Optimization of conditions for Growing *Brassica oleracea* and *Rhodiola imbricata* under Hydroponic System

Dissertation submitted in partial fulfilment of the requirement for the degree of

Masters of Science

IN

BIOTECHNOLOGY

BY

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Under the Guidance of

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MAY 2023

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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

Waknaghat, Solan

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DECLARATION

I hereby declare that the project work entitled "**optimization of conditions for growing medicinal plants under hydroponic system"** has been submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, solan is a record of an original work done by her under the supervision of **DR. HEMANT SOOD.**

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Supervisor's Certificate

This is to certify that the project report **on "Optimization of conditions for growing *Brassica oleracea* and *Rhodiola imbricata* under hydroponic system**" by **Yatshi Yadav** during her end-of-semester requirements for Masters of Science in Biotechnology from Jaypee University of Information Technology, Solan, was completed under my supervision on the topic mentioned above. This work has not been submitted for consideration or a degree to another institution.

Supervisor's name- **Dr. Hemant Sood**

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Acknowledgement

I take this opportunity to express my first and foremost gratitude to the "DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS" for showing faith in me and trusting my project titled "**Optimization of condition for growing *Brassica oleracea* and *Rhodiola imbricata* under Hydroponic System"**

I feel honoured in expressing my sense of gratitude and deepest appreciation to **Dr. Hemant Sood**, for believing in me and providing motivation, and guidance throughout the time. I thank her for being continuous source of inspiration and for giving insightful advice and suggestions. The help and support I got from her side helped me to successfully complete this project on time.

The in-house facilities provided by the department throughout the semester are also equally acknowledgeable. I would like to thank **Dr. Sudhir Kumar Syal**, the department's head at Jaypee University of Information Technology Department of Biotechnology and Bioinformatics. I would like to thank Mrs. Mamta Mishra and the other department employees for their essential assistance and support.

Yatshi Yadav (217819)

Date:

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ABSTRACT

Brassica oleracea L. var. sebacilla, commonly known as curly kale is a member of the Brassicaceae family of plants, which also includes a number of popular vegetable cultivars like cabbage, some broccoli, cauliflower, curly kale, Brussels sprouts, Savoy cabbage, and a species called gailan. Being rich in antioxidants, and anti-aging property,it is consumed as sautéed salad and its optimization is carried out. *Rhodiola imbricata* is a rich plant species found at the higher altitude of Trans-Himalayan region of India, and attracted quite a lot of attention recently because of its enormous medicinal and therapeutic potential .It is well known in both the scientific and medicinal communities for treating a huge variety of illnesses, the plant has an amazing array of qualities including antioxidant, antiaging, radioprotective, etc. Being a rare plant and hard to grow and is not easily accessible, its optimization is carried on in hydroponics system, in the field as well as *In-vitro*.

CHAPTER 1

INTRODUCTION

1.1 Brassica oleracea L. var. sebacilla

While some cultivars of cabbage (*Brassica oleracea*) are produced for their decorative qualities, or grown for edible leaves. The core leaves of kale plants do not form a head and have green or purple leaves (as with headed cabbage). The majority of the numerous domesticated varieties of Brassica oleracea are believed to be more related to cabbage than kale(4). It is native or local to coastal western and southern Europe. *Brassica oleracea* is a tall biennial plant and in its first year, it develops a uniform rosette of heavy leaves. If we Compare to other Brassica species, the leaves are thick and more fresh, it shows an adaptation that helps it to store minerals, water along with nutrients in its challenging habitat (21). It grows with numerous yellow flowers that are1 to 2 meters (3–7 ft) tall in its second year. Full maturation is shown in fig 1.

Classifications of*Brassica oleracea var. sabellica* (curly kale)

Kingdom: Plantae

Phylum**:** Magnioliophyta

Order**:** Brassicales

Family**:** Brassicaceae

Genus*: Brassica*

Species*: Brassica oleracea var. sabellica*

Fig 1 *Brassica oleracea* grown in field

1.2Rhodiola imbricata

Rhodiola imbricata is one of the species with radio-protective abilities and to-protective (14). The ability of *Rhodiola species*, which is a member of the Crassulaceae family, to increase physical fitness and treat fatigue, and gastrointestinal, cardiac, and central nervous system diseases, has been used widely in both traditional and in advance medicine.. It is extremely useful for further pharmacological research due to its medicinal qualities, antimutagenic, including anticancer, anti-inflammatory, and anti-aging effects. *Rhodiola* sp. pharmacological's significance in humans has been demonstrated by its adaptogenic capacity and therapeutic efficacy over high-altitude sickness, sleep disorders, or depression sexual dysfunction, and. Flavonoids, phenolic glycosides, tannin and some derivatives of phenyl ethanol, and phenylpropanoids are all present in *Rhodiola*(17). [Bioactive chemical concentrations and types are known to vary greatly between species]. *Rhodiola imbricata* is shown in fig 2.

Classification of *Rhodiola imbricata (*golden rice or arctic root)

Kingdom**:** Plantae

Phylum: Tracheophyta

Order: Saxifragales

Family: Crassulaceae

Genus: *Rhodiola*

Species: *Rhodiola imbricata*

Fig.2*Rhodiola imbricata* in pot

1.3 Therapeutic properties of *Rhodiola imbricata* and *Brassica oleracea* and their comparison

Both of the plants have high medicinal value, especially *Rhodiola imbricata* that is rich in radioprotective properties and curly kale which is rich n antioxidant properties. These plants are propagated in the hydroponic system. Therapeutic properties of *Rhodiola imbricata* and *Brassica oleracea* are as shown in table 1.

Table1: Therapeutic properties of *Rhodiola imbricata* and *Brassica oleracea.*

Content	***Rhodiola imbricata***	***Brassica oleraceae***
About plants	*Rhodiola imbricata* belongs to the family Crassulaceae which is found in higher altitude region of the Indian trans-Himalayan cold desert	A rosette of elongated leaves with waved to curly borders are produced by kale plants. The stem can grow as tall as 60 cm.
Common importance	*Rhodiola imbricata* Possess pharmacological, potent biological activities such as anti-inflammatory, antioxidant, antistress, etc.	They posses very high antioxidant activity and pro-healthy potential.
Bioactive compounds	*Rhodiola imbricata* Salidroside, tyrosol, rosrin, rrosavin, and rosin.	Brassica vegetables are rich in bioactive compounds namely flavonoids tannin including ascorbic acid, carotenoids, tocopherols, polyphenols, and glucosinolates

1.4 Rationale and Research Gap

1. Hydroponic systems have not been used for producing medicinal plants in India till date.

2. Production of plants containing phytochemicals, irrespective of the environmental complications (inhabitable climate, location, cultivating season, soil type, and nutrients)

3. Plant maturity and processing along with post-harvest storage is still a problem without any solution.

1.5 Research objective

1. To optimize the hydroponic system for growing *Brassica oleraceae*(curly kale) and *Rhodiola imbricata* (golden or arctic root).
2. Phytochemical analysis of *Brassica oleraceae* and *Rhodiola imbricata*.

CHAPTER 3

Review of literature

2.1 Physiology of *Brassica oleracea*

Using contemporary plant tissue culture techniques, micropropagation is a way to quickly multiply plant stock material to create multiple offspring plants(9). *Brassica oleracea* mainly has Phenolics, flavonoids, polyphenols, phenolic acids, carotenoids (zeaxanthin, lutein) alkaloids, phytosterols, glucosinolates, chlorophyll, terpenoids, and glycosides are only a few of the phytochemical compounds that these plants possess. These plants have great antioxidant potential in terms of actions that reduce lipids, chelate metals, reduce oxidative stress, and scavenge free radicals. Brassica oleracea is the most common vegetable species belonging to the mustard family, which also includes cauliflower, broccoli, kale, and Brussels sprouts. Kale, also known as leaf cabbage, or curly kale, is a cabbage cultivar known for its edible leaves (*Brassica oleracea*)(8). Kales are thought to be more closely related to wild cabbage than most other domesticated forms of Brassica oleracea. While there are several varieties of kale, they all cook and taste differently, curly kale is the most common variety, and as the name implies, the leaf edges are curly and wavy (8). Although it is a little tougher than other varieties, eating it raw benefits from light massaging with vinegar, salt, or lemon juice.

2.1.1 Properties

1. It is considered one of the most nutrient-dense foods.

2. They also have antioxidant, anti-inflammatory, anti-stress, and phytoremediation properties (16).

2.1.2 Traditional usage of *Brassica oleracea*

1. Young flowering shoots of *Brassica oleracea* can be eaten raw or boiled.

2. They are often consumed as a seasoning in soups and salads.

3. The leaves are eaten raw or freshly cooked.

4. They are often Prepared as kale smoothie and tastes similar to broccoli.

2.1.3 Ethnobotanical studies:

1. **Growth form:** It is a herbaceous plant growing up to 0.45m in height with a compact rosette growth form.
2. **Foliage:** The leaves are fleshy, wax-coated, and have white colour veins. The leaves are obovate, long to nearly linear in shape, and have a widely wavy or bifurcate leaf margin. Leaves that are older include 1–5 pairs of small lobes and a 50–30 cm long big terminal lobe (22).
3. **Flower**: The flower blooms in a fixed manner at the plant's tip. They are bisexual and range in colour from pale to bright white or yellow.
4. **Fruit**:Fruits occur in dehiscent or in siliques also known as dry pods. Each of the fruit contains up to 25 seeds and measures(height) 6-10cm 0.5cm. The seeds are brown,black and round along with finely reticulate venation, with a diameter of 0.15-0.2cm.
5. **Cultivation**:It may take up to ten days for seeds to sprout. Depending on the variation of plant, it may take 50 to 60 days for them to reach harvest size. The plants are ready to harvest when they reach 30 cm tall or the desired size.
6. **Ethnobotanical uses**: The thick midribs and petioles are rough and bitter and must be removed before eating. Leaves are primarily used as salads and vegetables (22).

2.1.4 Therapeutic properties of *Brassica oleracea*

1. Anti-cancer activity: *Brassica oleracea* serves a significant and advantageous function in the treatment of many forms of cancer that develop in various sections of the body. It is also efficient in the management of other disorders. Because of this, the pharmacological effects of broccoli are described in terms of sick states.

2. Anti-diabetic activity: In India, broccoli is a popular food that many people eat because it has been shown to have anti-diabetic properties. Both experimental animals and people have demonstrated the positive hypoglycaemic effects of *Brassica*

oleracea (20). In type 2 diabetes patients, brassica oleracea is proven to reduce complications and improve insulin resistance. Utilizing its antioxidant components, this is one of the novel techniques (18).

3. **Anti-inflammatory activity:** Diabetes can be prevented by flavonoids, which are abundant in *Brassica oleracea* and have anti-inflammatory and antioxidant properties. Sulforaphane may activate certain peroxisome proliferator-activated receptors that help maintain glucose homeostasis in hyperglycemic and oxidative conditions. They also protect against nephropathy, diabetic fibrosis, and vascular problems(12).

2.2 Physiology of *Rhodiola imbricata*

The goal of the current work is to establish a micropropagation procedure for *Rhodiola imbricata* as shown in figure 3 and its optimization in hydroponics system. It is a critically endangered medicinal plant that can be found in Indian Himalayan's Leh to Ladakh area. It also attempts to examine the antioxidant capability of in vitro and in vivo plants in addition to secondary metabolites with medicinal importance. For shoot in-vitro rooting of the micro shoots, here IBA bad Kn was used as plant growth regulators. By using HPLC, the secondary metabolites of *Rhodiola imbricata* with pharmaceutical value, including flavonoids, carbohydrates, proteins, total polyphenols, and anti-oxidant activity can be examined.

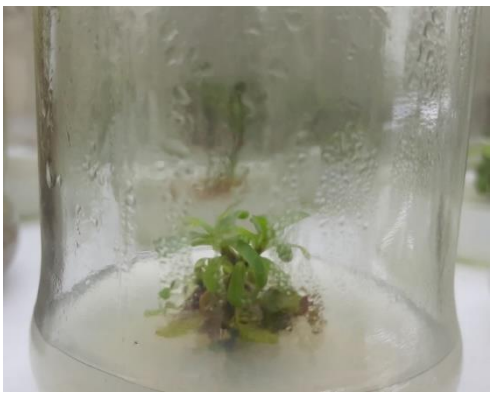

Fig 3. *Rhodiola imbricata* after subculturing kept in culture room

2.2.1 Therapeutic properties of *Rhodiola imbricata*

Different Rhodiola species, which are confined to the northern hemisphere's subarctic regions, have long been employed in traditional European and Asian countries medicines as **tonics, antidepressants, adaptogens, and anti-inflammatory agents**. The pharmacological properties of Rhodiola sp. have been thoroughly investigated in a way to establish the therapeutic applications of medicnal plants in contemporary medicines.(5)

Aside from adaptogenic properties, Rhodiola species appear to have anti-tumoral and anti-proliferative properties that protect tissues from free radicals and mild to moderately potent mutagens(6). One of the most important properties of *Rhodiola imbricata* extracts is their ability to prevent maturation. Aside from adaptogenic properties, Rhodiola species appear to have antitumoral and proliferation resistance properties that protect tissues from free radicals and mild to moderately potent mutagens. One of the most important properties of Rhodiola extract is that they have ability to prevent maturation(1).

2.2.2 Pharmacological activities:

This plant's roots and rhizomes have yielded 30 compounds, with major components including phenylpropanoids, terpenoids, flavonoids, phenolic acids, and phenylethanol derivatives. Pharmacological research has shown that *Rhodiola imbricata* has, cardio-protective, anti-stress, adaptogenic and anti-inflammatory properties. Plants composition have been shown to improve work productivity, physical endurance and long active hours(2). It has also been used to treat fatigue, haemorrhage, impotence, asthma, gastro-intestinal ailments, high altitude sickness prevention, depression and nervous system stimulation. To summarise, *Rhodiola imbricata* has a wealth of folklore and pharmacological evidence for treating stress and fatigue(10). There is also emerging evidence that this plant has radioprotective properties.

2.3 Methanol extraction of Brassica oleracea:

2.3.1 Extraction:

Extraction is the process of separating medicinally active tissues from inactive compounds in plant or animal tissues using a specific solvent in conventional extraction processes. The mature leaves of the plant were washed thoroughly running tap water. The leaves were afterwards left for drying. After drying, the leaves were grinded with the help of a grinder into a fine powder and stored in air-tight container.

2.3.2 Extraction method

The sample plant extract was obtained using a method known as Soxhlet extraction method. The leaves were air dried as shown in fig 4 . At first, about 20gm of powdered leaf extract was kept in a flask, then 80ml methanol and 20ml water(distilled) were added separately. Then the flask is kept on a shaker for 24 hours(15). Then it was filtered with whatman filter paper. methanol is used due to its high polarity which could produce high extraction yields. After this step, the extract was taken in a closed borosilicate jar and kept in the refrigerator for further storage as shown in fig 5.

Fig 4 Dried leaves of *Brassica oleracea*

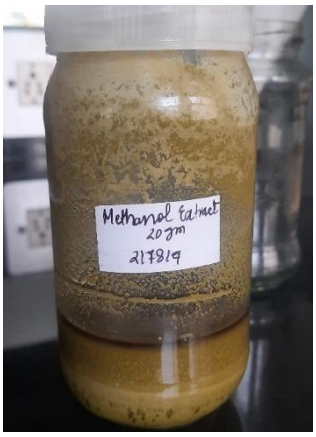

Fig 5 Methanol extract after 24 hours of shaking

2.4 Tissue culture media:-

Plants grow in materials referred to as growing media. Growing media is created expressly to aid plant growth. To develop a growth media, different elements are combined. A variety of plants are grown utilizing various growing media. It is also referred to as substrate, culture medium. They are compositions of different stock solutions, agar, sucrose and plant growth regulators which help in the growth of the plants.(13)

MS media: the plant growth media called Murashige and Skoog medium is employed in laboratories for micropropagation and cultivation of plant cell. It is frequently used for callus culture, suspension culture, organ culture. A combination of amino acids, vitamins and inorganic salts make the composition. It is extensively used tissue culture medium that has given rise to numerous modifications because it provides all the necessary macro- and microelements.

2.5 Phytochemical analysis

2.5.1Total Phenolic Content

There are important components of plants known as phenolic compounds which have redox characteristics that are responsible for antioxidant activity(18). The free radical scavenging is much easier due to hydroxyl groups present in plant extract. The Folin-ciocalteu reagent is used to test the phenolic compounds present in the plants. Afterwards the results were obtained by calibration curve of gallic acid(0-250 g/mL) and reported as gallic acid equivalents (GAE) per gram of dry extract.

2.5.2 Diphenyl-1-picrylhydrazyl (DPPH)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a method which is quick, popular, simple and inexpensive method for determination of antioxidant properties that includes the usage of free radicals to assess the potential of substances to work as hydrogen providers or free-radical scavenger (FRS).

CHAPTER 3

MATERIALS AND METHODS

3.1Chemicals used:

(a) For media preparation: different stock solutions which are compositions of chemicals are prepared in stock and then used for media preparations. These chemicals are basic requirements needed for plant growth. The composition is shown in table 2.

Table:2. Stock solution and their composition of different compounds

Stock solution and components	For MS medium (g/l)
STOCK SOLUTION A (10X)	
KNO_3	19.00
$MgSO_4.7H_2O$	3.7
KH_2PO_4	1.70
STOCK SOLUTION B (20X)	
NH_4NO_3	33.0
STOCK SOLUTION C (100X)	
$CaCl_2.2H_2O$	44
STOCK SOLUTION D (100X)	
Na_2 EDTA	3.726
$FeSO_4.7H_2O$	2.785
STOCK SOLUTION E (100X)	
KI	0.083
STOCK SOLUTION F (100X)	
H_3BO_3	0.62
$COCl_2.6H_2O$	0.0025
$ZnSO_4.7H_2O$	0.86
$CnSO_4.5H_2O$	0.0025
$MnSO_4.4H_2O$	2.23
$Na_2MoO_4.2H_2O$	0.025
STOCK SOLUTION G (100X)	
Myo-Inositol	10.00
Glysine	0.20

STOCK SOLUTION H (100X)	
Pyridoxine HCl	0.050
Nicotinic acid	0.050
Thiamine-HCl	0.010

(b) Plant growth regulators: Indolebutyric acid (IBA), kinetin(kn)
(c) Methanol
(d) Distilled water

3.2 Plant source

1. The ex-plants of *Rhodiola imbricate* were taken from the plant tissue culture laboratory of the institute. The stems of the plant were cut diagonally with the help of a surgical blade and inserted into MS media, hydroponic system, and soil.
2. *Brassica oleracea* seeds were taken from a local nursery and hardened into pots, hydroponic system, and in the field.

3.3 Preparation of media for *Brassica oleracea*

MS media was prepared using the prepared stock solutions prepared previously :-

a. Stock A: 100 ml/l
b. Stock B: 50 ml/l
c. Stock C-H: 10 ml/l
d. Sucrose: 30g/l
e. Agar: 9g/l

Plant growth regulators

IBA: 3mg/l

Kn: 1mg/l

1. Different compositions of MS media were added with the stock solutions separately.
2. Then 30 gm sucrose was added to the solutions.

3. Took the volume to 900 ml by adding clean and distilled water and checked the pH of the solutions (pH must range between 5.6-5.7).
4. The solutions volume were made up to 1000 ml with the aid of water(distilled) and added 9 gm of agar in the solutions.
5. The solutions were boiled before autoclaving to solubilize the agar.
6. The mediums were transferred to small jars and autoclaved for 30 min at 121^{o}C and 15 psi pressure.
7. After autoclaving the mediums were kept at room temperature for 2-4 days to check contamination.

3.3.1 Sub-Culturing

3.4.1 *Brassica oleracea* on MS media

(a) **Process of culturing**

1. Placed the objects (petri plates, scalpel, forceps, media jars, surface sterilizers) required in the procedure in Laminar Air Flow.
2. Switched on the UV light of the LAF 15-20 min before starting to work in it.
3. The explants (seeds) to be used in the procedure were taken directly from the culture room.
4. Washed to seeds under running water to remove dust particles. Surface sterilized the seeds with labolene (detergent) 2-3 times. Washed the seeds under running water to remove the labolene completely.

(b)Methodology for culturing:

1. Turned Off the UV light of the LAF and transferred the seeds in LAF.
2. Washed the seed with surface sterilizers 0.5% Bavistin and 0.1% $HgCl_2$ for 1 min and 30 seconds respectively by dipping in respective jars to disinfect the explants from any microbial growth.
3. The seeds were placed in the media jars with the help of forceps.
4. The jars were labelled properly.

5. Then the jars were moved to the culture room and kept at 25°C to observe the growth.

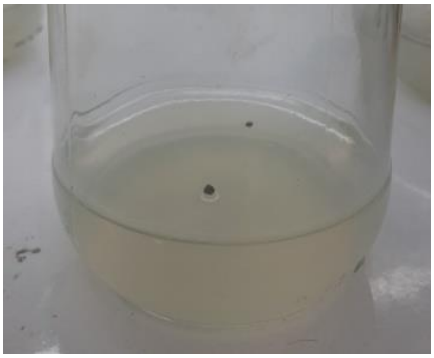

Fig 6 Inoculated *Brassica oleracea* seeds in MS media

3.3.2 *Brassica oleracea* in Hydroponic system :-

1. Thoroughly washed the seeds under running tap water with liquid detergent as teepol, again washed with distilled water
2. Few seeds were planted into Petri dish and kept at 25°C for growing in a hydroponic system.
3. Best propagation was recorded on a hydroponic system, Propagated plants were acclimatized and rooted in pots having soil and sand in equal proportions
4. Propagated plants were acclimatized and were rooted in the hydroponic system with equal proportions of coco peat .
5. The experiments were performed in vitro as well as in static conditions to observe the growth rate and the results obtained

Fig 7 Growth of brassica oleracea in hydroponic system after 1 month

3.4 Micropropagation of *Rhodiola imbricata:*

Using a modern plant tissue culture method, micropropagation is a technique for quickly replicating plant stock material to create numerous progeny plants.

Shoot cuttings inoculated on the medium with a fixed quantity of IBA and Kn.

3.4.1 Preparation of media

MS media was prepared using the stock solution prepared previously:

1. Stock A: 100 ml/l
2. Stock B: 50 ml/l
3. Stock C-H: 10 ml/l
4. Sucrose: 30g/l
5. Agar; 9g/l

1. Different compositions of MS media were added with the stock solutions separately.
2. Then IBA and Kinetin were added as a plant growth regulator.
3. Then 30 gm of sucrose was then added to the solutions.
4. The final volume was made up to 900 ml with the aid of distilled water and checked pH of the solutions (pH must range between 5.6-5.7).
5. The solutions were made to 1000 ml by adding distilled water along with adding 9 gm agar to the solutions.
6. The solutions were boiled before autoclaving to solubilize the agar.
7. The mediums were transferred to small jars and autoclaved for 30 min at 121^{o}C and 15 psi pressure.

8. After autoclaving the mediums were stored at room temperature for 2-4 days to check the contamination.

3.4.2 In-vitro propagation of *Rhodiola imbricata*

(a) **Methodology of culturing**

1. Placed the objects (petri plates, scalpel, forceps, media jars, surface sterilizers) required in the procedure in Laminar Air Flow.
2. Switched ON the UV light of the LAF 15-20 before starting to work in it.
3. The explants (shoots) to be used in the procedure were taken directly from the plant grown in the culture room.
4. Turned Off the UV light of the LAF and transferred the explants to LAF.
5. With the help of forceps and scalpels some of the explants were wounded.
6. The explants were placed in the media jars with the help of forceps.
7. The jars were labelled properly.
8. Then the jars were moved to the culture room and kept at 25°C to observe the growth of the shoots as shown in fig 9

Fig 8 Subcultured *Rhodiola imbricata* kept in culture room for propagation

3.4.3 *Rhodiola imbricata* in the hydroponic system:

1. Ex-plants were taken and planted into pots containing coco peat
2. The pots were then covered with glass jars
3. After stabilization, the jars were removed from top
4. The plants were transferred into hydroponic system as shown in fig 10

Fig.9 Established *Rhodiola imbricata* in hydroponic system

3.4.4 Hardening in pots for in field growth:

1. Ex-plants were taken and planted into pots containing coco peat
2. The pots were then covered with glass jars
3. After stabilization, the jars were removed from top
4. Plants were kept in greenhouse for observation and growth

Fig10*Rhodiola imbricata* in pots after hardening

3.5 Testing different Phytochemicals present in *Brassica oleracea*

1. For flavonoids

(a)**Alkaline reagent test:** Two-three drops of sodium hydroxide were added to two milli-liters of extract.

2.For tannin

(a)**Ferric chloride test:**2ml of aq. solution of plant extract was added to 3 drops of 10% Ferric chloride sol. which are present in light yellow in color.

3.For saponin:

(a)**Frothing Test:** 3ml of the aq. solution of the extract was

Combined with 10 mL of distilled water in a test tube and shaken vigorously for about 5 min.

4. For alkaloids:

Mayers reagent : a portion of the acidic solution present in the test tube was supplemented with a few drops of Mayers reagent, and the presence of a yellowish precipitate showed the presence of alkaloids(24)

5.Test for steroids (Salkowski'stest):

Concentrated sulfuric acid was carefully incorporated into the second portion of the previously mentioned solution so that the acid formed a lower layer, and the interface was examined for a reddish-brown color suggestive of a steroid ring.

3.5.1 Carbohydrate

(Fehling's test)- A mixture of equal volumes of Fehling's solutions I and II and 2 ml of the extract's aq. solution were combined in a test tube and heated in a water bath ab -out two minutes. The decreasing sugars were visible in the brick-red precipitate(12)

3.5.2 Protein estimation

Biuret Test-

Add 5 to 6 drops of copper sulphate solution and 2ml of sodium hydroxide to it. Allow the mixture to rest for 4 to 5 minutes after giving the test tube a little shake to thoroughly combine the ingredients. If bluish-violet color appears then there is protein present.

3.5.3 Quantitative Analysis of Carbohydrates

Take clean and dry test tubes and mark all the tubes as per the protocol.

- Pipette 0.2–1.0 ml of the glucose standard working solution into two test tubes.
- Fill each test tube to a capacity of 1 ml by adding distilled water.
- Place only 1 cc of water (distilled)in one test tube, label it "blank."
- After that, thoroughly combine 3 ml of Anthronereagentst into each test tube.
- Boil the test tubes in a water bath for 8 minutes.
- Rapidly cooled and read the 630 nm green to dark green color.

3.5.4 Quantitative Analysis of protein

Reagents:

Solution A is a mixture of 2% sodium carbonate and 0.1 N sodium hydroxide, whereas Solution B is a mixture of 0.5% copper sulphate and 1% sodium potassium tartrate.

50 ml of solution A + 1 ml of solution B form solution C.

Folin-ciocateau reagent- It can be purchased commercially from stores and must be diluted before usage.

Standard solution for protein:

In a volumetric flask, 200 mg of BSA is dissolved in 100 ml of water **Working standard:** Mix 10 ml of solution in 100 ml of distilled water. Take 0.2 ml of BSA working standard in different test tubes and make up the vol. up to 1 ml by adding distilled water. Take one test tube and add 1 ml of distilled water. This test tube serves as a blank. Now add 4.5 ml of reagent 1 and add 1 ml of the plant extract and then it was incubated for 10 minutes. After the incubation period add 0.5 ml of reagent 2 and then keep it for incubation for 30 minutes. Now measure the absorbance at 660 nm and make the graph. Measure the quantity of protein present in the given sample**.**

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

It was assessed whether plant extracts could directly react with and squelch free radicals. The stock solution of DPPH was made in methanol. The diluted test sample was added to the reaction mixture in 96-well plates for the DPPH test, which was then incubated at 36 °C for 30 min. Then absorbance was calculated at 515 nm. Here Gallic acid was used as a positive control.

3.5.6 Total Phenolic Content

The Folin and Ciocalteu reagent was used to determine the total phenolics of the extracts using the Singleton and Rossi method, though slightly modified. Using a spectrophotometer, the sample and standard were determined at 765 nm and compared with the reagent blank.0.6 mL of water was added together with the test sample (0.2 mL), folin-Ciocalteu's reagent in the proportion of 1:1 ratio, and the test sample.1 ml of sodium carbonate solution (8% w/v in water) were added to the mixture after 5 minutes, and the volume subsequently raised to 3 m l with water (distilled).Then the reaction was centrifuged for 30 minutes in the closed dark room,

and the blue colour absorbance in several samples was measured at 765 nm. The amount of phenol in liquid plant material was calculated as gallic acid equivalents (GAE)/g using a standard curve for gallic acid (5-500 mg/L). For each choice, a duplicate was produced.

3.5.7 Total Flavonoids Content

An aluminium chloride colorimetric technique was employed to measure the sample's total flavonoid content.The standard calibration curve for the measurement of total flavonoid concentration has been established using quercetin.5.0 mg Quercetin was added in 1.0 ml of methanol to create the stock Quercetin solution. The stock solution was then repeatedly diluted with methanol (5-200 g/mL) to generate the standard quercetin solutions. Separately, 0.6 mL of 2% aluminium chloride was added with 0.6 ml of diluted standard quercetin solutions. After this, the mixture was left undisturbed for 50-60 minutes at the room temperature. The absorbance of the reaction mixtures was determined using a spectrophotometer against a blank at 420 nm. The TFC of the samples was taken using the calibration plot and quantified as mg Quercetin equivalent (QE)/g of the dried plant.

CHAPTER 4

RESULT

***4.1 Brassica oleracea var. sabellica* (curly kale) in a hydroponic system:**

The curly kale was optimized in the hydroponic system for propagation and the growth was observed in 15 days and then in 1 month of interval. The seeds were germinated and then transferred into the hydroponic system. After transferred into hydroponic system the growth was observed and the results showed enormous shoot growth as shown in Fig11.

(a)

(b)

(c)

(d)

Fig11. The *Brassica oleracea* were planted into a hydroponic system till full maturation and then transferred into the field, subsequent growth was observed in fixed intervals of time (a):After germination (b) After 17 days (c) after 15 days (d) After 52 days

4.1.1 In-vitro growth of *Brassica oleracea:* The seeds were inoculated in MS media jar and were kept in tissue culture room. The seeds were observed from germination till full maturation and the results concluded from the observation was that the growth was low as compared to the hydroponic system. It took for more than a week for the seed to germinate as shown in Fig 12.

(a) (b) (c)

(d)

Fig12. Seeds of brassica oleracea were cultured into MS media contaiting jars and were kept in culture room for a period of time (a)Day 1, (b) After 17 days, (c) After 15 days, (d) After 40 days

4.1.2 Comparision of growth of *Brassica oleracea* in tissue culture and hydroponic system: Growth of *Brassica oleracea* in tissue culture as well as in hydroponic system is done with the number of days. The growth was observed from germination to full maturation. The results concluded from observation is that the growth in tissue culture is less when compared to the hydroponic system. The hydroponic system showed enormous growth as shown in table 3.

No. of days	***Brassica oleracea***			
	Shoot length(cm)		**No. of Shoots**	
	Tissue culture	**Hydroponic system**	**Tissue culture**	**Hydroponic system**
Day 1	0	0	0	1
Day 15	0.3	1	1	2
1 month	2.3	12	3	4
2 months	4.5	20	4	12
3 months	5.5	32	4	16
4 months	7	40	5	23

Table 3: Comparison of growth of *Brassica oleracea* in tissue culture and in the hydroponic system

4.1.3 Biomass data :

The leaves of curly kale were taken after full maturation and the length of the leaves were measured along with their weight. The data of the plant is as shown in table 4. The weight of the plant and for a single leaf is as shown in table 5

Plant	**1**	**2**	**3**	**4**	**5**
Length of leaves (cm)	8.5	29	21.5	21	23
No. of leave	25	41	26	29	23
Length of plant(trunk) (cm)	11	26	22	18	16
Length of plant(cm)	25	42	35	38	33

Table 4: Biomass of *Brassica oleracea* taken directly from feild

For single leaf	25gm
For whole plant	309.6 gm
root	51gm
Total weight	360.6 gm

Table 5: Weighing data of *Brassica oleracea*

4.2 In-vitro Optimization of *Rhodiola imbricata:*

The explants of *Rhodiola imbricata* were sub cultured in MS media jar and were kept in tissue culture room. The explants were observed from day 1 till full maturation and the results concluded from the observation was that the growth was low as compared to the hydroponic system. It took for more than 8 days for further growth . The growth was observed as shown in fig 13.

(a) Explants cultured into media

b) After 44 days

c) After 20 days

Fig13. Growth of *Rhodiola imbricata in* MS media jar kept in tissue culture room

4.2.1 Optimization of *Rhodiola imbricata* in hydroponic system:

The *Rhodiola imbricata* was optimized in the hydroponic system for propagation and the growth was observed in 15 days and then in 1 month of time interval. The explants were planted into plants for hardening then transferred into the hydroponic system. After transferring into hydroponic system the growth was observed and the results showed enormous shoot growth as shown in Fig14.

(a) Day 1

(b) After 15 days

(c) After 1 month

(d) After 1 month

Fig 14. *Rhodiola imbricata* in hydroponic system for propagation

4.2.2 Comparison of growth of *Rhodiola imbricata* in tissue culture and in hydroponic system: Growth of *Rhodiola imbricata* in tissue culture as well as in hydroponic system is done with number of days . The growth was observed from plantation to full maturation .The results concluded from observation is that the growth in tissue culture is less when compared to the hydroponic system . the hydroponic system showed enormous growth as shown in table 6

No. of days	***Rhodiola imbricata***			
	Shoot length(cm)		**No. of Shoots**	
	Tissue culture	**Hydroponic system**	**Tissue culture**	**Hydroponic system**
Day 1	1	1	1	1
Day 15	1.5	12	1	4
1 month	2	20	2	13
2 month	3	32	3	20
3 month	3.4	36	4	23
4 month	5	40	5	27

Table 6: comparison of growth of *Rhodiola imbricata* in tissue culture and in hydroponic system

4.3 Quantification of *Brassica oleracea*

4.3.1 quantitative analysis of carbohydrates: The quantification was carried out with the extract to calculate the total amount of carbohydrates present in *Brassica oleracea* and the R value obtained is 0.944 as shown in fig 16 .

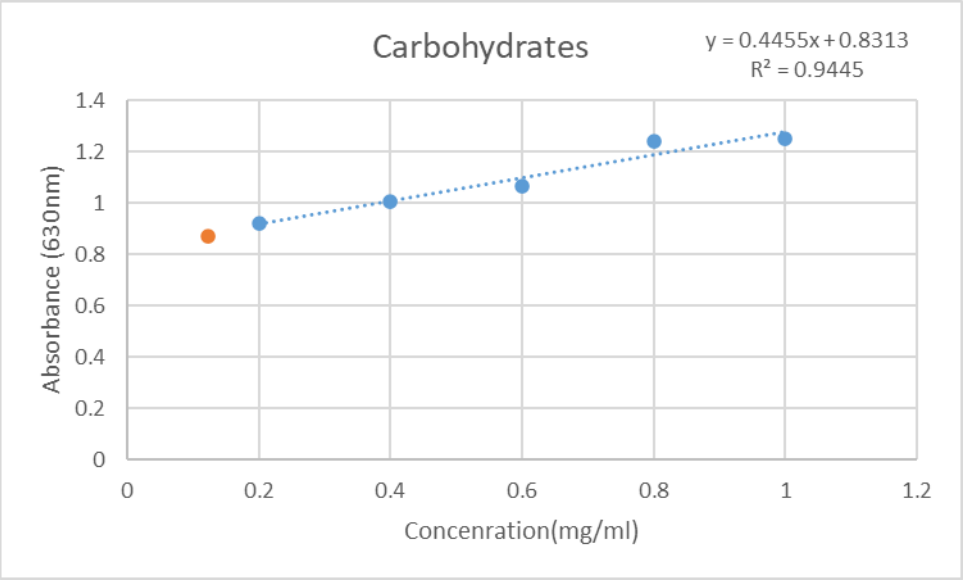

Figure 15: Quantification of Carbohydrate

4.3.2 Quantification of protein : the quantification for protein was carried out with the extract and the readings was taken from spectrophotometer .The readings showed that adequate amount of protein was present the sample as shown in the fig 17

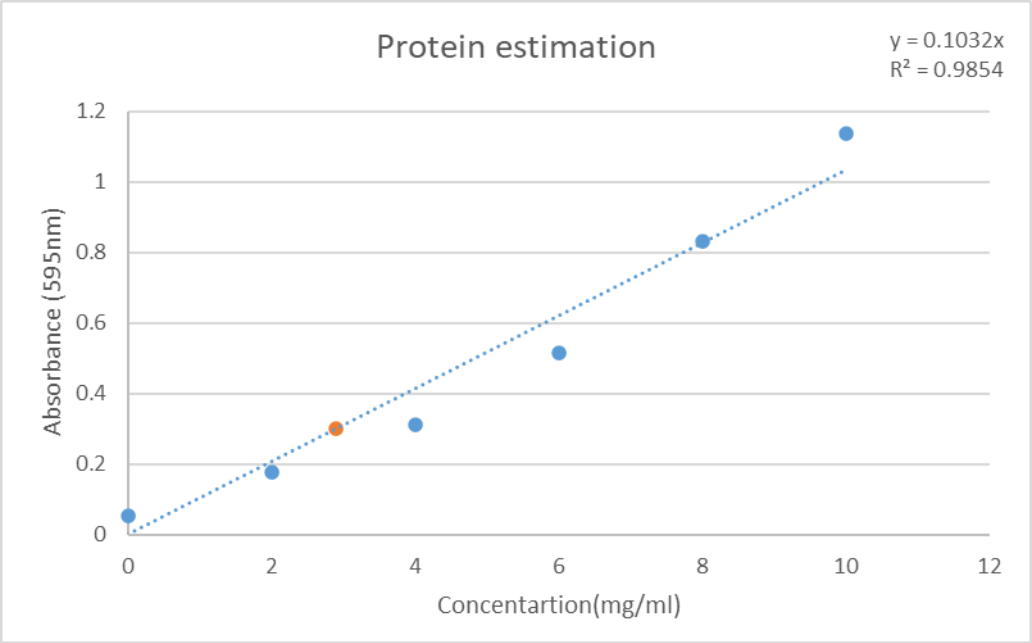

Figure 16: Quantification of Protein

4.3.2 Quantification of total phenolic content: Quantification was done with the extract prepared and standard curve was obtained , the OD was taken after running the sample in spectrophotometer. R value obtained is 0.962 . adequate amount of TPC was observed as shown in fig 18

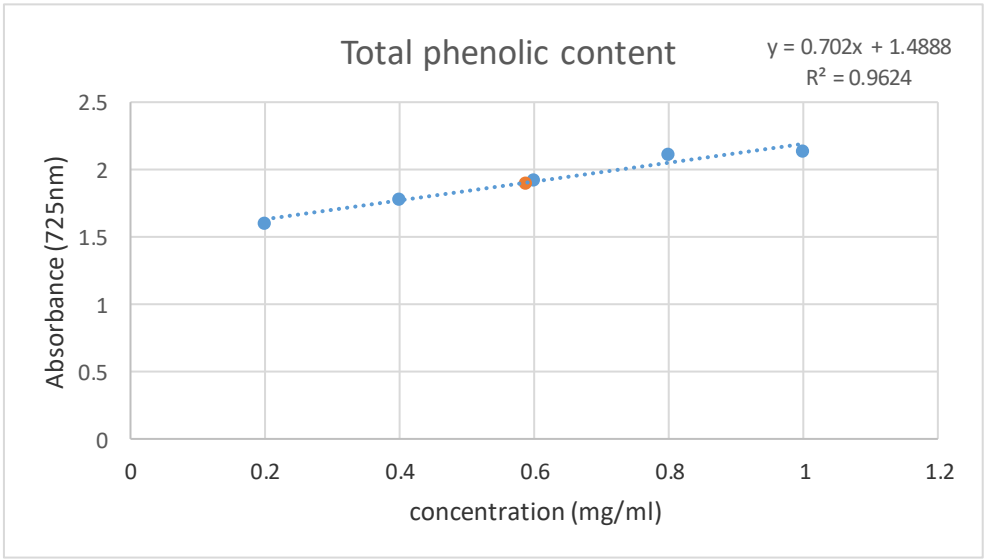

Figure 17: Quantification of Total Phenolic Content (TPC)

4.3.3 **Quantification of total flavonoid content** : Quantification of TFC was done with the help of extract prepared and then the sample was run in spectrophotometer, according to the graph plotted it was observed that even though there was flavonoid present in *Brassica oleracea* the readings showed negative value as shown in fig 19

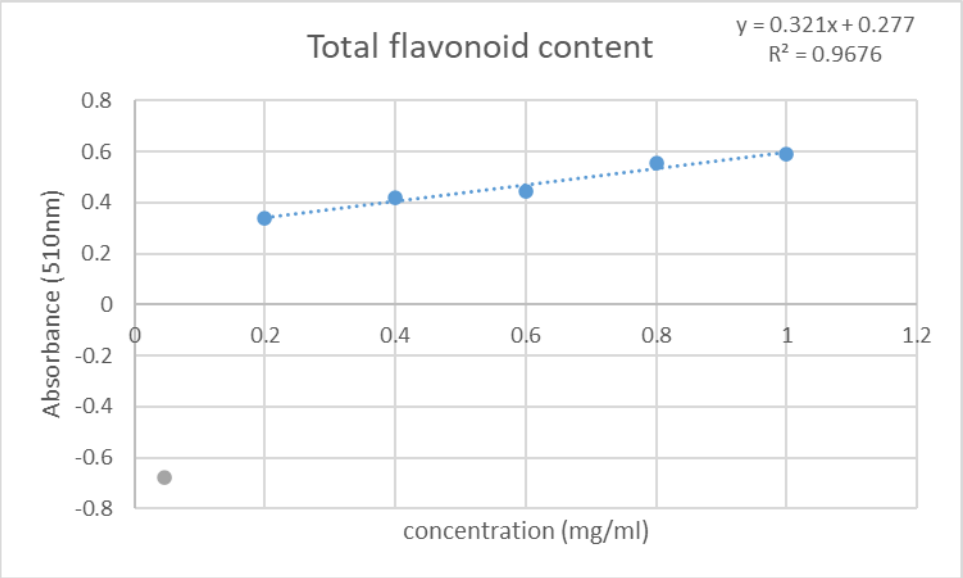

Figure 18: Quantification of Total Flavonoid Content (TFC)

Discussion

Optimization, comparison and advantage of hydroponic system for growing medicinal plants.In this study, a culture system was established which suits best for the growth of *Brassica oleracea* and *Rhodiola imbricata* in hydroponic system, that is quick and cheaply build, most importantly easy for control and monitoring. We are aware that William F. Gericke originally used the term "hydroponics"in 1929.More and more hydroponic systems were developed and optimized, among various crops, for rice, barley, lettuce, tomato and Arabidopsis(22) However, rather than being for medicinal plants, all of these hydroponic cultivation methods were for herbaceous plants.

As a result, this optimization system serves as the first hydroponic growth method for medicinal plants, particularly for plants with high pharmacological properties and limited accessibility. For the research of the optimization and phytochemical analysis, *Brassica oleracea* and *Rhodiola imbricata*, both of which have high pharmacological properties,(25) are employed as model plants. In this study, numerous growth markers were identified and plant growth and development were compared across in-vitro, soil grown and hydroponics. The plants develop normally in both soil and hydroponic environment and neither plant height nor biomass showed any significant differences. These findings suggested that the hydroponic culture technology is ideal for growing medicinal plants. The numerous additional benefits of our hydroponic system are in addition to the benefits of the hydroponics system, such as control over mineral and nutrition access to the root system. First off, this hydroponic system is easy to build, takes little time and costs less money. Second, switching the plants quickly and easily, from one hydroponic solution to another solution in fresh solution(nutrient) is made possible by the hydroponic system. The plant materials were initially grown for 5-6 weeks in a standard nutrient hydroponic solution before being moved to the field to monitor plant growth. The hydroponic system is efficient and rapid in processing in processing plant material, based on the results. Thirdly, the tissue samples that were cultivated in our hydroponic systems can be cultivated easily and damage free.

Conclusion

The hydroponics system can be built quickly, cheaply, and with standardized growth conditions for the production of medicinal plants and can be setup anywhere and can be controlled easily to grow medicinal plants that are not easily accessible. The model plants used are *Rhodiola imbricata* and *Brassica oleracea.*

These medicinal plants grow in soil, are profiled and compared to show the quality and adaptability of hydroponic systems, including biomass and phytochemical analysis and quantification. The results showed that the hydroponic system are best suited for growing medicinal plants as they can be easily transferred if required and do not take much maintenance and are cost-effective. The comparison was done for tissue culture, hydroponic system, and field grown showing that the hydroponic systems are way more convenient than the field grown method.

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