



Decalepis arayalpathra: Ethnobotany, Scientific Interventions and Prospects

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Abstract

Decalepis arayalpathra (J. Joseph & V. Chandrasekharan) Venter, (Syn. *Janakia arayalpathra* J. Joseph & V. Chandrasekharan), belonging to the family Apocynaceae, is a chasmophytic plant which grows in the crevices of rocks. Due to overuse of this species the number of plants has been decreased in the native habitats and is enlisted as a critically endangered plant. The in vitro established plantlets of *Decalepis arayalpathra* i.e., KMA 05 was obtained from CSIR-CIMAP, Lucknow. One of the samples of the KMA 05 clone was dried and submitted to Herbarium of Department of Botany, University of Delhi. The KMA 05 clones were multiplied, and acclimatization experimentations were performed. Phytochemical investigations of the root extract of in-vitro grown metabolites were done and elicitation experiment was performed using one of the phyllospheric bacterium, *Methylobacterium* sp. VP103. Biopriming experiments using *Methylobacterium* sp. VP103. On in vitro established plantlets was also performed. The successful establishment in green house conditions indicated that the silent growing harmless bacterium provided some internal strength to the plant in terms of balancing the endogenous auxin to cytokinin ratio which is important for the plants to grow in outer environments. The *Methylobacterium* sp. VP103 can be explored in future on molecular levels for its plant hormone production. The whole genome sequencing of these bacteria can be done to check for the presence of genes for cytokinin and auxin production.

Keywords: Apocynaceae; Chasmophytic; Alpha Amyrin; Elicitation; *Methylobacterium*; Biopriming

Abbreviations: CSIR-CIMAP: Central Institute for Aromatic and Medicinal Plants; IAA: Indole-3-Acetic Acid; ACC: Aminocyclopropane 1-Carboxylate; PDF: Peptide Deformylase.

Introduction

Decalepis arayalpathra [1] Venter, belonging to the family *Apocynaceae*, is a chasmophytic plant which grows in the crevices of rocks (Figure 1). This species is confined

to few forest segments and therefore considered as narrow endemic species [2]. The root of this plant is medicinally very important because of its role in making an anticancerous drug formulation against Ehrlich's ascites carcinoma [3].

According to recent reports, the tuberous root oil of this plant contains high amount (96.8%) of the flavor compound 2H4MB [4]. Due to overuse of this species the number of plants has been decreased in the native habitats and is enlisted as a critically endangered plant [2,5]. Scanty seed

germination and poor rooting on the stem cuttings are the few main physiological reasons for its decrease in number from the native habitats [6]. Genetic diversity of this plant is affected due to the self-compatible nature [2]. This species must be restored in its native wild habitats and the area is totally banned for collection of the plant material [7]. Only 2000 individuals in 12 populations are left which is a matter of great concern according to National Biodiversity Act [8].

Ethnobotany

The Kani tribes of Kerala call this plant as “Amruthpala”; “Amruth” meaning life giving and “pala” meaning milk [6]. In Tamil Nadu, it is locally called as “Palarasu” [5]. According to folklore, this plant was brought by Lord Hanuman to cure Laxmana. It is also being mentioned in the Oushadha Nighantu (The Dictionary of Medicinal Drugs, by Thayyil Kumar K [9] that Lord Hanuman drank the milky juice of this plant to gain strength; hence, this drug is also called “Mritha Sanjeevani” (a lifesaving drug) or ‘Sanjeevani Thampra Rasayani’ as mentioned in Oushadha Nighantu.

Scientific Interventions and Prospects

This plant was discovered by Joseph, et al. [1]. They named it as “*Janakia arayalpathra*” in which the generic name was given in honour of Dr. Janaki Ammal (a renowned

Indian botanist), and the specific epithet because of its leaves resembling those of *Ficus religiosa* and “Ficus” in Tamil being known as “arayal” and leaf as “pathra” [10].

Microbes have very easy access to its roots and the roots, become infected and rotten after rainfall. To study the fungal assemblages over healthy and infected roots, a comparative study indicated that *Trichoderma* species was present in abundance in the healthy as well as diseased root samples, while *Fusarium* and *Mucor* species were found to be present in the rotted samples, thus giving a possible indication towards the decline in population of this plant [11].

Tissue culture methods are very fruitful for those plants having problems in poor seed set and rooting on stem cuttings. Therefore, for this plant an efficient protocol for clonal propagation through seeds was established [12] at Central Institute for Aromatic and Medicinal plants (CSIR-CIMAP), Lucknow under DST project (Ref. no.: SR/WOS-A/LS-242/2012). The seeds were provided and authenticated by Dr. Sunderasen Velusamy (Plant Taxonomist at CSIR-CIMAP, Lucknow) (Figures 1-3). The in vitro established plantlets bearing accession no. KMA 05 were procured for further acclimatization and secondary metabolite production studies in the Department of Botany, University of Delhi.



Figure 1: Plant in the native habitat. (Photo courtesy: Dr. Sundaesan Velusamy).



Figure 2: Seeds of *Decalepis arayalpathra*; Ventral view of a single seed; Dorsal view of another seed. (Samples provided by Dr. Sundaresan Velusamy).



Figure 3: Specimen of the in vitro established plantlet bearing accession no. KMA 05.

Acclimatization or hardening is the last step of micro propagation, the strategy with which the tissue culture or in vitro grown plantlets survive in the outer environment. Many micro propagated plants fail to survive even after efficient shoot and root induction process in the outer environment. Due to poor acclimatization and hardening, the in vitro propagated plantlets fail to establish in the outer environment, and the only option is to grow them under laboratory conditions. But chromosomal aberrations are observed in laboratory grown plantlets [13]. Because of this, the phenotypic variation the amount of principal chemical compound expression gets affected. Therefore, it cannot be kept in the laboratory for a long time. The bio-priming of tissue culture grown plantlets in which plantlets are presensitized for acclimatization and survival in field conditions is a very efficient method which helps the plant to establish easily in the outer environment [14] without disturbing its genetic composition [15].

The plants always cross talk with the microbes present in the air as well as in the soil and recruit them for their own benefit according to their choice, this is called adaptive mechanism of plants in natural environment. The rhizosphere and phyllosphere of the medicinal plants are an enriched source of beneficial microbes which are plant growth promoters as well as antibiotic producers [16,17] and can be used for bio-priming. For *Decalepis arayalpathra* KMA 05 clones bioprimering experiments were performed using one of the phyllospheric bacterium, *Methylobacterium* sp. VP103 [18].

Methylobacteria are ubiquitous but are generally present in the air and are known for their uniqueness in recruitment by plants for their benefit according to their choice [19]. They get attracted to the volatiles especially to the methanol [20], which are produced by the pectin demethylation during cell [21]. These volatiles or by-products of plants are generally

released through the stomatal pores, hence the phylloplane is a good source for the presence of *Methylobacterium* [22] and this is present on the plant as an epiphyte [23], or as an endophyte [24]. It was shown by scanning electron microscopy images in the leaves of field established plantlets of *D. arayalpathra* KMA 05 clones that the *Methylobacteria* were present around stomatal region and it was confirmed through 16srRNA sequencing methods [18].

Although it is not confirmed yet, that it is present as an endophyte or the plant recruits as a part of its survival mechanism. Members of the Genus *Methylobacterium* are popularly known for the production of various plant growth promoting hormones, such as Indole-3-acetic acid (IAA), cytokinins or vitamins [25]. *Methylobacterium oryzae* was isolated from the stem tissues of rice and was a 1-aminocyclopropane 1-carboxylate (ACC) deaminase producing bacterium which utilized ACC as a nitrogen source [26]. The plant growth promoting bacteria which can utilize ACC as a nitrogen source would prove to be a good source for making bacterial based sprays and fertilizers, which would play a great role for ecofriendly agriculture. The plants which face stress (abiotic and biotic) produce ACC in good amount and the species of *Methylobacterium* which utilize ACC as a sole nitrogen source will help the plants indirectly in escaping with these problems [27]. *Methylobacterium* sp. VP 103 can be explored in future for its ACC deaminase production also and in turn used as a potential PGPR in fields.

It is approximated through ancient ayurvedic literatures, that 60% of the plants have important chemical constituents in their roots. These medicinally important plants which are used for making drug preparations are collected from their native habitats and, since the roots are the principal source of the drug, the whole plant gets uprooted [28]. *D. arayalpathra* shows poor rooting on stem cuttings; therefore, for vegetative propagation, the upper part of the plant cannot be used and gets wasted. Since it is a perennial plant, it takes 5 years for maturation of roots. *Agrobacterium rhizogenes* mediated hairy root cultures is one of the major solutions for obtaining the important compounds present in the roots [29].

For those plants which fail to produce hairy roots by infection of *A. rhizogenes*, the normal root culture system can be used, although in normal cultures, roots are produced at slow pace [28]. In many cases, the plants face the problem of rooting in liquid medium due to hyperhydricity and do not grow through traditional normal root cultures [30]. These plants can be grown in the laboratories on solid medium; however, growing on solid medium is not economical, although no alternative methods have been discovered so far. The above said methods offer opportunities for isolating drugs from the root extracts in the laboratory, without disturbing the native habitats. The major principal

metabolite 2H4MB (2 Hydroxy 4 methoxy Benzaldehyde) was shown to be produced from internode callus cultures and root cultures [31].

The hairy root culture methods are more appropriate and economical and can be used for those plants which are not recalcitrant to *A. rhizogenes* infection, as these cultures are genetically stable and can survive without supplementing hormones in the basal medium for a long time. The possible cause of failure to produce hairy roots is the production of stress hormone, ethylene that hampers the root initiation pathway [32].

The secondary metabolites can also be produced through callus culturing methods, as in these methods de novo expression of the chemicals take place which depends on the media combination and various physical parameters under laboratory conditions. Therefore, this is also a unique feature of a plant to produce callus. In all these processes, the characteristic feature of the plants, the de novo expression, plays an important role [33-36].

The aromatic compounds are always being used as an important remedy against microbial problems and are a safer option since ages. Instinctively, a naturalist is always in search of some good herbal extracts as well as compound to be applied in curing the routine diseases of mankind because no side effects are associated with this plant-based compounds. Natural products as a remedy to diseases are one of the major sources of new drug molecules today. The sources of these drug compounds can be prokaryotic bacteria, eukaryotic microorganisms, plants, and animals. According to literature survey, microbial and plant products occupy key position in the antimicrobial compounds discovered until now [37].

D. arayalpathra is a perennial plant, the roots of which after maturation produces milky latex which has a specific aroma similar to vanillin [38], and is known for its rejuvenating and curing properties since ancient times. The aromatic properties of its root are due to the major marker compound, 2H4MB which is also present in some other plants like *Utleria salicifolia* Bedd., *Hemidesmus indicus* [39,40], *D. hamiltonii* [41], *Mondia whitei* [42] and *Periploca sepium* [43]. 2H4MB has been reported for its anti-fungal [44], antibacterial [45], anti-acetylcholinesterase [46], anti-tyrosinase [47] and antileukemic [48] activity.

The root aroma of *D. arayalpathra* was also investigated by Verma, et al. [49], in which the hydro distilled volatile oil of the roots was revealed by the presence of 2H4MB (96.8%) along with some other minor or trace constituents. Gastric antisecretory and anti-ulcer activity of *D. arayalpathra* was also reported by Shine, et al. [50]. They observed that, in pylorus ligated rats the ethanolic extract of roots of *D.*

arayalpathra inhibited the pepsin secretion at a dose of 250 mg/kg and the gastric juice volume and acid output at a dose of 500 mg/kg and pretreated rats with, the extract (500 mg/kg) protected the rats with peptic ulcers.

Molecular docking is the mechanism through which the scientists can perform the protein drug interaction on computers and by checking their binding affinities they can screen the compounds to be used as ligands for validating the data [51-54]. Molecular docking experiments are increasingly needed by 'common' biologists for performing various other tasks such as screening for enzymatic substrates or validation of experimental data. Today, the available information on biological activity and proteins structure has increased to a level that makes the computational prediction of binding affinities for drug ligand complexes. This mechanism is time saving and economical also [55]. Molecular docking experiments was performed using one of the metabolite produced from in-vitro grown cultures of *D.arayalpathra* KMA 05 clones i.e., alpha amyirin [56] to dock with the peptide deformylase (PDF) protein of *Helicobacter pylori*. (<http://hdl.handle.net/10603/372219>) [57]. According to the results obtained alpha amyirin can be used as a lead compound in the preparation of anti-helicobacter pylori drugs.

D. arayalpathra KMA 05 is a tissue culture raised plantlet which is adversely affected by the outside environment and its hairy root cultures cannot be produced through *Agrobacterium* strains (MTCC-532 AND ATCC-15834). Previous attempts have been made at CSIR-CIMAP under DST project (Ref. no.: SR/WOS-A/LS-242/2012). Earlier micropropagation studies were carried out by Sudha et al. in which seed based plants did not survive under field conditions. In a study, *Agrobacterium rhizogenes* mediated transformation was also done [29] in which TR105 strain was successful in inducing hairy roots from the hypocotyl. The major compound, 2H4MB produced by the plants in native habitats was shown to be expressed in the normal root cultures (0.16%) [28]. In hairy root cultures its content was enhanced (0.22%) as reported by Sudha, et al. [29], stating that this could be due to the huge biomass of roots produced by *A. rhizogenes* (TR105) infection. Moreover, the hypocotyls give more biomass than cotyledons that might be due to the basipetal movement of auxins, which suggested that those explants having more accumulation of auxins are better for hairy root induction. The *Agrobacterium* strain MTCC-532 has been found to induce callus formation in *D. arayalpathra*. Callus induction studies were also carried out by Thangavel K [58] who showed that the callus biomass increased on solid media provided, but there was no production of 2H4MB.

Suspension cultures were also induced with these solid cultures, but there was no sign of 2H4MB production from these cell cultures also. Nine types of chemical compounds, i.e., α -amyirin acetate, 2-hydroxy,4-methoxy benzaldehyde, 4-methoxy salicylaldehyde, magnifcol (12,20(29)-lupadien-3-ol), β -sitosterol, 3-hydroxy-p-anisaldehyde, naringenin, kaempferol and aroma dendrin were isolated from the crude extracts of naturally grown old tubers by Chacko, et al. [59]. Out of these, the production of only one major compound, 2H4MB was studied in vitro through normal root cultures and in hairy root cultures, and the production of the same were enhanced. Remaining of the eight compounds are also medicinally important and their production and evaluation need to be undertaken in order to get a sustainable development of these metabolites. Antimicrobial and antioxidant activities of 2H4MB have been reported by Wang, et al. [60]. Antifungal activity of 2H4MB against seed borne pathogens were also reported by Mohana, et al. [61].

Conclusion

The *D. arayalpathra* KMA 05 clones can be exploited in future to produce the principal chemical component 2H4MB. One of the compounds alpha amyirin got enhanced when *Methylobacterium* sp. VP103 was used as an elicitor in tissue culture grown KMA 05 variety of *D. arayalpathra*. *Methylobacterium* sp. VP 103 potential can be checked at molecular levels. One of the phytochemical alpha amyirin expressed in the tissue culture grown roots can be exploited for its anti-*Helicobacter pylori* activities in future because of suppression of Peptide deformylase protein and molecular docking results at the scale 10 was more than 5.

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