

PLANTS AND PLANT SUBSTANCES AGAINST AIDS AND OTHER VIRAL DISEASES

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Summary

Considerable progress has been made in the field of antiviral drugs. However, current therapies, especially combination of antiretrovirals, are too expensive to be affordable in developing countries and are suffering from the emergence of drug resistance. Taking into account the large number and structural diversity of currently available plant constituents, the plant kingdom remains an exciting source for new antiviral agents. This review focuses on plant-derived compounds possessing activity against Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Cytomegalovirus (CMV), and Influenza Virus. If known, special attention is given to their mechanism(s) of action and cytotoxicity.

1. Introduction

Since the mid 1990s, we have witnessed a remarkable growth in the development of antiviral therapies for Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Cytomegalovirus (CMV), Hepatitis B Virus (HBV) and Influenza Virus infections. Nevertheless, there is a constant medical need for new antiviral drugs, since these are not always fully efficacious or well-tolerated and since drug-resistance is rapidly emerging. Moreover, most of the marketed antiviral drugs, especially the antiretrovirals, are too expensive to be used in developing countries, which still have to rely to a large extent on traditional medicine. It should be emphasized that traditional medicine may offer an inexhaustible source of biologically active compounds, and with respect to this review, of potentially new antivirals. Research on antiviral natural products is mainly focused on plants, since, among other reasons, they can be selected on the basis of their ethnomedicinal use. The ethnomedicinal use of plants, known to contain highly active antiviral constituents, is listed in Table 1 and the importance of this strategy is clearly demonstrated by the increasing number of reviews on antiviral plant products. This review intends to critically evaluate the current state of the art on plant-derived antiviral substances.

2. The plant kingdom as source of new antiviral agents

2.1. Opportunities and challenges

The plant kingdom is undoubtedly a successful source of drug leads. This has resulted in the use of a large number of medicinal plants to treat various diseases, and several

drugs in western medicine are based on the traditional use of such drugs. Well-known examples are taxol (paclitaxel) and camptothecin derivatives (topotecan and irinotecan) as antitumor agents (see *Plants as a Source of Anti-Cancer Agents*) and artemisinin derivatives (artemether and artesunate) as antimalarial agents (see *Traditional Medicinal Plants for the Treatment and Prevention of Human Parasitic Diseases*). Approximately one-third of the top-selling drugs in the world are natural products or natural product derivatives, indicating a higher hit rate for natural products compared to synthetic chemicals. A prevailing impression is that the plant kingdom has already been thoroughly examined for biologically active molecules. However, this is not the case, since the number of different plant species is estimated at over a quarter of a million, and only 10% have been tested for some type of biological activity. In addition, natural compounds offer structural diversity that is not rivaled by the creativity or synthetic ingenuity of medicinal chemists.

Despite this positive outlook, many large pharmaceutical companies have stopped exploring natural resource collections for several reasons. Firstly, because of concerns about the 'intellectual property' status of leads from natural resources. Secondly, because natural compounds do not fit well into modern high throughput screening (HTS) strategies. Extracts are generally seen as too difficult to assay or too time-consuming to be competitive with chemical collections. Furthermore, known structures are frequently 're-discovered' or if new lead compounds can be isolated, medicinal chemists are often reluctant to derive optimized drugs due to structural complexity and/or many chiral centers. Therefore, screening of extracts and subsequent isolation and characterization of biologically active compounds have been passed on to smaller companies or academia and involves a multidisciplinary team consisting at least of a pharmacognosist and a virologist. Furthermore, as estimated by the International Union for the Conservation of Nature, we are steadily losing a number of plant species each day. Recognizing that still very little is known about the biodiversity on Earth, high priority must therefore be given to preservation and inventorization of species, supported by ethnobotanical and ethnomedical information.

Family Name	Species	Traditional use	Country	Active substances	Class	Activity
Anacardiaceae	<i>Rhus javanica</i> L.	Gastric and duodenal ulcer and empyema	Japan and China	Moronic acid, Betulonic acid	Terpenes	HSV
Berberidaceae	<i>Podophyllum peltatum</i> L. and <i>P. emodi</i> Wall.	Condylomata acuminata	USA and Canada India	Podophyllin	Lignans	HSV
Cucurbitaceae	<i>Momordica charantia</i> L.	Bitter tonic, antipyretic, antirheumatic, anthelmintic and laxative	China, India, Thailand	MAP30	Proteins	HIV
	<i>Trichosanthes kirilowii</i> Maxim.	Mid-term abortion and treatment of choriocarcinoma	China	Trichosanthin	Proteins	HIV
Cupressaceae	<i>Cupressus sempervirens</i> L.	Vascular diseases	Mediterranean countries	Proanthocyanidin polymer fraction	Tannins	HIV
Ephedraceae	<i>Ephedra sinica</i> Stapf.	Common cold, fever, cough	China	Catechin, ...	Flavonoids	Influenza
Euphorbiaceae	<i>Croton lechleri</i> Muell.-Arg.	Wound healing, anti-inflammatory, antiviral and antitumor properties	Colombia, Ecuador, Bolivia and Peru	SP-303	Tannins	HSV
	<i>Euphorbia poissonii</i> Pax.			12-deoxyphorbol 13-phenylacetate	Phorbol esters	HIV
	<i>Homolanthus nutans</i> Baill.	Diverse medicinal purposes	Samoa	Prostratin	Phorbol esters	HIV
	<i>Ricinus communis</i> L.	Laxative	China, Vietnam	Lectin	Proteins	HIV
Guttiferae	<i>Calophyllum lanigerum</i> Miq. var. <i>austrocoriaceum</i>	Wounds	Malaysia	Calanolide	Coumarins	HIV
Hypericaceae	<i>Hypericum perforatum</i> L.	Depression and mental illness	Europe	Hypericin	Naphthodiantrones	HIV
Labiatae	<i>Scutellaria baicalensis</i> Georg.	Common cold, conjunctivitis, enteritis, burns	China	Isoscutellarein and isoscutellarein-8-methyl ether	Flavonoids	Influenza
Leguminosae	<i>Glycyrrhiza glabra</i> L.	Anti-inflammatory, antipyretic and laxative activities	China	Glycyrrhizin	Terpenes	Influenza
	<i>Phaseolus vulgaris</i> L.			Lectin	Proteins	HIV
Meliaceae	<i>Melia azedarach</i> L.	Anthelmintic, antilithic	China, India	Meliacine	Proteins	HSV

		and diuretic				
Menispermaceae	<i>Stephania cepharantha</i> Hayata	Inflammatory diseases, asthma bronchiale and alopecia areata	Japan	Cepharanthine FK-3000, cephacicine	Alkaloids Alkaloids	HIV HSV
Moraceae	<i>Maclura cochinchinensis</i> (Lour.) Corner	Chronic fever, skin infection and abnormality of the lymph nod	Thailand	Morin	Flavonoids	HSV
Myrsinaceae	<i>Maesa lanceolata</i> Forsk.	Hepatitis and bacillary dysentery	Rwanda	Maesasaponin V3	Terpenes	HSV
Myrtaceae	<i>Syzygium aromaticum</i> Merr. et Perr.	Allergic disorders	Molucca	Eugeniin	Tannins	HSV
Rosaceae	<i>Geum japonicum</i> Thunb.	Diarrhea, tonic	Japan	Eugeniin	Tannins	HSV
Sargassaceae	<i>Sargassum horneri</i> (Turner) C. Agardh	Savory food	Japan	Fucan sulphate	Carbohydrates	HSV
Simarubaceae	<i>Quassia Africana</i> Baill.	Antidysenteric and antidiarrhoeal	Central Africa	Simalikalactone D	Terpenes	HSV

Table 1. Traditional use of plants used for the isolation of antiviral active substances and discussed in this review.

2.2. Selection of plants

Four discovery approaches for new (antiviral) agents from plants can be envisaged: (1) random collection of plants followed by mass screening; (2) selection based on ethnomedical uses; (3) follow-up of existing literature leads and (4) chemotaxonomic approaches. Approaches (2) and (3) would seem to be the most cost-effective for finding plants with antiviral properties. Information on ethnomedical use of plants is well-documented in Ayurveda, Unani, Kampo, and traditional Chinese medicine. They have flourished as traditional medical systems and have the following elements of credibility: (1) they have a long historical written documentation, (2) they are based on theories, (3) they are based on well-organized educational systems, and (4) they are frequently revised based on scientific research. In Africa and South America, ethnomedical use of plants is less recorded. The plants that are used are often kept secret by the traditional healer, so that there is less scientific evidence. In this case, it is necessary to determine which part of the plant is used, how it is conserved, its posology, and how it is prepared.

A comparative study between random and ethnobotanical collections of plants for *in vitro* anti-HIV activity showed a five-times higher percentage of active leads for the ethnobotanical collection. On the other hand, the screening of extracts from randomly collected plants may produce more novel substances. When the capacity of a screening operation is limiting, selection of plants based on a combined analysis of ethnomedical, phytochemical, taxonomical, and toxicological data should be preferred.

2.3. Preparation of plant extracts

Once the plant is selected, the next step is its collection and botanical identification. It is important that plant collection involves a professional botanist who rigorously documents with appropriate voucher specimens, photographs, and written notes on collection site, season, status of the plant, etc. Stabilization is usually obtained by drying the plant material at ambient temperature in a shady place. The dried or stabilized plant material should then be powdered and subjected to a suitable extraction process.

Plant extracts are generally prepared by maceration or percolation of dried powdered plant material with water or organic solvents. Sometimes, a fractionation of the total extract is carried out prior to testing in order to separate polar from non-polar compounds and acid and neutral from basic substances. It is advisable to extract the plants and to dry the extracts at low temperature to avoid destruction of thermo-labile (antiviral) constituents. Non-polar extracts can be dissolved in organic solvents, such as dimethylsulfoxide (DMSO), and in methanol or ethanol. Since most plant extracts and purified compounds are readily soluble in DMSO, stock solutions of test samples can be prepared in 100% DMSO and stored until use. An added advantage is that DMSO eliminates microbial contamination of the test samples, so that sterilization by autoclaving, filtration or other methods is not necessary anymore. In view of automation and integrated high-capacity screening, choosing a single solvent for the stock solutions becomes an advantage. To avoid interference in *in vitro* cell-based test systems, the in-test concentration of DMSO should preferably not exceed about 1%.

For purification and isolation, the active plant extracts are further fractionated and each fraction is evaluated in an antiviral assay (see section 3), called bioassay-guided

fractionation. Two key-elements in a successful bioassay-guided fractionation are dereplication and selectivity. When screening natural products it is essential to identify the active constituent(s) at an early stage to avoid isolation of molecules with known structure and known activity. This 'dereplication' prevents a group from wasting resources by rediscovering known compounds. The combination of literature search and analytical power, such as GC-MS and HPLC with diode array detection, is a prerequisite before any bioassay-guided fractionation should be initiated. In anti-HIV and anti-HSV screenings, removal of sulfated polysaccharides and tannins from plant extracts must be considered. Tannins interfere with enzyme- and receptor-based inhibition assays giving false-positive results. However, caution should be taken with such an approach, as some leads could theoretically be missed. Once a new compound has been isolated and identified, it must be fully evaluated for its biological activity and toxicity. An important criterion for activity is selectivity of action, and this can only be investigated if the antimicrobial screens are run in an integrated manner, i.e. against a broad panel of related (other viruses) and unrelated organisms (bacteria, fungi, and parasites).

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Bibliography

Buckheit R.W., White E.L., Fliakas-Boltz V., Russell J., Stup T.L., Kinjerski T.L., Osterling M.C., Weigand A., Bader J.P. (1999). *Antimicrobial Agents and Chemotherapy* **43** (8), 1827-1834. [Buckheit *et al.* demonstrate that research on plant-derived molecules should be continued and even accelerated, since e.g. the calanolides represent a novel and distinct subgroup of the NNRTI family].

Cos P., Vanden Berghe D., De Bruyne T., Vlietinck A.J. (2003). Plant substances as antiviral agents: an update (1997-2001). *Current Organic Chemistry* **7** (12), 1163-1180. [This is a comprehensive review focused on the latest developments on plant substances as antiviral agents].

De Clercq E. (2002). Strategies in the design of antiviral drugs. *Nature Reviews drug discovery* **1** (1), 13-25. [This work describes the rationale behind current and future drug-based strategies for combating viral infections].

Kinghorn A.D. (2001). Pharmacognosy in the 21st century. *Journal of Pharmacy and Pharmacology* **53** (2), 135-148. [The importance of pharmacognosy in the development of new drugs is clearly demonstrated].

Pluyms W., Neamati N., Pannecouque C., Fikkert V., Marchand C., Burke T.R., Pommier Y., Schols D., De Clercq E., Debyser Z., Witvrouw M. (2000). Viral entry as the primary target for the anti-HIV activity of chicoric acid and its tetra-acetyl esters. *Molecular Pharmacology* **58** (3), 641-648. [This work redefines the primary viral target of L-chicoric acid from integrase to viral envelope gp120 glycoprotein].

Utsunomiya T., Kobayashi M., Pollard R.B., Suzuki F. (1997). Glycyrrhizin, an active component of licorice roots, reduces morbidity and mortality of mice infected with lethal doses of influenza virus. *Antimicrobial Agents and Chemotherapy* **41** (3), 551-556. [Emphasizes that immunomodulating compounds can be selected as potentially antiviral agents].

Vanden Berghe D.A.R., Haemers A., Vlietinck A.J. (1993). Antiviral agents from higher plants and an example of structure-activity relationship of 3-methoxyflavones. *Bioactive natural products: detection, isolation and structural determination*, Ed. Steven M. Colegate, Chapter 17, 405-440. New York, CRC Press. [In this book chapter the most important features of *in vitro* antiviral evaluation of plant extracts and plant-derived compounds are discussed].

Vermani K., Garg S. (2002). Herbal medicines for sexually transmitted diseases and AIDS. *Journal of Ethnopharmacology* **80**, 49-66. [Gives a nice overview of herbal medicines used for prevention and treatment of sexually transmitted diseases, including AIDS].

Vynograd N., Vynograd I., Sosnowski Z. (2000). A comparative multi-centre study of the efficacy of propolis, acyclovir and placebo in the treatment of genital herpes (HSV). *Phytomedicine* **7** (1), 1-6. [A randomized, single-blind, masked investigator, controlled multi-centre study showing that an ointment of Canadian propolis was more effective than acyclovir ointment in healing genital herpetic lesions].

Zak O., Sande M. (1999) *Handbook of animal models of infection: experimental models in antimicrobial chemotherapy*, 1136p. New York, USA: Academic Press. [This book is a reference work for scientists developing new antimicrobials].

Biographical Sketches

Paul Cos is a post-doctoral researcher of the Fund for Scientific Research – Flanders and works at the University of Antwerp. He graduated as chemist (1992), pharmacist (1995), industrial pharmacist (2001), and doctor in pharmaceutical sciences (2001) at the same University. His research is mainly focused on the evaluation of antioxidant and antimicrobial activities of plant extracts and pure compounds.

Louis Maes is Professor of Parasitology at the Faculty of Pharmaceutical, Biomedical and Veterinary Sciences of the Antwerp University. After a long career in the pharmaceutical industry, where he was involved in drug discovery and drug development of veterinary antiparasitic drugs, he is now responsible for teaching human and veterinary parasitology together with drug discovery research against human tropical protozoal diseases, in particular Leishmaniasis.

Dirk Vanden Berghe graduated as biochemist (1967), doctor in sciences (1972), and clinical biologist (1978) and became professor in microbiology at the University of Antwerp. He is laureate of the National Triannual Award for the battle against viral diseases. He is author of more than 250 international publications with peer review and he holds different international patents.

Nina Hermans is a PhD student at the Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium. She graduated as a pharmacist (1999) and industrial pharmacist (2003) at the University of Antwerp. She is currently working on a project examining oxidative stress and the potency of potential antioxidative plant substances *in vivo*.

Sandra Apers is a post-doctoral researcher (Fund for Scientific Research – Flanders) of the Laboratory of Pharmacognosy and Phytochemistry, working in the field of bioassay guided isolation of active plant substances. Research is mainly focused on antiviral, antifungal and antiprotozoal activities and activities related to chemoprevention. She is also involved in standardization of plant extracts which are clinically investigated.

Arnold J. Vlietinck graduated as Pharmacist (1964), Ph.D. in Pharmaceutical Sciences (1968), Industrial Pharmacist (1969) and Clinical Biologist (1972), all at the Catholic University of Leuven (KU Leuven). After a post-doc position at the University of Wisconsin-Madison he became professor in Pharmacognosy and Phytochemistry at the University of Antwerp (UA) in 1974. His research encompasses bioassay-guided isolation and identification of bioactive plant compounds and drug analysis. He is (co)-author of more than 300 publications and has been the thesis-advisor of more than 30 Ph.D. students. He is chairman of groups 13A and 13H of the European Pharmacopoeia (Strasbourg) and member of the Herbal Medicinal Products Committee (London) and coordinator of several research projects in Africa.