INTRODUCTION **CHAPTER-I**

1.1 General Introduction

The North-eastern states of India, namely Assam, Meghalaya, Manipur, Mizoram, Arunachal Pradesh, Tripura and Sikkim contribute to almost 50% of the plant biodiversity in entire India. Consequently, Assam and regions within it like Bodoland Territorial Region (BTR) are very rich in its medicinal plant and herbal diversity, with many plant species are only known by the natives residing in the region. The bioactive components of many of these plants are still to be studied, which may be useful in designing new drugs, furthermore 286 plant species have been documented till now with properties to treat up to 40 different kinds of diseases (Ghosh & Parida, 2015). It can be noted that apart from food 80% of the world population relies on medicinal plant or ethnic medicine in the form of plant extracts (Sharma & Das, 2018). Such traditional or ethnic medicines play oftentimes are primary form healthcare and treatment in developing countries which lack medical infrastructure and resources (Kasagana & Karumuri, 2011). Despite plant life especially wild ones having such importance for human society several medicinal plants have gone extinct without being explored or documented for its scientific importance. With decreasing biodiversity due to environmental and ecological exploitations many important species are declining and getting endangered day by day. Therefore, the conservation of these wild plant is of utmost importance.

The technique in *vitro* propagation of plants or plant tissue culture may be an ultimate solution for the conservation and mass propagation of any plant species. in this technique the plant tissue, cell or any organ is used and grown *in vitro* condition supplemented with required nutrient media and growth regulator inside controlled and aseptic condition.

1.2 Plant Tissue Culture Technology

Plant tissue culture is a technique used to grow plant cells, tissues, or organs in a controlled, sterile environment under optimal conditions of light, temperature, and nutrients. The process typically begins with the isolation of a small piece of plant tissue (such as a leaf, stem, or meristem), which is then cultured on a nutrient medium. The medium provides essential macronutrients, micronutrients, vitamins, and growth regulators (such as auxins and cytokinins) to promote cell division, differentiation, and regeneration. This method is widely used for clonal propagation, genetic modification, and conservation of rare or endangered plant species. This technique is very important for rapid mass propagation of disease free, and uniform plantlets, also this technique is widely used for conservation of endemic, and rare plants, and genome transformation for production of better-quality plants (Debnath et al., 2006; Altpeter et al., 2016). The concept of plant tissue culture was started in 1902 by a German Botanist Haberlandt also known as the father of plant tissue culture. He experimented on palisade tissue grown in Knop's salt solution supplemented with sucrose, resulting cell growth and development (Bhojwani & Dantu, 2013). It is very effective technique for production of secondary metabolites and extraction of bioactive compounds from plants, plant improvement through genome transformation, vaccine production (Espinosa-Leal et al., 2018). In this technique, nutrient compositions required for the plant propagation, different growth regulators are used for growth of the plant from any tissue, organs or cell. The cell differentiation, tissue development and response of the tissues varies according to the nutritional components and growth regulators used in the culture media. Sometimes the tissues used for propagation may undergo changes in the genomic configuration of the plant which may or may not cause the changes in the phenotype of the plant, this phenomenon is called as Somaclonal variation.

Any plant parts, or organs can be used for initiation of tissue culture, though meristem is difficult for shoot regeneration and micropropagation. The healthy, disease-free mother plant is required for successful culture initiation. Shoot tip explants are preferred due to its higher actively dividing cells (Tegen & Mohammed, 2016).

The process of tissue culture has various sequential steps, which include media preparation, explant preparation/explant surface sterilization, explant inoculation, plant development in the growth room, hardening etc.,

1.2.1 Different Stages of Plant Tissue Culture

1.2.1.1 Media Preparation

Media preparation consists different steps and considerations which are underlined in the following subsections.

1.2.1.2 Composition of Media

The plant tissue culture media comprises of different nutrient compositions and hormones. The response of plant cell, tissue, or organs purely dependent on the culture media compositions and different hormone ratio. The growth and development of the plant tissues may vary from species to species; hence the nutritional requirements also differ in different species, moreover the nutritional requirement varies from different tissue from different parts of the plant (Saad & Elshahed, 2012). Therefore, the selection of nutrient medium is responsible for successful *in vitro* propagation, cell differentiation and development. The preparation of culture media is dependent on the nature of plant species (woody plant, soft tissue, or herb species) and plant material (Tissue, organ, or cell) used for tissue culture.

Therefore, the nutrient composition and nature of culture media is formulated considering the requirement of the species. The nature of the media may be solid medium or liquid medium. Some important and commonly used tissue culture media are- White's media (Chatot et al., 1989), MS medium (Murashige & Skoog, 1962), B5 medium (Gamborg et al, 1968), N6 medium, Nitsch's media (Nitsch and Nitsch 1969), Synthetic media, Natural media, etc.

Table 1.1: Composition and concentration of some commonly used plant tissue culture media (Saad & Elshahed, 2012)

Composition and concentration of some commonly used plant tissue culture media (mg/L concentration)					
Components	**White's medium**	**MS medium**	**Gamborg (B5 medium)**	**Chu (N6 medium)**	**Nistch's medium**
Macronutrients					
$MgSO_4.7H_2O$	750	370	250	185	185
KH_2PO_4	--	170	--	400	68
$Na_2PO4.H_2O$	19	--	150	--	--
KNO_3	80	--	2500	2830	950
NH_4NO_3	--	1900	--	_	720
$CaCl_2.2H_2O$	--	1650	150	166	--
$(NH_4)_2SO_4$		440	134	463	--
Micronutrients					
HBO_3	1.5	6.2	3	1.6	--
$MnSO_4.4H_2O$	5	22.3	---	4.4	25

$MnSO_4.H_2O$			10	3.3	--
$ZnSo_4.7H_2O$	3	8.6	2	1.5	10
$Na2MoO_4.2H_2O$		.25	.25		.25
$CuSO_4.5H_2O$	0.01	0.025	0.025	---	0.025
$CoCl_2.6H_2O$		0.025	0.025	--	0.025
KI	0.75	0.83	0.75	0.8	--
$FeSO_4.7H_2O$		27.8		27.8	27.8
$Na_2EDTA.2H_2O$		37.3		37.3	37.3
Sucrose	20g	30g	20g	50g	20g
Organic					
Supplements					
Vitamins					
Thiamine HCl	0.01	0.5	10	1	0.5
Pyrodoxine HCl	0.01	0.5	1	0.5	0.5
Nicotonic Acid	0.05	0.5	1	0.5	5
Myoinositol		100	100		100
Others					
Glycine	3	2	--	--	2
Folic acid	---	---	--	--	0.5
Biotin	--	--	--	--	0.05
pH	5.8	5.8	5.5	5.8	5.8

1.3 Chemical constituents of medium/media:

Plant tissue culture media typically consists of a carefully balanced mix of essential nutrients, vitamins, plant growth regulators, and energy sources. The primary nutrients include both macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) and micronutrients (iron, boron, manganese, zinc, copper, and molybdenum) that are necessary for growth and development. Vitamins such as thiamine (B1), pyridoxine (B6), and niacin support metabolic processes and enhance cell division. Plant growth regulators (PGRs), like auxins (e.g., IAA, NAA) and cytokinins (e.g., BAP, kinetin), control processes like root and shoot formation. Sucrose is commonly used as an energy source, while agar or gelrite serves as a gelling agent to provide a solid surface for growth.

The pH of the medium is also adjusted to ensure optimal nutrient absorption and plant development. Together, these components provide the ideal conditions for in vitro growth, regeneration, and differentiation of plant tissues (Bhatia, 2015).

1.3.1 Macronutrients: Six elements, namely mangesium, phosphorus, sodium, potasium, calcium, and sulphur are the main essential macronutrients in tissue culture (Chimdessa Emiru, 2020) (Chatot et al., 1989).

1.3.2 Micronutrients: These nutrients are required for plants in small quantities, including manganese, iron, zinc, molybdenum, copper, and boron. In tissue culture media iron and copper is used in chelated form (Chimdessa Emiru, 2020) (Chatot et al., 1989).

1.3.3 Organic nutrients: The inorganic salts in media undergo dissociation and ionization. The K^+ ion and NO_3^- ion is contributed from KNO_3, KH_2PO_4, and NH_4NO_3.

1.3.4 Inorganic nutrients: Inorganic nutrients are crucial for supporting plant growth in tissue culture media, as they provide essential macro and micronutrients that regulate various physiological processes. The primary macronutrients include nitrogen (N), in the form of nitrate or ammonium, which is vital for protein and amino acid synthesis; phosphorus (P), typically supplied as phosphate, essential for energy transfer and nucleic acid formation; and potassium (K), which aids in osmotic regulation and enzyme activation. Secondary macronutrients like calcium (Ca), magnesium (Mg), and sulfur (S) are also important for cell wall structure, enzyme function, and protein synthesis. Micronutrients such as iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and boron (B) are required in trace amounts to support enzymatic functions, chlorophyll synthesis, and cellular metabolism.

1.3.5 Carbon energy sources: in the tissue culture process, the plants or tissues inside the culture media are heterotrophic, therefore they require carbon source from outside. Sucrose is commonly used as carbon source in tissue culture. In the sterilization sucrose is hydrolysed to glucose and fructose. The explants response is better in autoclaved sucrose media other than the media where sucrose is added using filter sterilization, therefore it is proved that hydrolysed sucrose that is glucose is more efficient source of energy (Gamborg et al., 1976).

1.3.6 Organic supplements: some organic supplements like amino acid, vitamins, activated charcoal, organic extracts, organic acids, and antibiotics.

1.3.7 Growth regulators: Plant hormones a group of organic compounds which promote growth, differentiation, and development of plant cells. There are 4 groups of these growth regulators which are used in tissue culture- auxin, gibberellins, cytoninins, and abscisic acid (Patau et al., 1957).

1.3.7.1 Auxin: Auxins help in cell division, callus formation, and cell elongation in plants. At high concentration of auxin callus formation occurs and at low concentration root formation is induced. IAA, IBA, NAA, 2-4-D, 2 4 5-T, 4-CPA, NOA, MCPA, Dicamba, Picloram are some auxins may be used in the tissue culture media. 2, 4-dichlorophenoxy acetic acid or 2,4-D is commonly used and most effective auxin used in the tissue culture media (Chatot et al., 1989).

1.3.7.2 Cytokinin: Cytokinins induce cell division, shoot differentiation in plants. They induce in RNA synthesis, thus help in protein and enzyme production. Some common cytokinins which may use in tissue culture media are BAP, BA,2ip (IPA), Kinetin, Zeatin, Thidiazuron. BAP and Kinetin are commonly used cytokinins in tissue culture media.

1.3.7.3 Gibberellins: There are about 20 different gibberellins which are commonly used as growth regulators. GA_3, are most used gibberellin used in tissue culture media. GA_3 enhances callus growth and elongation of dwarf plant. They inhibit shoot formation and adventitious root formation (Saad & Elshahed, 2012).

Both auxin and cytokinin is required for callus culture maintenance. Cytokinin concentration is required for shoot formation and multiplication and auxin is required for rooting in the explant. The required auxin to cytokinin ratio varies depending upon species to species (Saad & Elshahed, 2012).

1.4 Explant preparation/explant surface sterilization: Explant surface sterilization is most crucial and most important step for tissue culture, because it is very difficult to control the fungal and bacterial contaminants in the culture medium. For successful establishment of tissues in tissue culture first step is removal of the external and internal contaminants from the plant tissue or explant, the collected explant may contain microorganisms (Okoroafor, 2022). The microorganisms may be bacteria, viruses, fungi, yeast, these microorganisms cause contaminations in the culture medium causing tissue nacrosis, reduction in shoot proliferation and rooting, tissue mortality (Oyebanji et al., 2009). The sterilization method is different for different species, also for same species a single sterilization method may not work because the type of microorganisms is different in different season or place (Rezadost et al, 2013). There are some important chemical agents which are commonly used for explant surface sterilization, mercuric chloride, sodium hypochlorite, ethanol, calcium hypochlorite, silver nitrate, hydrogen peroxide. These are harmful for plant tissues, treatment of the tissues must in these agents should be for a very shorter time duration (Eliwa et al., 2024). Ethanol, sodium hypochlorite (NaOCl) is effective to reduce the fungal growth in the culture medium (Adetunji Amusa, 2007).

1.5 Explant inoculation: The surface sterilized explants were cultured in different basal media containing different growth regulator concentrations. The inoculated explants are typically incubated in a controlled environment, usually in a growth chamber with specific light, temperature, and humidity conditions. Depending on the culture conditions, the explants may undergo processes like callus formation, organogenesis, or somatic embryogenesis, leading to the development of new plants (Fehér, 2019).

1.6 Plant development in growth room: the growth parameters for different plants vary from species to species. after the explant inoculation in the culture media, the explants need to be kept in optimum condition of temperature and humidity inside growth room. The explants inside growth room should be monitored regularly for contamination and tissue responses. Subculture of the plant tissues are required time to time for the differentiation, multiplication, and root formation.

1.7 Hardening of micro plants: after complete growth of plant tissue to plant, the plantlets should be transferred to the field. The tissue cultured plants cannot directly transfer to the field because inside laboratory condition the growth condition like temperature, humidity, and intensity of light is optimum with high nutrition supply in the culture media. Therefore, these plants need gradual acclimatization process. The tissue cultured plants use glucose as the energy source instead photosynthesis. Therefore, the hardening of the plant is required to gradually adjust the plants for relying on photosynthesis (Lata et al., 2013). Hardening process is done in two steps-

1.7.1 Primary Hardening: The fully developed plantlets inside the growth room are taken out and washed followed by transplantation in the pots containing sterilized substrates and nutrients like- saw dust, coco pit, vermicompost soil, ash, charcoal chips, etc. the inert media are soaked in the basal medium (MS). The pots are the placed in the light for 2-3 weeks in a primary hardening room or laboratory. The inert substances are soaked in the media time to time when it dries, reducing the carbon source nutrient gradually. The relative humidity is maintained in this step (Deb & Imchen, 2009).

1.7.2 Secondary Hardening: the primary hardened plants are the transferred to the green house at constant temperature and humidity for one week. Then the plantlets need to be transferred to the seed trays with different combinations of coco-peat, compost, saw dust, vermiculite etc. after 3-4 weeks the plantlets are transferred to the fully sunlight place and kept for 2 weeks. Finally, the plants are ready to transplant in the field (Lata et al., 2013).

1.8 Different methods in Tissue Culture: Tissue culture can be performed through suspension culture; in this process the plant cells are grown in liquid culture media. Generally, this method is absorbed for large scale production, like in pharmaceticals. Adherent culture**;** semi solid media is used in this process where cells are adhered in the media. This process is used for studying cell differentiation, cell behaviour, and tissue formation (Sharma et al., 2022).

1.9 Advantages of tissue culture

Disease free plant: The plant propagated through tissue culture allows production of disease free, uniform, and free from other pathogens plantlets.

Rapid multiplication: Using this technique large number of plantlets can be produced in any season, in a short time.

Genetic modification: Plant improvement through gene manipulation for better quality plant production can be possible.

Conservation of important or endangered plant species: For conservation of endangered species tissue culture technique is very useful.

1.10 Disadvantages of tissue culture

High cost: The costs for production of plants through tissue culture process is costly.

Less genetic diversity: The plants produced through tissue culture results very less variability or no variation.

Contamination: Contaminations in the tissue culture media is a major issue in the tissue culture process.

Adaptation/Hardening: After successful multiplication and rooting of the explants *in vitro,* the *in vitro* plantlets struggle to adapt in the natural climate condition.

1.11 Somaclonal Variation: This term is seen in plants basically occurs at the time of plant tissue culture, chromosomal aberrations play an important role in this phenomenon. Somaclonal variations are mostly seen in the plants which are regenerated from the callus. The variation may be genotypic or phenotypic. The phenomenon has both advantageous or disadvantageous. The phenomenon of variation in the plant genome in the process of tissue culture of plant cells, tissues is called somaclonal variation (Rodriguez-Enriquez et al., 2013). Therefore, it can be considered that the variation occurs due to the genetic changes or mutations caused at the time of *in vitro* condition or by chemical compositions in the culture media. This phenomenon is undesirable, sometimes this process may lead to production of new cultivars with additional characteristics in the species, eg- disease resistance, pest resistance. Specific characters like disease resistant and other characteristics to upgrading commercial values in Sugarcane, potato, and Banana (Pérez et al., 2000). The chances of somaclonal variation may be reduced by-

1.11.1 Long term culture

1.11.2 Avoiding use of 2,4-D in the culture medium

1.11.3 Use of axillary shoots

1.11.4 Minimum number of subcultures.

The soma clonal variation may cause due to biotic stress and abiotic stress (cell differentiation, and dedifferentiation, growth of plant cell under extreme cold, salt, and drought condition). The term soma clonal variation is commonly known for variation of plants induced by the stresses. There may two scenes of variation, the variation may be pre-existing in the donor plant, and the changes may be induced by the stress which are still under discussion.

In fact, while it is well established that cells in adult plant tissues may not be genetically homogeneous, many observations have shown that a large fraction of variability is directly induced by stress.

1.12 Description of Plants: A total of five medicinal plants were selected for the standardization of *in vitro* culture technique and study of comparative antioxidant capacity of *in vitro* propagated medicinal plant with the wild plant, and study of bioactive compounds found in wild and *in vitro* propagated plants. The five medicinal plants selected for tissue culture are *Torenia crustacea, Lindernia pusilla, Phlogacanthus thyrsiformis, Enydra fluctuans, and Hygrophila auriculata.* Since there are no reports been published on the *in vitro* propagation and comparative antioxidant study and bioactive compound production study of wild and tissue cultured plants, these medicinal plants were selected for the experiment.

1.12.1 Taxonomic classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Linderniaceae

Genus: Torenia

Species: *Torenia crustacea* (L.) Cham. & Schltdl

Torenia crustacea also known as Malaysian False Pimpernel is a medicinal plant of the family of Linderniaceae. It is branched grows up to 30 cm tall. Stems are greatly branched. It is distributed worldwide in tropical and temperate areas. *Torenia crustacea* is found in moist, banks of ricers, in lawns, in fields and on roadsides. It is an annual flowering plant (*Capraria crustacea* Linnaeus, Mant. Pl. 87. 1767). The plant is known for its important ethnomedicinal properties throughout the world, *T. crustacea* is a common medicinal plant used in Indonesia and Malaysia, it is used to treat injury, fever, ear ache, anti-inflammatory, and skin diseases, to relief against itching, sores, dysentery, boils, ringworms, navel infection and the decoction is used as medicine after childbirth (Sirimat & Sakulsathaporn, 2019). The benzene extract of *L. crustacea* plant is potential natural antioxidant source. In the plant extracts flavonoid and phenolic compounds were found. The benzene extracts of *T. crustacea* possesses significant anti-inflammatory, anti- pyretic and analgesic properties (Rekha et al., 2019). Nhut et al. (2022) developed *in vitro* propagation of *Torenia fournieri* an important ornamental plant. In this experiment the maximum *in vitro* plants were obtained using the nodal explants in the MS medium supplemented with 2mg/L BAP with an average of 5.5 cm shoot length and 12 numbers of shoots per explant were obtained in the media after 60 days of the culture. Maximum rooting was obtained in the MS supplemented with 1mg/L IBA.

1.12.2 Taxonomic classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Linderniaceae

***Genus**:* Lindernia

Species: *Lindernia pusilla* (Willd.) Bold

Lindernia pusilla is an important medicinal plant of the family Linderniaceae, widely distributed in Indian Continent, Malaysia, Cambodia, Laos, Nepal, Myanmar, Sri Lanka, Thailand, New Guinea, Philippines, Vietnam East Asia. It si grassy found in bunds of paddy fields along with grasses in ponds, wet and dry places, low lands and hill countries. The stems are prostate, brancced from the root stock, grows upto 25cm, with long internodes. Leaves are ovate to orbicular ovate, flowers are annual. The herb is used traditionally to treat various diseases. *L pusilla* is used in Vamana Karma in Sri Lankan traditional medicine specially in natural and artificial poisoning. Some countries use this plant to promote breast milk and infections of finger nail. The plants of the family Linderniaceae contains terpenes, and alalgesic, anti-inflammatory, anti tumor activities (Ediriweera, 2015).

Rajila & Arunprasath 2020 developed *in vitro* propagation of an endemic plant *Lindernia madayiparense* using nodal explants. The callus induction was obtained highest in the MS supplemented with 0.5mg/L NAA& BAP. The well-developed shoot was induced for rooting in MS medium supplemented with 1mg of NAA (Rajila & Arunprasath, 2020). Similar study for *in vitro* propagation and *in vitro* flowering of an important aquatic ornamental plant *Lindernia antipoda* L. (Alston) was conducted by Jabir et al., 2026. In this experiment the most effective culture initiation was observed in the half strength MS medium supplemented with 1mg/L BAP, *in vitro* shoot multiplication and shoot length was best in the MS medium supplemented with 1mg/L BAP and 0.2mg/L NAA. In the MS medium supplemented with 0.2mg/L NAA highest *in vitro* flowering were obtained. The rooting was obtained in all the NAA concentrations. Finally, 90% of the *in vitro* plants were survived in the hardening using polythene propagator system (Jabir. et al., 2016).

1.12.3 Taxonomic classification

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Family: Nongmangkha

Order: Lamiales

Genus*:* Phlogacanthus

Species: *Phlogacanthus thyrsiformis* (Roxb. ex Hardw.) Mabb.

Phlogacanthus thyrsiflorus Nees is an important medicinal plant in the family of Acanthaeae. It is a shrub species, grows upto a height of 2.4 metres. The flowers are up to 30cm long, tubular, elongated thyrsoid, and panicles and the leaves are 13-35 cm long. The Meitei population of Manipur considers this shrub a holy plant. The plant is found throughout the season. Mostly it is found to be growing during December to April and distributed in entire NorthEast Region of India. It is also found in subtropical Himalayas, North Bengal, and Bhutan (Phurailatpam et al., 2014). The plant is used in cough and menoeehagia. The flower of the plant is used to reduce tumour growth and treating wounds and as a blood purifier. The flowers are also used to treat liver and kidney stones. The burnt leaves and fruits are used against fever (Das et al., 2017). The medicinal uses of this plant include treatment of cough, influenza, cold, child birth, irregular menstrual cycle, diarrhea, abortion, high blood pressure, skin problem, boils, body ache, small pox, constipations, sprains, and burns (Singh Ningombam & Kumar Singh, 2014). The leave extracts of the plant are used orally with water against loose motion by the Missing community. The leaves and flowers are consumed as vegetables by the people of Assam. The tribes of Upper Assam, India use the plant to treat helminthiasis. The leaves are been using to treat gout, rheumatism, fever, and allergies. The bioactive compounds such as ß-sitosterol, botulin, and lupeol were found in the leaf extracts (Deori et al., 2023). The leaf extracts showed antimicrobial properties against *Salmonella typhimurium* (MTCC 3231) and *Salmonella enteric* (MTCC 1164). Also showed an effective against Hela cells (Kumar et al., 2017). The flower of the plant contains saponins, flavonoids, phenols, steroids, tannins, and terpenoids (Deori et al., 2023). The extracts of *P. thyrsiformis* flower was proved to decreasing in the size of struvite stones in vitro and successfully removed calcium oxalate stones in Wistar rats in vivo (Das et al., 2017b). in an experiment it was proved that the *P. thysiformis* extract has no harmful effect on mice (Deori et al., 2023).

In the micropropagation of *Justicia adhatoda* best shoot induction of all the leaf stem and nodal explant was observed best in MS medium supplemented with 10mg/L NAA 0.1mg/L BAP, 0.1mg/L Kn, and 2 mg/L 2,4-D. maximum shooting rate was observed in the MS medium supplemented with 1mg/L IAA, 1mg/L BAO and 1mg/L Kn (Bhawna et al., 2017). Another similar experiment was conducted by Srikun 2017 for *in vitro* propagation of *Strobilanthes tonkinensis*. Successful explant surface sterilization was achieved in 10 min explant treatment with 1.2% sodium hypochlorite. Shoot multiplication was obtained best in the MS medium supplemented with 16 µM BAP after 8 weeks and maximum rooting was observed in the MS medium supplemented with 7.5 µM IBA. 100% of the *in vitro* grown were survived under the greenhouse condition (Srikun, 2017).

1.12.4 *Enydra fluctuans:*

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Species: *Enhydra fluctuans* Lour

Enydra fluctuans Lour commonly known as helencha or harchuk is a tropical herb bearing medicinal properties and it is consumed as vegetables. the herb belongs to the family of Asteraceae. It is a flowering semi aquatic herbaceous plant, found commonly around the country. The herb is prostate herb, linear oblong leaves, with opposite sessile, grows 1-3-inch-long. Stems are 0.3m-0.6m, rooting occurs at the nodes. The taste of the leaves is slightly bitter, used to cure skin diseases, inflammation, nervous affections, laxative, leukoderma, bronchitis, biliousness, and small pox. The herb contains β-carotene, cholesterol, glucoside, saponins, enhydrin etc, this plant bears numerous medicinal properties including antioxidant, Hepatoprotective, Analgesic, Antidiarrheal activity, and CNS Depressant. The leave extracts bear significant antibacterial against *Pseudomonas aeruginosa, Escherichia coli, Micrococcus luteus, and Staphylococcus aureus* (Ali et al., 2013). An experiment for *in vitro* propagation and shoots generation of *Petasites hybridus* (Asteraceae) using nodal explants was standardised by Wildi et al., 1998, in this experiment shoot induction was achieved in the MS medium supplemented with 17.6 µM BAP and 0.54 µM NAA. The 27% of leaf explant and 40% of the petiole produced shoots after 5 weeks of culture. Most favourable explant shoot multiplication was observed in the MS medium supplemented with 8.8 µM kn+0.54 µM NAA (Wildi et al., 1998). *In vitro* plant regeneration of a medicinal plant *Lychnophora pinaster* was standardised by Souza et al., 2007, in the $1/4^{th}$ strength of MS medium supplemented. Shoot induction was achieved in the $1/4^{th}$ MS medium supplemented with 2.76 µM BAP. Shoot elongation was observed best in the $1/4^{th}$ MS supplemented with 8.67 µM GA, and rooting was best in $1/4^{th}$ MS supplemented with 10.7 µM NAA, 100% of the *in vitro* grown explants were survived while hardening in the greenhouse (de Souza et al., 2007).

1.12.5 Hygrophila auriculata:

Kingdom*:* Plantae

Phylum*:* Tracheophyta

Class*:* Magnoliopsida

Order*:* Lamiales

Family*:* Acanthaceae

Species*: Hygrophila auriculata (*Schumach.) Heine

Hygrophila auriculata is a wild medicinal herb of the family Acanthaceae, commonly found in wet areas, river banks, rice fields and ditches in India, Sri Lanka, Malaysia, Burma, and Nepal. The stem is reddish brown, leaves are whorled, large lanceolate, subsessile edges are sharp, straight or curved thorns. Flowers are arranged in axillary whorls. The plant root, seeds, and aerial portions are used to treat jaundice, rheumatism, hepatic blockage, inflammation, urinary infection, pain, edoema, malaria, gout, etc. (Hussain et al., 2010). The plant is also known for its anticancer, antibacterial, anti-diabetic, hypoglycemic, with radical scavenging activity. Important bioactive components like leupol, terpenoids, butelin, fatty acids, and flavonoids are also found (Jebamalai et al., 2021). The ethanolic extract of *H. auriculata* showed anti-microbial activity against *Mycobacterium canis*, *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton mentagraphytes*, and the stem showed against *Trichophyton mentagraphytes*, *Mycobacterium canis*, and *Candida albicans* (Hussain et al., 2010). The plant also contains some important phytochemicals like tannins, flavonoids, alkaloids, and saponins. Some important bioactive components like Pentane,1,1-diethoxy-; Butane, 1, 1- diethoxy- 3-methyl- ; Propane,1,1,3-triethoxy-; 3,3 –Diethoxy-2-Butanone; 1,1,3-Triethoxybutane ; Benzene, [Ethoxy (1-Propenyloxy; Nonane, 3,7-Dimethyl-; Diethyl Phthalate; Octadecanoic acid, 2-oxo- methyl ester; Isopropyl myristate; 2- Hexadecan-l-ol, 3,7,11,15-tetram; 2,6,10-trimetyl, 14- Ethylene- 14-Pe; 2,6,10- trimethyl, 14-Ethylene-14-Pe; 7-Octadecyne,2-methyl-; Heptadecanoic acid, Ethyl ester; Phytol Isomer; Phytol,acetat; Tridecanol, 2-ethyl-2-methyl-; Squalene; 1,2-Benzenedicarboxylic (Jebamalai et al., 2021). The plant bears numerous therapeutic potentials to treat various diseases. The leaves are used to treat lumbago, Prameha, cough, arthralgia, and anal fistula. The roots are used to cure jaundice and calculus, again the seeds are used against blood problems. De decoction of the root and entire plant is effective against rheumatoid arthritis. The vegetable when consumed is effective against anaemia (Sarvananda L, 2018).

An experiment was conducted by Aasim *et al.,* 2019 for *in vitro* propagation of *Hygrophila schulli* an important medicinal plant which are primarily used as haematinic, diuretic, and liver tonic. Well-developed callus was obtained in the MS medium supplemented with 0.2 mg/l 2,4-D and 0.2 mg/l 2,4-D. MS medium supplemented with 1mg/L BAP showed highest explant multiplication (Hussain et al., 2010).

Research problem and gap

The preliminary studies including survey and review of literatures reflected that some of the medicinal plants bearing important medicinal properties found in the region in very less amount, and seasonal plant, therefore mass propagation of the plants is required. Some of the plants mass propagation is not possible by traditional method. Tissue culture or *in vitro* mass propagation may be an effective solution for this problem. From the study it was observed that the *in vitro* propagation technique, genetic stability study, study of active compounds in the wild and *in vitro* plant, and production of secondary metabolite is not done yet for these medicinal plants.

Therefor the objectives of the study are-

1.1 Standardization of *in vitro* propagation technique for

1.1.1 Torenia crustacea

*1.1.2 Lindernia pusilla (*Willd.) Bold

1.1.3 Phlogacanthus thyrsiformis

1.1.4 Enydra fluctuans

1.1.5 Hygrophila auriculata

1.2 Study of genetic stability in the genomes of *in vitro* propagated plants using RAPD assay

1.3 Study of antioxidant potential of wild and *in vitro* propagated medicinal plants

1.3.1 Total Phenol Content

1.3.2 Total Flavonoid Content

1.3.3 Total Antioxidant Capacity

1.3.4 DPPH radical scavenging assay

1.4 Comparative analysis of bioactive compounds in wild and tissue cultured medicinal plants using GCMS.