Pharmacia-JTPA
30 (3), 169-181, 1990

PHARMACOGNOSY- A HIGH TECH PHARMACEUTICAL SCIENCE

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SUMMARY

During the 1960s and 1970s pharmacognosy struggled to find its identity as a science. Now, through the rapid integration of computer technology, advanced separation and structure elucidation techniques, molecular biology and advances in bioassay development, coupled with the growing emphasis on potentiating and conserving the tropical rain forests, pharmacognosy stands at the forefront of the pharmaceutical sciences. Opportunities abound for pharmacognosy to contribute in a meaningful way for the benefit of mankind, providing efforts are focussed on addressing important medical and biological issues. A global effort to evaluate the biological potential of the flora of the tropical rain forests is proposed, and the integration of data sets of ethnomedical information as an adjunct for prioritization for novel bioactive compound discovery is emphazised.

Key Words: *Pharmacognosy; ethnomedicine; Tropical rain forests; Computer technology; Data analysis; Structure elucidaion; Biological evaluation;*

FARMAKOGNOZİ-BİR ECZACILIK BİLİMİ

ÖZET

1960 ve 1970 li yıllarda Farmakognozi bir bilim dalı olarak kimlik mücadelesi vermiştir. Şimdi, bilgisayar teknolojisindeki hızlı entegrasyon, gelişmiş ayırma ve yapı aydınlatma teknikleri, moleküler biyoloji ve biyoassay gelişmelerindeki ilerlemeler tropik ormanların korunması ve buralardan yararlanılması üzerinde artan duyarlılıkla birleşimce Farmakognozi Eczacılık bilimleri arasında ön sıraya çıkmıştır. İnsanlık yararına anlamlı bir şekilde kullanılacak Farmakognozi ile ilişkili fırsatlar önemli tıbbi ve biyolojik konular üzerinde yoğunlaşmıştır. Tropik ormanların floralarının biyolojik potansiyelinin değerlendirilmesi için global çaba öne sürülmüştür ve yeni biyoaktif bileşiklerin keşfi için öncelik alacak şekilde etnomedikal bilgilerin veri setlerini integrasyonu vurgulanmıştır.

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The term "Pharmacognosy" is derived from the Greek words "pharmakon", meaning drug, "co" meaning intense and "gnosia", meaning knowledge of. It is the oldest of the pharmaceutical sciences, and since prehistoric times, the "pharmacognosist" has been a person in the society whose role it was to identify crude drug materials both for their beneficial and toxicant properties and, in the former case, to assure the absence of adulteration. In many countries of the world this role in both academia and pharmacy practice continues today. By the mid-1960s, in American Pharmacy practice and education, the science was struggling, primarily because it lost a well-defined research focus (1).

In this article we will see how in the past ten years, as a result of the global concern about the tropical rain forests, the advent of a more chemical approach, the development of rapid **in vitro** bioassays and the evolution of computer technology, pharmacognosy has re-emerged as a vital science playing a leading role in the discovery of new drug candidates and biological tools. We will see how it is now positioned at the forefront of the application of many scientific technologies. In addition, we will explore the potential role of pharmacognosy in the early part of the next century. We will address some crucial questions: what is pharmacognosy? What is its' scope? What are the crucial "tools" for success? What are some of the possible directions for pharmacognosy research, and therefore, what is the future of this ancient science?

Let us begin by trying to define "pharmacognosy", a term for which probably everyone who works in this area has a different definition. That is an essential part of the problem, because many scientists who work with natural products are only just realizing that they too are pharmacognosists! I define pharmacognosy as "the study of biologically active natural products". There are no constraining factors with respect to either the nature of the study or the source of the biologically active compound.

Traditionally, pharmacognosy had as its foundation the writings of the ancient physicians associated with the civilizations inhabiting the valleys of the Tigris and the Euphrates. Successive dominant civilizations spread this knowledge all over Europe and the Middle East. At the same time, the Chinese, and independently the Indian, cultures were developing their own systems of traditional medicine. As social and scientific interactions increased so traditions of healing, and the knowledge contained therein, was transferred, initially through the merchants and traders who plied the oceans and crossed the great mountain ranges and deserts, but eventually by the ethnobotanists who accompanied exploratory voyagers to North, Central and South America, to Africa, to the Near and Far East, and to the remote islands of the Pacific. Substantial documentation concerning the use of these plants was accumulated, either written as notes on herbarium specimens, in diaries, or occasionally in more formal articles discussing the utili-

zation of indigenous plants.

Many valuable and highly regarded drugs were discovered as a result of studying some of these plants having exceptional use profiles (2). Some examples include the notorious mandrake, **Mandragora officinarum,** and henbane, **Hyoscyamus niger** which yielded scopolamine; Coca leaf, cocaine; opium, **Papaver somniferum,** which afforded morphine and codeine; South American curare, **Chondodendrum tomentosum,** from which tubocurarine is derived; Calabar bean, **Physostigma venenosum,** used as an ordeal poison, which gave physostigmine; digitalis, **Digitalis lanata,** yielding the digitalis glycosides; ergot, **Claviceps purpurea,** the fungus which infests rye, which yielded the alkaloids ergotamine and ergonovine; and **Rauvolfia serpentina,** which yielded reserpine and deserpidine (3).

However, during most of the last 30 years, the knowledge of medicinal plants has been little explored by the major pharmaceutical companies as a source of new medicinal and biological agents from potentially renewable and sustainable resources. Slowly that situation is changing, and it is pertinent now to look at the assets that we have available, and why it is that pharmacognosy, as a high-tech pharmaceutical science, must play a pivotal and expanding role in future drug discovery efforts.

I. Biodiversity and the Role of Pharmacognosy

Each nation has three forms of wealth: material, cultural and biological. If one accepts the current estimates that man developed on Earth approximately 3,5 million years ago, then coincidentally this was probably also the time of the greatest biological diversity. Not since the Mesozoic period 65 million years ago has biological diversity been as reduced as it is today. While much has been written on the impact of reduced botanical diversity and increased deforestation on the environment and the ozone layer, the immense, mostly untapped, potential of the biome (fauna and flora) has been largely ignored. But what is this potential? Unfortunately, since the number of species in the biome is not known, in statistical terms this becomes an impossible question. However, there are some approximations, for example it is believed that there are about 350,000 species of vascular plant, about 200,000 species of marine invertebrates, and about 1.5 million species of terrestrial animal, insect and arthropod species. The number of deep sea organisms is completely unknown.

It is well established that the tropical rain forests are the richest concentration of biota, be they plants or arthropods. For example, a one hectare plot in Kalimantan, Indonesia yielded 700 species of plants (4) and a single tree in Peru yielded 43 ant species in 26 genera. Yet every year 42 milllion acres of tropical rain forest are permanently lost. Species diversity is probably our planet's most important and irreplaceable resource. Once exter-

minated, species regeneration will take 5 to 10 million years. As Wilson has pointed out (5), failure to correct this loss of genetic diversity may be "the folly (for which) our descendants are least likely to forgive us." The biome is not the only loss when the tropical rain forest are destroyed, for just in terms of the potential for the discovery of new medicinal and biological agents there is the incalculable loss of knowledge of the use of plants and animals by the indigenous populations. While efforts continue to try to gather this information, the fact is that the information base is disappearing much faster than it is being collected.

There are three principal aspects to the evaluation of the biome for the potential to serve as sources of new medicinal agents and biological tools: i) the resources to acquire the materials for study, ii) the ability to be able test the samples and isolate and characterize the active principles, and iii) the ability to optimize availability. It is in these areas that pharmacognosy, formerly the old spinster of the pharmaceutical sciences, has now become a young and active, ultra-modern teenager. "Renaissance" is defined by Webster's Collegiate Dictionary as "a movement or period of vigorous artistic and intellectual activity". Pharmacognosy is experiencing such a period of revitalization at the present time (6,7).

II. Pharmacognosy Today

Pharmacognosy today is a highly interdisciplinary, essentially **collaborative** science, ensconced at the interface of anthropology, botany, taxonomy, chemotaxonomy, natural product chemistry, physical organic chemistry, phytochemistry, marine and terrestrial ecology, biochemistry, molecular pharmacology, plant physiology, animal physiology, cell biology, microbiology, enzymology, genetic engineering, chemical enginering, and economics. It encompasses a broad range of studies involving biologically active principles obtained from plants, fungi, marine organisms and animals, including the ethnomedical and ethnobotanical use of a plant material, the standardization of ethnomedical preparations, the detection of biological activity in an extract of the organism, the isolation of the active principle(s) through bioactivity-directed fractionation, the structure elucidation of the active principle(s), studies of the mechanism of action of the compound, biological and conformational studies, the metabolism of natural products, development of improved sources of the material, e.g. partial synthesis, biosynthesis, studies of the enzymes involved in the biosynthetic pathway, and cloning studies of the product-forming enzymes. In each of these areas of science, pharmacognosy is utilizing the most sophisticated technologies in order to stay at the forefront of drug discovery and studies related to biologically active natural products.

Fundamental to all of this work is COLLABORATION. The days of working as an individual scientist in this field are over. No single individual has all of the necessary skills to be successful in this area. Each of us must

find collaborators, either in our own institutions, or elsewhere in the world, to collect and identify organisms, to do the isolation and structure elucidation work involved, to establish and operate the biological assays, to conduct the biosynthetic studies or the analyses or cultivation studies. The formation of research groups is therefore becoming a very serious aspect of the future of the science, if we are to seriously work involved, to establish and operate the biological assays, to conduct the biosynthetic studies or the analyses or cultivation studies. The formation of research groups is therefore becoming a very serious aspect of the future of the science, if we are to seriously work for the betterment of mankind with the resources thet we have available. Let us now examine some of these individual component parts, see how it is that pharmacognosy is at the forefront of the utilization of the available tecnology, and establish how these component parts fit into an integrated, operative whole.

a. Information Gathering and Plant Selection and Collection

As indicated above, pharmacognosy has available an almost unlimited, albeit rapidly shrinking, research base from which to draw samples for biological evaluation. There are four ways to use this resource pool: a Random approach, in which collection of species is conducted in certain geographic areas; a Phytochemical approach, in which one may look for certain types of phytochemicals, e.g. indole alkaloids or flavonoids, and then test the isolates **vs.** controls to look for enhanced activity; a Taxonomic approach, where a certain genus or family may be studied; and lastly the Ethnomedical approach, where the collection of plants is based on folkoric or current Traditional Medicinal practice. For the purposes of new drug discovery from plants, I believe that the ethnomedical approach will be the most productive.

The ethnomedical approach requires prioritization of existing ethnomedical, chemical, biological and clinical data so that plant collection can initially address those plants which are viewed as being most likely to yield interesting new compounds for potential development. Modern pharmacognosy is addressing this problem through the use of the third form relational database NAPRALERT at the University of Illinois through which an initial list of potential plants can be scored for ethnomedical, **in vitro, in vivo,** and clinical data to identify those plants to be collected in the initial phases of a program. Collection of species, particularly if they have been identified as being of potential interest, is a relatively unappreciated aspect of natural product drug development (8). The key is to have a taxonomic leader who has available a group of local collectors who are capable not only of collection, but also, with their local knowledge, of providing authoritative identification and information on use or ecological implications. Accurate identification of herbarium specimens is essential, and the same philosophy of unambiguous identification also applies to marine and microbial organisms.

b. Biological Evaluation of Extracts

The biological evaluation of extracts of organisms is a vital aspect of the discovery process, and developments in the area of **in vitro** techniques have totally transformed the testing of extracts. Whereas it previously took weeks or months to test a hundred samples, it now takes, for some assays, only a matter of hours. Animal organ assays have an important role in screening if an appropriate enzyme or receptor is not available (9), but in many studies, whole cell, enzyme-based, and receptor-based assays are now quite routine, and the emphasis is shifting rapidly to genetically-engineered assays which evaluate the ability of a compound to interfere with a biological process in an exceptionally specific manner.

Whatever **in vitro** assays are used, certain criteria must be met for the assay to be effective. Ideally, the assay should be simple, so that it can be run without elaborate preparation or training of personnel. It should be accurate, i.e. capable of meaningful quantitation with a reasonable margin for activity. It should be reproducible, both on a day-to-day basis and on a lab-to-lab basis. It should be selective, i.e. that only an individual biological event or process is evaluated. It should be sensitive, probably down to about 0.0001 % of an active compound in an extract, based on the dried weight of the original organism. It should be fast and not require extensive delays for results to be obtained. It should be economical, particularly if thousands of samples are to be evaluated. Finally, it should be scientifically valid, that is both at the forefront of the biological category under study, and hopefully predictive of a therapeutic avtivity.

c. Isolation and Structure Elucidation

Once an active extract has been obtained, the focus of work turns to the most rapid and efficient method for detecting the active principle (s). One would like to combine the demonstration of activity with a concurrent indication of the nature of the active compound(s). Such techniques come under the general category of bioautography. Recently, we made some improvements in the agar diffusion assay (10) whereby, rather than placing the tlc chromatogram onto an agar dish, the agar gel containing the microorganism was applied onto a tlc chromatogram, and the microorganism allowed to incubate. A staining system was then used to identify the areas of growth inhibition.

Isolation of the active principle can now be conducted through a variety of chromatographic, solvent partition, size exclusion or other procedures (11), typically with sequential bioassay. This is bioactivity-directed fractionation. In the chromatographic area, preparative HPLC is quite routine, using both normal phase and reverse phase systems. For small scale preparative work there is over pressure layer chromatography (OPLC)(12,13)

and circular locution chromatography (CLC)(14), in which the circular chromatogram is rapidly spun with the solvent entering at the center of the chromatographic techniques, which essentially rely on the unequal partition of solutes between two solutes, are undergoing substantial innnovative changes. One of these is rotation locular countercurrent chromatography (RLCC)(15) and another is centrifugal partition chromatography (CPC). Effectively countercurrent distribution at higher gravity, this technique results in more efficient, more rapid preparative scale separations of a wide range of compounds, including biologically important, labile geometrical isomers under mild conditions (16).

One of the techniques that has the potential to be effective for the preliminary separation of crude plant extracts is super-critical fluid chromatography (17). Commercially, this technique is used in the decaffeination of coffee, but recent efforts have also indicated utility in phytochemical analysis.

It has become quite apparent in recent years that enantiomers or diastereoisomers may have quite different biological properties. This chiral biological selectivity is classically illustrated by the male fertility regulating activity of gossypol, where the (-)- isomer is responsible for the activity observed for the racemate; the (+)- isomer being inactive (18). Since enantiomerically pure (-)- gossypol is not a natural product, a novel, chiral HPLC stationary phase was needed in order to effect the chromatographic separation of the racemic form.

Structure determination has markedly benefitted from the tremendous recent advances in nmr spectroscopy (19). Since the number of new natural product skeleta that are discovered annually is relatively few, attention has now focussed on the unambiguous assignment of the protons and carbons in a molecule for two purposes: to provide better correlative information for future studies of compounds in a series, and to investigate the foci of biological interactions within a molecule. In the structure elucidation of natural products, advanced nmr and mass spectral techniques are being applied to solve structure problems which would otherwise take months or years.

Mass spectrometry has also undergone recent dramatic changes due to the availability of time-of-flight mass analysis, tandem mass spectrometry, the use of ion traps, and the development of a variety of new desorption techniques (20). The result is that more accurate mass ranges and more polar compounds can now be investigated. Of particular interest to the pharmacognosist are new matrices for FAB mass spectrometry (21,22) and plasma desorption using californium ionization (23) for particularly polar or moderate mass (up to 10,000 amu) samples. In addition, there is considerable interest in the use of MS-MS systems for the classification and development of fragmentation patterns, for various categories of polypeptides, lipopepti-

des, and polysaccharide systems (24). For metabolic studies, LCMS has proven to be a particularly effective technique, since polar and thermally labile compounds can be analyzed directly, even though they may originate from complex matrices (25).

X-Ray crystallography and molecular modeling techniques have progressed subsantially in the past few years, particularly in the area of biopolymers. These techniques are being used to compare the conformations of active species in the solid and gas phase, and nmr spectroscopy is used to abtain the corresponding information for the solution conformation, providing information related to the conformation at the active site. Thus, with the enhanced computer capabilities, a number of enzyme-substrate complexes, as well as numerous large proteins have been characterized. The next step will surely be to examine the dynamics of these processes. One exciting new development is technology which will allow for diffraction patterns to be obtained on a 100 picosecond time scale (26), suggesting that it might be possible to monitor dynamic biological processes by X-ray techniques.

In our own work, we have begun to study the integration of information on solution, solid and gas phase conformations with respect to biological activity, using the fertility-regulating agent pseudolaric acid B (27) and the cytotoxic agent savinin (28) as initial examples. In the latter case, it was clearly demonstrated thet flexible molecules can have vastly different conformations in the solid and solution state, though at present the implications of this observation for biological activity are not clear.

Jeffs and co-workers have been examining an important aspect of how antibiotics interact with cell walls, making extensive use of quantitative, as well as qualitative, twodimensional nuclear Overhauser effect correlation spectroscopy (NOESY) in order to examine interproton distances, and therefore the conformational constraints on the molecule, showing how L-Lys-D-Ala-D-Ala, as a model of putative cell-wall precursor, could be folded into the binding pocket of aricidin(29).

d. Biosynthesis and Biocatalysis

Classical studies in biosynthesis previously focussed on the use of the radioactive isotopes 3H and 14C. Now, quite different strategies for determining biosynthetic pathways are being used, where stable isotopes of carbon, hydrogen and nitrogen augment and enhance the level of detail that can be obtained about biosynthetic processes (30) and establish the cleavage and/or formation of C-C, C-O and C-N bonds can be detected through nmr techniques. Such procedures have been exceptionally useful in examining the alternative folding patterns of polyketide chains in the formation of complex polyketides, and also in determining the fate of oxygen atoms form a polyketide chain.

The enzymes involved in the pathways for the biosynthesis of fungel and plant secondary metabolites are being isolated, characterized and immobilized, ant the genes involved in the formation of secondary metabolites are now being cloned into bacteria leading to he possibility that enhanced production of economically significant entities will be achievable (31).

The possibility that plant tissue (callus or cell free suspension) cultures could provide an alternative, rapidly renewable resource for the production of crucial secondary metabolites has attracted a lot of interest in the past twenty years (32). Substantial success has been achieved in many studies, not only to produce the required metabolites, but also in dramatically raising yields. Indeed shikonin, from **Lithospermum erythrorhizon,** which is used in lipstick, is now commercially available through high yielding suspension cultures (33).

An important and rapidly developing area of pharmacognosy is biocatalysis; the study of the interaction of natural products and biological systems (bacteria, fungi, other micro-organisms) where the natural product is a xenobiotic to the system. Biocatalysis may attain selective transformations which would otherwise be chemically difficult or impossible, it may yield new analogues of bioactive compounds which can be evaluated, or it may serve as a model system for metabolic transformations, either plant, microbial or mammalian (34). Biocatalysts may be whole cell systems or relatively purified enzyme systems. They may be part of continuous culture technique, supported on an immobilized matrix or, more recently, cloned into bacteria.

III. Some Possible Directions for Pharmacognosy Research

The nature of the compounds, the amount of sample available, ant the instability, of the isolates will become major challenges to the isolation of active compounds and enzymes from organisms. Current examples include the effort to characterize neurochemicals and hormones, the chemicals of sight, and the inducers of plant, animal and microbial biological events such as plant growth regulation, the mechanisms of cell differentiation, and other biological "switches" . Success in these latter studies will be necessary if secondary metabolites are to be systematically generated in cell free or cell suspension systems.

Data gathering and analysis will be especially crucial from two perspectives in the future. Firstly, there will be the need to keep abreast of th burgeoning literature on biologically active natural products, and secondly there will need to be a substantially increased global effort to gather ethnomedical information from all over the world on the use of indigenous plants before the shaman and medicine men who presently have this information die or are assimillated into other societies (35).

The whole notion of how new lead compounds are sought will change in the next ten years. There will be an increased reliance on highly selective, fully automated, biogenetically engineered, enzyme and receptor based assays. There will be dramatic developments in the speed at which assays are conducted, and the relationship between an active extract and the isolation of an active principle through bioautography. One area in need of attention is the development of diagnostic, **biological,** field tests which can be used to evaluate organisms in the field for their biological activity. There has been some progress in marine pharmacognosy along these lines, but none for terrestrial pharmacognosy.

Integrated structure elucidation will be a reality. That is, a computerized database will be able to analyze data from spectroscopic sources (ir, uv, mass and nmr) and integrate these not only into a structure, but also to project a probable solution conformation.

In the area of biocatalysis, from the commercial point of view, there will be a broad array of new biological cleaners, new, semi-synthetic enzymes with modified substrate activity, antibodies which mimic transition states as catalysts for biological reactions will be used, and bacteria for the propagation of plant —derived enzymes for the production of secondary metabolites will become commen-place.

IV. Future of Pharmacognosy

What then is the future of pharmacognosy? While new, biologically, important, therapeutically relevant, natural products continue to be isolated and provide lead compounds for future development, while the search continues for natural, non-polluting insecticides and herbicides, and while efforts to commercialize the products of gene replacement technology continue to be successful, pharmacognosy will continue to thrive for many years to come. But in practice what does this mean? What are the future challenges in developing the chemistry and biology of natural products?

There are many additional aspects to be considered in contemplating the future of pharmacognosy. Perhaps the most important is to evaluate the potential origins of the medicinal agents of fifty years from now (36). Synthetic modification to existing drug entities for the purposes of enhancing and/or reducing activity is an attractive focus of drug development while the cost of the chemicals involved is relatively cheap. At some point, however, as competition between developed and developing countries for diminishing oil resources increases, and as the standard of living in developing countries rises, so the pressure on the prices of fine chemicals will dramatically increase. This will have an immediate effect on the price of all synthetic and semisynthetic drugs, and may result in prohibitively expensive drugs. Currently, many of the major pharmaceutical companies have a

substantial commitment to the development of new antibiotics for various infectious diseases and for cancer chemotherapy. But rationally, what is the economic and clinical future of a sixth or seventh generation cephalosporin? Aren't we developing compounds for a market that almost doesn't exist, and at costs that are prohibitively high?

The opportunities to develop new drugs or other economically useful compounds are extremely limited, they are two, synthetic or genetically engineered. In the latter category, terrestrial and marine plants, animals and microorganisms are our only available sources. Anything that we do that jeopardizes these resources also diminishes the potential availability of new medicinal and biological agents, probably permanently.

There is irrefutable evidence that the tropical rain forests and the oceans are an almost limitless source of biologically active compounds. New important drugs and biological tools will undoubtedly be found as a result of systematic investigation of these resources and the ethnomedical leads provided from the existing literature and the local shaman. All of the tools needed are available and ready, there is plenty of expertise, and some outstanding laboratories in several countries, and there is no shortage of pharmaceutical companies to convert a drug discovery to a finished product.

In reality though, if we are to optimize the development of the existing biome, a quite different approach is called for. As indicated previously, this beautiful planet has extremely limited resources. We have barely begun to investigate the potential for our civilization to be self-sustaining in terms of its herbicides, insecticides and pharmaceuticals. Now what is needed is a truly global effort in the next fifty years to thoroughly evaluate every available plant, animal, insect, fungus, alga and bacteria, whether marine or terrestrial, for new, biologically active compounds. Such an enormous undertaking will need to be coordinated by a major international agency, and will require funding by many governments, pharmaceutical companies and philanthropic organizations. It will be the biological equivalent of the human genome project. It is at least as important. For will the human genome project eliminate AIDS, herpes, malaria, heart disease and schistosomiasis? Once this project is underway, pharmacognosy can rightfully claim its place as the pre-eminent collaborative science for the health of mankind. At the present time we have a window of opportunity available. It is essential that we use it wisely.

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