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In Vitro Conservation and Enhancement of Secondary Metabolite Production in Leptadenia reticulata

Dr.Jitendra Malviya*, Dr. Vandana Sharma, Ruchi Choudhary and Aakash Gupta

Department of Life Sciences and Biological Science IES University, Bhopal,India

Abstract

The major component of the flora is medicinal and aromatic plants (MAPs), which offer physiologically active phytochemicals utilised in the pharmaceutical, cosmetics, fragrance, flavour, and perfumery industries. Indigenous peoples all over the world use them in a variety of traditional medical techniques to cure a variety of human diseases. L. reticulata is an important Asclepiadaceae medicinal plant. L. reticulata (Jivanti) is a versatile medicinal plant that has been utilised as a natural cure for a variety of ailments since ancient times. Many ailments can be helped by the L. reticulata plant, including haematopoiesis, emaciation, cough, dyspnoea, fever, burning feeling, and night blindness. It also aids in the improvement of breastfeeding as well as the treatment of skin infections and eye disorders. A large number of Indian scientists worked on phytochemical screening, pharmacological effects, and propagational tactics. Scientists are attempting to embark on a new route that will aid in the conservation of L. reticulata as well as the discovery of hidden qualities of this valuable medicinal plant through in vitro culture. This paper presents some of the major work done by Indian researchers in the fields of phytochemical ingredient separation and characterization, as well as plant tissue culture of L. reticulata (jivanti).

Keywords: *Leptadenia reticulata*, Secondary Metabolite, In Vitro Conservation

Introduction

Jivanti or Dodi is the popular name for L. reticulata, a versatile medicinal climber of the Asclepiadaceae family. It is an Indian origin plant that was renowned for its medical usefulness. Apigenin, rutin, p-coumaric acid, lupeol, -sitosterol, diosmetin, quercetin, luteolin, isoquercetin, and other key metabolites are found in the plant. The whole plant helps to balance the three doshas (Vatta, Pitta, and Kapha) and is known in Ayurveda as a Rasayana herb for its reviving and renewing effects. For the first time, a compound called 'Leptaden' (equal proportions of L. reticulata and Breynia patens) is used to prevent habitual abortion and other disorders in women. Mal-kanguni, a polyherbal formulation including L. reticulata and used as an antidepressant, is another polyherbal formulation containing L. reticulata.

Seeds are used to propagate L. reticulata in the wild, however poor seed setting and seed germination rates limit its spread. On the other hand, because of the plant's multi-purpose therapeutic benefits, its yearly demand soared, leading to overexploitation and habitat degradation. Between 2000 and 2005, the demand for medicinal plants on the domestic, national, and worldwide markets climbed by 15–16 percent each year. The National Medicinal Plants Board (NMPB) has prioritized L. reticulata, which has an annual need of 200–500 MT and a cultivation cost of US $ 494.88/h in 2016–2017. Due to limited distribution and seasonal availability, natural resources are unable to fulfill present demand. It has also been reduced by overexploitation via a variety of anti-social actions, making it an endangered species. Plant regeneration by tissue culture is an appealing method for both species conservation and exploitation of plant genetic resources (including bioactive chemicals). Somatic embryogenesis has been found in plants such as *Phaseolus vulgaris* and *Ecliptaalba* to regenerate true plants in very less time. SE development is controlled by a variety of circumstances, with plant growth regulators playing a critical part in morphogenesis. Because bipolar somatic embryo, the in vitro rooting step is skipped, and the creation of synthetic seeds is facilitated, this process offers significant benefits over other organogenic pathways. As demonstrated in Leptadenia pyrotechnica, an effective somatic embryogenesis approach is equally beneficial in genetic transformation investigations. Few studies have been published on shoot regeneration using leaf and nodal explants, as well as somatic embryogenesis using node and shoot tip, stem, and petiole. Because there are few data on the

potency of leaf explant for the creation of SEs, a speedy and efficient technique to increase the quantity of SEs and improve their growth is required.

L. reticulata contains significant phenolic compounds such p-coumaric acid, which is crucial for secondary metabolism since it may be converted to other phenolic acids, flavonoids, lignin precursors, and other secondary metabolites. In addition to preventing atherosclerosis, oxidative cardiac damage, UV-induced damage, neuronal injury, anxiety, gout, and diabetes, it has antioxidant, anti-ulcer,anti-inflammatory, antiplatelet, antimutagenic, and anti-cancer properties. It is produced via the phenylpropanoid pathway, and several studies have shown that cytokinins and auxins influence the expression of various enzymes, including phenylalanine ammonia-lyase. An improved HPLC method was developed for measurement of p-coumaric acid in L. reticulata in vivo plants, however the approach is expensive and only one sample can be tested at a time. This may be avoided by using high-performance thin layer chromatography, which is a quicker, easier, and more cost-effective method of analysing several samples at once. Previously, several L. reticulata metabolites were utilised as a qualitative and quantitative marker chemical for rutin, stigmasterol, 1--tocopherol acetate, p-coumaric acid, rutin, and quercetin.

Taxonomy of *Leptadenia reticulata*

L. reticulata (Jivanti), an Ayurvedic herb, belongs to the Apocynaceae plant family.

Its taxonomic position is detailed as follows:

Kingdom	Viridiplantae
Phylum	Streptophyta
Class	Magnoliopsida
Order	Gentianales
Family	*Apocynaceae*
Sub-family	*Asclepiadoideae*
Genus	*Leptadenia*
Species	*Leptadenia reticulata* (Retz.) Wight & Arn.

L. reticulata is known as Keerippaalai in Siddha medicine. Table 1 lists the numerous vernacular names used in India for L. reticulata. Leptadenia pyrotechnica (Forssk.), Leptadenia arborea (Forssk.), Leptadenia hastata (Pers.), and L. reticulata (Weight and Arn.) are the four species that make up the genus Leptadenia [Chavan JJ, *et al*., 2012, Chermahini, S.H *et al*. 2011]. L. pyrotechnica, for example, is a xerophyte herb having straight stems and mainly leafless leaves, whereas others are twining bushes with bear leaves. These three taxa are often referred to as a single species because of their taxonomic complexity [Chermahini, S.H *et al*. 2011, Chilton MD, 1982]. The medicinal capabilities of the majority of these Leptadenia species make them commercially valuable. One of the most significant therapeutic plants used in Ayurveda for enhancing vigour and life is L. reticulata.

Table 1. Vernacular names /Synonyms of *Leptadenia reticulata*

Language	Vernacular Names (Language)
Hindi	*Dori*
Bengali	*Bhadjivai*
English	*Jiwanti or Jeevanti*
Gujarati	*Methidodi, Dodi saka/Dodi Saag, Dori*
Marathi	*Haranvel, Hiranvel*
Kannada	*Hiriyahalle*
Sanskrit	*Madhusrava, Jivniya, Jivapushpa or Jivani*
Tamil	*Palaikkodi*
Telugu	*Kalasa*

Origin and Distribution of *Leptadenia reticulata*

Although the real origin of L. reticulata has yet to be determined, its depiction in Hinduism's earliest text (Atharvaveda) suggests that it most likely originated in India. “It may be found in Rajasthan, Gujarat, Punjab, the Himalayan ranges, the Khasi Hills, Sikkim, the Deccan Plateau, the Konkan mountains, Karnataka, and Kerala up to an altitude of 2000 m [Chermahini, S.H *et al*. 2011, Christie PJ ,1997]. Apart from India, it has been found in Africa's tropical and subtropical regions, as well as Burma, Nepal, Sri Lanka, the Malay Peninsula, Cambodia, the Philippines, Mauritius, and Madagascar.”[Chanda S, 2011, Chaturvedi P *et al.* 2014, Dash, S.K.*et al*.1972] This plant is used as a pot herb in Gujarat and Kathiawar [Chaturvedi P *et al.* 2014]. It was reported that the presence of L. reticulata in several regions after conducting an extensive field study in 12 different districts in the Western part of Rajasthan (Thar Desert). Hedgerows, open woodlands, and the lower slopes of hills were also home to this species [Devi CS *et al.*2012]. It is commercially grown in various regions of India due to its high demand [Chermahini, S.H *et al*. 2011, and Christie PJ 1997].

Morphology of *Leptadenia reticulata*

It's a branching, twining, and laticiferous perennial climber. Younger stems are greenish glabrous and mature stems are light yellowish with extensively broken bark. “Simple, opposite, ovate or ovate-oblong (3–9 cm 1.1 cm), cordate, and finely pubescent above, the leaves are rather large (4–7.5 cm long and 2–5 cm broad), simple, opposite, ovate or ovate-oblong (3–9 cm 1.1 cm), cordate, and finely pubescent above.” [Christie PJ 1997] The petiole can reach a length of 2.5 cm. The plant blooms lavishly (up to 270 flowers per plant), and buds open completely in 25–28 days. Flowers bloom for 4 to 5 days after peak anthesis, which occurs between 9:00 and 9:30 a.m. [Devi CS, 2012]. Flowering takes place from July to October, while fruits take place from September to December. Yellowish flower are with lateral cymes or subordinate umbellate cymes. The calyx has five lobes that are oval, sub-acute, silky, and covered with tiny hairs. With a small tube, the corolla is rotated and fleshy. The column of stem is not very long. Corona is gamopetalous, having five lobes and a spur from the interior of each lobe. The stigmatic head and filaments combine to produce a five-angled disc termed gynostegium, which is adnate to the base of the corolla tube. The anthers lack membrane appendages. The pollen grains are placed on the stigma's lateral side. Bicarpellary ovary is with limited placentation. Fruit is follicular, sub woody, turgid, 6.3–9 cm long, tapered, green, and follicular [AYUSH, 2008, Chermahini, S.H.*et al* 2011]. Fruits take 102–158 days to develop and can carry about 448 seeds. The seeds are ovate oblong and taper to a diameter of around 6 mm. There are no approved varieties available at this time [Devi CS, 2012]. However, this plant may be divided into two types depending on leaf morphology: plants and plants with narrow leaves. Germplasm with broad leaves was shown to be more prevalent than narrow-leaved genotypes, yielding more roots and other photochemical. The roots have longitudinal ridges and furrows and are rough and white in appearance. The roots are cylindrical, twisted unevenly, and ridged longitudinally. The length of the roots can be up to 1 m or more. The stem has longitudinal lenticels and is yellowish white in colour [Chermahini, S.H.*et al* 2011]. “According to Mammen *et al*. [Chaturvedi P *et al*. 2014], anisocytic stomata are seen in the leaf ‘slower epidermis, and the presence of smooth, uniseriate, and multicellular trichomes is distinguishing markers for diagnosing adulteration in *L. reticulata*. Rectangular cells make up the leaf's epidermal layer, while the mesophyll is made up of 3–4 layers of palisade and spongy parenchymal layers [Dixon RA, *et al.*1999]. Vascular bundles with lignified xylem and non lignified phloem were found to be arc-shaped.” The stem is made up of a single layer of elongated epithelial cells with trichomes in the cross section. Thin-walled parenchymatous cells can be found in the cortex under the epidermis. The cambium forms continuous ring wood by creating supplementary xylem and phloem [Dixon RA, *et al.*1999]. Stone cells are strewn about in Phelloderm. The stem of L. reticulata is distinguished microscopically by the outer phloem having lignified stone cells, non-articulated laticifers and intraxylary phloem.

Biotechnological Tools

Many medicinal climbers exist in nature, with essential secondary metabolites that are employed in pharmaceuticals. There is a need for a clear approach for improving these chemicals. Biotechnological tools have proven to be a godsend in this regard. Biotechnological instruments are procedures that are used to improve the quality of plants in modern times. Making a plant resistant to a specific disease, increasing the total supply of important phytochemicals, boosting plant tolerance to a variety of biotic and abiotic stressors, and so on are all examples of 'plant quality.'

Plant Tissue Culture

Controlled circumstances are necessary for the execution of any biotech technology. The first step in this direction is to cultivate the plant in vitro. This will provide plant homogeneity as a starting point for any research. In vitro cultivation may be regarded a requirement for the proper implementation of these approaches. Tissue culture techniques provide several benefits over conventional breeding, including the absence of environmental changes, infections, a high rate of multiplication, and metabolic process and cell growth management. Plant tissue culture technology is utilised in this case to preserve valuable plants by organogenesis, somatic embryogenesis, or genetic change. Slow growth conservation is also a critical strategy for saving endangered plants. Efforts were made in this approach to maximise the conservation duration with the fewest possible subcultures. Callus is a secondary metabolite that is induced and grown from the same plant part that generates them. It is commonly utilised in secondary metabolite research. In most cases, callus is used in elicitation experiments. Many companies, on the other hand, choose to harvest secondary products using plant cell culture techniques rather than killing the entire plant. Plants that are difficult to grow in nature or that synthesise key plant products in small quantities are grown using tissue culture techniques. These approaches have also made it easier to investigate the secondary metabolite biosynthesis pathway.

Many biotechnological approaches, including as genetic transformation, hairy root induction, elicitation, precursor feeding, and others, have been utilised to boost secondary metabolites in recent years. These biotechnological technologies are critical for medicinal plant conservation and genetic improvement. Improved production of high-value medicines, and other critical secondary metabolites is possible because to the combination of genetic engineeringand tissue culture. In vitro cell culture for the production of natural or recombinant substances has piqued the interest of researchers in recent years.

Genetic Transformation

Plants were first changed with genetic markers, then with economically significant genes, such as the gene of interest, in the transformation technique. The output of secondary metabolites can be increased by genetic transformation. Pest and disease resistance is also improved by genetic change. Transgenic techniques aid in the creation of insect, pest, and other disease resistance, as well as the battling of stresses like as drought, salt, and others. Genetic transformation, along with other biotechnological processes, meets global demand and contributes to plant biodiversity conservation in natural environments. More than 120 plant species have been successfully transformed using various transformation methods.

Hairy Roots

A gram-negative soil bacterium named*Agrobacterium rhizogenes*which causes hairy root syndrome, a neoplastic disease in which roots emerge at the wound site of afflicted plants. Stable transformation events cause these common hairy root signs. “T-DNA segment must be appropriately integrated into the nuclear genome of plants, including dicotyledons, gymnosperms, and certain monocotyledon species, for a successful transformation event. T-DNA genes RolA, RolB, and RolC are responsible for increased secondary metabolite

production. Rol genes also cause the transcription of defence genes to be activated. Following its insertion, the T-DNA, which consists of loci, was discovered between the specified TR and TL border sequences (25-bp repeats), providing the transformed roots with great genetic and biochemical stability." Hairy root clones have different morphology whichis caused by differences in T-DNA insertion sites and copy numbers.

Rol genes function in plants via a variety of signalling pathways. The calcium-dependent NADPH oxidase pathway is one, while phyto- alexin synthesis is another. Aside from these, other routes are active. "The success of rol genes in producing changes in secondary metabolite content varies by plant species and secondary metabolite type, and can range from 2- to 300 times. Hairy roots have a lot of genetic stability, which is very essential. We can circumvent the karyotype instability of in vitro cultures by utilising this characteristic. The existence of a heterogeneous mass of cells at various stages of development causes this instability. Root cultures derived from the gram-negative bacteria A. rhizogenes found to be highly beneficial, with increased secondary metabolite synthesis and good genetic stability." Hairy root cultures have two key characteristics: (1) a high capacity for biomass production and (2) a high steady production of secondary metabolites throughout time. There are research papers available that show that hairy root cultures maintain genetic stability in secondary metabolite synthesis following extensive subculture periods.

"Transgenic plants may also be produced by A. rhizogenes-mediated transformation using a binary vector system, in which a foreign gene is transferred to a second plas- mid during the transformation process. Foreign gene transfer using A. rhizogenes was first documented in 1984. The ability of A. rhizogenes to transfer genes opens the door to a slew of new strategies, including increased metabolite accumulation in transgenic roots, the production of recombinant proteins, and the discovery of new genes via RNA silencing and T-DNA activation tagging processes." In plants that are difficult to convert with A. tumefaciens, A. rhizogenes is a promising option. Gene silencing enables for loss-of-function analysis, whereas T-DNA activation tagging allows for gain-of-function alterations.

Elicitation

Secondary metabolites are produced in response to a variety of stimuli. Various plant species have different types of pressures and how they respond to them. There are three sorts of elicitors: biotic, abiotic, and physical. Certain metabolites' biosynthesis can be enhanced or even induced by elicitors. Endogenous elicitors are those generated by plant cells, whereas exogenous elicitors are those produced by microbes. "Elicitors include pectin, pectic acid, or cellulose, as well as chitin, chitosan, or glucans, which are components of plant and microorganism cell walls. They are known as biotic elicitors because of their biological origin." Elicitors induce signalling or mimic substances that cause phytoalexin accumulation in plants. Elicitation in plants is analogous to a similar artificial state that occurs when incompatible diseases attack. Many protective secondary metabolites were secreted in intact plants and cell cultures as a result of these interactions. "Elicitors, alone or in combination with two or more other substances, work synergistically to increase secondary metabolite yields or even generate new molecules. Elicitors do not always directly boost secondary metabolite synthesis; the capacity of the cells to release the needed metabolite into the surrounding medium is sometimes required for the secondary metabolite to be economically viable. Elicitors have varied modes of action; some cause stress by increasing the formation of ROS (reactive oxygen species), while others cause hypersensitive reactions. Plant plasma membranes have unique receptors that recognise elicitor chemicals. The elicitor-receptor interactions are thought to be responsible for the creation of signals that cause plant defence-related nuclear genes to be triggered. These elicitor-based techniques have made a name for themselves in the field of biotechnology, owing to their significant impact on the generation of secondary metabolites."

Biotechnological Studies of *L.reticulata*

"Plant tissue culture is a biotechnological technique that is commonly used as a substitute for obtaining sufficient actual planting materials for commercial production. Furthermore, many endangered medicinal plant species can be saved [Sastry, B.S *et al*. 1985, Seong ES *et al*. 2009], and plant secondary metabolites valuable in the pharmaceutical, cosmeceutical, and food sectors might be enhanced [Bhat SR *et al*. 1992]. To create active principles from in vitro grown cells, a special method is necessary [Bhat SR *et al*. 1992, Sharma, S.C.1976]. Type of explants, media composition, type of plant growth regulators, different growth conditions (temperature, light sources, and humidity), types of cultures (solid cultures and agitated liquid cultures), cell line section, and the use of elicitation technology are some of the factors that influence in vitro culture [Bhat SR *et al*. 1992, Saxena C, 1997, Siahsar B., *et al*.2011]. Direct organogenesis or indirect organogenesis, which involves callus interphase, can be used to regenerate plants in vitro." [Sastry, B.S *et al*.1985, Savithramma N *et al.*2011, Saxena C, 1997] The in vitro culture system that has been built will be particularly valuable for future genetic manipulation investigations or large-scale secondary metabolite synthesis. The growing interest in L. reticulata's medicinal potential has resulted in a number of biotechnological research projects throughout the world. The following section summarises the most notable study publications on L. reticulata's biotechnological features.

In Vitro Conservation Studies

Many significant medicinal plants are on the verge of extinction due to well-defined pharmacopoeia, rising urbanisation, indiscriminate collecting, and overexploitation of natural resources. Several technologies provide a viable alternative for plant variety studies, genetic resource management, and conservation in order to cope with the worrying situation. Plant tissue culture technique is one such crucial instrument, since it plays a key role in creating disease-free plants that are true to type, quick, and mass produced under controlled settings [Bhat SR *et al*. 1992]. This species has also been subjected to the micropropagation method for bulk multiplication. There are just a few studies on micropropagation by direct organogenesis known so far. According to Arya et al. [Sivakumar G *et al*. 2000], there is a pressing need to establish a non-traditional technique for mass multiplication, conservation, and long-term use of L. reticulata. "MS media with (25 mg/L each of adenine sulphate, arginine, citric acid, 50 mg/L ascorbic acid) containing 0.6 M indole-3-acetic acid (IAA) and 9 M benzyladenine was used to produce three to four shoots from a single node (BA). Sub-culturing on fresh medium containing 0.6 M IAA and 2.2 M BA multiplies the shoots much more. After being treated with 123 M of indole-3-butyric acid (IBA) and -naphthoxyacetic acid, individual shoots were rooted ex vitro. The rooted plants were placed in a net container filled with sterile soilrite. After 15 days, the hardened plants were transported to polybags and subsequently to the field. Hariharan *et al*. [Sivarajan, V.V *et al*.1994] described somatic embryogenesis and plant regeneration using L. reticulata leaf explants. On MS medium supplemented with 6-BA (2.0 mg/L) and –naphthalene acetic acid (NAA) (0.5 mg/L), embryogenic callus was successfully begun and established. The developing embryoids were sub cultured on hormone-free MS medium or medium with decreased hormone content. Later, the embryoids were germinated on MS medium with 1.0 mg/L kinetin (Kn). The plants were then transplanted to the field for hardening, and a 50% survival rate was reached. BA-induced somatic embryogenesis and plant regeneration from distinct L. reticulata explants were described in another work [Soniya EV *et al*.2002]. On MS media supplemented with 8.87 M BA and 2.46 M IBA, shoot tip and nodal segment were found to be morphologically active and effectively included embryogenic callus among the several explants tested. To help the embryo develop, the embryogenic calli were moved to a suspension culture. MS medium (1/2 strength) was shown to be the most effective in converting

embryos to plantlets, along with 1.44 M gibberellic acid (GA3) and BA (0.22 or 0.44 M). Plants were transplanted to net pots and then to the field, where they survived at an 80 percent rate [Soniya EV *et al*. 2002]. Plant regeneration by somatic embryogenesis has also been performed using L. reticulata stem explants [Bulgakov VP 2008]. The optimum medium for callus induction was MS medium supplemented with 3 percent sucrose, 2.68 M NAA, and 2 M BAP. For cell growth, MS liquid media outperformed solid medium. The produced shoots were rooted in half strength MS medium with 4.90 M IBA and planted in the field with a survival rate of 75%. On MS media with 5.0 mg/L 6 BA, several shoots were induced [Bulgakov VP 2008]. 1.5 mg/L BA and 0.5 mg/L kinetin were used to multiply the plants (Kn). For rooting, well-grown shoots were transferred to MS medium containing 200 mg/L IBA. Plant growth regulators have been found to have a considerable impact on L. reticulata morphogenesis in vitro [Srivastav, S. *et al*.1994, Srivastava M *et al*. 2013]. Multiple shoots and callus were generated at the base of nodal explants in MS basal media supplemented with IBA (1 mg/L) and Kn (10 mg/L). Later, NAA (1.5 mg/L), Kn (10 mg/L), or IBA (1 and 1.5 mg/L) with Kn (2 mg/L) were used to induce organogenesis in callus [Srivastava M *et al*. 2013]. Rathore *et al*. [Subramanian, P.S.*et al.*1977] found that MS medium with 5.0 mg/L of BA and ammonium sulphate was efficient in the proliferation of shoots. Another study found that MS medium supplemented with 0.25 mg/L BA and 0.25 mg/L Kn produced the optimum response for shoot multiplication. The highest rooting response was generated by full strength MS medium with 2 mg/L IBA. Surprisingly, stimulating roots with 200 mg/L activated charcoal in MS medium was similarly successful [Saxena C *et al*. 1997]. Sudipta et al. [Saxena C *et al*. 1997] investigated the influence of various carbon sources and natural additions on the in vitro morphogenesis of L. reticulata. They found that 2 percent sucrose followed by 2 percent table sugar had a significant impact on shoot multiplication rate and plant physiology." The 10 percent coconut water was shown to be the greatest natural addition for triggering the most numerous shoots. In addition, instead of sucrose and purified water, tap water and table sugar were used to lower the expense of culture medium. Picloram (2 mg/L) was shown to be beneficial in the formation of leaf explants' friable callus [Hassan AKMS et al. 2005]. Furthermore, a phytochemical screening research revealed that suspension cultures produce endogenous and exogenous secondary metabolites. Steroids, on the other hand, were created endogenously and were not found in the media.

Conclusion

The conservational characteristics of L. reticulata have been carefully investigated in this work. This multi-purpose medicinal plant has multiple potential medicinal properties and can thus be employed in modern therapeutic methods to treat a variety of human illnesses. With its renewing, rejuvenating, and lactogenic characteristics, L. reticulata can be employed as the principal ingredient in a variety of herbal preparations. There are various types of bioactive chemicals in this plant. "In order to test these methodologies in future applications, biotechnological technologies such as micro propagation, molecular markers, and cell culture are also addressed. The tissue culture data gathered will undoubtedly pave the way for the development of a low-cost tissue culture technology for propagating elite L. reticulata germplasm by micropropagation." Because of overexploitation, improper harvesting, and habitat degradation, L. reticulata is currently classified as a threatened endangered species. As a result, future study should concentrate on its environmental characteristics. In vitro generation of bioactive chemicals from L. reticulata will be supplemented by the use of innovative methods like bioreactors, genetical engineering, and cell culture. Despite the fact that the biological features of L. reticulate are well established, most investigations have focused on crude extracts and a few isolated molecules. Furthermore, many physiologically active chemicals are yet unknown. Modern technologies have a wide range of applications including transcriptomic analysis, RNA silencing and recombinant DNA technology. With the use of these methodologies, great progress will be made in

understanding the biosynthesis routes of these secondary metabolites, resulting in a large increase in their content.

Conclusion

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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