MICROPROPAGATION OF MEDICINAL PLANT IN BANGLADESH-A SHORT REVIEW

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Master of Science in Biotechnology

Department of Mathematics and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Abstract

Micropropagation of medicinal plants is widely applied in the ayurvedic and pharmaceutical industries to produce active compounds. Besides that, in-vitro regeneration is important in the production of high-quality plant-based medicine. This also helps to conserve the genetic material of several vulnerable medicinal plants. Micropropagation protocols have been developed for a wide variety of medicinal plants, including endangered and vulnerable plant species. These well-developed methods are now available to assist growers in meeting the demands of the pharmaceutical industry in the next century. This study reviews *in vitro* micropropagation methods of medicinal plants and gives a comprehensive knowledge of these plants.

Keywords: Micropropagation, medicinal plants, *in vitro*, regeneration, herbal medicine, Bangladesh.

Dedicated to my family and friends

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List of Acronyms

WHO	World Health Organization
FAO	Food and Agriculture Organization
IUCN	International Union for Conservation of Nature
MPDB	Medicinal Plants Database of Bangladesh
MS	Murashige and Skoog medium
B5	Gamborg medium
WPM	McCown Woody Plant medium
PGRs	Plant growth regulators
$HgCl_2$	Mercury(II) chloride
BAP	6-Benzylaminopurine
KIN	Kinetin
NAA	Naphthalene acetic Acid
IAA	Indole-3-acetic acid
GA_3	Gibberellic Acid
BA	6-benzyladenine
2,4-D	2,4-Dichlorophenoxyacetic acid
IBA	Indole-3-butyric acid
mg	Milligram
cm	centimeter
l	litter
pH	potential of hydrogen

Glossary

Term	Definition
Autotrophic	An autotrophic is defined as an organism that can produce its own food using light, water, carbon dioxide, or other chemicals.
Auxin	Growth promoting substances that contributes to the elongation of shoots.
Callus	A tissue arising from disorganized proliferation of cells either in cultures or in nature.
Cytokine	Substance affecting the growth and division of cells.
Explant	An explant is a part of the plant by which a whole plant can be produced through plant tissue culture technique.
Gibberellins (GA)	Substances that promote cell elongation, shoot growth, and are involved in regulating dormancy.
Medicinal Plant	Medicinal plants can be defined as the plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body.
Micropropagation	Micropropagation is a method of plant propagation using extremely small pieces of plant tissue taken from a carefully chosen and prepared mother plant, and growing these under laboratory conditions to produce new plants.
Plant breeding	Plant breeding is the science driven creative process of developing new plant varieties that goes by various names including cultivar development, crop improvement, and seed improvement.
Protoplast fusion	Protoplast fusion is a type of genetic modification in plants by which two distinct species of plants are fused together to form a new hybrid plant with the characteristics of both.
pH	A measurement of the degree of acidity or alkalinity on a scale of 1-14.
Plant Growth Regulators(PGRs)	Plant growth regulators (PGRs) are chemicals used to modify plant growth such as increasing branching, suppressing shoot growth, increasing return bloom, removing excess fruit, or altering fruit maturity.
Totipotency	Potentiality or property of a cell to produce a whole organism.

Chapter - 1: Introduction

CHAPTER-1

INTRODUCTION

A medicinal plant is one that has substances in one or more of its organs that can be used for therapeutic purposes or chemo-pharmaceutical semi-synthesis. When a plant is called medicinal, it means it can be used as a medication, a therapeutic agent, or an active ingredient in a medicinal treatment. For thousands of years, the majority of plant species have been used as a source of medicine in many countries around the world. Besides these plants also play an important role in the development of human cultures all over the world. Additionally, several plants are considered key sources of nutrition because of their therapeutic qualities. Medicinal plants include a wide range of natural antioxidants and are used to treat a variety of diseases all over the world. Some of these properties are antimicrobial, anti-cancer, anti-diabetic, anti-atherosclerosis, immunomodulatory etc.Herbal medicines are in high demand for basic health care in both developed and developing nations because of their extensive biological and pharmacological activity, higher safety margins, and lower costs. Surprisingly, around 80 percent of the population of developing countries (according to WHO) is now completely or temporarily dependent on herbal drugs for primary healthcare (Mohiuddin *et al.,* 2019).

There are 422,000 plant species available worldwide, according to Marinelli's estimation in 2005 (Uddin *et al*., 2020).Of these, over 50,000 plants are medicinal plants that are used across the world (according to FAO). Besides, many of the world's flowering plant species have been used medicinally. Around 50,000–80,000 flowering plants are used medicinally (Uddin *et al*., 2020). Moreover, plants are the source of at least 7,000 medical compounds in the modern pharmacopoeia (Mohiuddin *et al.,* 2019). Medicinal plants have a promising future and the trade in medicinal plants is increasing in both volume

and exports. The overall global market for the trade of herbal medicine has been estimated at $36 billion in 2020(Riaz *et al*., 2021).

1.1 Characteristics of Medicinal plants:

Depending on when and how these medicinal plants are applied, they can be divided into two categories.

a) **Synergic medicine**- The interaction of two or more drugs when their combined effect is greater than the sum of the effects seen when each drug is given alone. Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

b) **Preventive medicine**- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present.

1.2 Classification of medicinal plants:

The classification of medicinal plants can be presented in different ways depending on the criteria used. Some significant classifications are mentioned below:

a) **Classification according to the Active Constituents**:

According to the active constituents all herbs are divided into five major categories: Aromatic (volatile oils), Astringents (tannins), Bitter (phenol

compounds, saponins, and alkaloids), Mucilaginous (polysaccharides), and Nutritive (food stuffs).

b) **Classification According to the Usage**: The herbs are classified in four parts: medicinal herbs, culinary herbs, aromatic herbs, ornamental herbs.

c) **Classification According to the period of life**: Plants can be grouped into Annual herbs (bloom one season and then die), Biennial herbs (live for two seasons, blooming the second season only) and Prennial herbs (bloom each season and can last for many years).

1.3 Medicinal plants in Bangladesh

Bangladesh is a tropical country with a rich diversity of plant species in a diverse range of ecosystems because it lies in the transition zone between two mega biodiversity hot spots, viz., the Indo-Himalayas and the Indo-Chinese. The International Union for Conservation of Nature (IUCN) mentions that, Bangladesh's ecosystems are so well defined that they support a diverse range of biodiversity. Bangladesh presently has an abundance of medicinal plant genetic diversity. Besides, FAO (2004) mentions that greatly, the south-east hilly area and north-east terraces of Bangladesh are rich in medicinal plants. Bangladesh is the home of medicinal plants. It has highly favorable climate and soil conditions for the production of medicinal plants. Based on a literature review, there are approximately 5000 plant species, of which approximately 1500 plant species are expected to be medicinal. Until now, 747 plants have been identified as medicinally important (Uddin *et al*., 2020). In addition, nearly 255 medicinal plants are used for preparation in ayurvedic and unani medicine (Palash *et al*., 2021).

Major plant families in Bangladesh, which are listed, are presented in a diagram.

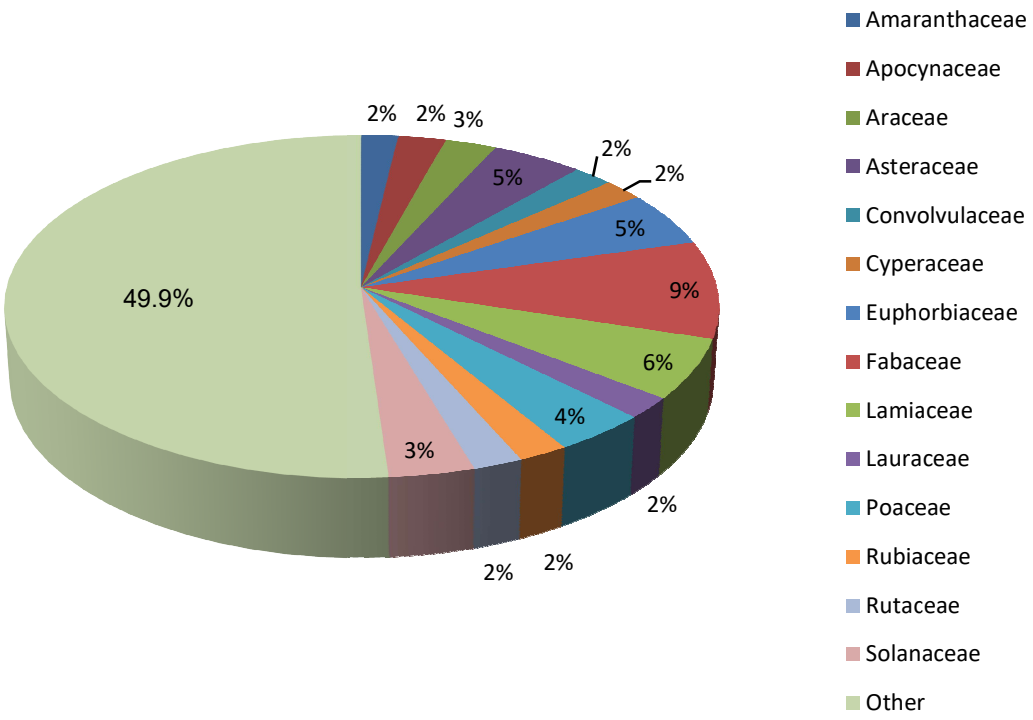

Figure 1: Pie chart demonstrates the most prominent plant families, expressed as percentage, from the MPDB 2.0.

A short brief about some important medicinal plants, their families, local names, and medicinal uses that have been reviewed are given below:

Family Name	**Scientific Name**	**Local Name**	**Utilized part**	**Medicinal uses**
Solanaceae	*Solanum surattense*	Kontikari, Wild Eggplant	Leaf, Stem, Whole Plant, Flower	Breathing trouble, heart diseases and pain
	Withania somnifera	Ashwagandha	Fruit, Leaf, Root, Roots, Whole Plant	Anti-inflammatory, anticancer, anti-stress and immune-modulator, adaptogenic, central nervous system, endocrine and cardiovascular activities

	Physalis minima	Bon-Tepari, Shod-Tepar, Kopal Futi, Futushkata	Fruit, Leaf	Constipation, Stomach Problems, Cleansing Of Gastric Tract
Rutaceae	*Clausena heptaphylla*	Panbahar	Leaf	Leaves are used for the treatment of dysentery and impotence.
	Aegle marmelos	Bael	Fruit, Root, Leaf, Dried Fruits, Unripe Fruit	To Keep Body Cool, Diarrhea, Dysentery, Constipation, Astringent, Repeat Fevers, Contagious Fevers, Frequent Urination (Diabetes), Blood Dysentery, To Increase Memory, To Prevent Stomach Upsets, Gonorrhea, Excessive Bleeding During Menstruation, Chronic Dysentery, Anti- Dandruff, Gastric Problem
	Feronia limonia	Koth Bel	Leaf	Dysentery And Other Bowel Troubles
Scrophulariaceae	*Scoparia dulcis*	Chini Shakkor, Maitta Orha, Chini Gura, Bon-Dhone, Chini-Dhonia, Mishri Dana.	Leaves, Whole Plant, Flower, Leaf, Fruit	Body Ache, Gastric Ulcer, Gastric Problems, Ulcer, Gonorrhea, Diabetes, Stones In Gall Bladder, Dysentery In Children, Anemia, Abscess, Fever, Dysentery, Jaundice, Burning Sensations During Urination, Severe Fever, Dysentry, Leucorrhea
Caesalpiniaceae	*Cassia obtusifolia* L.	Chakinda	Leaf, Seed, Root	Scabies, Stomach Pain, Frequent Urination
	Cassia alata	Dadmardan, Dadmari	Fruit, flowers	Ringworm disease, itching, cough, asthma, snake-bites, eczema, herpes and skin diseases
Araceae	*Acorus calamus* L.	Bois Gach	Leaves,	Typhoid, Snake Bite, , Mental

			Root, Leaf	Disorders, Being Possessed With 'Evil Spirits' Like Genies, Fever With Convulsions, Burning Sensations In The Body Of Adults And Children, Indigestion, Pneumonia, Cough, Mucus.
Asteraceae	*Tridax procumbens* L.	Tridhara	leaf	Leaves are used as a treatment against bronchial catarrh, dysentery, and diarrhea.
	Eclipta alba (L.) Hassk.	Keshraj, Keshuti, Kalokeshi	leaves, roots & areal parts	Liver ailments and shows effects on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement.
	Gynura procumbens	wonder plant, anti-Cholesterol plant and longevity spinach	leaf	kidney discomfort, rheumatism, Diabetes mellitus, constipation, and hypertension
Asclepiadaceae	*Hemidesmus indicus* (L.)	Anontomool, Ontomul	Root, Stem	Loss Of Strenght, Tuberculosis, Dizziness, Passing Of Semen With Urine, Impotency In Males, Loss Libido
Rubiaceae	*Paederia foetida* L.	Gondho-Vaduli, Gondho Pata, Gondho Vadal	Leaf, Whole Plant	Fractures, Loss Of Strength, Low Sperm Count, Rheumatism, Pain, Piles, Skin Allergy, Constipation, , Any Type Of Pain, Constriction Of Nerves Leading To Distortion In Hands Or Feet, Stoppage Of Urination, Paralysis, Body Pain, Internal Lesions, Stomach Problems, To Recuperate From Illness, Appetizer, Indigestion, All

				Types Of Pain, Fever
Aristolochiacec	*Aristolochia tagala*	Iswarmul	Roots and leaves	fever, dysentery, snakebite, rheumatism and toothache
Zingiberaceac	*Kaempferia galanga* L.	Akangi	Whole Plant	Loss Of Appetite, Excessive Libido, Leprosy, Piles, Acne, Coughs, Respiratory Problems, Tumor, Helminthiasis, Goiter, Stuttering, Muscle Spasms Or Pain(In Cattle)
	Curcuma aromatica Salisb	Bao Ada, Kor Ada	Leaves, Rhizomes	Rheumatic Pain, Bone Fracture
	Curcuma amada Roxb	Bao Ada	Rhizomes	Bone Fracture
Nyctaginaceae	*Boerhaavia diffusa* L.	Punornova, Punnainobboy , Gadha-Purnima, Purnolova	Leaf, Root, Whole Plant	Gonorrhea, , Burning Sensations In The Body, Loss Of Appetite, Dysentery, Heart Disorders, Stomach Pain Or Infections, Indigestion, Constipation In Cattle
	Mirabilis jalapa	Shondha Maloti, Shondhya Ful	Flowers	Gonorrhea, , Progressive Loss Of Eye Sight, Edema (Legs, Body)
Moraceae	*Ficus religiosa*	Ashathwa	fruits , Leaves, Bark	Extract of bark is antibacterial, astringent, relaxant and spasmolytic on smooth muscles and is used in diarrhoea, dysentery, gonorrhea, scabies and ulcers
	Ficus benghalensis	Bot Gach, Bot	Aerial Roots, Sap From Young Leaf, Root, Plant Exudate	Meho (Urinary Problem Arising From Endocrinological Disorder Or Diabetes), Excessive Urination, , Toothache, Loosening Of Tooth, Excessive Bleeding During Menstruation, Skin Diseases, Gonorrhea, Leaf, Abscess, To

			(Sap), Roots Of Ficus, Young Leaves, Bark	Lengthen Hair In Women, Piles
Verbenaceae	*Vitex negundo*	Nishinda, Eyrie Gach, Nishindir Gach	Leaf, Root, Fruit	Severe Insanity, Helminthiasis, Severe Fever, Jaundice, Scabies, Eczema, Skin Diseases, Headache, Dizziness, Debility, Dental And Skin Diseases, Fever, Malaria, Mosquito Repellent, Typhoid Fever, Sprain, Hurt, Bone Pain, Constipation, Poor Memory, Poor Eye Sight, Biliary Disorders, Bloating, Rheumatism In Joint, Edema, Spleen Disorders, Throat Infections, Piles,
	Rauvolfia serpentina L.	Borochanda, Shurjomukhi, Shorpogondha, Choto Chondro, Holud Korobi	Leaf, Whole Plant/ Root, Root, Flower, Seed	Skin Diseases And Sores Caused By Infection, Antidote/Snake Repellent, Hypertension, Mental Instability, Pimple, Snake Bite, Snakebite, Tigerbite, Sedative, Cough, Anti- Spasmodic, Malaria, Dermatitis, Continuous Vomiting, , Impotency In Males, Loss Libido, Constipation, Skin Diseases
Euphorbiaceae	*Phyllanthus fraternus* Webster	Bhui-amla	Leaf,shoot, root,	Gonorrhoea, leucorrhoea, dyspepsia, colic, diarrhea and dysentery, deobstruent, stomachic, febrifuge and anticeptic. leaves are used as poultice on swellings and ulcers, tender shoots are used in

				curing chronic dysentery, fresh roots are beneficially used in jaundice .The latex is also applied to offensive sores ulcers and mixed with oil it is used in ophthalmia
	Ricinus communis	Venna, Vella, Verenda, Venna Gach, Valla, Veron, Enda	Bark, Seed, Seed Oil, Leaf, Fruit, Oil From Whole Plant, Root, Venna	Diarrhea, Lesions On The Tongue, Physical Problems, Toxicity, , Headache, Joint Pain, Rheumatic Fever, Pain, Inflammation, Rheumatic Pain, Coughs, Mucus, Fever, Stomachache, Bloating, Decreased Eyesight, Conjunctivitis, Analgesic, Constipation, Body Pain
Compositae	*Stevia rebaudiana* Bert.	Stevia	Leaf	Diabetes
Fabaceae	*Abrus precatorius*	Laal Koonch, Josthimodhu, Laal Koonchvv, Kuch, Deshi Jasthimodhu, Kuch Pata	Root, Leaf, Stem	Gonorrhea, , Hoarseness Of Voice, Cold, Coughs, White Dysentery, Wounds, Diabetes, Liver Disorders
Amaranthaceae	*Achyranthes aspera* L.	Upomargo, Apang, Udvut Nangra, Lal Chorchora, Dhanshisari, Coscoria, Chet	Root, Stems, Leaf, Whole Plant, Stem	Tooth Infections; Irregular Menstruation, Gonorrhea, , Dermatitis, Chronic Dysentery, Menorrhagia, Blood Dysentery, Eczema, Abdominal Pain, Diabetes ,Vomiting Tendency, Coughs, Obesity, Respiratory Tract Disorders, Pain, Wounds, Gastrointestinal Disorders (Leaf), Jaundice (Root)
Mimosaceae	*Mimosa pudica*	Shada	Leaf, Root	Abscess, Wounds, , Jaundice, Skin

		Lojjaboti, Laal Lojjaboti, Lojjaboti, Salai, Ajing Kiu		Diseases, Snake Bite, Leucorrhea, Any Type Of Tooth Problem, Malaria, Fever With Convulsions, Diarrhea, Rheumatic Pain, Swelling Due To Injury, Rheumatism, Insect Repellent, Tooth Diseases
Acanthaceae	*Adhatoda vasica nees*	Bashok	Leaf, Bark	Colds, Coughs, Fever, Ear Lobe Infection, Porcine Diseases, Jaundice, Pneumonia, Pain, Gonorrhea, Chronic Asthma, Leprosy, Mucus, , Chest Pain
	Phlogacanthus thyrsiflorus	Ram Basak	Fruit and leaf	Fevers, coughs, bronchitis and asthma and as mild bronchial antiseptics
Liliaceae	*Aloe vera* L.	Ghritakancha n, Ghrito Kumari,	Leaf, , Soft Pulp Within Leaf, Whole Plant	Skin Disorders, Gonorrhea, Burns, Constipation, Hypertension/Anxiety, Weakness, Strong Headache, Diabetes, Head Warm And Dysentery, Stomach Upsets, Leucorrhea.
Plantaginaceae	*Bacopa monnieri* (L.) Penn	Brahmi Shak	Leaf, New Stems	Abnormal Brain Functions, Breaking Down Of Voice, Leprosy, Swelling Due To Injuries, Blood Toxicity, Edema, Fever, Memory Loss
Lamiaceae	*Ocimum sanctum*	Tulshi	Leaf, Root, Flower, Seed	Puerperal Fever, Symptoms: Wasting Away Of Body, Bilious, Pain, Bronchitis, Burn, Dry Cough

Table 1: A short brief about some important medicinal plants, their families, local names, and medicinal uses that have been reviewed

1.4 Micropropagation

Micropropagation is the practice of vegetative growth and rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. It is based on the principle of totipotency, which refers to a plant's potential to develop into a new plant from its cells and tissues. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction. This technique is very useful for mankind by conservation of elite, endangered plants and eco- friendly production of drugs. The application of tissue culture and rapid propagation methods are becoming increasingly widely used in both developed and developing countries.

Most medicinal plants are still collected from the wild population or their natural habits due to climatic and cultural issues. As a result of the increased global demand for medicinal plants, their natural habitat has been over-exploited. Besides, When propagated conventionally, many of these plants take a long time to multiply, have a low rate of fruit set, poor seed germination, and are frequently protected or listed as endangered. According to the 1997 IUCN Red List of Threatened Plants, 12.5 %, or approximately 34,000, of the world's vascular plant species are threatened with extinction, including 7% of the Apocynaceae family and 5% of the Vitaceae family (Walter & Gillett *et al*., 1998). Later, the 2004 IUCN Red List includes 11,824 plant species, 8,321 of which are threatened and day by day it’s increasing. A conservative estimate of IUCN’s Threatened Plants Unit shows that about 60,000 plant species (25%) would become either extinct or nearly extinct by the year 2050 (Uberoi *et al*., 2010). The subject of threatened plants in Bangladesh with their importance of inventory was first highlighted by Khan (1991) with a tentative list of 12

threatened vascular plants in Bangladesh (Rashid *et al*., 2014). Later, IUCN Red List of Threatened plants included 24 vascular plant species (IUCN, 1997). The IUCN has assessed 600 plant species on their "Red List" until April 2022 in Bangladesh. Two species of Ptericlophytes, three species of Gymnosperm, and 555 species of Angiosperm are among the 600 plant species.

According to The IUCN categories and criteria, the details are giving below:

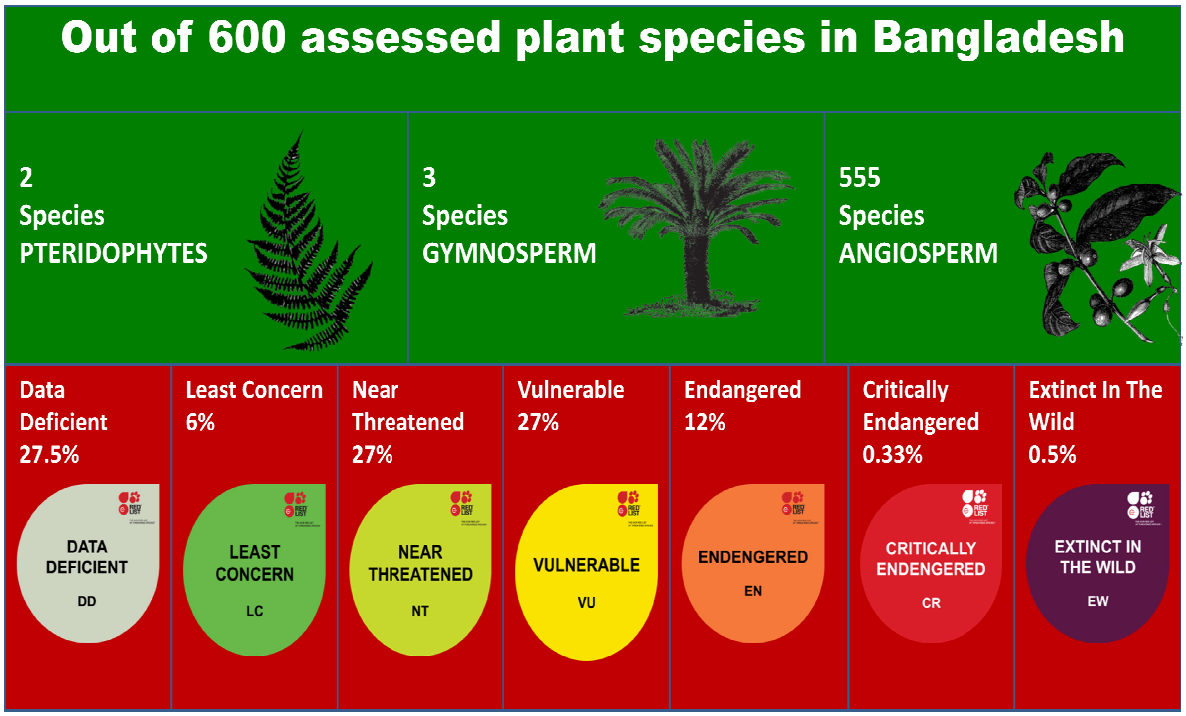

Figure 2: This chart demonstrates the classification and percentage of species at high risk of global extinction, from the IUCN website.

So, to deal with this vulnerable situation, it is important to undertake immediate conservation of these important plant species using biotechnological approaches such as micropropagation. Day by day, the interest in mass propagation of medicinal plants in vitro has distinctly increased.

The advantages of *In vitro* micro- propagation of medicinal plant are listed below:

- Higher rate of multiplication.
- Environment can be controlled or altered to meet specific needs of the plant.
- Plant available all year round (independent of regional or seasonal variation)
- Production of plants with changed genotype (tetraploids, haploids, hybrids).
- Production of secondary metabolites.
- New and improved genetically engineered plant can be produced.
- Conservation of threatened plant species.
- Preservation of genetic material by cryopreservation.

1.4.1 Stages of Micropropagation

Micropropagation generally involves five stages:

Stages	Methods Involved
Stage 0	Mother plant selection, maintenance and preparation for culture initiation
Stage I	Culture initiation and establishment
Stage II	Shoot multiplication
Stage III	Rooting of the shoots
Stage IV	Transfer of plantlets in the greenhouse environment

Table 2: Stages of Micropropagation

1.4.2 Advantages of Micropropagation

The micropropagation technique has proved beneficial in many ways. Following are the advantages of micropropagation in plant production:

- This is an alternative method for vegetative propagation with enhanced multiplication rate.
- Large quantities of identical plants can be obtained from a single plant tissue within a very short time period.
- The shoot multiplication has a very short cycle and each cycle results in a logarithmic increase in the number of shoots.
- The small-sized propagules can be stored and transported easily.
- The germplasm stocks can be maintained for several years using this technique.
- It helps in the production and maintenance of pathogen-free plant varieties.
- In a dioecious plant, the seed progeny yield is 50% male and 50% female. This method helps in obtaining the desired sex of the plant.
- Millions of plantlets can be maintained in the cultural vials.
- Genetic uniformity of the propagules can be maintained through this technique.
- It is a cost-effective process.
- New varieties of species can be propagated.
- A requirement of less space and human resources.
- This method is independent of season and can be carried out anytime.
- Assists in the regenerating genetically modified cells after protoplast fusion.
- Often produces healthier plants, leading to quicker growth compared to those plants produced by a conventional method.

1.4.3 Disadvantages of Micropropagation

The disadvantages of micropropagation are given below:

- The plants produced are not autotrophic.
- It cannot be implemented in all the crops.
- The plants find a problem acclimatizing to the natural environment.

1.5 Objectives

The present study was conducted to achieve the following objectives:

- A short review on micropropagation of medicinal plants.
- Identification of the best explants for medicinal plants.
- Identification of the best Plant growth regulators *(*PGRs*)* for shooting and rooting of medicinal plants.
- Acclimatization technique of the regenerated plantlets.

Chapter -2: Materials and methods

CHAPTER- 2

MATERIALS AND METHODS

2.1 Search:

The search engine that was used for the review was Google Search by using search terms (e.g. “medicinal plant”, “herbal drug”, “herbal medicine”, “aromatic plant”,)and keyword combinations (e.g. “micropropagation”, “in vitro”, “regeneration”, and “Bangladesh”) to retrieve the abstracts of relevant articles. For literature focusing on the micropropagation of medicinal plants, retrieved full articles from full text databases (ScienceDirect, Springer, Researchgate, Google scholar, Sci-hub).The journals that were accessed to retrieve articles were Banglajol, Bangladesh Association for Plant Tissue Culture & Biotechnology, Journal of Agriculture and food, etc.

2. 2Article analysis:

To retrieve the data, approximately 100 articles (published in international and local journals until 2021) claiming information on the micropropagation of medicinal plants in Bangladesh were checked. To review, I personally compiled information on medicinal (therapeutic) uses of medicinal plants, explant source, medium, PGR types, concentrations for shoot and root, acclimatization, and survival rates from articles published in various journals and universities and MPDB 2.0(Medicinal plants database of Bangladesh) website.

Chapter -3: Result

CHAPTER- 3

RESULTS

3.1 Explants source

The small pieces of plant part or tissues that are aseptically collected and grown in a nutrient medium are referred to as explant. Explant is a material used as an initial point for micropropagation. The most commonly used explants are shoot tips and nodal buds. Besides, seed, cotyledons, internodal, vine, apical and axillary buds, leaf, rhizome tip; peptiole and root are also used as explants for micro propagation of medicinal plants.

Mainly micropropagation success depends on the age, size, and position of explants because not all plant cells have the same ability to express totipotency (shidu *et al*., 2010). The age of the explant is a high priority. It's advised by researchers, young parts should be used as explants for culture because younger tissues correlate with better physiological responses in lab experiments. Also, large size of explants can increase chances of contamination and small size of explants like meristems can sometimes show less growth (shidu *et al*., 2010).

The nature of the explant to be used for in vitro propagation is, to a certain extent, governed by the method of shoot multiplication to be adopted (Bhojwani *et al*,. 1996). It is known that, when the goal is to produce virus-free plants from an infected individual, starting with sub-millimeter shoot tips becomes a requirement. If the stock plant has been virus-tested or if virus control is not needed, Nodal segments are the most suitable explant. Explants of small shoot tips have a low survival rate and grow slowly initially.

Nodal segments are widely used explants for micropropagation of medicinal plants in Bangladesh. A great number of Bangladeshi authors used nodal segments and shoot tips, petiole, leaf, or internodes as explants, in their experiments; they discovered that nodal segments produced the best outcomes that they reported in their articles. For Example, Hossain and his colleagues used nodal segment and shoot tip as explants for micropropagation of Ashwagandha **(***Withania somnifera*) that belongs to the Solanaceae family and were reported to have various medicinal properties. They demonstrated that nodal segment responded better than shoot tip, whereas shoot initiation was 90% from nodal segment and was 78% from the shoot tip (Hossain *et al*., 2019). Similar result was reported for *Physalis minima* (Afroza *et al*., 2009) *Scoparia dulcis* (Rashid *et al*., 2009), *Clausena heptaphylla* (Majumder *et al*., 2016), *Tridax procumbens* (Jesmin *et al*., 2013), *Gynura procumbens* (Azad *et al*., 2017), *Ficus benghalensis* (Rahman *et al*., 2004), *Ocimum sanctum*L (Jamal *et al*., 2016), *Phlogacanthus thyrsiflorus (*Hassan *et al.,* 2011), *Phyllanthus fraternus* Webster (Hassan *et al*., 2011), *Mimosa pudica* (Hassan *et al*., 2010), *Paederia foetida* (Amin *et al*., 2003), *Cassia alata* (Hasan *et al*., 2008), *Adhatoda vasica* Nees (Khalekuzzaman *et al* ., 2008) .

Besides that, nodal explants respond better than other explants, viz. petiole, leaf, or internodes. Banu and her colleagues used nodal, petiole, and leaf as explants for micropropagation of *Gynura procumbens*, whereas shoot initiation was 99% from nodal segment and was 95% from petiole and leaf. Therefore, the nodal segment responded slightly better than the other two, like the petiole and leaf (Banu *et al*., 2017). A similar result was observed in *Rauvolfia serpentina,* where nodal explants responded better compared to leaf explants (Khan *et al*., 2018). Nodal, internodes and leaves were used for micropropagation of Punarnava (*Boerhaavia diffusa*).Among them nodal explants responded better compare to other explants(Biswas *et al*.,2009).

Further, internode explants were found to be more responsive (100% in various plants) to multiple shoot induction. Internode was used as an explant for micropropagation of wild eggplant (*Solanum surattense*) (Rahman *et al*., 2011). Similar observations were reported for *Adhatoda vasica* Nees (Azad *et al*., 1998) and *Achyranthes aspera* (Sen *et al*., 2013).

Generally, pieces of the cotyledon, hypocotyl, stem, leaf, or embryo are usually used for callus induction. Some authors reported better results with cotyledon, leaf, and rhizome tips as explants than with shoot tip and nodal segments for micropropagation of valuable medicinal plants. For example, Cotyledons and shoot tips were used for the regeneration of Bael *(Aegle marmelos)*. From these two explants, Cotyledon was found to be superior for maximum multiple shoot induction as well as maximum number of shoots per explant (Das, R. *et al.,* 2008).

Leaf and nodal segment were used for micropropagation of Brahmi Shak (*Bacopa monnieri* L.) which is an important medicinal herb, leaf showed the best response towards shoot regeneration (Rahe et al., 2020). Similar results were found for the micropropagation of Basok (*Adhatoda vasica* Nees) (Khan *et al*., 2016).

Rhizome tip explants have also been addressed in medicinal plants. It was found to give a better response than rhizome buds in terms of shoot proliferation in *Curcuma amada Roxb* (Ferdous *et al.,* 2012).

In conclusion, the nodal segments are one of the most used and the best explants for various medicinal plants as they produce the highest number of shoots per explant. Additionally, cotyledon, leaf, and rhizome tips have also been explored by different authors in different medicinal plants and found their desire result.

3.2 Culture initiation and establishment

For initiating the culture, there are four steps, which are as follows.

- Isolation of the explant
- Surface Sterilization
- Washing
- Establishment of explant on an appropriate culture medium

One of the most important steps in successful *in vitro* micropropagation is explant sterilization. Sterilization is important to reduce contamination and to obtain disease-free explants. Disinfecting agents, like, mercuric chloride, savlon, Tween 80, Tween 20, ethanol, sodium hypochlorite, calcium hypochlorite, etc. are mostly reported in these medicinal plants. Generally, sterilization depends on plant species and explants used for micropropagation. Explants can be directly treated with the disinfectant if they are hard and large enough to be easily handled, such as mature seeds, mature endosperm or nodal explants, whole seeds, decoated seeds, or stem pieces, that are surface sterilized. When immature ovules, embryos, or endosperm are to be used as explants, the standard procedure is to surface sterilize the ovary or ovule, then dissect off the explant under aseptic procedures to protect the inoculum's soft tissues from the sterilizing agent's damaging effects. It is observed that a few drops of Tween 80 (0.1 percent), Tween 20, savlon, and liquid detergent are used for sterlinging several medicinal plants whose nodal segments, shoot tips, seeds, cotyledons, and leaves have been used as explants for micropropagation. Mercuric chloride, 70% ethanol, and Bavistin are used whose internode, rhizome tip, young twigs, cotyledonary nodes, and rhizome sprout have been used as explants.

A detailed documentation of the sterilizing agent with respect to different medicinal plants and their explants is presented below:

Medicinal plant	Explant	Agent	Reference
Solanum surattense (Wild Eggplant)	internodal explants	$HgCl_2$	Rahman *et al*., 2011
Withania somnifera	Shoot tip and nodal segment	savlon	Hossain *et al*., 2019
Physalis minima	nodal segments and shoot tips	0.1% Tween-20	Afroza *et al*., 2009
Clausena heptaphylla (Panbahar)	Shoot apex and nodal explants	liquid detergent	Majumder *et al*., 2016
Aegle marmelos/Wood Apple(Beal)	cotyledons and shoot tips	Tween-80 and Savlon	Das.R.*et al.,* 2008
Feronia limonia	Seed	detergent	Islam *et al.,* 2010
Scoparia dulcis(Bondhane)	Shoot tips and nodal segments	1% Tween 80	Rashid *et al*., 2009
Cassia obtusifolia L./ Chakunda	shoot tips	Tween-80	Hasan *et al.,* 2008
Cassia alata L.	nodal explant	Tween-80	Hasan *et al*.,2008
Acorus calamus L.	rhizome tip	$HgCl_2$	Ahmed *et al*.,2007
Tridax procumbens L.	Shoot tip and nodal segment	tween-20	Jesmin *et al*., 2013
Eclipta alba	Nodal segments	mild detergent	Yesmin *et al*., 2015
Gynura procumbens	nodal and shoot tip	Savlon	Azad *et al*., 2017
Paederia foetida L. (Gondhabadali)	Shoot tips and nodal segments	savlon	Amin *et al*.,2003
Aristolochia tagala	Young twigs	$HgCl_2$	Biswas *et al*.,2007
Curcuma aromatica Salisb	rhizome sprout	70% ethanol	Sharmin *et al*.,2013

Boerhaavia diffusa L.	Nodal segment	liquid detergent	Biswas *et al*.,2009
Mirabilis jalapa	nodal explants	liquid detergent	Rahman *et al*.,2021
Ficus religiosa	Shoot tips and nodal explants	liquid detergent	Hassan *et al*., 2009
Ficus benghalensis	Shoot apices	Savlon	Rahman *et al*., 2004
Vitex negundo (Nisinda)	Shoot apex of twigs	$HgCl_2$	Islam *et al.,* 2009
Rauvolfia serpentina L.	nodal and leaf explants	Tween-20	Khan *et al*., 2018
Phyllanthus fraternus	Shoot tips and nodal explants	mild detergent	Hassan *et al*.,2011
Ricinus communis	cotyledonary nodes	$HgCl_2$	Alam *et al*.,2010
Stevia rebaudiana	Nodal segment	Savlon	Karim *et al.,* 2008
Abrus precatorius	Juvenile twigs	$HgCl_2$	Biswas *et al*.,2007
Achyranthes aspera (apang)	nodes	liquid detergent	Sen *et al*., 2013
Mimosa pudica	shoot tip and nodal explants	liquid detergent	Hassan *et al*.,2010
Adhatoda vasica nees	shoot tip and nodal explants	Tween-80 and Savlon	Khalekuzzaman *et al*., 2008
Phlogacanthus thyrsiflorus	Shoot tip and nodal explants	liquid detergent	Hassan *et al.,* 2011
Aloe vera L.	Shoot tip	detergent	Razib *et al.,* 2016
Bacopa monnieri	leaf and nodal	detergent	Rahe *et al*., 2020
*Ocimum sanctum/*Tulsi	The nodal and shoot tip explants	tween-20	Jamal *et al*., 2016

Table 3: A detailed list of the sterilizing agent with respect to different medicinal plants and their explants

After isolation of the explant, an appropriate culture medium is needed for establishing the micropropagation. It is observed that MS (Murashige and Skoog, 1962) media is widely used for micropropagation of medicinal plants. But some authors used different kinds of culture media for their experiments. Rahman and his colleagues used three basal media: Murashige and Skoog (MS)

medium, Gamborg (B5) medium, and McCown Woody Plant medium (WPM) for micropropagation of wild eggplant (*Solanum surattense)*. Of the three basal mediums that were used, the MS medium was the most effective on *in vitro* shoot regeneration. The best rooting of wild eggplant was also observed in MS medium (Rahman *et al*., 2011).

It is known that tissue loses its green pigments over time in culture and must depend on an external source of carbon. So it's essential to have an utilizable carbon source in the culture medium. The most commonly used carbon source is sucrose. Glucose and fructose are also known to promote the growth of some tissues. In terms of medicinal plants, it is observed that 3% sucrose is commonly used as a carbon source.

The pH of the medium for micropropagation of medicinal plants is usually adjusted between 5.7 and 5.8 before autoclaving.

3.3 Shoot Formation

Shoot formation by culture of explants or calluses on media containing growth hormones (mainly cytokinins, but sometimes auxins also) is needed for successful plant regeneration. Plant growth regulators (PGRs) are mainly chemicals that are used to modify plant growth. It regulates various physiological and morphological processes in plants. Different types and concentrations of growth hormones are reported to be used for shoot formation.

To achieve in vitro multiplication, different methods are used:
(1) Multiplication by axillary and apical shoots or direct regeneration
(2) Through callusing or indirect regeneration.

3.3.1 Direct regeneration

In vitro organs are produced directly from explants in direct regeneration. The effects of auxins and cytokinins on the shoot multiplication of various medicinal plants have been reported.

For shoot multiplication of wild eggplant (*Solanum surattense)*, Rahman and his team used BAP, KIN, and NAA as growth hormones in their experiment. It is observed that BAP was more effective compared to KIN, and also, BAP alone was superior to a combination of BAP and NAA. The highest mean number of shoots per explant (100%) was recorded on MS media containing BAP (Rahman *et al*., 2011).Similar result was reported for micropropagation in various medicinal plants, such as, *Physalis minima (*Afroza *et al*., 2009), *Feronia limonia* L (Islam *et al.,* 2010), *Scoparia dulcis* (Rashid *et al*., 2009),(Hassan *et al*., 2009), *Tridax procumbens* (Jesmin et al., 2013), *Gynura procumbens (*Azad *et al*., 2017), *Paederia foetida* (Alam *et al*.,2010)(Binto *et al*.,2020), *Curcuma aromatica Salisb(*Sharmin *et al*.,2013), *Mirabilis jalapa(*Rahman *et al*.,2021), *Ricinus communis* (Alam *et al*.,2010), *Achyranthes aspera*(Sen *et al*., 2013), *Adhatoda vasica nees*(Khalekuzzaman *et al*., 2008).

A combination of BAP and NAA was found to be the best treatment for various medicinal plants. For micropropagation of Beal (*Aegle marmelos)*, this combination showed the maximum multiple shoot induction and number of shoots per explant (Das *et al.,* 2008). Similar results were reported for *Eclipta alba (*Yesmin *et al*., 2015), *Aristolochia tagala (*Biswas *et al*., 2007), *Boerhaavia diffusa* L (Biswas *et al*., 2009), *Mimosa pudica (*Hassan *et al*., 2010), and *Phlogacanthus thyrsiflorus (*Hassan *et al.,* 2011).

Majumder and his colleagues used BAP and IAA as PGRs for the micropropagation of Panbahar (*Clausena heptaphylla*) and reported that within 20 days, direct multiple shoot buds developed and the longest shoot buds (5.60 cm) were developed in this combination (Majumder *et al*., 2016). Some authors also used this combination and got maximum results in their experiments, such as, with *Gynura procumbens (*Banu *et al*., 2017) and *Ficus religiosa (*Hassan *et al*., 2009).

For the micropropagation of Nisinda *(Vitex negundo),* Islam and his team used a combination of BAP and KIN. The combination of BAP and KIN produced the highest induction (95%), as well as the highest number of shoots per explant (19.33±1.25) and shoot length (6.6±0.22 cm) (Islam *et al*., 2009).

Some authors combined cytokinins and gibberellic acid in their experiments. Hasan and his colleagues used a combination of BAP and GA_3 for the micropropagation of *Cassia alata* and reported the best development and elongation of multiple shoots (Hasan *et al*., 2008). Similar results were found for the regeneration of *Phyllanthus fraternus*, and combinations of BAP and GA_3 were found to be more suitable than combinations of BAP with NAA or IAA (Hassan *et al.,* 2011).

BA alone was effective for the micropropagation of some medicinal plants. Rahman and his team used two cytokinins (BA and KIN) and BA proved better than KIN and it was found to be the most effective cytokinin for shoot induction as well as shoot proliferation in *Ficus benghalensis (*Rahman *et al*., 2004). BA provided the best responses observed for *Paederia foetida* L (Amin *et al*., 2003) and *Ocimum sanctum* (Jamal *et al*., 2016).

The combination of BA and NAA showed superior results for a variety of medicinal plants, including *Kaempferia galanga* (Rahman *et al*., 2005),

Curcuma amada Roxb (Ferdous *et al.,* 2012), and *Adhatoda vasica nees* (Azad *et al*., 1998).

Ahmed and his team did micropropagation of Bois Gach *(Acorus calamus)* and used BAP alone, KIN alone, combination of BA and KIN, combination of BA and NAA, combination of BAP and IAA, combination of KIN and NAA, combination of KIN and IAA, NAA alone, IBA alone, combination of NAA and IBA. The highest percentage of shoot proliferation (95.86%) and the longest shoot length (2.85 cm) were recorded after the treatment of BAP. The combination of KIN and NAA showed the highest percentage of shoot proliferation (98.99%), outperforming BAP and NAA or BAP and IAA (Ahmed et al., 2007).

A detailed documentation of growth hormones and their concentrations in relation to various medicinal plants is presented:

PGRs	Medicinal plants	PGRs concentration	% of shoot regeneration	Number of shoots/explants	Reference
BAP	*Solanum surattense*	0.5 mgl^{-1}	100%	58.2 ± 4.6a	Rahman *et al*., 2011
	Physalis minima	1.0 mgl^{-1}	95%	Nodal segments= 32±0.46 Shoot tips= 29±0.5	Afroza *et al*., 2009
	Feronia limonia	0.2 mgl^{-1}	88%	10.0 ±3.0	Islam *et al.,* 2010
	Scoparia dulcis	1.0 mgl^{-1}	85.3%	65.3±0.34	Rashid *et al*., 2009
	Scoparia dulcis	0.1 mgl^{-1}	94%	Shoot tips= 12.4±0.60 Nodal segments= 16.4±0.82	Hassan *et al*., 2009
	Tridax procumbens L	1.0 mgl^{-1}	90%	Shoot tips= 10.5±0.27 Nodal segments=	Jesmin *et al*., 2013

				12.2±0.32	
	Gynura procumbens	4.0 mgl^{-1}	97.31%	6.22±0.51	Azad *et al*., 2017
	Paederia foetida	1.0 mgl^{-1}	84%	1.60±0.12	Alam *et al*., 2010
	Paederia foetida	1.0 mgl^{-1}	-	4.40±0.98	Binto *et al*., 2020
	Curcuma aromatica Salisb	1.0 mgl^{-1}	95.33 %	13.20 ± 0.69	Sharmin *et al*.,2013
	Mirabilis jalapa L	3.0 mgl^{-1}	80%	8.1	Rahman *et al*.,2021
	Ricinus communis L	3.0 mgl^{-1}	85%	12.56±0.46	Alam *et al*.,2010
	Achyranthes aspera L.	3.0 mgl^{-1}	100%	4.33±1.7	Sen *et al*., 2013
	Solanum surattense	0.5 mgl^{-1}	100%	58.2 ± 4.6a	Rahman *et al*., 2011
BAP+ NAA	*Aegle marmelos L*	(2.0+0.2) mgl^{-1}	91.23%	Cotyledons= 22.7±0.5 Shoot tips= 20.5±0. 5	Das *et al.,* 2008
	Eclipta alba	(1.0+0.1) mgl^{-1}	90%	18.40±0.67	Yesmin *et al*., 2015
	Aristolochia tagala	(2.0+0.5) mgl^{-1}	79%	3.60 ± 0.09	Biswas *et al*.,2007
	Boerhaavia diffusa	(2.0+0.2) mgl^{-1}	93%	12.51 ± 0.45	Biswas *et al*.,2009
	Mimosa pudica	(1.5+0.5) mgl^{-1}	88.2%	20.4 ± 1.20	Hassan *et al*.,2010
	Phlogacanthus thyrsiflorus Nees	(1.0+0.5) mgl^{-1}	84.2%	12.4 ± 0.66	Hassan *et al.,* 2011
BAP+ IAA	*Clausena heptaphylla* (Roxb.)	(2.0+1.0) mgl^{-1}	90%	4.20 ± 0.18	Majumder *et al*., 2016
	Gynura procumbens	(1.0+0.5) mgl^{-1}	99%	20.1 ± 1.96	Banu *et al*., 2017
	Ficus religiosa	(0.5+0.1) mgl^{-1}	78%	18.8 ± 1.39	Hassan *et al*., 2009
BAP+ KIN	*Vitex negundo*	(1.0+0.5) mgl^{-1}	95%	19.33±1.25	Islam *et al.,* 2009
BAP+ GA_3	*Cassia alata*	(1.0+2.0) mgl^{-1}	80%	Shoot tips=7.0±1.0 Nodal segments =8.0±1.0	(Hasan *et al*., 2008)
	Phyllanthus fraternus	(0.5+0.1) mgl^{-1}	88%	16.8 ± 0.95	Hassan *et al*.,2011
BA	*Paederia foetida*	1.0 mgl^{-1}	95%	7.80 ± 0.56	Amin *et al*., 2003
	Ficus benghalensis	0.5 mgl^{-1}	90%	12.65 ± 2.16	Rahman *et al*., 2004

	Ocimum sanctum	0.2 mgl^{-1}	91%	6.0 ± 0.14	Jamal *et al*., 2016
BA+ NAA	*Kaempferia galanga*	(1.0+0.1) mgl^{-1}	100%	20.50±1.80	Rahman *et al*., 2005
	Curcuma amada Roxb	(8.0+1.0) mgl^{-1}	99.8%	10.6 ± 0.26	Ferdous *et al*., 2012
	Adhatoda aasicd Nees	(1.0+0.1) mgl^{-1}	100%	10.3± 1.14	Azad *et al*., 1998
KIN+ NAA	*Acorus calamus*	(2.0 +0.05) mgl^{-1}	98.99%	8.34±1.44	Ahmed *et al*.,2007

Table 4: A detailed documentation of growth hormones and their concentrations in relation to various medicinal plants for direct shoot formation

However, it has been noticed that a combination of cytokinin and auxin is the most commonly used treatment as a growth hormone for shoot formation in a variety of medicinal plants. For medicinal plants, BAP alone is the most commonly used cytokinin for shoot formation. Also, a combination of BAP and NAA, BAP and IAA, BAP and KIN, BA and NAA, or BA alone, is reported to be used in various medicinal plants. In exceptional cases, it is seen that combinations of cytokinin and gibberellic acid, such as, a combination of BAP and GA3, are also used as growth hormones for the micropropagation of medicinal plants.

3.3.2 through callusing or indirect regeneration

Callus culture is the culture of dedifferentiated plant cells induced in growth medium with suitable hormones. Different growth hormones are used to promote callus induction and development. Sometimes, cultures of callus in the callus initiation media encourage shoot initiation in due course of time. But in other cases, cultures need to be transferred to a fresh medium with different hormonal supplementation so that shoot initiation is supported by the callus. In both cases, new plants can be successfully regenerated following root induction.

A combination of BAP and NAA was found to be effective for callus induction in various medicinal plants. For the micropropagation of Chinigura (*Scoparia dulcis*), Hassan and his team used a combination of BAP and NAA and a combination of BAP and IAA. It was found that BAP with NAA was more effective than BAP with IAA for callus induction, and in this combination, a deep green compact and nodular callus developed after four weeks of culture. Then the callus was subcultured in the same combination of growth hormones, and it produced a huge number of shoots after six weeks (Hassan *et al*., 2008). A similar result was reported for the micropropagation of *Stevia rebaudiana* (Karim *et al*., 2008). Also, Hassan and his team used BAP alone, BAP with NAA, and BAP with IAA for callus induction of *Paederia foetida*. From all these combinations, BAP with NAA was found to be more effective than BAP alone or BAP with IAA. Callus developed on the cut surface of the nodal explant between seven and fifteen days of culture, and after three weeks of culture, a yellowish green compact and nodular callus formed. When the same callus was subcultured on BAP alone for six weeks, shoot buds began to form (Hassan *et al*., 2012). A similar result was reported in Gondho-Vaduli (*Paederia foetida*) (Ghosh *et al*., 2019). For in vitro propagation of Josthimodhu (*Abrus precatorius)* through induction of indirect organogenesis, a combination of BAP and NAA was used as growth hormones by Biswas, and a yellowish-green nodular callus was induced at the cut surface of the nodal segments. The callus differentiated into adventitious shoots when KIN was combined with BAP and NAA and subcultured (Biswas *et al*., 2007).

Combination of BAP and KIN was used for regeneration of Ashwagandha (*Withania somnifera)* by Siddique and his team. It was observed that callus was initiated from explant in this combination and shoot was also formed in the same combination when it was subcultured (Siddique *et al*., 2004). Similar

result was reported for micropropagation of *Bacopa monnieri* (Rahe *et al*., 2020).

2, 4-D was found to be the best treatment for some medicinal plants. Hasan and his colleagues used 2, 4-D as a growth hormone for callus induction and regeneration of Chakunda (*Cassia obtusifolia*), and from seven to ten days after inoculation, callus was formed at the cut surfaces of shoot tips. For shoot regeneration, calli from shoot tips were subcultured after combining KIN and the medium. A combination of 2, 4-D and KIN was found to be an ideal treatment for shoot induction as well as elongation (Hasan *et al.,* 2008). A similar result was observed in Dadmardan (*Cassia alata*) (Hasan *et al.,* 2008).

Some authors reported that combinations of 2, 4-D and KIN, as well as, combinations of 2, 4-D and NAA, were reported as growth hormones for the indirect regeneration of medicinal plants. Hossain and his team used a combination of 2, 4-D and KIN for the micropropagation of Ashwagandha (*Withania somnifera*) (Hossain *et al*., 2019). Sen and his team used a combination of 2, 4-D and NAA for the micropropagation of Apang (*Achyranthes aspera)* (Sen *et al*., 2014). In both cases, callus was formed in these combinations, and the best response was reported. Shoot formation occurred when they both used a combination of BAP and KIN in their medium.

The combination of BAP, IAA, and NAA was the most effective treatment for Basok *(Adhatoda vasica Nees)*. Callus initiation started after 14 days and gave the best response (100%) when Khan and his team used this combination. Shoot formation was observed after transferring the green and light green calli on medium supplemented with BAP alone (Khan *et al*., 2016).

A combination of BAP and IAA was used as a growth hormone for the micropropagation of chinigura *(Scoparia dulcis)* by Majumder and his colleagues. The maximum amount of green or light green compact callus was

reported in this combination. When it was subcultured in the same medium, the highest number of multiple shoot buds was developed (Majumder *et al*., 2016).

BAP alone was found to be the best for callus induction and multiple shoot formation of Sarpagandha (*Rauvolfia serpentina*). Khan and his team achieved the best results by utilizing BAP in the culture medium.Callus initiation occurred within 13–22 days of inoculation and multiple shoots were produced from the callus within 7 days in the same medium (Khan *et al*., 2018).

A detailed documentation of Growth hormone and their concentration in respect to different medicinal plants and is presented:

Medicinal plants	Calli/ Shoot	PGRs combinations	PGRs concentration	Number of shoots/explants	Reference
Scoparia dulcis	callus	BAP+ NAA	(1.5+0.5) mgl^{-1}	Green compact	Hassan *et al*., 2008
	Shoot	BAP+ NAA	(0.5 + 0.1)mgl^{-1}	29.2 ± 1.74	
Paederia foetida	callus	BAP+ NAA	(1.5+0.5) mgl^{-1}	Yellowish green compact	Hassan *et al*., 2012
	Shoot	BAP	0.5 mgl^{-1}	14.4±1.29	
Paederia foetida	callus	BAP+ NAA	(1.5+0.5) mgl^{-1}	Yellowish green compact	Ghosh *et al*., 2019
	Shoot	BAP	0.5 mgl^{-1}	14.4±1.29	
Stevia rebaudiana	callus	BAP+ NAA	(1.0+1.0) mgl^{-1}	Brownish to Light green	Karim *et al.,* 2008
	Shoot	BAP+ NAA	(1.0+1.0) mgl^{-1}	12.00	
Abrus precatorius	callus	BAP+NAA	(5.0+0.5) mgl^{-1}	Light yellowish green ,nodular callus	Biswas *et al*.,2007
	Shoot	BAP+KIN+ NAA	(3.0+0.5+0.5) mgl^{-1}	6.87 ± 0.26	
Withania somnifera	callus	BAP+ KIN	(1.0+2.0) mgl^{-1}	Light green to dark green	Siddique *et al*., 2004
	Shoot	BAP+ KIN	(1.0+2.5) mgl^{-1}	4.35±0.95	
Bacopa monnieri	callus	BAP+ KIN	(1.0+2.5) mgl^{-1}	Light green	Rahe *et al*., 2020
	Shoot	BAP+ KIN	(1.0+2.5) mgl^{-1}	12.6 ± 0.21	
Cassia obtusifolia	callus	2,4-D	2.0 mgl^{-1}	Creamish friable.	Hasan *et al.,* 2008
	Shoot	2,4-D +KIN	(2.0+0.2) mgl^{-1}	20.0±0.0	
Cassia alata	callus	2,4-D	1.5 mgl^{-1}	Greenish friable	Hasan *et al.,* 2008
	Shoot	2,4-D+kIN	(1.5+0.5) mgl^{-1}	6.0 ±1.0	

Withania somnifera	callus	2,4-D +KIN	(2.0+0.2) mgl^{-1}	Greenish friable	Hossain *et al*., 2019
	Shoot	BAP+ KIN	(2.0+0.2) mgl^{-1}	6.00±5.20	
Achyranthes aspera	callus	2,4-D+NAA	(2.0+0.5) mgl^{-1}	Luxuriant greenish+ Friable	Sen *et al*., 2014
	Shoot	BAP+ KIN	(4.0+0.5) mgl^{-1}	4.83±0.17	
Adhatoda vasica nees	callus	IAA + NAA+ BAP	(0.05+0.05+1.0) mgl^{-1}	Green	Khan *et al*., 2016
	Shoot	BAP	10.0 mgl^{-1}	10.6 ± 1.82	
Scoparia dulcis	callus	BAP+IAA	(1.5+0.5) mgl^{-1}	Green compact	Majumder *et al*., 2011
	Shoot	BAP+IAA	(1.5+0.5) mgl^{-1}	28.1 ± 0.30	
Rauvolfia serpentina	callus	BAP	2.0 mgl^{-1}	Green	Khan *et al*., 2018
	Shoot	BAP	2.0 mgl^{-1}	12.5	

Table 5: List of growth hormone and their concentration in respect to different medicinal plants for indirect shoot regeneration

However, it is seen that a combination of cytokinin and auxin is used as a growth hormone for callus and shoot formation of various medicinal plants. Sometimes callus and shoot formations occur in the same growth hormone-containing medium, and sometimes have to add or remove growth hormones from the medium. It is reported that the combination of BAP and NAA is the most commonly used combination for various medicinal plants. A combination of BAP and KIN or BAP, IAA, and NAA or BAP is also used for medicinal plants. Besides, combinations of 2, 4-D and KIN or 2, 4-D and NAA have also been reported to be used for micropropagation of medicinal plants.

3.4 Rooting of the shoots

In shooting medium, adventitious and axillary shoots produced in the presence of cytokinin are usually lacking of roots. The shoots must be placed to a rooting medium that is different from the shoot multiplication medium in order to produce entire plants. Auxins are mostly used in root induction, and their effects vary depending on the type and concentration used in various medicinal plant species.

Rooting of shoots is the best achieved using different concentrations of auxins during regeneration. Approximately, 70 to 80 articles on the micropropagation of different medicinal plants are available in Bangladesh until 2021.It is observed that, IBA and NAA are the most commonly used auxins for rooting of various medicinal plants that is reported in Bangladesh.

IBA was found to be an ideal treatment for root induction in medicinal plants. Hossain and his team used different concentrations of IBA and NAA for root induction of Ashwagandha **(***Withania somnifera).* It is observed that the highest percentage of root formation (85%) was observed in IBA and the lowest percentage of root induction (40%) was recorded in NAA. The average length of roots (3.5 cm) was also reported in the IBA (Hossain *et al*., 2019). Similar results was found in various medicinal plants, such as, *Aegle marmelos (*Das *et al.,* 2008), *Cassia alata(*Hasan *et al*.,2008)(Hasan *et al*.,2008), *Acorus calamus(*Ahmed *et al*.,2007), *Tridax procumbens(*Jesmin *et al*., 2013), *Eclipta alba(*Yesmin *et al*., 2015), *Gynura procumbens(*Banu *et al*., 2017)(Azad *et al*., 2017), *Paederia foetida(*Hassan *et al*., 2012), (Ghosh *et al*., 2019),(Alam *et al*., 2010), (Amin *et al*., 2003), *Aristolochia tagala(*Biswas *et al*.,2007), *Kaempferia galanga(*Rahman *et al*., 2005), *Curcuma amada Roxb(*Ferdous *et al*., 2012), *Boerhaavia diffusa(*Biswas *et al*.,2009), *Ficus benghalensis(*Rahman *et al*., 2004), *Vitex negundo(*Islam *et al.,* 2009), *Rauvolfia serpentina* (Khan *et al*., 2018), *Phyllanthus fraternus(*Hassan *et al*.,2011), *Stevia rebaudiana* (Karim *et al.,* 2008), *Abrus precatorius(*Biswas *et al*.,2007), *Achyranthes aspera(*Sen *et al*., 2013), *Mimosa pudica(* Hassan *et al*.,2010), *Adhatoda vasica nees(*Khalekuzzaman *et al*., 2008), (Azad *et al*., 1998),(Khan *et al*., 2016), *Aloe vera(*Razib *et al.,* 2016), *Bacopa monnieri(*Rahe *et al*., 2020).

For various medicinal plants, NAA was the most effective for root induction. Rahman and his team used NAA for root induction of wild eggplants (*Solanum surattense)* in their experiment. It is observed that root initiation occurs within 7

days. Within 21 days after culture, an efficient root system was observed and got the best result (100%). Similar results were found in various medicinal plants, such as *Physalis minima (*Afroza *et al*., 2009), *Feronia limonia (*Islam *et al.,* 2010), *Scoparia dulcis (*Rashid *et al*., 2009), *Cassia obtusifolia (*Hasan *et al.,* 2008), *Curcuma aromatica Salisb (*Sharmin *et al*., 2013), *Ricinus communis (*Alam *et al*., 2010), and *Ocimum sanctum (*Jamal *et al*., 2016).

A combination of IBA and NAA was found to be the most useful treatment for some medicinal plants. Hassan and his used this combination for the root initiation of Chini Gura (*Scoparia dulcis).* It is reported that in vitro-raised shoots were rooted on medium containing this combination of growth hormones (Hassan *et al*., 2008). Similar results were also reported in *Scoparia dulcis (*Hassan *et al*., 2009), *Ficus religiosa (*Hassan *et al*., 2009), *Achyranthes aspera (*Sen *et al*., 2013), and *Phlogacanthus thyrsiflorus (*Hassan *et al.,* 2011).

A combination of IBA and KIN was reported to be the best level of treatment for root induction and growth of Anontomool *(Hemidesmus indicus) (*Siddique *et al*., 2006).

IAA was found to be the most effective for rooting microshoots on its own. Binto and his colleagues used IAA as growth hormones for the micropropagation of Gondho-Vaduli (*Paederia foetida).* It is observed that, root initiation from the explants was recorded after 14 days and 21 days of culture (Binto *et al*., 2020). A similar result was reported for the micropropagation of Shondha Maloti (*Mirabilis jalapa*) *(*Rahman *et al*., 2021).

A combination of IBA and IAA was also reported to use as growth hormone for some medicinal plants, such as, *Clausena heptaphylla (*Majumder *et al*., 2016) and *Scoparia dulci* (Rashid *et al*., 2009*).*

A detailed report of Growth hormone and their concentration in respect to different medicinal plants is presented below:

PGRs	Medicinal plants	PGRs concentration	% of root regeneration	Number of roots/plant	Reference
IBA	*Withania somnifera*	2.0 mgl^{-1}	85%	5.00±3.50	Hossain *et al*., 2019
	Aegle marmelos	1.0mgl^{-1}	80.42%	4.0 ±1.0	Das *et al.,* 2008
	Cassia alata	1.0mgl^{-1}	83.3%	5.0 ± 1.0	Hasan *et al*.,2008
	Cassia alata	1.0mgl^{-1}	80%	7.0±1.0	Hasan *et al*.,2008
	Acorus calamus	1.0mgl^{-1}	40.51%	3.15±2.25	Ahmed *et al*.,2007
	Tridax procumbens	0.5mgl^{-1}	92%	0.24±12.0	Jesmin *et al*., 2013
	Eclipta alba	0.1mgl^{-1}	96%	8.70 ± 0.42	Yesmin *et al*., 2015
	Gynura procumbens	0.1mgl^{-1}	100%	15.7	Banu *et al*., 2017
	Gynura procumbens	4.0mgl^{-1}	86.33%	4.3 ± 0.3	Azad *et al*., 2017
	Paederia foetida	0.5mgl^{-1}	81.2%	13.6±0.24	Hassan *et al*., 2012
	Paederia foetida	0.5mgl^{-1}	81.2%	13.6±0.24	Ghosh *et al*., 2019
	Paederia foetida	0.3mgl^{-1}	85 %	4.20±0.36	Alam *et al*., 2010
	Paederia foetida	0.1mgl^{-1}	95%	3.50 ± 0.32	Amin *et al*., 2003
	Aristolochia tagala	0.5mgl^{-1}	80%	1.17 ± 0.12	Biswas *et al*.,2007
	Kaempferia galanga	0.2mgl^{-1}	100%	12.4±1.23	Rahman *et al*., 2005
	Curcuma amada	4.0mgl^{-1}	98%	12.7	Ferdous *et al*., 2012
	Boerhaavia diffusa	1.0mgl^{-1}	100%	10.48 ± 0.06	Biswas *et al*.,2009
	Ficus benghalensis	0.1mgl^{-1}	100%	6.84 ± 0.12	Rahman *et al*., 2004
	Vitex negundo	0.5mgl^{-1}	80%	15.0±0.82	Islam *et al.,* 2009
	Rauvolfia serpentina	0.2mgl^{-1}	80%	10	Khan *et al*., 2018
	Phyllanthus fraternus	0.5mgl^{-1}	82.4%	11.8±0.59	Hassan *et al*.,2011
	Stevia rebaudiana	0.1mgl^{-1}	95%	6.25	Karim *et al.,* 2008
	Abrus precatorius	1.0mgl^{-1}	70%	3.23 ± 0.27	Biswas *et al*.,2007
	Achyranthes aspera	3.0mgl^{-1}	82%	10.0± 9.82	Sen *et al*., 2014
	Mimosa pudica	0.5mgl^{-1}	85.2%	13.4±0.24	Hassan *et al*.,2010
	Adhatoda vasica nees	1.0mgl^{-1}	80%	4.0 ± 0.3	Khalekuzzaman *et al*., 2008
	Adhatoda vasica nees	0.2mgl^{-1}	100%	3.4± 0.31	Azad *et al*., 1998
	Adhatoda vasica nees	1.0mgl^{-1}	80%	4 ± 1.70	Khan *et al*., 2016

	Aloe vera	1.0mgl^{-1}	90%	12.60 ± 0.45	Razib *et al.,* 2016
	Bacopa monnieri	0.25mgl^{-1}	100%	14.0 ± 0.23	Rahe *et al*., 2020
NAA	*Solanum surattense*	0.5mgl^{-1}	100%	12.5 ± 1.2	Rahman *et al*., 2011
	Physalis minima	0.3mgl^{-1}	98%	37.50±3.98	Afroza *et al*., 2009
	Feronia limonia	0.5mgl^{-1}	80%	-	Islam *et al.,* 2010
	Scoparia dulcis	0.5mgl^{-1}	21.5%	21.5±0.76	Rashid *et al*., 2009
	Cassia obtusifolia	2.0mgl^{-1}	80%	6.0±0.4	Hasan *et al.,* 2008
	Curcuma aromatica Salisb	0.5mgl^{-1}	100%	9.40 ± 0.98	Sharmin *et al*.,2013
	Ricinus communis	1.0mgl^{-1}	87.5%	10.5±0.81	Alam *et al*.,2010
	Ocimum sanctum	0.1mgl^{-1}	100%	5.2 ± 0.03	Jamal *et al*., 2016
IBA+ NAA	*Scoparia dulcis*	(1.0+1.0) mgl^{-1}	85.2 %	13.4 ± 0.24	Hassan *et al*., 2008
	Scoparia dulcis	(0.5+0.5) mgl^{-1}	85.20%	16.4±0.82	Hassan *et al*., 2009
	Ficus religiosa	(2.0+1.0) mgl^{-1}	81.4%	5.8 ± 0.66	Hassan *et al*., 2009
	Achyranthes aspera	(2.0+0.5) mgl^{-1}	100%	17.0±0.75	Sen *et al*., 2013
	Phlogacanthus thyrsiflorus	(0.5+0.5) mgl^{-1}	82%	11.8 ± 0.59	Hassan *et al.,* 2011
IBA+ KIN	*Hemidesmus indicus*	(4.0+1.0) mgl^{-1}	80%	2.25±0.2	Siddique *et al*.,2006
IAA	*Paederia foetida*	0.5mgl^{-1}	-	4.33	Binto *et al*., 2020
	Mirabilis jalapa	0.5 mgl^{-1}	%	8.1	Rahman *et al*.,2021
IBA+ IAA	*Clausena heptaphylla*	(1.0+0.5) mgl^{-1}	91 %	13.01 ± 0.21	Majumder *et al*., 2016
	Scoparia dulcis	(1.0+0.5) mgl^{-1}	100%	14.00 ± 0.22	Majumder *et al*., 2011

Table 6: detailed reports of growth hormones and their concentrations for root formation with respect to different medicinal plants.

However, generally, auxins are used for root induction. It has been noticed that IBA is the most reported auxin that is used for root induction of various medicinal plants. In addition, NAA is reported to be used for several medicinal plants. A combination of IBA and NAA or IBA and IAA or IAA alone is also

used for root induction of medicinal plants. It is known that cytokinin is commonly responsible for a lack of root formation. But in exceptional cases, it is seen that a combination of Auxin and cytokinin is also used for root formation. A combination of IBA and KIN is reported to be used for root induction.

3.5 Acclimatization and Transfer of micro propagated plantlets to the soil

The method of transplanting and acclimating micropropagated plants to the soil environment is a very essential step for adaptation of any tissue culture regenerated plantlets and this is no exception for medicinal plants. Successful acclimatization reduces the percentage of dead or damaged plants, resulting in improved plant growth and establishment. Rooted plantlets are generally removed out from the medium and soaked in water to remove any remaining agar particles sticking to the root system. The plantlets are then placed in a pot containing sterilized soil and sand mixture (3:1).To provides high humidity around the plants, a transparent polythene bag is placed over the potted plantlets. After around two weeks, the polythene bags are removed for three to four hours each day to allow the plants to acclimate to natural humidity. After a month of being transferred, these plants were moved to larger pots and kept in a greenhouse.

Successful acclimatization , field transfer and maximum survival rate (90-100%) of the regenerated plantlets were reported for several medicinal plants, such as, *Solanum surattense* (Rahman *et al*., 2011), *Withania somnifera* (Siddique *et al*., 2004), *Physalis minima* (Afroza *et al*., 2009), *Feronia limonia* (Islam *et al.,* 2010), *Tridax procumbens* (Jesmin *et al*., 2013), *Eclipta alba* (Yesmin *et al*., 2015), *Gynura procumbens*(Banu *et al*., 2017),(Azad *et al*., 2017), *Paederia foetida*(Alam *et al*., 2010).

In addition, satisfactory acclimatization, field transfer and medium survival rate (70-85%) of the rooted plantlets were reported in various medicinal plants, such as, *Scoparia dulcis (*Majumder *et al*., 2011), *Cassia obtusifolia (*Hasan *et al.,* 2008), *Ocimum sanctum (*Jamal *et al*., 2016), *Abrus precatorius (*Biswas *et al*., 2007), *Aloe vera (*Razib *et al.,* 2016), *Phlogacanthus thyrsiflorus (*Hassan *et al.,* 2011), *Adhatoda vasica nees(*Khan *et al*., 2016),(Khalekuzzaman *et al*., 2008), *Mimosa pudica(*Hassan *et al*.,2010).

On the other hand, some authors reported just above 50% survival rate of the regenerated plantlets. For example, Rahman and his team noted well-rooted micropropagated plantlets of Bot (*Ficus benghalensis*) were acclimatized and successfully established in pots with a 65% survival rate under field conditions (Rahman *et al*., 2004) and Sen and his team reported similar survival rate for Apang (*Achyranthes aspera) (*Sen *et al*., 2014).

Chapter 4: Conclusion

Chapter -4

Conclusion

Medicinal plants have been used as a source of medicine in almost all cultures since time immemorial. Herbal medicines help the majority of people around the world, particularly in developing countries. Medicinal plants are also a wide source of pharmaceutically important compounds, as well as cosmetic and nutritional applications. Many plant species have yet to be identified, and their medical characteristics are also unknown; even traditional medicinal cures are being lost. More research and conservation of all plant species are required to maintain nature's natural medicines. For that, plant tissue culture or micropropagation could be used, which has huge potential for the production of high-quality plant-based medicines. Protocols have been developed for the clonal multiplication of medicinal plants. It also provides a successful and rapid technique that can be used for commercial propagation, ex-situ conservation, and large-scale production for sustainable use in the industry. The protocol would also provide a more homogeneous source of medicine. So, there is a greater need for research in these sectors to conserve and maximize the use of medicinal plants.

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