NEW AVENUES IN DRUG DISCOVERY AND BIOACTIVE NATURAL PRODUCTS

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Natural Medicine

(Volume 2)

New Avenues in Drug Discovery and Bioactive Natural Products

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CONTENTS

PREFACE

Natural products always play an important role in the process of drug discovery and the development of modern medicine. Drug discovery aiming to find robust and viable lead candidates is a challenging and time taking process that involves the participation of experts from various fields. Screening of natural products to a new molecule or new biological effect of an existing drug candidate requires expertise, experience and modern techniques. Natural products and their structural analogues are used as a lead in the drug discovery process and have always played an imperative role in pharmacotherapy. However, drug discovery from natural products is always challenging because of technical issues, such as screening, isolation, characterization, identification and optimization, which results in a decline in their pursuit by pharmaceutical companies. Over the past few decades, the demand for natural products has been increasing, and researchers have concentrated on drug discovery from natural sources, predominantly focusing on traditional medicinal information. Plants are an abundant source of chemically vastly diverse secondary metabolites, which upon screening, exhibited different biological functions and are still far from being comprehensively investigated. Scientific awareness in natural product-based drug discovery leads to concentrating the focus on finding a new strategy for isolation, identification, characterization, screening of natural products and resupply of biologically active compounds being developed, which are the necessity of the future. Drug discovery from medicinal plants can be considered a hot spot of research. A large number of drugs were obtained from the medicinal plant, and several others have been discovered by using phytochemicals as lead. Plants have historically proven their value as a major source of bioactive molecules, and still represent an imperative tool for the discovery of novel drug leads. Intrinsic complexity involved in drug discovery from plant sources necessitates extremely integrated interdisciplinary approaches. Scientific developments and technological advancement, including sophisticated analytical tools, engineering strategies, in silico approach, *etc*., are opening up new opportunities to reestablish natural products as a key source for drug discovery and find new drugs quickly. Volume 2 of this series of the book entitled "Natural Medicine: New Avenues in Drug Discovery and Bioactive Natural Products" is devoted to the current research in drug discovery from natural sources, modern analytical and scientific approaches, the bioactivity of isolated phytochemicals and their analogues. This volume is a collection of important research on this field which will strengthen scientific advancement on natural product-based drug discovery and will be helpful for the scientific community.

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CHAPTER 1

Role of Analytical Methods in Herbal Drug Discovery

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Abstract: Nature contains a huge array of unique phytomolecules, many of which have previously evolved into lead compounds and have been transformed into herbal formulations or are currently undergoing clinical studies. Plant-based pharmaceuticals account for 25% of all drugs on the market, either directly or indirectly. The herbal drug sector has gotten a lot of attention in recent years, which has resulted in its exponential rise. People increasingly prefer herbal treatments to synthetic drugs, highlighting their safety and efficacy. Standardization of these herbal medications has become a significant component of the process involved in herbal drug development, not just for Asians but also for westerners, due to increased interest. The role of various analytical methods, such as chromatographic methods, spectroscopic methods, metabolomics, DNA barcoding, and so on, has been explored in this chapter. These methods aid in not only authentication but also quality assurance during the herbal medicine research process.

Keywords: Analytical methods, Drug discovery, Herbal drugs, Phyto molecules, Standardization.

INTRODUCTION

Most active components in today's medicines are derived from nature, a rich source of new compounds. Medicinal plants have had a significant impact on global health. The therapeutic characteristics of many plants are still unknown to us, and have yet to be investigated; nonetheless, despite enormous developments in modern medical science, they continue to play a vital role in health sciences [1]. In this world, there are around 400,000 species of plants [2].

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Raja Chakraborty & Saikat Sen (Eds.)

Plants are thought to manufacture up to 200,000 phytochemicals among their many and varied species [3]. Higher plants are considered to be the source of around 25% of all contemporary are even in use in the present day. There are no beneficial synthetic alternatives that have the same potency and pharmacological specificity to a specific condition [4]. About 60% of currently marketed medicines and those on the edge of final clinical trials, such as antitumoral and antibacterial treatments, are derived from natural compounds, primarily from higher plants [5]. In recent years, some traditional plant-based medicines have seen their market share significantly eroded by the introduction of synthetic alternatives, while others have been given new investigational or therapeutic status [6]. According to a recent analysis, approximately sixty percent of the anticancer and anti-infective agents that are currently either commercially available or in the late stages of clinical trials are derived from natural product sources [7]. Plants provide primary health care to about 65–80 percent of the world's population in poor areas [8]. Antimicrobial, antiparasitic, anticancer, antiviral, anti-inflammatory, immunomodulatory, and neuroprotective compounds can all be found in marine natural products [9, 10]. More than 50,000 natural products of microbial origin are also used in drug development [11]. It is also seen that there is an increased interest in higher plants as potential sources of novel lead structures, as well as the production of standardized phototherapeutic compounds with evince efficacy, quality as well as safety [12, 13]. Between 2000 and 2005, natural ingredients were responsible for the launch of more than 20 new pharmaceuticals around the world [14]. Because of their natural origins and lesser side effects or dissatisfaction with synthetic drug results, there has been the utilization of a number of preparations originating from traditional herbal medicines in both developing and developed countries for thousands of years. However, whether they are single plants or groupings of herbs in composite formulae, all herbal medicines are extracted by utilizing boiling water during the process of decoction, which is one of the distinctive qualities of eastern herbal medicine preparations. This could be one of the reasons for the major challenge in terms of quality control for eastern herbal medicines when compared with western medicines. Traditional medicine has not been formally acknowledged in most nations, despite its long history, widespread use, and popularity and widespread use in the recent decade. As a result, education, training, and research in this field have received little attention and funding. Traditional medicine's safety and efficacy studies are insufficient in quantity and quality to meet the requirements required to support its use globally. The paucity of research data is owing to a lack of suitable or acceptable research techniques that aid in the evaluation of traditional medicines, in addition to healthcare regulations [15, 16]. Patients were previously treated individually, and drugs were prepared in accordance with their requirements; however, there is a change in the landscape, and herbal medicines are now being manufactured on a large scale. Manufacturers are confronted with various issues, such as the availability of high-quality raw materials, the authentication of raw materials, the availability of standards, proper standardization methodology of single drugs and formulations, quality control parameters, and so forth. The existence of therapeutically relevant elements in plant chemistry is frequently linked with a large number of inert compounds (coloring agents, cellulose, lignin, *etc*.). For their specific pharmacological activity, the active ingredients are isolated from the plants and refined for medicinal use. As a result, quality

management of herbal crude medications and their ingredients is critical in today's medical system. Adulterated herbs arise due to a lack of suitable standard standards for the standardization of herbal preparation and various cases of substandard herbs. Standardization of herbals is required to meet the rising wave of curiosity [17 - 21]. As a result, every individual herb must be verified for quality to ensure that it meets all quality requirements and consistently offers the desired qualities. Quality, efficacy, performance, and safety are all assured through standardization since it ensures that items are consistent in their characteristics. However, it has been found that the pharmaceuticals available in the marketplace are commonly contaminated and do not meet the quality requirements established for genuine drugs.

DEVELOPMENTS IN MEDICINAL PLANT RESEARCH AND ANALYTICAL TECHNOLOGY

Instead of a single component or simple mixture of multiple components, traditional medicine, and herbal items are typically made from plants and contain hundreds of unknown constituents. As a result, a large number of the components are scarce. Usually, the active ingredients which are found responsible for eliciting the pharmacological effect are not known to the investigators or the public. Usually, an assumption is made that there is the presence of a number of active components, both macro and micro, which are responsible for the potential therapeutic effects, and hence multiple component examinations are more justifiable for the process of quality control. Furthermore, herbal medications are a complex matrix of chemicals with no one active element responsible for total efficacy. As a result, quantitative measurement of several components which are active is one of the most direct and also a crucial tool for quality assurance. Rarely do phytochemical research succeed in identifying and characterizing all secondary metabolites contained in a plant extract, despite the availability of sophisticated analytical instrumentation techniques given in Fig. (**1**) [22]. Moreover, only about 10% of plants originating from the higher species have been described chemically to a significant degree. The complexity of the chemical makes the quality control process far more difficult. However, chemical profiling of plant-based products is necessary for greater scientific as well as clinical acceptance along with global placement. Traditional medicines are not officially recognized in most countries, despite their long history and widespread use. Traditional medicines' safety and efficacy evidence do not satisfy the standards required to justify their widespread usage over the world [15].

All phytochemical elements of traditional medicines must be identified to verify the credibility of pharmacological research and comprehend their bioactivities and probable adverse effects [20, 21, 23]. We also know that traditional medicines work because of a complicated mixture in their crude form, making it difficult to assess their relationship. Biological assays can be connected to a chemical

CHAPTER 2

Genome Mining: Recent Trends of Drug Discovery in Natural Products

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Abstract: Drug discovery relies on screening for bioactive components in natural sources, such as bacteria, fungi, and plants. Bioactive natural products and secondary metabolites provide the main source for developing new therapeutics, serving as new antibiotics and anticancer agents. With identifying the first biosynthetic genes over the last few decades, scientists now have the tool at their disposal to better understand the genetics and logic behind these biosyntheses. In addition, in this genomic age, one can have access to an increasing number of genomes for reproducible research outcomes. Genome mining, in conjunction with synthetic biology, has become significantly more relevant to drug discovery. In the current chapter, the main aim is to explore how the recent trends of genome mining and synthetic biology approaches can be used to discover novel natural products while highlighting both the benefits and drawbacks of these techniques. We also consider the adjacent methods for the validation of data after genome mining. By reviewing the literature, an attempt was made to finally summarize all-natural drugs obtained by genome-mining from fungi, bacteria, and other microorganisms.

Keywords: Bacteria, Fungi, Genome, Genome mining, Natural products, Synthetic biology, Therapeutics.

INTRODUCTION

In the medical field, natural products stand out as an outstanding resource of bioactive substances serving as a nutraceutical and pharmaceutical products [1]. As per Newman and Cragg, naturally occurring compounds and their derivatives have accounted for a majority of new drugs approved over the past three decades

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Raja Chakraborty & Saikat Sen (Eds.)

[2]. In fact, several of these bioactive compounds come from microorganisms, including bacteria and fungi [3], which have historically been important for synthesizing antibiotics and other medicines [4]. In particular, bacteria originating from the marine environment can produce secondary potential metabolites that have anticancer and antifungal efficacy due to their cytotoxic properties [5]. In light of the large problem of antimicrobial resistance occurring in the current situation and the inability to produce new chemical entities simultaneously, it is vital to address this issue by investigating novel agents from natural products which might have lesser side effects, more efficacious and probably organic in nature [6, 7]. Due to these reasons, genomics has proven a useful tool recently to track down the new gene clusters that could be used for natural product biosynthesis and to identify potential drug targets. Since the advent of genome sequencing technologies, the pharmaceutical industry has surely taken an interest in discovering new metabolites that had previously been lost due to the advent of combinatorial chemistry [8]. The advancements in genomics, bioinformatics, and chemical analytics have paved the way for more innovative approaches to genomics-based discovery [9].

GENOME MINING

"Genome mining" is the term used to describe any bioinformatics analysis of natural products for the detection of biosynthesis pathways and their interactions [10]. By applying genome mining to sequenced organisms, previously unknown gene clusters involved in natural product synthesis, genes encoding those enzymes, and their products can be identified experimentally and effectively [11].

Bioinformatics and computing are an integral part of genome mining. An overwhelming majority of DNA sequences and associated annotations have now been deposited in public databases. The continued development of computers and networks is essential in storing and handling these resources. Having identified all the genes within a new genome, they can be compared to the genes in public databases with known functions. There are numerous websites that deliver raw and annotated genomic data, as well as bioinformatics tools to compare the sequences. It is relatively easy to identify the pathways in which natural products are synthesized if the sequences of the proteins involved in the biosynthesis are stored in databases. By comparing their sequences with parent genes and with the availability of these enzymes and their functional pathways, it is possible to identify homologs of these enzymes. Nonetheless, it is important to remember that many enzymes are chemically similar but follow slightly different pathways; as a result, they yield very different outputs [12].

Moreover, genome mining is well-supported by synthetic biology, which consists of constructing new biological systems. Combining both approaches facilitates the identification of novel bioactive compounds in bacteria and fungi [13]. In this way, transcriptional levels can be controlled, and also metabolic functions can be associated with them [14]. In addition, genome mining is primarily used to uncover new biosynthesis gene clusters (BGCs). These genes encode polyketide synthases (PKS) and non-ribosomal peptide synthases (NRPS), a pair of key enzymes in the biosynthesis of natural products [15]. Additionally, such an approach allows the comparison of target gene clusters to known gene clusters to help predict those genes' functional and structural properties [16]. Despite early discoveries of gene clusters responsible for the synthesis of natural products based on genome mining, web-based tools and databases have been integrated to enhance this approach over the past decade [17]. In response to this progress, three web applications, namely "Antibiotics and Secondary Metabolite Analysis Shell" (antiSMASH), "Prediction Informatics for Secondary Metabolomes" (PRISM), and "Integrated Microbial Genomes Atlas of Biosynthetic gene Clusters" (IMG/ABC) has been developed. AntiSMASH is an online tool that allows the analysis of gene clusters to be linked to a series of algorithms for analyzing compounds [18]. This approach predicts sequences and offers a more detailed analysis of identified gene clusters, which is then used to predict the stereochemistry structure of amino acids. PRISM is an online tool for predicting secondary metabolomes based on genomic information. This approach generates correspondence for known natural drug classes and hypothetical new ones by comparing genetic information with 57 virtual enzymatic reactions [19]. In March 2015, the IMG/ABC project was launched as an open-source microbial BGC database. The database is able to associate predicted metabolites (SMs) with predicted BGCs and analyze both SMs and BGCs [20]. It, therefore, makes it possible to locate BGCs that are similar to those in the database and those that should be identified [21].

In addition to these general thoughts, the current chapter aims to emphasize how genome mining can lead to the discovery of novel natural products and discuss the available benefits and drawbacks of this method. The chapter also discusses the associated technologies for data validation after genome mining. As a last step, a comprehensive literature survey was executed to describe the entire novel drugs discovered in living organisms such as bacteria, fungi, and related organisms based on genome mining approaches, focusing on examples from recent literature.

History of Genome Mining

New DNA sequencing techniques provide high throughput, low cost, and high accuracy, enabling molecular analysis to analyze a broad range of organisms.

CHAPTER 3

Approaches and Challenges in Developing Quality Control Parameters for Herbal Drugs

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Abstract: Herbs have been used as medicines from ancient times in the world. In the present scenario, awareness and acceptability towards herbal medicines have been raised tremendously due to their easy availability and few or no side effects. Unfortunately, due to the lack of stringent regulatory guidelines for herbal drugs, standard quality degradation may be associated with these herbal medicines through either intentional or unintentional adulterations, spurious drugs, the substitution of drugs with other drugs, *etc*. Hence, it becomes mandatory to control the quality standards of herbal medicines as they are being used for the betterment of human health. Improvements in various domains of herbal medicine have helped developed countries, such as USA, UK, Australia and European countries, adopt this ancient and enriched medicinal system leading to the "Herbal Renaissance". Herbal medicines, however, are associated with a number of shortcomings such as quality assurance, safety, efficacy, purity, lack of appropriate standardization parameters, lack of accepted research methodology and toxicity studies. Despite the availability of numerous traditional quality control methods (*e.g*., thermal methods, HPTLC, HPLC, SFC) for herbal medicines, owing to the lacunae, there is a prerequisite for newer approaches in fostering quality parameters of herbal drugs. Chromatographic and spectral fingerprinting, DNA fingerprinting and metabolomics can be used as newer approaches to the authentication and standardisation of medicinal botanicals. Currently, the computational *In-Silico* technique for standardization of phytochemicals is in trend because of the number of pros like less time consumption, fast, and improved efficiency of the entire process with excellent reproducibility.

Keywords: Adulterations, DNA fingerprinting, Herbal medicines, *In-silico*, Quality-control.

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Raja Chakraborty & Saikat Sen (Eds.)

INTRODUCTION

Herbal drugs are natural plant-derived products that may be either herbs, minerals, vitamins, immunomodulators, antibacterials, or any other medicinal agent. They can be administered orally as solid or liquid or applied topically over the skin or on the scalp or hair. Herbal medicines are used for various therapeutic purposes due to ampule number of beneficial phytoconstituents present in them like alkaloids, flavonoids, essential oils, terpenoids, phenolics, saponins, carbohydrates, *etc* [1]. The use of herbs to prevent, treat, or alleviate any disease is since thousands of years ago. Our ancestors were reported to use many part plants to treat the disease [2]. Herbal drugs are widely and easily acceptable by worldwide patients owing to their safety and efficacy as compared to alternative medicines, which are associated with adverse effects in some way. The use of herbal medicines has increased tremendously over the past two decades. They are being used for simple colds and flu to even complicated psychological problems like dementia, depression, and anxiety also. The best part of these medicines is their broad applications (acute as well as chronic disease), lack of side effects, no need for premarketing assessments, *etc* [3].

WHO also recommended specific guidelines for the assessment of herbal medicines with the view to their safety, efficacy and quality [4]. More than 80 percent population of the world rely on herbal medicines these days depending on the country as a developing country has more belief on herbal medicines due to their low cost, easy availability, safety, efficacy *etc*.

Owing to their origin from the natural sources herbal medicines are contemplated as safe and comprise a major part of traditional medicine and basic component of Ayurvedic, Homeopathic, Naturopathic, Neutraceuticals and many other medicine systems [5].

HERBAL MEDICINES

Herbal medicines are defined as a whole plant or any part of the plant, like seed, fruit, leaf, root, *etc*., that possesses therapeutic efficacy and is capable to prevent, treat, alleviate disease or provide beneficial effects to health as supplementary/nutritional products [6]. As per World Health Organization (WHO), herbal medicines are natural products that are either whole plant or their part or their secretion or exudates and can be used for health benefits or to treat any disease [4].

The most primitive evident data from around five thousand years back are documented for herbs for medicinal use in countries like India, China, Greek, Egypt, Rome, Syria, Arab countries, *etc*. The herbal medicines are hence

considered to be consequential of enriched ethnicities of primaeval cultures and systematic legacy [7]. Initially, herbs were used by human beings as such or left to wrap the wound/injury, or applied over the skin topically as juice or paste of the plant parts or were eaten or drunk in the form of juice or decoction. Herbal medicinal therapy is being adopted by many patients who need to account for clinicians owing to inconvenience, high cost, delayed approach to professional healthcare, side-effects, *etc* [8]. In rural areas, some hypotheses also supported the herbal medicines like cultural issues and the belief that the "mother of the disease also is the mother of cure/treatment", hence where the disease originated, the treatment also lies there only [6]. Therefore, they depend on the local herbs only for any disease. Still, there are so many regions of various developing countries, which have no reach to alternative medicines as the population of these regions primarily rely on herbal medicines solely, leading to the failure of government efforts to provide healthcare facilities by manufacturing healthcare centres in such rural areas [9].

Furthermore, herbal medicines as natural products are alleged to be healthier than allopathic medicines, which include the number of excipients in them [10]. However, in cases of adverse effects associated with allopathic medicines, finding reports is very expensive compared to the herbal toxicity report finds. This is because it is difficult to track mechanisms linked with the adverse effects of allopathic medicines.

Since the last few decades, herbal medicines have been used by people without any prescription [11]. Herbal drugs are blessed with the pros of ample availability, low cost, rare or minimum side effects, better patient compliance, better potency and efficacy. While intricacy in standardization and slow pharmacological response are some drawbacks linked with herbal drugs, making them difficult to use in emergencies. Seeds, fruits, flowers, roots, barks, leaves, and exudates have been used as herbal medicines to combat health issues since ancient times. Numerous pharmacological activities have been proven to date with herbs. Some of them include antipyretic, antimicrobial, antidiabetic, anti-depressant, anti-malarial, anticoagulant, anti-ageing, anti-fertility, anti-arthritic, spasmodic, anti-anxiety, analgesic, anti-inflammatory, vasodilatory, anti-tuberculotic, anti-hypertensive, anti-cancer, hepatoprotective, anti-asthmatic, anti-migraine, immunomodulator, effective against acne and other skin problems, impotency, menopause, gall stones, Alzheimer's disease, jaundice, dementia, *etc* [12]. Herbs, such as herbal medicines alone or in combination with an alternative system of medicine, may be used significantly by people. They are also the source of numerous lead molecules with pharmacological activity, hence turning out to be nerve centers for researchers in drug discovery [13].

CHAPTER 4

DNA Fingerprinting for Identification and Standardization of Herbal Drugs

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Abstract: Herbal medicines are emerging as the bliss of the modern era for the treatment and welfare of the population owing to their safety, and minimum or lack of side effects. Keeping this perspective in mind, the quality of herbal medicines is equally important as allopathic medicines. To thwart the adulteration of herbal medicines with substandard or similar low-grade herbs is the prime objective of standardization for the maintenance of the quality of herbal medicines. Assessment of quality and purity of crude drugs using several parameters such as morphological, microscopical, physical, chemical & biological evaluation can be performed through standardization technique. A number of conventional standardization methods are available, but owing to their lackings in one or more aspects, modern standardization techniques are being opted for by many researchers nowadays. Amid all the novel standardization techniques, DNA fingerprinting method is the most important in the quality control of herbal medicines on account of its accuracy and consistency. DNA fingerprinting defines barcode-like DNA fragment patterns generated by multilocus probes after the electrophoretic parting of genomic DNA fragments. Hence, this chapter primarily emphasizes on DNA fingerprinting method as a tool for the standardization of herbal medicines.

Keywords: Fingerprinting, Herbal drug, Identification, Isolation, Standardization.

INTRODUCTION

Herbal drugs basically include natural plant-derived products that may be either herbs, minerals, vitamins, immunomodulators, antibacterial, or any other medicinal agent. They can be administered orally as solid or liquid or applied

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Raja Chakraborty & Saikat Sen (Eds.)

topically over the skin or on the scalp or hair. Herbal medicines comprise plenty of valuable phytoconstituents such as alkaloids, flavonoids, essential oils, terpenoids, phenolics, saponins, carbohydrates, *etc* [1]. Global acceptance of herbal medicines has increased enormously due to their safety and efficacy compared to alternative medications that are allied with adverse effects in some way. Herbal medicines are expected as innocuous and embrace a principal share of traditional medicine and are an elementary constituent of Ayurvedic, Homeopathic, Naturopathic, Neutraceuticals, and many other medicine systems on account of their derivation from natural sources [2].

As per World Health Organization (WHO), herbal medicines are natural products that are either whole plant or their part or their secretion or exudates and can be used for health benefits or to treat any disease [3]. WHO also endorsed definite guidelines for the assessment of herbal medicines with the view to their safety, efficacy and quality [3].

The last two decades are the witness of unprescribed use of herbal medicines by individuals on account of easy availability, low cost, fewer side effects, safety and better therapeutic efficacy. However, in disparity to these benefits of herbal medicines, a few limitations are also allied with herbal drugs like complexity in standardization, delayed pharmacological response, *etc*., limiting their use in emergency conditions [4].

In contrast to the 20th century which was a conquest for synthetic drug molecules by replacing traditional natural products, the 21st century became the rebirth century for herbal drugs. In the present scenario, many pharmaceutical industries are also approaching researchers for the newly discovered lead molecules from herbs. Moreover, many allopathic compounds are combined with herbal extracts or plant-derived therapeutics for better therapeutic effects. Though the developed countries were very much dependent on modern medicines in the 20th century, in the present era, along with the developing countries, who always primarily rely on herbal products, developed countries are also showing their interest in herbal products.

Many developed countries also market herbal medicines as neutraceuticals or nutritional supplements owing to their amazing health benefits [5]. Cosmeceuticals industries are also growing rapidly in the present era with no adverse reactions associated with these herbal cosmetic products.

Currently, herbal medicines are being utilized principally as a source of drug discovery to identify the lead molecules from plants. Though a lot of success has been gained in this direction with natural products still, some drawbacks like safety and quality concerns of these natural products, the high cost of collection of

herbs, lack of reproducible results, *etc*., led the researchers back further in the path [6].

Unfortunately, the Covid-19 pandemic originated in Wuhan, China [7], which is also one of the reasons for this tremendous rise in global herbal share; due to the unavailability of specific treatments for covid-19, many herbal products were utilized as immunity-boosting agents to cope with such an unknown dangerous viral infection. Since SARS and H1N1 influenza patients' inclination towards herbal medicines had drastically increased, the Covid-19 pandemic made this rise a boom [8].

Seeing the current golden scenario for herbal medicines, it can be estimated that herbal medicines will be a major shareholder in the future medicine market worldwide.

CONVENTIONAL METHODS FOR STANDARDIZATION OF HERBAL DRUGS AND FORMULATION

Since herbal medicines are allied with the major challenge of standardization which is complicated with the herbal drugs, stringent norms are urgently needed regarding standardization of herbal medicines. Standardization and quality control of herbal materials with appropriate incorporation of recent scientific methods and traditional acquaintance are imperative for converting herbal materials into medicines. Herbal drugs are medicinally important, and chemical constituents present in them are responsible for therapeutic activity; hence quality and purity of herbal material are of utmost importance [9].

The intricacy of standardization of herbal drugs due to the presence of a mixture of phytoconstituents, and the risk of adulteration of herbal drugs with substandard herbs in the industry mandates the specific standardization of herbal drugs and formulations.

European Medicinal Agency, Food and Drug Administration, World Health Organization, USP Herbal Medicine Compendium, Hong Kong Chinese Materia Medica Standard, British Pharmacopoeia, Indonesian Herbal Pharmacopoeia, American Herbal Products Association, Indian Pharmacopoeia, Ayurvedic Pharmacopoeia has prepared guidelines for evaluation of the quality of herbs and herbal derived drugs [10].

In India, guidelines for the evaluation of herbal drugs are provided by various regulatory authorities like the Ministry of AYUSH, the Indian Council of Medical Research (ICMR) and the National Medicinal Plants Board.

CHAPTER 5

Advanced Drug Discovery Techniques for Identification of Natural Resources as Potential Therapeutic Agents

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Abstract: Emerging disease threat and the mortality rate associated with it has triggered the need for the identification of treatment and prevention of such diseases. Natural products are known for here therapeutic value and can act as a prominent source for the identification of new drugs for these diseases. Many different strategies have been developed to identify and obtain newer drugs from natural resources. Natural products are a potential source of drugs for many diseases due to their structural diversity and already reported biological activity. Lead compounds for many lethal diseases, such as the recently emerged infectious disease COVID-19 have been identified using computational techniques which may help to curb the COVID-19 outbreak. Omics-based techniques such as proteomics, genomics, metabolomics, transcriptomics, *etc,* have become one of the most helpful techniques for discovering drug products from natural resources. CRISPR is another such technique that combines bioinformatics, genomics and synthetic biology. It is a DNA-targeting genome editing tool that has aided medical research. Other than these, many more drug discovery tools such as multi-omics, combinatorial biosynthesis, artificial intelligence and 3D printing have been a boon for identifying natural products with diverse chemical structures and therapeutic indexes. Advanced computational techniques have helped develop potential drug candidates with desired therapeutic activity. This chapter focuses on recent computational techniques employed to discover drugs from natural resources.

Keywords: Artificial intelligence, Combinatorial biosynthesis, Computational technique, CRISPR, Multi-omics, 3D-Printing.

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INTRODUCTION

Many of the new drugs and medicinal therapeutic agents find their source in well-known natural products. Most of the medicinal plants contain molecules which are structurally diverse and have a wide range of therapeutic properties. The information about these properties of medicinal plants is helpful in the development of drugs obtained from these natural products. Earlier, the therapeutic implications of these naturally obtained active substances were discovered by various trial and error methods. Thus, these active compounds were isolated and structurally modified with the help of advanced drug discovery techniques to develop newer biologically active compounds. The drugs which are difficult to isolate, purify or chemically synthesize were tested with the help of techniques such as high throughput screening and artificial intelligence [1]. With the advancement in drug discovery techniques, many of the secondary metabolites can also be selected for further modification with the help of knowledge of biological targets. These biologically active compounds are a prominent source of lead compounds and pharmacophores, which can be explored for further modification into therapeutically active compounds with the help of various omics and multi-omics techniques [2].

PREVALENT USES OF NATURAL DRUG PRODUCTS

Plants are emerging as a potential source of many clinically active agents, and have helped identify many therapeutically active agents. However, natural drug products provide a source of many structurally diverse low molecular-weight drug candidates with diverse medicinal uses Table (**1**). The most ancient record of natural drug products used in the treatment of cough, cold and inflammation are oils from *Cupressus sempervirens* (Cypress) and *Commiphora species* (myrrh), and these are still used today [3]. Although, there are also many other medicinally useful plant products apart from these, the chemical structures of a few of them are given in Fig. (**1**).

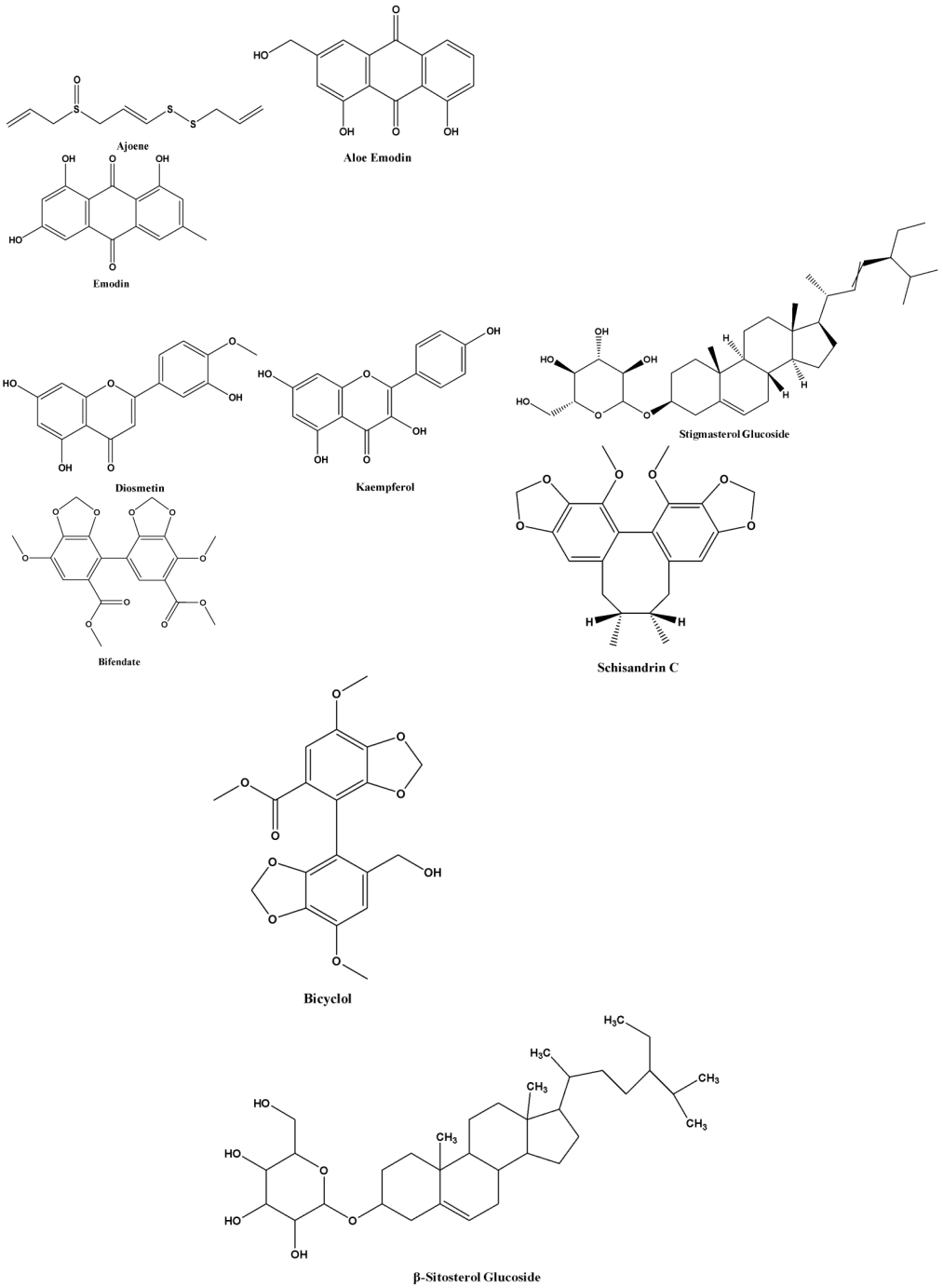

Fig. (1). Chemical scaffolds of some medicinally useful plant metabolites and their derivatives.

CHAPTER 6

Isolation, Identification and Characterization of Glycosides

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Abstract: Nature has bestowed our universe with a vast wealth of restorative and curative plants. In the 21st century, medicinal plants' therapeutic effects have been considered a promising future drug/medicine for managing health care. The phytochemicals in herbs are the active ingredients that possess therapeutic activity and are considered medicine or drug. The ever increasing demand for phytochemicals from plant origin, either as pure compounds or as standardized extracts, provided unlimited opportunities for new drug leads. Seeking therapeutic drugs from natural products is of particular interest throughout the world. Plants synthesize secondary metabolites, which include alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins and volatile oils. One of the important phytochemicals to be discussed in this chapter is glycosides. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzymatic or acid hydrolysis leading to one or more sugar moieties along with non-sugar moieties. The former is called glycone, and the latter is aglycone or genin. Based on sugar moiety linkage, they are classified as C-glycosides, O-glycosides, S-glycosides and N-glycosides. Due to the complexity of plant chemical constituents, pure phytochemicals must be obtained using proper extraction and isolation techniques. This chapter focuses on the analytical methodologies, which include the extraction, isolation, identification and characterization of glycosides from medicinal plants.

Keywords: Glycosides, Identification and characterization, Phytomolecules, Plant isolation.

INTRODUCTION [1, 2]

Compounds isolated from nature have long been known to possess biological profiles and pharmaceutical potential far greater than anything made by man; however, natural products are disreputably cumbersome to isolate and very challenging to synthesize.

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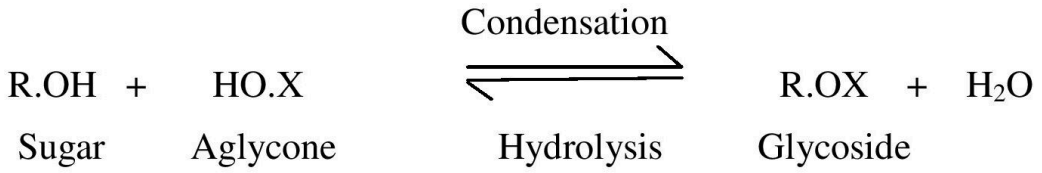
Numerous medicinal plants contain complex organic molecules which are conjugated with sugar moieties, mostly monosaccharides. The number of these sugar molecules could be one or more; such compounds are called glycosides and exercise therapeutically significant effects on humans and animals. Therefore, more advanced knowledge of natural glycosides is required by researchers and students in this field and by those who are dealing with the practical application of natural phytochemicals. In the plant kingdom, a variety of chemical constituents occur in a glycoside form (conjugation with sugar), and plant glycosides are a highly important target for modern scientific studies and plant utilization.

Glycosides may be defined, in general, as the organic compounds obtained from various species of plants and animals. The structural diversity of plant glycosides is caused by the large varieties and the stereochemical configurations of their sugar components. Plant glycosides are a highly important target for modern scientific studies and plant utilization. Glycosides are innate organic compounds; each is made up of two distinctly different chemical compounds joined together by glycosidic linkage. Among these two components, one is a sugar called a sugar moiety, and the other is a well-defined organic compound called a non-sugar moiety. The sugar moiety is also known as glycone, while the non-sugar moiety is known as aglycone or genin. Glycosides can be easily broken down to release the two components, as shown below:

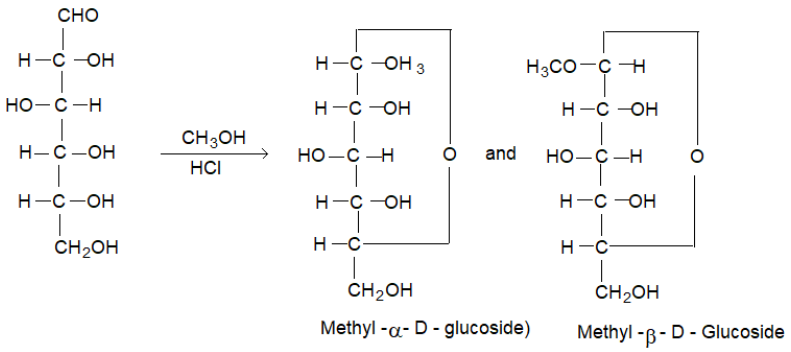
Glycoside----------> Glycone (sugar moiety) + Aglycon or genin (non-sugar component).

These compounds, in turn, are readily hydrolyzed to yield one or more sugars, and the non-sugar component



Chemistry of Glycosides

Chemically seen, the glycosides are acetals in which the hydroxyl of the sugar is condensed with a hydroxyl group of the non-sugar component, and the secondary hydroxyl is condensed within the sugar molecule itself to form an oxide ring. Hence the glycosides are considered sugar ethers. Emil Fischer, by passing dry hydrogen chloride in methyl alcohol, obtained the simplest glycosides α-methyl glycoside and β-methyl glycoside.



On hydrolysis of α-methyl glycoside, α-glucose and methyl alcohol and of β –methyl glycoside, β-glucose and methyl alcohol are obtained. Based on the type of glucose, glycosides are either α-glycosides or β-glycosides. If the sugar is glucose, a glycoside is called glucoside, while with other sugars, they are called glycosides. Glycoside can be hydrolyzed by enzyme, acid, alkali, or sometimes only with moisture. Enzymes hydrolyzing α-glycosides are called α-glycosidases examples-invertase and maltase, enzymes hydrolyzing β-glycosides are called β–glycosidases examples -Myrosin, linarase, amygdalase, cellobiase and gentianase.

These enzymes commonly take place along with the glycosides in the same plant and consequently, the compounds can be hydrolyzed when the two come in contact through extraction and isolation procedures, therefore, suitable measures are necessary to inactivate the enzymes. In some drugs, as in digitalis, original glycosides, *i.e*., glycosides as such, are more active and the action of the enzyme is undesirable, and steps should be taken to prevent it. In cyanogenetic as well as isothiocyanate glycosides, hydrolysis yield, *i.e*., genins, are active, and the action of enzymes is enviable.

Aglycones: In the glycoside molecule, the aglycone or genin is responsible for the pharmacological action (cardiotonics, purgatives, analgesics, anti-rheumatic, anti-ulcer and demulcent, *etc*.) of the glycoside. Aglycones fit into a range of different classes of distinct chemical compounds. Accordingly, the glycosides can be grouped as cyanogenic glycosides (amygdalin, prunasin), glucosinolates (glucocapparin), lactone or coumarin glycosides (daphnin, digitoxin), iridoid glycosides (swertiamarin, therapagoside), lignan glycosides (arctium), quinone glycosides (barbaloin), phenolic glycosides (sennoside A), flavonoid glycosides (quercetin), steroidal alkaloid glycosides (solanine), xanthone glycosides (mangiferin), and triterpenoid glycosides or saponins (glycyrrhizin).

CHAPTER 7

Mikania Species: Revealing Phytochemicals from the Pandora's Box

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Abstract: Natural medicines and traditional remedies are pretty valuable. Ayurveda, Traditional Chinese medicine, and Unani have all been practised in various parts of the world and have grown into well-organized medical systems. Secondary metabolites such as alkaloids, flavonoids, and tannins have already established their anti-microbial, anti-diabetic, and anti-cancer attributes. *Mikania* is one such plant genus used in folk medicine, which belongs to the Asteraceae family and is native to Central and South America. Still, it is extensively dispersed in Southeast Asia and Pacific Islands. Phytometabolites, *viz*., mikanolides and achalensolide, have emerged as potent antineoplastic agents. Sesquiterpene lactones such as deoxymikanolide and mikanolide possess anti-microbial activities. Apart from sesquiterpenes, several phenolic compounds comprising (+)-isolariciresinol and protocatechuic aldehyde were found in the aerial parts of *Mikania micrantha*. Antifungal activity of essential oil containing β-caryophyllene, δ-cadinene, and α-cubebene was characterized by GC/MS and isolated from *Mikania scandens*. Various steroids and diterpenoids obtained from *Mikania cordata* exhibited potent analgesic activity. This plant also contains germacrene D, β-pinene, and α-thujene, characterized by GC/MS. Many phenylpropanoids, sesquiterpenes, and diterpenes obtained from *Mikania laevigata* were characterized using NMR and mass spectrometry. Lupeol, lupeol acetate, and kaurene diterpenes were derived from *Mikania glomerata* and validated using RP-HPLC methods.

Keywords: Alkaloids, Coumarins, Flavonoids, *Mikania* species, Phytosterols, Phytoconstituents, Traditional use, Terpenoids.

INTRODUCTION

Many medicinal plants have been utilized as a kind of treatment for various ailments through still unexplained processes for ages throughout the world. As a

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result, the number of herbal medications grows every year, and the phytotherapeutic sector is worth billions of dollars [1]. Furthermore, most chemicals found in plants have the potential to induce significant adverse effects. As a result, it is critical to correctly identify and safely separate the primary components' chemical structures from employing active medicinal herbs. Furthermore, herbal medications offer unique properties that single compounds do not have since they are complex combinations of chemicals. Long-term therapy is quite familiar with phytomedicines since they take a long time to achieve a significant enhancement and appear to have a cumulative impact [2]. Furthermore, many phytomedicines have been phytochemically identified, but their mechanism of action is unclear, complicating the identification of a single compound. *Mikania* is the most extensive genus in the Asteraceae family, with over 430 species in tropical areas [3]. The herbaceous, annual, or perennial members of this genus are known for their scandent habit. Among these species, colloquially known as “guaco,” the most investigated *Mikania* species are *M. micrantha*, *M. scandens*, *M. glomerata*, *M. laevigata, M. cordata, M. cordifolia, M. vitifolia, M. hirsutissima, M. lindbergii, M. periplocifolia, and M. holwayana*. The most common variety of *Mikania* in India is *Mikania micrantha* (Fig. **1**). Furthermore, this genus has remarkable pharmacological properties like antispasmodic, sudorifics, antisyphilitics, anti-ulcer, anti-inflammatory, analgesic, antispasmodic, anti-microbial, anti-allergic, antipyretic, tonic, anticoagulant, cytotoxic, and many other properties, making it an important aspect to explore more [4, 5].

Fig. (1). Whole plant, aerial parts, and anterior and posterior view of leaves of *Mikania micrantha*.

GEOGRAPHICAL DISTRIBUTION AND TRADITIONAL USES

Asia and the Pacific, Australia, South, North, and Central America are all home to this genus (Fig. **2**). There are several species of *Mikania*; among them, the most

common species, along with their traditional uses, are listed in Table **1**.

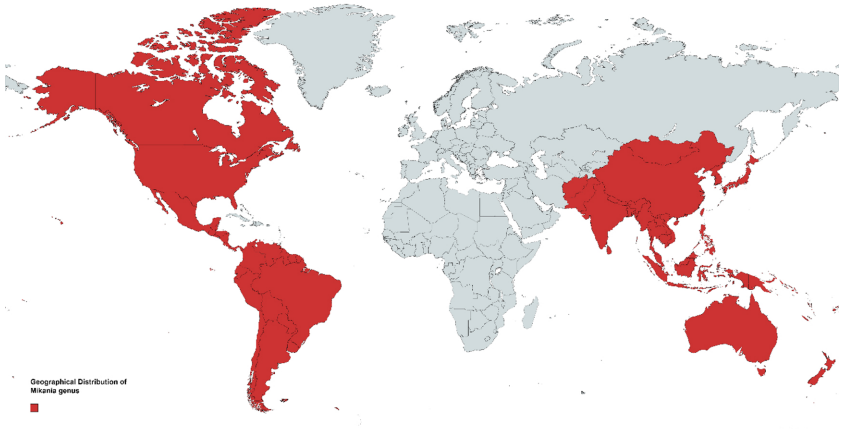

Fig. (2). Geographical Distribution of *Mikania* genus.

Table 1. Biological source, distribution, and traditional uses of various *Mikania* species.

Sl. no.	Scientific Name	Synonym	Geographical Distribution	Parts used	Traditional Use	Ref.
1	*M. micrantha*	Bitter vine, climbing hemp vine, Chinese creeper	India, Sri Lanka, Bangladesh, Malaysia, and other countries of Southeast Asia as well as the Pacific islands	Leaves	Venomous biting of insects reduces skin rashes and itches	[6]
2	*M. scandens*	Climbing hempweed	United States of America, India	Leaves	Stomach ulcers, wounds, and bruises	[7, 8]
3	*M. glomerata* and *M. laevigata*	Guaco	Native to South Brazil	Arial parts	Snake bites and respiratory troubles	[9]
4	*M. cordata*	*Eupatorium cordatum*	India, Sri Lanka, Bangladesh	Leaves and stems	Wounds and bruises	[10]
5	*M. cordifolia*	*Cacalia sessilifolia*	South America (Brazil, Columbia) and Mexico	Arial parts	Anti-inflammatory, anti-asthmatic, anti-parasitic, and analgesic	[11]
6	*M. hirsutissima*	*Mikania hirsutissima* DC	Southwest region of South America	Arial parts	Treatment for rheumatism, diarrhoea, and gout	[12]

CHAPTER 8

Implementation of *In-Silico* Drug Design to Find Natural Product-Based SARS-CoV 2 Spike Protein Inhibitor

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Abstract: COVID-19 has been a threat to the whole world due to its massive amount of infectivity. The causative SARS- CoV virus has an extremely small biological footprint. However, it has the ability to bind with the host cell, which, in this case, the human upper respiratory tract, with the intervention of a minimal amount of enzymes and energy. For this anchoring, this virus uses a specially designed protein known as the spike protein or S-protein which also gives the virus its unique shape. Unfortunately, even after the discovery of the vaccine, the number of people getting it is still significant. This is due to the ability of the virus to prevaricate immunity through constant mutation. Therefore, the search for an antiviral drug is still necessary. While there are only a few identified targets of anti-SARS-CoV drug designing, the S-protein can be unique for two reasons; first, it can be both virostatic and can be used as a post-exposure prophylaxis measure. Here, in this book chapter, we look into several drug-designing techniques that can be utilized for designing a molecule that can prevent the first stage of infection, and that is to attach with the ACE receptor of the host cell using the S-protein. Both ligand-based and structure-based designs have been taken into consideration, with a special focus on lead molecules obtained from natural products.

Keywords: Drug design, Molecular docking, QSAR, SARS-CoV2, S- protein.

INTRODUCTION

The COVID-19 pandemic has seen disproportionate responses in nearly all countries, leading to inequitable impact. Science has unearthed SARS-CoV-2, which made unexpected and unparalleled progress in the improvement of vaccines, but still, there is great uncertainty as the pandemic progresses. Partially, a great dependence on the continuing fruition of SARS-CoV-2 is created, based on the comportment of citizens, how to retort, development of the vaccines and treatments emphasizing on bringing the pandemic to an end fruitfully as well as

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learning how to diminish the bearings. Vaccines that offer long-lasting protection are effective in preventing both SARS-CoV-2 transmissions, and will not bar the pandemic or permit the world back to normal. Vaccination, along with the drug entities, has tried to reduce the mortality rate, but considering the longer term, new chemical entities need to be entitled. The beliefs about immunity longing can bring about concurrent threats. SARS-CoV-2 can doubtlessly never be eradicated. The vaccines remain anonymous since the duration of immunological efficacy is unknown. With such ambiguities, it cannot be presumed that the contemporary scientific progress in diagnostics, vaccines and treatments will end the pandemic. Vaccines and drugs can slash hospitalizations and potentially also deaths. Combining vaccines and antiviral drugs could become a powerful tool for controlling. Fig. (**1**) shows the cumulative cases in different countries, whereas Fig. (**2**) explores the cumulative deaths around the world. The data has been collected from the WHO coronavirus update [1].

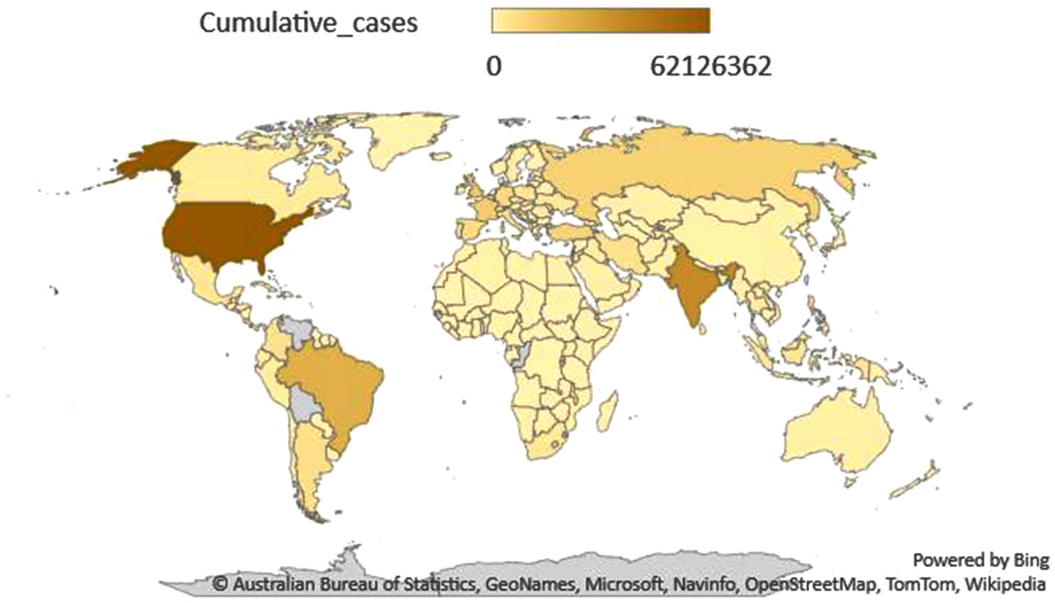

Fig. (1). Cumulative cases around the world as of 13.01.2022.

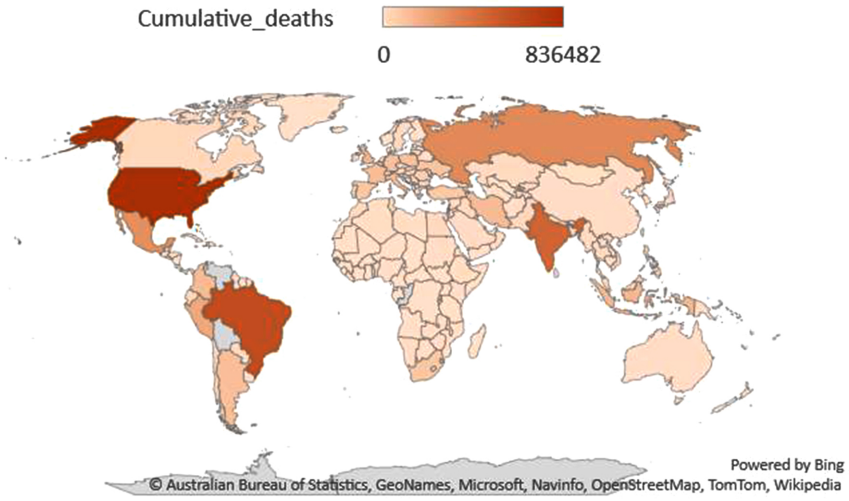

Fig. (2). Cumulative deaths around the world as of 13.01.2022.

Natural products have been an intrinsic part of enduring socialization because of their medicinal properties. The past discoveries of bio-actives based on serendipity and these compounds provide insight for the generation of analogues with desired physicochemical properties. Natural products with proper therapeutic potential are in abundance. However, there has been a remarkable revolution in the field of computer-aided drug design as many unproductive researches have been deterred and money, time and energy saved. Computer-aided drug design generates various aspects of research. The drug discovery processes from idea to market include seven steps: disease selection, target selection, lead compound identification, lead optimization and preclinical trial testing, clinical trial testing and pharmacogenomics optimization. The pragmatic basis of CADD includes quantum mechanics and molecular modelling studies like structure-based drug design, ligand-based drug design, database searching and requisite affinity based on the knowledge of a biological target. Cure with the help of medicinal plants is as prior to the evolution of mankind itself. The efficiency of computational approaches as the experienced tools for facilitating drug discovery and development has been acknowledged for a long in the case of natural products. Scientists are bombarded with data produced by advanced technologies. Thus, collating these data would be beneficial in improving the world's health population and well-being.

STRUCTURE OF THE SARS-COV2 S PROTEIN

The spikes of SARS-CoV-2 are trimers of S protein, which belongs to a group of class I viral fusion glycoproteins. The SARS-CoV-2 S protein translates a

CHAPTER 9

Phytochemicals of Promise in the Management of Type 2 Diabetes Mellitus

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Abstract: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) is typically characterized by insulin resistance, wherein there is a cross-talk involving obesity and inflammation. Symptoms of T2DM include polyuria, polydipsia, and weight loss, often accompanied by polyphagia, blurred vision, growth impairment, *etc*. The disease burden is often complicated by secondary complications like retinopathy, nephropathy, peripheral and autonomic neuropathy, increased incidence of atherosclerosis, peripheral and cerebrovascular diseases. Advances in understanding the pathophysiology involved in disease progression have led to the exploration of novel targets like glucose transporters, incretin-based therapies, SGLT-1/SGLT-2 modulation, AR/PTP1B dual inhibitors, PPAR-based therapies, NFκB and modulation of IRS for the management of T2DM. Various *in vitro* and *in vivo* studies have strongly suggested the potential of phytochemicals for the treatment of different pathological conditions associated with hyperglycemia and its complications. Different secondary metabolites have shown promising results in the treatment of different metabolic disorders, including T2DM, by different mechanisms of action. This chapter aims to provide an overview of some of the major phytochemicals, currently investigated for their potential activity against T2DM along with the mechanisms contributing to glycemic control.

Keywords: Glucose transporters, Hyperglycemia, Insulin resistance, Type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) has emerged as one of the major concerns for the global population because of its disease progression. As reported by the International

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Diabetes Federation in the IDF Diabetes Atlas, 9th Edition, 2019, around 463 million population globally are suffering from Diabetes mellitus, and the number will reach up to 578 million in 2030 and 700 million in 2045, as shown in Fig. (**1**). Around 4.2 million deaths were reported in 2019, which were associated with DM. In India only, the disease burden may reach staggering numbers, which is unprecedented.

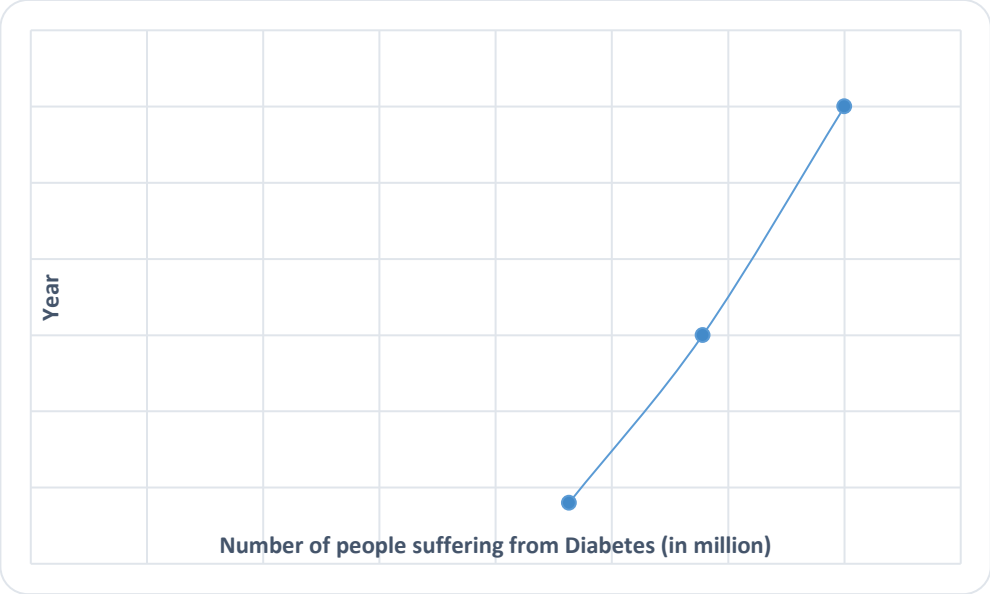

Fig. (1). Epidemiology of diabetes mellitus from 2015 to 2050.

Diabetes mellitus is a complex metabolic disorder characterized by a hyperglycemic state resulting from defects in insulin release, insulin activity, or both. The cells in the body need glucose as their source of energy, and the body regulates the amount of glucose that needs to be present in the system to maintain normoglycemic conditions. The body performs complex metabolic actions to regulate the desired normoglycemic condition. Any pathological condition that may result in failure of maintaining the normoglycemic state can cause hyperglycemia or hypoglycemia. Pathological conditions caused by inadequate insulin secretion or diminished insulin activity, or both of them result in hyperglycemia, whereas excessive insulin secretion may result in hypoglycemia, as seen in cases of insulinoma [1]. What makes DM such a threat is its association with different disease conditions, like obesity and inflammation, that arise with disease progression.

Chronic hyperglycemia is one of the major initial characteristic traits of DM, which include polyuria (frequent urination), polydipsia (sensation involving intense thirst despite drinking large amounts of fluid), weight loss accompanied by polyphagia (a condition with excessive physical hunger), blurred vision. Chronic hyperglycemia is often accompanied by growth impairment and susceptibility to specific infections [2]. The acute hyperglycemic condition leads to a sudden uncontrollable rise in blood glucose levels, known as diabetic ketoacidosis, whereas a much slower progression of uncontrollable rise in blood glucose level is known as a hyperglycemic hyperosmolar nonketotic syndrome (HHNS). Both these acute conditions prove to be fatal if left unchecked [3]. The disease burden of DM is often complicated by several other secondary microvascular complications, such as neuropathy, nephropathy, retinopathy and secondary macrovascular complications, such as peripheral and autonomic neuropathy, increased incidence of atherosclerosis, peripheral and cerebrovascular diseases [4].

The National Diabetes Data Group (NDDG) initially recommended the classification of DM based on its pharmacological therapy in 1979. In 1985, WHO also endorsed the classification by NDDG and classified DM into two major categories. However, the current classification of DM was published in 2003 by the recommendation of ADA expert panel. Four major categories of DM were proposed; **i)** Type 1 DM, **ii)** Type 2 DM, **iii)** Gestational diabetes mellitus and **iv)**other specific types of DM. Type 1 DM, formerly known as insulin-dependent DM, is caused by the destruction of β cells of the Pancreas. This is caused by autoimmune destruction of the β cells and results in the inability of the body to produce sufficient insulin. Type 2 DM, previously known as non-insulin dependent DM, is a hyperglycemic condition caused by insulin secretion and action. T2DM patients do not present with the destruction of inadequate β – cells, as seen in cases of T1DM. T2DM is the more common form of DM and has been seen in 85-90% of the total diabetic population. Gestational DM is first identified during pregnancy, and the hyperglycemic condition is often resolved with pregnancy. The other specific type of DM that has been around is Maturity Onset Diabetes in Young adults or more commonly, known as MODY. This type of DM is associated with the early onset of DM in adults younger than 25 years, and hyperglycemia in MODY is caused by mutations of specific genes, responsible for insulin secretion. MODY is further classified into 6 other sub-categories, based on the specific gene that is mutated [5].

Although T2DM has been the most identified among the different classes of DM, it is known that not all populations or persons present with the same risk as others. It has been established that people with different ethnicities, genetics and life styles are affected differently by T2DM. T2DM has been frequently associated

CHAPTER 10

Discovery of Potential Lead Molecules from Medicinal Plants using *in silico* Molecular Docking Approaches

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Abstract: Natural products have historically made a significant contribution to traditional and modern medicine and are considered one of the most successful new structural leads and effective drug development sources. Secondary metabolites and their structural analogues produced by suitable chemical or biological modifications have helped significantly to discover new lead molecules from herbal medicines. Moreover, 34% of prescription drugs are from plant-based or semi-synthetic derivatives, which include anti-cancer, anti-Alzheimer's, anti-hypertensive, anti-psychotic, infectious diseases and immunosuppressive molecules. More than 80% of people worldwide rely on herbal medicinal products and approximately 60% of clinical medicines are prepared from natural resources. This book chapter deals with the introduction to the phytochemical investigation, the significance and systematic steps involved in the phytochemical investigation, characteristics of phytoconstituents, selection of solvents, solvents influence and extraction procedure of natural products and treatment of plant residue after extraction. Further, the application of *in silico* molecular docking studies for the phytoconstituents detected from medicinal plant extracts to identify novel possible lead compounds against disease therapeutic targets. The key steps that enable natural product-based drug development and discovery with recent technological advancements are summarized.

Keywords: Drug discovery, Medicinal plants, Molecular docking studies, Phytoconstituents, Therapeutic targets.

INTRODUCTION

Traditional medicinal herbs are becoming popular due to their outstanding prope-

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Raja Chakraborty & Saikat Sen (Eds.)

rties as a rich source of bioactive compounds. Traditional medicinal plant knowledge has been passed down from ancestors to the next generations through oral transmission without any documentation. Therefore, traditional medicinal plants have a high value that must concern us [1]. Herbal remedies are still used in most developing countries because they are readily available, and cultivation and collection processes are eco-friendly, less expensive, safer to use, and highly effective [2, 3]. Indian traditional medicinal systems like Ayurveda, Siddha, Unani and folk (tribal) medicines place a high value on natural products (NPs). Morphine was the first alkaloid isolated in 1804 from the poppy plant (*Papaver somniferum*). Biological medicinal products have already made fruitful contributions to modern medicine [4, 5]. However, therapeutically important natural product-derived drugs are against diabetes, cancer, malaria, hypertension, ulcers, migraine, *etc* [6, 7].

NATURAL PRODUCTS ROLE IN MODERN DRUG DEVELOPMENT

Natural Products (NPs) and their structural analogues have played an essential role in pharmacotherapy, particularly for cancer, Parkinson's, Alzheimer's, diabetes, hepatitis, *etc*. Nonetheless, NPs that present challenges to the drug discovery process, such as screening, isolation, purification, characterization and lead optimization, are essential in the pharmaceutical industry. Several technical and scientific advancements, including enhanced analytical techniques, genome mining, metabolomics, engineering strategies and improvements in microbial culture, have addressed such difficulties over the past several years and opened new possibilities. As a result, NPs are becoming more popular as a potential source of novel therapeutic compounds used for the management or treatment of various diseases [8]. Some essential plant-derived drugs like atropine, codeine, morphine, thebaine, digitoxin, vincristine, quinine and artemisinin are still being used. For several years, NPs based compounds and their derivatives have been recognized to find lead molecules using powerful new technologies such as separation, isolation and purification [9 - 11]. Medicinal plants play an important role, especially in traditional medicinal practices.

Ayurveda: Ayurveda is mainly based on the five essential elements of life or pancha mahabhutas, *i.e*., earth, water, fire, air and ether, which all living bodies possess [12]. The Ayurvedic medicine method is generally recognized as the world's oldest written medical system and is often believed to be more effective than modern therapies, the dosage forms of Ayurvedic formulations, such as churna, asava, arista *etc*., are used for treatment or management of diseases that occur due to imbalance of kapha, vata and pitta. Multi-component mixtures of plant materials, animal products, minerals and metals also make up these dosage types [13].

Siddha: Siddha system of medicine is a traditional system of healing, mainly practised in the Southern parts of India. In Siddha, herbs are used primarily along with animal and mineral sources to treat various diseases, including colds, coughs, fever, psoriasis, eczema, alopecia, diabetes, *etc* [14]. Hence, the Siddha system is part of a way of life that deals with the relationship between the body and mind for maintaining physical, mental and moral health [15].

Unani: The basic theory of the Unani system is based on the Hippocratic idea; there are four senses of humour in the body, *i.e*., the heart produces red blood, the lungs produce white phlegm, the liver produces yellow bile, and the kidneys produce black bile. This theory was closely related to four elements: earth, fire, water and air [16]. Unani medicines are drawn from plants, animals and minerals/metal sources to treat several diseases, such as antibacterial, antidiabetic, antiulcer, anti-HIV, hepatoprotective, antitumor, antiarthritic, nootropic, anti-inflammatory, *etc* [17].

PHYTOCHEMICAL INVESTIGATION OF NATURAL PRODUCTS

A natural product is a chemical compound derived from plants, animals and mineral sources. Numerous herbs and plants derived drugs have been used for centuries as natural remedies and may lead to basic research on potential bioactive compounds that are now being studied as anti-cancer anti-Alzheimer's and antimicrobial agents [18 - 20]. Between 1981 and 2010, a detailed analysis of new drugs licensed by the US Food and Drug Administration (FDA) [21] indicated that 34% of small-molecule-based pharmaceuticals were used NPs or natural product derivatives such as statins, tubulin-binding anti-cancer treatments, immunosuppressants, *etc* [22 - 25].

Nature has been a rich source of medicinal products for millennia, with many useful drugs derived from plant sources. Once a hit has been verified in biological screening, the extract must be fractionated to separate the active chemicals, which generally necessitates the use of bioassays at each stage of the purification process [26]. Medicinal plants have long been recognized as a source of compounds with medicinal promise, and they continue to be valuable resources for finding new drug leads today. In previous decades, the pharmaceutical industry mostly relied on synthetic chemical libraries as a source of medication discovery [27]. However, natural compounds may have the additional benefit of drug development derivatives' innate affinity for biological receptors. Natural products have these characteristics, which allow them to engage biological targets more effectively than synthesized compound libraries, which have more planar and fewer stereo-chemically complicated features [27]. This suggests that such chemicals are likely to be substrates for one or more of the numerous transporter

CHAPTER 11

Role of Andrographolide and its Analogues in Colon Cancer

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Abstract: Colon cancer is the third most frequently occurring and second most death-causing disease in the entire world. Even though there is rapid development in the treatment, there is a high increase in the death rate of colon cancer. The current treatments available for colon cancer are not specific and are mainly associated with numerous toxic effects. Hence, developing novel drug treatments with desired characteristics to treat colon cancer patients is in huge demand. In case of novel drug discovery for various kinds of dreadful diseases like cancer and neurodegenerative diseases, medicinal and plant-derived components also play a significant role. Here, in this review, we have chosen andrographolide, a potent bioactive compound that shows multiple pharmacological actions such as anti-inflammatory, antiviral, antioxidant, hepatoprotective and neuroprotective effects. In addition, it also shows anticancer effects against various types of tumours along with colon cancer. In this review, we

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Raja Chakraborty & Saikat Sen (Eds.)

aimed to describe in detail the effect of AG and its analogues in colon cancer by identifying its possible drug targets. Thereby, it develops a chance to qualify AG as a novel drug candidate for colon cancer in the near future.

Keywords: Andrographolide, Analogues, Colon cancer, Modulation, Potential targets.

INTRODUCTION

Cancer is a rapidly growing health concern and one of the most deaths claiming diseases in the entire world. There are about 200 various kinds of cancers identified. However, each cancer's signs, symptoms and staging varies and depends on the size, location and biopsy [1]. Among the several cancers, colon cancer is the third recognized as well as the second most death-causing disease in the entire world. Moreover, it is evaluated that near about 60% of death will increase by the end of the year 2035 [2]. Importantly this data will vary and not be the same in all countries; it actually depends on the economic status of each and every country.

The major risk factors associated with the disease include being overweight, obesity, diabetes mellitus, inflammatory bowel disease, decreased physical activity, excess alcohol consumption, smoking, a diet with low fiber, vegetables, and fruits and alteration in gut microbe environment [2]. Along with this, socioeconomic status, age, race and gender are important in generating colon cancer. Earlier reports revealed that more than 90% of the cases of colon cancer till now reported were above the age of 50 years. In addition, there are three times increased risk of generating cancer in the population of age more than 65 years. This concludes age is one of the most important risk factors for the disease [2, 3]. Moreover, the data obtained from the American Cancer Society discloses that the men population has a higher chance of acquiring colon cancer as well as mortality in comparison with the women population. Besides, in women, cancer generates often in the right side region, and it is more vigorous than the development of tumor in the left-sided region [4]. Race is also one of the prominent risk factors that promotes colon cancer development. Earlier reports suggested that non-Hispanic black people develop colon cancer more commonly as compared to non-Hispanic white individuals, where the incidence of cancer is low [5, 6]

In spite of well-developed treatment techniques, cancer is still a huge health concern in both developed as well as developing countries. The current treatment methods followed can't eradicate the tumour completely, since most colon cancer cases are identified at a very advanced stage with metastases; this results in the mortality rate increasing drastically year after year. At present, surgery,

chemotherapy and radiation are the only options to treat colon cancer, but they remain far from providing satisfactory results. In chemotherapy treatment, 5-Flurouracil (5-FU) is primarily used as an individual drug or in combination with various other anticancer drugs such as oxaliplatin, irinotecan and capecitabine [7]. However, the available treatments are expensive and can't reach low-income patients. Furthermore, these treatments are primarily associated with severe toxicity and soon gain resistance by the cancer cells [8 - 10]. Hence, there is an urgent need to identify potential treatments for colon cancer with minimal toxicity and high specificity.

There are several sources of drug discovery, such as medicinal plants, synthetic, bacteria, marine and fungi. Among all the sources, medicinal plants and their secondary metabolites play a prominent role in identifying a novel treatment for various kinds of disorders [1]. From ancient times, human beings are much associated with plants and their derived materials for treating different ailments [11]. Due to the availability of a never-matched chemical library and negligible toxicity, the medicinal plant and its derived secondary metabolites always act as a better source of drug discovery for treating dreadful diseases like cancer and neurodegenerative diseases [12 - 16].

Several studies suggest that medicinal plants and their secondary metabolites always serve as a major source of anticancer compounds. The data obtained from Food and Drug Administration also reveals that 40% of drugs approved are of natural origin or their derivative compounds, in that 74% are approved for anticancer application only [16]. In this context, Andrographolide (AG), a medicinally active diterpenoid compound isolated from *Andrographis paniculata* extract that shows multiple pharmacological activities such as antioxidant, hepato protective, anti-arthritis, anti-inflammatory, immunomodulatory, anti-viral and anti-hepatitis effects [17]. Andrographolide shows an effect on various cancers *via* targeting multiple signalling molecules, such as Janus tyrosine kinases-signal transducers, phosphatidylinositol 3-kinase, metalloproteinase, cyclin-dependent kinases, NF-κB, P53, p21 and Heat shock protein 90 [18]. In addition, andrographolide also anticancer effect by inducing apoptosis in colon cancer cells *via* ROS generation and caspase-3, mTOR mediated pathway [19]. From the earlier studies, it was also reported that AG and its analogues show significant biological activities, including anticancer effects against various cancer cells such as breast cancer, cervical, liver, leukemia, and non-small cell lung cancer [18]. Moreover, they also target colon cancer cells *via* modulating multiple signaling pathways. This review aims to discuss the importance of andrographolide and its structural analogues in colon cancer.

CHAPTER 12

Screening of Natural Resources Through High Performance Liquid Chromatography

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Abstract: Currently, analytical techniques play a crucial role in drug discoveries and isolation processes. Different advanced methods are available for screening natural resources into desirable forms. Among them, chromatographic techniques are the most reliable and applicable for small quantities of samples. Chromatography is a technique for separating mixtures that depend upon differential affinities of solutes between two immiscible phases. One of the phases is a fixed bed of a large surface area while other fluid flows through or over a fixed phase. The greatest utility of chromatography lies in its ability to separate mixtures of solutes so that several individual substances may be quantities isolated in a pure state. Separation and identification of a natural substance can be challenging. Natural substances are a mixture of different components with different physiochemical properties. They are available as natural sources in large to small quantities in the form of a mixture. Herein, we are trying to explain different types of chromatographic techniques and types of strategies adopted to identify and isolate natural substances.

Keywords: Analytical techniques, Chromatographic column, Elution, Natural substances, Retention time.

INTRODUCTION

Different types of natural resources (NRs) are available for humans in different forms. Animal derivatives are Carbohydrates, Fats and Protein. Plant-derived

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Raja Chakraborty & Saikat Sen (Eds.)

alkaloids, flavonoids, Glycosides, Coumarines, Steroids and terpenoids inspired most of the active ingredients in medicines [1]. The high-performance liquid chromatography (HPLC) technique is a very powerful and versatile chromatographic technique for the separation of natural resources (NRs) [2 - 4]. The technique is widespread and has been adapted to the analysis of a broad range of NRs, generally without the need for complex pretreatment.

The technique provides high separation efficiency, rapidity and simplicity. By varying operation modes and chromatographic packings, the separation and purification of various types of phytochemicals could be achieved.

The chromatographic technique is simply based on the principle where sample components in the mixture are applied onto the surface or into the solid, and the stationary fluid phase (column) separates from each other while moving with the help of a mobile phase.

The chromatography technique based on [3, 5 - 7]:

- **Stationary phase(Column)**: This phase is always made up of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support"
- **Mobile phase**: This phase is always made up of "liquid"
- Separated molecules(sample components)

Nowadays, chromatography is accepted as an extremely sensitive, and effective separation method. HPLC technique, which has many superior features, especially its higher sensitivity, rapid turnover rate, and use as a quantitative method, can purify Carbohydrates, Fats, Protein, alkaloids, and flavon Glycosides, Coumarines, Steroids and terpenoids.

Types of chromatographic techniques:

1. **Affinity chromatography**: It is based on the specific affinity between a substance to be isolated and a molecule that it can specifically bind (a ligand). The specific protein which makes a complex with the ligand is attached to the solid support (matrix) and retained in the column. The bound protein leaves the column by changing its ionic strength by altering pH or adding a salt solution. Generally, the purification of enzymes, hormones, antibodies, nucleic acids, and specific proteins is performed by this technique [2].

2. **Column chromatography**: This technique mainly has a column (stationary phase) through which the sample is separated with mobile phase; their flow through inside column material placed on a fibreglass support is ensured. The samples are accumulated at the bottom of the device in a time and volume-

dependent manner. The purification of biomolecules was done by this method [2, 3].

3. **Ion-exchange chromatography:** Ion-exchange chromatography is based on electrostatic interactions between charged protein groups and solid support material (matrix). The matrix has an ion load opposite the protein's to be separated. Positively charged ion exchange matrices are called anion-exchange matrices and adsorb negatively charged proteins [2, 3].

4. **Gel-permeation (molecular sieve) chromatography**: Chromatographic technique is based on the difference in molecular weight or size, and is one of the effective methods used to isolate and analyze bio-macromolecular substances. Larger molecules pass through spaces between porous particles, and move rapidly inside the column. Molecules smaller than the pores are diffused into pores, and as molecules get smaller, they leave the column with proportionally longer retention times. Column material such as dextran, agarose, and polyacrylamide is used. This technique is basically used to determine proteins [2, 3].

5. **Paper chromatography**: In the paper, chromatography support material consists of a layer of cellulose highly saturated with water. In this method, a thick filter paper comprised the support, and water drops settled in its pores, making up the stationary "liquid phase." The mobile phase consists of an appropriate fluid placed in a developing tank. Paper chromatography is a "liquid-liquid" chromatography [2, 3].

6. **Thin-layer chromatography** is a "solid-liquid adsorption" chromatography in which the stationary phase is a solid adsorbent substance coated on glass plates. The solvent travels up the thin plate soaked with the solvent by means of capillary action. The separation of analytes is achieved. This upward travelling rate depends on the polarity of the material, solid phase, and solvent nature [2, 3].

7. **Gas chromatography**: Gas chromatography is a "gas-liquid" chromatography and its carrier phase consists of gases such as He or N_2. The mobile phase, which is an inert gas, is passed through a column under high pressure. The sample to be analyzed is vaporized and enters a gaseous mobile phase. The components contained in the sample are dispersed between the mobile phase and stationary phase on the solid support. The technique is simple, highly sensitive, and accurate for the separation of very minute molecules [2, 3].

8. **High-pressure liquid chromatography (HPLC)**: HPLC using normal phase conditions, followed the logic of atmospheric open-column chromatography in which the stationary phase acted as the polar phase, and the mobile phase was nonpolar, an organic solvent. Normal phase HPLC focused on the separation of

CHAPTER 13

Herbal Medicines: From Bench to Bedside

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Abstract: Mother nature has provided us with a vast array of plants to be utilized as medical remedies. Since ancient times, natural products have been and continue to be a fertile source for the development of novel medicines and therapeutic leads, and various research data conclude that more than 50% of the drugs in the market are derived from natural resources, highlighting the importance of herbal medicine in today's era. Recent research on medicinal plants has led to the rediscovery of long-known effects at the cellular and molecular levels. The absence of standardized procedures in older investigations shows up in situations of biological activity. Furthermore, traditional medicines with plant origins may be used as additional treatment alternatives to treat various illnesses. More research is needed for a solid knowledge of the herbal medicine system combined with current science that may enlist functional leads foundation for deadly disease states at preventative, diagnostic, promotive, and curative levels. Due to the many adverse effects of conventional treatment consisting of synthetic drugs, there is a surge in demand for complementary and alternative therapies, mainly herbal botanicals. With the paucity of empirical evidence on the mechanistic routes of herbal medications used till now, the medical world has had difficulty accepting them. As a result, there has been a surge in interest in deciphering the mechanisms of action of herbal compositions. Our work will highlight the various phytochemicals, such as flavonoids, alkaloids, glycosides, volatile oils, resins, organic acids, amino acids, tannins, proteins, and trace elements polysaccharides present in various plants that can be employed as a treatment option to cure major diseases.

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Keywords: Alkaloids, Amino acids, Anti-cancer, Anti-inflammatory, Anti-oxidant, Clinical trials, Extraction, Flavonoids, Glycosides, Health conditions, Herbal medicines, Neurodegenerative, Organic acids, Phytochemicals, Polysaccharides, Proteins, Resins, Tannins, Trace elements, Volatile oils.

INTRODUCTION

Plants have been identified as a crucial source for modern medicines, such as the emergence of digoxin from foxglove, morphine from the opium poppy, quinine from the bark of cinchona and aspirin from willow bark [1]. Herbal medicines, often known as nutraceuticals, are becoming increasingly popular across the world, with many individuals turning to these items for the treatment of a variety of health problems in a variety of national healthcare systems. Natural medicines have clearly risen in popularity and acceptability in both developing and developed nations over the last decade, with herbal medications being available not just in pharmacy stores but also in grocery stores and supermarkets [2 - 4]. As a result, herbal medicines are frequently seen as a balanced and reasonable approach to treatment, and people who use them as home remedies and over-the-counter pharmaceuticals spend money on them. This helps to explain why herbal medicine sales are rising and account for a significant amount of the worldwide pharma industry [4]. Herbal goods include extracts from roots, gums, leaves, seeds, bark, flowers, or berries that contain phytochemicals such as polyphenols and carotenoids, as well as alkaloids, phenolic acids, glycosides, flavonoids, lignans, and saponins, all of which are thought to have health advantages [5]. Plants have been found to supply a variety of essential metabolites, including lipids, carbohydrates, nucleic acids, and a variety of secondary metabolites like alkaloids, terpenoids, and phenolic chemicals. Anti-allergic, anti-inflammatory, anti-atherogenic, antimicrobial, antiviral, hepatoprotective, antibacterial, anticarcinogenic, cardioprotective, antithrombotic, and vasodilatory are some of the biological features of various herbal medicines Table (**1**) [6]. Herbs have long been employed in healing rituals by indigenous societies such as African and Native Americans. Other sophisticated traditional medical systems, such as Siddha, Ayurveda, Unani, and traditional Chinese medicine, have effectively utilized herbal medicines [7]. However, since the development of more predictable synthetic medications with immediate effects and availability, herbal medicine has experienced a dramatic decrease in the West.

On the other hand, many emerging countries continued to reap the benefits of herbal medicine's vast expertise. A huge majority of people in India still utilize Siddha and Ayurvedic remedies [8]. Traditional medicine plants that are utilized as food or raw material are more likely to produce pharmacologically active chemicals. Plants are also high in vitamins, biomolecules, and minerals, all of

which are essential for a healthy body [9]. Herbal therapy has been widely used to treat common problems like allergies, colds, upset stomachs, and toothaches. The existence of metabolites, which are organic substances classed as main or secondary, is responsible for the pharmacological actions of plants. Primary metabolites, including protein, glucose, lipids, polysaccharide, starch, and nucleic acids, help the human body grow and develop. On the other hand, plants create secondary metabolites such as alkaloids, saponins, flavonoids, steroids, terpenoids, tannins, glycosides, volatile oils, and so on to protect themselves from microbial infections or insect invasions [10].

Around 80% of India's rural population uses medicinal plants or traditional medical methods. The Indian herbal sector is projected to utilize about 960 plant species, and the industry's sales are more than Rs 80 billion [11]. AYUSH items, which account for 3% of total Indian pharmaceutical exports, are included in herbal exports [12]. Raw materials account for 70% of the herbal sector's exports, which are expected to be worth Rs. 10 billion per year. Finished items, such as herbal extracts, account for 30% of the export. In recent years, India has exported a significant number of medical plants and herbs, but the nation's contribution towards the worldwide herbal export market is less than 1% [13, 14]. Herbal medication use is on the rise, and the market is expanding steadily. The Indian herbal medicinal sector has an annual revenue of around Rs. 2,300 crores, compared to the pharmaceutical industry's turnover of Rs. 14,500 crores, with a 15% growth rate. India produces over 1,25,000 tons of castor seeds each year, making it the second-largest producer [15].

Table 1. Various herbal plants that have recorded medicinal utilization.

Plant Name	Family	Usage	Reference
Allium sativum	Liliaceae	Anti-hyperlipidemic Anti-hypertensive	[16]
Andrographis paniculata	Acanthaceae	Anthelmintic Hepatoprotective Dyspepsia	[16]
Aconitum heterophyllum	Ranunculaceae	Fever Reduction Cough Suppressant	[17]
Berberis aristata	Berberidaceae	Laxative Astringent Menorrhagia	[17]
Crocus sativus	Iridaceae	Antitumor Anti-spasmodic	[18, 19]
Garcinia Cambogia	Guttiferae	Anti-fungal Anti-obesity	[20]

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

L

M

N

O

P

R

S

T

U

V

W

X

Raja Chakraborty

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In recognition of his excellence in teaching, he was awarded the APJ Abdul Kalam award for Teaching Excellence in 2018 by Marina Labs Chennai. He was also recognized with a reward from the Journal of Forestry for the best-cited article of the year. Dr. Chakraborty has completed a significant networking project on Scented Rice of North East India, under the sponsorship of the Department of Biotechnology (DBT) of the Indian government.

Dr. Chakraborty was born and raised in Tripura, India, where he completed his basic education. Growing up, he witnessed the utilization of plants and herbs by the people of Tripura for their health needs, which inspired him to contribute his research to the field of natural chemistry in respect to traditional knowledge, aimed at serving both beneficiary and economic healthcare purposes.

Saikat Sen

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