OPTIMIZATION OF HARDENING CONDITION FOR *RHODIOLA IMBRICATA* AND *VALERIANA JATAMANSI*

A THESIS

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IN

DEPARTMENT OF BIOTECHNOLOGY AND BIO INFORMATICS

UNDER THE SUPERVISION

OF

Dr. Hemant Sood

BY

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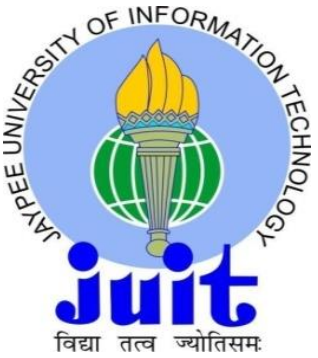

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CERTIFICATE FROM SUPERVISOR

This is to ensure that the work entitled "**Optimization of hardening condition for *Rhodiola imbricata* and *Valeriana jatamansi***" put together by "Ankush Verma" in fractional satisfaction for the honour of M.Sc Degree Program in Biotechnology "at Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor

Name of Supervisor - Dr. Hemant Sood

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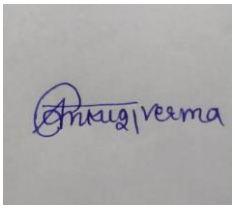
Date:- 12-07-2021

DECLARATION

I certify that

- The work contained in this thesis is original and has been carried out by me under the guidance of my supervisor.
- The work has not been submitted to any other organization for any degree or diploma.
- Whenever, I have used materials (data, analysis, figures or text), I have given due credit by citing them in the text of the thesis.



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In gratitude
Ankush Verma (197803)

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LIST OF ABBREVIATIONS

IBA	Indole-3-butyric acid
KN	Kinetin
BAP	6-Benzylaminopurine
NAA	Naphthalene acetic acid
MS	Murashige and Skoog
TDZ	Thidiazuron
CCR	Cinnamyl -CoA reductase
CAD	Cinnamyl alcohol dehydrogenase
PAL	Phenylalanine ammonialyase

ABSTRACT

Forest are great source of plants and herbs having high medicinal properties and they are used from the ancient time for the treatment of the various disease. *Valeriana jatamansi* and *Rhodiola imbricata* are one of the high therapeutic value plants. *Valeriana jatamansi* family-Valerianaceae) called locally muskbala. It grow at higher altitude ranging from 3000-4500 m. The variety Rhodiola contains around 24 distinct species, which is available in the trans Himalayan range at the height of 4000-5000 m. An individual from this family has been utilized in customary or present day medication and it is known for Anti-apoptotic Radioprotective, Antioxidant, Antihemolytic, Anticancer activity and Antiviral properties due to the plant contain large no of secondary metabolite like salidroside, rosavin, rosasarin , whereas *Valeriana jatamansi* is the great source of flavonegloside, terpinoid, terpenes, valepotritates. Its essential oil contain large number of bioactive compound which are highly expensive and high demand in pharmaceutical and in perfumery industry so present study is carried out to multiply the plants of *Rhodiola imbricata* and *Valeriana jatamansi* under in vitro condition and optimize parameters for increasing their survival rate under field condition. The percentage of survival of *Rhodiola imbricata* was not so significant i.e 60% , whereas approximately 100% survival rate was achieved in *Valeriana jatamansi* by optimizing all possible parameter for the hardening of these important medicinal herb, so these study holds future avenue for commercialization of these important medicinal plants

CHAPTER -1

INTRODUCTION

1.1 *Rhodiola imbricata*

A stone yield family belong to Crassulaceae which contain more than 1300 species and they are distributed in 33 different generation which includes *Rhodiola* (Golden root; Rose root; Arctic root) and is generally present particularly in the South Africa and Northern Hemisphere. This family generally proliferates at a height 3500-5000 m in depleted soil and recognized to have restorative potential (Mishra et al., 2007). *Rhodiola* comprises about 80 species or more than that which is broadly scattered in cool districts of the North Hemisphere at an elevated height (Yidong Lei et al., 2003). In conventional prescriptions of some regions of Tibet & different districts, different *Rhodiola* plant is utilized for the curing of disease over more than 100 yea ago (Xiong.1995 & Rohloff 2002).

R. Heterodonta, R. Wallichiana, R. tibetica, R. imbricata, R. sinuate, and R. quadrifida are total six types of *Rhodiola* are found in India (Chaurasia and Gurmet, 2003). *Rhodiola imbricate EDGEW* comes under the family of crassulaceae and recently known by the name of *Sedum roseu,* which is dioecious and an enduring herb, also called as rose root because freshly cut rootstock give rose like scent. Normally in the India is called as Golden root, Rose root, the Arctic root and the Shrolo Stone and Himalayan Rhodiola(Chaurasia and Singh, 1996). The pieces of rhizomes plant along with roots are commonly utilized. The origin of *R. imbricata* has recently received considerable recognition. *R. imbricata* is a cold-tolerant, adaptogenic medicinal herb.

Rhodiola. imbricata EDGEW. [Synonym:- *Sedum.imbricatum* Walp and *R. imbricatum EDGEW*] (ordinary names:- The Golden root; the rose root; and the cold root or also called as Shrolo) an dioecious plant, confined to the Northern part of the globe and beginning at the hilly districts of South and West China (Chaurasia and Singh, 1996).

Within India, the locality of Jammu & Kashmir and some upper part of Himachal Pradesh, some part of Arunachal Pradesh and nearly whole Himalayan ring there distribution can be noticed. Tibet and its nearby area are one of spots which are having a wealthy creation of *Rhodiola* spp. (Kumar et al., 2010). The species are discover in some part of Russia, Canada & in some part of US. *R. imbricata* and *R. heterodonta* found in the region of Ladakh From the 5 distinct valley of Ladakh namely the Suru, the Zanskar, the Nubra, and the Indus & Changthang, plant are found in Indus; Changthang; Nubra; and Zanskar valley. In the the Changla pass (among Nubra & Changthang), Khardungla pass (among Indus & Nubra basin), the Pensi--la pass (among Zanskar and Suru basin) good population of the Rhadiola can be

found and so on are available inside the middle regions of these valleys (Chaurasia et al., 2007). *R. imbricata* recognized as a Sanjivani but late, *R. heterodonta, R.quadrifida*, described so on. Every one of these accounted to have high restorative qualities like cancer prevention agent, mitigating, infection. The sort of condition where these plants develop is extremely unforgiving having very low temperature of approximately 10ºC and elevation 3900–5100 m amsl (Figure 1).

R. imbricata is aromatic plant having a bulky rhizome which is brilliant from external surface & contain light pink colour within the rhizome, 11-36 cm long which contain rose fragrance rootstocks. Leaves 1.2-4 cm long, oval to limit ecliptics about whole flower light yellow in blocked bunch, contain lots of seed. Blooming & fruit formation in the plant occurred in month 7 -8 (Chaurasia et al., 2007).

Classification

Kingdom	Plante
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Rosales
Family	Crassulaceae
Genus	*Rhodiola*
Species	*imbricata* EDGEW

Figure 1 - *Rhodiola imbricata* at Valley Of Flowers. [online] Available at: <https://valleyofflowers.info/flowers.

Investigation of various *Rhodiola* class exposed the six gatherings of dynamic standards in their compound syntheses (Khanum et al., 2005).

- Phenylethanol subsidiaries: like tyrosol & Rhodiolaoside
- Flavonoids: it contains rhodionin &acetylrhodalgin
- Monoterpenes: like rosiridol.
- Triterpenes: like daucosterol & β-sitosterol.
- The nearness of triandrine, p-coumaric & its glucosides p-cumaric corrosive, a caffeic corrosive & β-sitosterol, daukostero & salidroside likewise been distinguished in the callus tissues societies (Tayade at el., 2015).

For the dealing of constant shortcoming and ailment because of contamination, *Rhodiola* species is being utilized as customary medications from the past 1000 or more than this. In Tibet and different areas (Xiong, 1995 & Rohloff, 2002). *R. imbricata* is a significant customary therapeutic plant and is broadly utilized as the foodstuff crops for domestic animal in Himalayan desert locales. *R. imbricata* is utilized for curing of cough, cold, some lung issues, aspiratory objections and the loss of vitality in Tibetian arrangement of conventional medication (Chaurasia et al. 2007). Current investigations demonstrated that this plant fluid, the ethanolic and the hydro-alcoholic root separates have high cell and the immune modulatory potential (Mishra et al., 2012), radio protective viability & the immune

stimulatory action & adjuvant action (Mishra et al., 2010), radiomodulatory (Arora et al., 2008), adaptogenic movement (Tulsawani et.al., 2011), the cytoprotective activity has been seen (Kanupriya et al., 2005), the cell reinforcement potential (Kanupriya et al., 2005), metal chelating action and free-radical action (Arora et.al., 2008), restriction (C-H-R) introduction, cold, post-stress and hypoxia (Gupta et al., 2010), and limitation (C-H-R) hypothermia initiated by pressure and recuperation after pressure (Gupta et al., 2009), capability to cure dermal injury (Gupta et al., 2007), hepatoprotective action, antiproliferative action of the concentrates in HT-29 person colon disease cell and radical searching (Senthilkumar et al., 2013).The referenced pharmacy exercises, *R. imbricata* is used by the pharmaceutical sector to produce prescriptions to build endurance, profit in work, life span and to reduce weakness, stress. Therefor popularity of *RhodIola* material worldwide for the pharmacy use.

1.2 *Valerina jatamansi*

Plants are used as important and healthy natural source for the medicine and an agent of medicinal, agricultural, environmental services throughout the diversified cultures throughout recognised civilizations. Widespread use of herbal remedies and medical preparations is get from the commonly of traditional herbs and the medicinal plants in the ancient text including the Vedas, Sacred Kuran and in the Bible.

Use of the traditional medicines and medicinal plants is the basis for ensuring good health in the majority of developed countries by the creation of numerous pharmaceutical and chemical therapies by Herbal preparations. Herbal remedies are more common recently in the treatment of different conditions as a result of increased knowledge about the preservation of personal health by natural products. As a consequence, global research has been carried out in the field of herbal medicines trade. The international medicinal plants market in 2000-02001, which continues to rise at a rate of 7% annually, was more than 62 trillion US dollars

The Indian Coded Scheme includes, for example, Ayurveda, Unani and Sidha, a wide range of treaties on recognised medicinal products.The Ayurvedic medicinal scheme, which accounts for a large part of healthcare systems and for 84 per cent of the internal market, does not exclude medicinal plants, but actually uses as many as 1000 single drugs and more than 8

ooo (Anonymous at el., 1978), respectively use Sidhat Unaani and Amchi (Tibetan Medicines System) (Puspangadan at el., 2001).

The efficacy of these medicines depends primarily on the proper use of authentic material and its availability. The Indian System of Health and Homeopathy's domestic market amounts to Rs.4000 crore, and is expanding every day Just Rs. 3500 crore are available on the Ayurveda drug market. In addition, there is increasing demand in the domestic and international market for natural products, including medicinal value products, dietary supplements and cosmetics.

In the forest, medicinal plants and herbs have been the traditional source over the centuries. This status cannot be maintained much further as, on the one hand the forest area has been constantly decreasing whereas daily medicinal crop requirements have increased. This led to non-scientific use of medicinal plants in the forests. Some medicinal plants are already at risk and facing a extinction danger. The production, protection and preservation of plants needed so that's the way to reclaim its natural population in the nature and sustainable supply of crude substances as well as for easing natural resources pressure. However, for cultivating crops, the standard of products that affirm the good agricultural practises recommended by the World Health Organisation must be maintained (WHO).

Good agricultural practise of medicinal plants includes the manufacture of toxins and raw materials free of heavy metals. This is particularly important in medical plants, as their products are already in use in disease improvement and patients with low immune systems use. The organic culture of these plants is extremely important among good agronomical practises. This type of products is more appropriate on national and the international markets as it would also mean farming in natural circumstances. This will particularly affect medicinal plants, which are used as raw or pulp, decoction, powder, extracts, etc. This is especially important.

Organic agriculture in India is not a recent technique. The use of animal dung as manure was degraded in ancient literature such as Righveda. The value of green manure practised pre-1000 B.C. (Figure 2).

Classification

Kingdom-	Plante
Division	Mangnoliophyta
Order	Dipsacales
Family	Valerianaceae
Genus	Valeriana
Species	Jatamansi

Figure 2 - Plant of Valeriana jatamansi

Organic agriculture is one of the most environmentally friendly oroad methods of production. The organic method of production is focused on unique food production principles that are specifically formulated and aim at achieving socially and ecologically sustainable agro-ecosystems. It is based on the minimization by the use of on-farm resources of external

inputs. The use of synthetic and pesticide fertilisers is prevented while the Codex Alimentarius Commission defines organic farming as a holistic management system for production which promotes and improves agro-economic health, including biodiversity. It stresses the application of management methods in favour of the use of agricultural inputs. In contrast to synthetic materials these techniques can be carried out by using agriculture, biology and mechanics to perform some particular role within the device.

As the pharmaceutical industry has an enormous demand for this plant, so this work carried out hardening of tissue culture plant via using organic mixture and most of the organic modes for the hardening of these tissue culture plantlets under field condition. Therefore, taking into account its multiple uses, studies were carry out on these 2 medicinal plants *Rhodiola imbricata* and *Valeriana jatamansi*

CHAPTER – 2

REVIEW OF LITERATURE

2.1 *Rhodiola imbricata*

In india there is a 10 type of *Rhodiola*

1.*Rhodiola hetrodonta*

Morphology- Toothed *Rhodiola* is arhizomatous lasting with beefy grayiesh green leaves. Alternately masterminded on up branch upstanding stem. Leaves are expansive spear like triangular with sporadic toothed marginis the distinguishing highlight of species thick cymes of star formed red yellow stalkless bractless blossom sprout pre-summer to late-spring male bloom have red or purple anther female have carpel with purple tips (Figure 3).

Figure 3 - *Rhodiola hetrodonta* (Flowersofindia.net. 2020. Rhodiola Heterodonta - Toothed Rhodiola. [online]. Available /Toothed%20Rhodiola. 25 May 2020)

Common name - Toothed *Rhodiola*

Habitat - It develops in cool, bumpy areas

Family - Crassulaceae

2 *Rhodiola quardifida*

Morphology: Flowers are light /dark ruby coloured and very small sized

Flowers are generally 2mm in size (Figur 4) and (Figure 5).

Leaf size 2mm Petal with stamen:-1mmn, Acter gland 2mm in size, Pistillodep- 1mm.

FAMILY- *Crassulaceae*

Figure 4 - Flowering and dried root *of R.quardifida*(. *Rhodiola Quadrifida* Extract(Id:9177950). Buy China Botanical Extract, Plant Extract, Herb Extract - EC21. [online] Available at: <https://www.ec21.com/product-details/Rhodiola-Quadrifida-Extract--9177950.html>

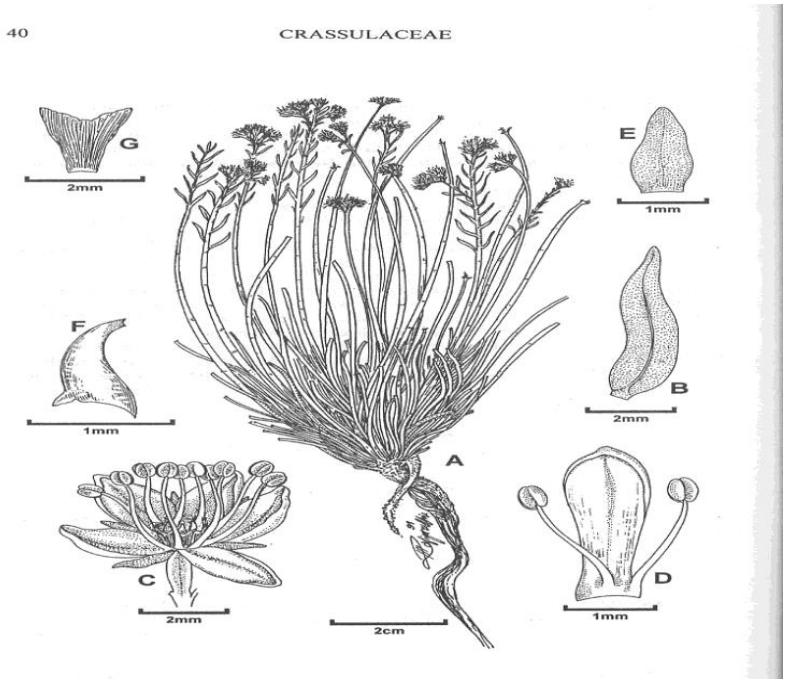


Figure 5 - Different part of plant with size

3.*Rhodiola tibetica*

MORPHOLOGY: Blooming stems numerous in every tuber, straightforward, straight, bald, blossoming stems persevering, 6-24 cm elongated. & 1.4-3 mm nearly broad. Stem foliage are interchange, whole, obtuse, bare, elliptical, elongated praise, 0.4-1.4 cm long & 3-5 mm wide. Blossoms born in several - bloomed, and compound cyme which is at the highest point of stems. The bracts look like the leave of stem. Blossoms are unisexual and 3-5 merous, tail 1.2-3.2 mm long. Sepals intertwined at bottom, whole, obtuse, direct lanceshap 1.3-2.1 × 1.6-2 mm in the male. 2.5-4.1 × 0.6 -0.9 mm occure in the female Petal are bare, gruff, elongated, 3.1-4 ×1.7-1.6 mm in the male. 3.4--4.3 × 1.6-2.6 mm in female (Figure 6).

Figure 6 - *Rhodiola tibetica* (Available at: <https://www.flowersofindia.net/catalog/slides/Tibetan%20Rhodiola.html> [Accessed 25 May 2020].)

COMMON NAME - Tibetan *Rhodiola* or red sedum

FAMILY - *Crassulaceae*

HABITAT - The Tibetan *Rhodiola* is a perenial herb discovered developing on rock and bumpy inclines in the Himalayas, this develops at heights of 3100-4900 m in the Himalayas, which are ranging from the Afghanistan to India & China.

FLOWERING - from June to August, the seeds mature from August to September

4.*Rhodiola wallichiana*

MORPHOLOGY::The plant has various erect stems, 15-30 cm tall, secured with various covering plump straight leaves. Leaves are 2.5-3 cm long, remotely toothed. Blossoms are light yellow, indiscriminate, with spear formed petals. Petals are around 8 mm long, twice the length of the sepals. Organic product is red. (Figure 7).

Figure 7 - *Rhodiola wallichiana*(pale yellow coloured flower) Sites.google.com. 2020. *Rhodiola wallichiana* - Efloraofindia. [online] Available

COMMON NAME - Wallich's *Rhodiola*

FAMILY - *Crassulaceae*

HABITAT - Wallich's *Rhodiola* is found in the Himalayan region, from Kashmir to Bhutan, at heights 3000-4800 m.

FLOWERING - June - September.

5. *Rhodiola fastigiata*

MORPHOLOGY: Old blossoming stem are steady, 5-16 cm long, 0.7-3 mm wide. Stem leave sare substitute, whole, pointed or obtuse, direct applaud barely praise, 5-11 Mm long and 1-1.6 mm wide (Figure 8).

HABITAT: It is a lasting herb, which is found on the rocks & rock cervices and lush slants of the Himalayas, from the regions of Pakistan to China, at heights of 6004-5600 m. The species name fastigiata implies bunched and corresponding to one another.

Figure 8 - *Rhodiola fastigiata*(Specialplants.net. 2020. *Rhodiola fastigiata* Seed. [online] Available at: <https://www.specialplants.net/shop/seeds/Rhodiola_fastigiata)

COMMON NAME - Clustered *Rhodiola*

Family - *Crassulaceae*

FLOWERING - Blossoms emerge on the stems, in conservative corymblike cymes, 5-14 bloomed. Bracts resemble with stem leaves. Blossoms are either yellow or may red. tail 1-5 mm long and Sepals basally combined Sepals basally melded, whole, obtuse, triangular-praise or barely applaud, 2.4-3.4 x 0.6-1.6 mm. The Petal are whole, gruff, barely elliptical, Barely, Obovate direct oblanceolates 2.5-5 x 1.5-2.8 mm.

Blossoming: June-August.

6.*Rhodiola himalensis*

HABITAT - Himalayan *Rhodiola* is found in the Himalayan region, from Kashmir to China, at elevations of about 3400-4800 m.

MORPHOLOGY - Himalayan *Rhodiola* is an enormous conspicuous plant with erect verdant stems 10-35 cm tall. Stems are secured with thin elliptic or upset spear formed plump leaves (Figure 9).

: 

Figure 9 - flower of *R.himalensis*(Flowersofindia.net. 2020. *Rhodiola himalensis* - Himalayan Rhodiola. [online]

FAMILY - *Crassulaceae*

FLOWERING - On the stems are borne groups of dull red, pinkish to yellow blossoms. Blossoms groups are 3-5 cm over. The blooming stems emerge from an erect root-stock which is secured with old vanished stems Blossoming: June-August.

7.*Rhodiola cretinii*

HABITAT - Various. Moron's *Rhodiola* is found in the Himalayan region, from Nepal to Sikkim & south east Tibet, at heights 3700-4300 m.

MORPHOLOGY - Idiot's *Rhodiola* is a little succulent tangle shaping lasting herb with crawling stems (Figure 10)

Figure 10 - *Rhodiola cretinii* Zhiwutong.com. 2020. *Rhodiola cretinii* (Hamet) H. Ohba Subsp. Sino-Alpina (Fr?D.) H.Ohba_.[online] Available at: <http://www.zhiwutong.com/dan_tu/36/29546.)

COMMON NAME - Cretin's *Rhodiola*

FAMILY - *Crassulaceae*

FLOWERING - Blooming stems are basic, erect, 2-12 cm tall. Leaves are exchange, restricted elongated or obovate-elliptical, 7-10 x 1.5-2.5 mm, edge whole or shallowly 3-5-adjusted toothed, tip adjusted, gruff, or pointed. Blossoms are borne in bloom groups, around 1 cm over, at the highest point of the blossoming stems. brings down unisexual, inconsistent 5-merous in male plants, once in a while 4-or 6-merous. Sepals straight to subulate, 3-4.5 mm, tip gruff. Petals are greenish-yellow to greenish-red, direct altered lanceshaped, straight spoon-formed, barely elliptic, or straight obovate, 3.5-6 x 1-1.5 mm, tip obtuse. Stamens are 10. Carpels are erect, barely ellipsoid, 5-7 mm,

Blossoming: June-Augus.

8.*Rhodiola sinuate*

MORPHOLOGY: *Trifid sinuate* is a little plant, frequently developing on trees or developing on stones, with verdant groups of white to pale pinkish blossoms, and meaty elliptical to applaud normally pinnately-lobed leaves. Blossoms are borne at branch-closes, in spread groups regularly encompassed by longer leaves (Figure 11). Petals are straight lance

shaped 5 mm, twice the length sepals. Leaves are truly factor, 2.5-5 cm, with 3-5 inconsistent spreading gruff flaps, or now and then not lobed; stems a few, erect 8-30 cm, smooth

Figure 11 - *Rhodiola sinuate* (Sites.google.com. 2020. Rhodiola Sinuata - Efloraofindia. [online

COMMON NAME - Trifid Sedum

HABITAT - Trifid Sedum is found on rocks, open slants in the Himalayan region, from Bhutan to kashmir, at heights 1200-4300 m

FLOWERING - August-September**.**

9. Rhodiola bupleuroides

MORPHOLOGY: Bhutan *Rhodiola* is an enduring herb, 7-37 cm tall, with scarcely any stems. Blossoms are red to dark purple, in huge verdant, level bested, spread, frequently careless bunches, continued top of verdant stems with expansive applaud to elliptic leaves. Petals are lanceshaped, twice the length of the sepals. Leaves are 1.3-5 cm long, whole to remotely toothed, regularly with a heart-molded or eared base (Figure 12).

Figure 12 - Rhodiola bupleuroides([online] Available at: <https://www.flowersofindia.net/catalog/slides/Bhutan%20Rhodiola.)

COMMON NAME - Bhutan *Rhodiola*

FAMILY - *Crassulaceae*

HABITAT - Bhutan *Rhodiola* is found in the Himalayas, in Pakistan, some upper part of Nepal & Sikkim at elevations of about 2740-3600 m.

FLOWERING - July-September.

10.*Rhodiola imbricata*

MORPHOLOGY - *Rhodiola imbricata* is an enduring and dioecious herb of 25 to 30 cm stature. Root stocks are fragrant having an aroma of like rose blossoms. It is a herb which has a thick rhizome 11-36 cm, rhizome is of pink colour from inside and brilliant from the exterior region, rose scented huge root stock, also leaves as 1.4-4 cm long, oval to limit elliptical, almost whole; blossoms light yellow in blocked group; organic products are 3-6 and many seeded. It's blooming and its fruiting occurs in the month of 7 to 9 (Chaurasia .et.al., 2007).

Figure 13 - R.imbricata .Valley Of Flowers. [online] Available at: <https://valleyofflowers.info/flowers

HABITAT :Plant develops fiercely basically on rough slants, wet spots, higher goes at an elevations of 15000-19000 ft amsl in the Himalayan cold desert and in the Eurasia mountain locales and in high Cold scopes . In the valley of Indus and Leh i.e. Indian trans-Himalaya it is normally founded (Chaurasia et al., 2007). *R. imbricata* founds in the Sinai Himalayas, in Nepal at Qinghai, Xizang, it founded in the bumpy area Himalaya (Kanupriya et al., 2005). In the three distinct valleys of Zanskar, Indus and Changthang of Ladakh area of the Trans-Himalayas the significant no of *R. imbricata* is present (Chaurasia et al., 2007).

FAMILY: Crassulaceae

Utilized: The Tibet and nearby locality, for the treatment of undying shortcoming and disease because of contamination, the *Rhodiola* species is being utilized as customary medications from last thousands years (Rohloff et al., 2002). *R. imbricata* is a significant conventional therapeutic plant and it is generally used as a nourishment crop . Foundations of *R. imbricata* is being utilized for the curing of cough, lung issues, fever, pneumonic grumblings and the loss of vitality.

Importance of *Rhodiola imbricata*

- *Rhodiola* has for quite some time been known as an adaptogen, a characteristic substance that expands your body's protection from worry in vague manners.

- It discovered noteworthy enhancements in side effects of pressure, for example, weakness, fatigue and uneasiness

- *Rhodiola* rosea has likewise been recommended to have stimulant properties by adjusting the synapses in your mind

- usually recommended stimulant sertraline, which is sold under the name Zoloft.

- Improves Brain Function

- It Can Improve Exercise Performance

- Anticancer Properties
- Cytoprotective activity
- Radioproctive activity
- Antioxidant
- Anti-hemolytic avivity
- Antiviral activity

2.1.2 Micropropagation of *R.Imbricata*

- Under the lab conditions callus establishment and shoot recovery was recorded as standout in MS media having a BAP as 1 mg/L and the IBA as 2 mg/L, while utilizing the MS media which is have cytokines e.g BAP and KN as 2 mg/L brought about the directly shoot development & expansion. MS media contain BAP and IBA as 4 mg/L came about in vitro root enlistment inside 25 to 30 days. 75 to 80 % appeared by in vitro established plantlets in the wake of external environment. (Sharma et al., 2017).

- Callus culture of *Rhodiola imbricata* built up over various light situation: RGB (40% green 40% red: 20% blue), 100% red, 100% blue, 100% green, and the 100% white as the control. The outcomes indicated that callus societies developed below the red light aggregated most extreme measure of biomass (7.43 g/L) till to 21 day of culture, when contrasted with another light situation. Greatest explicit development rate was (0.126 days-1) multiplying period (132.66 hours) was found in callus societies developed in red light state. Switch stage elite fluid callus enlistment recurrence (93 %) was found in MS medium enhanced with mixture of 5 mg/L BAP and 5mg/L NAA (kapoor et al., 2018).

- The competent plans for the *R. Rosea* micro propagation had been developed by (Tasheva et al., 2010), on the mediums of Murashige and Skoog (MS), improving microshoots on the hormone free medium contain 1/2 MS (BAP) by 5 μM 6-benzylaminopurine and 2,5 μM α-naphthylacetic (NAA) and demonstrated that no additional pre-planting treatments are needed for the compelling in-vitro germination of R. rosea This technique results in a strong return on in vitro plants.

2.1.3 Production of secondary metabolite

The rhizome and roots collect numerous pharmaceutically dynamic optional metabolites which have a place with various concoction gatherings (Table 1)

Table 1- Secondary metabolites of *Rhodiola rosea*

Chemical group	Reference
phenylpropanoid glycosides	Zapesochnaya and Kurkin 1983, Brown *et al.* 2002, Tolonen *et al.* 2003
phenylethanol derivates	Troshchenko and Kutikova 1967, Brown *et al.* 2002
flavonoids	Kurkin *et al.* 1983, 1984, Brown *et al.* 2002
terpenes	Kurkin *et al.* 1986. Beloy *et al.* 1994, Brown *et al.* 2002, Rohloff 2002
phenolic acids	Brown *et al.* 2002
coumarins	Furmanova *et al.* 1995
lactones	Furmanova *et al.* 1995

2.1.4 Cinnamyl alcohol glycosides and salidroside biosynthetic pathway

The results of phenylpropanoid digestion are both the salidroside and the cinnamyl liquor glycosides, got from the phenylalanine which subsidiary of shikimic-chorismic corrosive way. Compound which guides the carbon to a blend of the phenyl propanoid metabolites recognized like phenylalanine smelling salts lyase that is PAL, Buddy is one of the most broadly considered chemical in phenylpropanoid path, if it is not in all optional digestion. It changes phenylalanine into the cinnamic corrosion. Also the path leaves the principle biosynthesis way of phenylpropanoid, which can prompt the coumarins, flavonoids or then again lignins and some of the lignans. Nonetheless, similar kinds of chemicals take part in future bio-synthesis of cinnamyl liquor glycoside (Figure 14)

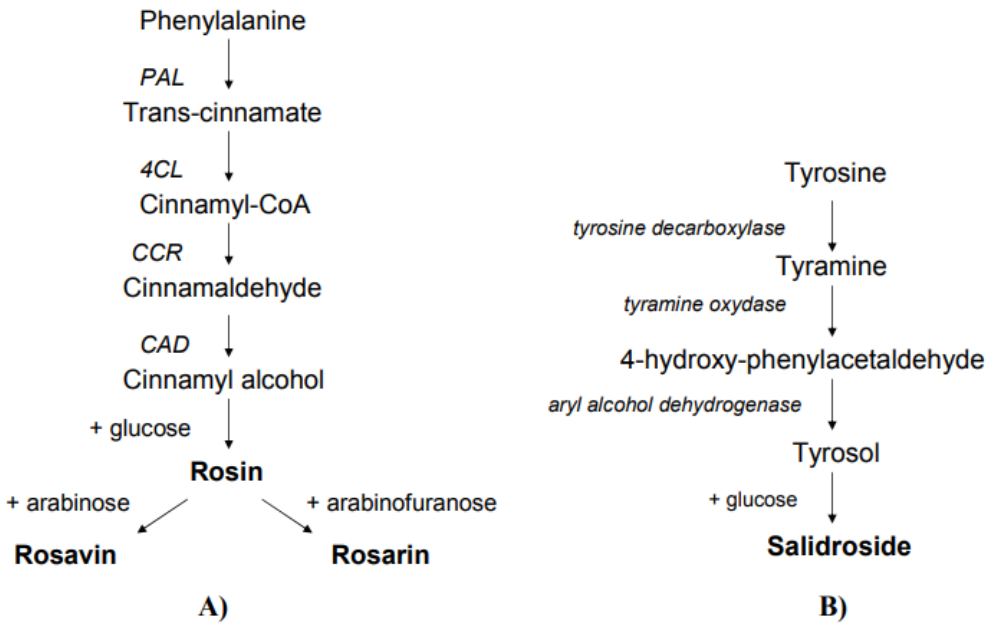


Figure 14 - Cinnamyl alcohol glycosides and salidroside biosynthetic pathway

2.1.5 salidroside

Salidroside secures the human erythrocytes by the cancer prevention agent action nature and the caspase-3 restraint in the portion sub-ordinate style. It also shield the hematopoietic un-differentiated cell from the oxidative worries by the initiating PARP1, the DNA fix protein which is efficiently linked to the apoptosis of cell (Li et al., 2014). After effects of the various medical preliminaries has uncovered that it has different capacities, as an e.g the hostile to chilly, against the exhaustion, hostile for anoxic, hostile to an infection, hostile for the microwave radiation, and also hostiles to the tumour.

It additionally has different restorative properties, for example, forestalling ailment related with mature age, fortifying and improving work productivity. As a result of its natural acclimations movement, a significant job is assumed in medicinal services, in military, and sports and aviation. The biosynthesis pathway and its guideline of p-tyrosol has not totally comprehensive (Figure 15).

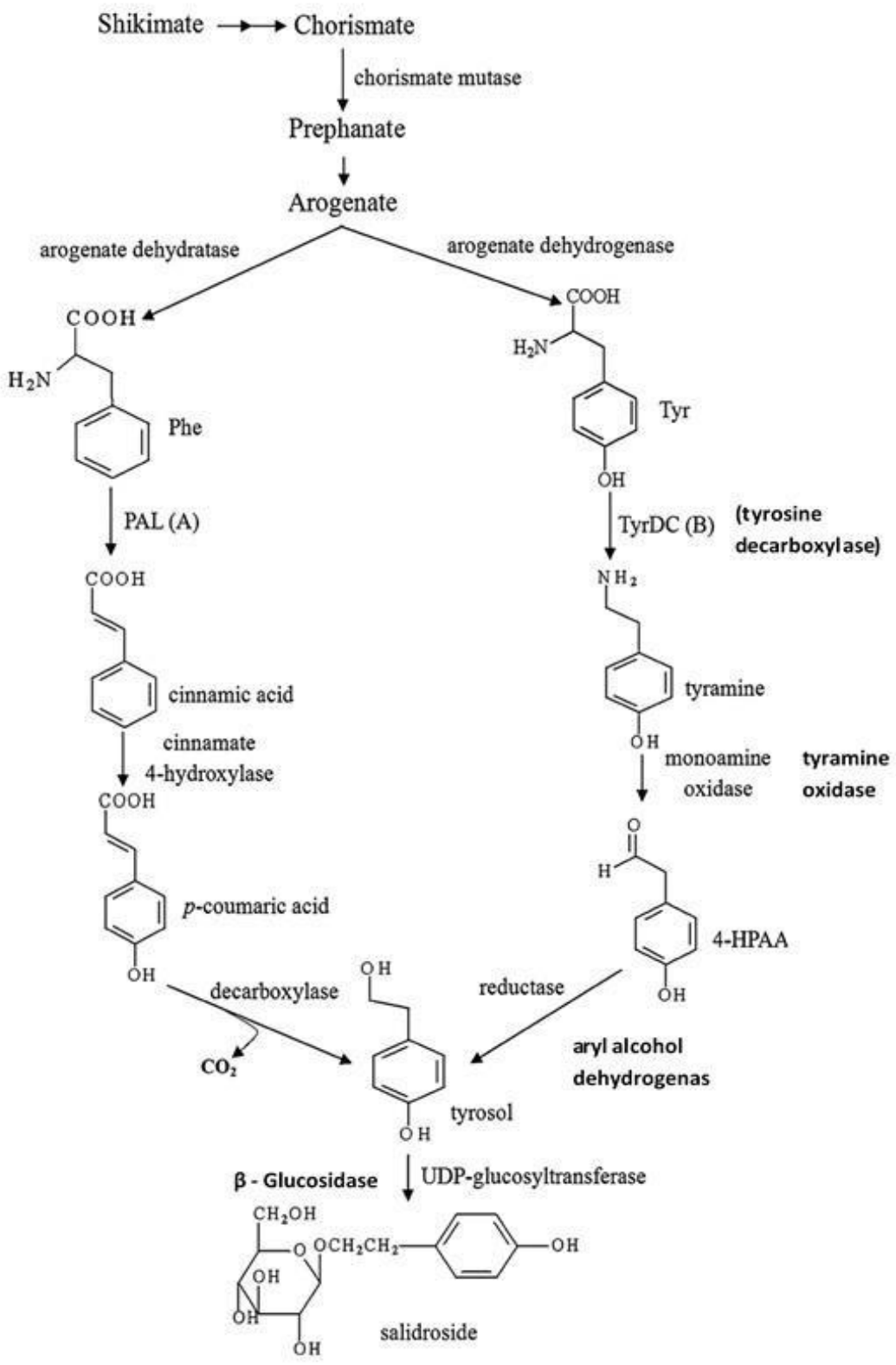


Figure 15 - Showing . Suggested pathway liable for the Salidroside biosynthesis

2.1.6 Rosavin

The phenylpropanoids usually present in Rhodiola spp are cinnamoylglycosides such as rosavines.They were first isolated and identified by Zapesochnaya and Kurkin and named. Rosavin is recognised as an anti-stress and adaptogenic motor function stimulant (Zapesochnaya et al., 1995).Its antidepressant potential and UV-protective properties (Goldstein et al., 2008) have been recorded. Extremely reduced neovascular reaction also induced into mice skin after L-1 sarcoma grafting, at the maximum dose (Bany et al., 2008)

Shalidroside and cinnamyl alcohol glycosides are all components of the synthesis of phenylpropanoids originating from the pathway of shikimic chorismic acid, phenylalanine Phenylalanine ammonium lyase is the enzyme that guides carbon to phenylpropanoid metabolites (PAL).PAL is, if not in all secondary metabolism, the most thoroughly studied in the phenylpropanoid pathway. PAL Phenylalanine is converted to cinnamic acid.The pathway leaves at this stage the principal phenylpropanoid biosynthesis route leading to coumarins, flavonos or lignins and lignans Cinnamyl-CoA reductase reduces this ester to cinnamaldehyde (CCR).The amount of cinnamaldehyde is also reduced to cinnamyl alcohol by cinnamyl alcohol dehydrogenase (CAD). There is still no description of the enzymes involved in the production of cinnamyl alcoholic glycosides. Rosin is formed by a glucose conversion, the simplest roseroot glycoside (Figure 16).It is generated from rosin by the connection of rosavin arabinose and rosarin arabinofuranose.Further glycosides may be formed depending on the type of sugar and site to which it is associated.

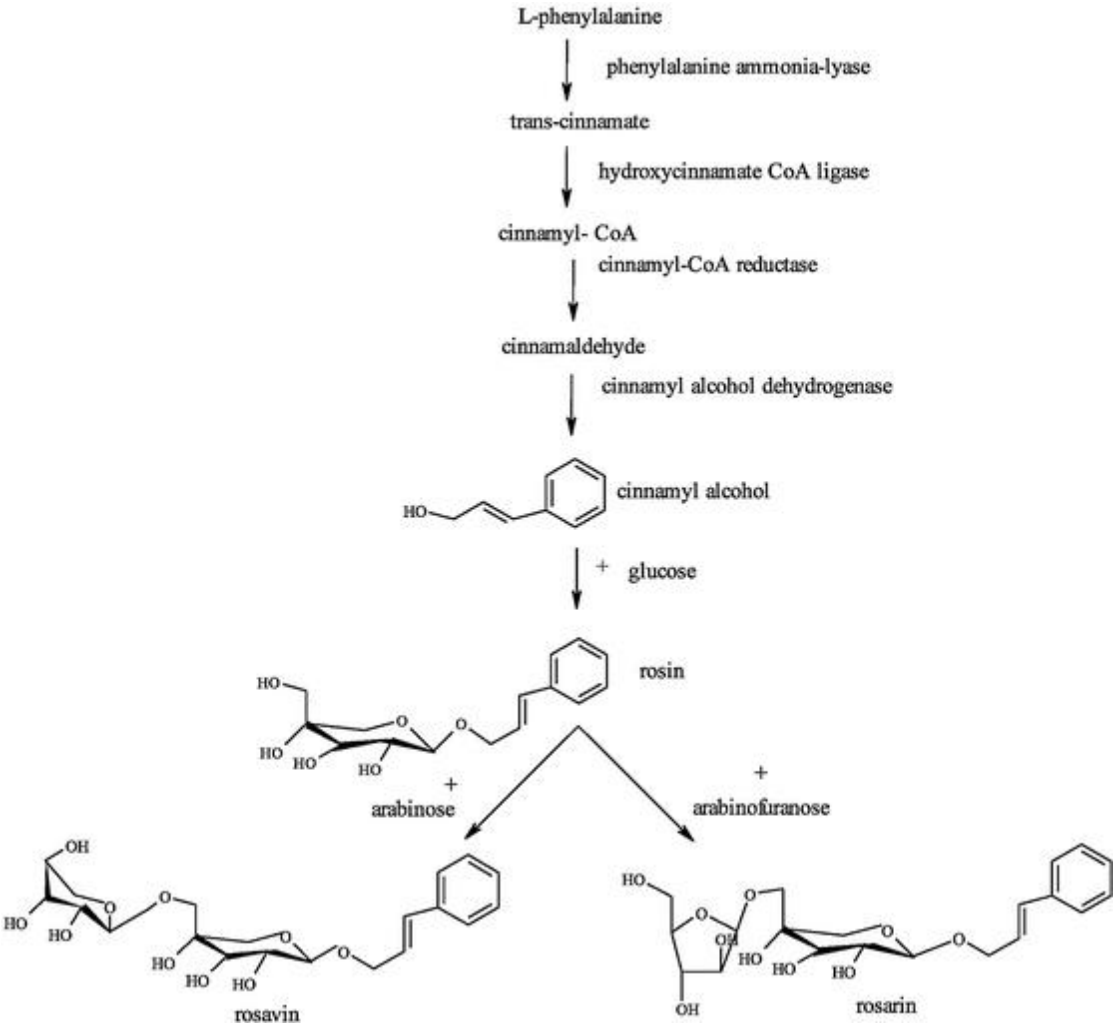


Figure 16 - The synthesis of the Rosavin, the Rosin and the Rosarin (Grech-Baran .et.al., 2015).

2.2 *Valerina jatamansi*

Valeriana jatamansi (Bennet et al., 1987), who belonged to the Valerianaceae family and is commonly referred to as Valeriana indian, Mushkbala, Tagar, etc. It is a perennial grass with a short, sometimes heavy rootstock that smells. Tufted stem is typically approximately 45 cm tall. Basal feeds have a diameter of about 3.5-8.5 cm, are deeply corded-ovate, often toothed or sinuated with long size petiol. Stem leaves just a few, a lot less, whole or pinnate. White or pink flowers born on almost leafless branches. compressed, glabrous or hairy fruit (Stainton et al., 1984).

The species is present in the temperate Himalayas up to an altitude of approximately 1200-4000 m between kashmir and Bhutan and between 1200-2000 m in hills of Chasi and Jaintia (Anonymous et al., 1976). The economic elements of the rhizomes and roots of this plant are the medication. These are medically used for hysterical fits, hypochondriasis, anxious restlessness, mental distress, etc. Pulverized medicine and sugar combined for urinary disturbances is recommended (Anonymous, 1976; Maheshwari and Singh, 1989).The therapeutic effects of *V. jatamansi* are caused by a new group of iridoids known as valpotria monoterpenic derivatives, useful as reassurance products and sedatives (Wagner et al., 1980).

14 plantations in separate parts of the Poland (Debska et al., 1956) trial conducted of The three species of the *Valeriana officinalis*. The yield of seed was 200 kg/ha under unfavourable spring, but in favour of summer and autumn, with roots at 2,350 Kg/ hac. The oil was between 0.25 and 1.25 percent and 10.95 to 1492 units of organic activities.

The root output improved by 2 -4 times by an effective weeding control in *V. officinalis*. No herbicides have affected the vital root quality of more than 20 herbicides studied. The most powerful way for Patoran to leave no residues on the roots is to increase the chlorophyll content of the leaves (Yekshin and Bukina et al., 1973).

Sowing the seeds V. officlnalis @ 3 Kg/ha was shown by(Czabajska et al 1976) to be a strong yield flower, essential oil and valepotriates, as a result of the seedlings that were planted. However, dilution of the direct plants produced no better results. The best date for seeding was mid-August, however good Spring or autumn weather patterns might be used.

(Gorbunov et al., 1979), in relation to their introduction to cultivation, some of the Valeriana spp germinated higher at 3-4°C, 7-9°C (18-20°C) or 3-4°C, compared with 18-20°C (18-20°C). The plants cultivated were promisingly propagated in the vegetation. The polyploid species *V. cardamines and V. erlophylla* is found to be more appropriate than the diploid species *V. alpesrtris, V. tiliifolia*.

The hydroponic processing of V. officinals was attempted by (Babakhanyan at el., 1979). Air dry root yield and productivity in the plants of hydroponically active substances is 1.5 times higher than in the plants to be cultivated.

(Sharma et al., 1979), cultiver and forest exploitation on the hills of Chakrata, lJttar Pradesh, introduced the exotic strain of *V. offlcinalis* (USSR). In summer/monsoon, they announced strong increase of transplants. In addition, 40-50 fresh root weights per plant and 0.76% to 0.35% essential oil were noted.

(Lomazov et al., 1984) analysed drying of Vallerian roots at 40°C and at 60°C at air speed 1 and 4 m/sec, respectively. The effect of the NPK on value yields was examined in V. *bureinalis* by(Bosetto et al., 1987) and achieved highest yields in both the soil and hydroponic culture at 150 kg N and 75 kg P and K ha.

The best way to grow lemon grass (Cymbopogon flexuosus) was investigated by (Thomas et al., 1989), who examined transmitted, slip-transplanting and planting seedlings; he also observed that planting slip and semen transplanting substantially gave a higher yield of weed and oil relative to straight-seedling.

The dry matter generation, uptake of nutrients and quality study in *Tagetes minuta* was studied by (Senthilkumar et al., 2003). They observed the development of dry matter.A maximum of 25 percent of organic and 75 percent of inorganic sources was combined with the nutrient contents, the uptake of nutrients and the essential oil contents. The relationship effect was however considered to be irrelevant. Trans-ocimene were higher in Tagetes oil in relation to critical consistency.

2.2.2 Medicinal value

The medicine and essential part are the rhizome and roots of the plant. Essential oil has poor action of antibacterial and ampiprotozoa (Chopra et al., 1956). The calming and sedatives of valeriana preparations are caused by the newly found group of compounds known as valpotriates (Anonymous et al., 1976). (Wagner et al., 1980) and (Nahrstedt et al., 1984) have also identified sedative and spasmolytic behaviour based on comparative research into Valeriana extract sedative effects

The new roots and rhizome juice is better than dry ones.The medicine has lost its properties through drying. In insomnia and in some cardiac preparations fresh juice is used as narcotic.

Valeriana jatamansi as a holy plant while identifying economically significant medicinal plants in the Kanatel forest of Tehri-Garhwal. It is used at such ceremonies in preparation of ubtan (as cosmetic). The woollen robes are held to prevent the invasion of insects. There are also people who use the rootstock to prepare hair oil. This is contained as an ingredient in the essential ayurvedic preparations 'Shringiadikwath' and 'Jwardikashyai.'The insect repelent behaviour in the roots of v. hardwickii was reported by (Atkinson at al., 1980).

Valeriana spp. has a central nervous system with depressant activity and is used in hysterical fit therapy. Additional nervous disturbances, headache, hysteria, liver distress, flatulence. The root extract has an effect which is stimulating to treat epilepsy, antispasmodes, cholera, eye disorder, shell shock and neurosis. The fruity juice and the roots are more powerfull, the drug's characteristics loose during the drying process (Manandhar at.el., 1980) (Oshima et al., 1986).

(Pandey at al., 1988) documented a couple of Arunanchal Pradesh mon's healing plants in small-scale forest produce like *V. wallichii* and the rhizome with a blazing coal and inhaled smoke. Sometimes uses as an inscense.

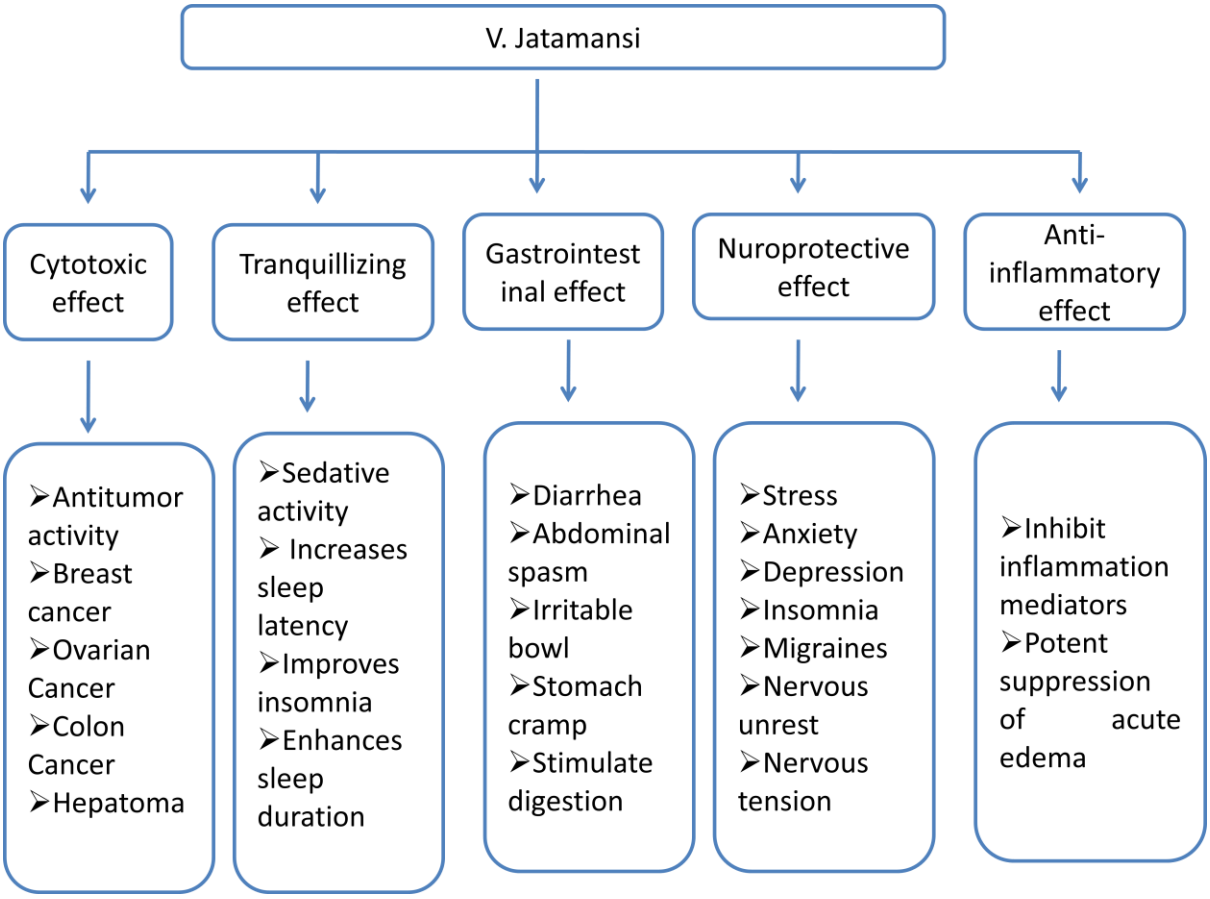


Figure 17 - Pharmaceutical properties of Valeriana jatamansi

2.4 Research gap

- Late progressing study in plant uncovered crucial activity in the rhizome and root of a plant which are tried in the various types of solvents. In any case, the dynamic standards in this concentrate despite everything stayed unrevealed
- Till date no rapid method developed for the micropropagation and production of secondary metabolite yet developed in the plant which can be up scaled moreover large scale production is yet not been reported so that we can reclaim its natural population
- Till date no such research conducted in which bacteria used enhace the micropropagation potential of *Rhodiola imbricata* and secondary metabolite

Objective of study

1:- To carry out multiplication of in vitro grown shoot of *R. imbricata* and *V. jatamansi*

2:- To optimise hardening condition for *R. imbricata* and *V. jatamansi*

Chapter 3

MATERIALS AND METHODS

3.1 *Rhodiola imbricata*

3.1.1 Selection of plant

R. Imbricata plants were derived from in vitro tissue culture chamber in Jaypee University of Information Technology in Waknaghat, HP, India at 15 ± 2 ° C with relative humidity of 70 percent, a photoperiod of 16 hours daily / 8 hours, and 3000 lux of florescent tubes (Philips, India) (Figure 18).

Figure 19 - *R. imbricata* in vitro cultivated tissue cultures in plant tissue culture chambers Of Biotech Department, Jaypee University of Information Technology, Waknaghat, India,

3.1.2 Media preparation and culturing

The tissue media of plants consist of a number of things separated into micro and macro components. In the laboratory, MS media for cultivation of plant crops were used and the culture conditions for micropropagation in *R.imbricata* were assessed. A number of 6-benzylaminopurine (BAP), Kinetin (KN), Gibberellic acid (GA3), Indole-3-butyric acid (IBA were combined and used. To this end, a number of different doses were used. A 3% (w/v) solution was applied to the MS media. The pH for any medium was altered to 5.7, and the gelling agent used was agar-agar 0.8 per cent (w/v). At 121°C and 15 lb/in pressure for 15 to 20 (Figure 20)

(A) (B)

(B)

Figure 20 - Establishment of culture (b) Multiplication of culture

3.1.3 Hardening tissue cultured plants

In vitro shootings were rooted and rooted plants used to harden after 7–8 periods of subculture. Harding was favoured for plant having strong growth. The plants were taken from culture tubes carefully. The roots are softly cleaned with tidy warm water for any sign of agar to be eliminated. The plants was then placed in small pots with a proportionate soil mix and sand(1:1). Since the stomata did not work properly and a low cuticle formed, a jar/polybag was used to preserve a high moisture content (fig 21). Moisture of 60–70%, from 18–21°C, After seven days of the hardening, container was removed for brief intervals (30 minutes) to acclimate plants. The exposure time has been expanded by 1 hour per day. Humidity is maintained time to time

Figure 21 - Optimization of humidity during hardening of plant via coverning with jar

In this, I try different potting mixture for the hardening of plant For hardening, plantlets having good growth were preferred. 5 sample were taken and all plants have different length The plants were taken from culture tubes carefully. the roots are softly cleaned with tidy warm water for any sign of agar to be eliminated. Then out of 5 the 3 are transfer to small pots which contain a proportionate mixture of soil + sand and mixture of cocopeat, perlite, vermicompost in 1:1:1 ratio and remaining 2 plantlets were transformed in the pot containing mixture of cocopeat, perlite,vermicompost and sand in 1:1, they covered with jar/polybag to maintain the level of humidity because the stomata are not properly working and low cuticle developed. Conditions were optimized to 60–70% humidity, 18–21 °C, After seven days of hardening, the container was removed for brief intervals (30 minutes) to acclimate the plants. The exposure time has been expanded by 1 hour per day. Humidity is maintained time to time (Figure 22).

Figure 22 - Plants harden under different potting mixture (a) sand+mixture of cocopeat, perlite and vermicompost in 1:1 (b) sand +soil+mixture of cocopeat,perlite and vermicompost.

3.2 *Valeriana jatamansi*

3.2.1Micropropagation

In present study in vitro propagation carried out in MS media with different combination of plant growth regulator (PGRs). In vitro shoot multiplication is carried out by using microshoot (Figure 23). In MS media supplemented with KN 1.5 and IBA 1 mg/l and culture were incubated for 4 weeks duration of time for their good growth.

Figure 23 - In vitro multiplication of *Valeriana jatamansi*

3.2.2 Hardning

The in vitro grown plants have been transported to potting mixture which contain sand and soil (1:1) along with potting mixture cocopeat, vermiculite, perlite in (1:1:1). they cover with jar/polybag to keep the strong moisture level because the stomata are not properly working and low cuticle developed. Optimized conditions of moisture 60-70 percent, 18-21°C, seven days after hardening. The container was take off for brief intervals (30 minutes) to acclimate the plants. The period to expose has been expanded by 1 hour per day. Humidity is maintained time to time. The field studies are carried at Rihana Village, located about fifteen kilometres from Jaypee University of Information Technology waknaghat (Figure 24). The field of experimentation is sub-temperate. The atmosphere and the climate during the season was usually May, June as the hottest months hit the highest temperature up to 37°C, while the lowest temperature reached up to 2°C during December. Rainfall during monsoon mainly occurred (July-August). When the plants were transplanted vermicompost along with manure and cow dung was tested on plant for their survival, Different combination of organic mixture were used for hardening of plant (Table 2).

Figure 24 - Set up prepared for hardening of the plants

Table 2- Different treatment of organic potting mixture used for hardening of plant

NAME OF SOIL AND COMBINATION	**TEST**
FIELD SOIL	**T1**
FIELD SOIL+OAK LITTER	**T2**
FIELD SOIL+SAND	**T3**
FIELD SOIL+ VERMICOMPOST	**T4**

Chapter 4

RESULTS AND DISCUSSION

4.1 *Rhodiola imbricata*

4.1.1 Establishment of hardening

In 2 different potting mixture, I try to harden the tissue culture raised plant at 1000 m having poting 1:1 (sand and soil) mixture which usually grow at the height of 4000-5000 m. It has been observed that plants are grown during the 1& 2nd week plant seemed to be adapting. The potting mixture contain different combination of soil, sand, mixture of cocopeat, Perlite and vermicompost (Table 3). It has been found that T1 potting mixture not giving best result in hardening of plants whereas T4 is giving best result in hardening of plants where I achieved approximately 50% survival in 1 replicate and 60% in another replicate and plant growth show in (Figure 25).

Table 3- Effect of different organic potting mixture on growth and development of *Rhodiola imbricate.*

Test	**length**	**No.of stem**	**No of sub stem**	**No. of leaf before hardning**	**No.of leaf after hardning**	**Plants after 20 days**	**Media mixture used**
T1	2.5-3 cm	6	7	6	0	Plant are not able to survive	Sand +soil+mixture of cocopeat, Perlite and vermicompost
T2	13.5-14.5cm	4	16	32	13	Transfer to another mix because	Sand +soil+mixture of cocopeat,

						they are week but still died	Perlite and vermicompost
T3	11-8 cm	3	6	21	4	Transfer to another mix because they are week but still died	Sand +soil+mixture of cocopeat, Perlite and vermicompost
T4	4.5-5.5cm	3	8	49	44	Still good condition	Sand +mixture of cocopeat, Perlite and vermicompost
T5	6.2cm with callus	1	9	112	83	Colour change from green to pale yellow	Sand+mixture of cocopeat, Perlite and Vermicompost

(A) (B)

Figure 25 - Harden plant of *Rhodiola imbricata* (A) After 4 week (B) After 6 week

4.*2 Valeriana jatamansi*

The same test and trial were carried out on *Valeriana jatamansi*. The in vitro grown plants have been transported to potting mixture(Table 4) which contain sand and soil (1:1) along with potting mixture cocopeat, vermiculite, perlite in (1:1:1). Plant were harden with different organic potting mixtureas (Table 5). The plant were survive approximately 95% in one replicate and approximately 100% in another replicate. Maximum height of plants was observed after 2 month in the field soil and the vermicompost composition 17 cm which is followed by oak leaf 14.5 cm, sand 12.3 cm and at last field soil achieve 11.02 cm. After 4 month that maximum height of plants was observed in the field soil and the vermicompost composition 38 cm which is followed by oak leaf 29 cm, sand 26.05cm and at last field soil

achieve 21.12 cm (Figure 26). Optimization protocol are so significantly produce or increase biomass of plant into approximately 12 folds Data in table 6. maximum biomass was observed in the field soil and the vermicompost composition 110g which is followed by oak leaf 87g,sand 74 g and at last field soil it achieve 6 (Table 6).

Table 4- Different concentration of potting mixture used for hardening

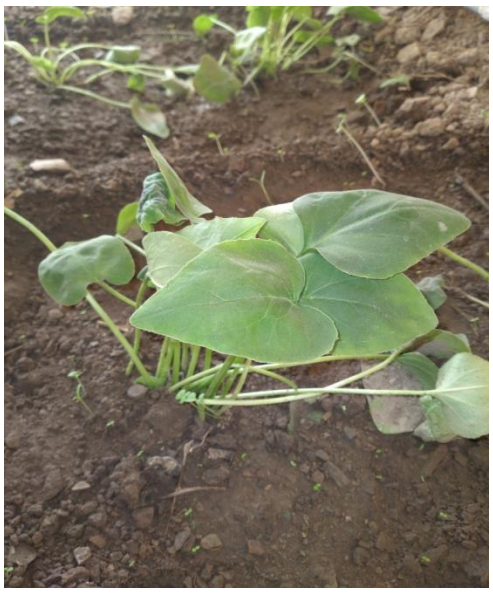
Ratio	Potting mixture
1:1:1	Soil+ sand + potting mixture cocopeat, vermiculite, perlite in

Table 5- Different potting mixture for hardening of *Valeriana jatamansi*

Treatment of plant	**Height of plant in cm**	
	After 2 month	**After 4 month**
FIELD SOIL (T1)	11.02	21
FIELD SOIL+OAK LITTER (T2)	14.5	29
FIELD SOIL+SAND (T3)	12.03	26.05
FIELD SOIL+ VERMICOMPOST (T4)	17	38.05

Table 6- Biomass of *Valeriana jatamansi*

Treatment of plant	**Biomass of plant in g**	
	During the plantation	**After 4 month**
FIELD SOIL	9	66
FIELD SOIL+OAK LITTER	10	87
FIELD SOIL+SAND	8	74
FIELD SOIL+ VERMICOMPOST	9	110



(a) Young plant (b) Vegetative stage

Figure 26 - *Valeriana jatamansi* at different stage (a) Young plant (b) Vegetative stage (c) Flowering stage

The present work carried out in this study has observed the result on similar line as reported by (Sharma et al., 2016). He tests for callus induction, calli-shooting regeneration, direct shoot-organogenesis and rooting were done with 10 different medium combinations of various levels of 6-bizylaminopurine (BAP), Indole-3-butyric acid (IBA), kinetine (KN), gibberellicic acid (GA3) and thidiazuron (TdZ) induction. (Bhardwaj et al., 2018) focuses on developing the *Rhodiola imbricata* micropropagation protocol. Different cytokininins and auxins have been studied to proliferate and root microshoots in vitro. (Kaur et al., 2000) Strong medium supplemented with either benzyladenine alone or with naphthalene acetic acid became developed in conjunction with the use of explanatory medium. The buds cultivated with BA and IAA or NAA in a nutrient medium that after three or four weeks developed roots on the same medium. (Das et.al., 2012) For Valeriana jatamansi Jones' large-scale development a reproducible and effective callus-mediated shoot regeneration system has been developed. Murashige and Skoog Effect (MS), augmented by varying concentration of IBA, NAA, 2,4-D with respect of multiplication of plant. Whereas very less data available on organic hardening plant in othere plant species.

Chapter 5

Conclusion

These two important medicinal plants *Rhodiola imbricata* and Valeriana jatamansi are of high commercial value so we carried out in vitro multiplication as per the optimization protocol which were established in the lab at JUIT, Waknaghat. *Rhodiola imbricata* plants are transcendently used due to there high therapeutic properties, *Rhodiola* is encouraged to increase vitality, endurance, force and mental ability, to enhance athletic success, to resist stress and to better treat depression, anxiety and other symptoms. *Valeriana jatamansi* contain significant number of chemical components found in plants roots and rhizomes suchas iridoids, liganoids, valerianadoids and valpotriates to cure various diseases. anxiolytic, anti-inflammatory and the neuroprotective activities as well as anti-cancer activity and antioxidants. So these plants needed to be produce in large amount after their transplantation under field condition, so the present study has found that T4 (Sand + Mixture of cocopeat, perlite and vermicompost) mixture give best hardening of *Rhodiola imbricata* and treatment T4 (Field soil+ Vermicompost) give the best hardening of *Valeriana jatamansi.* So this study provided the methodology for commercial production of these important medicinal herb so it Compensate rising demand of pharmaceutical industry.

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