Chapter 1

The Evolving Role of Natural Products from the Tropical Rainforests as a Replenishable Source of New Drug Leads

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Additional information is available at the end of the chapter

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1. Introduction

More than 80, 000 of the 250, 000 species of flowering plants have been reported to be used by human civilizations for medicinal purposes [1]. This figure could be higher as information on the native uses of plants as medicines was mainly passed on verbally from one generation to another and has mostly stayed unregistered. Some of the traditional knowledge might be lost as some practitioners were secretive and reluctant to reveal enough information. Information on the ancient uses of plant materials as medicines can be found in archeological finds, archives, ancient documents, history books and pharmacopoeias. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60, 000 years ago [2]. The World Health Organization (WHO) estimated that as much as 80 per cent of the world's population relies on traditional forms of medicine, chiefly plants [3]. The tropical rainforest plants are biologically and chemically diverse resource as they synthesize a wide spectrum of organic molecules as defense agents against diseases, pests and predators. They have been shown to be the source of chemicals of diverse structures with promising biological activities and perhaps the most valuable source of therapeutic agents due to their rich biodiversity [4]. Tropical rainforests have been called the 'world's largest pharmacy', because over one quarter of natural medicines have been discovered within them; additionally, about 70% of the drugs used today are models of natural products. Plants have always been a rich source of natural products and historically provided many major new drugs. Natural products or natural product-derived compounds signify great structural variety, which is not usually found in

synthetic compounds. Of the 1184 new chemical entities reported through 1981 to 2006, 60% were derived from or based on natural products. Twenty-five percent of all prescription medications are made using the various types of secondary metabolites from living organisms, mainly plants [5-9].

One hundred twenty-one prescription drugs are made from higher plants. This does not include antibiotics from microorganisms. Among the world's 25 best-selling pharmaceutical agents, 12 are natural product-derived [10], and natural products are playing key role in drug discovery programs of the pharmaceutical industry and various research organizations [11-13]. Half of these plant-based medicines come from the tropics, and 74% of these came from native folklore. Seventy percent of the plants identified as having anti-cancer characteristics by the US National Cancer Institute are found only in the tropical rainforests. Quinine, the first known antimalarial, comes from a neotropical tree, and curare, used as a poison for arrow tips by indigenous peoples in the Neotropics, is also useful for heart conditions. As new medical discoveries are being made all the time obviously, the tropical rainforests and their resources such as natural products find place in every part of our life. Plants have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites.Therefore, natural products play a leading role in the research of leads for the development of drugs for treating human diseases [14]. The investigation of biological and chemical properties of natural products for the past two centuries has not only produced drugs for the treatment of several diseases, but has instigated the development of synthetic organic chemistry, and the arrival of medicinal chemistry as a major route to discover efficacious and novel therapeutic agents. Structural alteration of natural compounds or synthesis of novel compounds, based on designs following a natural compound scaffolding, have offered us a lot of vital new drugs in the fields of medicine, agriculture, and food spheres [15-16].

2. History of drug discovery and development

Medicinal plants have been used by mankind since ancient times as the main source of medicines. Originally the choice of plants to treat various ailments was on an irrational basis as it was strongly influenced by ideologies of the primitive societies which were deeply rooted in mysticism and superstition. They believed, for example, a person became ill because evil spirits dwelled in his body. Plants with unpleasant odor and taste, accompanied by enchantments and ceremonial dances, were used to get rid of the demons and the person would then recover from his illness. However, the selection of plants for medicinal purposes became more rational when it was influenced and shaped by new philosophies and religions [17]. In the search for remedies from plants many different theories of disease were proposed and the choice of plants was based on these ideas. Although the ancient peoples were ignorance on the etiology of disease and the mechanism by which the medicinal agents made the disease disappear, they tried to establish a relationship between them. One of the earliest theory was the concept of plant signature which claimed that God provided visual sign (signature) in plants that permits us to recognize and utilize them to cure diseases [18]. Signature plants were

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probably first recognized in ancient China, where there was classification that associated plant features to human organs. 'Yang' was linked to strong acting plants: diseases of upper half of the body were treated with upper parts of plants, whereas 'Yin' was related with plants possessing modest action and those with sweet, salty, sour and bitter tastes; underground parts of the plant were used to treat the diseases of lower parts of the body [19]. Signature plants emerged for medicinal uses in western cultures during the Middle Ages. Paracelsus Doctrine of Signature, published in the 16th century, was a theory based on signature plants [17].

The signature plants provided a way of reducing the complexity of selecting plants to treat diseases. However, the traditional medicine practitioners of many cultures did not depend on the Doctrine of Signatures alone for knowledge of the proper use of a medicinal plant. A wide range of plants were experimented by trial and error to treat diseases and discovered that certain plants were effective against a number of diseases. The 'crude clinical trials' were actually a screening process but they could also possibly have produced many adverse effects, casualties and possibly thousands of deaths resulted from the toxicity of some of the plants. Several thousands of plants had been screened and based on their long-term use by human, one might expect that plants presently in use in traditional medicine produced some beneficial effects and have low human toxicity. If a plant showed acute toxic effects following its use to treat illness it would then not be used at all. However, if a plant had chronic toxic effects it would be less likely be noticed and it would continue to be used [20]. Moreover, there was lack of reporting system to document these effects as most information was passed on only by word of mouth. The information derived from the empirical knowledge and theories on the use of plants, although has been regarded with interested skepticism, had contributed towards the early development of medicinal agents from plants. The traditional knowledge and practices on the use of medicinal plants for treating diseases rely exclusively on practical experience and observation passed on verbally from one generation to the next with little documentation. It is necessary to understand the contemporary relevance of empirical knowledge and the interpretation of this knowledge using modern methods in the chemical and biological studies of substances [20].

During the course of history, natural products have offered a variety of compounds that have extensive applications in the fields of medicine, food and agriculture [21]. Higher plants in particular have been the basis of medicinal agents for centuries, and presently they play key roles in the primary health care of 80% of the world [22]. Medicinal agents and natural products therefore, are also a significant feature in the health care systems of the residual 20% of the population living in developed countries, with more than half of all the drugs in clinical use having sourced from natural products [23].The earliest history of the identification of novel bioactive compounds from natural sources can be traced back in 1804 when a young German pharmacist had successfully isolated morphine from the seed pods of the poppy, *Papaver somniferum* [24]. Since then, it marked the birth of the study on purification and the effects of drugs from natural products. This classical example represents most of the drug discovery process from natural products, where majority of new drugs have been discovered by direct isolation from natural sources and molecular modification of natural products. The phenotypic screening and single target or bullet-based approach have been the dominant paradigms to

discover natural small organic molecules from natural resources as new leads or models for the development of synthetic molecules for the discovery of drug targets.

Plant-based traditional medicine systems have been in existence for centuries in countries like China [25], India [26], the Malay Archipelago, and medicinal plants are used widely in African traditional health systems [27-28]. Many phytomedicines are registered and extensively used in Europe, and more than 600 botanical items have been legitimately documented in various editions of the United States Pharmacopoeia[29], however existing rules forbid most from being marketed as drugs. According to the WHO, majority of population still rely on plant-based traditional medicines for primary health care [30] and 80% of 122 plant derived drugs were related to their original ethnopharmacological purposes [31]. The knowledge associated with traditional medicines has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals. Until the 1950s almost all drug research relied heavily on vascular plants as sources of medicines. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs [32]. Most of the early discoveries of plant-derived drugs were by examining the use of these plants in traditional medicine. The phytochemicals isolated directly from plants were used unmodified as drugs. Morphine, isolated from opium poppy (*Papaver somniferum*) was the first plant-derived drug. Other examples include reserpine, an antihypertensive drug, from the root of *Rauvolfia serpentina*. the cardiac glycosides digoxin and digitoxin from *Digitalis lanata*, and the anticancer agents, vincristine and vinblastine from *Catharanthus roseus* [33]. The anticancer area, in particular, has made great use of natural products such as vinblastine and vincristine (vinca alkaloids), etoposide and teniposide (podophylltoxin analogues), paclitaxel (taxol), and camptothecin–derived topotecan. In the past ten years, 62% of the new anticancer-agents have been natural products or based on natural products models [31].

3. Conventional approach of drug discovery from natural products

History had shown that serendipity played a major role in the finding of novel drugs. One classical example that warrant a repetition was the discovery of penicillin, an antibiotic produced by *Penicillium notatum*, by Alexander Fleming in 1928. Henry Harris once said that 'Without Fleming, no Chain; without Chain, no Florey; without Florey, no Heatley; without Heatley, no penicillin.' After several years, the main active component was finally determined to be benzylpencillin which lysis the staphylococcal colonies on an agar plate [34]. The impact of the aforementioned example was so great that more scientists at that time started to look into natural products as the main source of leads. Since then, more structured strategies in natural products drug discovery were implemented in choosing plants for biological screening such as the ethnobotanical approach, chemotaxonomic approach or the phylogenetic survey screening, phenotypic screening and the single target or bullet-based approach.

The selection of plants based on traditional knowledge serves as the initial biological screening in a drug discovery program. The selection of plant samples for biological screening is

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generally based on the indigenous uses of plants. The ethnobotanical approach is more targeted and the most successful of the plant-surveying methods. The indigenous uses of plants offer important clues or information on the biological activities of those plants which are based on accumulated past knowledge and experiments by ancient civilization. Undeniably, some of the effectiveness of these plant extracts have been refuted due to the placebo effect in the patients, it is still one of the best approach for identifying leads. For example, plants used traditionally against diseases possibly caused by viruses are collected for anti-HIV screening [33]. The ethnobotanical field searches have generated many lead compounds which have been identified as drug candidates for the development of many therapeutic agents such as antiviral, antifungal and anticancer drugs. There are many important discoveries that lead to the development of more effective and safer drugs. Prostratin, a powerful antiviral agent, was found in the aqueous extract of *Homalanthus nutans* which is used in Samoa to treat yellow fever. A novel compound that kills parasitic worms in the stomach was isolated form *Curcuma comosa* which is used in Thailand to ease stomach pains and other gastrointestinal disorders. The extracts of ipecacuanha root, found in the genus of the Rubiaceae family, was used in the Aztec and Mayan cultures to treat amoebiasis and emetine, the bioactive compound responsible for the activity had been isolated from the plant [35]. Due to the adverse side-effects, a synthetic compound dehydroemetine was synthesized. Codeine which is found in the opium poppy, *Papaver somniferum*, is widely used for the treatment of moderate pain. The use of codeine can be traced back hundreds of years far back in the Egyptians and the Ancient Chinese history. Another plant extract known as ma huang or 'Yellow Hemp' has been used by the Chinese to treat asthma and hay fever for thousands of years [36]. It was not until 1926, when the active ingredient, ephedrine was introduced into the Western medicine as an orally active bronchodilator to treat acute asthma. Another important example is the use of cinchona bark from the species *Cinchona succirubra* by the Quechua, South American Indians in the treatment of fevers. The active ingredient, quinine was the first effective treatment for malaria caused by *Plasmodium falciparum*. Other plant that possess antimalarial activity was reported by the Chinese herbalist known as *Artemisia annua* or sweet wormwood which had been described in a 4th century text. Studies have shown that the active compound responsible for the antimalarial properties was artemisinin [33, 37].

Phylogenetic survey or chemotaxonomic method is a plant-collecting method where researchers select relatives of plants identified to yield useful compounds. The ecological survey is a selection made on the basis of defensive features of the plants against predators, indicating that they produce chemicals capable to exert an effect on animals. The non-targeted method (haphazard collection of plant samples) is practiced by researchers particularly in areas involving biological diversity, nonetheless the success rate is poor [33]. Taxol, derived from *Taxus brevifolia*, is a notable example of anticancer drug discovered by a random screening program. In phenotypic screening, pure compounds or extracts from plants are screened through *in vitro* followed by *in vivo* or *vice versa* to monitor the desirable change in phenotype before an effort is made to determine the biological target. Meanwhile the single target approach requires a molecular structure (active site or receptor) that will undergo a specific interaction with the so called plant extracts or pure compounds when they are administered to treat a certain disease. While these two pharmacological approaches are still commonly

practiced in most of the universities and research centers in developing countries, the chances of finding bioactive compounds from plants are highly serendipitous. To minimize the time consumption and tedious laboratory work, a directed screening is principally carried out on medical folkloric plants.

Methods adopted in the natural products isolation or bioassays screening have been rather straightforward. Crude extracts obtained from the dried or fresh plants are extracted with aqueous or organic solvents such as water, methanol, chloroform, hexane, and ethyl acetate. The practical of this isolation method can be found since the Mesopotamian and Egyptian civilizations where natural products extraction had been a common practice to produce perfumes or pharmaceutical products. Simple soxhlet extraction, maceration, hydro-distillation, and other solvent extractor techniques have been employed in the past and present, albeit large quantity of plant samples are generally required to obtain pure compounds. Conventional chromatography methods such as column and planar chromatography which rely on the differential partitioning between the mobile and stationary phases play important roles in drug discovery from natural products. Before new chromatographic methods were developed, isolation of minor compounds from plant extracts was relatively difficult. In recent time, the weaknesses using these conventional methods have been gradually solved by the replacement with more advanced instruments such as high-throughput gradient flash and medium or high pressure liquid chromatography (HPLC). In general, methods of isolation can be divided into a few categories including extraction, precipitation, and chromatography. Before the invention of sophisticated analytical spectroscopy techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS), infrared spectroscopy (IR), and X-ray crystallography, determination of molecular structure of pure compounds from natural products had been the major obstacle in drug development. People had been using different sets of chemical transformations and measurement of physical data such as melting point and molecular weight for the compounds structure determination. Issues regarding chirality have been an on-going debate among the scientists when deciding on the structure of the isolated natural compounds.

When Fischer and Daniel Koshland suggested the lock and key model and induced fit theory, the chirality of natural compounds had become an important issue that needs to be resolved before the real mechanism of action can be proposed. Since single target approach relies heavily on the interactions between the bioactive compound and protein target; possibly specific enzyme or receptor, the real identities of the natural compounds need to be accurately determined. Nowadays, it is obligatory to include the proper and accurate structural elucidation data of the pure compounds before the pharmacological activity can be published in any research journals. Such remarkable improvements in the spectrometry techniques have definitely amplified the speed of drug discovery process from natural products. A single plant extract may consist of a considerable pool of metabolites. Attempt to isolate the right bioactive compound out of the hundreds mixture of other compounds is quite tedious and time consuming; similar to finding a needle in a haystack. Two conventional approaches that deserved a mention here are the extract-library screening and bioassay-guided isolation. These two techniques, popularized in the 1980s, are commonly used for screening, hit identification, and hit-to-lead development from the natural products, however received a competitive

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disadvantage when compared to the approaches using the synthetic chemical libraries [38]. The screening of extract-library normally carried out on cell cultures to study the cellular responses under controlled conditions. The extract that shows certain activity will then be subjected to a series of isolations until the pure active compound is obtained. A more recent bioassay-guided isolation method requires a step-by-step separation of extracted components based on differences in their physicochemical properties and assessing the biological activity where the process continues until the right bioactive compound is successfully isolated. One best example is the use of TLC plate, in which spraying with reactive media that respond with the compounds from extract will produce a color implying the presence of an active compound and further isolation work is required to obtain the compound. While these two methods are in disadvantage position compared to the more well-defined high throughput screening on synthetic chemical libraries, lately it received a renewed interest from both the academic institutions and drug industries. The practicality of discovering new drugs by conventional methods has been constantly receiving negative perception from the funding agencies and private companies. Considering the number of people who actually have the access to high throughput screening chemical libraries or other high throughput bioassays, authors truly believe that the use of the aforementioned conventional methods are still highly relevant in our society. Without a doubt, these old practices have definitely set a good platform in natural products drug discovery program.

4. De-emphasize of natural products research in the 80's

The interests in finding bioactive natural products from the tropical rainforests at several major pharmaceutical companies had generally declined in the early 1980s when microorganisms and fungi which were easy to collect and culture provided alternative sources. Dwindling interests on plants as source of drugs were also due to low success rate in the discoveries of plant-derived drugs, and advances in synthetic organic chemistry and biotechnology that offered more opportunities to design new drugs in the laboratory [33, 39]. Many pharmaceutical firms had abandoned exploring traditional knowledge of plants in mid-1980s in their search for new drugs.. The reductionist approach in finding bioactive natural products from the tropical rainforests had declined due to the many major hurdles faced by them such as difficulties in obtaining sufficient supply of high quality natural products screening libraries, ownership issues and research in this field is lengthy, expensive, highly complex and ineffective with low success rate. On the other hand, the process of drug development is often a risky and costly endeavor. Drug discovery and development process requires between 10 to 20 years and was estimated to cost US$ 802 million per drug in 2004 [40]. Most recently it was estimated that the cost of inventing and developing a drug could exceed US$1 billion or more. The average drug developed by a major pharmaceutical company costs at least $4 billion, and it can be as much as US$11 billion [41]. Natural products drug discovery has been marginalized in favor of the rational design of synthetic compounds to target specific molecules after the advent of high throughput screening (HTS), combinatorial chemistry and advancement in the knowledge of molecular mechanisms, cell biology and genomics.

Historically numerous complications linked with natural products, particularly plant-derived products, added to decreasing attention in their development within the pharmaceutical industry. A few years ago, there were major problems with obtaining authentic plant materials. Difficulty in the access to genetic resources and traditional knowledge by the multinationals and pharmaceutical firms and the absence of a legal framework for a fair and equitable sharing of benefits, arising from the commercial and other utilization of genetic resources with the resource countries resulted in the departure of many drug companies from investing in natural products from tropical rainforest plants as a source of drug leads. It was convenient to collect plants and exhibit that their extracts possessed interesting biological potential. However, when investigators returned to approve the potential and ultimately to develop and commercialize the product, failure resulted often due to inadequate records and loss of the original collections of plant. There were also problems regarding the assessment of biological activities of natural products, which commonly are complex combinations of materials. The interactions among the components of the mixtures, either the antagonism by a material of another's activity or the combined effect/addition of activities, often delivered unclear results. Identification and purification of active components from intricate natural products encompassing lots of numerous chemical substances, regularly of almost similar physical and chemical properties, were slow and expensive. Once the active component was isolated and purified, its chemical structure still needed to be documented. Moreover, natural product materials are often poor pharmaceuticals; their chemical stability may be minimal; they might have poor solubility or bioavailability characteristics; they may not formulate properly, etc., therefore not ensuing Lipinski's Rule of Five [42]. All these difficulties have stood stern challenges. The most accountable problem for restricted importance in plant-based natural product materials for pharmaceutical discovery and development has been hesitation regarding the availability of amounts of pure chemical substances. Quantities are required initially to generate information to understand and assess real prospective of the material for pharmaceutical application. Ultimately, the most restrictive thought is the amount required to fulfill market demand should a pharmaceutical develop into an effective drug. The market demand can vary from a scale of hundreds to thousands of kilograms yearly. It is recognized that the complete synthesis will not provide the intricate natural product to satisfy this market demand [43].

Subsequent to the 'Golden Age of Antibiotics' and the worldwide reassurance to discover new antibiotics, several main pharmaceutical companies at the time initiated natural product discovery (NPD) programs which highlighted not only on antifungal and antibacterial targets but also on infectious illnesses. These platforms provided compounds for the treatment of hypercholesteremia, tissue rejection in organ transplantations, cancer and microbial infections [44, 45]. Though majority of the larger pharmaceutical industries removed their NPD programs during the 1980s and early 1990s, it was the advent of programmed high throughput screening (HTS) which enhanced the impetus of biological testing and combinatorial chemistry started to be endorsed as a better method to producing 'drug-like' materials for HTS. As a result, several pharmaceutical companies sold their gatherings of screening extracts [46, 47] as it was believed that orthodox extract-derived monitoring resulted in the constant rediscovery of previously isolated compounds and that the structural intricacy of natural products required whole synthesis and derivatization which is both synthetically and economically difficult.

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Owing to supply difficulties, the time required to develop a natural product from an extract to a pharmaceutical was believed to be excessively lengthy; HTS machineries rely on combinatorial chemistry to yield huge compound libraries. During the past two decades 'customary natural product chemistry' has predominantly been substituted by molecular target-based drug discovery, using great combinatorial libraries to discover effective 'hits' [45]. However, developments in technology and sophisticated instrumentation for the rapid identification of novel natural products and structure explanation keep enhancing the natural product discovery [44]. Since 1980s, it was thought that combinatorial chemistry would be the upcoming basis of numerous new carbon skeletons and drug front runners or novel chemical entities (NCEs). This has unquestionably not been the case because there has only been one combinatorial NCE permitted by the U.S FDA in that time frame, the kinase inhibitor sorafendib (approved by the FDA, 2005) to treat renal carcinoma [48]. Combinatorial chemistry has definitely altered the progress of new chemical leads following in the synthesis of structural analogues [48]. At that instance, combinatorial libraries included hundreds to thousands of novel compounds; however through the late 1990s synthetic chemists came to know that these libraries needed the intricacy of the complex natural products produced by nature [48]. The idea of diversity-oriented synthesis (DOS) was applied in which synthetic chemists would synthesize compounds analogous to natural products or that are based on natural product topologies. These compounds are currently being verified in a large variety and number of biological screens to define their role (s) as leads to new drug entities [48]. The examination of the degree of NCEs authorizations shows that natural products still add to or are involved in ~50% of all small molecule testing from 2000–2006 [49]. This is endorsed by the statistical data where out of 7000 natural compounds, 20 drugs have been discovered with a hit rate of 0.3% whereas HTS of synthetic compound libraries only achieved below 0.001% hit rate. Nonetheless the pharmaceutical industries have used considerable resources to both combinatorial chemistry and HTS [49]. Of the 1184 NCEs covering all diseases/sources/countries, 30% were discovered to be synthetic. It is also noteworthy that 52% of these compounds are whichever a natural product, a chemical modification or a mimic of a previously existing natural product pharmacophore [49]. Notwithstanding recompenses and the past accomplishments, majority of large pharmaceutical companies have reduced the use of natural products in drug discovery screening. This has been due to the apparent shortcomings of natural products and the expectations related with the use of assortments of compounds prepared by combinatorial chemistry approaches.

5. Revival of natural products research in the 90's

However, there was a revival of traditional knowledge-driven drug development in the later part of the eighties and this was partly due to the advances in chromatographic and spectroscopic techniques which have had a tremendous impact on the isolation and structure elucidation of the constituents of medicinal plants and the development of series of bioassay methodologies which were fast, easy to perform, quantitative and could selectively detect biologically active molecules at very low levels [50]. The phytochemicals can also be used as

starting materials for the partial synthesis of drugs, as lead structures for molecular modification or as models to synthesize new drugs with improved therapeutic effects. In order to conduct preclinical and clinical trials and further develop a promising lead into a marketed drug, sustainable supply is necessary. The continuous supply problem is the major challenge for plant natural product drug discovery and development. Nevertheless, contemporary methodologies are accessible to overwhelm the difficulties. Advancement in technologies like biotechnology, fermentation, total chemical synthesis, sampling strategies and nanoscale NMR for structure determination are all vital to the accomplishment of natural products as drug leads. The novelty in the field of natural products, will result in a new wave revival of novel drugs in the upcoming future [51]. The past few years, though, have seen a reintroduced attention in the use of natural compounds and, more significantly, their part as a basis for drug development. The contemporary apparatus of chemistry and biology particularly, the numerous '-omics' technologies now let researchers to detail the precise nature of the biological effects of natural compounds on the human body, in addition to discover probable interactions, which grasps much potential for the development of novel treatments against numerous devastating diseases, including cancer and dementia. The recent revival of interest in natural products research is mainly due to disappointing results of combinatorial chemistry and HTS in delivering potent chemical leads. There is a new hope for the discovery of drug leads from the tropical rainforests. One reason why natural products are advantageous is that they provide complex molecules not accessible through synthesis. For example, taxol and rapamycin could not be synthesized cost effectively by standard medicinal chemistry, even including combinatorial chemistry approaches. Combinatorial synthetic methods are unlikely to produce molecules of the complexity of rapamycin and taxol.

Natural products have pointed the way to the future. A number of noteworthy advances in science and industry have been inspired by the quest of capturing the value of natural products. In 1982, the US National Cancer Institute began again to look for new drugs from the rainforests. There are good reasons for such renewed interest because they have already provided tangible evidence of their potential with remedies for a range of medical problems ranging from childhood leukemia to toothaches. Some examples of rainforest plants responsible for 25 percent of the drugs used in the clinic are included in Table 1. Most developments in capability and technology are nurturing a revival in natural products research and are either directly or indirectly addressing the historical obstacles to development of natural products [52-56]. There are three foremost reasons for the revitalization of natural product research; i). The potential of combinatorial chemistry to fill drug development projects with de novo synthetic small-molecule drug candidates is unsatisfied. ii). The applied problems of natural products drug discovery are being overawed by advances in separation equipment and in the speed and sensitivity of structure explanation. iii). A convincing case is being made for the essential usefulness of natural products as foundations of drug leads.

Possibly the strongest motivation for development of new natural products is the improvement in bioassay technology over the last few years. We now have automatic, specific and selective bioassays in which materials, comprising natural products preparations, can be evaluated quickly and cost-effectively. In reality, although the bioassay technology develop-

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ment facilitates faster and accurate substances evaluation, the availability of those evaluating substances has become very limited. Once the biological activity has been confirmed by a suitable bioassay or primary screen, the current separation and structure elucidation technology allow us to isolate, purify and determine the chemical structure of the active constituents in few days, or at maximum, a few weeks. The development of separation methods are mainly linked with high performance chromatography techniques [52]. The biodiversity of the earth is quickly declining and we frequently come across many articles in newspapers and journals on the rate, consequences, cause, etc., of loss of biological diversity. This will lead to the loss of chemical diversity and loss of potential uses of those chemicals have for mankind. Besides medicines, foodstuffs, fibers for clothing, building materials, etc. are chemicals derived from nature. The loss of those important organisms and their respective chemical diversity is the driving force for the revival of natural products research [56]. Globalization of world's economy increases the interest in pharmaceutical development. Since the discovery and development of new pharmaceuticals help to uphold the competitiveness of the industry, natural products research has been revisited as a way to increase the effectiveness of discovery and development [55].

No	Drug	Origin	Use
1	Quinine	Cinchona tree (S. America)	Treat malaria
2	Neostigmine	Calabar bean (Africa)	Used to treat glaucoma and provides a blueprint for synthetic insecticides
3	Novacaine, cocaine	Coca plant (South America)	local less addictive anesthetics
4	Turbocurarine	Curare liana (America)	Native people used to poison arrow tips. Used as relaxants to treat muscle disorders like Parkinson's and Multiple Sclerosis.
5	Vincristine, vinblastine	Rosy periwinkle (Madagascar)	Hodgkin's disease and Pediatric leukemia
6	Cortisone	Wild yams (Central America)	Active ingredient in birth control pills
7	Calanolide A	*Calophyllumlanigerum* (Borneo)	Reverse transcript inhibitor (Anti HIV)
8	Michellamine B	*Ancistrocladuskorupensis* (southwestern Cameroon)	Reverse transcript inhibitor (Anti HIV-1, and HIV-2)

Table 1. Drugs derived from rainforest plants

Many new approaches have been employed in the isolation and purification of NP, using various advanced fractionation techniques which includes the counter-current chromatography [57, 58] and structure elucidation by spectroscopic techniques (1D, 2D NMR spectroscopy, FTIR, UV, mass spectrometry, etc.) [59, 60]. The arrival of more compatible high-throughput screening technique provided an opportunity to screen natural products mixture within the quick span of time. Singh and Barrett [59] reported that pure bioactive compounds can be isolated from fermentation broths in 2 weeks and that the structures of more than 90% of new

compounds can be elucidated within 2 weeks. Advanced NMR techniques permit to solve complex structures using less than 1 mg compound. Quinn et al. [61] showed that it was possible to prepare a screening library for highly diverse compounds from plants from analysis of the Dictionary of Natural Products, where the compounds were pre-selected based on drug-like in their physicochemical properties. It will be interesting to see if such a collection proves to be enriched in bioactive molecules. Besides the above, many alternative methods are also being explored in efforts to increase the speed and efficiency of using natural products in drug discovery. Multifaceted approach integrating traditional and modern techniques, i.e. combining botanical, phytochemical, medicinal chemistry, HTS, organic and combinatorial chemistry synthesis producing natural products-like compounds, biological, computational, molecular, multi-target approach, metabolomics, proteomic and genomic techniques will continue reviving the interest in using natural products as leads for the discovery of new drugs.

6. Modern approaches in drug discovery from natural products

The arrival of novel technologies in mass spectrometry, NMR and other spectroscopic techniques, bimolecular target or cell-based screening, early hit characterization and the utilization of computational methods have improved the impact of natural products in the HTS based drug discovery. Natural product extracts frequently contain a large number of constituents comprising those, which are difficult to separate. The unambiguous structures of pure compounds can be determined by the combination of conventional techniques like ultraviolet absorption spectroscopy (UV), IR, MS and NMR. In rare cases, where there is a difficulty in determining the absolute configuration, the single crystal X ray analysis is employed. The conventional separation techniques are time consuming and tiresome. The direct hyphenation of a proficient separation technique with efficient spectroscopic techniques such as HPLC-FTIR can be used to support the dereplication process [62]. HPLC-FTIR has been used to detect functional groups in main constituents of mixtures but has not found extensive application owing to limitations in compatibility; i.e. obtaining optimum separation accompanied by adequate detection [62].

One of the new technologies in drug discovery from natural products is the use of capillary electrophoresis (CE) in screening program first developed by Cetek Corporation and Cubist. The assay is able to identify active natural product compounds/ extracts and by detecting any shift in the protein when a ligand binds to it due to the conformational and surface charge changes. The CE technique can distinguish between weak and strong binding compounds in extracts prior to determining their concentration [63]. This technology has been applied to Cetek's internal drug discovery program in finding novel natural product compounds that inhibit the cancer target, HSP90, a molecular chaperonin that is responsible for maintaining the correct folding and stability of proteins [64]. Another interesting example which was recently reported by Wang, et al.showed that a CE method in conjunction with liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been successfully applied in the screening of plant extracts, successfully identified a natural compound called baicalin from *Radix scutellariae* as a new protein kinase an inhibitor [65]. In an another separate study, Zhao

and Chen, developed a simple and effective neuraminidase-immobilized capillary microreactor facbricated by glutaraldehyde cross-linking technology for screening of the neuraminidase inhibitors from traditional Chinese medicines. Six out of eighteen natural products including bavachinin, bavachin, baicalein, baicalin, chrysin, and vitexin have been found as potent inhibitors from the screening[66]. Some important aspects of CE that deserve a recognition in this chapter is its ease of use, versatility and high resolution separation components, high separation efficiency, and its low amount of sample and reagent consumption.

Flow injection analysis-NMR (FIA-NMR) encompasses a sample, which is injected as a plug into a fluid stream and then swept into the NMR detector coil. In a FIA-NMR, the mobile phase is used as a hydraulic push solvent which transfers the injected sample from the injector port to the NMR flow cell. The scout scan that is used to determine the location of the solvent peaks is obtained by the spectrometer once the pump stops. Finally a signal is sent to the solvent pump so that the old sample from the NMR flow-cell can be flushed out [67]. HPLC-NMR-MS is a novel and most advanced spectrometric method that is used in the de-replication of natural product extracts [68]. Despite being the most effective method, the benefit of the above said hyphenated methodology is the matching of the MS data to the NMR spectrum. In addition, the information of the functional groups (e.g., hydroxyl and amino moieties) that are delivered by the HPLC-NMR is readily identified by MS techniques. The advent of higher field magnets and cryo probes had proven HPLC-NMR to be a strong and effective spectroscopic instrument and applied to the crude extracts (NMR and UV profile from PDA HPLC detection). There is a significant improvement in the profiling sensitivity and de-replication of natural products due to the utilization of higher field magnets and the recent developments of the micro coil HPLC-NMR and capillary NMR (CapNMR) which has allowed for smaller amounts of samples to be examined in the order of 40–120 μL [69-71]. The micro coil HPLC-NMR is best suited for online HPLC-NMR which uses the on flow, stop flow or time silencing experiments to separate components present in greater concentrations and analyses the same [71-74], whereas the Capillary NMR uses the non-deuterated solvents in an off-line HPLC separation thereby offering a wide range of solvents with a low cost. The isolated compounds are re-dissolved in deuterated solvents and then injected into the CapNMR flow probe using volumes of around 6 μL with ^{1}H-NMR spectra acquired for sample quantities in the order of 2–30 μg, thereby increasing the sensitivity with a prospect to classify the novel low level secondary metabolites [75].

Besides the above, the information obtained from the 1D and 2D NMR spectra is sufficient to classify the compounds in addition to the provision of a 'high-fidelity' snapshot of the constituents in the extract, thus providing the information which paves way for rational decisions about the top method of fractionation or to proceed with isolation further. Many recent publications have been reported using this approach [71-73].The technique and utilization of HPLC-NMR in natural products identification/classification is well recognized in the literature but applications of its uses have mainly dealt only with the chemical profiling of plants [76-78]. Numerous modes of HPLC-NMR (mostly on-flow and stop-flow modes) combine the resolving power of chromatography, which is interfaced with the structural

understanding provided by NMR. The reductionist approach has not been very successful in discovering effective drugs to treat complex diseases, such as cancer, metabolic, cardiovascular and neurological diseases. Single-target drugs may not always induce the desired effect to the entire biological system even if they successfully inhibit or activate a specific target.There are limitations in the use of reductionist or mono-target approach in drug discovery. The approach yields only a limited understanding of complicated pathogenesis and multi-target pathologies of systemic diseases such as cancers, cardiovascular diseases and neurodegenerative disorders. There is difficulty in identifying relevant interventions to target such complexities. Bullet-based or mono-target drug intervention cannot effectively combat the complex pathologies of systemic diseases as they are regulated by complex biological networks and depend on multiple steps of genetic and environmental challenges to progress. Recently there is a growing interest to use innovative approaches to drug discovery from natural products by network pharmacology which integrates systems biology and pharmacology [79]. The integrated multidisciplinary concept of multiple targets, multiple effects and complex diseases in network pharmacology have enriched our understanding of complicated pathogenesis and multi-target pathologies of systemic diseases and reduced difficulty in identifying relevant interventions to target such complexities. The '-omic' technologies in system biology have now been widely used to correlate and elucidate multiple targets and network of human diseases and drug actions [80]. The concept of network pharmacology is especially useful in accurately translating and interpreting the therapeutic effects of herbal medicines into modern biochemical and biological meanings. Herbal medicines may serve as valuable resources for network-based multi-target drug discovery. The concept of network pharmacology is especially useful in accurately translating and interpreting the therapeutic effects of herbal medicines into modern biochemical and biological meanings. The efficacy of the multi-target drugs from herbal extracts are developed followed by identification of their major bioactive components and redevelopment of a completely new multi-component formulations composed of the major bioactive components in order to reach a synergistic and optimal combination [81].

Combining natural product chemistry and metabolomics approaches in drug discovery is a new strategy to discover new drugs. There are few reports in the scientific literature, which discuss the unison of classical natural product chemistry approaches with metabolomics to identify novel bioactive natural products. These have generally focused on the study of plants [82]. The identification of bioactive natural products from plants remains a multifaceted task because of their high chemical diversity and complexity. By measuring the metabolome of different extracts or fractions of a plant and combining these data with its corresponding biological activity, signals related to the compounds related to the displayed activity can potentially be determined. Systems biology is a most promising field encompassing tools in the post-genomics revolution such as transcript omics, proteomics, glycomics and fluxomics with an intention to characterize all gene and cell products completely inclusive of mRNA, proteins, glycan structures and metabolites. Metabolomics aims at constructing balanced observations using highly reproducible tools followed by the analysis of data to locate the correlations between the available data. The profiling of all the low molecular weight metabolites of an organism is not possible and hence this emerging field of metabolomics combines analytical chemistry, biochemistry and computational biology permitting the analysis of

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thousands of metabolites in any biological system. The principal analytical platforms are the Mass spectrometry with gas chromatography (MS-GC), liquid chromatography (LC) or capillary electrophoresis (CE) and NMR spectroscopy. A balanced extraction procedure to efficiently extract all the primary and secondary metabolites from tissues and the body fluids is used to obtain them in the natural form prior to the analysis in the various solvents used. The metabolite extraction procedures are more complicated and complex due to the diversified nature of small molecules present and due to the unavailability of a single analytical technique and platform which helps in analysis of all the metabolites simultaneously. Several separation techniques and methodologies need to be applied to achieve complete analysis of the metabolites [83]. Simultaneous analysis of hundreds of compounds is achieved by various tools in informatics that extracts information from the data, removing the background noise, detection and integration of peaks throughout large data sets and normalizing and transforming the resulting data matrices prior to any statistical analysis [84]. Metabolomics has a restricted access to the ability of identifying the signals with respect to the chemical nature. About 60 to 80 % of all detected compounds are unknown [84-85] even today and the metabolic discipline has created a large mass of spectral NMR library to tackle this problem. These unknown secondary metabolite structures can be one among the undiscovered resources of the natural products, fingerprinting, foot printing, profiling or target analyses are common terms used in this field. Fingerprinting aims to take a 'snapshot' of the organism where the signals cannot necessarily be used to detect/identify specific metabolites and depends strongly on the technique used. The signals are assigned to a metabolite irrespective of its nature to be a known or a novel compound. The term target analysis aims to determine and quantify a specific metabolite of interest [81].

The microarray is a new technology recently developed that has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body.DNA microarray technology can analyze and compares changes in DNA or protein. A chromosomal change in an abnormal individual could be identified when DNA from this individual is compared with DNA (control) from a healthy individual. It is very precise and useful in that it is capable to detect much smaller changes compared to conventional karyotyping technique. This competent technique enabled us to understand the elemental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body. Microarray technology has been utilized extensively in pharmacogenomics where comparative analysis of the genes from an unhealthy and a normal cell will help the identification of the biochemical constitution of the proteins synthesized by the abnormal/unhealthy genes. The information obtained from the analysis then could be utilized for synthesis and design of drugs which fight the abnormal proteins and reduce their effect [86].Kwon and his colleagues have developed an *in vitro* approach utilizing a multi-enzyme containing microarray system for high-throughput synthesis of polypeptide derived product and their subsequent full polyketide-based library screening of the human tyrosine kinase (TK), on a single glass microarray. The TK inhibitors are expected to treat chronic myeloid leukemia, gastrointestinal stromal tumours and breast cancer [87, 88].

A drug discovery program aims to find novel bioactive natural products, which possess some form of potent biological activity. However the isolation of known and undesirable natural products with no pharmacological interest or chemical is inevitable. The term dereplication which is a process of identifying known compounds responsible for the activity of an extract prior to bioassay-guided isolation becomes popular among the natural products researchers [89, 90]. Dereplication strategies generally involve a combination of bioassay, separation science, spectroscopic methods, and database searching and can be regarded as chemical or biological screening processes. There are a number of ways in which natural product programs approach dereplication, which is based upon availability of screening methods/instrumentation, time and the cost to identify possible 'biological leads or novel compounds' from a crude extract. At present there are many advanced methodologies and protocols that distinguish novel entities from known natural compounds at an early stage of a drug discovery program or in a natural product isolation strategy [90]. The dereplication process can be easily done by screening the compounds through the commercially available databases, reducing the time taken for structure elucidation of known compounds. One example is the Chapman and Hall Dictionary of Natural Products [91]; The Dictionary of Marine Natural Products (on-line) (subset of the Dictionary of Natural Products) containing over 30, 000 compounds [92]; MarinLit- The Marine Natural Products Database containing up to date bibliographic data on marine organisms with the number of references from 1, 200 journals/books and data for ~21, 000 compounds [93]; AntiMarin is a more recent database, in which the number of methyl groups, the number of sp3-hybridised methylene or methine protons, alkene, acetal, ether and formyl groups can be searched [94, 95]. Besides, SciFinder Scholar and SCOPUS are important research discovery tools (Chemical Abstracts on-line) [96, 97] and NAPRALERTTM is a database of all natural products, including ethnomedical information, pharmacological/ biochemical information of extracts of organisms *in vitro*, *in situ*, *in vivo*, in humans (case reports, non-clinical trials) and clinical studies [98]. The availability of these scientific databases such as the ones mentioned to the research and academic institutions, is a fundamental and crucial step in a well-governed natural product program. With the rise in the number of novel drug targets, computational methods such as high throughput virtual screening, ligand docking tools, ADME (absorption, distribution, metabolism and excretion) profiling and other modern computational tools and softwares have been applied to accelerate the drug discovery process. Some of the common natural products libraries and databases as listed below allow the prompt screening of large number of natural compounds to be done in short period of time against a variety of drug targets. Dictionary Of Natural Products (*http://dnp.chemnetbase.com/intro/index.jsp*); UCSD Marine Natural Products Database (*http://naturalprod.ucsd.edu/*); Natural Products Alert (*http://napralert.org/) ;* ZINC (*http://zinc.docking.org/browse/catalogs/natural-products*) ; InterBioScreen (*http://www.ibscreen.com/products.shtml*); AnalytiCon Discovery (*http://www.ac-discovery.com/);* Molecular Diversity Preservation International (*http://www.mdpi.org/*). Computer-generated models of proteins including novel enzyme and receptor targets apart from the protein crystal structures that are deposited in Protein Data Bank (*http://www.pdb.org/*) can be easily generated by homology modeling subsequently followed by simple molecular docking to examine the interactions between the natural compounds and

the protein targets. Bioassays can then be conducted selectively on the natural hits or leads retrieved without the neccesities of wasting the precious amount of the compounds and avoid expensive and time consuming experimental methods. Examples of molecular docking softwares currently available are AutoDock, AutoDockVina, FlexX, FRED, GOLD, eHiTS, and Lead finders. Some examples using high throughput virtual screening including the work of Wang et al. where ten natural compounds have been successfully identified as flacipain-2 (FP-2) inhibitors [99] and Liu et al. who have identified a natural product-like STAT3 dimerization inhibitor by structure-based virtual screening [100].

Other bioinformatics tools such as ligand and structure-based pharmacophore screening have also been reported to be successful in assisting the process of drug discovery from natural products. Chen et al. have proposed a three-dimentional quantitative structure-activity relationship pharmacophore model based on known mTOR inhibitors. Virtual screening using the best pharmacophore model successfully retrieved 20 natural products as potential mTOR inhibitors scaffolds[101]. It is also important to mention here that from the previous study by Doman showed that out of 365 molecules suggested by docking, 127 (34.8%) of them inhibited the activity of enzyme protein tyrosine phosphatase-1B (PTP1B) while only 85 (0.021%) out of approximately 400, 000 molecules were retrieved from high-throughput experimental assay. That is around 1700-fold enrichment of hit rate from structure-based docking over random screening [102].On the other hand, incorporation of chemoinformatic tools in drug discovery from natural products allow the compounds to be screened for their ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties before they are enrolled in any drug development programs. Screening of natural compounds using the Pfizer 'Rule of 5' allows the researchers to remove any molecules that do not obey the rules. Since these rules were derived from a set of experimental observations of thousands of known drugs and drug-like molecules, a trained medicinal chemist/biochemist can easily use the *in silico* data as a guide in determining the potential drug-like natural compounds followed by the synthesis of further analogues so that they would have a favorable drug-like properties. A good drug-like molecule in general obey the following rules (i) molecular weight ≤ 500, (ii) calculated logP ≤ 5, (iii) number of hydrogen bond donors ≤ 5, and (iv) number of hydrogen bond acceptors ≤ 10. The introduction of *in silico* screening and natural products facilities for high-throughput screening in academic labs as well as in drug companies reduce the cost from random screening of very large collections of compounds. *In silico* or virtual screening helps to filter down the number of compounds used in real screens [103]. On the other hand, bioinformatic tool such as the Dictionary of Natural Products gives structural information on 150, 000 different compounds that could be used in virtual screening, even though the compounds would still have to be physically available for any predicted activity to be confirmed through testing in a relevant assay. Finally, clustering of chemically related scaffolds can be very useful in guiding the synthesis of new compounds, but obviously there is a delay and expense in the synthesis.

An academic collaboration has established the Drug Discovery Portal (see http://www.ddp.strath.ac.uk/) in an attempt to combine the techniques of virtual screening of

chemically diverse natural products and their synthetic analogues with the rapid availability of physical samples for testing, This allows a wide variety of compounds from academic laboratories in many different institutions in a database that can be used for virtual screening. Academic biology groups also propose new and novel protein structures as targets for virtual screening with the Portal's database (and with conventional commercially available databases). When hits are predicted from the *in silico* screening, the compounds can be obtained from the originating chemist for confirmatory tests. Often, there is an immediate link to expertise for the preparation of analogues to help start a lead optimization program. Nevertheless, access to the Portal is freely available for the academic group. The continued expansion of the chemical database means that there is a valuable and growing coverage of chemical space of many novel chemical compounds. Although the compounds in the Portal's database will generally have already been disclosed in a thesis or in a chemistry journal, very few of them have been previously tested for biological activity. This is a common feature of known natural products: of the 150, 000 structures in the CRC Dictionary of Natural Products only 1% of them have been investigated. The introduction of metabolomics technologies in natural product discovery processes will be beneficial on multiple levels. By increasing the number of identifications in our metabolomics data, compounds with novel structures can be easily obtained and tested for any disease under investigation. Furthermore, multi-parallel analysis using metabolomics technologies will also enhance the throughput of chemical characterization processes of many different species from natural resources. Since natural product chemists have collected a lifetime of compound libraries of active and also inactive pure compounds, these data can be used to construct the mass spectral and NMR spectral libraries, undoubtedly help the biological interpretations of metabolomics data to be done with less difficulty. The advancements in analytical instrumentation and sophisticated hyphenation of separation techniques with high sensitive detectors have allowed for greater detection of small molecule compounds measurable in biological systems (i.e., primary and secondary metabolites). These technologies can be used to advance the discovery of natural product chemistry to identify potential novel drugs candidates which will assist in sustaining health and the fight against disease and illness. In the case of NMR of crude extracts, patterns can be easily visualized and interpreted using the multivariate data analysis. This can be carried out in a comparative manner distinguishing differences between relatively similar extracts or it can be linked with a specific (generally *in vitro*) biological activity. Ultimately this enables the construction of a complex database of the metabolome [104-106].

7. Scientific interests and recent advances in natural products research from the tropical rainforests

Plant derived natural compounds have continuously captivated scientists globally on grounds of being biocompatible, and are considered as potentially safe and effective therapeutic agents. Table 2 shows examples of natural products derived from plants that have become well known pharmaceuticals [107-116].

No.	Drug/Chemical	Action/Clinical Use	Plant Source
1	Betulinic acid	Anticancerous	*Betula alba*
2	Bromelain	Anti-inflammatory, proteolytic	*Ananascomosus*
3	Camptothecin	Anticancerous	*Camptothecaacuminata*
4	(+)-Catechin	Haemostatic	*Potentillafragarioides*
5	Cissampeline	Skeletal muscle relaxant	*Cissampelospareira*
6	Codeine	Analgesic, antitussive	*Papaver somniferum*
7	Colchicine	Antitumor agent, anti-gout	*Colchicum autumnale*
8	Curcumin	Choleretic, Antitumor agent	*Curcuma longa*
9	Demecolcine	Antitumor agent	*Colchicum autumnale*
10	Deserpidine	Antihypertensive, tranquillizer	*Rauvolfiacanescens*
11	L-Dopa	Anti-parkinsonism	*Mucunasp*
12	Digoxin	Cardiotonic	*Digitalis purpurea*
13	Etoposide	Antitumor agent	*Podophyllumpeltatum*
14	Glasiovine	Antidepressant	*Octeaglaziovii*
15	Irinotecan	Anticancer, antitumor agent	*Camptothecaacuminata*
16	Kheltin	Bronchodilator	*Ammivisnaga*
17	Monocrotaline	Antitumor agent (topical)	*Crotalaria sessiliflora*
18	Neoandrographolide	Dysentery	*Andrographispaniculata*
19	Podophyllotoxin	Antitumor anticancer agent	*Podophyllumpeltatum*
20	Quinine	Antimalarial, antipyretic	*Cinchona ledgeriana*
21	Qulsqualic acid	Anthelmintic	*Quisqualisindica*
22	Sanguinarine	Dental plaque inhibitor	*Sanguinariacanadensis*
23	Scopolamine	Sedative	*Datura species*
24	Sparteine	Oxytocic	*Cytisusscoparius*
25	Taxol	Antitumor agent	*Taxusbrevifolia*
26	Teniposide	Antitumor agent	*Podophyllumpeltatum*
27	Tetrandrine	Antihypertensive	*Stephaniatetrandra*
28	Theophylline	Diuretic, brochodilator	*Theobroma cacao and others*
29	Topotecan	Antitumor, anticancer agent	*Camptothecaacuminata*
30	Trichosanthin	Abortifacient	*Trichosantheskirilowii*
31	Vinblastine	Antitumor, Antileukemic agent	*Catharanthusroseus*
32	Vincristine	Antitumor, Antileukemic agent	*Catharanthusroseus*
33	Scopolamine	Sedative	*Datura species*
34	Taxol	Antitumor agent	*Taxusbrevifolia*

Table 2. Natural products derived from plants [107-116]

7.1. Anti-inflammatory agents

Natural products (and conventional medicines) offer great anticipation in the identification of bioactive compounds and their development into drugs for the treatment of inflammatory diseases. Plants have been the source of several conventional medicine systems through the

world for centuries and continue to deliver mankind with new medicines to treat anti-inflammatory conditions. Previously, the plant-derived medicines were dispensed in the form of crude drugs like tinctures, teas, powders, poultices, and other herbal preparations. This eventually serves as the basis of the current modern drug discovery [117]. Numerous records can be found in conventional medicine concentrating on the relief from pain and inflammation. People who are suffering from inflammation in the ancient times were treated with phyto-chemicals eventually lead to the discovery of the first anti-inflammatory, analgesic drug aspirin. The discovery of aspirin was based on the previously known analgesic and antipyretic features of the bark of willow-tree since 400 BC by the Greeks and Romans. In 1899, aspirin or also known as acetylsalicylic acid was introduced as the first potent drug for the treatment of rheumatic disease [118]. Hippocrates said 'let your food be your medicine'. Another very well studied natural anti-inflammatory agent is curcumin which is derived from the turmeric root. Turmeric is a yellow spice intrinsic to Asia, commonly relished as both a food and a dye. Turmeric appears as yellow in curry powder, and is a viscous compound found in turmeric rhizomes. Many scientific reports have shown that curcumin exhibits strong anti-inflammatory powers and extremely effective at relieving pain, and most important of all it is nontoxic. Like the NSAID's, curcumin inhibits the pro-inflammatory mediator cyclo-oxygenase-2 (COX-2) although it is not selective to a single isoform. Besides, curcumin also affects the activity of other important factors in inflammation such as NF-kappa β, PPAR Gamma transcription factors, and 5-LOX [119]. By inhibiting the activity of all these features of inflammation, curcumin delivers much superior anti-inflammatory and pain-relieving activity than most drugs.

Ginger root which is a common spice found in food, also comprises a number of scientifically confirmed pain relieving mediators. Ginger consists a protein-digesting enzyme called zingibain, which seems to relieve arthritis pain by reducing inflammation. In reality, the anti-inflammatory activity of ginger relates positively with aspirin. Ginger root also contains two groups of compounds called as the shagaols and gingerols. These compounds are strongly antioxidants, therefore serving to stop cells from untimely destruction because of exposure to environmental toxins and by-products of metabolism. Still more, these compounds are strong anti-inflammatory agents, and are recognized to relieve inflammation throughout the body [120]. Provided that oxidation and inflammation are part and parcel of all chronic degenerative diseases, ginger can play an important role in disease risk reduction. On the other hand, plants such the Amazon bark cats' claw and the common spice rosemary are also found to exhibit potent anti-inflammatory and pain-relieving characteristics. In toxicity studies, these plants were proven to be highly nontoxic. Besides, plants such as hops also contain well known anti-inflammatory mediators. This herb is commonly found in beer brewing, comprises a group of compounds called the humulones which were shown to inhibit phorbol ester-induced COX-2 expression in mouse skin by blocking activation of NF-kappaB and AP-1: IkappaB kinase and c-Jun-N-terminal kinase as respective potential upstream targets [121].

Propolis has been used in folk medicine for thousands of years and gain wide recognition for its possible therapeutic uses, because of the wide range of the biological and pharmacological activities. Among the well-known and one of the major properties the anti-inflammatory effect.

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Though there are good numbers of studies focused on the biological activities of propolis together with its botanical sources, studies on Chinese propolis are insufficient. The anti-inflammatory effects of the ethanol extracts from Chinese propolis (EECP) and poplar buds (EEPB) from *Populus canadensis* were investigated *in vitro* for their modulating effects on the RAW 264.7 cells and the inflammatory cytokines production and by measuring nuclear factor (NF)-κB activation in TNF-α or IL-1β stimulation HEK 293 cells using reporter gene assays. Their possible modulatory effects on LPS-induced endotoxemia and acute pulmonary damage as acute inflammatory signs were also tested in mice. Both *Populus Canadensis* EECP and EEPB displayed a strong free-radical scavenging action and significant *in vitro* anti-inflammatory effects by modulating key inflammatory mediators of mRNA transcription, inhibiting the production of specific inflammatory cytokines, and blocking the activation of nuclear factor (NF)-κB [125]. A group of scientists investigated the antioxidant capacities and anti-inflammatory activities of ethanol extracts of leaves of *Cassia alata, Eleusine indica, Carica papaya, Eremomastax speciosa* and the stem bark of *Polyscias fulva*, collected in Cameroon. The ethanolic extracts displayed robust antioxidant activities against both hydrogen peroxide and superoxide anion reactive oxygen species. The highest antioxidant activities was observed with the *Cassia alata*. The effect of plant extracts on $\gamma\delta$ T cells and in DC was evidenced by the dose dependent reduction in TNF-α production in the presence of *Cassia alata*, *Carica papaya, Eremomastax speciosa Eleusine indica,* and *Polyscias fulva*. $\gamma\delta$ T cells proliferation was affected to the greatest extent by *Polyscias fulva* [126].*Celastrus paniculatus* Willd., an important medicinal plant widely used in Ayurveda, is enriched with remarkable nervine, cognition enhancing, and other therapeutic properties. Antioxidant and anti-inflammatory activities of the aqueous, methanol, and chloroform extracts of *C. paniculatus* seeds were evaluated using DPPH radical scavenging assay, Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power assay (FRAP), and lipoxygenase inhibition assay, respectively. Total phenolic content was also determined. Almost all the assays suggested chloroform extract to have the strongest antioxidant property and the highest phenolic content. However, aqueous extract showed maximum anti-inflammatory activity [127].

7.2. Anticancer agents

Plants have a long history of use in the treatment of cancer. The medicinal herbs that are traditionally used for anti-cancer treatment and that are anti-angiogenic through multiple interdependent processes (including effects on gene expression, signal processing, and enzyme activities) include *Goniothalamus species (*Custard-apple family*), Artemisia annua* (Chinese worm wood), *Viscum album* (European mistletoe), *Curcuma longa* (curcumin), *Scutellaria baicalensis* (Chinese skullcap), resveratrol and proanthocyanidin (grape seed extract), *Magnolia officinalis* (Chinese magnolia tree), *Camellia sinensis* (green tea), *Ginkgo biloba,* quercetin, *Poriacocos, Zingiber officinalis* (ginger), Panax ginseng, *Rabdosia rubescens* hora (Rabdosia), and Chinese destagnation herbs [128]. The exploration of anticancer agents from plant sources has started since the 1950s. More than 3000 plants species have been reported to be involved in the development of anticancer drugs [129]. Numerous compounds from tropical rainforest medicinal plant species with potential anticancer activity have been identified.

Positively, most of the new plant secondary metabolites and their derivatives have been utilized in clinical cancer trials [130-132]. Of all available anticancer drugs between 1940 and 2002, nearly half were natural products or their derivatives with another 8% considered natural product mimics [131]. The anticancer agents from plant origin which are currently in clinical use can be classified mainly to four classes: the first class is the vinca alkaloids e.g. vincristine, vinblastine, vindesine, vinorelbine; second class the podophyllotoxin and its derivatives which includes etoposide, teniposide; the fourth is taxanes under which come the paclitaxel, docetaxel and the fifth class is the camptothecin and its derivatives (e.g. topothecan, irinothecan). Other groups include anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin). As a matter of fact, half of the anti-cancer drugs which are globally accepted and in use today were either natural products or their derivatives and were developed based on of knowledge gathered from naturally existing small or macromolecules [133, 134]. In addition to this there is numerous active biomolecules identified in fruits and vegetables and can used in anticancer treatment. These agents include the curcumin which is mainly isolated from turmeric, resveratrol found in red grapes, peanuts and berries, the genistein exist in the soybeans, the diallyl sulfide and S-allyl cysteine in allium, the allicin of garlic, the lycopene in tomato, the capsaicin in red chilies, beside the diosgenin in fenugreek, 6-gingerol in ginger, ellagic acid in pomegranate, ursolic acid naturally found in apple, pears, and prunes. Similarly the catechins in green tea, eugenol in cloves, indole-3-carbinol in cruciferous vegetables, and beta carotene in carrots [128, 135].

Vinblastine and vincristine were isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae) (formerly *Vincarosea* L.) and have been used clinically for over 40 years[136]. The vinca alkaloids and several of their semi-synthetic derivatives block mitosis with metaphase arrest by binding specifically to tubulin resulting in its depolymerization [137]. Podophyllotoxin was isolated from the resin of *Podophyllum peltatum* L. (Berberidaceae) but was found to be too toxic in mice so derivatives were made with the first clinically approved drug being etoposide [138]. The epipodophyllotoxins bind tubulin, causing DNA strand breaks during the G2 phase of the cell cycle by irreversibly inhibiting DNA topoisomerase II [138]. Paclitaxel was originally isolated from *Taxus brevifolia* Nutt. (Taxaceae) and was clinically introduced to the U.S. market in the early 1990s [139, 140]. The taxanes, including paclitaxel and derivatives, act by binding tubulin without allowing depolymerization or interfering with tubulin assembly [141, 142]. Camptothecin was isolated from *Camptotheca acuminata* Decne. (Nyssaceae) but originally showed unacceptable myelosuppression [139, 143]. Interest in camptothecin was revived when it was found to act by selective inhibition of topoisomerase I, involved in cleavage and reassembly of DNA [143]. The taxanes and the camptothecins together account for approximately 30% of the worldwide accepted and marketed anticancer drugs in 2002, costing over US$ 2.75 billion [143]. Besides these, irinotecan was isolated from the same *Camptotheca acuminata* tree in China for metastatic colorectal cancer and 9AC for ovarian, stomach cancer, and T-cell lymphoma. Like camptothecin, this drug is also currently in clinical trials and have already been approved by the FDA [144]. Numerous derivatives of all four compound classes have been synthesized, some of which are currently in clinical use. All of these natural products have led to significant biological discoveries related to their unique mechanisms of action.

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7.3. Immunomodulatory agents

Immunomodulators are used to enhance or suppress host defense responses in the treatment of those diseases in which defective immune responses play an important role in determining disease outcomes. These include primary and secondary immunodeficiencies that accompany long-standing infections, as well as debilitating diseases like cancer, rheumatoid arthritis, systemic lupus erythematosus and long-standing infections, leading to acquired immune deficiencies. Immunosuppressive drugs are commonly used in the treatment of inflammation and allergic disorders and rejection of transplanted organs, while immunostimulant drugs are highly desirable for the treatment of immunodeficiency and infectious diseases [145]. Many therapeutic effects of plant extracts have been recognized and recommended based on their impact on human immunity [146]. A good numbers of herbal preparations such as *Phyllanthus debelis, Tinospora cordifolia, Trogonella foenumgraecum*, *Pouteria cambodiana*, *Centella asiatica*, *Panax ginseng* and *Picrorhiza scrophulariiflora* have been found to display a wide range of immunomodulatory effects [147-150]. The idea of modulation of the immune response to relieve the diseases has existed in early system of medicine together with Ayurveda and Unani. Additionally, plants have been widely used as a source of medicine in these systems to support health and to uphold body's resistance against infections [151]. Phytochemicals such as polysaccharides, lactones, alkaloids, diterpenoids and glycosides, isolated from several plants have been reported to contain potential immunomodulatory agents [151, 152]. Several types of immunomodulators have also been identified from isolates and extracts of bacteria and fungi, mammalian proteins such as interferons, interleukins and cytokines and some synthetic chemicals [153]. Several types of immunomodulators have been identified, including isolates and extracts of bacteria and fungi, mammalian proteins such as interferons, interleukins and cytokines and some synthetic chemicals [153]. Natural products and their derivatives represent a new class of novel immunomodulating agents. However, only little information is available about the immunological effects exerted by medicinal plants from the tropical rainforests which have been used traditionally for treatment of various ailments.

Plants belonging to family Meliaceae, such as, *Azadirachta indica, Mumronia pumila, Melia azedarch, Cedrela lilloi* and *Trichilia elegans* etc. show strong anti-inflammatory and anti-rheumatic properties [154] *C. lilloi* and *T. elegans* constrained the phagocytic capability and oxidative metabolism by opsonized zymosan as stimulus in peritoneal macrophages [155]. Lima et al. [156] reported that *Pisumsativum* agglutinin (PSA) prompts immunomodulatory effects by activating spleen lymphocytes *in vivo*. Our previous study on the screening of 20 medicinal plants for their phagocytic properties have indicated that the methanol extracts of some plants including *Phyllanthus amarus* exhibited strong immunomodulatory effects on polymorphonuclear neutrophils and macrophage cells [157]. The standardized methanol extracts of *P. amarus* and *P. urinaria* and their biochemical markers phyllanthin and hypophyllanthin, were able to modulate the innate immune response of phagocytes especially on the chemotactic migration of phagocytes, phagocytic ability and on the release of reactive oxygen species (ROS) [158]. The withanoloid, coagulin-H from *Withania coagulans* was found to have extremely potent IL-2 inhibitory activity. A complete suppression of PHA-activated T-cell was observed at 2.5 μg/mL concentrations and this suppression activity was similar to that of

prednisolone. Similarly the IL-2 production was inhibited (IC_{50} = 0.35 mg/mL). Molecular docking technique revealed the better interaction of coagullin-H at amino acids at the receptor binding site of the IL-2 protein compared with prednisolone [159]. The natural compound cheiradone, from a *Euphorbia* species was identified to interfere with angiogenesis process, inhibiting the *in vivo* and *in vitro* vascular endothelial growth factor (VEGF) in stimulated angiogenesis process. All stages of VEGF-induced angiogenesis were inhibited with an IC_{50} values that range between 5.20 and 7.50 μM. In addition to this activity it inhibited VEGF binding to VEGF receptor-1 and 2 with IC_{50} values of 2.9 and 0.61 μM respectively. However, cheiradone had no effect on fibroblast growth factor (FGF)-2 or epidermal growth factor (EGF) activity. [160]. Recently, a study using a transgenic mouse model of melanoma exhibited that the anticancer effects of popular kampo medicine were mediated by an improved antigen-specific antitumor cytotoxic T-lymphocyte response [151, 161].

8. Conclusions

Despite the scientific interests and advances in the past few decades of natural products research, to date efforts to discover new bioactive agents from the flora for use as chemical leads in the development of new drugs have not experienced the expected progress. There is a need to strategize research approach which should be based on the integration of human and technology resources available and the establishment of smart partnerships between academic and research institutions, industries and multinational drug coorporations. We need to learn from the past successful research experiences to increase the chances of discovering new drugs from the tropical rainforests. The right research strategy has to be used but it will take more than a few decades for countries of the tropical rainforests to establish their research and drug design capacity and be a serious competitor in the pharmaceutical markets. We have to be up-to-date with the newly emerging technologies which are already playing highly significant roles in natural product research such as the advances in high-throughput screening methodologies, the development of molecular biology and biotechnology and the use of virtual technology in rational drug design. The role of combinatorial chemistry in drug discovery and the future impact of genomics, proteomics and metabolomics in medicinal plant research should also be given due consideration. The potential of rainforests natural products to become new drugs is still on the horizon. The recent adoption of the Nagoya Protocol of the Convention on Biological Diversity on 29 October 2010 in Nagoya, Aichi Province, Japan, on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization should encourage more pharmaceutical firms to venture into natural research to discover new drug leads [162]. The protocol provides a transparent legal framework for the effective implementation of a fair and equitable sharing of benefits between participating parties arising out of the utilization of genetic resources. However, the number of plants that have been studied extensively to search for new drugs is very few. A very small fraction, which is less than 5% of tropical forest plant species have been scanned for their chemical composition and medicinal values [163]. The figures are not unusual as systematic drug discovery programmes from plants are largely carried out by multinational drug corporations or research groups of

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the industrialized countries which possess the technology resources and well-equipped research facilities but have little access to the tropical plant genetic resources. In tropical countries rather most tropical countries there is little and minor concern for systematic research effort, to screen plants for new drugs, and the reason is the capacity to investigate these resources to their full potential and other aspects is very limited in these countries. Neverthless instead of this, several commercial activities such as clearing of forests for agricultural purposes and timber extraction predominate and are rapidly destructing the plant genetic resources.

Chemical prospecting in the tropical rainforests for potential drugs is still progressing. The prospective for drug discovery from plants and other natural sources is huge, but little time remains to discover this speedily diminishing resource. Given the quick devastation of tropical habitats, particularly the rainforests, and the degradation of marine ecologies, this deficiency of information is alarming. The wealth of potential drugs has two potential sources: either from the rainforest or from the laboratory. We need to stress here that the future of drug discovery lies in neither of these options alone, rather it embodies both the rainforest and the laboratory. Combinatorial laboratory technique, which is a relatively newly emerging technique, is growing in proficiency with support from pharmaceutical companies. And as new combinatorial libraries emerge, the ability and chance to quickly synthesize and derivative a biologically active compound increases. But even with this technology, there cannot be a substitution for the biodiversity that can be found within the boundaries of the rainforests.

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