The A B C of Modern Pharmagognosy

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Abstract

Pharmacognosy has evolved considerably during the past two hundred years and in recent years has regained importance, partly because of the renewed interest in natural products as lead molecules for the development of 'orthodox' medicines, but also because of the growth in industrialised countries of the use of complementary medicinal products. New developments in pharmacognosy which are being developed to meet the challenges posed by the current situation can be categorised as A analysis, B biological testing and C collaboration.

The need to define and trace source material to optimise guarantees of quality, and thereby safety and efficacy, of phytopharmaceuticals requires that any sample has to be defined analytically beyond the macroscopical, microscopical and chemical parameters that are traditionally used. There is much intraspecific variation in chemical content so the composition must be defined, not only by established techniques, but also by computer-aided analysis of the profiles of the complex mixture of compounds present, which can now be generated by techniques such as near infra-red spectroscopy.

The introduction of in vitro bioassays has been the most important change in pharmacognosy research over the last twenty years. These small scale assays enable bioassay-guided fractionation to be utilised to identify active compounds in extracts. However, there are dangers in such a reductionist approach when searching for new compounds to treat a particular disease state and it is best to use a portfolio of tests, backed up by in vivo studies.

Collaboration between scientists of different disciplines is now a necessary part of any worthwhile research project because of the explosion of knowledge and shortage of resources. The pharmacognosist can be a valuable bridge between specialists and has much to offer to help pharmaceutical knowledge of phytomedicines to advance.

INTRODUCTION

Pharmacognosy, a term coined about 200 years ago, is derived from the Greek, meaning 'knowledge of drugs'. Although the term strictly should apply to all drugs, the fact was that most of the drugs used in the early nineteenth century consisted of plant material, and the term evolved to become restricted to the analysis of drugs and medicines from natural sources, especially as a means to check their authenticity and purity. During the next hundred years, medicines increasingly became based on single chemical substances, either produced synthetically or isolated from plants, and the importance of pharmacognosy as an analytical science decreased in mainstream pharmacy. In the last fifty years, it has increasingly been regarded as an aspect of drug discovery as well as drug analysis and nowadays is an interdisciplinary subject which is defined here as 'the study of substances of natural origin with properties which are relevant to the science and practice of pharmacy' (Houghton, 1997). Traditionally the subject has been mainly concerned with the flowering plants and their products, especially isolated secondary metabolites but it should be noted that other groups of organisms, such as those of marine origin and fungi, are considered by many as legitimate material for study by

pharmacognosists. However, this review restricts itself to the study of flowering plant material.

A Brief History

Before about 1950, most pharmacognosy that was taught and practised concentrated on the botanical characteristics of plant material that were retained after processing into the crude drug form. These features comprised macroscopical and microscopical characteristics to identify drugs, to distinguish between related species and to detect adulteration. Where the drug was in powdered or finely broken form, microscopy was the only method of much use and major contributions to microscopy were made by pharmacognosists (e.g. Wallis, 1965) over this period.

Between about 1950 and 1980, advances in chromatography and spectroscopy enabled the isolation and structural characterisation of phytochemicals on a much larger scale than previously. Pharmacognosists were in the forefront of this development and the work of Stahl in pioneering thin-layer chromatography as a quick, cheap and easy way of characterising the chemical composition of crude drugs merits special attention (Stahl, 1969). A large amount of work also was carried out to identify the compounds present in crude drugs with the view to producing novel lead molecules for the development of new pharmaceuticals. There were some spectacular successes, such as the introduction of the Catharanthus alkaloids as anticancer agents, but much of the research was carried out in universities and the pharmaceutical industry was generally more interested in synthetic compounds. However the elucidation of the constituents of crude drugs also enabled more meaningful quantitative assays to be developed so that efficacy and safety could be more accurately predicted. Overall, however, these thirty years were characterised by a decline, and in many places a total eclipse, of pharmacognosy as a subject of academic or industrial interest.

From 1980 onwards, however, there has been something of a resurgence of interest in pharmacognosy. Although the situation is rather complex, it can be summarised as being due to two major factors. The first of these is a renewed interest in plants and other living organisms as a source of lead molecules, particularly aided by the introduction of biological tests which need only small amounts of material. The other factor is the unexpected growth in sales and usage of phytomedicines throughout the industrialised world. This has raised many concerns about quality, safety and efficacy and has resulted in a realisation of the need for analytical methods appropriate to the unique features of these products.

Thus, at the beginning of the twenty first century, pharmacognosy is facing new developments, which raise new challenges, but which also present new opportunities because pharmacognosy is interdisciplinary and can often have a wider perspective than more specialised sciences. This paper considers, what is sometimes called in English the ‘A, B, C’, i.e. the basics, of modern pharmacognosy namely **A**nalysis, **B**ioassays and **C**ollaboration (although **C**hemistry could also be included).

ANALYSIS IN MODERN PHARMACOGNOSY

As mentioned above, there has always been a strong analytical component to pharmacognosy. Optical microscopy was the most important method in analysis of the botanical aspects of crude drugs but transmission of the nature of the objects viewed relied on the drawing skills of the microscopist and was time-consuming. In addition, a large amount of experience was required before a microscopist could identify crude drugs microscopically, especially where closely-related species were concerned. The advent of photography enabled photographs to be taken through the microscope but it did not make much of an impact in pharmacognosy, although low power electron microscopy provided some fascinating and beautiful images, which conveyed a three-dimensional aspect of microscopical features not possible with the light microscope. Nowadays, however, as in many other areas of life, the application of computers has had a profound effect and images can now be captured electronically and processed to emphasise points of interest.

Another use of computers has been to analyse a set of significant features which, taken together, present a profile unique for each drug in a collection. This requires much less skill on the part of the microscopist and only the ability to identify particular types of cell or cell contents. The presence or absence of these for an unknown is recorded on a data sheet whose entries are then fed into a computer and compared by the computer against a databank, giving a list of drugs whose profiles in the databank agree most closely with the input data (Jolliffe and Jolliffe, 1978).

Chemical constituents have played a role of ever-increasing importance in the analysis of crude drugs, both qualitatively and quantitatively. The absence or presence of a particular constituent, or group of constituents, may be a very useful marker as to the identity of a species and full use has been made of chromatography, especially TLC, in this respect (Wagner et al., 1984). These methods are widely used in pharmacopeias. Quantitative analysis of individual constituents, which is so necessary for determination of levels of impurities as well as for gauging the likely effect of the drug through the amount of active ingredient, has also been greatly facilitated by the introduction of HPLC. Other high resolution separation science methods, such as capillary electrophoresis, have also been applied to crude drugs but multidimensional chromatography, and especially linked techniques such as liquid chromatography/mass spectroscopy (LCMS) are providing a new dimension in analysis.

These most recently-introduced methods combine high resolution with high sensitivity and are therefore able to generate large amounts of data on the constituents being measured, whether the DNA profile (genomics), the pattern of proteins produced (proteomics) or the array of other secondary metabolites (metabolomics). Profiles of complex mixtures based on other physico-chemical characteristics such as the Near Infra Red (NIR) spectra and mass spectra are also being used in the same way so that different samples, even of the same species, will yield different profiles. Processing the vast amount of data produced by these techniques can only be done economically with the aid of a computer to perform cluster analysis and other related techniques. Such techniques have the advantage in that, as well as facilitating differentiation between species, it is possible to identify distinct populations within a species so that plant material can be more easily sourced and also tracked from field to finished product. The use of these techniques in pharmacognosy should be able to overcome many of the analytical problems of herbal medicines where a complex mixture of substances is present and no single compound, or group of compounds, can be held responsible for the overall activity.

BIOLOGICAL TESTING

Although the ultimate test of the efficacy of a drug or medicine is a series of well-designed and conducted clinical trials, safety and economic reasons preclude these in initial studies into the activity of plant extracts, particularly where it is wished to determine the active constituents. Animals were used frequently in the past for both the standardisation of existing medicines obtained from plant extracts and in the discovery of new active compounds. The use of animals is now discouraged for economic and ethical reasons and because of pressures from society. Most animal methods, at least if macro-organisms are concerned, are not suitable for the testing of large numbers of samples, so it was difficult to employ them in bioactivity-guided fractionation and isolation of active compounds.

The introduction of small-scale bioassays was therefore a very necessary event and is, arguably, the most important development in the last twenty years in drug discovery generally, not only in the realm of natural products. These bioassays are designed to use very small amounts of test material, give results within a few days at the most and many samples can be processed over a short time so they are consequently often referred to as High Throughput (HT) bioassays. Microtitre well plates, in which large numbers of samples can be simultaneously tested, are very widely used. In many industrial and commercial laboratories where the same test system is used for large numbers of samples, this process is automated and robotised to a very large extent This

method has been used on a large scale by the pharmaceutical industry but many academic groups are also involved in the development of bioassays and in their use to isolate active compounds and they have been described in a variety of recent papers and books (Bohlin and Brun, 1999; Gebhardt, 2000; Hostettman, 1991; Houghton, 2000a).

Results can be quantified by a variety of methods according to the test employed. Visual observation can be made, especially for the growth of microorganisms over a range of concentrations, but, more commonly, a reaction or biological process takes place which results in the production of a colour of fluorescent substance and the concentration of the product can be measured by colorimetry or fluorimetry. Where microtitre well plates are used, a special type of spectrophotometer, called a plate reader, can measure the absorbance or fluorescence very quickly of all the wells simultaneously. Other quantifiable parameters used include radioactivity (using compounds labelled with ^{3}H or ^{14}C) or immunoassays. These bioassays are based on systems utilising either cells, enzymes or receptors and measurements are made of quantifiable parameters which can be recorded by highly sensitive equipment so that levels of activity may be tested. Common approaches are listed in Table 1.

Such activity generates a large amount of data and this necessitates the use of electronic processing of results and automatic rejection of samples with activity below an amount specified for the particular test.

The use of such small scale assays has several applications in modern pharmacognosy. The most important one is the elucidation of the compounds responsible for the reputed or observed activity in an extract. This is widely employed in industry, and to some extent in academia, in the screening of plant extracts collected randomly or based on a wide diversity. It is also used when the use of a plant in traditional medicine is investigated scientifically, when tests are chosen which relate to the disease or condition concerned. It should be noted that there is a large gap between activity demonstrated in an in vitro test and its clinical efficacy and it is generally recognised that it is better to have a battery of tests relating to a clinical condition, rather than one test only (Table 2). In King's College during the last five years, the Centre for Bioactivity Screening of Natural Products has been established and this gives interested parties access to over 100 bioassays, many of which are grouped according to particular disease states.

An application of bioactivity testing which is only beginning to be considered is its use as a dimension of standardisation (Houghton, 2000b). This is particularly important in the case of herbal drug extracts which are known to exert a clinical effect based on the activities of a number of types of component, thus the conventional chemical quantification based on one 'active' is not a very reliable indicator of efficacy.

CHEMISTRY

Some may be surprised that the 'C' in A B C of the title was not for chemistry. In the UK, and, from conversations with colleagues from other places, in many other industrialised countries, chemistry is not a popular subject either with students of pharmacy, with parts of the pharmaceutical profession or with the general public. This could be an argument for stressing its importance but, to some extent, both topics given emphasis here, i.e. analysis and biological activity, are based on the chemical structure and amount of the constituents present in the plant. Too often, chemistry has become an end in itself when surely the aim of a pharmaceutical chemist is to contribute in some way to the understanding and use of current or future drugs and pharmaceuticals. Formerly relatively small molecules were the focus of attention, although they could have very complicated structures, but it is now possible to turn attention to large compounds such as peptides, proteins, carbohydrates or hybrids between these classes and this is an exciting new area of natural product chemistry.

COLLABORATION

In modern scientific research, teamwork is essential for worthwhile progress to be made. Any particular project can only go forward to best effect when there is synergy

within a mix of original thinkers, specialists with expertise in design and facilities for techniques, workers to carry out the experiments and those who can provide the funds. Often this will involve crossing traditional boundaries between research groups and disciplines and, unfortunately, in many centres a protectionist reaction against such moves impedes real progress so that individual groups may be working on different aspects of a topic but may not be aware of the other's work or be prevented from disclosing results. The result is, for example, that phytochemistry is carried out without any relation to biological activity or that the biological activity of a plant extract may be investigated without any help, to the pharmacologists involved, from phytochemists in elucidating the compounds responsible.

The international nature of scientific research is one of its attractive features and those of us from the developed, usually richer nations, have an obligation to provide opportunities for training and introducing new techniques to scientists from the developing world. This is particularly important in the case of plants of pharmaceutical interest since the majority of uninvestigated species are found in this portion of the world. London is probably a rather special case, because of historical and cultural reasons, but over 40 different nationalities have spent time in our research laboratories over the last twenty years and an international mix is a feature of most active groups in Europe and North America.

Pharmacognosy has always been a multidisciplinary subject, which has used the skills and expertise of other branches of science. In the past botanists, chemists, pharmacologists and agriculturalists were the main contributors but increasingly there are other interfaces with social scientists (e.g. anthropologists), clinicians (as more clinical trials are being performed), toxicologists and the 'new biologists' who have expertise in molecular biology and are helping in the discovery of the mode of actions of drugs at the subcellular and genetic level.

CONCLUDING REMARKS

Any living organism has to adapt to a changing environment to survive and, if pharmacognosy is to continue to play a useful part in pharmaceutical science, it will have to learn how to develop its traditional base of knowledge and skills to take in the new technologies and interdisciplinary aspects of the twenty first century. To use the literacy allusions of the title, the basic alphabet remains the same but the words that are used will include new ones as well as older ones and it is important that all working pharmacognosists know how to spell correctly!

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Tables

Table 1. Some common parameters used in small scale bioassays

Technique	Applications	Example
QUANTIFIABLE		
UV/visible light absorbance	Production of colour by metabolism of compound.	MTT test for cell viability (mitochondrial conversion to coloured compound).
	Staining of particular features	SRB assay for cytotoxicity– protein from living cells stained red
	Enzymatic conversion to coloured compound	Test for oestrogen binding in genetically-modified cells expressing β-galactosidase
	Measurement of light intensity	Chemiluminescence test for phagocytic activity.
Fluorescence	Staining of particular features	Used in DNA analysis
	Immunoassays using a fluorescent label	Competitive binding assays to receptors
Radioactivity	Conversion of labelled precursor	Test for viability of *Mycobacterium tuberculosis*
	Radioimmunoassay techniques	Synthesis of eicosanoids, estimating virus titre in HIV
NON-QUANTIFIABLE		
Visual observation	Growth of microorganisms	Tests for antibacterial, antifungal, antiviral effects

Table 2. Examples of batteries of bioassays used to test for possible activity in aclinical condition

Clinical condition/ /traditional use	**Relevant in vitro assays**	**Examples and references**
Wound healing	Antibacterial assay Fibroblast growth stimulation Anti-inflammatory tests Antioxidant tests Regrowth of damaged cultured cell layer	Investigations on *Buddleja* leaves (Mensah et al., 2001)
Ailing memory/Alzheimer's Disease (AD)	Choline-esterase inhibition Anti-inflammatory assays Antioxidant tests Oestrogenic receptor binding GABA-receptor binding	Sage as a potential treatment in symptoms of AD (Perry et al., 2000, 2001).
Arthritis and other inflammatory conditions	Neutrophil enzyme release e.g. myeloperoxidase (MPO) reduction Eicosanoid synthesis - inhibition of COX and 5-LOX enzymes Reactive oxygen species - reduction in production, inhibition of enzymes, decrease in effect	Investigation of *Nigella sativa* oil (Houghton et al., 1995)