Journal of Advances and Scholarly Researches in Allied Education
Vol. 16, Issue No. 5, April-2019, ISSN 2230-7540

A Study on the Phytochemical Properties of Leptadenia Reticulata

Tejal A. Rajput*

Adhoc Assistant Professor, Faculty of Science, Gokul Global University, Sidhpur, Gujarat

Abstract – Leptadenia reticulata (Retz.) Wight & Arn. (Apocynaceae), is a traditional medicinal plant species widely used to treat various ailments such as tuberculosis, hematopoiesis, emaciation, cough, dyspnea, fever, burning sensation, night blindness, cancer, and dysentery. In Ayurveda, it is known for its revitalizing, rejuvenating, and lactogenic properties. This plant is one of the major ingredients in many commercial herbal formulations, including Speman, Envirocare, Calshakti, Antisept, and Chyawanprash.

The therapeutic potential of this herb is because of the presence of diverse bioactive compounds such as α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentricontanol, a triterpene alcohol simiarenol, apigenin, reticulin, deniculatin, and leptaculatin. However, most biological studies on L. reticulata are restricted to crude extracts, and many biologically active compounds are yet to be identified in order to base the traditional uses of L. reticulata on evidence-based data. At present, L. reticulata is a threatened endangered plant because of overexploitation, unscientific harvesting, and habitat loss.

The increased demand from pharmaceutical, nutraceutical, and veterinary industries has prompted its large-scale propagation. However, its commercial cultivation is hampered because of the non-availability of genuine planting material and the lack of knowledge about its agronomical practices. In this regard, micropropagation techniques will be useful to obtain true-to-type L. reticulata planting materials from an elite germplasm to meet the current demand. Adopting other biotechnological approaches such as synthetic seed technology, cryopreservation, cell culture, and genetic transformation can help conservation as well as increased metabolite production from L. reticulata.

Keywords: Jivanti, Leptadenia reticulata, traditional medicine, herb, therapy, rasayana, galactagogue, pharmacology, biological activities, medicinal plant

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INTRODUCTION

The utilization of plants for their therapeutic value has been known to mankind from times immemorial and has played an essential role in the various traditional systems of medicine including Ayurveda, Homeopathy, Siddha, Unani, Naturopathy, and Chinese, Tibetan, and Native American medicine.

As per the estimation of the World Health Organization, 80% of the world population is currently using herbal medicines for primary health care needs. At present, drug discovery research is mainly focused on natural plant resources and their compounds. Most of the currently available therapeutically active drugs are discovered based on the knowledge available from various traditional disease treatment practices.

Awareness, health consciousness, thoughts on prevention being better than cure, and natural ways of healthy living has further propelled the use of herbal products. The exponential growth of nutraceutical and cosmeceutical consumers has increased the demand for plant raw materials. Ayurveda has a science called Rasayana, which deals with the improvement of general health, vigor, and vitality. Among various herbs used in Rasayana, *Leptadenia reticulata* (Jivanti) has a unique place in lieu of its revitalizing, rejuvenating, and lactogenic properties.

L. reticulata is used for treating various ailments such as hematopoiesis, emaciation, cough, dyspnea, fever, burning sensation, night blindness, and dysentery. This plant is used as one of the ingredients in many herbal formulations. The therapeutic potential of this herb is because of the presence of diverse bioactive compounds such as

α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentricontanol, a triterpene alcohol simiarenol, apigenin, reticulin, deniculatin, and leptaculatin.

The wild resources are unable to meet the current demand because of restricted distribution and seasonal availability. Hence, cultivation remains the only sustainable alternative. Further, wild resources of *L. reticulata* have been depleted by overexploitation through various anti-social practices, and thus, it has been listed as an endangered species [16]. Although farmers and industries are ready to cultivate the herb, its low percentage of germination, non-availability of genuine plant materials, and a lack of knowledge about its cultivation practices pose a challenge for its commercial cultivation.

However, the higher market value and global demands for *L. reticulata* have forced farmers to consider cultivating it in recent times. Today, various medicinal plants are commercially cultivated to meet the ever increasing global demand for plant metabolites used by the pharmaceutical industries.

Nevertheless, various crop improvement strategies are yet to be adopted to develop superior varieties of medicinal plants. The use of micropropagation approach will certainly help in the large-scale production of elite genetically and chemically uniform planting material.

In spite of intensive conventional research effort and vast agronomic practices, the desired *L. reticulata* production target has not been achieved thus far. *L. reticulata* is often adulterated with many other herbs, including *Holostemma ada-kodien*, *Dendrobium ovatum*, *D. macraei*, *Flickingeria macraei*, *Cimicifuga foetida*, and *Ichnocarpus frutescens.*

Thus, the authenticity of *L. reticulata* is another great challenge in the herbal medicine industry. The commercial cultivation of any herb requires a thorough understanding of its botanical, chemical, geographical, growth, and developmental characteristics. Further, better management of plant nutrition, diseases, and pest problems will boost the commercial yield of a crop. The present review was undertaken to compile the available scientific data on the botanical, agronomical, phytochemical, pharmacological, and biotechnological aspects of *L. reticulata*. This data will certainly enable researchers to explore novel drug molecules from this multi-utilitarian herb.

PHYTOCHEMICAL PROPERTIES OF LEPTADENIA RETICULATA

Although the true origin of *L. reticulata* has not been identified so far, the description in the oldest scripture of Hinduism (*Atharvaveda*) indicates that it probably originated in India. In India, it is mainly found in Rajasthan, Gujarat, Punjab, the Himalayan ranges, Khasi Hills, Sikkim, Deccan Plateau, Konkan ranges, Karnataka, and Kerala up to an altitude of 2000 m.

Apart from India, it is also reported to be distributed in the tropical and subtropical parts of Africa, Burma, Nepal, Sri Lanka, Malay Peninsula, Cambodia, the Philippines, Mauritius, and Madagascar.

People in Gujarat and Kathiawar use this plant as a pot herb. After intensive field survey in 12 districts in Western Rajasthan (Indian Thar Desert), Panwar and Tarafdar reported the occurrence of *L. reticulata* from various districts. This species was also found on hedges, in open forests, and on the lower slopes of hills. Because of its high demand, it is commercially cultivated in some parts of India.

Mature stems are pale yellowish with deeply cracked barks, and younger ones are greenish glabrous. The leaves are quite big (4–7.5 cm long and 2–5 cm wide), simple, opposite, ovate or ovate-oblong (3–9 cm × 1.1 cm), cordate, and finely pubescent above. The petioles are up to 2.5 cm long. The plant flowers profusely (up to 270 flowers per plant), and complete opening of flower buds takes 25–28 days. Peak anthesis is observed between 9:00 and 9:30 a.m., and flowers open for 4 to 5 days. Another dehiscence occurs between 11:00 a.m. and 1:00 p.m. on the fourth day during which the flowers are nearly at the wilting stage.

Flowering occurs between July and October and fruiting between September and December. Flower is yellowish with lateral or sub auxiliary umbellate cymes. Calyx is five-lobed; lobes are ovate, sub-acute, and silky with small hairs on the surface. Corolla is rotate and fleshy pubescent with short tube. Staminal column is short. Corona is five-lobed, gamopetalous, spreading with spur from the interior of each lobe. Stamens are five and adnate to the base of the corolla tube; filaments fuse with the stigmatic head to form a five-angled disc called gynostegium. Anthers are without membranous appendages. Pollen grains are arranged on the lateral side of stigma. Ovary is bicarpellary with marginal placentation. Fruit is follicular, sub woody, turgid, approximately 6.3–9 cm long, tapering, and green.

Fruits mature in 102–158 days and contain up to 448 seeds. Seeds are ovate oblong, tapering about 6 mm in diameter. Presently, there are no certified varieties available.

However, based on leaf morphology, this plant is mainly categorized into two variants i.e., broad-leaved plants and narrow-leaved plants. The broad-leaved germplasm was evaluated to be

predominant than narrow one and yields higher amount of roots and other phytochemicals. The roots are rough and white in color with longitudinal ridges and furrows. Roots are cylindrical and twisted irregularly with longitudinal ridges. Root length varies up to 1 m or more. Stem is yellowish white in color with longitudinal lenticels.

The epidermal layer of the leaf is composed of rectangular cells, and mesophyll consists of 3–4 layers of palisade and spongy parenchymal layers. Arc-shaped vascular bundles with lignified xylem and nonlignified phloem were observed. The cross section of the stem consists of single layer of elongated epithelial cells having trichomes. The cortex below the epidermis contains thin-walled parenchymatous cells. The cambium produces secondary xylem and phloem producing continuous ring wood. Phelloderm contains scattered stone cells. Lignified stone cells found in the outer phloem, intraxylary phloem, and non-articulated laticifers are the microscopic distinguishing characters of the stem of *L. reticulata.*

Figure 1: Morphological view of *L. reticulate*

CLIMATE AND SOIL

The plant grows well in tropical and subtropical climate and requires moderate rainfall and relative humidity. This plant also grows in arid regions, which are characterized by sandy soil, low organic matter, and rainfall deficit. Black soil is found to be good for cultivation; however, red laterite soil is also suitable for its satisfactory growth. Open sunlight and support is necessary for healthy and vigorous growth.

PROPAGATION TECHNIQUE

Plants reproduce vegetatively from stem cuttings, roots, and vines. Evaluation of various planting materials such as stem cuttings, root cuttings, and vine cuttings revealed that among all parts propagation using healthy and strong stem cuttings is the most successful. Maintenance of high humidity around the cutting was found to be a critical factor to reduce the evaporative loss of water from cuttings. High humidity was maintained by covering the planting material with clear plastic bags.

After rooting, the plastic bag can be removed. Treating the cuttings with root-promoting compounds is found to be a valuable tool in stimulating root formation. Newly rooted cuttings should not be planted directly. The plants can instead be transplanted into a container or a bed before transferring them to a permanent location to increase the chances of survival. Although the fruits contain fairly large quantity of seeds, the number of seedlings was less because of low germination rate and limited availability.

Fruits turn ripe during November to December. Seeds are collected before the fruits dehisce, and they are dried and stored. After soaking in water for 4–5 h, the seeds are sown on nursery bed with thick layer of sand. About 1–1.5-month-old seedlings were transferred to the main field.

As per the agronomic study carried out by the Department of Horticulture, University of Agricultural Science, Bengaluru; Dhanvanthari Vana, Department of Forestry, Government of Karnataka; Bengaluru University, Bengaluru; State Department of Horticulture, Hulimavu; and Biotechnology Centre, Bannerghatta Road, Bengaluru, the period of February-March is suitable for planting the cuttings.

The cuttings of 12–15 cm long with 3–4 nodes were treated in antifungal agent and root-inducing hormones to get better rooting response. Rooted cuttings were transferred to polybags filled with Farm Yard Manure (FYM) and red earth in the ratio of 1:1 after 45 days. Three-month-old saplings intact in the soil were transferred to the plot prepared in the main field.

SPACING OF PROPAGULES

Intensive study was conducted at field level for yield and biomass by planting at different levels of spacing. Sapling survival rate at a spacing of 2 m × 1 m of pit size 45 cm^3 and nearly 5000 plants planted per hectare was found to be ideal for maximum yield.

PREPARATION OF LAND AND FERTILIZER

The lands were ploughed three to four times and the soil was made finer. The plot of convenient size was prepared with good irrigation facility. Pits of 45 cm^3 filled with FYM and top soil in the ratio of 1:1 with a spacing of 2 m × 1 m is ideal for plantation. The months of February and March are more favorable for planting the cuttings. It is also suggested that the pit should be dug deeply to facilitate the growth of the root. Cuttings can also be planted directly in polybags or seed pan filled

with mixture of sand, FYM, and red earth in the ratio of (1:1:1) for better results.

Different fertilizers such as arbuscular mycorrhizae (AM) (100 g soil/plant), FYM (8–10 tons/ha; 5.77 g/plant), Hexameal (an organic manure; 40 q/ha; 2.31 g), nitrogen, phosphorus, potassium (NPK): full dose (60:40:30 kg/ha), and NPK: half dose (30:20:15 kg/ha) were evaluated for optimum growth.

IRRIGATION

As irrigation plays an important role, an adequate amount of water must be supplied for the overall growth and development of the plant. Among the different methods tested, such as sprinkler irrigation, surface irrigation, and drip or tickle irrigation, furrow irrigation twice a week for two to three months after planting in the field is preferred. Later, the irrigation may be done at an interval of 8–15 days. Drip or tickle irrigation, where water is supplied directly to the roots of the plants in small amounts, may be a good second choice.

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Manual control of weeds and earthing up at regular intervals of one to two months is found to be beneficial.

CROP PROTECTION

Control measures need to be undertaken against certain common infectious diseases and pests. Powdery mildew is reported to be a serious problem affecting the plant during the winter months. Various fungal diseases like leaf spot and leaf blight are also commonly observed. Infestation by leaf-eating caterpillars, aphids, and mites is commonly noticed which can be controlled manually or by spraying prophylactic sprays of monocrotophos (0.15%), dicofol (0.2%) etc. To protect the crop from termites, the soil mixture should be treated with phorate granules before transplanting them. The use of chlorpyrifos 20 EC in 20 mL L^{-1} water solution is beneficial to control termite attack.

Methyl parathion dust 20 kg ha^{-1} and nuvacron 1 mL L^{-1} are used to control the manifestation of grasshoppers in the rainy season and aphids and ladybird beetles in the winter season, respectively. Leaf wilting is occasionally noticed at various stages and can be controlled by phytosanitary measures and drenching the affected vines with 0.15% carbendazim.

INTERCROPPING

Being a climber, *L. reticulata* needs a host plant or a stalk to support its growth. Healthy growth is noticed when the plant is grown in partial shady areas than in completely open areas. Therefore, it should be preferably intercropped at the base of certain common trees for support. Hence, intercropping is recommended for this plant to achieve the benefit of economizing water cost and controlling diseases and pests.

Pests are less abundant when planted as intercrops than monocrops. The requirement of huge land space and physical support for climbing are other factors that increase the cost of cultivation when planted as a monocrop.

HARVESTING

The crop can be maintained in the field for 10–15 years. The harvesting is preferably done twice a year without removing the root, which can serve as a future planting material or a root stalk. It is reported that higher yield can be obtained when harvested after 18 months.

At this stage, the fresh yield of dry roots and biomass was found to be the maximum. Fruiting of *L. reticulata* takes place between December and February, maturation continues until May, and dehiscence takes place between June and July. The favorable season for harvesting is between January and February when the leaves dry up. After harvesting, the roots and leaves are cut into required size and dried retaining the moisture content at 10% for storing. Six to seven hundred kilograms of dry weight root per hectare per year yield was reported.

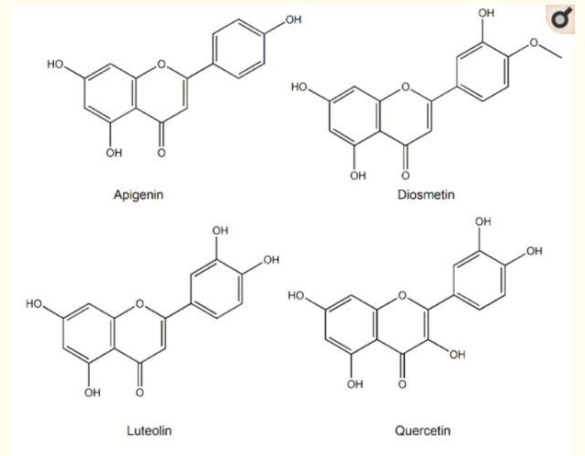

Figure 2: The structures of flavonoids and phenolic acids found in *L. reticulate*

In recent times, phytochemists and biologists are focusing on the isolation and identification of specific lead molecules of *L. reticulata* and understanding their therapeutic significance. The phytochemical composition and the content of bioactive compounds vary within the plant parts. Moreover, various factors such as geographical topographies, climatic condition, growing patters, and harvesting duration influence the accumulation pattern of biochemical constituents in plants. In addition, the practice of extraction

procedures can lead to discrepancies in the composition of plant compounds.

To date, there are only a few studies related to the identification, isolation, and characterization of individual phytocompounds of *L*. *reticulata.* However, accurate documentation of well-characterized phytocompounds will benefit in proper understanding of their biological activities. In *L. reticulata*, quite a few classes of chemical compounds have been reported including terpenoids, phenolics, flavonoids, steroids, and esters. In this section, the phytochemical composition of *L. reticulata* is discussed in detail considering the above facts.

L. reticulata extract (Leptaden tablet) provides a good remedy for new mothers suffering from breast milk deficient or absence. This medicine has a galactagogue effect and also useful in the treatment of habitual abortions.

An experimental research in guinea pigs using radioimmunoassay suggested that Leptaden inhibits F2 alpha biosynthesis. This helps in preventing abortion, since any increase in prostaglandins causes abortion or premature delivery. The effect of leptaden therapy is more beneficial over the combined treatment with progesterone. Also, it has been concluded that Leptaden therapy when done alone proved beneficial for the management of threatened abortio.

The researcher also described Leptaden as a non-hormonal and safe herbal drug. In case of threatened abortion, Leptaden can be used without hormonal treatment and requires no tests. It was found to be safe for both the mother and the child without any toxic side effects. leptaden can also be used to reduce uterine cramps of threatened abortion. This is probably because of the anti-prostaglandin effect of Leptaden. Leptaden tablets are also used for the treatment of uterine hemorrhages.

CONCLUSION

The agronomic practices, including vegetative propagation, crop management, and harvesting conditions, are well documented to support its improved large-scale cultivation in the field. Some of the scientific practices such as selection of elite planting material, well-timed planting, timely application of soil nutrients, appropriate use of growth regulators, irrigation, weed elimination, and harvesting stage are essential for sustainable cultivation and production.

Furthermore, the utilization of biotechnological tools such as micropropagation, molecular markers, and cell culture are also emphasized in order to validate these methods in future applications. The compiled tissue culture data will certainly pave a way toward developing a low-cost tissue culture methodology for propagating elite germplasm of *L. reticulata* through micropropagation. At present, *L. reticulata* is a threatened endangered plant because of its overexploitation, unscientific harvesting, and habitat loss. Therefore, future research should focus on its conservational aspects.

Application of novel techniques such as cell culture, genetic engineering, and utilization of bioreactors will also supplement the in vitro production of bioactive compounds from *L. reticulata*. Although the biological properties of *L. reticulata* are well understood, most studies were restricted to crude extracts and few pure isolated compounds. Moreover, many biologically active compounds are yet to be identified. Most of the phytochemicals described as constituents of the title plant are rather widespread in plants and not particularly characteristic for *L. retiulata*.

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Corresponding Author

Tejal A. Rajput*

Adhoc Assistant Professor, Faculty of Science, Gokul Global University, Sidhpur, Gujarat