ethephon to the rooting medium, excessive callusing was observed in explants in all the treatments. Addition of 40 µM/L $AgNO_3$ to ethephon containing medium resulted in improvement in root initiation and elongation. Ethylene production was monitored in all the treatments with IAA/$AgNO_3$/ethephon and it was observed that the treatment with IAA (2.88 µM/L) alone showed a greater increase in ethylene production when compared with $AgNO_3$, ethephon and their combinations.

Latter for efficient rooting of *in vitro*-derived micro-shoots by using phloroglucinol (PG), activated charcoal and $CoCl_2$ was investigated by Reddy et al. (2001). Transfer of *in vitro* derived shoots to MS medium supplemented with 8.8 mM/L (indole-3- butyric acid, IBA) and 1.43 mM/L IAA resulted in root induction. IBA was found to be a good root-promoting agent, dipping of explants in 4.4 µM/L IBA for 30 min and subsequent inoculation on MS basal medium was also beneficial for root induction. Maximum of 2 to 3 roots were formed when the microshoots were rooted on medium containing 16.11 µM/L NAA (Anitha and Pullaiah, 2002), whereas Reddy et al. (2002) found that TRIA at 5 and 10 µg/L was playing an effective role in root induction of *D. hamiltonii*. Giridhar et al. (2003) found that the shoots rooted on medium containing 9.8 mM IBA showed 80 to 90% survival rate under field conditions. The efficient root formation when the elongated shoots of *D. hamiltonii* inoculated on medium supplemented with various phenolic compounds along with 9.8 mM/L IBA within 5 to 6 weeks was reported by Giridhar et al. (2005a). They found that phloroglucinol and salicylic acid interaction with IBA stimulated maximum *in vitro* rooting of shoots.

Germplasm maintenance by employing encapsulation technology was also investigated in this endangered plant. Plantlet regeneration from encapsulated nodal segments of *D. hamiltonii* was investigated by Sharma and Shahzad (2012). A gelling matrix of 4% sodium alginate and 100 mM $CaCl_2 \cdot 2H_2O$ was found most suitable for the production of ideal Ca-alginate beads. Maximum shoot re-growth (77.00±2.09%) was recorded on MS basal medium supplemented with 5.0 µM/L BAP, 0.5 µM/L IAA and 30.0 µM/L adenine sulphate. Microshoots, recovered from encapsulated nodal segments (capsule) were best rooted on half-strength MS medium containing 2.5 µM/L NAA. This method was helpful for short-term storage and conservation of the plant germplasm.

Conclusion

The scientific research on *D. hamiltonii* suggests that this plant has huge biological potential. It is strongly believed that the detailed information regarding phytochemistry and various biological properties presented in this paper providing evidence for use of this plant in curing various ailments. A number of phytochemicals were isolated from the roots of *D. hamiltonii*. The phytochemicals exhibited different structural characteristics with various pharmacological actions. There is a demand to standardize the active principle of this plant in the form of modern standardized drug. To develop new drugs it is important to know the mode of action of that particular active principle in human beings. This could be achieved by molecular modeling studies involving the interaction of bioactive molecules with respective target sites. So, it is important to study the interaction of active principles obtained from *D. hamiltonii* with that of their respective target receptors and their mode of action by various clinical trials and by molecular diagnostic studies, etc. In future by this studies, *D. hamiltonii* can use as a source to pharmaceutical industry to develop new medicine of commercial importance. Seed germination, *ex vitro* and *in vitro* plant regeneration methods were also mentioned in the present paper which will help successful conservation and commercial cultivation of this species. The *in vitro* techniques developed were helpful in genetic transformation studies to further enhance the active principle present in this plant and to develop resistant hybrids to all environmental stress. These techniques can also be useful to develop callus and cell suspension cultures to study elicitation and biotransformation studies for commercial production of active principles *in vitro*.

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ABBREVIATIONS

^{13}C NMR, Carbon 13 NMR; **^{1}H NMR,** proton NMR; **2,4-D,** 2,4, dichlorophenoxyacetic acid; **2iP,** isopentenyladenine; **AC,** activated charcoal; **Ads,** adeninesulphate; **$AgNO_3$,** Silver nitrate; **ALP,** alkaline phosphatase; **ALT,** alanine transaminase; **AST,** aspartate transaminase; **BA,** N6-benzyladenine; **BAP,** 6-benzylaminopurine; **CAT,** catalase; **CFTRI,** central food technological research institute; **$CoCl_2$,** cobalt chloride; **CTX,** cyclophosphamide; **DNA,** deoxyribonucleic acid; **EAT,** Ehrlich's ascites tumour cells; **ED,** effective deposit; **GA_3,** gibberellic acid; **GAE,** gallic acid equivalent; **GC,** gas chromatography; **GC-MS,** gas chromatography mass spectroscopy; **GOT,** glutamate oxaloacetate transaminase; **GPT,** glutamate, pyruvate transaminase; **GPX,** glutathione peroxidase; **GR,** glutathione reductase; **GSH,** glutathione; **GST,** glutathione S-transferase; **HDL,** high density lipoproteins;

HMB, 2-hydroxy-4-methoxy benzaldehyde; **HPLC,** high performance liquid chromatography; **HPMCD,** 4,(2,hydroxypropan,2,yl),1, methylcyclohexane,1,2,diol; **IAA,** indole-3-acetic acid; **IBA,** indole-3- butyric acid; **IC,** concentration of extract that provide inhibition; **KN,** kinetin; **LC,** lethal concentration; **LDH,** lactate dehydrogenase; **LDL,** low density lipoproteins; **LOO,** lipid peroxide; **LPO,** lipid peroxidation; **MIC,** minimal inhibitory concentration; **MID,** minimum inhibitory dose; **MMPs,** matrix metalloproteinases; **MS,** mass spectroscopy; **MS medium,** Murashige and Skoog medium; **NAA,** *α*-naphthaleneacetic acid; **NMR,** nuclear magnetic resonance spectroscopy; **NO,** nitric oxide; **O_2,** superoxide; **OH,** hydroxyl; **PAA,** phenyl acetic acid; **PG,** phloroglucinol; **Rf,** resolution or retardation factor; **ROS,** reactive oxygen species; **SGOT,** serum glutamate oxaloacetate transaminase; **SGPT,** serum glutamate pyruvate transaminase; **SOD,** super oxide dismutase; **SRAE,** aqueous extract of swallow root; **SRPP,** pectic polysaccharide from swallow root; **TBARS,** thiobarbituric acid reactive substances; **TC,** total cholesterol; **TDZ,** thidiazuron; **TG,** triglycerides; **TLC,** thin layer chromatography; **TRIA,** triacontanol; **VLDL,** very low density lipoproteins; **WBC,** white blood cells.

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