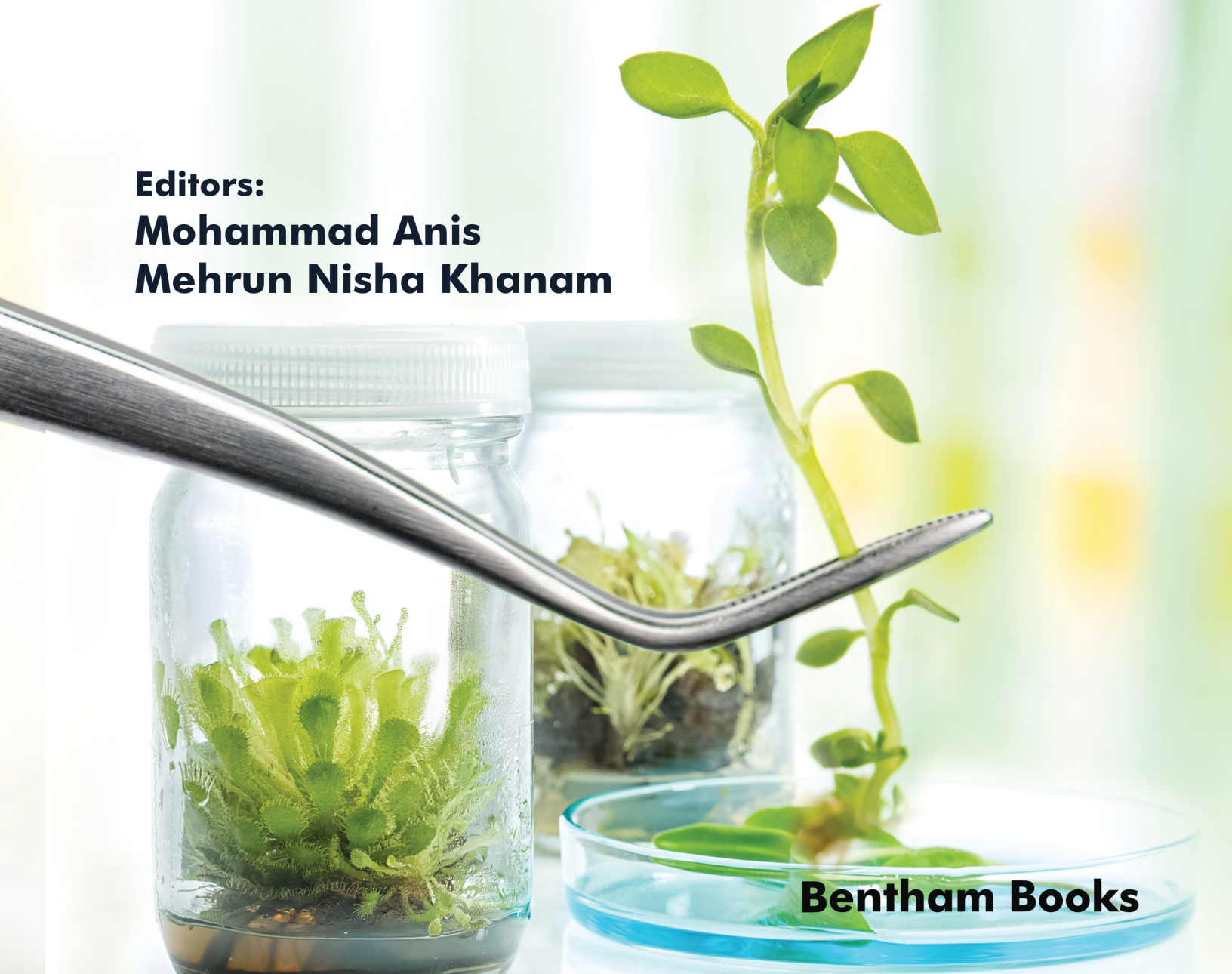
IN VITRO PROPAGATION AND SECONDARY METABOLITE PRODUCTION FROM MEDICINAL PLANTS:

CURRENT TRENDS (PART 2)

Editors:
Mohammad Anis
Mehrun Nisha Khanam



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(Part 2)

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PREFACE

The extinction of plant species is progressively taking place due to their being trapped in the vicious circle of ever-increasing industrialization, deforestation, global warming, climate change and also unscrupulous human activities. This has led to many species being listed in the Red Data Book or /in the various threat categories of IUCN. Of the total 3000 medicinal plants reported from India, over 1700 species of medicinal value are found in the Indian Himalayan region; nearly 47% are endemic to this region and about 62 species fall under different categories of threat. The situation warrants the acceleration of efforts to develop methods for germplasm preservation. The importance and applications of plant cell and tissue culture in plant science are vast and varied. The last few years of our research investigations have led to the emergence of this technique. Utilizing the biotechnological tools, many tissue culture protocols have been developed for rapid and mass multiplication of valuable medicinal plants to increase planting stock so as to meet the market demand. The rapid increase in knowledge of nutrition, medicine, agriculture, and plant biotechnologies has effectively changed the concept of food and health causing an overwhelming revolution.

India is known for its diverse climatic zones which are habituated of diverse flora having medicinal value, thus there is a wide scope for India to lead global herbal market. The National Medicinal Plant Board of India has recognized more than 7000 medicinal plants, which are currently used in different systems of medicines. The Ayurveda market in India has been valued at INR 300 billion in 2018 and is expected to reach INR 710 billion by 2024. Plants are active biochemical factories of a vast group of secondary metabolites which are indeed the basic source of various commercial pharmaceutical drugs. There are possibilities for year round production of biomass with reduced cost and time. Elicitation and precursor feeding are two important strategies of the *in-vitro* techniques to enhance metabolite production to meet the demands of mankind. Utilization of the existing genetics resources and understanding the biosynthesis, transport, accumulation and modulation of important secondary metabolites are critical issues linked to its improvement.

Overall, the rapid propagation of elite plants will provide high dividends to farmers and the associated herbal industry.

This book provides comprehensive coverage of the fundamental principles, current practices and trends in the field of pharmaceutical industry and provides baseline data for further research in the field. We are grateful to all the contributors and hope the book will be beneficial to students, researchers, scientists and other concerned stake holders who are working in the respective fields. MA acknowledges the much needed moral support of his wife, Humera Anis. We would also like to place on record our sincere thanks to Mr. Mohammad Zohaib Siddiqui for preparing the layout of the contents.

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CHAPTER 1

Bioactive Components in *Senna Alata* L. Roxb

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Abstract: *Senna alata* is an ethnomedicinal plant. The crude extracts of the plants are said to have a large number of medicinal properties due to their phytochemicals. In the present study, we made an attempt to isolate and screen the phytochemical constituents present in the species. In order to determine the bioactive constituents present in *S. alata,* and the effect of drying on the loss of bioactive constituents, studies on a set of pharmacognostical parameters were conducted on seeds, shade and sun-dried leaves of *S. alata* as per US pharmacopeia and WHO guidelines. The results of the present studies showed the presence of various important bioactive molecules that are responsible for the medicinal properties of the species. The phytochemical analysis of seed extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, resins and glycosides in all the extracts, while coumarins, phenols, terpenoids, phlobatannins and quinines are completely absent in all the seed extracts. Preliminary phytochemical investigations from shade and sun-dried leaf extracts showed alkaloids, flavonoids, anthraquinones, saponins, glycosides and tannins in high amounts in all the extracts, resins and phenols are present in moderate amounts. Terpenoids and phlobatannins are present only in fresh leaf extracts. Studies were also conducted on the physicochemical and organoleptic properties of leaves of *S. alata* that help in the identification and standardization of the leaf extracts for manufacturing of plant-based drugs of *S. alata.*

Keywords: Bioactive components, Leaf extracts, Preliminary phytochemical screening, *Senna alata*, Sun-dried, Shade dried.

INTRODUCTION

Ever since the origin of the human race, plants have been used as medicine because of their potent therapeutic value. Plants have been a source of therapeutic agents for thousands of years, and the majority of drugs or their derivatives used in the present day have been isolated from plants. Since ancient times, conven-

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-tional medical systems have been known to play a key role in the primary healthcare needs of the human race [1]. Almost all known civilizations around the world, including the Chinese, Indus Valley, African, and Egyptian, have their own ancient system of medicine that includes various types of naturally occurring compounds derived from medicinal plants.

Indian Vedic literature such as Rig Veda and Atharvana Veda (4500-1600 BC) also mentioned the use of several plants as a source of medicine. Ancient ayurvedic practitioners such as *Charaka*and *Susrutain,* and their respective books *Charaka Samhita* and *Susruta Samhita* referred to the use of more than 700 herbs as medicine and ancient medical systems such as *Ayurveda, Unani, Homeopathic and Siddha* have been surviving over 3000 years, by using plant-based drugs or their preparations and formulations for curing diseases.

World Health Organization [2] defined medicinal plants as follows: "medicinal plant" is any plant in which one or more of its organs possess compounds that can either be used for the therapeutic purposes or act as precursors for the synthesis of practicable drugs. The term "herbal drug" is used for the part/parts of a plant, *viz.* leaves, flowers, fruits, roots, bark, and seeds that are used for the preparation of therapeutic compounds. These definitions distinguish medicinal plants whose bioactive ingredients and therapeutic values have been demonstrated scientifically from plants that are considered medicinal but have not been established scientifically. WHO [3] further defines a medicinal formulation as any medicinal plant preparation obtained by subjecting the crude plant material to physical processes such as extraction, purification, fractionation, concentration, or biological processes that can be used for immediate consumption.

Many people from both developing and developed countries across the world do not have adequate access to basic needs, including food, water, clean environment, and medical and health services. The main concern of public health is still the intense need for basic health care, which is lacking even at the elementary level. According to WHO, more than half of the world's population do not have access to basic healthcare needs as poor people are unable to access the present healthcare services due to their non-affordability. Therefore, the challenge for governments in both developed and developing countries in the near future lies in food and medical security that should necessarily double the production of food and medicine in the next 50 years to meet the needs of the growing population. Medicinal plants not only offer access to medicine to poor people at an affordable price but also help in generating income, employment, and foreign exchange in developing countries, thus contributing significantly to the national economy. It is estimated that plant-derived drugs account for about Rs. 2,00,000 crores in the world market.

During the past century, the formulation and large-scale production of synthetic drugs have brought a revolutionary change in health care across the world. Nevertheless, more than 70-90% of people in both developing as well as developed countries rely on traditional practitioners and herbal medicine as a source of primary medicine [4], which attracted the attention of researchers towards medicinal plants globally. In modern pharmacopoeia, not less than 25% of drugs are derived from plants and many other drugs are synthetic analogues of standard compounds that are already isolated from medicinal plants. Even today, about 121 such active compounds are in use in the pharmaceutical industry [5] and more than 100 herbal-based drugs are under clinical study [6].

Even though modern medicines are effective, they have several disadvantages, including high cost, reducing immunity, causing severe side effects, and physical dependence. On the other hand, plant-based medicines are natural, cost effective, and have minimum or no side effects, that is, leading to an increase in the number of people turning towards herbal medicine, thus being used in achieving the goal of "Health for all" in a cost-effective manner [7]. This interest in phytomedicine can lead to the exploration of about 500 different plant species in the last few decades, and many species are still being studied.

Escalating faith in herbal medicine is one of the several reasons for the increasing need for recognition of medicinal plants [8]. Medicinal plants play a vital role in various traditional, complementary, and alternate systems of medicine as they contain a broad range of secondary metabolites, such as alkaloids, flavonoids, tannins and terpenoids [9, 10], which are found to play a key role in the regulation of diseases in human beings. The presence of these phytochemicals is responsible for the antioxidant, antimicrobial, and antipyretic effects of these medicinal plants [11]. WHO states that medicinal plants are the best source for obtaining a variety of herbal formulations. Hence, plants with such medicinal properties should be studied for a better understanding of their therapeutic properties, efficacy, and safety issues [12].

Plant metabolites can be divided into two groups as primary metabolites that are directly involved in the growth and metabolism of the plant and secondary metabolites are organic compounds which are the byproducts of primary metabolism that are not generally used by plants for metabolic activities. These secondary metabolites serve as interspecific defenses when the plant interacts with their counter biotic and abiotic partners in the environment [13]. These secondary metabolites are structurally and functionally diverse in nature and can be classified as alkaloids, flavonoids, glycopeptides, phenolics, peptides, steroids, terpenoids, and volatile oils [14]. These secondary metabolites act as precursor

CHAPTER 2

Plant Tissue Culture: A Potential Tool for the Production of Secondary Metabolites

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Abstract: Plants are an immense source of phytochemicals with therapeutic effects and are widely used as life-saving drugs, and other products of varied applications. Plant tissue culture is a unique technique employed under aseptic conditions from different plant parts called explants (leaves, stems, roots, meristems, *etc*.) for *in vitro* regeneration and multiplication of plants and synthesis of secondary metabolites (SMs). Selection of elite germplasm, high-producing cell lines, strain enhancements, and optimization of media and plant growth regulators may lead to increased *in vitro* biosynthesis of SMs. Interventions in plant biotechnology, like the synthesis of natural and recombinant bioactive molecules of commercial importance, have attracted attention over the past few decades; and the rate of SMs biosynthesis has increased manifold than the supply of intact plants, leading to a quick acceleration in its production through novel plant cultures. Over the years, the production of SMs *in vitro* has been enhanced by standardising cultural conditions, selection of high-yielding varieties, application of transformation methods, precursor feeding, and various immobilization techniques; however, most often, SM production is the result of abiotic or biotic stresses, triggered by elicitor molecules like natural polysaccharides (pectin and chitosan) that are used to immobilize and cause permeabilization of plant cells. *In vitro* synthesis of SMs is especially promising in plant species with poor root systems, difficulty in harvesting, unavailability of elite quality planting material, poor seed set and germination, and difficult to propagate species. Thus, the present article reviews various biotechnological interventions to enhance commercially precious SMs production *in vitro*.

Keywords: Biotecnology, Callus secondary metabolites, Phytomolecules, Plant tissue culture, Suspension cultures.

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INTRODUCTION

Plants are renewable sources and form an important part of our daily diet, and provide essential primary metabolites (*e.g.*, carbohydrates, lipids and amino acids) [1] and phytochemicals (low molecular weight compounds-SMs) for different industrial applications like pharmaceuticals, nutraceutical, textile, construction and cosmetic sectors [2]. The majority of the world population's health and wellness relies on plant-derived components. Therefore, plants with medicinal properties are considered important to support the transition to a bio-economy that is less dependent on fossil resources. The SMs not only play a pivotal role in plants' adaptation to their environment but also represent an important source of active pharmaceuticals [3] and are synthesised by plants to defend themselves against exogenous stresses, both biotic and abiotic. A study [4] proposed the concept of SMs that were known as opposed to primary ones and an entire volume of "plant biochemistry" series named as "endproduct" [5]. It is known that higher plants are a rich source of phyto-pharmaceuticals and are used in the pharmaceutical industry. Some of the plant-derived products include drugs like morphine, codeine, cocaine, pilocarpine, belladonna alkaloids, colchines, phytostigminine, L-DOPA, berberine, reserpine, capsaicin, podophyllotoxin, shikonin derivatives, ajmalicine, vincristine and vinblastine [6] and steroids like ginsenosides, anti-cancer (taxol), diosgenin, digoxin and digitoxin. Significant synthetic substitutes of these drugs with the same efficacy and pharmacological specificity are yet to be found [7].

Previously, chemical synthesis for the production of SMs was achieved through field cultivation; however, the plants originating from particular biotypes were difficult to grow outside their ecosystems and thus led scientists and biotechnologists to consider plant cell, tissue and organ cultures as an alternative to produce secondary metabolites. The major advantages of *in vitro* synthesis of bioactive secondary metabolites within controlled conditions include: these are climatic and soil stipulations independent, minimal inferences of negative biological parameters affecting the SM production, possible choice of elite germplasm with respect to the presence of SMs, computerization of cell growth control, metabolic processes regulation, and cost price, which can be decreased with increased production. Plants produce alkaloids, flavonoids, lactones, glycosides, quinines, phenylpropanoids, resins, tannins, terpenoids, saponins, sesquiterpene, and steroids [8]. The first large-scale production of commercial plant cells application was carried out in stirred tank reactors to synthesis shikonin by cell cultures of *Lithospermum erythrorhizon* [9, 10].

SECONDARY METABOLITES

Plants are capable of producing different organic molecules called secondary metabolites, having unique carbon skeletons with basic properties. SMs are not necessarily for a cell (organism) to live but also for interaction with its environment. These are organ, tissue and cell-specific with low molecular weights and often differ amid individuals from the same population with respect to their type. SMs protect plants against stress; and are used as drugs, flavors, fragrances, insecticides and dyes and hence are of great economic value. SMs have evolved as molecules imperative for organisms producing them, the majority of these interfere with the pharmacological targets, and thus make them significant for several biotechnological applications.

Primary *vs* Secondary Metabolites

Primary metabolites (PMs) are compounds that are universally present in all plants, but are not species-specific and, thus might be identical in some organisms. These are directly involved in metabolic activities like growth, development, nutrition and reproduction of a plant whereas secondary metabolites are produced in other metabolic pathways that, although important, but are not essential to the functioning of the plant. Whereas, SMs are species specific and, therefore, unique for each species. The major differences between PMs and SMs are listed in Table **1**.

Table 1. Comparison between primary & secondary metabolites in plants.

Basis for Comparison	Primary Metabolites (PMs)	Secondary Metabolites (SMs)
Function	These are directly involved in the metabolic pathways of an organism required for its growth, development, and reproduction.	These are not directly involved in the growth, development, or reproduction of the organism but are essential in ecological and other activities.
Synonym	Also known as central metabolites.	Also known as specialized metabolites.
Phase of growth	Are produced during the growth phase of the organism, called ‘trophophase’.	Are produced during the stationary phase of the organism, called ‘idiophase’.
Quantity of production	Synthesized in large quantities.	Synthesized in small quantities.
Process of extraction	These are easy to extract.	These are difficult to extract.
Specificity	They are not species-specific and thus may be identical in some organisms.	These are species-specific and thus are different in different organisms.

CHAPTER 3

In Vitro Propagation and Secondary Metabolite Production from *Withania Somnifera* (L.) Dunal

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Abstract: *Withania somnifera* (L.) Dunal, commonly known as ashwagandha or Indian ginseng, is an important medicinal plant that belongs to the family Solanaceae. Ashwagandha has been used from time immemorial in different systems of medicine and extensively used in the Indian system of medicine, and there is discussion of this plant in different ayurvedic scripts like Charaka samhita, Ashtanga sangraha, *etc.* The plant is extensively used for anti-aging and general well-being, and also has anti-cancer potential. Ashwagandha is also known for its antioxidant, anti-inflammatory, and other therapeutic activities. In the recent days of Covid-19, the plant has been extensively used as an immunostimulant. The plant has great potential for its raw materials, especially for the extraction of bioactive molecules like withanolide-A, withaferin-A, withasomniferin, withanone, *etc.* The conventional mode of propagation could not meet the required commercial demand for either the pharmaceutical industries or the traditional practitioners. The conventional method of obtaining biomass is influenced by a large number of environmental factors, where biomass quality and quantity of bioactive molecules have shown variation. To overcome this, biotechnological approaches such as plant tissue culture techniques have been established for large-scale cultivation using micropropagation and also other techniques like a callus and cell suspension culture, shoot culture, adventitious root culture, and hairy root culture have been extensively used for *in vitro* production of bioactive molecules from ashwagandha. With the advent of metabolic engineering, biosynthetic pathway editing has made it possible to obtain higher yields of desired metabolites. The present chapter focuses on the *in vitro* propagation, biosynthesis of withanolides, and tissue culture strategies for obtaining high biomass and metabolites. The chapter also focuses on different elicitation strategies, metabolic engineering approaches, and the development of elite germplasms for improved metabolite content. The chapter also identifies research lacunas that need to be addressed for the sustainable production of important bioactive molecules from ashwagandha.

Keywords: Ashwagandha, *Agrobacterium rhizogenes*, Callus culture, *Withania somnifera*, Withanolide-A, Withaferin-A.

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INTRODUCTION

Withania somnifera (L.) Dunal (Solanaceae) is one of the important medicinal plants which has been cited in different traditional systems of medicine. It is a woody shrub which is rich in diversified phytochemicals. It is commonly distributed and grown in various parts of Africa, tropical regions of Europe, and Asian countries, especially in India, where it is cultivated for its rich pharmacological potential [1]. It is commonly called ashwagandha in Sanskrit for its characteristic smell of horse ("ashwa" means horse and "gandha" means characteristic smell) [2]. Studies suggest that every part of the plant is rich in various kinds of metabolites (Fig. **1**). Phytochemicals like withanolide-A, stigmasterol, withanine, withananine, vitoindosides, sitoindosides, and ashwagandhanolide were reported from the roots of ashwagandha. In leaf, bioactive compounds like withaferin, withanone, withanolides-B, D, E, Z, 27-deoxywithaferin-A, 2, 24-dienolide, trienolide (steroidal lactones), and withanoside-IV and many varieties of phytochemicals were reported [3]. Some of the pharmacologically significant molecules from ashwagandha are shown in Fig. (**2**).

Ashwagandha is rich in secondary metabolites, which contribute to various pharmacological activities. Studies reveal that withaferin-A has great potential as an anti-cancer agent, and it induces apoptosis in human melanoma cells [4]. And also, a synergistic effect, along with X-ray irradiation, enhances apoptosis in U937 cell lines (human myeloid leukemia cells) [5]. It is observed that ashwagandha has great potential in combating arthritis [6]. In collagen induced arthritis, root extracts of ashwagandha showed efficient anti-oxidant properties and helped in the production of antibodies for arthritis [7], proving the anti-inflammatory activity as well [6]. The cardioprotective efficiency of ashwagandha was also explored. Reports suggest that isoproterenol induced myocardial infarction is efficiently suppressed by the hydro-alcoholic root extracts of Ashwagandha [8]. There are many reports which prove the antimicrobial activity of ashwagandha. Significant antibacterial [9 - 11] and antifungal properties have been demonstrated [12] from ashwagandha extracts. Initial studies on ashwagandha as a potential anti-covid 19 agent were in discussion. The multi pharmacological potential of ashwagandha showed promising results with respect to *in-silico* studies and showed good binding efficiency with target proteins [13, 14].

The market value for ashwagandha was rising and reached a million-dollar market due to the rise in demand for raw materials for the extraction of phytochemicals. To meet the increasing demand for the supply of raw materials, conventional methods employed for the production of ashwagandha will not be sufficient.

Moreover, the seed viability for the cultivation of ashwagandha is very poor and has less germination efficiency when stored for a prolonged time [15]. So, there is a need for alternate propagation methods. For industrial-scale production and the maintenance of regenerated plants, the *in vitro* approach of propagation is the most useful. Several biotechnological techniques, including plant cell culture, hairy root culture, multiple shoot culture, different elicitation strategies, metabolic engineering approaches, and the creation of transgenic variants, can be used to produce plant secondary metabolites.

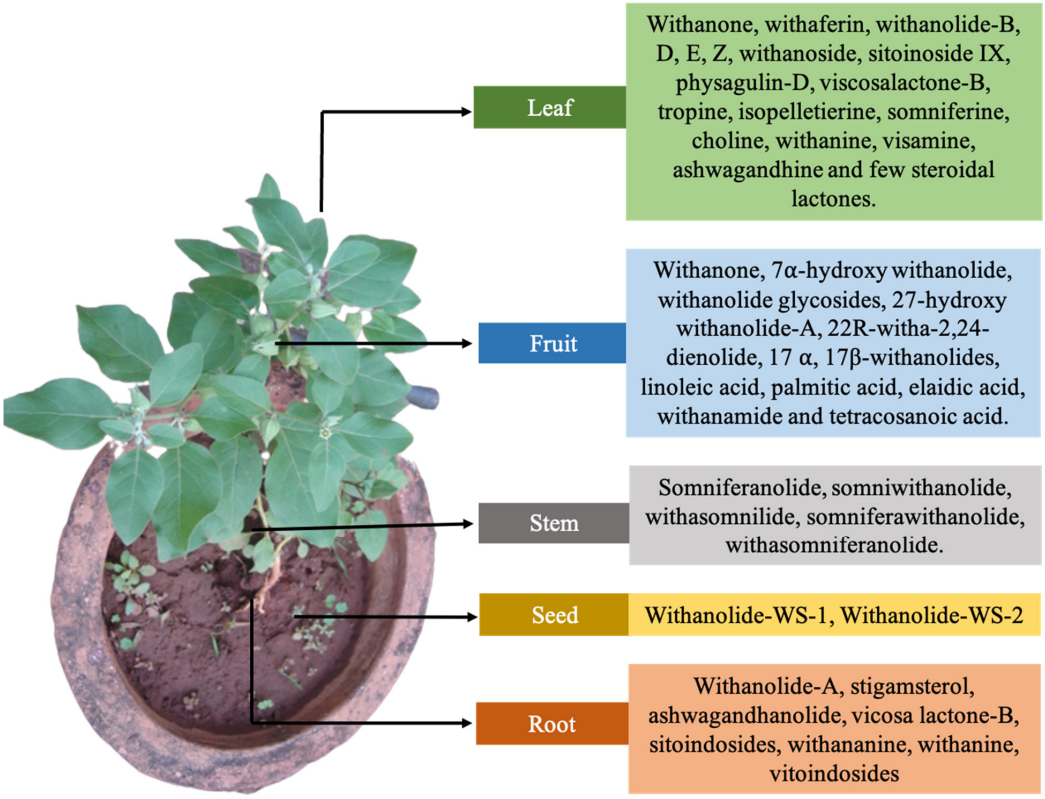

Fig. (1). Phytochemical profile of various parts of *Withania somnifera* (L.) Dunal.

In view of these, the present review focuses on various *in vitro* techniques used for micropropagation, secondary metabolite production, optimization of cultural conditions for withanolides, and withaferin production, and their elicitation strategies using various biotic and abiotic factors. The biosynthesis and metabolic engineering studies related to bioactive molecules production from ashwagandha are also emphasized in this study.

CHAPTER 4

In Vitro Propagation and Phytochemical Screening of Some Important Medicinal Plants of Northern India-A Review

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Abstract: Plants are indispensable for the preservation of human life. They supply us with oxygen, food, fuel, and shelter while also holding a crucial role in disease treatment, such as cancer, diabetes, and tumors. Medicinal plants are harnessed across various cultures and nations as medicinal precursors. In today's era, biotechnological methods like tissue culture are vital for selecting, multiplying, and conserving medicinal plant genotypes. Regeneration under *in vitro* conditions notably enhances the production of high-quality plant-based medicines. Plant tissue culture techniques offer a unified approach for producing standardized phytopharmaceuticals, yielding consistent plant material for physiological characterization and active phytoconstituent assessment. While many medicinal plants are successfully regenerated under *in vitro* conditions, there are certain species that continue to be cultivated in soil, with their large-scale development through micropropagation remaining uncommon. The micropropagation technique employed for cloning these medicinal plants involves the utilization of various concentrations of plant growth regulators within a media variant (MS 1962). The process of plant regeneration is achieved through both organogenesis and embryogenesis, facilitated by the supplementation of auxins and cytokinins. In this context, this chapter provides a concise overview of the integrated micropropagation culture system designed for the effective propagation of medicinally significant specimens.

Keywords: Diseases, Microoperation, Medicinal plants, Murashige and skoog media.

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INTRODUCTION

Plants play an indispensable role in supporting life on Earth. They have historically and will continue to provide essential resources such as daily sustenance, animal feed, fuel, shelter, recreation, and medicinal herbs for a significant portion of the global human population. The significance of medicinal plants lies in their biologically active compounds, which serve as the true agents of healing in medical processes. Throughout history, medicinal plants have been utilized for addressing various diseases, a practice evident in traditional usage recorded in both Vedic and post-Vedic texts. However, over the past two centuries, there has been a notable decline in the utilization of plant-based therapeutic systems, owing to the increasing emphasis on synthetic drug production. The advancement of modern medicine, marked by the discovery of antibiotics and corticosteroids, led to a decline in the utilization of plant-based remedies. Nonetheless, the adverse effects associated with their usage within the body have sparked a renewed attraction towards herbal medicine [1]. The World Health Organization (WHO) reports a growing global interest in traditional medicine, fostering a widespread availability of plant-derived drugs in health food stores across the globe, including affluent nations [2]. Unfortunately, this trend has triggered extensive harvesting of diverse wild plants, giving rise to significant challenges such as resource depletion and the endangerment of rare and vulnerable species. The resurgence of public interest in phytomedicines has led to heightened demand, coupled with the rapid expansion of pharmaceutical enterprises. However, this has resulted in rushed, unsustainable harvesting of medicinal plants for commercial purposes [3, 4]. In the Indian industry, over 95% of medicinal herbs are sourced from the wild. Various factors contribute to the extinction of these medicinal plants, including habitat destruction due to increased human activities such as settlements, agriculture, and development projects. Other factors encompass the introduction of non-native weeds, ecosystem stress from pollution and poisoning, global warming, greenhouse effects, and inappropriate chemical and pesticide usage. The illicit trade of rare and unique plant species, coupled with the degradation of forest regeneration capacity, has accelerated species extinction. Consequently, it is crucial to take action by initiating an effort to conserve existing germplasm before it becomes irretrievable. The objective of this chapter is to provide a concise overview of significant medicinal plants in North India, complemented by an outline of their phytochemical alterations Table **1**.

Table 1. List of some important medicinal plants occurring in Northern India.

S. No.	Botanical Name and Family	Common Name	Explants	Life from	Ailments Treated	*Ex-vitro* Cultivation
01.	*Acorus calamus*	Vai	Rhizome	Herb	Diarrhoea, stomach pain, cough and swellings.	Multiple shoot regeneration (Mass propagation)
02.	*Aconitum heterophyllum* Wall. ex Royle	Patris	Roots	Herb	Roots are dried and grinded, boiled in water, then taken orally or used to cook rice which is eaten to cure joint problems. Treatment of skin diseases and healing of wounds.	Callus culture
03	*Gentiana kurroo* Royle	Neelikant	Apical Meristem	Herb	Roots and rhizomes are bitter tonic. Antiperiodic.	Rapid Micropropagation
04.	*Ajuga bracteosa* Wall Ex Benth.	Jain-a-adam	Leaves	Herb	Diuretic, diarrhoea and treatment of wounds.	Callus induction and multiple shoot induction
05.	*Meconopsisaculeta* Royle L.	GuleNeelam	Seeds	Herb	N/A.	*In vitro* plantlet regeneration
06.	*Artemisia absinthium* L.	Teethwan	Leaves	Herb	Anthelmintic, abdominal pain, fever and indigestion.	*In vitro* Mass Multiplication\ Regeneration
07.	*Arnebia benthamii* Wall. ex G.Don	Kahzaban	Shoot tips	Herb	Enhances lactation in women, cough and throat infection, root extract is mixed with oil to control hair fall.	Clonal multiplication (Mass propagation)
08.	*Artemisia amygdalina* Decne	Virteethwan	Whole plant	Herb	Abdominal pain, anthelmintic, and high fever.	Suspension culture

CHAPTER 5

Phytochemistry, Antioxidants, Antimicrobial Activities and Edible Coating Application of *Aloe Vera*

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Abstract: *Aloe vera* (L.) Burm. f. is a medicinal plant that has gained widespread interest due to the distinctive biological activities associated with its biologically active phytocomponents. To combat the difficulties caused by microbe resistance, it is urgently necessary to investigate potent antimicrobials as a natural alternative to synthetic chemicals. This challenging task is attracting a lot of interest from the scientific community worldwide. The previous antimicrobial results of *A. vera* indicated its broad spectrum to treat a variety of infectious diseases, which will support the development of new herbal antimicrobial agents and avoid the side effects of conventional antibiotics as well as preserve the fruit quality and extend the shelf-life of various vegetables and fruits To take advantage of the prospective uses of this plant, the current review offers insight into the phytochemical composition, and its production-limiting factors, antimicrobial and antioxidant properties, as well as the promising use of *A. vera* in postharvest fruit-coating.

Keywords: *Aloe vera*, Antimicrobial, Anthraquinone, Antioxidant, Fruit coating, Polysaccharide.

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INTRODUCTION

Aloe vera (L.) Burm. f. (also known as *Aloe barbadensis* Miller) is a medicinal plant belonging to the family Xanthorrhoeaceae that is assumed to have its origins in the arid regions of Southern Europe, Africa and Asia [1]. *A. vera* has acquired popularity due to its beneficial phytochemicals, which have potent therapeutic medicinal properties. It is frequently used in traditional medicine to treat wounds, minor burns, and skin irritations, as well as internally to treat numerous ailments, including constipation, coughs, ulcers and diabetes.

The name *A. vera* is derived from "Alloeh" (an Arabic word that means "shining bitter substances") and "vera" (Latin word for "true"). Moreover, because of its medical properties, the plant has earned the names "survival plant", "medicine plant" and "lily of the desert" [2]. *A. vera* (Fig. **1a**) is a flowering succulent Crassulacean acid metabolism (CAM) xerophyte, that develops water-storage tissue in the leaves inside the parenchymatous tissues to enable the plants to survive in dry environments. The plant has a bright yellow tubular flower. It is a perennial plant with whorled-shaped, green fleshy leaves along the stem.

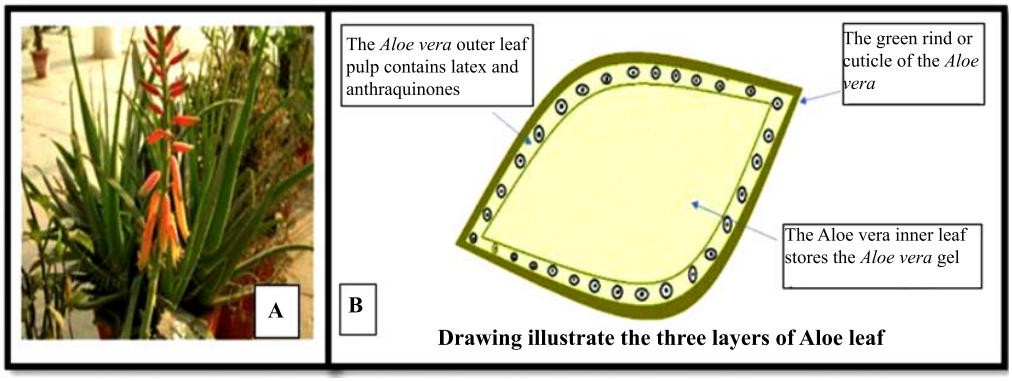

Fig. (1). (**A**) *Aloe vera* plant and (**B**) diagram of leaf layers. Source: This figure was reconstructed based on Boudreau and Beland [9].

The elongated and pointed leaves of the plant, which resembles a cactus and has common green leaves, have a thick outer rind and an inner full of pulpy substance [3]. It can be cultivated in saline soils as well as those irrigated with saline water [4 - 6] due to its salt tolerance capacity that is ascribed to its high K/Na ratio. Additionally, the plant can survive under severe as well as prolonged drought due to its storage-succulent leaves and drought-avoidance mechanisms [7]. The green dagger-shaped leaves of *A. vera* are the most used portion of the plant, and each leaf has three distinct layers (Fig. **1b**). Acemannan, the primary bioactive polysaccharide found in leaves, appears to be the greatest bioactive

polysaccharide. The mucilaginous layer or inner gel contains 98% of water and is responsible for the majority of the plant's pharmacological purposes. The middle layer is made up of latex, an anthraquinone- and glycoside-rich bitter yellow sap that has laxative properties [8]. Finally, the outer, thick green covering or rind, which is composed of 15–20 cells and serves as protection, is also photosynthetically active; thus, synthesizing carbohydrates and proteins.

A. vera phytochemicals have been shown to have a variety of biological properties, including antioxidant [10, 11], anti-inflammatory [12], antimalarial [13], antiviral [14], anti-fungal and antimycoplasmic activity. This plant is used in a variety of industries, including cosmetics, pharmaceuticals, healthcare and food processing, and is particularly effective at inhibiting bacteria resistance as a promising natural antimicrobial alternative agent. Similarly, the anti-oxidant properties of its gel are related to the activities of glutathione peroxidase, superoxide dismutase enzymes and phenolic derivatives [15]. Furthermore, the gel encourages cell formation, improves skin healing, increases water retaining capacity and provides a cooling effect; additionally, as a drink, it maintains mucous membranes [16]. Contrary to the high incidence of fresh fruit and vegetable food-borne diseases, awareness of the health benefits and high nutritional value of fresh fruits and vegetables expands their use, leading to selection of different approaches for the microbiological protection of fruits and vegetables at the highest levels. Edible coatings, modified atmosphere packaging and radiation preservatives are the most significant innovative technologies [17]. Coatings, such as *A. vera*, which recently has attracted much attention in reducing microorganism proliferation and early germ count in fresh products, are regarded as a pleasant environment that are economical and easy to apply after harvest [18]. It is interesting to note that *A. vera* gel (AVG), which is crucial to maintaining the consistency of all fruit's bioactive components, can be used as a natural substitute for synthetic fungicides. Proteins, soluble sugars, polysaccharides, minerals and vitamins make up the majority of the AVG components, though *Aloe* species have relatively low levels of lipids (0.07 - 0.42%) [19, 20]. Compared to a lipid-based coating, a polysaccharide-based coating has a low water vapor barrier. Thus, the hydrophobic characteristics of AVG can be improved by inserting a lipid source in the composite and subsequently improving the barrier performance of the coating [21]. Additionally, the strong, orderly configuration of the polysaccharides is an excellent mechanical and gas barrier. Despite various findings on the constituents of polysaccharides in Aloe pulp, it is generally agreed that acetylated glucomannan molecules are essential for the dense mucilage-like properties of crude aloe gel. When applied to fruits and vegetables *A. vera* forms a semi-permeable coating which creates a barrier around the treated fruits and can alter the environment. This barrier restricts exchange of gas and water vapor, which slows down the metabolic processes and delays fruit ripening [22].

CHAPTER 6

Micropropagation and Phytochemical Studies on *Oroxylum Indicum* (L) Kurz – A Review

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Abstract: *Oroxylum indicum* (L) Kurz is a medicinal forest tree with therapeutically active principles owing to its anticancer, anti-inflammatory, antimicrobial, antiulcer, anti-arthiritic, and anti-angiogenic properties and known to be employed in ayurveda, Unani and folk medicine. Due to the possession of biologically active constituents, the tree is uprooted for the isolation of phytoconstituents and preparation of drugs from different parts of a tree and is over-exploited by pharmaceutical industries. Hence the tree is becoming an endangered species. In view of the above, this medicinally important tree species needs conservation and also thorough study on its medicinal properties. *In vitro* culture methodologies have to be employed for large-scale production and to know the importance and the activity of various chemical components of this valuable medicinal tree, as this knowledge plays a vital role in the conservation and synthesis of active principles with specific activity to treat various ailments. The present review focuses on the published data on conservation and also phytochemical studies of *O. indicum* to highlight the traditional usage of this tree species in various health disorders and also to conserve the tree using various *in vitro* culture techniques for its large-scale production.

Keywords: *In vitro* culture studies, Micropropagation, *Oroxylum indicum*, Phytochemical constituents.

INTRODUCTION

Medicinal plants have been used by human beings from time immemorial to cure various ailments and the important bioactive compounds extracted from these were used in ayurvedic, siddha and Unani medicinal systems to treat various ailments. Natural products obtained from medicinal plants are the important ingredients of therapeutics used in traditional medicine as they are easily accessible in the healthcare system of rural people from underdeveloped to developing countries, and these are preferred over western medicine as they are inexpensive and without any negative impact. Most of the world countries depend

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on traditional medicine for healthcare needs, and hence many important medicinal plants are disappearing from natural ecosystems at an alarming state and becoming endangered species due to over-exploitation by pharmaceutical industries and have been enlisted in RED DATA BOOK, which need to be conserved not only for their sustainability in the nature but also for their vital therapeutic properties. Among them, *Oroxylum indicum* (L) Kurz is one of the endangered medicinal tree species with a wide range of medicinal values with an urgent need for its conservation [1].

Oroxylum indicum is an important medicinal forest tree known by names **Syonaka** or **Sonpatha** with therapeutic properties such as anticancerous [2], immunomodulatory, antinflammatory [3], analgesic, anti-tussive [4], antidiabetic [5], anti-helminthic, anti-leucodermatic, anti-rheumatic, anti-anorexic, antimicrobial [6, 7], antioxidant [8], and anti-angiogenic activities [9]. Its stem bark is used in the preparation of a **Khakhi** dye, **Agarbathi** and has got an enhancing effect on silk production of *Bombyx mori* and employed in tribal and folk medicines to alleviate a number of diseases [10], and is the main component of ***Chyavanaprasha*** and ***Dasamoolam*** [11]. In view of its wide range of therapeutically active properties, the total plant is uprooted and over-exploited from wild populations. Due to its large-scale utilization and indiscriminate collection by pharmaceutical industries, the species is depleted from natural populations and becoming endangered and has been pushed into the **red-listed plants** [12].

In order to multiply and conserve the species, *in vitro* culture technology can be employed for rapid clonal propagation. Conservation of rare and endangered medicinal plants can be achieved by applying *in vitro* multiplication [13] and has been used for the conservation of many medicinally important plants such as *Wrighitia tomentosa, Givotia rottleriformis,* and *Oroxylum indicum*, which are endangered through micropropagation technique [14 - 16]. Hence, with the aim of conserving the species and also to evaluate its medicinal properties, we have made an attempt to conserve and propagate the species of *O. indicum*. This review not only discusses efficient micropropagation methods using various explants but also focuses on screening methodologies for analyzing medicinal potentiality in various parts of *O. indicum*.

O. indicum is known as the ***broken bones tree*** or ***mid-night horror tree.*** The tree is deciduous with large bipinnate or tripinnately compound leaves with ovate or elliptical leaflets. The tree is a night bloomer adapted to chiropterophily with many large bell-shaped flowers with five stamens (Bignoniaceae), and produces fruits of 1.0 meter long flat curved capsule enclosing many flat, thin winged seeds (Fig. **1a-d**).

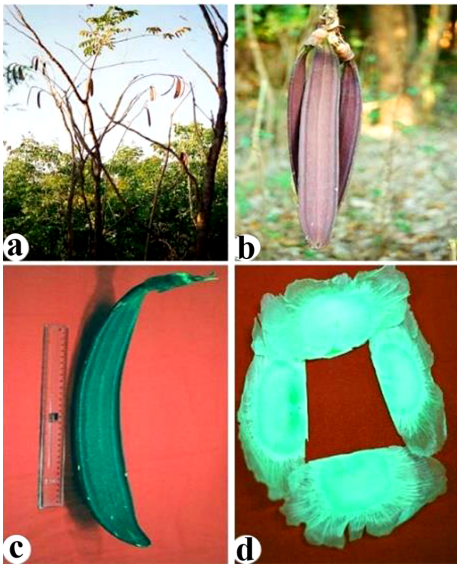

Fig. (1). Showing habit of *Oroxylum indicum*. **a**) Plants growing in Mallur forest, **b**) Bunch of Fruits, **c**) A single pod about 1.0 m long, **d**) Seeds with wings (Samatha *et al.* 2013).

In vitro culture of medicinal plants can be employed for the large-scale production of disease free plants and secondary metabolites for standard plant-based medicines irrespective of the season, which is found to be advantageous over conventional methods. The micropropagation methods increase the multiplication rate and scope for the production of *true-to-type* and pathogen-free plants, and have been reported in many medicinal plants [17].

In vitro micropropagation is advantageous over conventional methods as it paves the way for conservation of rare endangered and threatened plant species with desired characteristics, production of disease-free plants viable for recalcitrant seeds and production of secondary metabolites.

The natural propagation of the *O. indicum* takes place by seeds with 30% of *in-vivo* seed viability, which germinate in the rainy season. This plant has been placed on the list of endangered species of India due to its indiscriminate exploitation for its therapeutic value and problems related to its natural propagation. It has been reported that the estimated demand for the plant material of *O. indicum* in Southern India is 500 Kg/year [1]. Since the demand for *O. indicum* by the pharmaceutical industry is increasing, it is on the verge of extinction. The RAPD analysis of the species has been reported for its conservation and collection strategies; the genetic diversity in different accessions

CHAPTER 7

Exploring Plant Tissue Culture in *Ocimum basilicum* L.

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Abstract: *Ocimum basilicum* is a well-known, economically important therapeutic plant that belongs to the family Lamiaceae. Basil is marvelous in the environment as the complete plant has been used as a conventional remedy for domestic therapy against numerous illnesses since ancient times. *O. basilicum* exhibited interesting biological effects due to the presence of several bioactives such as eugenol, methyl eugenol, cineone and anthocyanins. *O. basilicum* possesses antimicrobial, anti-inflammatory, hepatoprotective, hypoglycemic, immunomodulator, antiulcerogenic, antioxidant, chemomodulatory and larvicidal activities. The oil of this plant has been found to be valuable for the cure of wasp stings, snakebites, mental fatigue, and cold. The demand of this multipurpose medicinal plant is growing day by day due to its economic importance, pharmacological properties and its numerous uses in cooking and folk medicine. Thus seeing the exciting biological activities of *O. basilicum*, micropropagation could be a fascinating substitute for the production of this medicinal plant because numerous plantlets can be achieved in fewer times with the assurance of genetic stability. An overview of the current study showed the use of the plant tissue culture technique for micropropagation, which is very beneficial for duplicating and moderating the species, which are problematic to regenerate by conventional methods and save them from extinction.

Keywords: Bioactives, Eugenol, Lamiaceae, Micropropagation, Medicinal, Pharmacological, Therapeutic.

INTRODUCTION

Ocimum species are outstanding commercially significant healing plants on the globe [1]. It belongs to the family Lamiaceae. The genus *Ocimum* L. comprises almost 150 species, having an abundant dissimilarity in plant morphology, essential oil and chemical composition [2]. The term basil is supposed to be originated from the Greek word “Basileus”, meaning “Royal or King”. It is

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frequently referred to as King of the Herbs [3]. *Ocimum basilicum* is universally recognized as "Sweet basil" and is used in both Ayurvedic and Unani systems of medication [4]. *Ocimum basilicum* in English is known as Basil or Sweet Basil [5], whereas in Hindi and Bengali [6], it is named Babui Tulsi. This plant is extensively developed as a decorative as well as a field crop all over Burma, India and numerous Mediterranean nations, including Turkey. It is a tropically dispersed genus with two-thirds of the 160 species reported from Africa and the remaining one-third from Asia and America. Around nine species are reported from India distributed in tropical and peninsular regions. In India, 300 hectares of basil is cultivated, and the total yield is approximately 300 tons of oil in the states of Uttar Pradesh, Haryana and Punjab [7]. This herbaceous plant is also extensively cultivated in France, U.K., U.S.A and Egypt [8].

Ocimum basilicum is a widespread herb rich in perfumed imperative oil and is cherished for its spicy, mildly peppery flavour with a trace of mint and clove. This plant is utilized as a cookery herb [9] and has been used as a food ingredient for flavouring baked foods and meat products [10]. The plant is diaphoretic, antihelminthic, carminative, antipyretic and stimulant [11]. *O. basilicum* is used to cure many ailments, such as migraines, cold, diabetes, tension, fevers, feminine spasms, cardiovascular infections, nerve torment, and abdominal pain reliever [12, 13]. The *O. basilicum* is utilized as a characteristic coagulant for the management of material wastewater [14] and as a biosorbent for copper and chromium uptake with its high biosorption limit of the seeds [15, 16].

O. basilicum majorly comprises a number of bioactives such as methyl eugenol, α-linalool, β-linalool, estragole 1, 8-cineole, linalool, estragole, Camphor, limonene and thymol. Methyl eugenol is the foremost compound of *O. basilicum*. It has been observed that 1,8-cineol (5.61%), methyl eugenol (18.74%) and Linalool (52.42%) are the chief phytoconstituents, whereas myrcene, neral and borneol are the minor compounds present at 5%, 8%, and 9% w/w respectively [17, 18]. *O. basilicum* possess several pharmacological properties such as anti-thrombotic [19], anti-hyperlipidemic, antiplatelet property [20], anticonvulsant [21], anti-aging, antiviral, anticancer and anti-microbial [22], immunomodulatory [23] and cytotoxicity effect [24], anti-inflammatory [25] and also antioxidant [26, 27].

The actual difficulty during the utilization of Lamiaceae species for pharmacological desires lies in the genomic and proteomic variability [28]. Propagating the Lamiaceae family through conventional modes is *via* seed, but poor seed viability and low rate of germinating seeds limit their proliferation to a huge extent [29]. Therefore *in vitro* micropropagation is the best alternative technique for the rapid multiplication of species to obtain a high offspring uniformity. *In vitro* micropropagation can guarantee a large-scale production of

several true-to-type plants in precise conditions in a small period of time without adverse effects on habitats [30].

The vanishing of this medicinal important plant in some areas is growing step by step; therefore, to generate attentiveness to the therapeutic significance of this plant to stop its disappearance is important. Moreover, speedy economic development and urbanization have led to overexploitation and damage of valued natural resources, together with numerous therapeutically imperative herbaceous plants [31]. Hence enthusiasm for utilizing *in vitro* culture procedures for quick and expansive scale proliferation of therapeutic plants has definitely improved. The miscellaneous species of genus *Ocimum* were exposed to *in vitro* studies by means of diverse explants, such as nodal segments [32] *via* leaf segments [33]. *In vitro* flowering has also been reported [34].

EVALUATION OF HEREDITARY STABILITY OF TISSUE CULTURE RAISED PLANTLETS *VIA* MOLECULAR MARKERS

Genetic uniformity is the preservation of the hereditary structure of a specific copy through its lifespan era [35] and is a vital pre-necessity in the multiplication of plant species, and is affirmed through molecular investigation [36]. *Ocimum* species exhibited hereditary along with biochemical differences because of interspecific hybridization. Therefore, it is essential to establish the best micropropagation procedure for the establishment of hereditarily identical plants before it is ready for profitable purposes. Moreover, there is a need to frequently check the clonal fidelity of micro propagated plantlets to confirm their true-to-t-e-type nature in order to avoid variations, which, if introduced, can proliferate very rapidly and lead to damage to the desirable characters of the parental genotypes. A lot of factors may possibly affect the firmness of the *in vitro* raised plantlets, such as genotype, time of culture period and nature of explants.

The biochemical stability of micropropagated plantlets has been established using various valuable tools such as GC profiling, molecular markers and flow cytometry [37]. To examine the hereditary uniformity and unsteadiness of *in vitro* culture-derived plantlet, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) have been commonly utilized because of easiness, rapid execution and requirement of a minute quantity of DNA devoid of little prior information regarding the genome [38]. The utilization of more than one marker has been more significant for the examination of the hereditary strength of plants, as they target various regions of the genome [39].

As assessed, fingerprinting sketches of *in vitro* cultured and donor plants of *O. basilicum* by utilizing ISSR and RAPD markers check the true-to-type nature of the plants [40]. It has been observed that all banding profiles generated during

CHAPTER 8

Plant Tissue Culture: A Perpetual Source for the Production of Therapeutic Compounds from Rhubarb

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Abstract: Plants are interesting natural resources that have had a close association with mankind since their existence. Their utility ranges from simple food, fodder, varied commercial and industrial products, and above all, as efficacious medical agents to cure various human health ailments. Amongst this vast reservoir of natural economical wealth, Rhubarb (*Rheum* Linn; Family: Polygonaceae), a perennial herb represented by about 60 extant species occurring across Asian (mostly restricted to China) and European countries, is one of the oldest and best-known medicinal plant species which finds extensive use in different traditional medical systems. Over the past several decades, and owing to the pharmacological efficacy of Rhubarb, the plant species has been subjected to different natural and anthropogenic pressures in the regions of its occurrence, rendering it threatened. In this context, the present chapter provides the basic account of Rhubarb while giving a gist of its therapeutic potential vis-à-vis major bio-active secondary chemical constituents. Additionally, the focus has been given to the *in vitro* production system of this wondrous drug for its sustainable conservation and meticulous utilization while highlighting various attributes of the technique of tissue culture such as somatic embryogenesis, cell suspension cultures, hairy roots, *etc.* , as projected potential approaches for desirable benefits from the genus *Rheum*.

Keywords: Conservation, Pharmacological efficacy, Phytochemicals, Polygonaceae, Rhubarb, Threatened, Tissue culture.

INTRODUCTION

RHUBARB: A General Account

Throughout the ages, nature has been the home for basic human needs. In particu-

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lar, man has been using plants as food and fodder besides benefitting from various services plants could afford. Owing to the close association with plants and the experiences gained thereby, humans have identified a rich treasury of medicinal wealth among the diverse flora inhabiting the earth alongside other creatures. This knowledge traversed through generations while getting enriched with every passing generation with the addition of new plants and improvement in the ways this medically rich repository could be utilized. Certainly, the same information ultimately gave rise to the widely practiced and majorly accepted (in developing and underdeveloped countries) traditional medical systems, which include Ayurveda, Chinese, Homeopathic, Naturopathy, Siddha, Tibetan, Unani systems, *etc*. The WHO has estimated that about 75% of Asians and Africans still believe and use this ethnomedicinal knowledge to cure varied ailments [1, 2]. They employ conventional measures in using single or conjugate cheap herbal extracts with higher safety and minor adverse effects. Importantly, previous decades have seen the use of plants and plant products as efficacious chemotherapeutic and/or chemopreventive agents for the effective treatment of various diseases. Pertinently, sumptuous investigations have shown that the medicinal properties of this flora are due to the presence of special metabolites literally known as secondary chemical constituents, that have a very wide diversity in their form and function. These natural products (NPs) have indeed provided a platform for renewed attention from both practical and scientific viewpoints for the evidence-based development of novel phototherapeutics and nutraceuticals.

Earth holds a vast reservoir of angiosperm taxa, among which Rhubarb is one of the important plant species with a complex history of use and trade over centuries and across borders. This perennial species has remained as a source of fascination vis-à-vis its role in cathartic therapy and as a tonic without considerable warnings in eighteenth- and nineteenth- century America and Europe. Indeed, in therapeutic history, and in all probability, there hasn't been a contemporary to the medicinal Rhubarb (*R. officinale*) owing to its efficacy and wide use among immense number of people [3]. Rhubarb, besides being an important object of botanical, horticultural and commercial interest, has seized considerable attention of both theoretical and clinical physicians by vigorously helping with the major medical requirements in the eighteenth century [4]. Nevertheless, there were certain misperceptions associated with the existence of true medicinal Rhubarb. Pertinently, it was clear in the second half of the nineteenth century that high valued Rhubarb roots were native to west China highlands, northern Tibet and southern Mongolia. Moreover, other extant species of Rhubarb with comparatively lesser medical efficacy are known to occur in countries like Bhutan, India, and Nepal, besides South East Europe and South West Asia [3, 4]. The perennial *Rheum,* commonly known as Rhubarb, finds wide use as a medicinal herb for ages in the traditional medical system of China. This

Polygonaceous member has limited most of its species to China vis-à-vis its distribution centers in north-western and western China. It is called "Chun-tza" and "Ta-huang" in traditional Tibetan and Chinese medical systems, respectively [5]. Indeed, owing to its wide distribution in China, the common names for them are readily found in Chinese and/or Tibetan languages; suffix 'huang' is used with different species of this wondrous drug. On the other hand, Rhubarb species also receive their names based on their utility (such as 'ornamental Rhubarb', *etc.*) or the country of their occurrence (such as 'Turkish Rhubarb', 'Chinese Rhubarb', 'Indian/Himalayan Rhubarb' or 'Russian Rhubarb').

Rhubarb as a genus includes familiar ethnomedically important plant species which are mostly confined to the mountainous regions of the Qinghai-Tibetan Plateau (QTP) and its adjacent areas [6]. The literature reports 60 extant congeneric species of this perennial herb, which exhibit wide distribution from temperate to alpine regions growing at an elevation of 500 to 5400 m asl. Owing to wide habitat tastes, the genus *Rheum* is found across Asian and some European countries with 19 species of them reportedly known to be endemic to China [7]. Moreover, earlier investigations have reported the existence of 10 species [8, 9] in the Indian subcontinent, which were later restricted to a mere 8 (*R. acuminatum* Hook. f. & Thomson, *R. australe* D. Don, and *R. webbianum* Royle., *R. globulosum* Gage, *R. moorcroftianum* Royle, *Rheum nobile* Hook. f. & Thomson, *R. spiciforme* Royle, and *R. tibeticum* Maxim. ex Hook. f.) [10]. Pertinently, we have reported *R. moorcroftianum* for the first time from Kashmir Himalaya [11]. The widespread diversity in habitat characteristics, which in turn has shaped and thereby resulted in varied morphological attributes of this dynamic vegetable and medicinal herb, has been employed by various investigators to classify Rhubarb into different sections. The pioneering work on such distinguishing traits of *Rheum* was done in 1936 [12], the authors divided this perennial herb into nine different sections based on the morphology, pollen exine structure and trnL-F region (of cpDNA). Nonetheless, around four decades later, this sectioning faced modifications by researchers [13] who firstly accredited only 5 sections from the above classification while adding two more sections *viz.* sect. Acuminata (based on the morphology of leaf) and sect. Globulosa (based on the inflorescence). Furthermore, aerobiology (study of pollen grains) came to the rescue, and, as of now, 8 sections are recognized, accepted and acknowledged within the genus *Rheum* that are based on six different types of pollen grains the species produce [14]. The pollen grain types include: Verrucate-rugulate (sect. Nobilia); verrucate-perforate (sect. Globulosa); rugulate (sect. Spiciformia); microechinate-perforate (sects: Rheum, Palmata, Deserticola and Spiciformia); microechinate-foveolate (sects; Rheum, Palmata, Acuminata, Deserticola, Orbicularia, Nobilia and Spiciformia); and finely-reticulate (sects; Rheum and Palmata). The miniature pollen grains present a great taxonomic significance in families like

CHAPTER 9

In Vitro Plant Regeneration from Nodal Segments and Biochemical Fidelity Analysis of *Operculina Turpethum*, a Threatened Medicinal Plant of Odisha

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Abstract: Trivrit [*Operculina turpethum* (L.) Silva Manso], belonging to the family Convolvulaceae, is a perennial, herbaceous and creeping vine. It is a medicinal plant which is widely used in traditional systems of Indian medicine. The roots, undamaged bark, stem and leaves possess immense medicinal properties and are used in the treatment of various ailments, including bronchitis, skin diseases, tuberculosis, cough, asthma, rheumatism, jaundice, ulcer, gastrointestinal disturbances, *etc.* The plant is enlisted as threatened species in different states of India, particularly in Odisha, due to indiscriminate destruction of forests, shrinkage of natural habitats, and unsustainable harvesting and collection for medicinal uses. Thus, there is an urgency for its protection and conservation. To scale up the production of *O. turpethum*, aiming at its conservation, micropropagation can be an alternative in order to circumvent the limitations of conventional propagation of the plant. Keeping this in view, an efficient protocol for plant regeneration of *O. turpethum* by axillary shoot proliferation from nodal segments was optimized. Multiple shoots were induced from mature nodal explants by axillary shoot proliferation on Murashige and Skoog's (1962) (MS) medium augmented with different types and concentrations of plant growth regulators. The highest number of shoots (13.3) proliferated on MS + 3.0 mg/L meta-Topolin. *In vitro* regenerated shoots were rooted on ½ MS medium containing 0.5 mg/L indole-3-butyric acid. *In vitro* regenerated plants with well-developed roots were successfully acclimatized in the small pots containing sterile garden soil and sand (1:1), followed by transfer to the large pot containing garden soil. Finally, plants were successfully

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[#] Kumari Monalisa and Shashikaanta Behera have equal contribution

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established in the field. The biochemical fidelity, in terms of secondary metabolites, was checked for tissue culture raised-field established plant vis-à-vis mother plant.

Keywords: Axillary shoot proliferation, Biochemical fidelity, Medicinal plant, Nodal explants.

INTRODUCTION

Operculina turpethum (L.) Silva Manso (Trivrit), belonging to the family Convolvulaceae, is a large, perennial, and herbaceous climber that exudates milky juice [1, 2]. It is commonly known as transparent wood rose as well as Indian jalap in English. This plant is regarded as an important medicinal herb in the Ayurvedic system of medicine. Trivrit has two varieties known as *Aruna or Shweta* [*O. turpethum* (L.) Silva Manso (syn. *Ipomoea turpethum)*] with whitish coloured root and Shyama (*Ipomoea petaloideschois)* has blackish coloured root [3]. *O. turpethum* root, stem, and leaf are rich in medicinal properties and are used for the treatment of various ailments [4]. Due to the presence of resins containing glycosides like turpethinic acid A, B, C, D, E, and scopoletin [5, 6], the roots of *O. turpethum* with its undamaged bark show thermogenic, purgative, antidiabetic, antipyretic, anti-inflammatory, antioxidant, anti-cancer, antimicrobial, antiproliferative, and stimulant properties [7, 8, 9]. The oil extracts from the root bark are used for the treatment of skin disorders and diseases (particularly of scaly nature) and also play a vital role in the treatment of cough and asthma [10, 11]. In addition to this, the root is also used as the chief ingredient for the treatment of ulcer and related gastrointestinal disturbances [12, 13]. Stem extracts of *O. turpethum* show antioxidant, antibacterial, hepato-protective, and anti-clastogenic effects due to the presence of a chemical EH4 (N-p coumarytyraminse) [14, 15]. Besides these, the alcoholic extracts derived from the fresh fruits of *O. turpethum* are reported to have antibacterial activities [16].

O. turpethum has been enlisted as a threatened (endangered or vulnerable) medicinal plant in different states of India, including Odisha, due to overexploitation, habitat destruction, and unsustainable trading of its stem and roots [17, 18]. The conventional methods for multiplication of *O. turpethum* are done through vegetative propagation and seeds which are time-consuming and require some pre-treatment like mechanical scarification that possesses restrictions and limitations [19]. Therefore, conventional methods of propagation are inadequate to meet the demand for raw materials. Because of above-mentioned problems, there is a need for special techniques (*e.g*., development of reliable *in vitro* plant regeneration protocol) for the conservation and protection of this threatened medicinal plant. Two earlier reports on plant regeneration of this plant using nodal segments and cotyledonary nodes as explants are not sufficient.

Therefore, the objective of this study was to develop an efficient, reliable *in vitro* plant regeneration protocol by axillary shoot proliferation of nodal explants and assessment of their biochemical fidelity in terms of phytochemical analysis.

MATERIALS AND METHODS

Collection and Surface Sterilization of the Explants

A healthy *O. turpethum* plant maintained at the Department of Botany, Ravenshaw University, Cuttack, Odisha, India, was used as the source of explant. Healthy, young, and tender shoots of the plant were collected from the vine. The nodal segments of 1.0-1.5 cm (excluding 3 nodes from the tip portion) were used for the plant regeneration experiment.

The nodal segments were washed under running tap water for about 25 mins, followed by treatment with 5% (v/v) aqueous solution of a liquid detergent, 'Teepol' for 20 mins (Reckitt Benckiser Ltd., HP, India). Then explants were rinsed with distilled water 4-5 times. Before inoculation, the nodal segments were surface sterilized under aseptic conditions inside a laminar airflow cabinet with 0.1% (w/v) aqueous solution of mercuric chloride ($HgCl_2$, Himedia, India) for 6 mins. After surface sterilization, nodal segments were rinsed thoroughly 5-6 times with sterile double distilled water.

Culture Medium and Culture Conditions

The surface sterilized mature nodal explants were inoculated in different culture media including Murashige and Skoog's [20] (MS) medium or MS medium supplemented with N^6-benzylaminopurine (BA; 0.5-5.0 mg/L), meta-Topolin (mT; 0.5-5.0 mg/L), Zeatin (Z; 0.5-5.0 mg/L), and Kinetin (KIN; 0.5-5.0 mg/L) for the regeneration of multiple shoots by axillary bud proliferation.

For root induction, the 3.0-4.0 cm long shoots were excised from the primary cultures and cultured individually in the culture tubes (Borosil, India) containing half- (½) or full-strength MS medium alone or ½ MS or MS medium supplemented with Indole-3-butyric acid (IBA; 0.5-2.0 mg/L).

All media were supplemented with 3% (w/v) sucrose and gelled with 0.7% (w/v) agar. The pH of the medium was adjusted to 5.8 ± 0.1 prior to autoclave at 121 ºC for 17 mins. All the cultures were maintained at 25 ± 1 ºC with a photoperiod of 16h/ 8h under illumination of 35-50 µmol m^{-2} s^{-1} photon flux density provided by cool white fluorescent tubes (Phillips, India).

CHAPTER 10

Tissue and Cell Culture of Tea (*Camellia sp.*)

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Abstract: Tea(*Camellia sp.*) is a non-alcoholic drink consumed across the globe. Upon consumption, it provides refreshment and enormous health benefits. Tea possesses antioxidant compounds which prevent human health from several diseases and disorders as well. Micropropagation and somatic embryogenesis are two distinct cell and tissue culture methods which have been utilized for a long time for the production of secondary metabolites having economical and industrial values. Micropropagation is a clonal propagation method accomplished by selection of explants and establishment of culture in basal media followed by shoot multiplication, development of callus, rhizogenesis, hardening and acclimatization by transferring plantlets from the laboratory to an open environment in the greenhouse or in the field. Somatic embryogenesis is the development of embryos from somatic cells, not from the zygotic cells. It consists of induction, multiplication, development and maturation of the embryo. Globular, heart and torpedo, these three distinguishable developmental stages are visible in somatic embryogenesis. Numerous genes associated with cell division, organ formation and specific cellular processes related to somatic embryogenesis have been identified. Tea possesses several secondary metabolites which have versatile functions. Caffeine, theobromine and theophylline are typical secondary metabolites which impart characteristic taste and flavour to tea. In addition, polyphenols, catechins, proanthocyanin and flavonoids act as antioxidant compounds and possess several health benefits. Various cell and tissue culture methods have been adopted for the biosynthesis of secondary metabolites on laboratory and industrial scales. These methods can be adopted on a larger scale, from experimental laboratory investigation to the industrial setup for the discovery of novel metabolic compounds for their potential applications as medicines and in commercial sectors.

Keywords: Antioxidants, Callus, Micropropagation, Somatic embryogenesis, Secondary metabolites, Tea.

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INTRODUCTION

Tea is a popular beverage consumed worldwide. It is prepared with leaves of *Camellia sinensis* (L.) O. Kuntze. Besides holding medicinal properties and being economically important, it is a living representation of ancient cultural practices that originated in China and gradually spread throughout the globe. Owing to its large demand, micropropagation serves as a better alternative to traditional propagation methods for commercial use. Micropropagation technique was developed in 1946 by Ball, who is regarded as the father of micropropagation [1 - 4]. It is also worthwhile to mention the contribution of a pioneer scientist in tissue culture [5] and the initiation of a systematic study on micropropagation of tea [6].

MICROPROPAGATION OF TEA

In regard to woody and perennial plants like tea, which exhibit recalcitrancy and have long gestation periods, micropropagation offers a rapid, cost-effective production [7, 8]. There have been several works on the micropropagation of tea in the past [9 - 11]. The main objective of micropropagation is to achieve plants with desirable characteristics, increased productivity and with developed resistance to drought, pest, salinity, acidity, alkalinity, frost and other factors that limit plant growth. It can be achieved by – (i) enhancing axillary bud breaking, (ii) production of adventitious buds directly or indirectly *via* the callus, and (iii) somatic embryogenesis directly or indirectly from explants [12 - 14].

In this book, we will classify the micropropagation of tea into four broad stages which are discussed below:

Stage I: Selection of explants and establishment of culture through explants.

Stage II: Shoot multiplication: Initiation and multiplication of callus.

Stage III: Rhizogenesis.

Stage IV: Hardening & Acclimatization: Transfer from *in vitro* to *ex vitro* condition.

Stage I: Selection of Explants and Establishment of Culture Through Explants

Explant material forms the basis of all micropropagation works. Factors like the availability of the explant material throughout the year, its origin, and its type are to be considered. Apart from this, sterilization of explants, as well as aseptic laboratory conditions, are necessary to establish *in vitro* propagation successfully. For *in vitro* culture of tea, different kinds of explants such as meristems, shoot tips

[15, 16], parts of stem *viz.* nodal segments [17], stem segments, epidermal layers of stem segments, stem segments without epidermal layer for shoot regeneration, zygotic embryos, mature and immature cotyledons for the induction of adventitious buds [18, 19] were used. Generally, shoot tips were used for tea micropropagation through either apical meristem culture or shoot apex culture. Shoot tip and axillary bud culture were reported as effective, easier, simpler and quicker in securing the growth of shoots [20]. While comparing the effect of both methods in C. *sinensis* var. *assamica* and C. *sinensis* var. *sinensis*, it was found that the shoot tip culture was more effective than axillary bud culture in the former variety, while axillary bud culture was more effective than the shoot tip culture in the latter variety.

Stage II: Initiation, Multiplication, and Elongation of Shoots

By the late 1980s of the twentieth century, enhancement of the rate of multiplication was well established in the micropropagation of tea. Several types of basal media were used, *viz.* Heller's medium, MS (half or full strength) [21], B5 medium [22], and WPM [23], in which MS was considered the most common and efficient basal media for shoot proliferation and multiplication, while half-strength MS was reported to be as effective as the full strength MS [24 - 27]. Basal media supplemented with PGRs, commonly 1 mg/l BAP and IBA, IAA, Kinetin, NAA, GA3, or 2,4-D, were used in varying composition and concentrations by several researchers. In a study, it was reported that shoot tips and nodal segments of tea, when cultured in a media composition of MS with BAP (3.0 mg/l) and IBA (0.05 mg/l), showed the highest shoot elongation [28]. They also showed that seeds without seed cover gave an early response to shooting formation compared to seeds containing seed coat. TDZ was reported to be a significant cytokinin like growth factor for micropropagation of tea with high proliferation rates [29]. Several researchers [30 - 33] have found that Picloram {2,4,5-trichlorophenoxyacetic acid (2,4,5-T)} can significantly aid in the shoot elongation of tea plants. A protocol for somatic embryogenesis, a micropropagation technique, was developed [34] in which efficient embryo induction from mature cotyledons was found in MS media with BAP (3 mg/l) and NAA (0.1 mg/l) while media with BAP (2 mg/l) and NAA (3 mg/l) induced callus of leaf explants effectively.

Growth adjuvants for tissue culture of tea include coconut milk [35, 36], yeast extract [37], casein acid hydrolysate, serine and glutamine as nitrogen sources [38] and sucrose as carbon sources [39]. Also, 3% (w/v) sucrose as a carbon source for micropropagation of tea shoots were used.

CHAPTER 11

In Vitro Strategies for Isolation and Elicitation of Psoralen, Daidzein and Genistein in Cotyledon Callus of *Cullen Corylifolium* (L.) Medik

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Abstract: In recent times, natural herbal products/biomolecules are gaining immense impetus, over modern synthetic allopathic medicines, for curing serious human ailments as the former are proving their better efficacy, causing no or minimum side effects. Consequently, many pharmaceutical industries are coming forward for exploring novel drugs based on medicinal plants. *Cullen corylifolium* (L.) Medik., a well-known traditional medicinal herb of China and India, is extensively used in Ayurvedic medicine to cure several skin diseases such as psoriasis, leprosy and leucoderma. Besides, it also has properties like antioxidant, anti-cancer, anti-inflammatory, hepatoprotective, anti-diabetic, anti-mycobacterial, and anti-helminthic due to the occurrence of a number of important furanocoumarins and isoflavonoids. Furanocoumarins and isoflavonoids are biosynthesized *via* the phenylpropanoid pathway in the plant parts of *C. corylifolium* and are extensively used as anticancerous agents. The prominent marker compounds occurring in *C. corylifolium* are psoralen, genistein and daidzein produced mainly in the green seeds. These are highly expensive and occur in very low amounts. *In vitro* cell, tissue and organ culture can be used as an alternative, controllable, sustainable and eco-friendly tool for rapid multiplication of cells for the synthesis and elicitation of bioactive compounds. In addition, various strategies such as precursors feeding, hairy root culture, biotic and abiotic elicitors, cell suspension cultures, cloning and overexpression of genes involved in biosynthetic pathways of secondary metabolites. are also available for the enhancement of bioactive secondary metabolites. The present review aims at the screening of high-yielding elite plant parts, biosynthetic pathways of psoralen, daidzein and genistein, and various strategies employed for their elicitation and isolation in *C. corylifolium*.

Keywords: Biosynthetic Pathway, *Cullen corylifolium*, Callus, Daidzein, Elicitation, Genistein, Green seed cotyledons, Isolation, Key enzyme genes, Psoralen.

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INTRODUCTION

Medicinal plants have been reckoned as potential sources of important biomolecules [1] that play a very crucial role in treating several serious ailments and saving numerous precious lives. In recent times, the natural compounds, due to their synergistic activity, less or no toxicity and more compatibility, are gaining much attention in the pharmaceutical industries for novel drug designing and several other therapeutic uses. Incidentally, such compounds occur in very low amounts in plant cells, and their enhancement involves a series of biosynthetic pathways, key enzyme genes and different abiotic and biotic stressors. With the onset of modern phytochemical and analytical tools, few past decades witnessed an immense progression in the discovery of natural products-based drugs, target diseases and their mechanism of action. Considering variable climatic conditions, the Indian subcontinent is a habitat for diverse flora that contributes immensely to herbal medicines at national and international markets. The ever-increasing demand for herbal products is encountered by collections from natural habitats, making them susceptible being threatened and even extinct in some cases. However, the plant cell, tissue and organ culture technique offers a sustainable approach for germplasm conservation as well as enhanced synthesis of bioactive compounds. It is thus imperative to develop a cost-effective and efficient in vitro protocol to cope up with the needs of the pharmaceutical and nutraceutical industries. Besides, in *vitro* elicitation of secondary metabolites facilitates the yield of higher amounts of pharmaceutically desirable compounds with limited resources without sacrificing the entire plants. Callus derived from various plant parts is considered the best source material for the incessant and rapid proliferation of cells for the elicitation of biomolecules [2]. Enhancement of biologically active secondary metabolites can be achieved following different methods, *e.g*., through precursor feeding, hairy root culture, using biotic and abiotic elicitors, cell suspension cultures, cloning and overexpression of genes involved in biosynthetic pathways of secondary metabolites. Biotic, abiotic and phytohormonal elicitors may induce the synthesis of bioactive compounds [3 - 5]. Though the mechanism of action of elicitors is not much explored, it is believed that elicitors interact with the receptors present on the plasma membrane, which activate the intracellular signal transduction system, including G proteins, calcium ions (Ca^{2+}) and other secondary messenger molecules with the mitogen-activated protein kinases (MAPKs) pathway for the biosynthesis of bioactive compounds [6, 7].

Cullen corylifolium (L.) Medik. (syn. *Psoralea corylifolia* L.) (2n = 22) is a traditional medicinal herb of China and India and extensively used in Ayurvedic medicine [8]. It belongs to the family Fabaceae which comprises almost 751 genera and 19,500 species [9]. The genus *Cullen* consists of 32 species. It is

known by various vernacular names such as purple fleabane or West Indian satinwood (English), Babchi or Bakuchi (Hindi), Kusthahantri or Sitavari (Sanskrit), Babechi or Bawachi (Urdu), *etc*. It is widely distributed in the North East tropical Africa, South West Arabian Peninsula, and tropical and subtropical Asia. In India, it is dispersed in Punjab, Rajasthan, Uttar Pradesh, Madhya Pradesh, Chhattisgarh and Tamil Nadu. *Cullen corylifolium* (2n = 22), is an annual herb growing up to the height of 30–180 cm. Fruit is one seeded, elongated and smooth. Mature seeds are glabrous, pitted, compressed, dark brown and non-endospermic. It blooms from July to August and seeds ripen from September to October. Interestingly, it is extensively utilized in Ayurvedic medicine to cure several skin ailments, such as leukoderma, psoriasis and leprosy [10 - 12]. Due to the presence of coumarin, furanocoumarin, flavononoid, isoflavonoid, flavone, meroterpene, chalcone and coumestan, this medicinally important plant possesses potent antioxidant, anti-cancer, anti-inflammatory, hepatoprotective, anti-diabetic, anti-mycobacterial, and anti-helminthic properties [13 - 17]. Furanocoumarins and isoflavonoids are synthesized in the different locations of *C. corylifolium* and are widely employed as anticancerous agents. Biosynthesis of anticancerous agents such as furanocoumarins and isoflavonoids occurs in different locations of *C. corylifolium*. The major bioactive marker compounds of *C. corylifolium,* such as psoralen, genistein and daidzein, are biosynthesized mainly in green seeds *via the* phenylpropanoid pathway [18].

BIOSYNTHETIC PATHWAYS OF PSORALEN, DAIDZEIN AND GENISTEIN

Psoralen, a furanocoumarin, is the major marker compound of *C. corylifolium* synthesized from umbelliferon *via* the phenylpropanoid pathway [19]. Phenylalanine acts as a precursor molecule to form cinnamate by the action of *phenylalanine ammonia lyase* (*PAL*), followed by the formation of p-coumaric acid, which is further converted into umbelliferone (7-hydroxycoumarin) through ortho-hydroxylation. The crucial step of biosynthesis is prenylation, which is catalyzed by *umbelliferone dimethylallyl transferase* and converts umbelliferone to demethylsuberin [20]. The *marmesin synthase* and *psoralen synthase* are two different cytochrome P450 (CYP450) enzymes that finally catalyse the conversion of demethylsuberosin to psoralen [21, 22]. A schematic representation of psoralen biosynthesis is shown in Fig. (**1**).

CHAPTER 12

Genetic Improvement of Pelargonium, an Important Aromatic Plant, through Biotechnological Approaches

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Abstract: Pelargonium is one of the most recognized aromatic herbs due to its wide distribution around several countries and its perfumery and aromatherapy properties. The present chapter aims at exploring the current scientific study on the various species of Pelargonium along with its significance. The essential oil of Pelargonium contains more than 120 monoterpenes and sesquiterpenes obtained from the steam distillation of herbaceous parts. Citronellol, geraniol, rhodinol, 6, 9 –guaidiene, and 10-epi-γ eudesmol are the principal components responsible for its oil quality. Traditionally, propagation of pelargonium is done through cuttings from its mother plant material. However, the tissue culture approach is one of the reliable techniques for propagation and conservation, not influenced by environmental conditions. More likely, tissue culture approaches used are somatic embryogenesis, callus culture, direct regeneration, meristem culture, and hairy root culture. Transcriptome analysis has also been carried out in *Pelargonium graveolens* to understand the metabolic pathway. In order to accomplish the maximum oil production and better geranium varieties through genetic engineering, *Agrobacterium* mediated transformation systems have been developed. These standardised genetic transformation procedures were used to over-express, silencing, and heterologous expression of desired genes in Pelargonium to understand the outcome and succeed with enhanced essential oil production with better quality for the ultimate benefit.

Keywords: Biosynthesis, Essential oil, Genetic transformation, Pelargonium, Tissue culture, Terpene.

INTRODUCTION

Pelargonium (Geraniaceae family) is one of the most recognized aromatic and medicinal herbs due to its wide distribution around several countries. Pelargonium

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has been renowned for its perfumery and aromatherapy properties. Besides being used in cosmetics, it is highly denuded to cure a number of diseases due to antibacterial, antifungal, antioxidant, anti-inflammatory and anticancer activities [1 - 5]. The aerial parts are used in folk medicine as a food and tea drinks additive and for relieving some gastrointestinal, topical, dental, and cardiovascular disorders and are effective in preventing haemorrhoids. This genus comprises 750 species, showing variation in floral morphology, chemical constituent and life forms [6, 7]. The essential oil of Pelargonium is obtained from steam distillation of herbaceous parts; they contain a mixture of more than 120 monoterpenes, sesquiterpenes and other low molecular weight aromatic compounds [8]. The most abundant monoterpenoids of geranium oil are citronellol, geraniol, caryophyllene oxide, menthone, linalool, β-bourbonene, iso-menthone, and geranyl formate (Fig. **1**) [9, 10]. However, the chemical composition of the oil is variable in different species, which could be due to differences in cultivars used, climate conditions, origin, time of harvest, fertilizers used, *etc.* Some of the Pelargonium species are rich in different aroma compounds mentioned in Table **1**. The commercial value of geranium oil depends on its quality, determined by the total rhodinol content and ratio of citronellol and geraniol [11]. Other two constituents of geranium oil, *viz.* 6, 9 –guaidiene and 10-epi-γ eudesmol, provide olfactory value, which are commercially utilized in the perfumery, cosmetic and aromatherapy industries worldwide. This wide area of importance of geranium essential oil enlarged the market demand (600 tons/per) at the estimated cost of $ 225/kg [12]. Propagation of Pelargonium was done by cuttings (10 to 15 cm in length) from top young shoots of mother plant material in sandy soil. Only a few species are capable of producing seeds, while most Pelargonium species do not produce or produce non-viable seeds and hence the occurrence of Pelargonium crops throughout the year is restricted [13 - 15]. However, alternative approaches for propagation and conservation are adapted by researchers through tissue culture, such as clonal propagation that involves somatic embryogenesis, callus culture, direct regeneration, and meristem culture. Most of the conventional breeding programmes cannot be applied for the genetic improvement of geranium owing to the vegetative mode of propagation; biotechnological approaches are more likely to be successful. *Agrobacterium* mediated transformation with relevant genes could provide avenues to make better geranium varieties for commercial cultivation [16].

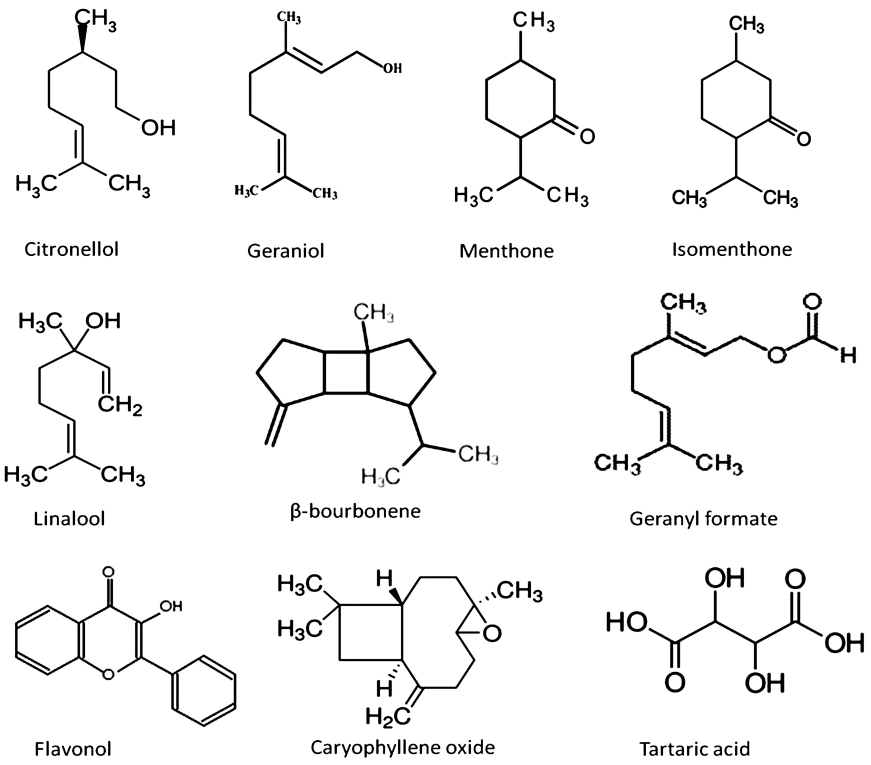

Fig. (1). Structure of some important compounds in *Pelargonium* species.

Table 1. Major oil constituents of some reported *Pelargonium* species.

***Pelargonium* Species**	**Major Constituent**	**References**
P. graveolens	Citronellol (27%), Geraniol (25%)	[51]
P.graveolens(South African)	Isomenthone (65.8-83.3%).	[7]
P. radens	Isomenthone (81.5%)	[7]
P. tomentosum	Isomenthone (61-62%), menthone (25-27%)	[7]
P. vitifolium	Citronellic acid (77-85%)	[62]
P. citronellum	Geranic acid (36%), Neral (17.4%) and Geranial (27.2%)	[7]
P. papilionaceum	Citronellic acid (96.2%)	[7]
P. grossulariodes	Isomenthone (13%), Citronellol (12%) geraniol (16%), methyl eugenol (11%)	[8]
P. capitatum	Citronellol (76.6%)	[7]

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Mohammad Anis

Prof. Mohammad Anis, is a Ph.D., has 38 years of teaching and research experience. He has served as dean, Faculty of Life Sciences and Chairman, Department of Botany at the Aligarh Muslim University, India. He has made significant scientific contributions in cytogenetics, morphogenesis and tissue culture. Prof. Anis has been the recipient of prestigious BSR Fellowship of the University Grants Commission, Government of India in 2018.

He visited the University of Georgia, Athens, USA (1994), the Frankfurt University, Germany (1998) and Institute of Plant Biotechnology, Nitra, Slovak Republic (2006). He is a recipient of Lifetime Achievement Award (2016) by Venus International Foundation, Chennai; Vigyan Ratan Samman (2010) of Council of Science and Technology, U.P.; Prof. P. Maheshwari Medal of Indian Botanical Society (2013); UGC Mid-Career Award (2015) and Prof, HE Street Memorial Award (2019). He has been a Visiting Professor to King Saud University, Riyadh from 2016-2019. He was elected President of Indian Botanical Society in 2020.

Prof. Anis has published over 220 research papers in reputed journals with "h" index 47; citations 6550, "i10" 148 and supervised research for over 25 PhDs, 10 M Phils and scores of MSc Projects. He has published three books on Plant Tissue Culture from Springer. He is a life member of National Academy of Sciences, India and holds fellowships of Indian Botanical Society (FBS), Indian Society of Genetics and Plant Breeding (FISG), International Society of Plant Morphologist (FISPM) and Linnean Society of London (FLS).

Mehrun Nisha Khanam

Mehrun Nisha Khanam is a Ph.D., earned her doctorate degree in botany from the Aligarh Muslim University, Aligarh in 2019 on the topic "Studies on Interactive effects of different PGRs on In-vitro regeneration and Biochemical Yield in Allamanda cathartica L." She then worked as assistant professor at the Department of Biotechnology and University Centre for Research and Development, Chandigarh University, Mohali (Punjab), India, before joining the Research Centre for Plant Plasticity, Seoul National University, Republic of Korea as Post-Doctoral Researcher in 2022.

She has published several research papers in journals of repute like Plant Cell, Tissue and Organ Culture (2018), Industrial Crops and Products (2020) and in-vitro cellular and develop Biol-Plant (2020) and received Prof. HC Arya Gold Medal during IBS Conference at Ranchi University, 2017.