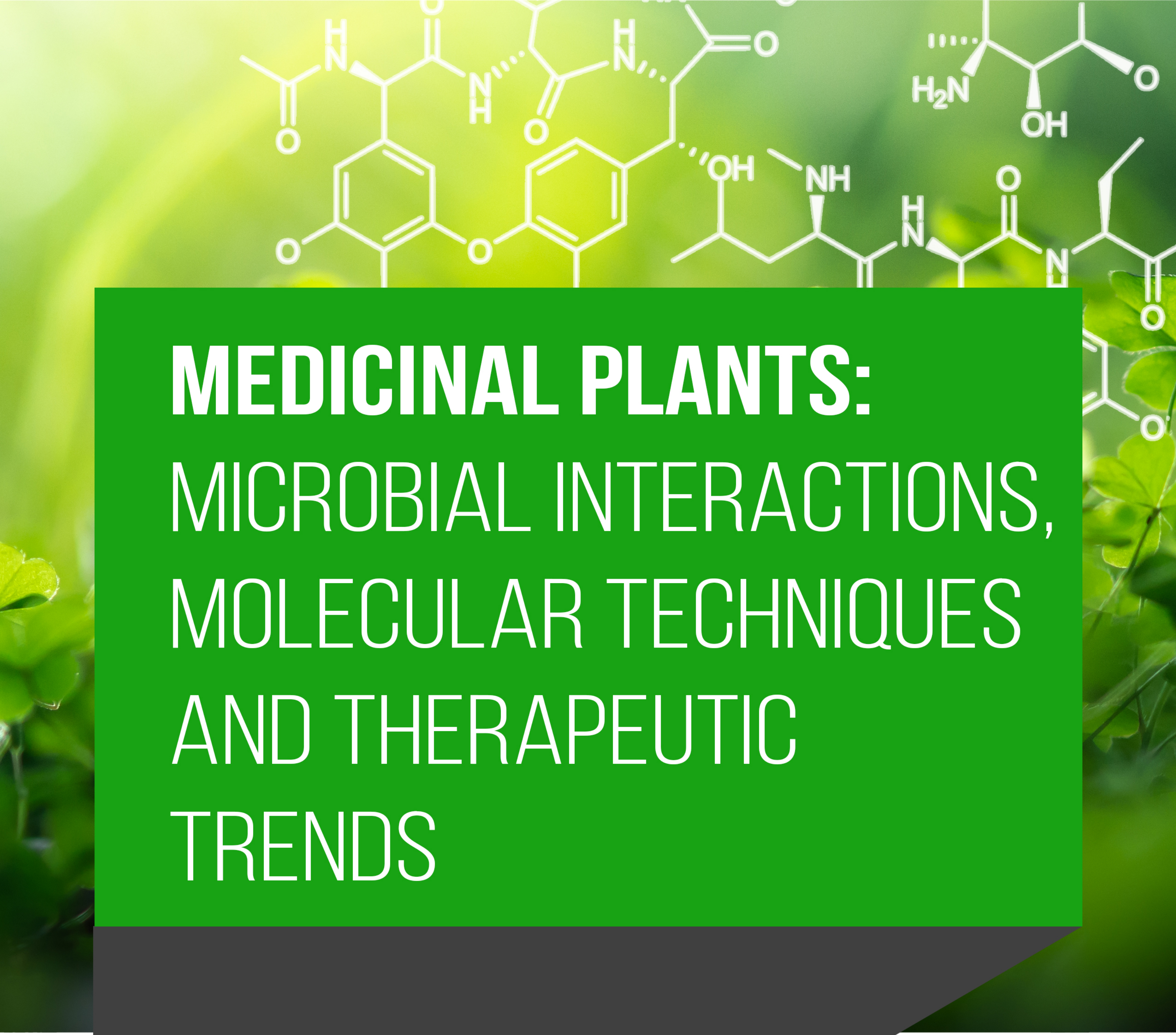

MEDICINAL PLANTS: MICROBIAL INTERACTIONS, MOLECULAR TECHNIQUES AND THERAPEUTIC TRENDS

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Medicinal Plants: Microbial Interactions, Molecular Techniques and Therapeutic Trends

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FOREWORD

Writing forward of this valuable book is a matter of pleasure and honour for me. This book, "Medicinal Plants: Microbial Interactions, Molecular Techniques and Therapeutic Trends" is divided into three sections focusing on medicinal plants and their therapeutic trends and techniques, which can be used to improve medicinal plants. The first section deals with the plant-microbe interaction strategies, the diversity of microbes related to medicinal plants and the role of these microbes in the value addition of medicinal plants. Microbial Phyto-therapeutics and microflora of medicinal plants in hydroponic systems are providing current trends in the respective field. The second section of the book focuses on molecular techniques available for the genetic improvement of medicinal plants. Latest cutting-edge tools such as genome editing and modern breeding tools and techniques are discussed to manipulate medicinal plants for better growth, yield and quality. In the third section of the book, nano strategies and nanoengineering opportunities and challenges are described which are involved in increasing the therapeutic tendencies of medicinal plants. Medicinal plants and their affiliation with the treatment of a variety of diseases along with the production of bioactive clinically administered drugs urge the need for persuasive therapeutic potencies through recent approaches. Detailed chapters on the contribution of nanotechnology in elucidating the pharmacological profile of medicinal plants are explored in this section. Therapeutic enhancement through most employed metallic and carbon nanostructures, nano-elicitors for the production of augmented bioactive secondary metabolites and the role of nanocarriers in achieving the best possible efficacy of the bioactive medicinal ingredients are comprehensively discussed in this section to cover the recent and up-to-date approaches in the nano-manipulations of the medicinal plants.

I am really convinced by the knowledge and expertise of the editors who have done a great job in compiling and formatting a comprehensive and valuable book on the importance, use, and improvement of medicinal plants. I hope that the book will get a broad readership ranging from students to scientists, academia and industry. I appreciate the editors, authors and publishers for their effortless hard work in writing, formatting, compiling and publishing this tremendous and valuable book.

Asif Ali Khan (T.I.)
MNS University of Agriculture
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PREFACE

"Medicinal Plants: Microbial Interactions, Molecular Techniques, and Therapeutic Trends" is one of the efforts and contributions to bring together the advancements in technologies going on with respect to medicinal plants in the search for healthcare agents. This book will look into the ongoing practices and recent innovations involved in enhancing, modulating, and isolating active therapeutic phytodrugs. The importance of medicinal plants can be realized from the fact that more than half of the clinically administered drugs are derived from natural medicinal plants. This book has been divided into three sections, each section focusing on the recent strategies employed for phytochemistry and therapeutic explication. The book will cover modern-day approaches like microbial-plant interaction, genome editing and nano-engineering of medicinal plants for increasing the therapeutic potencies of the plants that have not been assembled in one book yet. Given that, the book will give a comprehensive scenario of the recent scientific approaches explored for increasing the production of phyto-metabolites and therapeutic attributes. Detailed chapters on the contribution of microbial interactions, genetic alterations and nanotechnology in elucidating the pharmacological profile of medicinal plants have been presented. Hope the readers will find it functional and exemplary useful in the subject of medicinal plants.

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SECTION 1: MICROBES AND MEDICINAL PLANTS

CHAPTER 1

Association of Plants and Microbes: Past, Present and Future

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Abstract: Changing climatic conditions, biotic and abiotic stresses along with use of synthetic fertilizers have deteriorated soil quality and crop yield. Microorganisms are natural inhabitants of soil and plant surfaces that form a stable dynamic system with the host plants. The plant microbiome assists in plant growth by solubilizing minerals, recycling nutrients and inducing defense responses by mitigating environmental stresses. These plant-associated microorganisms can be used as functional moieties to enhance overall plant productivity and reduce negative impacts on the ecosystem. The plants and microbes are contemplated as natural partners that harmonize various functional traits, however, the magnitude of friendly or hostile consortium depends on the kind of microorganisms involved. Before the scientific advent of advanced technologies, conventional approaches such as culturing on media, microscopic observations and biochemical tests provided awareness of how these two communicate. Later on, contemporary molecular-based tools like polymerase chain reaction (PCR), microarrays, enzyme-linked assays (ELISA), and nucleic acid-based methods (next-generation sequencing, *etc.*) surfaced. This chapter will comprehend different types of aboveground and subsurface microbes associated with the plants, their impact on sustainable agriculture and high-throughput technologies used to investigate the plant-microbe relationship.

Keywords: Aboveground, Agriculture, Associations, High-throughput approaches, Microbes, Subsurface.

INTRODUCTION

Plants are ubiquitously colonized by microbiome including archaea, protists, fungi and bacteria which exhibit pleiotropic effects on plant health. Biotechnological

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advancements have enabled us to explore plant microbiome structure and their interaction with the host [1]. Owing to different areas of colonization, microbial species are classified into different groups; for instance, organisms that inhabit the external plant parts are known as epiphytes, while those that occur on the inner side are endophytes. Rhizosphere exists in areas closest to the roots while phyllosphere organisms colonize the leaf surfaces [2]. Owing to the plant exudation occurring at various developmental stages, the root region is explicitly teamed with microbes that are associated with plant health [3].

The plants build various kinds of relationships with the associated microbes. In mutualism, both host and microbial specie acquire benefits from each other by augmenting nutrient and mineral accessibility, heightening immunity against pathogens, and susceptibility to stress conditions. Further, the plant exudates such as sugars, vitamins and other growth factors also promote the colonization of microbes and thus, it can also be termed a synergistic relationship [4].

Commensalism states the symbiotic connection in which the commensal gets benefits, whereas the other partner is neither harmed nor benefitted from the relationship. Typically, the plant by-passes a nutrient to the microorganism that is living in its vicinity [5]. Amensalism is a sort of reciprocal action where the effect of one organism's activity negatively influences the other. Parasitism is a life-long, co-existing association in which a parasite favorably feeds on its host. In this relationship, if the host defense mechanism is vigorous then the interaction will favor the host over the parasite, however, in the opposite scenario, the host becomes ill and eventually die [5]. Thus, microbial heterogeneity makes it essential to understand the interactome association of microbes with the root systems. Due to the plant-microbe interaction, these are also known as holobionts or meta organisms [6].

Microorganisms that can adapt to particular stress conditions are beneficial for plants in different ways [3]. Beneficial microorganisms are normally inoculated in the soil, as a substitute for chemical treatments, to stabilize soil structure, control pests and diseases, mitigate the negative effects of agrochemicals and ameliorate agricultural practices. These inputs could be in the form of biocontrol agents, bioherbicides and biofertilizers. Recently, the use of inoculants has been intensified due to the availability of multifunctional strains that can improve the yield at minimal cost. Rhizobia is one of the extensively used microbial inoculants that form a symbiotic association with legumes and fulfil the requirement of plant nitrogen by influencing the process of biological nitrogen fixation [7]. Likewise, plant growth-promoting bacteria (PGPB) regulate phytohormones synthesis, and phosphate accessibility and elicit the plant's defense mechanism against environmental stresses. Furthermore, some microorganisms also influence crop production through pests and disease management [8].

The recent interest in advanced technologies has warranted in-depth analysis and characterization of microbes that colonize plants. Genome sequencing, metagenomics, proteomics and other contemporary techniques have enabled us to have a clear view of the dynamic belowground and aboveground microorganisms [9]. Hence, the current chapter highlights different microbial interactions, their impact on crop productivity and the advancements that have been adopted in this field.

THE TWO-DIMENSIONAL MICROBIAL COMMUNITIES

Aboveground Microbes

Microbes colonize different aerial parts constituting anthosphere microbiome (flowers), phyllosphere microbiome (leaves), spermosphere microbiome (seeds) and cardosphere microbiome (fruits) [2]. Endophytic and epiphytic organisms colonize plant tissues and acquire nutrients through the aerial tissues *i.e.,* flowers, fruits and leaves and so, their growth and development largely depend on the nutrition present within the organs [10]. However, the phyllosphere organisms exhibit unstable environments with variable physicochemical limitations like UV radiation, desiccation and temperature and they act as commensal-like organisms [11].

These organisms can be visualized in different host species. For instance, in a study conducted on grapes, it was noticed that the phyllosphere was dominated by *Pseudomonas, Acinetobacter, Pantoea, Bacillus, Sphingomonas* and *Curtobacterium* while the endophytes were inhabited by *Burkholderia, Bacillus, Ralstonia* and *Mesorhizobium* [12, 13]. In another study, *Devosia* and *Pedobacter* dominated the potato phyllosphere while *Pantoea* and *Bacillus*were observed in the lettuce phyllosphere. *Acinetobacter* was reported as the dominant community in the stems and leaves of tomatoes [13]. *Alternaria* was dominantly observed in the stem while peel samples, calyx, were populated by *Penicillium*. Hence, it can be proposed that the microorganisms existing in a particular plant depend on the nutrients, host tissue and the physicochemical properties of the soil [14].

Epiphytes can develop resistance towards immunological and antimicrobial chemicals produced by plant tissues and competing microbes. The quorum-sensing (QS) signals are also linked with these organisms such as in the tobacco phyllosphere [15]. Additionally, these organisms can also produce exopolysaccharides that increase resistance to desiccation along with different enzymes and phytohormones. In short, the association between the plant and

CHAPTER 2

Microbiomes in Phytotherapeutics: Pros and Cons

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Abstract: This chapter highlights the significance of microbiomes especially plant microbiomes in the field of therapeutics. The Plant microbiome comprises epiphytes and endophytes inhabiting the surface as well as inside of the tissues of the host. These microbial communities occupy a well-defined habitat and perform various activities developing certain interactions with the host such as commensalism, mutualism, and parasitism. For the establishment and functioning of the plant microbiome, plant root releases exudate according to the nutritional requirement of particular microbial species. In response to the stimulus, microbes chemotactically move towards the roots, colonize and move to other parts of the plant. Microbes also adopt certain mechanisms not only to colonize and multiply in specific hosts but also to promote the growth of the host by secreting various plant growth hormones and exopolysaccharides. The numerous compounds produced by microbes make plants tolerant of biotic and abiotic stresses. The microbial communities in plant microbiome have an active role in maintaining the health, ecology and physiology of the host. As a major portion of the world's population is dependent on phytotherapeutic medicines according to the World Health Organization, the pharmacological characteristics of major medicinal plants such as *Aesculus hippocastanum* and *Ginkgo biloba* are described in detail. This chapter highlights the significance of the core role of the microbiome associated with plants in the synthesis of various medicinal compounds. The phytotherapeutic potential of plant microbiome revealed that endophytes and epiphytes isolated from various plant species showed great potential for the production of antimicrobial as well as anti-inflammatory substances. The medicinally rich compounds such as antibacterial proteins, phenols, saponin glycosides, flavonoids, terpenoids, carbohydrates and fatty acids isolated from plant-associated microbes have various applications in the treatment of fetal diseases and also exhibit anti-inflammatory action. Certain public concerns are raised about the side effects of medicinal plants used in phytotherapeutics. A relevant case study about public concerns along with preventative measures such as rigorous testing is provided in this chapter.

Keywords: Epiphytes, Endophytes, Microbiota, Phytomicrobiomes, Phytotherapeutic potential.

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INTRODUCTION TO MICROBIOME

The word microbiome is derived from two Greek words, *mikros* which means small and *bios* which means life. The microbiome is defined in terms of the microbial community that occupies a well-defined habitat and performs multiple activities. These microbial communities' dwell on the host surface, colonize in various tissues, inhabit both inter and intra-cellular host organisms and have an effect on the health and functions of the host [1]. Nowadays, an engineering approach named host-mediated microbiome selection is applied to improve the health and functionalities of the host. According to this approach, the host is artificially inoculated with beneficial microbes to improve the microbiome [2].

BRIEF HISTORY OF MICROBIOME

The history of microbiomes in the field of microbiology dates back to the seventieth century. According to Whipps, a microbiome is a distinguishing microbial community inhabiting a definite habitat that has distinctive physical and chemical properties. According to this definition, the term microbiome covers microorganisms and their diverse activities [3].

From the historic perspective, the field of microbiome research has derived from the field of microbial ecology. Microbiome research is an interdisciplinary field that links multiple fields such as biotechnology, bioinformatics, bioeconomy, plant sciences, food sciences, medicine and mathematics [4]. The advanced field of microbiome research explains the concept of holobiont theory which is about host-microbial interactions. According to this theory, microbes have a symbiotic relationship with their host and these are transmitted from one generation to the other. The - host-microbial interactions have an impact on the health of holobiont in a particular environment and any mutations in hologenome result variations in microbiota or host genome [5, 6].

MICROBIOME VS MICROBIOTA

The two terms microbiomes and microbiota are often used interchangeably for microbial communities but they are distinct from each other. The term microbiota refers to living microorganisms such as pathogenic, commensal and symbiotic microorganisms living in a particular habitat. Generally, the microbiota includes viruses, archaea, bacteria, fungi and protists [7]. Microbiome involves the collective genome of the microorganisms that occupy a specific habitat. The term microbiome covers all the biotic and abiotic factors and the theater of activities such as the production of metabolites by the microorganisms while inhabiting a particular environmental niche. The structural elements, extracellular DNA and all mobile genetic elements such as phages viroid and prions that are considered nonliving microorganisms are included in the microbiome but not in the microbiota [8].

DIVERSE ARRAY OF HOST AND MICROBES INTERACTIONS IN THE MICROBIOME

The microbes inhabiting a specific microbiome enjoying a symbiotic relationship with the host. Symbiotic microbes are defined as microbes that live in symbiosis with other organisms and these are further classified as mutualists, commensals and parasites. Mutualism is a relationship in which both organisms are benefited. In commensalism, one organism is benefited and the other one is neither benefited nor harmed. In parasitism, one organism is benefited and in return, it causes harm to its host [9]. The microbes belonging to a microbiome are both potentially useful and harmful, for example, the human gut microbiome is mostly consisting of symbiotic or commensal communities while few species become pathogenic under specific conditions [10]. The symbiotic microbes are further categorized into two types endosymbionts and ectosymbionts. Endosymbionts are the microbes that live inside the tissues and cells of their host while ectosymbionts reside on the surface of the host [11].

DIFFERENT TYPES OF MICROBIOMES

Marine Microbiome

There are different types of microbiomes such as animal, plant, human, terrestrial and marine microbiomes. Marine animals share water bodies with an enormous number of microbes forming a marine microbiome. Scientists are studying the symbiotic relationship of marine organisms for years but the advancements in technology have provided a new paradigm to discover the correlation between diverse microbial life and sea animals. In marine microbiomes single host symbiont systems are majorly studied. The Advanced marine microbiome studies involve the association between diverse microbes and a variety of marine animals as hosts.

Microorganisms dwelling on or within marine animals are playing an immense role in the production of oxygen, nutrient recycling, and degradation of organic matter [12]. An excellent example of a marine microbiome is a coral reef. The coral polyp lives in symbiotic association with the algae. The coral polys provide shelter to the algae and in return algae gives color to the coral colonies. Algae perform photosynthesis and are the source of oxygen, organic products and host nutrition [13].

CHAPTER 3

Soil Bacteria-Medicinal Plants Interaction

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Abstract: Regulation of biogeochemical cycles depends on soil micro biota in which numerous and distinct types of bacteria are involved. These bacteria share a common environment in the soil and interact with the plants at three different levels *i.e*. endosphere, phyllosphere, and rhizosphere, resulting in improved soil fertility and plant health. The study of medicinal plants is ignored in Pakistan, though there exists a large number of different avenues for research in this field. Studying the medicinal plant-bacteria relationships in the era of new-generation sequencing paves new ways for understanding their association and facilitates improvement in sustainable production. Answers to new queries like "How bacteria respond to climatic changes" and "How do they interact with one another and with medicinal plants for growth and development" along with the exploration of rhizospheric bacteria in the future for enhancement in the production of secondary metabolites in medicinal plants might be a new vista unlocked for the sustainable agricultural practices. In this chapter, we focused on the role of soil bacteria-medicinal plants interaction in growth, nutrient acquisition, environmental stress alleviation, and quantity and quality of medicinal compounds present in these plants.

Keywords: Alleviation, Bacteria, Interaction, Medicinal plant, Rhizosphere, Soil, Stress and sustainable.

INTRODUCTION

Medicinal plants being a rich source of biologically active chemicals, are considered to be safer for humans as well as the environment than synthetic chemistries for serious diseases' treatments [1] There is a long history of using plant-based remedies throughout Europe and Asia [2]. Around six hundred medicinal plants are listed in Chinese *Materia Medica*, with the first usage of

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medicinal herbs in China in 1100 BC [3, 4].The use of plant-based medications for a wide range of human ailments is expanding because of the increase in population, prices, and hazardous impacts, as well as pathogens of infectious diseases are developing resistance day by day against synthetic medicines. To address the demand for plants with medicinal value, their large-scale cultivations using contemporary farming technologies are being practiced by most of the Asian farmers. The development and quality of pharmaceutically significant plants are being hampered by a variety of plant pests and diseases. Furthermore, pesticide abuse has a negative impact on the quality of therapeutic plant products. All of these factors need the development of novel medicinal plant cultivation technology. Plant growth-promoting rhizobacteria (PGPR) are bacteria that colonize plant rhizosphere and stimulate plant growth by a variety of methods such as nitrogen fixation, quorum sensing, siderophores synthesis, phosphate solubilization, and so on [5]. Because PGPR can be used to replace chemical fertilizers, insecticides, and other chemicals, its popularity has increased rapidly.

Before we get into the interactive strategies of bacteria and medicinal plants, it is important to understand the fundamentals and history of this amazing science. The utilization of plant growth-promoting bacteria can be traced back to Theophrastus (372–287 B.C.) who proposed blending diverse soil samples to repair faults and add vitality to land [6]. The technique behind mixing of various soils can be verified by microscopic examination. Virgil was the first to chronicle the establishment of legumes on cultivable ground [7]. Studies confirmed the existence of rhizosphere bacterial colonization in grasses as well as ascertained that these soil bacteria transformed atmospheric nitrogen and made it available for the plants [8]. The term “rhizobacteria” was coined after study on the radish crop, and it is defined as a population that colonises plant roots competitively, accelerates their development, and decreases plant illnesses by increasing plant immunity [9]. Belligerent colonisation and plant growth promotion properties, as well as biocontrol ability, are just a few of the qualities directly linked to PGPR [10]. PGPR interact with plants in all positive, negative, and neutral ways [11]. These are known as iPGPR and ePGPR. Mostly ePGPR could be found between root cortex cells, rhizoplane, and rhizosphere. ePGPR include bacteria from Agrobacterium, Azotobacter, Caulobacter, and Chromobacterium genera [12]. Endophytes [rhizobium spp.], and Frankia species live in specialized nodular structures of root cells [13, 14]. Plant growth promoting rhizobacteria promote nodule formation and fixing of atmospheric nitrogen in a variety of crops like soyabean (*Glycine max* L. Merr) [15]. Crop plant growth and productivity can be enhanced directly or indirectly by PGPR. Rhizosphere colonization is responsible for the generation of siderophores [16].

This chapter focuses on developing an understanding about medicinal plants, bacterial diversity associated with them and plant-bacterial interactions to get the current state of knowledge in this broad field of study.

BACTERIAL DIVERSITY ASSOCIATED WITH MEDICINAL PLANTS

Research on rhizobacteria from significant medicinal herbs is important because they significantly influence the crop production, synthesis of important plant metabolites, and the quality of medicinal products [17]. Many microbes can produce phyto-therapeutic chemicals [18]. This data is immensely important in the development of biofertilizers' industries for commercially cultivated medicinal plants. The soil microorganisms in the rhizosphere of medicinal plants growing on the desert ecosystem (*Calendula officinalis* L., *Solanum distichum* Schumach. & Thonn. and *Matricaria chamomilla* L.,) contain a high number of G +ve [Gram positive] bacteria that are important for disease suppression. There was a host-specific assortment of microorganisms as well as extremely distinct diazotrophic communities detected in all three plants. The findings revealed that plant species had an essential role in structural and physiological diversity. Additionally, *Bacillus* strains help the plant to develop and increase the synthesis of flavonoids. Significant variations were reported in the endophytic bacterial range in three different species of medicinal plants [19]. The microbial diversity in the rhizosphere of various medicinally significant plants was studied, and 50 strains were categorized into seven genera, with *Corallococcus* and *Myxococcus* being the most common genera [20].

Farming of *Rehmannia glutinosa*, a valuable medicinal plant continuously on the same plot of land reduces its production [21]. A change in the soil microbial population because of *R. glutinosa* cultivation could be one of the major reasons for the limits associated with monoculture cropping [21, 22]. Though, plant, soil and myco-floral interactions are critical for *R. glutinosa* production and quality in a sequential cropping system [23], in the intercropping treatments, addition of Gram-negative bacteria and a decrease in allelochemicals promoted peanut development and enhanced peanut production. There were 120 morphologically different isolates categorised into 21 phylotypes after root endophytes and rhizosphere bacteria were identified [24]. The majority of the isolates were Firmicutes and Gamma-Proteobacteria. The most common species were *Stenotrophomonas* and *Pseudomonas*, which accounted for 27.5 percent of all isolates. *Agrobacterium, Pseudomonas, Enterobacter, Acinetobacter, Pantoea, Sphingobium, Serratia,* and *Stenotrophomonas* were among the 103 bacterial communities isolated and described from the roots and rhizospheric region of Hypericum silenoides Juss [24]. About 123 bacterial species belonging to alpha- and gamma Proteobacteria were isolated from the roots and rhizospheric soil of

CHAPTER 4

Soil Fungi-Medicinal Plants Interaction

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Abstract: Medicinal plants are a natural source of therapeutic compounds and secondary metabolites; therefore, their demand is increasing day by day. Since the last thirty decades, their cultivation as well as preservation with the help of biofertilizers or pesticides is a point of great concern. The rhizosphere is an important area around the roots. It is a habitat for many kinds of microorganisms like fungi. This soil microbial performs a variety of beneficial functions for the growth of plants such as nitrogen fixation, solubilization and removal of toxins. Endophytes are also an important class of microbial flora that helps in the absorption of water and nutrients for the plant. Additionally, they also make plants able to cope with environmental stresses. Fungal endophytes supervise photosynthesis. Certain therapeutically important plants including licorice and white ginger lily can also perform antimicrobial activity depending upon the endophytic composition they have. These types of plants having antimicrobial activity are of great significance as they act as eco-friendly biopesticides.

Keywords: Endophytes, Fungi, Medicinal plants, Rhizosphere, Secondary metabolites.

INTRODUCTION

Medicinal plants have similar parts as other plants but they are characterized by some medicinal values. Herbal medicines relatively use different parts of herbal plants [1]. Medicinal plants used for herbalism have a profound tradition of their application besides conventional medicine. About 30,000 years ago, the use of medicinal plants in curing different types of diseases had been started throughout the world [2]. Medicinal plants contain chemicals in their different parts that can help in the treatment of different diseases in humans [3]. The total number of medicinal plants in the world is quite surprising. The use of herbal medicines is the oldest and still used system of medicines. Plant medicines also known as botanical medicine or Phyto-medicines (WHO) refer to herbal materials which

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contain complete plants, or parts of plants like leaves, flowers and roots [4]. It is expected that from lichens to trees, about 70,000 plants are used for medicinal purposes. In developing countries, 80% of people depend on traditional medicines based on plants.

The Rhizosphere, the narrow zone of soil surrounding and influenced by plant roots, is a natural habitat for numerous beneficial microorganisms and represents a biologically complex ecosystem on Earth [5]. This biologically active zone is critical for plant-microbe interactions and, as a consequence, for nutrient cycling, plant growth, and resistance of plants to diseases [6]. During positive plant-microbe interaction, rhizosphere colonization by soil microorganisms is beneficial for both plants and microorganisms. Both partners derive benefits from this intimate association and vitalize each other [7]. The large number of rhizo-deposits released by the plant roots are the key determinants of microbial activity and develop a community structure in the rhizosphere [8]. The rhizosphere microbes utilize the rhizome carbon deposits as a major energy source for their growth and development [9]. Consequently, plant roots can manipulate the rhizosphere microbiome for its benefit by selectively stimulating microorganisms with traits that are beneficial to plant growth and health [10, 11]. Mutual interdependence and the interplay between the rhizosphere microbiome and the plant result in the overall quality of plant productivity.

The rhizospheric microbial forms vary in diversity, which include bacteria, fungi, nematodes, viruses, arthropods, oomycetes, protozoa, algae, and archaea. A beneficial effect of the number of rhizosphere fungi concerning plant growth promotion has long been known [12]. These plant growth-promoting fungi (PGPF) include species of the genera *Aspergillus, Fusarium, Trichoderma, Penicillium, Piriformospora, Phoma*, and *Rhizoctonia,* which have the natural ability to stimulate various growth-related traits of plants [13]. Many studies on dicots and monocots have shown that PGPF mimics the well-studied plant growth-promoting rhizobacteria (PGPR) in their interaction with the host plant [14]. For example, treating seeds with PGPF inoculum can improve the germination and seedling vigor of different plants. They can also induce longer and larger shoots (Fig. **1**). Some may exert an effect on root development and performance. There are PGPFs that may stimulate early and vigorous flowering of plants [15].

Plants have always been used as medicines; from the beginning of life, plants were the first and most important source of medicine. Herbal plants show anticancer activity. Plant-based medications are often low-cost, accessible, and exhibit little reaction after treatment. . Herbal medicines are usually low in cost, abundant and show very little toxicity or side effects in treatment. For a long time, traditional Chinese medicines have been used for the cure of cancer in China and all over the world. In the treatment of cancer, a lot of molecules have been identified that are extracted from plants and their synthetic derivatives. The compounds extracted from plants do not have risk factors or side effects on human health [16].

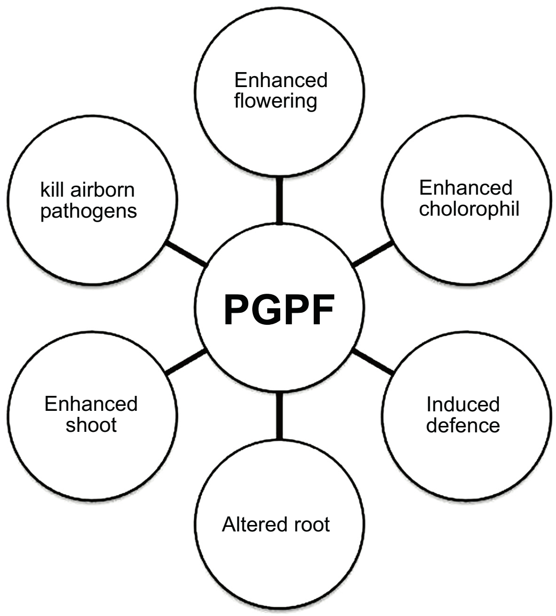

Fig. (1). Role of Plant growth promoting fungi (PGPF) in plants.

Many secondary metabolites were produced by plants which are a further source of many drugs. With the development of these drugs, many therapies are also discovered in this regard. These compounds have epigenetic properties as they have this ability in their genome which helps them to combat cancer. They have u tumour suppressor genes. Substances like mistletoe plant extract, taxanes, podophyllotoxin derivatives, vinca alkaloids, and camptothecin, have inhibitory activity on the growth of all the cancer cell lines and are extracted from marine species. Two of them have activity against mammary epithelial cells, and they also help in the discovery of new compounds and therapies. The healing properties of plants rely on their components for example antibiotic, antipyretic, and antioxidant effects. Natural yield extracts from living organisms (plants and animals) have some active ingredients, an alternative to chemotherapeutic treatment or appropriate with chemotherapeutic treatment [17].

CHAPTER 5

Endophytic Bacteria: Value Addition in Medicinal Plants

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Abstract: Plants and microorganisms have long evolved together and our most recent discoveries using advanced techniques have allowed us to understand the chemical interface within the plant further explaining the relationship between them. As we discover the interaction between the plants and the associated microorganisms, it has been clearer to us that there has been a synergy more evident than that of antagonism among them. A lot of chemicals or metabolites are being released favouring both the host as well as the microbes during this contact. Such an interaction which leads to the release of certain metabolites can be managed and manipulated in bringing about positive effects for the biosphere and environment. One instance of this positive effect can be the use of medicinal plants and their microbe-facilitated associated metabolites which may be regulated through the application of different endophytic microorganisms. If we can control the release of different metabolites from plants particularly from those of medicinal plants, we can harvest significant benefits for human and animal health as we are utilizing endophytes for their role as biofertilizers. The food for medicine concept has been emerging and requires quick and efficient identification of metabolites as well as chemicals that may be used in addressing multiple diseases in human beings and other animals.

Keywords: Chemical interface, Endophytic bacteria, Metabolites, Medicinal plants.

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INTRODUCTION

Human beings have long been depending on different natural resources for their survival, among which plants have played a significant role. Plants alongside being the food have also served as fibre as well as medicine needed for many types of diseases. It is a blessing to have these plants from which a multitude of diseases are cured through the metabolites released by these plants [1-4]. The field of medicine has already progressed in identifying many of these chemicals, and harvested them successfully from a wide variety of plants. Several classes, and purposes have already been defined for the said metabolites [5, 6] and more recently when COVID-19 struck, the focus was again shifted towards plants and their metabolites in finding a quick cure and remedies [7, 8]

In a more recent history, it has been observed that plants as well as microorganisms work in synergy in producing different metabolites [9 - 11]. For instance, some of the microorganisms when associated with different plants lead to the production of certain chemicals; which sometimes are released by the plants while on other instances, these metabolites are produced by the microorganisms. The advancements in technology have deciphered the chemical interface between host plants and microorganisms, and we can trace back to the original source of these metabolites. For example, microbes release certain types of metabolites for their metabolism, in their defence, in their host's defence, in facilitating their hosts, and in accomplishing their niche. Similarly, plants produce different primary and secondary metabolites serving various purposes: plant metabolism, recruiting beneficial microbes, facilitating nutrient uptake, and restricting different molecules' entry among others. The chemical interface for both entities is complex, with the combined responses, to which we are recently exposed.

The microorganisms produce different primary compounds; alcohols, organic acids, amino acids, nucleotides, and polysaccharides, and secondary compounds; bacteriocins, peptide antibiotics, polyketides, and cyclic lipopeptides among others. On the other hand, plants produce carbohydrates, lipids, proteins, and nucleic acids among the primary chemicals, while alkaloids, phenolics, sterols, steroids, essential oils and lignin in the secondary chemicals. All these secretions within the plant or in the environment possess multiple functions all the way from plant health to animal, microbial and environmental health.

A class of plants used for their therapeutic functions termed usually medicinal plants have a long history and have been serving animals. Either these medicinal plants or the microbes associated with them lead to the production of the chemicals which are directly or indirectly utilized for the cure of certain diseases. The role of microbes particularly those of endophytes has been receiving an increased focus due to the fact that they are in a closer interaction with the host plants and may allow us to regulate such chemicals for our use. This chapter focuses on the identification of these endophytes, their niche and roles, alongside putting forward their role in medicinal plants' metabolite regulation.

WHAT ARE ENDOPHYTES

Different niches of microorganisms have been defined and discovered as we go along further discoveries in this realm (Fig. **1**). Particularly in the plant sphere, the microbes can reside in the bulk soil, rhizosphere, phyllosphere, and endosphere. The phyllosphere can further be divided into different plant parts, and recent discoveries have put forward the microbiome incident in the flowering parts. Microorganisms are present in all parts of the plant especially those of roots, stem and leaves; where the majority of them are incident in the roots [12, 13]. The presence of endophytic microorganisms in the roots or the rootzone ensures that they are in close interaction with the plants, thus helping the plants in overcoming different kinds of stresses which may include biotic and abiotic issues. It has been widely established that there have been vertical as well as horizontal transfer of microbes *i.e.*, from one generation of the plants to the next, or from one plant to another plant [14 - 16]. Although the seeds which are dehydrated may have the presence of endophytic microbes [17, 18] which may reside in a dormant phase before they become functional when the plants need them and provide them with particular carbohydrates or exudates.

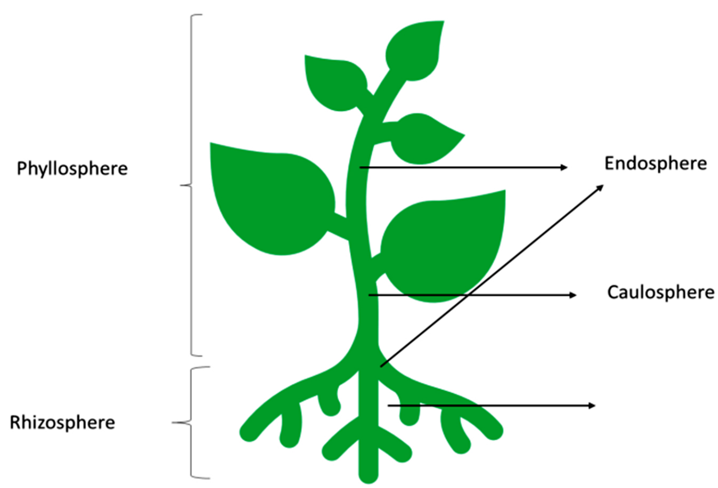

Fig. (1). Incidence of plant microbiome in different spheres of the plant.

Endophytic bacteria are further classified into culturable and non-culturable where the culturable can be cultured in laboratory media, however, the non-culturable

CHAPTER 6

Probiotic Bacteria and Plants

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Abstract: Probiotics are microorganisms, when consumed, give health benefits due to improvement in the activity of gut microflora. Various health claims are associated with probiotics *e.g*. modulation of the immune system, mitigation of lactose intolerance, protection from infections and maintenance of healthy gut microflora. They have also been demonstrated to be helpful in treating a wide range of illnesses, including cancer, inflammatory bowel syndrome, diarrhea brought on by antibiotics, and infantile diarrhea. *Streptococcus, Bacillus, Enterococcus, Escherichia coli, Bifidobacterium, Lactobacillus*, and several strains of the fungus *Saccharomyces* are significant probiotic bacterial genera. In fibrous parts of plants and probiotic bacteria, the bacteriocins play a major synergistic antimicrobial role. Prebiotics are non-digestible plant materials *i.e*., complex carbohydrates, fermented in the colon, thus yield short chain fatty acids and energy, and enhance the growth of probiotics. Inulin and fructans are important plant prebiotics. The indirect health benefits of prebiotics are immunomodulatory characteristics, mineral absorption, cancer prevention, and modulation of the metabolism of gut flora, and the prevention of constipation and diarrhea. Many fruits, tuber crops, root crops as well as vegetables contain a huge reservoir of prebiotic carbohydrates. The function of probiotic microbes in foods includes modulation of the immune system, normalization of gastrointestinal activity, and the inhibition of the growth of pathogenic microbes and harmful metabolites. The function of prebiotic food material is to promote the growth of healthy bacteria in the intestinal tract. This chapter highlights the potential need of probiotics and prebiotics in our diet, and it also discusses their health benefits, mode of action, sources, food applications, distinct types, and future perspectives.

Keywords: Bacteriocins, Fructans, Inulin, Metabolites, Probiotics, Prebiotics, Synergistic effect.

INTRODUCTION

The definition of probiotics is "constituents of microbial cells or microbial cultures which when consumed exert beneficial impact on human health and

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well-being" [1]. Regular use of probiotics in the diet has shown numerous health benefits to human health; they play a major role in maintaining regular digestive processes and nourishing the health of animals as well. Nevertheless, numerous uncertainties with respect to regulatory, microbiological, and technological aspects of probiotics exist [2]. After birth, gastrointestinal flora is gained quickly, it is necessary for maintaining normal human homeostasis and remains quite stable during the entire life. During the development of gastrointestinal flora, the association between the host and this microflora gives rise to the development of a distinct and unique intestinal immune system. Major challenge challenge in this host mucosal immune system is to differentiate between benign microorganisms and pathogens by triggering protective immunity without much inflammatory response, which can disturb the stability of the gastrointestinal mucosa [3]. The use of irradiation, immunosuppressive therapy, antibiotics as well as other means of treatment, can alter the composition of gut microflora and has adverse effects on their growth. Hence, to prevent diseases and to reestablish the equilibrium of gut microflora, an attractive option is to introduce beneficial microbial strains into the gastrointestinal tract [4].

Prebiotics include fiber, resistant starches, poly and oligosaccharides, as well as sugar polyols are non-digestible carbohydrates that exert a beneficial impact on the growth and maintenance of microflora present in the gut. Prebiotics are recognized for their potential to nutrify microbes harboring in the gastro-intestinal tract while significantly boosting metabolic activity, thus strengthening the immune system and improves nutrient absorption as well as digestion in the host while retarding the proliferation of pathogenic microorganisms [5]. Prebiotics can survive in the acidic medium/environment, and they are very resistant to various enzymes of the digestive system present in small-intestines. This ability makes prebiotics a very efficient tool to enhance the multiplication and growth of helpful gut microbes that ferment the prebiotics, which in turn leads to the production of vitamins, short-chain FAs (Fatty acids) and various fragmented substances [6]. Anyhow, different microorganisms utilize prebiotics differently because each microbe has its own nutritional requirement to survive in the gastrointestinal tract. In general, gut microflora utilizes prebiotics as nutritional supplements for their metabolic activities and proliferation, so in food industries they have been used widely as supplements of functional foods in various formulations [7]. In this sense, this chapter discusses the need and potential for probiotics and prebiotics in our daily diet, and it also discusses their health benefits, mode of action, sources, food applications, distinct types, and future perspective.

OVERVIEW OF PROBIOTICS

Probiotics are live microbes which can be used in the formulation of various products like dietary supplements, drugs, and foods. Probiotic is somewhat a new term which is specifically used for the bacteria related to beneficial effects on human health as well as animals. Meaning of the term probiotic is “for life” and an Expert Committee defined this term as “live microbes which when ingested in certain quantity exert positive impact on health beyond providing general nutrition” [8]. Probiotics which are commonly used in feed and food are *Lactobacillus* and *Bifidobacterium*. Among other microorganisms which are used as probiotics are yeast (*Saccharomyces cerevisiae*) and few species of *Bacillus* and *Escherichia coli*. For food fermentation Lactic-acid producing bacteria are being used since primeval time, they can perform dual functions by providing health benefits and acting as food fermenting agent [9].

HEALTH BENEFITS OF PROBIOTICS

In addition to meeting basic dietary needs, probiotics provide a number of positive health effects. Health benefits provided by probiotics are strain-specific, so there is no specific strain that would exhibit all projected health benefits and within the same specie, not all strains are effective against specified health conditions. In dairy products, the most used cultures of probiotics which have proven human health benefits are *L. casei*, *Bifidobacterium* spp. and *L. acidophilus* while *Saccharomyces cerevisiae* and some species of *Bacillus* and *E. coli* are also used [9]. Furthermore, probiotics are aimed to aid the naturally occurring gut microflora of the body. Antibiotic-related diarrhea can be prevented by using different probiotic preparations or they can be used as part of the treatment for such ailments. Recently, it has been reported that the functional properties of probiotics of potential importance are anti-carcinogenicity, antimutagenicity, and antigenotoxicity. Experimental data indicated that consuming fermented dairy items is linked with minor chances of colon cancers, which implied that probiotics are capable to inhibit carcinogens and procarcinogens and probiotics also inhibit the growth of those bacteria which can convert procarcinogens to carcinogens thus decreasing the risks of cancer [10].

MECHANISM OF ACTION OF PROBIOTICS

Probiotics have been recognized for providing numerous potential health-beneficial effects. The list of claimed effects of probiotics ranges from mitigation of constipation to prevention of major diseases like cardiovascular ailments, cancer, and inflammatory bowel disease. Among these claims, some claims are considered well recognized like relief from lactose intolerance or reducing intestinal transit time, whereas some claims require further scientific research like

SECTION 2: GENOME EDITING OF MEDICINAL PLANTS

CHAPTER 7

Medicinal Plants and Molecular Techniques

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Abstract: Medicinal plants provide a substantial source of bioactive compounds which serve greatly in the pharmaceutical industry. Before revolutionary advancements in medicines, traditional biotechnology approaches have been used in the breeding of significant therapeutic plants. The challenge is to incorporate effective, efficient, and resilient breeding techniques to enhance the production of phytochemicals by medicinal plants. Genetics and biotechnology can aid in the rapid advancement of therapeutic plants by assessing genetic diversity, conservation, proliferation, and overproduction. Hence, the use of advanced technologies is crucial for selecting, multiplying, and preserving medicinal plants.

Keywords: Conservation, Genetic diversity, Medicinal plants, Molecular techniques, Proliferation, Pharmaceutical industry.

INTRODUCTION

Medicinal plants are a significant group of plants that have been overlooked by humans and plant breeders for many years. They are an important source of novel medications all around the world [1]. In Europe, more than 1300 therapeutic plants are used, 90% of which are accumulated from wild assets; in the USA (United States of America), regular sources represent around 118 of the main 150 professionally prescribed drugs [2]. Besides, up to 80% of individuals in underdeveloped countries depend just on herbal medicines for their essential treatment, while more than 25% of suggested medications in developed nations come from wild herbal plants [3]. The usage of medical plants is quickly expan-

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ding globally, due to the strong demand for herbal medications, healthcare products, and bioactive components of medicinal plants.

In recent decades, the adverse side effects of chemical medications have prompted a greater focus on medicinal plants and their utilization in diet, pharmaceuticals, and other fields [4]. Considering the importance and need for these natural chemicals, genetic modification of this crop to increase secondary metabolite yield is critical. Plant biotechnology and genetic engineering approaches can help to compensate for the time it takes to develop therapeutic plants. Recently, “green” extraction techniques, have developed interest as they cause the elimination of toxic substances and volatile organic solvents.

Multiple molecular practices like DNA fingerprinting techniques have been established for the identification of traditional medicinal plants, these techniques are RFLP, AP-PCR, RAPD, DALP, AFLP, PCR-RFLP, ISSR, SCAR, and isothermal amplification) [5], DNA microarray [6], DNA barcoding, and Forensic informative nucleotide sequencing (FINS). Formerly, morphological traits were used to identify medicinal compounds, as described in Shengnong Bencaojing (200 AD) [7]. Morphological traits were graphically illustrated in Bencao Gangmu (1,593 AD) [8]. With the advancement of technology, a variety of identification methods have been developed. The development of molecular tools in the 1990s was a turning point in the history of traditional medicine identification. DNA barcodes have recently been proposed for identifying living species, including therapeutic compounds [9]. Nowadays, morphological and microscopic characteristics are coupled to identify therapeutic materials initially. To improve the accuracy of identification, these procedures are combined with chemical profiles acquired from thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or liquid chromatography/mass spectrometry (LC/MS) [10]. The subject of the possible application of herbal fingerprinting techniques has been brought by the expanding market for herbal remedies, the growth of Latvia's foreign commerce, and the absence of acceptable analytical procedures. Strategies for collecting the chromatographic fingerprints of four medicinal plants, for example (*Hibiscus sabdariffa L., Calendula officinalis L., Matricaria recutita L., Achillea millefolium L.)* that are taxonomically and evolutionarily distinct were established using high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) [11].

DNA Fingerprinting

The DNA polymorphism is investigated using DNA fingerprinting. Capillary electrophoresis [12] agarose gel electrophoresis [13], or polyacrylamide gels [14] are commonly used to detect polymorphism patterns. Due to post-harvest processing and storage, the quality and quantity of the DNA in medicinal materials may be compromised as compared to fresh materials [5]. DNA fingerprints that do not require DNA amplification, such as restriction fragment length polymorphism (RFLP), are ineffectual due to the lower yields and poor integrity of extracted DNA. Hence PCR-based fingerprinting is preferred by scientists. Some of the available fingerprints are arbitrary primed PCR (AP-PCR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), direct amplification of length polymorphism (DALP), inter-simple sequence repeat (ISSR), PCR restriction fragment length polymorphism (PCR, RFLP), and sequence-characterized amplification region (SCAR). Modern techniques have been used for population structure and genetic diversity in medicinal plants, such as in Iran, SCoT markers was used for the analysis of medicinal plant *Melissa officinalis* [15].

Polymerase Chain Reaction

PCR is an *in vitro* technique used for the amplification of DNA fragments using digestive enzymes [16]. Two oligonucleotide primers *i.e.* forward and reverse primers are used to initiate a DNA polymerase which is a thermostable enzyme that copies each strand of DNA throughout the PCR process [17]. A three-step reaction occurs when the DNA double helix is denatured, oligonucleotide primers are annealed to each complementary strand, and DNA polymerase synthesizes new strands at their best temperature. PCR is conducted in thermocyclers that are completely programmable to alter the reaction temperature at each step.

Arbitrarily Primed PCR (AP-PCR)

A whole-genome fingerprinting technique "arbitrarily primed PCR" (AP-PCR) was initially reported in 1990. It is identical to RAPD, except it uses two sets of primers greater than eighteen nucleotides of arbitrary sequence. The dry roots of Oriental ginseng (*Panax ginseng*) were successfully distinguished from those of American ginseng (*Panax quinquefolius*) using 3 primers (M13 forward and reverse, and Gal-K primer) [18]. A similar technique was used to categorize additional medicinal species, such as Kudidan (*Elephantopi Herba*), Pugongying (*Taraxaci Herba*), and Dangshen (*Codonopsis Radix*) [5].

Random Amplified Polymorphic DNA (RAPD)

Another whole-genome fingerprint is random amplified polymorphic DNA (RAPD). The approach of RAPD is analogous to AP-PCR, except for the usage of a single 10-nucleotide primer with random sequence resulting in amplification at different sites of the genome that are evaluated by gel electrophoresis. RAPD can

CHAPTER 8

Genetic Transformation in Medicinal Plants

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Abstract: Secondary metabolites including terpenoids, terpenes and enzyme cofactor have significant importance in medicinal use. Extraction of plant-based compounds is quite challenging by conventional methods. Biotechnological methods like genetic engineering and *In Vitro* techniques, proteomics, genomics and biochemical pathways are being employed to serve the purpose. Different organic compounds including enzymes, recombinant proteins, vaccines, antibiotics and anticancer have been successfully extracted through the genetic transformation of tomato, rice, corn, soybean and *Nicotiana tabacum.* This report describes different biotechnological approaches with a special focus on tissue culture and genetic transformation methods for the investigation of medicinal plants and their important role in our economic industry.

Keywords: Genetic transformation, Metabolic engineering, Plant secondary metabolites, Tissue culture.

INTRODUCTION

Plant-based products have a significant importance in various walks of life including food, medicine, pharmacy and industry [1]. Their safe application, especially in pharmaceutical industry as compared to the other products, enhances

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their importance [2]. Though medicinal plants are very important but cultivated at a small scale in comparison with food crops like rice, wheat, sunflower, soybean and potato [3]. Medicinal plants are known to contain secondary metabolites like Terpenoids, alkaloids, saponin, alkaloids, and terpenes.

Their demand is increasing at the current time owing to their safe and effective utilization [4, 5]. Fast and accurate strategies are required for the production of secondary metabolites in bulk amounts [6]. Exploration of medicinal plants and various techniques like proteomics and metabolomics, *In Vitro* culturing and genetic transformation methods, have now become a central attention to scientists [6, 7].

Different *In Vitro* techniques like callus culture, meristem culture and suspension culture are being used to exploit the beneficial compounds from medicinal plants [8, 9] like monoclonal antibodies, recombinant antibodies, and hormones [10-15].

Callus Culture

Callus is an undifferentiated mass of cells and is one of the most important sources of the regeneration and multiplication of medicinal plants [11]. Callus culture is well known to be used for the extraction of secondary metabolites and different biologically active compounds like anthraquinone, rosmarinic acid, and baicalin through downstream processing [16, 17]. *Opuntia ficus-indica* (cactus pear) and *Beta vulgaris* (Red beet), *Leucophyllum frutescens*, and *Poliomintha glabrescens* are some of the examples through which secondary metabolites are being extracted from callus cultures [18, 19].

Suspension Culture

Suspension cultures are made through the callus, where callus culture is introduced into the liquid media subjected to shaking and transferred to the liquid phase in bioreactors. Suspension culture is a special technique for developing transgenic products as it comprises decontamination and simple downstream processing in an economical way. It is a well-established way for production of secondary metabolites as cells are allowed to grow in optimum environmental conditions [20]. Uniform quality and yields are obtained through suspensions including high valued compounds such as taxol, ginsenosides and resveratrol [20-23]. *Thalictrum rugosum*, *Vitis vinifera* and Taxa species are few examples for the extraction of medicinally important compounds [21].

Organ Culture

Organ culture is another *In Vitro* culturing technique that is used to bypass the limitations of the undifferentiated cultures of callus and suspension cultures. This technique involves the direct culturing of whole organs like roots, buds, trichomes and even seeds. They've established a useful biological strategy for investigating the biosynthesis of variety of bioactive chemicals, including nicotine and tropane alkaloids, ginsenosides, anthraquinones, artemisinin alkaloids, hyoscyamines and cannabinoids [24, 25]. Hairy root is one of the organ cultures that is also being utilized in conservation of endangered species [34]. Plant tissue culture is directly responsible for increasing the *In Vitro* synthesis of important plant chemicals using strategies such as optimization of nutrient media, biotransformation strategies, use of eliciting molecules and *Agrobacterium* mediated transformation [26, 27].

MOLECULAR ELUCIDATION OF MEDICINAL PLANTS FOR SECONDARY METABOLITES PRODUCTION

A wide range of biotechnological methods are available to explore secondary metabolism. Interpretation and metabolite engineering of pathways have been used to boost the supply of desired products or to create novel medicinally important products such as antidiabetic and anticancer compounds [28]. Molecular elucidation entails a number of methodologies and phases, that govern secondary metabolite pathways such as transcription factors and ending with the cloning of the regulatory genes [29].

Targeting specific biochemical pathways of metabolites, enzyme catalysts, biosynthetic reactions, genes encoding the biosynthetic enzymes and regulatory factors are all part of molecular elucidation.

Overexpression, gene silencing and mutations are used extensively to control production of certain metabolites in the plant cells [30]. Through quantitative trait loci and genome-wide association analyses, high-throughput sequencing technology is being utilized to discover responsible genes for certain traits by using reference genome (Fig. **1**).

Different strategies have been used to increase production of secondary metabolites' in medicinal plants [9, 10, 31, 32]. Downregulation or functional deletion of genes is one method for reducing the production of an undesired group of chemicals while increasing the concentration of desired secondary metabolites (Fig. **2**). The underlying idea behind these methods is to eliminate an enzymatic step in a pathway by lowering the quantity of the associated mRNA or protein [33].

CHAPTER 9

Genome Editing and its Applications in Plants

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Abstract: Regularly interspaced short palindromic repeats/cas9 system (CRISPR-Cas) is a well-developed and frequently used genome editing system, which comprises a Cas9 nuclease and a single-guided RNA (that is an RNA-guided technique). Cas9 recognizes and cuts a specific DNA sequence by base-pairing with it, generating double-strand breakage (DSBs) that initiate cellular DNA repair mechanisms that result in alterations in the DSB regions or adjacent. CRISPR/Cas9 technology has transformed genetic modifications since its inception, and it is now routinely used to improve the genomics of large numbers of crops. CRISPR/Cas system is used for targeted modifications to improve plant growth, yield and tolerance to biotic and abiotic stress along with developing transgene-free gene-edited crops. The limitations of using the CRISPR/Cas9 technology, as well as ways for enhancing its responsiveness, are also investigated. This chapter also describes the introduction of CRISPR-edited DNA-free plants, which may be more acceptable than some other genetically-engineered organisms. The prospective uses of the CRISPR/Cas9 technology, as well as conventional breeding possibilities, are highlighted in this introductory chapter.

Keywords: CRISPR/Cas9, Conventional breeding, Genome editing, Genetically-engineered organisms.

INTRODUCTION

Because of the rapid advancement of decoding methods, the genome of many key crops has already been sequenced and analyzed [1]. Depending on these facts, we may breed better crops, identify the activities of genes that control essential agricultural features, and generate high-yielding crops [2].

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The function of a gene is frequently studied using mutants, although the most common method of obtaining such genetic variants in living animals is the use of chemicals or physical techniques, such as genetic alterations with T-DNA or gene silence using RNA interference [3].

The adoption of those technologies has significantly sped the separation and subsequent integration of gene products into the genome of improved varieties of significant crop plants. Due to the lack of selection methods, the changes produced by these processes are unanticipated and frequently lead to a significant impairment of gene function, making mutant screening and detection time-taking [4]. Given that the genomes of important agricultural plants contain a large number of valid gene data that has yet to be assessed, the development of flexible, efficient, and accurate methods for altering target genes is essential. Synthetic sequence-specific nucleases (SSNs) were used to generate double-strand breakage in specific genomic areas to achieve these criteria [5]. When base pairs are inserted, deleted, or replaced, DNA lesions are repaired, and the desired gene is created. This method is now typically referred to as "genetic modification." The main two repair mechanisms are non-homologous end joining (NHEJ) and homologous recombination (HR). In somatic cells, the error-prone NHEJ is used to restore DSBs rather than a more precise HR [6]. Since HR is a more complex and involved method that requires inserting both donor DNA and the kSSN expressing cassette into plant cells. An illustration of plant genomic alteration. The ZFN, TALEN, and the more modern improved type II CRISPR/Cas, notably the CRISPR/Cas9 system, are the most often utilized plant genome editing techniques [7] (Table **1**).

Table 1. Successful reports of gene Editing in various crop plants.

Crop Species	Gene Editor	Target Gene	DNA Repair Type	Target Trait	References
Maize	ZFNs	*ZmIPK1*	HR	Herbicide tolerant and phytate reduced maize	[21]
Maize	ZFNs	*ZmTLP*	HR	Trait stacking	[22]
Rice	ZFNs	*OsQQR*	HR	Trait stacking	[23]
Rice	TALENs	*OsSWEET14*	NHEJ	Bacterial blight resistance	[24]
Wheat	TALENs	*TaMLO*	NHEJ	Powdery mildew resistance	[25]
Maize	TALENs	*ZmGL2*	NHEJ	Reduced epicuticular wax in leaves	[26]
Sugarcane	TALENs	*COMT*	NHEJ	Improved cell wall composition	[27]

(Table 1) cont.....

Crop Species	Gene Editor	Target Gene	DNA Repair Type	Target Trait	References
Sugarcane	TALENs	*COMT*	NHEJ	Improved saccharification efficiency	[28]
Soybean	TALENs	*FAD2-1A, FAD2-1B*	NHEJ	High oleic acid contents	[29]
Soybean	TALENs	*FAD2-1A, FAD2-1B, FAD3A*	NHEJ	High oleic, low linoleic contents	[29]
Potato	TALENs	*VInv*	NHEJ	Minimizing reducing sugars	[30]
Rice	TALENs	*OsBADH2*	NHEJ	Fragrant rice	[31]
Maize	TALENs	*ZmMTL*	NHEJ	Induction of haploid plants	[32]
Brassica oleracea	TALENs	*FRIGIDA*	NHEJ	Flowering earlier	[33]
Tomato	TALENs	*ANT1*	HR	Purple tomatoes with high anthocyanin	[34]
Rice	CRISPR/Cas9	*LAZY1*	NHEJ	Tiller-spreading	[35]
Rice	CRISPR/Cas9	*Gn1a, GS3, DEP1*	NHEJ	Enhanced grain number, larger grain size and dense erect panicles	[35]
Wheat	CRISPR/Cas9	*GW2*	NHEJ	Increased grain weight and protein content	[35]
Camelina sativa	CRISPR/Cas9	*FAD2*	NHEJ	Decreased polyunsaturated fatty acids	[36]
Rice	CRISPR/Cas9	*SBEIIb*	NHEJ	High amylose content	[37]
Maize	CRISPR/Cas9	*Wx1*	NHEJ	High amylopectin content	[38]
Potato	CRISPR/Cas9	*Wx1*	NHEJ	High amylopectin content	[38]
Wheat	CRISPR/Cas9	*EDR1*	NHEJ	Powdery mildew resistance	[39]
Rice	CRISPR/Cas9	*OsERF922*	NHEJ	Enhanced rice blast resistance	[40]
Rice	CRISPR/Cas9	*OsSWEET13*	NHEJ	Bacterial blight resistance	[41]
Tomato	CRISPR/Cas9	*SlMLO1*	NHEJ	Powdery mildew resistance	[42]

The use of ZFNs and transcription activator-like effectors in plants was previously limited due to the complexity of the synthesis of proteins, which required a long process. ZFN and transcription activator-like effector systems are more difficult to build than clustered regularly interspaced short palindromic systems, which just require a Cas9 protein and a manufactured sgRNA for DNA binding [8]. The development of the sgRNA sequence allows for the acquisition of added gene targeting properties, and it is this advancement that allows the CRISPR/Cas9

CHAPTER 10

Genome Editing in Medicinal Plants for Abiotic Stress Tolerance

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Abstract: In the changing climate scenarios, living organisms have been facing several biotic and abiotic stresses. Abiotic stresses are the major factors posing huge threats to plants. Drought, heat, and salinity are bigger problems emerging in the world due to climate change. For adapting various climatic conditions, plants trigger several biochemical and molecular mechanisms. At the biochemical level, secondary metabolites play an important role in the survival of plants in uneven situations. Modulation of expression of genes and stress response elements is observed under stress. To cope with abiotic stresses in medicinal plants, the understanding of the biochemical and molecular mechanisms is very important. The use of modern biotechnological approaches along with conventional breeding may be helpful in developing tolerance against abiotic stresses in medicinal plants. This chapter highlights various abiotic stresses, their mechanisms and strategies to develop tolerance against these stresses.

Keywords: Abiotic stress, Adenine base editor, Biotechnological approaches, CRISPR/Cas, Drought, Homologous recombination, Light, Pentatricopeptide repeat proteins, RNA interference zinc finger nucleases (ZFNs), Site-directed sequence editing, Salt stress, Secondary metabolites, Temperature, Transcription activator-like effector nuclease(TALENS), Ultraviolet.

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INTRODUCTION

Medicinal plants, being complicated organisms, are subjected to various physiological and chemical conditions that have a profound effect on plant production, hindering high performance and endangering their existence [1]. Ultraviolet (UV) radiation, drought, high salinity, heavy metals, temperature (high or low), *etc*. are important among the various variables and together they are referred to as abiotic stressors [2]. Any increase in the frequency of abiotic stress affects the plant from germination to maturity [3].

Medicinal plants are useful flora that has chemically active elements in many of their parts and produces a physiological reaction when utilized to cure a variety of diseases. Medicinal plants have been used in countries such as Sri Lanka, China, Thailand, India, Japan, Nepal, and Pakistan since time immemorial [4]. The earliest evidence of medicinal plants' use dates back to 2600 BC from Mesopotamian culture [5]. To meet the ever-growing demands of the pharmaceutical industry, some medicinal plants should now be grown commercially [6]. Plants are at risk of abiotic stressors such as salt, drought, high and low temperatures, heavy metal toxicity, high and low light, nutrient deficiencies, ozone, and UV-B radiation due to continuous changes in the environment. Abiotic stressors are linked to decreased crop productivity and quality [7]. Abiotic stress depletes agricultural production and identified as a major barrier to food security worldwide [8]. The genome-editing technique is used to create a long-term solution to abiotic stress. The adoption of genomic engineering methods for making specific changes in the genome of a plant to improve adaptability, increase yields, and allocate resilience to various stresses has facilitated technological advances. Some of the most important methods for modifying plant genomes include homologous recombination (HR), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), pentatricopeptide repeat proteins (PPRs), the CRISPR/Cas9 system, RNA interferance (RNAi), cisgenesis, and intragenesis. CRISPR/Cas has revolutionized biological research and has greater use in agriculture than the other genome editing techniques. CRISPR / Cas is a powerful way to modify genomes to achieve desirable traits. The candidate genes must be chosen carefully to get the desired features.

Medicinal Plants and Secondary Metabolites (SMs)

In many regions of the world, traditional medicine has been discovered from plants for thousands of years. Plants are categorised as medicinal because they generate secondary metabolites that are advantageous to human health. Along with having medicinal value, these plant's secondary metabolites are crucial for plants to respond to environmental changes [9]. Different categories of medicinal plants may have different therapeutic effects. Alkaloids, terpenoids, and phenylpropanoids are among the second metabolites studied to improve therapeutic value. In cells, these chemicals are made up of series of enzyme-catalyzed processes.Secondary metabolites are chemical compounds unrelated to regular plant growth and development [10]. Natural pharmacologically active compounds with antimalarial, antidiabetic, hepatoprotective, antiulcer, anti-inflammatory, and antibacterial properties are among the secondary metabolites. When there are severe environmental conditions, they are produced in large quantities in various parts of the plant. Natural mutations have a significant impact on the control of several secondary metabolic processes. Stressful situations induce secondary metabolite's concentration to rise in medicinal plants because they have an impact on the genes engaged in secondary metabolic pathways.

Natural Factors that Affect Secondary Metabolite's Development Include

A local and temporal strategy carefully controls the integration and collection of SMs, which is also affected by biotic and abiotic environmental factors. Variations in SMs biosynthesis are caused by adverse abiotic circumstances, such as droughts or floods, excessive light or heat, poor soil quality, or the presence of harmful substances [11].

Light

An essential abiotic component needed by plants for photosynthesis, development, and the buildup of secondary metabolic products is exposure to photons of different durations and intensities [12]. High irradiance can occasionally promote plant growth. SMs production and different plant species have their own ideal light set (quality and quantity), capable of creating high yields for SMs [13].

Ultraviolet Light

In medicinal plants, exposure to ultraviolet (UV) light has a typical impact on SMs. For instance, the UV-B radiation of Chrysanthemum enhanced the content of flavonoids and phenolic acids [14]. While UV light exposure generally has a good impact on the production of SMs, excessive UV-B and UV-C radiation has a negative impact on plant growth, development, photosynthesis, and other critical functions [15]. Response of different medicinal plants under UV stress is described in Table **1**.

CHAPTER 11

Genome Editing for Biotic Stress Resistance in Medicinal Plants

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Abstract: Plants are continually subjected to a range of physical and biological stressors throughout their growth period. Insects and pests, like other biotic stressors, have created significant concerns about lower productivity, which jeopardizes agricultural production. Genome engineering, also known as genome editing, has emerged as a cutting-edge breeding technique capable of altering the genomes of plants, animals, microbes, and humans. Since ancient times, humans have used medicinal plants for food, medicine, and industrial purposes. Both traditional biotechnology and more recent next-generation sequencing (NGS) methods have been used successfully to improve natural chemicals derived from plants with medical potential. To modify the genome at the transcriptional level, protein-based editing approaches like zinc-finger nucleases (ZFNs) and transcription activator-like end nucleases (TALENs) were previously frequently employed. CRISPR/associated9 (Cas9) endonucleases are a powerful, resilient, and precise site-directed mutagenesis method in transcriptome gene editing. CRISPR/Cas9 genome editing employs specially created guide RNAs to detect a three-base pair protospacer adjacent motif (PAM) sequence situated downstream of the target DNA. The current review compiles current research published between 2010 and 2020 on the use of CRISPR/Cas9 genome-editing technologies in traditional medicines, describing significant innovations, difficulties, and prospects, as well as noting the technique's broader application in crop and lesser species. The CRISPR/Cas9 genome editing method has been utilised successfully in plants to boost agricultural productivity and stress tolerance.

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Despite this, only a small number of medicinal plants have been altered using the CRISPR/Cas9 genome editing technique because to a lack of appropriate transformation and regeneration techniques, and also a lack of comprehensive genome and mRNA sequencing data. However, a variety of secondary metabolic activities in plants (*e.g.* alkaloids, terpenoids, flavonoids, phenolic acids, and saponin) altered lately using CRISPR/Cas-editing through knocking out, knocking in, and point mutations, modulation of gene expression, including targeted mutagenesis.

Keywords: Biotic stress, CRISPR/Cas9, Diseases, Insect/Pest, Stress resistance.

INTRODUCTION

Previously, zinc-finger nucleases (ZFNs) and transcription activator-like endonucleases (TALENs) were described as genome editing tools for manipulating the transcriptome at the transcriptional level [1]. ZFNs and TALENs both use a protein-based nuclease called Fok1 to knock out genes by inducing double-stranded breaks (DSBs) in the target DNA [2]. Additionally, tools have become less effective as a result of the presence of that nucleotide sequence and the ability of both proteins to dimer in the right orientation for the given spacer length [3].The clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) technology provides several benefits over ZFNs and TALENs, including simplicity of use, ease of multiplexing, and capacity for large-scale library production [4].

The development of the CRISPR/Cas9 system does not need protein dimerization, and now allows very effective gene modification *via* the use of Tailored gRNAs that identify a three-base pair PAM region that is located downstream of the target gene [5]. CRISPR/Cas9 is an efficient and simple technique for gRNA genome editing. This technique is well-known for two significant benefits: I) the point mutation is entirely silenced, while RNAi dosage variation results in partial suppression; II) genomic modifications are durable and readily maintained in progeny, making them very useful in plant breeding [6].

Genome editing facilitated by CRISPR/Cas9 is used to generate site-specific breakage in DNA strands inside the gene, that are then repaired *via* the cell NHEJ or HDR machinery [7]. However, random addition or removal (indels) mediated by NHEJ mostly affects the open reading frame sequences that code for proteins or eliminates non-coding sequences cis-regulatory elements (promoters, regulators, *etc.*) [8]. Plants and plant-derived products have been utilized as a significant supplier of foods, medicines, health supplements, and also biomaterials for humans from time immemorial [9].

Natural compounds derived from plants have been identified as possible therapeutic targets for reducing human mortality and morbidity [10]. More than a few preclinical studies have taken conducted and proven the effectiveness of medicinal herbalism against a variety of disorders [11]. Numerous biotechnological treatments have been used to alter mechanisms in plants that lead to the synthesis of medicinal, nutritional, and industrial secondary metabolites [12]. Next-generation sequencing (NGS) methods have shown tremendous potential for evaluating the genetic diversity and metabolic pathways of medicinal plants. The breaks are repaired utilizing the host cell's repair process, either by a pathway of non-homologous end joining (NHEJ) or through Homologous recombination, which is a process that leads to the change of genes by utilizing a donor fragment. Genome editing uses four different kinds of nucleases to enhance plant resistance mechanisms that include mega nucleases ZFNs, TALENs, and the CRISPR–Cas9.

The production at an incredible rate of CRISPR/Cas9 research demonstrates that CRISPR technique requires lower financial resources with a greater percentage of success for gene amplification and manipulation than other current methods. CRISPR/Cas9 is a very effective tool for enhancing agronomic characteristics in plants [13]. *Streptococcus pyogenic* CRISPR/Cas9 system (SpCas9) has swiftly become indispensable in a variety of fields in plant study, and also in a variety of other fields of research. An sgRNA is used for this mechanism along with Cas9 to target DNA regions [14, 15].

The PAM element in a target gene reduces the possible target sequences. This constraint is irrelevant in the context of targeted mutagenesis [16]. CRISPR/Cas is a kind of bacterial defense mechanism that is particularly effective against bacteriophages, that may be used to modify specific genes in a variety of species. Diseases have posed a significant danger to our agriculture and manufacturing. Innovative biotechnological breeding procedures, like targeted genome modification, have also been touted as a good toolset for producing therapeutic plants with optimal secondary metabolite profiles [17]. The molecular mechanism of CRISPR-Cas9 editing tools as efficient genome editing approaches in herbal medicines, with an emphasis on recent innovations, limits, and possible future prospects are shown in Table **1**.

BIOTIC STRESSES IN PLANTS

Plants are susceptible to a large variety of pathogens that cause a variety of diseases. As a substitute, various herbicides, insecticides, and fungicides have been utilized, harming the environment directly and indirectly [29, 30]. Additionally, these compounds have an impact on the sequence of metabolic events in plants [31]. Thus, CRISPR/Cas9 technique is used to confer resistance

SECTION 3: NANO-ENGINEERING OF MEDICINAL PLANTS

CHAPTER 12

Medicinal Plants: Traditional Trends to Modern Therapeutics

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Abstract: Medicinal plant therapies are becoming more common, as more people seek natural cures and health approaches devoid of synthetic chemicals' adverse effects. The biological and pharmacological potential of plants is studied and utilized all around the globe for various purposes including the treatment of infections and diseases owing due to bioactive compounds in plants produced as a result of secondary metabolism. The study of medicinal plants is helpful in clinical trials to find pharmacologically useful chemicals, and this method has produced thousands of valued medicines. Opium, aspirin, quinine, and digoxin are some examples. Plants possess a large number of bioactive compounds. On the basis of their chemical structure, they are divided into four classes: alkaloids, flavonoids, tannins, and terpenes. Plants can now be turned into "factories" that create therapeutic proteins, vaccines, and many more products for use in the production of biotech pharmaceuticals, medications, and therapies. This chapter discusses the diversity and importance of medicinal plants in various sectors as well as highlights the successful drug products produced by the said entities and their future trends.

Keywords: Commercial value, medicinal plants, modern Phyto-therapeutics, plant-based drugs, secondary metabolites.

INTRODUCTION

Medicinal plants are defined as plants or herbs imparting therapeutic characteristics or that have a positive pharmacological effect on the human or animal body. Recently, there has been a lot of focus on using environmental friendly and bio-friendly solutions derived from plants for the inhibition and cure of many diseases. According to WHO, herbal remedies are used by 80% of individuals around the globe for various aspects of their healthcare. According to

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WHO, some 21,000 species of plants are considered to have the potential for disease prevention and cure [1].

Numerous plants have been utilized for a variety of medicinal purposes from the beginning. Indian Vids, Unani Hakims, and the Mediterranean and European cultures have all used plants as medicine for over 4000 years, according to evidence. Traditional medical knowledge is still valued around the world. Increased therapeutic use of plants is a consequence of insufficient drug supply, out-pricing of medications, adverse consequences of most of the man-made compounds, as well as resistance development against medications that are being used for various infectious diseases [2].

DIVERSITY OF MEDICINAL PLANTS

Over 50,000 species of plants are employed for pharmaceuticals and healthcare goods, accounting for over one-tenth of all plant species. The distribution of therapeutic plants, on the other hand, isn't constant around various countries. For instance, India and China contain the most medicinal plants, with 7500 and 11,146 plant species, respectively, accompanied by the USA, Thailand, Philippines, and many other countries, with share of medicinal plants ranging from 6% in Malaysia to 16% in India compared to total species of plant. Fig. (**1**) shows the diversity of plant species around the world. Not only do some plant families have more medicinal plants than others, but they also have a higher proportion of vulnerable species [3].

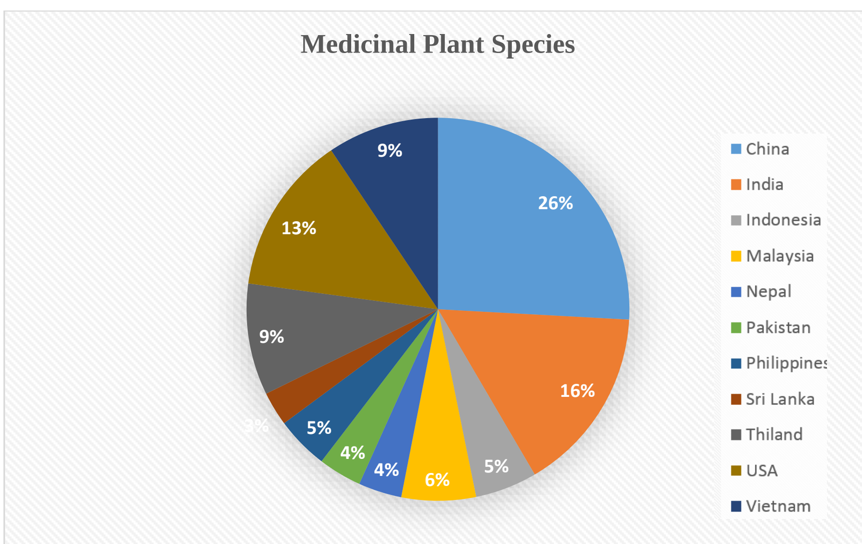

Fig. (1). Percentage of medicinal plant species present around the world.

Approximately seventy-five percent of the population gets their health care primarily from plants and plant extracts. Over one-third of all species of plants were used for medicinal purposes at some point. In developed countries such as America, plant pharmaceuticals are predicted to make up up to 25% of overall drugs, but in developing economies such as China and India, they might account for nearly 80%. As a consequence, the strategic value of medicinal plants in India exceeds that of the entire world. Two third of plant species being used in Western medicine are found in these nations, and the indigenous nation's primary care is based on traditional medicine [4].

MEDICINAL PLANTS IN PAKISTAN

In Pakistan, at least 12% of the flora is utilized medicinally. Baluchistan and Northern Pakistan is the hotspot of many medicinally important genera, including Allium, Cousinia, and Astragalus. Pakistan's plant hotspots are found over 13 natural regions, ranging from mangrove forests to alpine pastures [5]. Examples of some of the species having medicinal importance include *lantago ovata, Berberis lyceum, Papaver somniferum, Zingiber officinale, Foeniculum vulgare,* and *Cichorium intybus*. *Zingiber officinale* and *Foeniculum vulgare* are often used to treat gastrointestinal issues. *Berberis lyceum* is a valuable species used to treat diabetes, joint problems, and liver ailments. *Piper nigrum, Berberis lyceum, Plantago ovata, Butea monosperma, Rubia cordifolia, Zingiber officinale*, and *Nigella sativa* are among the other prominent species with a high usage value [6].

Traditional Plants

Plants as a source of medicines are thought to be safer, as they exhibit little or no adverse effects, than their synthetic counterparts. Medicinal plants are believed to contain a wide range of constituents that are employed to generate pharmacopoeial or synthetic medicines. Moreover, since some plants are considered as an essential source of nourishment, they are prescribed for various therapeutic applications [7]. In addition to medication, plants are used in organic dyeing, pest management, culinary, fragrance, herbal teas, as well as many other purposes. Certain plants and herbs are utilized in various parts of the world in order to prevent homes and other places free from flies, ants, fleas, and mice. Medicinal herbs have become key sources of biopharmaceuticals [8].

Herbal plants like black pepper, myrrh, cinnamon, ginseng, sandalwood, aloe, clover, barberry, and safflowers are known for treating sores, wounds, and sores. Basil leaves, chives, fennel seeds, cilantro, mint, apple, oregano, lemon balm, thyme, sage, and rosemary stand among the most useful medicinal herbs. Many plants are consumed to purify the blood, to help transform or improve a longstanding condition by getting rid of metabolic toxins. 'Blood cleansers' is

CHAPTER 13

Nanotechnology in Medicinal Plants

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Abstract: Nanoparticles have immense applications in plants from mass propagation to phyto-drug extraction and augmentation. Alongside, nanoparticles are also manifested as potential drug vehicles for carrying curative agents to the targeted tissues or part, accompanying control delivery of drugs to the infected site. Advancement in nanotechnology directed towards the transformation of metallo-drugs at the nanoscale brings new dimensions in therapeutics from the treatment of multidrug-resistant microbes to chemotherapies of tumors. With the nano-advancement, not only metals and their oxides are transformed at the nanoscale but also the potential phyto agents, proteins, and hormones are transformed into nanosized entities which change the entire fundamentals of therapeutic and curative practices. A lot of changes in medicine, drug delivery system and drug formulation as commenced just because of nanotechnology. The current chapter highlights nanotech advancements in the area of medicinal plant propagation, drug augmentation and extraction methodologies along with their limitations and future prospects.

Keywords: Nano-vehicles, Nanotized Herbs, Plant Therapeutics, Pharmacotherapy, Phyto-drugs.

INTRODUCTION

Plants are natural madicines for the treatment of numerous ailments and are a fundamental element of human societies throughout history. Medicinal plants not only account for the economic resilience of poor people in developing countries but also play a critical role in traditional as well as modern medicine [1]. Diversion of modern society towards natural products has been associated with low or no side effects which were obvious in conventional medication [2]. Complementary and alternative medicine (CAM) gained extensive scientific as well as commercial consideration. According to the World Health Organization, about 80% of people worldwide rely on herbal medicines to accomplish their primary healthcare needs. Like in Europe, 90% of wild resources are harnessed

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for therapeutic purposes which account for the use of more than 1300 medicinal plant species. Similarly in the United States, about 79% of top prescriptions belong to natural resources [3]. The Himalayan region, parts of Bangladesh, Nepal, India, Afghanistan, Pakistan, Myanmar, Bhutan and China are acknowledged as a hotspot of medicinal plant diversity [4].

Presently, France, the UK, the USA, Italy and China are found to be the biggest global market for medicative plants. In Asia, Pakistan is ranked as 7th medicinal plant producer [5]. Around 600 species are reported for their uses as traditional medicine in Pakistan and more than 75% of the local population count on herbal medicines for most of their health issues. Substantial use of medicinal flora is in medicine cosmetics, dietary supplements and food manufacturing [6]. The majority of the indigenous population is still dependent on plant-based remedies. Other reasons for the increasing use of herbal medicines in all cultures are due to efficacy, unique targeting ability, minimal side effects and safety, which are questionable in the case of synthetic medicines [7]. This development directed towards an increase in demand for herbal medicines. Industrialized and developing countries scrutinize herbal products for the past 40 years. Moreover, herbs were redeemed with the advancement in technologies as a prospective source of new drug candidates. This entire condition led to the use of common as well as endangered medicinal plants.

PHYTOCHEMICALS IN PHARMACOTHERAPY

The plant produces diverse bioactive compounds with unusual chemical scaffolds. Bioactive compounds in medicinal plantsplay a key role in anti-analgesic, antifungal, antioxidant, antibacterial, anti-dysentery, antiviral, anti-malarial, anti-inflammatory, and anticancer activities. The polypharmacological effect of herbal constituents increased the activation of pro-drug, bioavailability, synergistic action and interference with cellular transport processes. These phytoconstituents have a lot of therapeutic effects *i.e.* against skin disorders, dyspepsia, diabetes, aphrodisiac, deflatulent, polyurea, jaundice, and haematological disorders. Bioactive compounds are plant secondary metabolites having both toxicological and pharmacological effects on animals and mankind. These bioactive compounds are derived from primary plant metabolites, mainly performing photosynthesis. Two main pathways are involved in the biosynthesis of these principal compounds *e.g*., the mevalonic acid pathway and shikimic acid or aromatic amino acid pathway [8]. A diverse group of secondary metabolites are named as active compounds *i.e*., alkaloids, oils, steroids, glycosides, enzymes, phenolics, resins and terpenoids. The presence of one or more active compounds is ascribed to the medicinal values of plant crude extracts/drugs.

Medicinal plant-based drugs normally belong to one of the following groups *i.e*. taurine, choline, fatty acids, polyphenols, prebiotics, caffeine, polysaccharides, carotenoids and phytoestrogens [9, 10]. Major classes of phytoconstituents with disease-preventing functions are anticancer, dietary fibre, immunity boosters, antioxidants, and neuro-pharmacological and detoxifying agents. Each class of these phytoconstituents has a wide array of chemicals with variant potencies. The function of phytochemicals can be decided by the type of functional group present on its side chain. Bioactive compounds of medicinal plants are endowed with boundless opportunities for new drug leads [11]. A substantial quantity of newly registered drugs have been derived from natural sources (plants) like paclitaxel and docetaxel from *Taxus brevifolia,* artemisinin from *Artemisia annua,* etoposide and teniposide from *Podophyllum* species [12]. About 1073 chemical entities had been approved from 1981-2010, which belonged to the small molecules group. Out of these 1073, 64% were either extracted or inspired by natural sources, while only 36% were purely synthetic [13]. Indispensable plant-based bioactive compounds which were notably used in pharmacotherapy are listed in Table **1**.

Table 1. Bioactive compounds used in pharmacotherapy.

Common Name	Scientific Name	Family	Drug Name	Therapeutics	References
Pacific yew tree	*Taxus brevifolia*	Taxaceae	Paclitaxel and its derivatives	Anticancerous	[14]
Periwinkle / Vinca rosa	*Catharanthus roseus*	Apocynaceae	Vinblastine and Vincristine	Anticancerous	[15]
Happy tree/cancer tree	*Camptotheca acuminata* Decne	Nyssaceae	camptothecin and its analogs	Anticancerous	[16]
Common snowdrop	*Galanthus nivalis* L	Amaryllidaceae	Galanthamine	Alzheimer's disease	[17]
Wormwood or sweet sagewort	*Artemisia annua* L	Asteraceae	Artemisinin	Antimalarial and anti-cancerous	[18]

Various compelling plant-based bioactive compounds *i.e*., Podophyllotoxin, Paclitaxel, and Vinblastine were unable to meet the growing demands of the market. Because these species were slowly grown in their natural environment, some were even endangered due to over-exploitation. Moreover, the natural accumulation of these compounds is very low, over a long growth period. Besides this, they were also produced in very low concentrations in their natural green source [19, 20] The promising protective approach to avoid the extinction of these endangered medicinal herbs has been the cultivation of these plants under controlled conditions [21].

CHAPTER 14

Metallic Nanoparticles Synthesized Through Medicinal Plants: Therapeutic Improvement

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Abstract: The field of nanotechnology has developed new medicinal nanoparticles that have various uses in pharmaceutics and healthcare. Distinct macroscopic and microscopic entities including plants, fungi, microalgae, bacilli, and seaweed have been used to biosynthesize nanoparticles. Naturally-occurring chemicals like flavonoids, tannins, alkaloids, steroids, and saponins are abundantly present in plants. A potentially unharmful method to produce nanoparticles can be through extracts of different plants. As plant extracts carry many specialized metabolites, they can act as stabilizers and reducers in bioreduction reactions that take place in metallic nanoparticle production. The production of metallic nanoparticles by biological techniques is an easier, cheaper, and more environmentally sound option in comparison with other physical and chemical techniques that are extremely toxic and unsafe for biological use. Greener nanoparticles like Co, Cu, Ag, Pd, Au, ZnO, Pt, and Fe_3O_4 have been biosynthesized using medicinal plants. These nanoparticles have various uses in pharmaceutics ranging from gene delivery, drug delivery, pathogen detection, tissue engineering, and protein detection. Not only that but, metallic nanoparticles can also potentially be remedies to different acute diseases including hepatitis, human immunodeficiency virus, malaria, and even cancer. Improvements in drug delivery and tissue engineering have been made possible by nanotechnology and this has greatly facilitated translational level studies that relate to pharmaceutics. In this chapter, green syntheses of metallic nanoparticles through medicinal plants along with their uses in therapeutic improvements are described.

Keywords: Medicinal plant, Metallic nanoparticles, Nano-packaging, Nano-degradation, Phytotherapy, Reducing agent.

INTRODUCTION

Phytotherapy employs plant extracts as medicines to cure diseases. The majority of phytochemical formulations have been in use long before contemporary medicines, since the Stone Age. Improvements in the phytotherapy domain by

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different techniques and procedures have enabled the engineering of new drugs through conventional formulations. Conventional healthcare approaches include several concepts which are focused on the balance between the atmosphere and body functions [1]. Medicinal plants are being used for centuries; however, their existence has been imperiled recently thanks to contemporary approaches to alternative medicine. Approximately 85 percent of the global populace utilizes phytotherapy and plant-derived chemicals and medicines for treating a variety of illnesses [2]. A majority of phytoconstituents have been utilized for manufacturing medicinal goods since the chemicals produced in plants have a variety of recognized advantages [3]. These chemicals, also called specialized metabolites, are not direct contributors to the developing stages of a plant but rather are involved in the defensive mechanism of plants.

Secondary metabolites assist in plant-surrounding interactions thus giving plants a greater survival rate. The development of these metabolites has enabled intimate interactions of plants with a variety of prokaryotes [4]. Many works of research conducted on plants suggest that these specialized metabolites could be used as raw materials in artificial or chemically synthesized biologically active pharmaceutical compounds. Secondary metabolites have been discovered to be structurally comparable to chemical messengers or hormones. This offers a diverse spectrum of plant-based therapeutically important compounds. Furthermore, such metabolites are prevalent in combinations, this increases the absorption and effectiveness of the compounds while minimizing crop resistance [3]. Species possessing powerful therapeutic qualities include *Curcuma longa* (reduces inflammation and risk of cancer), *Withania somnifera* (increases bile pigment concentrations), *Aloe barbadensis* (reduces inflammation), *Ocimum tenuiflorum* (reduces toxicity), and *Zingiber officinale* (aids in digestion) [5].

Garcinia indica, Magnolia champaca, creeping rootstalk of *Curcuma aromatica, Vernonia anthelmintica* seedlings, and *Nyctanthes arbourtristis* foliage, are well-known plant species utilized in fighting different infections [6]. *Ruta graveolens* and *Ambrosia arborescens* foliage has been used as excellent blood purifiers. The bloom and foliage of *Borago officinalis* are used for treating sore throats, coughs, and other related problems. When compared, most components of *Matricaria recutita L*. are reported for alleviating abdominal pain as well as similar gastrointestinal problems [7]. Specialized metabolites include alkaloids, flavonoids, isoprenoids, and phenols hold great therapeutic potential. Isoprenoids are compounds present in nature that act as transmitters among symbionts, multiple plant bodies, or plant species with pollinators [8].

Isoprenoid-rich plant species have several therapeutic characteristics, including chemoprevention, bactericidal, fungicidal, virucidal, and inflammation-reducing activities [9]. Peppermint camphor, cannabinoid, and qinghaosu are examples of isoprenoids that have been widely utilized in pharmaceutical medicines. Paclitaxel, a well-known chemotherapeutic drug recognized for its mitosis-inhibiting properties in cancerous tissues, is an isoprenoid derived from*Taxus brevifolia* [10]. Alkaloids are nitrogenous plant compounds often produced as wound responses. Therefore, alkaloids have been widely used in medicine development in contemporary healthcare. Researchers have reported bactericidal, inflammation-reducing, and antioxidant properties in alkaloids derived from diverse plants like *Rutaceae* or *Solanaceae*. Cocaine, galantamine, caffeine, calotropin, quinine, and nicotine and phenols are the most common type of specialized metabolite present in a broad variety of plant species [11].

Secondary metabolites in addition to their role in providing pigment and flavor are extensively researched in regard to their antioxidative, inflammation-reducing, antibacterial, immune-modulating, and disinfecting characteristics. Phenols derived from plants are well-recognized for their ability to induce oxidative stress and potentially interfere with intracellular processes. Plant phenols like ellagic acid and glucose ester are present in berry plants like strawberries and have been shown to have chemotherapeutic effects on prostate, mouth, and colorectal carcinoma. This further insinuates the biological importance of plant-based phenolic compounds [12]. Flavonoids are plant polyphenolic compounds essential for the coloring of flowers. Flavonoids are frequently utilized for medicinal use because of their virucidal, anti-allergy, cancer-fighting, oxidation-inhibiting, and inflammation-reducing abilities [8]. Quercetin, a pharmaceutically important flavonoid, possesses oxidation-inhibiting and inflammation-reducing properties that could be utilized for preventing tissue injuries [13].

Limonin and naringin are citrus-based flavonoids that show chemotherapeutic effects and can scavenge free radicals [12]. Specialized metabolite classes such as aetheroleums, glycosides, tannins, and saponins have been in use in conventional medicine. Other metabolites like flavonoids and alkaloids have been reported to act as reducers and stabilizers to sustainably produce nanoparticles. Following nanoparticles have increased capability of reducing metal salts plants that contain a high ratio of secondary metabolites. Microscopy, Zeta potential, Dynamic Light Scattering, and Ultraviolet-Visible spectroscopy are some of the techniques that are commonly used to study nanoparticles. Nanoparticles that are created are coated with different required medicinal metabolites that have various benefits. They are easily used in food, remediation, medicinal, agricultural, environmental, and pesticide industries due to increased surface area and small size, and high reactivity ratio. Synthesis of both plant-based nanoparticles and green carbon dots that could be applied as nanotherapeutics agents have been made possible due to advancements in the field of green nanotechnology. Insufficient data on toxic

CHAPTER 15

Carbon Nanostructures and Medicinal Plants

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Abstract: It has been a decade since the widespread usage of carbon nanostructures (CNSs) in biomedical research. A few examples are the use of CNSs in medication, for protein administration and in instruments to provide nucleic acids to treat cancer and other chronic diseases. The near-infrared optical characteristics of CNSs allowed them to be used in diagnostics and in non-invasive and very sensitive imaging equipment. In recent years, the scientific and industrial sectors have paid increasing attention to the physical and chemical properties of various nanomaterials. Structure, electronics, water, and more may all be derived from them. This chapter will focus on carbon nanomaterials and related nanostructures, which are designed to give the most up-t--date research results. There is a broad acceptance of traditional medicine in many societies, with over 60 percent of the world's population and over 80 percent of the population in developing countries depending on medicinal plants for medical reasons. Among the many reasons for this are the ease of use, affordability, and low cost. It is believed that nanotechnology will play a significant role in medicinal plant research and drug delivery in the near future. These nano-drug delivery devices may boost the activity of medicinal plants, but also solve some of their limitations. Nanocarriers aiding in the treatment of cancer, diabetes, and other life-threatening illnesses by delivering herbal chemicals will also be discussed in this chapter.

Keywords: Biological cargoes, Carbon nanostructures (CNSs), Laser ablative therapy, Multiwalled carbon nanotubes (MWCNT), Nanocarriers.

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INTRODUCTION

Buckyballs' discovery in 1985 sparked a fast expansion of the carbon family. Fullerenes, carbon nanotubes, graphene, and nanodiamonds all have different shapes and forms (Fig. **1**) owing to the occurrence of three hybridization types of carbon: sp^3 (the most common), sp^2 (the second most common), and sp^1 (the third most common). Physical and chemical qualities have attracted growing attention from the scientific and industrial worlds in recent years. Structure, electronics, energy, water, and more are all possible uses of them. Carbon nanomaterials and related nanostructures will be covered in this special issue, which is intended to provide the most recent research findings in this field. In addition to CNTs and graphene, the articles that have been approved span a wide range of carbon-based materials including amorphous carbon films, carbon nanocomposite, graphite-like layered materials, carbon nanofibers, and so on. They cover a broad variety of subjects relevant to the production and uses of these nanocarbon materials in the disciplines of mechanics and tribology; thermal management; power generation through photovoltaics; energy storage *via* lithium-ion batteries; and medication delivery *via* photocatalysis. Carbon nanomaterials' electrical, optical, and surface adsorption characteristics have been calculated theoretically using density functional theory in two studies as well [1].

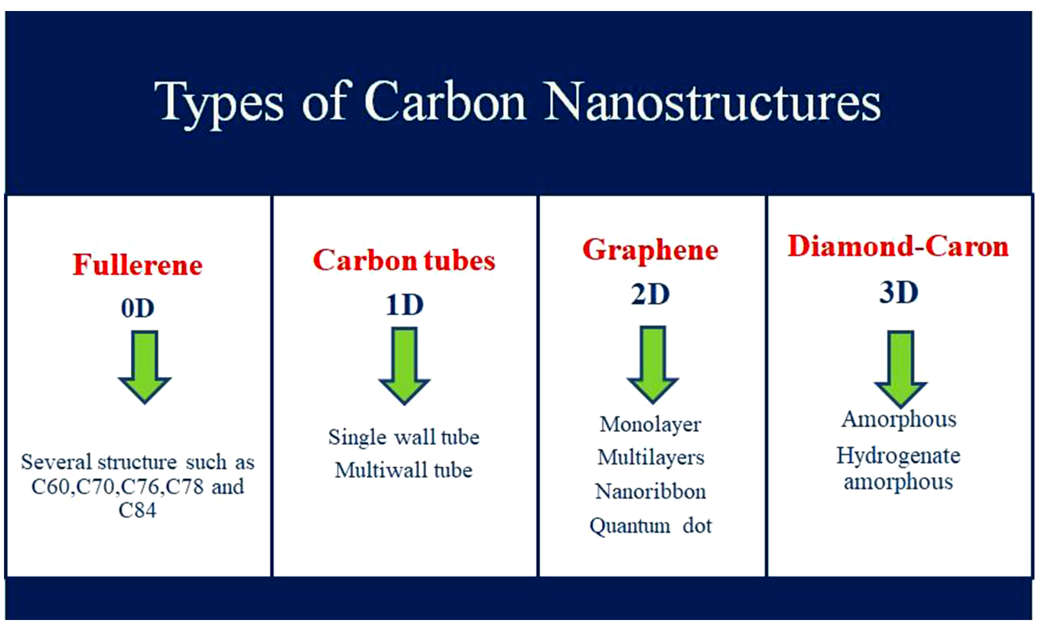

Fig. (1). Various carbon nanostructures.

Carbon Nanotubes

A Carbon Nanotube is a tube-shaped carbon-based material with a nanometer-wide width. To put it in perspective, that's one billionth of a millimetre, or a thousand times smaller than the width and length of an average human hair [2].

For example, carbon may be used to build structures with diverse characteristics by combining in different ways. Carbon's sp^2 hybridization creates a multilayer structure with weak van der Waals bonding and strong in plane limits. Around an average hollow, a few to several dozen MWCNT cylinders with regular periodic interlayer spacing are arranged in concentric cylinders. For multiwall nanotube pictures analysed in real space, we found a wide variety of interlayer spacings (0.34 to 0.39 nm) [3].

There is a continuous hexagonal lattice with carbon molecules at the corners of the hexagons in graphite's layer.

The structures of carbon nanotubes may be used to classify them:

- Nanotubes with one wall (SWNT)
- Nanotubes with several walls (MWNT)

CARBON NANOTUBES SYNTHESIS

Carbon nanotubes are typically synthesised using one of three techniques: arc discharge, laser ablation, or chemical vapour deposition (CVD). Each approach may be tailored to the researcher's individual study objective.

1. Arc Discharge
2. Deposition of Chemical Vapors
3. Laser ablative therapy

Arc Discharge

MWNTs and SWNTs were first synthesised using an arc discharge. The anode and cathode of an arc discharge are typically made of two high-purity graphite electrodes [4]. In an environment of 400 mbar of Helium, a DC current (about 100 A°) was sent through two high-purity graphite separated (by about 1–2 mm) electrodes, causing them to melt. A carbon rod is formed at the cathode of an experimental arc discharge equipment after a length of time of arc discharge. MWNTs may be made using this process, but SWNTs can also be made by adding a metal catalyst, such as Fe, Co, Ni, Y or Mo, to either the anode or the cathode of the reactor [5].

CHAPTER 16

Nano Elicitors and Bioactive Plant Metabolites

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Abstract: Nature has given plants the ability to produce a wide variety of secondary metabolites including alkaloids, phenolics, terpenoids and saponins. These metabolites provide them a defense mechanism against biological and non-biological stress factors. On the other hand, the same metabolites have proved to be effective against different dreadful human diseases. The efficacy of such metabolites ranges from antimicrobial to anticancerous effects. Bioactivity-guided characterization is one of the useful strategies that have been employed to identify, purify and characterize active components. These bioactive components have proved useful in future drug discovery. Elicitors are defined as signaling metabolites with the ability to induce biochemical and physiological processes in plants resulting in the activation of plants defense mechanisms. Elicitation is a useful tool as it leads to the generation of stress conditions and hence the accumulation of bioactive secondary metabolites in plants. Various strategies have been adopted to enhance the production of bioactive secondary metabolites including plant cell and tissue culture and use of signaling metabolites. Nowadays, nano-elicitors have emerged as an effective tool to enhance the production of pharmacologically important compounds. Various classes of nanoparticles (NPs) have been reported to be utilized as nano-elicitors like metallic NPs, metallic oxide NPs and carbon nanotubes with positive effects on phytochemical profile. The possible mechanism of nanomaterials as elicitors is the interaction with plant genomes by increasing the expression level of genes involved in the biosynthesis of active metabolites. Despite triggering biosynthetic potential of plants, certain negative effects have been observed in plants' primary metabolism like lower chlorophyll content, a decrease in cell viability, a decline in sugar content and suppressed seed germination. Thus, there is a need to develop biocompatible nanoparticles for use as nanoelicitors in plants to avoid the negative impacts of the used entities.

Keywords: Bioactive metabolites, biocompatibility, carbon nanotubes (CNT), nano-elicitors, nano-tubes.

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INTRODUCTION

Phytochemistry is the foundation of medicinal practices based on herbal products [1]. A good grasp of plant biochemistry can lead to a better comprehension of its potential medicinal value. Primary metabolites' role in essential life functions, such as growth, reproduction, cell division, respiration, and storage, has been described by modern chemistry [2]. By-products of activities like the Krebs or citric acid cycle, glycolysis, photosynthesis, and related pathways produce these metabolites.

Phytochemicals are a group of biochemical molecules produced by the plant cell due to the primary metabolic pathways. Albrecht Kossel, the Nobel Laureate in Physiology or Medicine in 1910, coined the term “secondary metabolite.”Czapek, on the other hand, classified them as end-products after thirty years. Secondary metabolites, according to Czapek, are formed as a result of secondary nitrogen metabolism changes. Progress in analytical techniques, such as chromatography, enabled the isolation of an increasing number of these compounds in the mid-twentieth century, laying the groundwork for establishing phytochemistry as a study.

Phytochemicals with antiviral, antibacterial, and antifungal properties can protect plants from infections. They also produce UV-absorbing chemicals, which protect the leaves from the harmful effects of direct sunlight. Some forage grasses, such as clover or alfalfa, have been found to have estrogenic characteristics and interact with the fertility of grazing animals. These metabolites are natural sources of biologically active compounds that are widely utilized in commercial and healthcare industries. Plants have attributed a significant role in both Western and conventional medicine systems. For thousands of years, plant-derived therapeutic agents have been a part of the evolution of human healthcare [3].

Classes of Bioactive Metabolites

Plant metabolites are classified into various groups based on their chemical structures. These classes include:

Phenolics

Compounds with hydroxylated aromatic rings, with the hydroxy group connected directly to the phenyl, substituted phenyl, or other aryl groups, are phenolic compounds. With over 8000 known structures, phenolic compounds are extensively dispersed in plants and are among the most abundant secondary metabolites [4]. Plant phenolics are primarily responsible for defense against predators, pathogens, parasites, and ultraviolet radiation. They are universally found in all plant parts and are an integral part of the human diet [5].

Phenolics are well-known components of plant meals (cereals, legumes, fruits, vegetables, olive oil, chocolate, *etc*. and beverages (beer, tea, wine, coffee, and so on), and are partially responsible for the organoleptic characteristics of these foods. For instance, they add to the astringency and bitterness of fruits. Flavonoids, phenolic acids, tannins, and the less well-known stilbenes and lignans, are all examples of plant phenolics [6]. There are two types of phenolic acids: benzoic acid derivatives like gallic acid and cinnamic acid derivatives like coumaric, caffeic, and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, and it is frequently esterified with quinic acid, as in chlorogenic acid, the most abundant phenolic compound in coffee. Ferulic acid, which is found in cereals and is esterified to hemicelluloses in the cell wall, is another frequent phenolic acid [7].

Flavonoids

Flavonoids are a type of polyphenol that is abundant in human diets. The flavan nucleus is its basic structure, consisting of 15 carbon atoms grouped in three rings (C6-C3-C6). Flavones, flavonols, flavonols, flavanones, isoflavones, and anthocyanins are the six subgroups of flavonoids [8]. Because of the degree of methoxylation, glycosylation, prenylation, and hydroxylation in each subgroup, structural diversity in each subgroup is relatively high. Quercetin, a flavonol abundantly found in broccoli, onion, and apple; catechin, an anthocyanin found in tea; cyanidin-glycoside, an anthocyanin found in berries; naringenin, a flavanone abundantly found in grapefruit; and isoflavones, glycitein, and daidzein in soybean.

Tannins

Tannins are another important class of polyphenols involved in human diets and can be categorized into two classes. Gallotannins and ellagitannins are two types of hydrolyzable tannins with a core of glucose or another polyol esterified with gallic acid or hexahydroxydiphenic acid. The various options in oxidative linking are primarily responsible for their structural diversity. Many oligomeric compounds with molecular weights of 2,000 to 5,000 Daltons result from intermolecular oxidation among molecules.

Flavan-3-ol oligomers or polymers are linked *via* an interflavan carbon bond in condensed tannins. They are sometimes referred to as proanthocyanidins because, when heated in a low pH alcoholic solution, they break down into anthocyanidins *via* an acid-catalyzed oxidation reaction [9].

CHAPTER 17

Nanocarriers: Promising Vehicles for Controlled Bioactive Drug Delivery in Current Medical System

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Abstract: Nanomaterials have been widely employed in the medical profession in recent decades, thanks to the rapid development of nanotechnology. Their distinctive physical and chemical qualities, such as minimal size, functionalized surface characteristics, stable interactions with ligands, high carrier capacity, and ease of binding with both hydrophilic and hydrophobic substances have made them ideal platforms for the target-specific and controlled delivery of micro-and macromolecules in disease therapy and have revealed an excellent potential pertaining to clinical entities with the goal of fine-tuning bioavailability, bioefficacy, and pharmacokinetics. The absorption, post-administration stability as well as bioavailability of bioactive drugs and other medicinal substances are the key issues. Some critical medications have low gastrointestinal absorption and permeability in their active form, are inactivated by pH and temperature fluctuations and cause catastrophic off-target and undesirable side effects. Certain investigations have also indicated that active efflux mechanisms affect the absorption of some presently integrated compounds with structural alterations across the intestinal wall. Furthermore, intestinal bacteria and/or enzymes break down fragile structures of active substances into a variety of metabolites, each of which has different bioactivity than the original chemical compound. Nanocarrier-mediated distribution improved their solubilization potential, changed absorption paths, and reduced metabolic breakdown by gut bacteria and enzymes. Combining nanobiotechnology with current therapeutic techniques has shown to be effective in bringing innovative and previously rejected bioactive substances to the market for treating a myriad of diseases and disorders. As a result, we predict that nanotechnology will play a larger role in illness detection and treatment in the future, perhaps helping to overcome bottlenecks in current medical approaches. This chapter focuses on a comprehensive discussion of strategies and applications of nanoengineered delivery systems along with the pharmacokinetic properties and drug-delivery mechanism of

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these nanocarriers. Probably associated drawbacks, challenges, future advancements, and scopes of nanocarriers in clinical care are also highlighted.

Keywords: Controlled release, Dendrimers, Liposomes, Nanocapsules, Nanogels, Nanocrystals, Nanosuspensions, Nanowires, Polymeric micelles, Quantum dots.

INTRODUCTION

Despite the fact that drugs, medicines, and novel bioactive molecules of synthetic or natural origin are beneficial to human health in diseased conditions, the main issues related to these disease-modifying agents of synthetic or natural origin are related to their post-administration instability, off-target, and unwanted side effects. Any disease-modifying agent's therapeutic outcome is determined by how well its pharmacokinetic profile improves after therapy. In the modern period, nanotechnology is tackling all of these difficulties by bridging the physical and biological sciences through the use of nanostructures and nanophases in a variety of sectors of study such as nanomedicine [1, 2]. Nanomedicine and nano-delivery systems are a relatively novel but rapidly growing discipline in which microscopic materials are utilized either as diagnostic tools or for the delivery of therapeutic drugs to specific sites in a controlled manner.

Nanotechnology proved to have a variety of benefits in treating chronic human diseases as it permits the exact delivery of drugs to some particular regions. Medication delivery, chemotherapy, biosensors, and tissue engineering are all using nanoparticles right now in biomedicine. It also covers the use of nano-dimensional materials in live cells, *e.g.*, nano-robots, nano-sensors for diagnostic and sensory applications, and the actuation of materials in living cells. Nanomaterials with diameters ranging from 1 to 100 nanometers, affect the frontiers of nanomedicine, ranging from biosensors to microfluidics, microarray testing to tissue engineering, and drug delivery [2, 3]. In nanotechnology, to produce nanomedicines, a curative agent at the nanoscale level has been utilized. Nanoparticles are typically tiny nanospheres composed of materials created at the atomic or molecular level [3, 4]. Because of nanoscale structural dimensions, they may travel more easily throughout the human body than larger materials and can easily permeate through the tissue system thereby allowing for facile drug absorption by cells to bring out a significant activity at the desired area. The structural, mechanical, chemical, magnetic, biological, and electrical characteristics of nanoscale particles are all different.

Nanomedicines have attracted considerable attention in recent years due to their ability to encapsulate pharmaceuticals or bind bioactive chemicals to nanostructures and deliver them to specific tissues more accurately in a controlled fashion. Furthermore, nanostructures also facilitate the transport of water-insoluble medications to their target area, as well as reducing drug decomposition in the gastrointestinal tract. Because the nanostructures have standard absorptive endocytosis absorption mechanisms as well as enhanced oral bioavailability, they are substantially more rapidly absorbed by cells than large particles ranging in size from 1 to 10 m. Nanostructures last a long time in the circulatory system, allowing combination drugs to be administered at precisely the right amount. As a result, they have fewer negative effects and cause fewer plasma fluctuations [4, 5]. The positive zeta potential, as well as the hydrophobicity of these nanoscopic particles, aids their absorption from the gastrointestinal tract. Other mechanisms found to be supportive in enhancing the absorption of bioactive molecule include electrostatic communication between positively charged nano-vehicle surfaces and negatively charged mucin, amplified transcytosis, and receptor-mediated endocytosis, interaction with junction proteins to modulate tight junctions, the microfold cells mediated phagocytosis of nanoparticles, and chylomicron aided absorption by enterocytes intervened by lipases for lipid nanocarriers. Also, there are many processes by which these nanosystems release their protected bioactive components once, inside the body *e.g.* desorption of adsorbed/surface-bound various components, matrix erosion, enzymatic degradation, matrix diffusion, dissolution, or a combination of either of the processes [5, 6].

A promising approach to nanotechnology in medicine is the synthesis of a variety of nanosized carriers in the size range of 10 to 100 nm (Fig. **1**). The uptake of loaded molecules across the gastrointestinal mucosa is influenced by the particle size, surface characteristics, and shape of the nanoparticles. Polymer nanocarriers (nanocapsules, polymeric micelles, *etc*.), molecular complexes (Cyclodextrins Inclusion Complexes and Phytosomes), lipid-containing nanocarriers (nanostructured lipid capsules (NLC), solid-lipid nanocapsules (SLN), lipid nanospheres, micro- and nanoemulsions, micelles) are all examples of nanosystems for enhanced, controlled, and site-specific delivery of bioactive compounds [6, 7]. Because of the hydrophobicity as well as hydrophilicity inside the polymeric system, polymer-based nanocarriers may accept a wide range of medicinal compounds. Natural or manufactured biodegradable polymers are commonly used as the carrier material in polymer-based nanocarriers.

Natural ones either polysaccharides or proteins are recommended since they have a lower level of toxicity. Plant-based polysaccharides such as pectin, gum arabic, alginate, starch, and its derivatives, cellulose and its derivatives, and animal-based polysaccharides such as xanthan gum, chitosan, *etc*. are used to formulate polymeric nanoparticles. Polyglycolic acid, polylactic acid, poly-cyanoacrylate alkyl esters, polyvinyl alcohol, polylactic-glycolic acid, *etc*. are examples of synthetic polymers. Polysaccharide nanoparticles having distinct characteristics

CHAPTER 18

Phyto-nanoformulations for the Treatment of Clinical Diseases

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Abstract: Plant-derived drugs or formulations have always been explored because of their lesser side effects and toxicities compared to synthetic drugs and they have been widely used as traditional and complementary medicines for the management of many diseases including cancer. The major challenges faced were the absorption of the plant-derived drugs, their stability, bioavailability, and transport to the intended sites inside the body. Recent progress in nanotechnology has helped to minimize these limitations and hence phyto-nanoformulations are slowly growing in preclinical trials as well as clinical use. The use of various nanostructures such as nano-micelles, lipid nanoparticles, carbon nanotubes, polymer nanoparticles, and nanoliposomes and various types of drug delivery vehicles such as polybutylcyanoacrylate, polylactic-c--glycolic acid, and lactoferrin has immensely helped in increasing the effectiveness of phytochemical drugs by increasing their stability, better pharmacokinetics and reducing the toxicity and side effects. Phyto-nanoformulations having natural product components such as curcumin, piperine, quercetin, berberine, scutellarin, baicalin, stevioside, silybin, gymnemic acid, naringenin, capsicum oleoresin, emodin, and resveratrol have been shown to improve the condition of patients diagnosed with diseases such as neurodegenerative disorders, diabetes, infections, and cancer. Phyto nanoformulations can also be used to treat disorders of the brain where the blood-brain barrier is impervious to the drugs. These phyto-nanoformulations have been shown to target several molecular cell-signaling and metabolic pathways. This chapter covers the compositions of phyto-nanoformulations and how they have been used to control several diseases.

Keywords: Nano micelles, Neurodegenerative disorder, Nanoemulsions, Pharmacokinetics, Phyto-nanoformulation.

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INTRODUCTION

Phytochemicals are secondary metabolites in plants that are largely involved in creating pigmentation and flavor and these metabolites also constitute the phytoimmune system. It is a common observation that plants that have more pigmentation generally contain a higher level of phytochemicals and nutrients such as phenols, steroids, allicin, carotene, terpenes, pyrimidines, and nucleic acids. Plants are a better source of medicinal and nutritive compounds and these compounds can be obtained easily and at a cheaper cost compared to lab synthesized compounds. The toxic side effects of synthetic drugs have forced researchers to look for plant-derived phytochemicals as complementary medicines and natural or semisynthetic medicines. These goals have motivated the agriculture, pharmaceutical, and food industries to focus on phytochemicals for drug leads. Many plant extracts have antioxidant properties that have health benefits such as enhancing vision and vascular health, decreasing the severity or chances of cardiovascular diseases, and preventing diabetes, cancer, and microbial diseases. Garlic and onion extracts have been used in traditional medicines as antimicrobial [1], antitumor [2], antihypertension [3], and cholesterol [4] reduction agents [5 - 8]. Carotenoids from the carrot have been shown to control colon cancer [9], inflammation [10] and oxidation [11]. Nanotechnology has helped to push the limitations of traditional methods of treatment [12]. The diverse nanostructures and vehicle systems have increased the stability and effectiveness and reduced the side effects of the drugs [13]. The nanoformulation of phytochemicals has beneficial effects as these have a greater surface area to volume ratio, delivery of drug to the precise location, controlled and well-regulated release of therapeutic drug, and better bioavailability [14] and hence nanoformulation and nanonization of phytochemicals are a potential replacement for conventional drug delivery system [15]. Phyto-nanoformulations alone or in combination with other drugs have shown a promising proficiency and potential for the treatment of cancer, neurodegenerative disorders, diabetes, metabolic disorders, and microbial infections.

PHYTO-NANOFORMULATION IN CANCER

Polymeric Nanoparticles

Cancer is a group of various pathological abnormalities that lead to a painful death and for poor and developing countries, the compromised health infrastructure presents a further challenge in controlling the disease [16]. Cancer becomes hard to control when it undergoes metastasis or develops drug resistance [17, 18]. The conventional approaches to treat the disease are chemotherapy, radiotherapy, and surgery but these therapies sometimes seem limited in their efficiency especially when cancer cells get resistant to the drugs or have disseminated in different parts of the body [19].

Nanoparticles are structures having a minimum one dimension lesser than a hundred nanometers and they can be grouped into nanospheres and nanocapsules [20]. Drugs are added to the matrix of the nanosphere or embedded into the inner cavity of the reservoir of the nanocapsules. Nanoparticles can be made up of natural or synthetic polymers and are sometimes known as polymeric nanoparticles [21]. The surface of nanoparticles can be modified by PEGylation to enhance their intake into the cells [22]. Chitosan surface coating increases stability and polyethylene glycol suppresses the protein expression in cell lines of breast cancer [23]. P glycoprotein is suppressed in PEG MDA-MB 453 and it has the capability to suppress resistance to the anticancer drugs in breast cancer therapy. The coating of PEG and chitosan on the BioPerine nanoparticles improves the intake inside the cells and fabricated BioPerine nanoparticles have better uptake than free BioPrine [24].

Some of the important nanoformulations that have improved the efficiency of phytochemicals are carbon nanotubes, liposomes, polymeric nanoparticles, lipid nanoparticles, lipid bilayers, dendrimers, micelle, nanocapsules, and functional gold nanoparticles [25].

Liposomes

These are vesicles made up of a lipid bilayer which have an aqueous inner core separated from a bulky outer part and it has a size of 10 nm-1 µm or sometimes higher [26]. The aqueous environment in the core serves as drug or other molecules' carrier. To target the specific sites on the cell, stealth liposome [27] is used whereas to improve the transportation of the drugs across various barriers, transferosomes [28] are used. Some of the advantages of liposomes are biocompatibility, amphiphilic nature, and biodegradability. The surface of the liposomes can be modified and ligated for the targeted delivery of the cargo [29]. The liposomes loaded with betulinic acid have been shown to target the HepG2 cells [30]. Icariin-loaded phytosomes have lesser IC50 compared to the pure form of Icariin [31]. Phytosomes are also useful for targeting ovarian cancer and as an inducer of apoptosis. Liposomal targeting of breast cancer cell lines using curcumin has shown a promising result. The half-life of liposomal curcumin is improved compared to the pure form of curcumin [32].

Solid lipid nanoparticles have solid and lipid cores and particle size ranges from 10-1000nm. Loading and targeting efficiency were improved by using solid lipid nanoparticles [33]. The lower particle size range has been used in biomedical applications as solid lipid core can be stabilized by interfacial surfactants such as

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Dr. Zulqurnain Khan is an Assistant Professor at the Institute of Plant Breeding and Biotechnology (IPBB), MNS University of Agriculture, Multan (MNSUAM), Multan, Pakistan. Dr. Khan received his PhD in Biotechnology in 2017 from the University of Agriculture, Faisalabad (UAF) in Pakistan. His study focuses on employing genome-editing techniques (TALEs, TALENs, Cas9, dCas9 and multiplexed CRISPR-Cas9) in model plants and cotton to develop resistance to Begomoviruses. He is also employing CRISPR-Cas9 technology to enhance cereal crop genetics for abiotic and biotic stress tolerance. He is also employing gene editing and nanotechnology for genetic improvement in medicinal plants. Dr. Khan has been using medicinal plants with high nutritious value as functional foods. His work on nutrigenomics is ready to get published in a book "Nutrigenomics: Trends, Challenges, and Opportunities". He has edited two books on genome editing, one book on cotton breeding, and authored over twenty book chapters. Recently, Dr. Khan has authored a book on "Engineering Disease Resistance in Plants Using CRISPR-Cas". In addition, he has several review and research papers published in international peer-reviewed journals. Dr. Khan was awarded an IRSIP scholarship by the Higher Education Commission (HEC) of Pakistan. He worked as a visiting researcher at Professor Caixia Gao's group at the Institute of Genetics and Developmental Biology (IGDB), Chinese Academy of Sciences (CAS), in Beijing, China. Dr. Khan has presented his work at various international forums/conferences at China, Egypt, Kazakhstan etc. Since 2018, Dr. Khan has been teaching several courses on biotechnology, breeding, molecular biology, genetics and genome editing.