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Pharmacognosy: The Changing Scenario

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ABSTRACT

Pharmacognosy, one of the oldest scientific disciplines is now undergoing major change. Currently plant based drugs are researched and formulated in modern framework of medicine rather than in the form of galenical preparations or conventional dosage. Since last two decades, as herbal wave continues to dominate drug discovery and development, pharmacognosy has gained paramount importance. Recent advances in extraction, chromatography, hyphenated techniques, screening of natural product as well as application of biotechnological tools in natural product research has necessitate sound knowledge of pharmacognosy. Rapid progress of biotechnology has opened new avenues for pharmacognosist to hasten natural product research. Newer approaches, which are more superior in sensitivity as well as specificity than conventional one, are gaining popularity. On this background one needs to understand basic science of pharmacognosy and its newer relation to modern techniques. This review consolidates current trends and newer technologies and attempts to bring into focus the changing needs and scope of pharmacognosy to boost modern enthusiastic natural product researcher to work more on herbal medicines.

KEYWORDS - Pharmacognosy, Natural products, Standardization, Traditional medicine, Molecular markers, High-throughput screening.

INTRODUCTION

Pharmacognosy is the scientific study of structural, physical, chemical and sensory characters of drugs from animal, vegetable and mineral sources. As late as the beginning of the 20th century, the subject had developed mainly on botanical side being concerned with history, identification, collection, preparation and storage of botanical drugs. Currently plant based drugs are researched, dispensed, formulated and manufactured in modern framework rather than in the form of galenical preparations or conventional dosage forms. Hence, it has becomes an important interface among various branches of pharmaceutical sciences. It is now emerging as interdisciplinary science that incorporates inputs from chemistry, biology and biotechnology directed towards natural products based drug discovery (1). Undoubtedly plants have many molecules which have yet to be discovered. This has open many research opportunities to pharmacognosists ranging from characterizing biologically active principles, designing suitable analytical methods for quality control and standardization, activity based screening and drug development. Thus to participate effectively in natural product drug discovery a pharmacognosist should have complete understanding of various disciplines including botany, organic and analytical chemistry, regulatory control, fundamentals of pharmacology, biochemistry, genetics, horticulture and commerce. This review consolidates current trends and newer technologies and attempts to bring into focus the changing needs and scope of pharmacognosy. On this background pharmacognosist should be multifaceted to adopt rapidly changing scenario. To work more on herbal medicines one should not underestimate the subject.

Traditional medicine and globalization

Traditional medicines are and will indomitable arena of pharmacognosy. To integrate and work with globalization, traditional medicine must reassess and open itself to the requirements of scientific rationality, convert itself in its diagnostic and therapeutic approach methods as well as in its deontology. It will thus ensure its influence, productivity, and progress as well as enhance its therapeutic efficiency and competitiveness. This requires the use of modern medicine's diagnostic means and therapeutic control—namely, laboratory analysis, various diagnostic tests, conventional radiography and tomography, tomodensiometry (scanner), magnetic resonance, and all current and future medical techniques. Secondly it should involve chemical and pharmacological study of medicines in order to determine their components, its active principles, its toxicity, and posology. This will lead to their commercialization nationally as well as internationally. An introduction to light and heavy pharmaceutical industries, which will give the medicines a rational galenic form for their conservation, use and large-scale production. The public authorities should be involved in-

1. Setting-up traditional medicine and traditional pharmacopoeia institutes for chemical, pharmacopoeia and clinical research.
2. Setting-up the national organization of traditional health practitioners.
3. A regular evaluation of the abilities of these organizations through joint (both systems) bodies to

ascertain the real impact of their action on people's health.
4. Providing legal recognition of this type of medicine.
5. Action at the grass root level to protect traditional knowledge.
6. The creation and upkeep of protected ethno-botanic gardens to perpetuate vegetal species used in African pharmacopoeia.

Traditional medicine: future need

WHO has been promoting traditional medicines as a source of less expensive, comprehensive medical care, especially in developing countries. Eighty percent of the world's population relies on medicinal plants for their primary health care. Such herbal medicines are easily available, cheaper, time tested and considered safer than some of modern synthetic drugs (2). Recently WHO introduced guidelines on research and evaluation of traditional medicine and practice. This guideline has a major objective of developing traditional medicine leads into standardized and scientifically validated drugs. This guideline aims to ensure quality and safety of botanicals before being evaluated for its efficacy. On this background pharmacognosy is playing paramount role in evolution of novel medicines taking lead from natural products.

Natural products: current status and challenges

Natural products have been our single most successful source of medicines. Each plant is like a chemical factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever. There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, while several other drugs are simple synthetic modifications of the natural products (3).Of the 20 best selling non-protein drugs in 2001, seven were either derived from natural products or developed as a result of leads generated by natural products. Despite the long history of success in discovering drugs from natural sources, natural products have fallen out of favor in current high throughput screening. In 2002, spending on medicines exceeded $ 400 bn worldwide (4). Completion of human genome project and role of genomic and proteomics have revolutionized natural products based drug discovery. Over 50% of the best-selling pharmaceuticals in use today are derived from natural products. In natural product drug discovery the conventional approach of extraction, isolation, separation, identification, characterization and test for the desired biological activity suffers from problems like lower yields, dereplication, difficult separation and inconsistent biological activity (5). However with the introduction of innovative technologies like high throughput screening (HTS) and recent advances in extraction, chromatography, electrophoresis and spectroscopy has revolutionized the entire scenario of pharmacognosy. Wider accesses to previously untapped biodiversity and application of molecular techniques for producing compounds from natural sources ensure that it will be beneficial in long run for discovering new lead molecules. Reverse pharmacognosy and advance docking component can be integrated into a program for drug discovery (6). This approach can be utilized to find new applications for identified compounds. This changing scenario defies the current available workforce, which is either chemistry intensive or biology intensive. These newer technologies demand manpower with multidisciplinary skills. Pharmacognosist being an interface between chemist and biologist has the potential to address these techniques effectively and efficiently. Collection, cultivation, extraction, standardization and quality control of plants are major aspects of routine pharmacognosy, which are rapidly changing because of application of newer techniques.

Modernization in extraction techniques

Accustomed methods of extraction are maceration, percolation and Soxhlet extraction that now replaced by new methods with several advantages. These techniques suffered from drawback of using concentrated organic solvents, time consuming, labor intensive, higher sample input and higher repeatability. Nowadays these extraction techniques are dominated by advanced techniques like Supercritical Fluid Extraction (SFE). SFE offers exciting opportunities and make use of supercritical fluids as extraction medium. Supercritical fluids properties can be exploited to maximize the extraction of plant constituents, minimize the degradation of thermo labile component and left no solvent residues. It has high repeatability, accuracy and high selectivity. Solvents used for natural product extraction in SFE are nontoxic, inert, provides clear extracts, has shorter extraction time and uses less organic solvents than conventional extraction methods.

SFE has been successfully used for extraction of volatile oils from herbs and effective constituents from bulb of *Allium sativum* were extracted by supercritical-CO_2 fluid (7). The remarkable highest vindoline concentration was extracted from the leaves of *Catharanthus roseus* using CO_2 as extracting solvent and it was further identified by HPLC and LC/MS (8). Numerous reports are listed on solubility and sepratibility of natural product like steroids, alkaloids, various anticancer agents, oils from seeds, caffeine from coffee beans in various supercritical fluids like CO_2 , ethane, NO_2, ethylene. Piperine from black pepper, essential oils from nutmeg, and capsicine from chillies has been extracted with supercritical CO_2 and yields up to 99 % of active material (9). Extraction and quantitation of digoxin and acetyldigoxin from the *Digitalis lanata* (10), *Curcuma longa* (11), *Colchicum autumnale* (12) and coumarins, lignans and prenylflavonoids were carried out by SFE (13). The highest yield of naringin is also obtained by extracting with SFE, the major flavonoid from the peel of *Citrus paradise* (14) *and* Alkylamides from *Echinacea angustifolia* (15). SFE has been used successfully in combination with enzyme immunoassay analysis, anion exchange disk sorption, and gas chromatography. Simultaneous use of ultrasound as a co-adjuvant enhances the efficiency of SFE (16). Computerization and automation of extraction processes has accelerated pace of natural product drug discovery.

Need for standardization - The time when traditional medicines were developed, the technology and concept of standardization and quality control was quite different. According to herbalist, Bob Brucea, "Standardization does

have advantages. It produces a consistently strong product with guaranteed constituents". Due to process of evolution, commercialization and environmental effect, identification and quality control of botanicals become more difficult which is of prime importance in pharmacognosy. Chromatographic techniques like TLC, HPTLC and HPLC uses chemical markers which may not be therapeutically active and has its own limitations. Secondary metabolites that are used as marker may change due to environmental factors and hence correct identification of botanicals is difficult task. Marker compound must not be present widely (like primary metabolites) and should neutral to environmental or other effects. Each herb contains large number of compounds, so it is not possible to analyze for presence or absence either quantitatively or qualitatively for all compounds. These serious difficulties in testing for active principles or chemical constituents are well known. Various national and international guidelines have suggested that test material should be free from pesticides, heavy metals and aflatoxins. Estimation of these contaminants requires advanced analytical techniques. Secondly countries with ethnobotanical practices are engaged in developing monographs of their indigenous plants. Monographs preparation involves chemical as well as biological markers. Marker development requires advanced analytical tools. Due to faster dereplication rates these techniques will able monograph preparation of even those botanicals whose chemical identification or characterization was not possible. These markers developed by these techniques will further be useful in stability testing and biological standardization. According to general guidelines for methodologies on research and evaluation of traditional medicines by WHO, first step in assuring quality, safety, and efficacy of traditional medicines is correct identification and this can be done very successfully with the application of molecular markers.

Molecular markers

Pharmacognosy, since 1990 has become molecular science, as molecular markers prove important tool in revolution of biochemical constituents and macromolecules, viz. proteins and deoxyribonucleic acids (DNA). Molecular markers identify plant at genomic level and establish new standards in standardization and quality control of botanicals. Hence, are more suitable and ubiquitous to most of plants. Molecular markers have highly polymorphic nature, show codominant inheritance, occur frequently in genome, unbiased to environmental conditions or management practices and easily available, highly reproducible and allow easy exchange of data between laboratories (17). Use of various molecular markers such as Random Amplified Polymorphic DNAs (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), Microsatellites and PCR- based DNA markers such as Sequence Characterized Amplified Regions (SCARs), Sequence tagged-sites (STS) and Inter-simple Sequence Repeat Amplification (ISA), Amplified Fragment Length Polymorphic DNAs (AFLPs) and Amplicon Length Polymorphisms (ALPs) are now a days not extremely new in plant research.

Application of molecular techniques

To generate breeds for disease and pest resistance plant in order to get better quality of crude plant material, Marker-assisted selection (MAS) has been useful. Sequence Characterized Amplified Region (SCAR) analysis was done to authenticate *Panax* Species and their adulterant (18). Molecular techniques are more superior in sensitivity as well as specificity than conventional techniques (19). RAPD also plays an important role in assessment of genetic variability and in strain identification like *Xanthomonas oryzae* (20) and differentiation of *Lycium barbarum* from its related *Lycium* species (21). Similarity index analysis revealed that *C. pilosula* samples from the same province generated similar DNA fingerprints, while samples of different provinces displayed different DNA fingerprints. This method is sensitive and valuable tool for locality authentication of Chinese herbal medicinal (22). Identification of fresh and processed Japanese green tea was based on, polymorphisms generated by STS-RFLP analysis using a combination of codominant DNA markers (23). RAPD, HPLC and ELISA proved useful for Genetic and alkaloid analysis *Papaver* species and their F1 hybrid by (24). Negi M. S. successfully studied genetic variation and relationship among and within *Withania* species by using AFLP markers (25). RFLP and Sequencing can be used for the identification of Botanicals and Potential Contaminants (26). Thus molecular techniques can be used in pharmacognosy for cultivation of medicinal plants, identification, and detection of adulterants and to discourage its fraudulent commercialization in herbal formulations. Newer biotechnological tools like cloning, sequencing, gene expression, gene manipulation can be used to increase yield of secondary metabolite. With the evolution of these molecular approach role of pharmacognosy is likely to be more challenging in forthcoming years.

Hyphenated techniques

Nowadays, traditional analytical methods are replaced by modern methods, which encompass hyphenation techniques, high throughput technologies, miniaturization and robotics. Hyphenated techniques are the most powerful techniques available for selection, identification, extraction, chromatographic separation, and resolution of mixtures. These will be the most useful tools for pharmacognosists involved in quality control, stability testing and in natural product screening programs. Hyphenation- combination of two or more techniques is one such approach followed by modern analyst. Natural products being chemically complex can be easily identified and characterized using appropriate hyphenated techniques. These techniques facilitate rapid and efficient screening of extracts and their online characterization. Being automated and highly sensitive these techniques require very less amount of sample and analysis can be done in no time. Mass spectrometer being universal detector when coupled with chromatographic techniques has resulted in better and enhanced sensitivity, which has led to identification and elucidation of complex mixtures. Magnetic sector mass spectrometers with higher accelerating voltage have increased sensitivity and can handle complex molecules like pterostilbene (27).

For rapid phytochemical investigation of plant extract, to perform efficient screening of extracts, one can use combined techniques such as HPLC coupled to UV photodiode array detection (LC/UV) and to mass spectrometry (LC/MS or LC/MS/MS) or LC/NMR or LC/TPS-MS-MS (28). Disposable solid-phase extraction cartridge coupled to an NMR flow- probe (SPE-NMR) provides optimal conditions for chromatography and possibility of automating (29). Extraction, separation and detection efficiency of present methods can be greatly enhanced by using combinations like HPLC-electro spray ionization -MS-MS analysis (30), HPLC-UV and HPLC-positive-ESI-MS analysis (31). Electro spray ionization (ESI) and tandem mass spectrometry (MS/MS) are used for structural determination of natural compounds (32) and lipids (33). HPLC can also combine with electrochemical detection to determine tea catechins (34). Electrophoresis when combined with mass spectrometry techniques MCE-MS (Microchip electrophoresis-mass spectrometry) was proved useful for analysis of basic drugs (35) and free amino acids. Anion exchange chromatography with integrated pulsed amperometer is utilized for sugar detection (36). Thus in phytochemistry, mass spectroscopy plays a central role in structure elucidation (37).

Natural product screening

Although Clinical trials and experiments involving whole animals are important in natural product screening but because of financial, ethical and time limitations importance of *invitro* screening is gaining popularity. As advances in biotechnology progresses various genetically modified cell lines, cultures and organisms become available for screening purpose that have shorten the screening time (38). Combinatorial synthesis allows production of compound libraries in an expeditious and organized manner immediately applicable for high-throughput screening. HTS is nothing but process of assaying large number of potential effectors of biological activity against target, which can be applied to combinatorial chemistry, genomics and protein and peptide libraries assuring that lead finding is not a rate limiting step in new drug development. Not only chemically but also biologically obtained libraries of highly diverse sets of an overwhelming number of compounds can now routinely be generated and screened in high throughput (HTS) assay (39). Ability to rapidly identify active compound in complex mixture of natural product extract, lead optimization as well as lead characterization are important factors in the natural product screening. The problem of separation of complex mixture can be elegantly overcome by gradient liquid chromatography followed by online biochemical detection parallel with chemical characterization commonly referred as high resolution screening (HRS) (40).

High-throughput screening

Apart from compound libraries from combinatorial chemistry, the major source for high-throughput screening (HTS) programs in drug discovery is natural products. HTS is gaining widespread popularity over last few years because of rapid selection from large number of samples with minimum human involvement, miniaturization with high density microplate with 384 and 1536 wells which allows to decrease cost and increase throughput (41). Today More than 500,000 assays per day are possible with development of 20,000 well formats for HTS system development (42). Within pharmaceutical industry, significant resources have been applied to the identification of new drug compound leads through the use of HTS to meet the demand for rapid analytical characterization of biologically active samples. The technique of high performance liquid chromatography- electro spray ionization mass spectrometry (HPLC-ESI-MS) has been utilized, and the application of this technique specifically for the integration of natural product sample mixtures into modern HTS is reviewed (43). Ultra-HTS (uHTS) assays require an accurate and reliable means of fluid handling in the submicroliter volume range. This relates to the design of instrumentation for dispensing fluids, as well as assay plates. With the development in the area of biotechnology and availability of genomic information significantly increases the number of potential targets available for drug discovery, although the function of many targets and their relationship to disease is unknown. In a chemical genomic research approach, ultra-high throughput screening of genomic targets takes place early in the drug discovery process, before target validation to generate drug leads (44).

Nuclear Magnetic Resonance

Various types of automation i.e. macroautomation and microautomation are now not new for various research laboratories which has improved quality as well as reduced errors. Macroautomation/ robotics are a possible way to improve efficiency and to cope with the high workload in various laboratories. Technology has advanced to such an extent that it is now possible to do many tests with the concept Total Laboratory Automation (TLA) (45). NMR, already some 50 years old, has long been an invaluable analytical method in pharmaceutical industry for verification of chemical synthesis and compound characterization. When combined with HTS gives unmatched screening sensitivity, information of structure and nature of molecular binding and justifies the growing interest in this dynamically expanding field of new drug development. A novel and sensitive NMR method for rapid, efficient, and reliable biochemical screening, named 3-FABS (three fluorine atoms for biochemical screening) requires the labeling of the substrate with a CF (3) moiety and utilizes (19)F NMR spectroscopy for the detection of the starting and enzymatically modified substrates. This method allows for high-quality screening of large compound or natural product extract collections (46). NMR-based screening has the advantage of directly detecting both the affinity and binding location of potential lead compound. Another method, RAMPED-UP NMR, (Rapid Analysis and Multiplexing of Experimentally Discriminated Uniquely Labeled Proteins using NMR) which generates simple spectra that are easy to interpret and allows several proteins to be screened simultaneously in one tube (47).

Fluorescence-based techniques

Assay miniaturization, which can provide greater throughput, as well as significant cost savings through reduced reagent costs, can be done efficiently by digital imaging detection method that can measure fluorescent or luminescent signals

in these miniaturized formats (48). Fluorescence-based assay technologies play an increasing role in high-throughput screening. They can be classified into different categories: fluorescence polarization, time-resolved fluorescence, fluorescence resonance energy transfer, and fluorescence correlation spectroscopy. Alternative analytical technique for high-throughput screening, which is known as confocal fluorescence coincidence analysis has been in use for screening of compounds (49). Novel cell-based assays are now being adapted for high-throughput screening, providing for in situ analysis of a variety of biological targets. The implementation of fluorescence resonance energy transfer (FRET)-based biochemical and cell-based assays in 3456-well NanoWelltrade mark assay plates using key components of Aurora's ultra-high-throughput screening system is a new technique in HTS (50). New strategies that eschew 2D micro plate technology, including technologies that enable mass screening of targets against large combinatorial libraries, have the potential to greatly increase throughput and decrease unit cost, state-of-the-art micro plate-based HTS technology and includes a discussion of emerging miniaturized systems for HTS which focus on new methods of encoding combinatorial libraries that promise throughputs of as many as 100,000 compounds per second (51). Fluorometric micro volume assay technology (FMAT) is a fluorescence-based platform for the development of nonradioactive cell- and bead-based assays for HTS. This technology is plate format-independent, and while it was designed specifically for homogeneous ligand binding and immunological assays, it is amenable to any assay utilizing a fluorescent cell or bead (52). High-throughput bioassay-guided fractionation (BGF) is an automated, high-throughput analytical tool for the unambiguous characterization of the active component(s) of a combinatorially derived reaction mixture.

Capillary electrophoresis

Capillary electrophoresis (CE) is a versatile micro/macro analytical technique gaining widespread usage for the separation and analysis of natural substances. CE has several advantages over thin layer chromatography, gas chromatography and high-performance liquid chromatography, such as low capillary cost, reduced operating costs, small sample amounts, low production of waste materials and short analysis time. CE appears to be an excellent separation methodology when coupled to biosensor detector. CE has several advantages like fast analysis times, requires extremely small sample volumes, complete automation in sample handling and data treatment. Although only a few applications of CE-based biosensors have been described up to the present, it is anticipated that this hyphenated technique could have a considerable expansion in the coming years (53). Electrophoretogram can be used for identification of drug as well as adulterants in Chinese formulation and thus can play important role in quality control of medicine (54). Micellar electrokinetic capillary chromatography (MEKC), which allows combined use of CE and other techniques, can be used in natural product research. Synthetic chemical drugs found as adulterant in Chinese medicines, can be studied by capillary electrophoresis/UV absorbance (CE/UV) and capillary electrophoresis/electrospray ionization mass spectrometry (CE/ESI-MS) (55). In Chinese traditional medicine detection of active constituents and its chemico-physical characteristics as well as constituents analysis of herbal medicines can be efficiently done with advances in CE (56). High performance capillary electrophoresis (HPCE) method is established for separation and quantitative analysis of active constituents in herbal formulations, based on the mode of capillary zone electrophoresis (57).

OVERVIEW AND CONCLUSION

Standardization and quality control of plants, is of growing concern over ensuring purity of raw material before processing. Yet alternative medicines based on plant substances are extremely popular, even though their safety and efficacy have not been scientifically proven. Now a days routine pharmacognosy has changed demanding interdisciplinary research. While stressing the dangers of inadequately regulated herbal remedies, we should not forget that plants have provided a multitude of life-saving drugs. To cope up with the limitations in drug discovery from natural sources there is a need for modernizing the techniques. Rapid extermination of various species has created major problem in front of natural product researchers. There are several challenging tasks for upcoming pharmacognosist. Plant tissue culture with biotechnology approach can work effectively with cells and tissue culture, cultivation, artificial propagation, conservation of gene pool and other new techniques. Before decade, pharmacognosy and biotechnology seems unusual combination of terminology and discipline. With the application of these modern emerging techniques in the field of pharmacognosy is likely to change the face of pharmaceutical research, drug development and discovery. In future, lead finding for new drug development, better quality control and standardization processes for phytopharmaceuticals, nutraceuticals and dietary supplements, tissue and cell culture, gene therapy and development of comprehensive monographs are some of the major challenges in the future of pharmacognosist. Pharmacognosist being an interface between chemist and biologist should have working knowledge of these techniques so as to assist natural product based drug discovery effort in effective way.

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