

“What is all knowledge too but recorded experience, and a product of history –?”

(Thomas Carlyle, 1795 – 1881)

Abstract

Many scientific methods of analysis have been developed for the investigation of the constituents and biological activities of medicinal plants during the 50 years since the inaugural meeting of the Gesellschaft für Arzneipflanzenforschung (GA). The chromatographic (e.g., TLC, GLC, HPLC), spectroscopic (e.g., UV, IR, ¹H- and ¹³C-NMR, MS), and biological (e.g., anticancer, anti-inflammatory, immunostimulant, antiprotozoal, CNS) techniques utilized for medicinal plant research are briefly reviewed. The con-

tribution that advances in scientific methodology have made to our understanding of the actions of some herbal medicines (e.g., *Echinacea*, *Ginkgo*, St John's wort, *Cannabis*), as well as to ethnopharmacology and biotechnology, are briefly summarized. Plants have provided many medicinal drugs in the past and remain as a potential source of novel therapeutic agents. Despite all of the powerful analytical techniques available, the majority of plant species has not been investigated chemically or biologically in any great detail and even well known medicinal plants require further clinical study.

Key words

Medicinal plant research · 1953 – 2003 · chemical and biological methodologies

Introduction

The participants of the first GA meeting at Bad Camberg in 1953 could scarcely have imagined the scientific advances that would take place in medicinal plant research during the subsequent 50 years. The topics discussed included pharmacopoeial plants, standardization, pharmacology of belladonna, crataegus, digitalis and valerian, sparteine in therapy, and paper chromatography [1]. The first 25 years of GA meetings, summarized in 1977 by Professor Otto Sticher, featured specific medicinal plants (e.g., digitalis, ergot, rauwolfia), active constituents (e.g., anthraquinones, bitters, terpenoids), biological activities

(e.g., cardiac, psychotropic), plant cell cultures (e.g., digitalis), and chromatographic techniques [2]. The proceedings of the 50th GA meeting in 2002 illustrate just how far scientific methodologies have advanced for medicinal plant research [3]. Small amounts of complex mixtures of natural products from extracts of terrestrial and marine species can now be investigated for the chemical structures of individual compounds with specific biological activities. This meeting attracted 676 participants from 70 countries and the published 575 abstracts cover a wide range of medicinal plant research. The ability to attract such an international group of scientists to discuss their research is in part due to the significant advances that have taken

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place in methodology enabling scientific progress to be achieved.

As a research student in the 1950's it took 48 hours to develop a paper chromatogram and several hours of careful measurements to record a UV spectrum. Medicinal plant research was severely limited by the lack of analytical techniques. The influence that the evolving chemical and biological methods of analysis have had on medicinal plant research has been reviewed [4] and a more detailed record can be followed through the annual publications of the GA congresses. Henry Ford (1863–1947), the American motor manufacturing pioneer is credited with the quotation “history is bunk” but many believe that lessons can be learned from the past, and since the GA is celebrating 50 years of research into medicinal plants it is perhaps fitting that we look to the past so that we may have a clearer perspective for the future.

Chemical Techniques

Chromatography

TLC had a tremendous impact on medicinal plant research from the 1960's onwards. Egon Stahl developed TLC for routine laboratory use and within a few years he separated a wide range of compounds from medicinal plants [5]. GLC, developed in the 1950's, also became established during the 1960's as a useful method for the analysis of essential oils, alkaloids and other natural products [6]. In the next decade, HPLC became available for the separation of natural products with widely differing polarities. GLC and HPLC have proved to be useful for quantification of components within mixtures and automated procedures have facilitated multiple separations, allowing the isolation of individual compounds. Other techniques, e.g., droplet counter-current and centrifugal chromatography, electrophoresis and ionophoresis, have also been applied in medicinal plant research. The separation of plant products by column chromatography has been accelerated by the application of medium and high pressure techniques.

Spectroscopy

By the 25th GA meeting in 1977, UV, IR, MS and ¹H-NMR were proving to be of great value for chemical structure determination of natural products that had been separated by chromatographic methods. At that meeting the relatively new technique of ¹³C-NMR was reviewed for interpreting the structure of natural product molecules and illustrations given for indole alkaloids and flavonoids [2]. Since then, enormous refinements and developments have taken place in ¹H- and ¹³C- NMR spectroscopy, particularly in increased sensitivity of analyses [4]. 2D-NMR spectroscopy, either homonuclear (e.g., ¹H-¹H COSY) or heteronuclear (e.g., HMQC, HMBC) correlated spectra and NOE spectroscopy have added considerably to our ability to determine chemical structures [4], [7]. During the 1960's, MS became a standard technique for medicinal plant research so that molecular weight and elemental composition from accurate mass determinations were possible. Analysis of molecular fragmentations during MS was of enormous value in chemical structure determinations, particularly within groups of closely related compounds from natural product extracts. More powerful instrumentation and

differing methods of ionization (e.g., CI, FD) have added further sophistication to MS analyses. Unequivocal proof of chemical structure determination is now routinely available by X-ray crystallography. Many natural products are optically active and ORD and CD enable absolute chirality to be determined.

Chromatographic-spectroscopic techniques

From the 1980's onwards, several hyphenated techniques (e.g., GC-MS, GC-FTIR, LC-MS, LC-NMR) have been developed, adding even further to the sensitivity of separations and chemical structure determinations. Hyphenated techniques are of use in the quality assurance of medicinal herbs, and when coupled on-line can help to minimize replication of known compounds during isolation procedures [8], [9].

Biological Techniques

Pharmacological evaluation of natural products in the 1950's and 1960's required gram quantities of isolated compounds and the test procedures involved whole animals or isolated organs. However, chemotherapeutic screening of plant extracts using antibacterial, antiviral and antifungal tests could be achieved using small amounts of plant material. Hence, during the 1960's and 1970's, programs for the chemotherapeutic screening of plants were initiated, e.g., the anticancer program of the National Cancer Institute (NCI). During the past 25 years, in particular, there has been a continued development of fast and sensitive *in vitro* test methods using specific enzymes, e.g., cyclooxygenase (COX), 5-lipoxygenase (5-LO) and receptors, e.g., 5-hydroxytryptamine (5-HT), opiate. The strategy of bioassay-guided fractionation, and isolation using chromatographic separation techniques revolutionized what could be achieved in medicinal plant research. There has been an explosion of research output in widely different areas of biological activity and the research emphasis has changed from being compound-led (e.g., alkaloids, flavonoids), following the chemical interests of researchers, into biological activity-led [3], [4], [10], [11], [12]. It is not possible in this brief article to do justice to the many research papers that have been published since 1953 and hence the following few examples, selected from work on higher plants only, are a personal choice yet illustrative of a much wider endeavor of scientific output.

Anticancer activity

In the 1960's and 1970's, scientists in the USA (e.g., S. M. Kupchan, N. R. Farnsworth) investigated plants for anticancer activity and J. L. Hartwell compiled an ethnomedical list of some 3000 species of plants possibly linked to cancer treatment [13]. The NCI investigation of plants for potential anticancer activity used L1210 lymphocytic leukemia cells in mice and other animal models. By the 1960's this program of research had grown and the tests were changed to P-388 lymphocytic leukemia in mice and KB carcinoma of the human nasopharynx *in vitro*. By 1986, some 35,000 plant species had been screened [13]. Screening procedures were again reviewed and the fast screens were replaced by slower-growing human tumors grafted onto athymic mice [10]. The NCI program resulted in new clinical drugs including etoposide, teniposide, and taxol, further adding to the *Catharanthus* alkaloids vinblastine and vincristine discovered by the Eli Lilly Company. Another important initiative of the NCI

natural products program in the 1980's was to extend drug discovery to other areas, in particular, to focus in the search for HIV-antiviral compounds from plants [13].

Anti-inflammatory and immunostimulatory activities

For many years, potential anti-inflammatory activity has been assessed by the ability of test substances to reduce swelling in rat paws previously injected with carageenan or another irritant. The scientific understanding of the cascade of inflammatory mediators from arachidonic acid enabled new test procedures to be developed and applied to medicinal plant research, e.g., by H. Wagner and colleagues. Activities of plant extracts can now be assessed by inhibition of specific enzymes, e.g., phospholipase A₂, COX-1, COX-2, and 5-LO [7], [10], [11], [14]. Another probe that can be used for testing for anti-inflammatory effects is to inhibit complement. Complement proteins are synthesized in the liver and hydrolytic cleavage results in a series of polypeptides affecting vascular permeability, vasodilation, and chemotaxis of lymphocytes. Cytokines, e.g., interleukins (IL), tumor necrosis factor (TNF), interferons (IF), are small proteins produced by immune cells and they act as immune messengers, increasing production of phagocytic granulocytes, monocytes, and lymphocytes. Cytokine expression is regulated by various transcription factors including NF- κ B and NF-AT. One procedure is to test the ability of substances to inhibit these transcription factors in order to suppress production of immunostimulatory cytokines [15].

Scientific interest in the ability of plants to stimulate the immune system started in the 1980's, e.g., by H. Wagner and colleagues. Tests for phagocytic activity were developed with human granulocytes or macrophages *in vivo* in mice. Other assays developed involved lymphocyte proliferation, NK cell activity, TNF and complement activation. Bioassay-guided fractionation techniques have led to the isolation of a wide range of active natural products and have provided scientific explanations for the anti-inflammatory and immunostimulatory activities of some medicinal plants [7], [10], [11].

Antiprotozoal activities

Protozoal infections bring death and suffering to millions of people living in tropical countries. Malaria, once thought to have been eradicated, has returned with a vengeance as strains of *Plasmodium falciparum* continue to develop resistance to antimalarial chemotherapy. Artemisinin, the active principle of the Chinese medicinal herb *Artemisia annua* has proved to be clinically effective in the treatment of cerebral malaria and has highlighted the plant kingdom as a source of new antimalarial drugs. Furthermore, many of the world's population continue to rely on their local traditional medicinal plants for the treatment of serious infections, including malaria. In 1979, an *in vitro* assay for *P. falciparum* was developed and by the mid 1980's applied to bioassay-guided fractionation of extracts from plants used in traditional medicine. Plant extracts can also be tested *in vitro* for activity against other protozoal diseases including amoebiasis, giardiasis, leishmaniasis and trypanosomiasis. Numerous natural products, including terpenoids and alkaloids, have antiprotozoal activity *in vitro* providing a scientific basis for the use of some traditionally used medicinal plants [16].

CNS activity

A number of neurotransmitters, e.g., acetylcholine, glutamic acid, dopamine, have been identified through biochemical and pharmacological research. In the 1990's, receptor-radioligand *in vitro* assays were developed to measure and characterize the interactions of ligands with receptors and their subtypes. These fast and sensitive assays use radioligands, e.g., ³H-, ¹²⁵I-labeled, that bind to specific receptors; test compounds are assessed by their ability to bind in competitive assays. Such assays form part of the high-throughput screening tests used by the pharmaceutical industry. As an example of academic-industrial collaboration, 10 Chinese medicinal plant species associated with CNS uses were screened using receptor-radioligand assays for 18 receptors, and bioassay-guided fractionation led to a series of active compounds [17]. In order to ascertain whether preselection of plants increased the possibility of obtaining active compounds, some 300 species of higher plants obtained from China, Asia, W. Africa, and S. America, and selected on the basis of their medicinal use, were screened in a bradykinin assay (BK II) expressed in CHO cells. The results were compared with those obtained from 300 species not preselected for medicinal uses; 20 species of the selected group showed significant activity in comparison with 2 species in the other group [17]. Early herbals claim that sage (*Salvia* species) enhances memory and hence some species have been investigated for their potential in the treatment for Alzheimer's disease. Extracts of a *Salvia* species inhibited acetylcholine esterase and the active principles were identified as 1,8-cineole and α -pinene [18].

Herbal Medicines

Despite all of the advances made in medicine, herbal medicines remain popular with the public in "developed" countries. Public skepticism of the inability of allopathic medicines to be free from adverse effects, or to cure chronic conditions, have contributed to consumer demands for high quality herbal medicinal products (HMP's). Advances in chemical and biological techniques during the past 50 years have resulted in scientific evidence to substantiate the use of many herbal products and have enabled manufacturers to produce standardized HMP's. In 1997, total sales of HMP's in Germany were reported to be US \$ 1.8 billion [19]. The regulatory control of HMP's varies in Member States of the European Union and has led individual countries to amend them and to consider proposals for future European legislation. In 1987, the European Parliament called for "– a scientific approach to phytopharmaceuticals –". The European Pharmacopoeia Commission responded by elaborating modern pharmacopoeial monographs on herbs for inclusion in the European Pharmacopoeia. The following examples illustrate how scientific methods, utilizing techniques developed since the first meeting of the GA, have provided evidence for the use of HMP's and have helped in the production of pharmacopoeial monographs.

Echinacea

Several species of *Echinacea* are used to stimulate the immune system and to help prevent infections such as the common cold [20]. A series of immunostimulating compounds including cichoric acid, alkamides, polyacetylenes, glycoproteins, and polysaccharides has been characterized. Biological tests, *in vitro* and

in vivo, including stimulation of phagocytosis and of cytokines (IL1, IL6, IFN α/β , TNF α), antiviral activity, formation of prostaglandins and leukotrienes have been reported.

Ginkgo

Ginkgo biloba is used for the treatment of cognitive deficiency and the active constituents are flavonoid glycosides and terpene lactones (e. g., bilobalide, ginkgolides). Pharmacological and clinical trial data for Ginkgo have been reviewed [19]. Systematic reviews and meta-analysis of relevant randomized double-blind, placebo-controlled trials support modest effects in the treatment of dementia. Further high quality clinical studies are required.

St John's Wort

Hypericum perforatum herb products, standardized on their hypericin (naphthodianthrone) and hyperforin content (prenylated phloroglucinol), are available for the treatment of mild to moderate depression. Clinical data support this use, but there is still a need for further trials [19].

Cannabis

Two major cannabinoids, tetrahydrocannabinol (THC) and cannabidiol (CBD), together with minor cannabinoids have been identified as the active principles of *Cannabis sativa*. A CNS receptor (CB₁) for THC was cloned in 1990, a second receptor (CB₂) from macrophages and spleen was cloned in 1993, and the natural ligand has been identified as anandamide, the ethanolamide of arachidonic acid [21]. THC and CBD stimulate the release of PGE₂ from synovial cells and inhibit leukotriene synthesis from human polymorphonuclear cells *in vitro*. Despite these advances in science, the medical use of *Cannabis* has remained problematical and it is the vociferous claims of people using it, albeit illegally, for neurological conditions including multiple sclerosis (MS), chronic pain and drug-induced emesis, that have moved medical and political opinion. Some clinical trials have been carried out and others are in progress in the UK for treating the symptoms of MS and relieving pain. Future clinical developments may use extracts of plant material, isolated cannabinoids, or synthetic analogues. Synthetic cannabinoid analogues, e. g., nabilone for neurogenic pain and emesis, are in current clinical use.

Ethnopharmacology

Indigenous plants are used worldwide as medicines, particularly in the developing countries [22]. However, the world continues to lose plant species, e. g., through agriculture and urbanization, and there is international concern as evidenced by the Convention on Biodiversity. The knowledge base on the use of indigenous medicinal plants is constantly being eroded and scientists need to be able to catalogue the actual uses of medical plant species and to develop research programs. The requirements of the poorer countries have to be carefully considered in such research and any program of development of pharmaceutical drugs with industrial partners must have well defined financial and legal agreements. The plethora of chemical and biological tests, including high-throughput screening, makes this an appropriate time for such research. Structures of indigenous medical systems

in Mexico, for example, their role in drug discovery and development, and the potential contribution of ethnopharmacology, have been reviewed [23], [24].

Biotechnology

The GA meeting of 1974 featured plant cell cultures and their potential role as alternatives for the production of therapeutic substances and biotransformation of natural product molecules, e. g., 12 β -hydroxylation and acetylation of digitalis glycosides [25]. Within a few years the biotechnological application of plant tissue cultures became a most active area of scientific investigation [26]. High yields of secondary metabolites, e. g., anthraquinone, berberine, shikonin, have been achieved from plant cell cultures but only low yields of pharmaceutically required drugs such as the *Catharanthus* alkaloids vinblastine and vincristine, morphine and hyoscyamine. Nevertheless, it has proved possible to have large scale production of plant cells in bioreactors. Plant cell cultures have proved to be extremely valuable research tools for the scientific investigation of the enzymes involved in biosynthetic pathways, particularly through the work of M. H. Zenk and colleagues [27], [28], [29]. Biosynthetic sequences are now known for a number of indole, isoquinoline and tropane alkaloids, and berberine was the first alkaloid to have its full biosynthetic pathway elucidated at the enzyme level [28].

Some plant cell cultures do not necessarily produce the same secondary products as the parent plant and can be manipulated to produce "unnatural" compounds, hence they may be of interest in drug development, e. g., production of immunostimulant polysaccharide from cultures of *Echinacea purpurea* [30]. Genetic engineering techniques have added considerably to scientific research with plant cell cultures. The gene encoding for the enzyme strictosidine synthetase has been cloned and expressed in *E. coli* so that the transformed bacterial cultures fed with tryptamine and secologanin produce strictosidine, the key intermediate for the biosynthesis of many biologically active indole alkaloids. It is now possible to produce pharmaceutical proteins in plants (e. g., vaccines) and in microorganisms (e. g., insulin) [29]. Recombinant DNA technology is a powerful tool for generating novel compounds, e. g., cloning and expression of *Streptomyces* genes have enabled genetic engineering to produce different biosynthetic pathways generating "unnatural" and potentially bioactive compounds.

Conclusions

The new millennium has seen the publication of several reviews on medicinal plant research (e. g., [29], [31]) and these should be consulted for a broader perspective. In the context of 50 years of the GA, there have been many significant developments of analytical techniques. These methods have been applied to medicinal plant research and have enabled a greater scientific understanding of bioactive constituents. As chromatographic, spectroscopic and biological techniques have developed so have sensitivity and specificity, such that today much information may be obtained from relatively small amounts of plant material [4], [9]. There is still a need for novel medicinal drugs, e. g., in chemotherapy (to

combat drug-resistant microorganisms, to develop better tolerated anticancer drugs), CNS disorders (e.g., Alzheimer's and Parkinson's diseases, schizophrenia, epilepsy, stroke, pain management). Even with the potential of gene therapy, it would be facile to imagine that this will be the only future route for the medical treatment of disease. Since plants have provided many drugs in the past, and they remain a rich source of novel compounds based on Nature's combinatorial natural products chemistry over millions of years of evolution, they should continue to be investigated as sources of novel therapeutic agents [13], [32]. The majority of plant species has not been investigated chemically or biologically [32], and bioassay-guided fractionation, dereplication techniques and the powerful methods of structure determination will continue to help this research in the future. It may be predicted that there will be a continued demand for high quality, safe and effective HMP's also requiring continued scientific investigation. Biotechnological advances have seen the transfer of genes between species, the ability to increase yields of selected constituents, and the production of "unnatural" compounds. The potential for the production of natural products within bioreactors remains a distinct commercial possibility for the future.

It is impossible to imagine what techniques will be developed in the next 50 years but it is predictable that these new techniques will be used in medicinal plant research. If the last 50 years have taught us anything, it is that researchers of medicinal plants will need to cooperate with scientists in different disciplines. What will be the future role of the GA? I believe that it will continue to be at the forefront of medicinal plant research on a worldwide basis. Future meetings will continue to stimulate research in the many different aspects of medicinal plants and continue to be a forum for independent scientists. The GA, hopefully, will retain its broad scientific base promoting multidisciplinary research and not just be concerned with the current perceived wisdom of the day. If there were one wish granted to me for the future of medicinal plant research, it would be that there will be greater cooperation between scientists and clinicians.

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