The Potential of African Plants as a Source of Drugs

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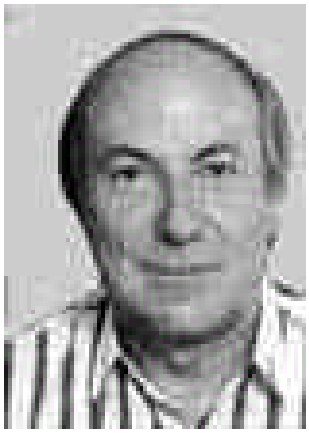

Abstract: African plants have long been the source of important products with nutritional and therapeutical value. Coffee originates from Ethiopia, *Strophanthus* species are strong arrow poisons and supply cardenolides for use against cardiac insufficiency, the *Catharanthus roseus* alkaloids are well-known antileukaemic agents – just to mention a few examples. Research is continuing on the vegetable material from this continent in an endeavour to find new compounds of therapeutic interest. An outline is presented here covering the results obtained by the Institute of Pharmacognosy and Phytochemistry of the University of Lausanne during 15 years' work on African plants. The strategy employed for the study of these plants is outlined, covering all aspects from the selection of plant material to the isolation of pure natural products. Different bioactivities have been investigated: the search for new antifungal, molluscicidal and larvicidal agents has been the most important axis. Results are also included for antibacterial, cytotoxicity, anti-inflammatory testing.

INTRODUCTION

Plants have a long history of use on the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines. Many of these plants have been documented (for example, [1-5]) and an African Pharmacopoeia has been published by the Scientific Technical Research Commission of the Organization of African Unity, starting with volume 1 in 1985 [6]. The available knowledge on the use of plant preparations in traditional medicine is enormous but if this is not rapidly researched, indications as to the usefulness of this vegetable treasurehouse will be lost with succeeding generations.

Africa, together with Madagascar, is reputed for the extraordinary richness of its flora, totalling several tens of thousands of species. Environmental degradation provides a threat to biological diversity but the sub-Saharan region still boasts a wide variety of indigenous species. Based on carful observation and a judicious choice of plants, it is possible to discover very interesting new natural products [7]. Biologically-active compounds can be isolated from African medicinal plants by bioassay-guided fractionation procedures, in which various screening methods are employed to locate the desired activities in the crude extracts and in the fractions issuing from the different separation steps. This approach and investigations of other aspects of the chemistry, botany and pharmacology of African medicinal plants were the subjects of a symposium held in Zimbabwe in 1996 [8]. This meeting provided a timely reminder of the potential of African ethnomedicine and a summary of the important work on African plants which is presently underway both in Africa and in other continents.

This article will attempt to summarize the results obtained after more than 15 years' work on African plants by the Institute of Pharmacognosy and Phytochemistry of the University of Lausanne.

However, before proceeding with this survey, and to provide a background, a look will be taken at a selection of plants from Africa which have already provided useful products, mainly in the field of medicine (aloes will not be mentioned here

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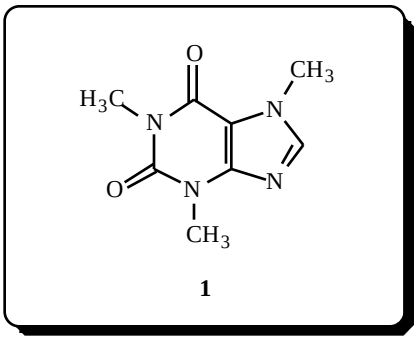
because they are the subject of a separate review by Dagne in this volume). It is, of course, impossible to include all the plants of note but an idea will be given of the potential which is available for the discovery of new, commercially-important natural products.

To start with, mention must be made of the traditional xanthine-containing fruits which provide coffee and cocoa. Classical examples, such as the Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae) will be given and, finally, recently publicised preparations, with Vuka-Vuka as an example, will be presented.

Coffea arabica (Rubiaceae)

The coffee tree *C. arabica* originates from the highlands of south-west Ethiopia. The beverage coffee is produced from the seeds of *C. arabica* and certain other varieties (*C. canephora, C. liberica*). The use of coffee was introduced into Islamic countries by the Arabs and then its consumption spread to Europe via the Venetians. Despite strict controls by the Arabs on its cultivation, live plants were introduced into Brazil (among other countries) and this latter is now the principal producing country. Coffee is made from the kernel of the dried ripe seed, deprived of most of the seed coat and roasted.

Prepared coffee contains 1-2% of the purine alkaloid caffeine (1,3,7-trimethylxanthine, **1**), probably combined with chlorogenic acid and potassium. The composition of coffee seeds and roasted coffee has been extremely well studied and hundreds of constituents have now been identified. Coffee owes its stimulant properties to caffeine. Caffeine stimulates the central nervous system and has weak diuretic activity. It also has positive inotropic activity and produces tachycardia. Interestingly, caffeine activates lipolysis.



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Cola nitida (Sterculiaceae)

Kola trees are 10-15 m in height and grow in equatorial West Africa (from Mali and Sierra Leone to Nigeria and Gabon). The most common species is *C. nitida* but *C. acuminata* and *C. verticillata* are also used. Kola nuts are chewed (masticated) fresh in their regions of origin. Commercial kola consists of the dried cotyledons of the seeds. These are usually picked before they reach maturity.

Kola nuts contain 2.5% caffeine, together with 5-10% flavan-3-ols (catechin, epicatechin). During preparation, oxidation and polymerization of these polyphenols produces an insoluble phlobaphene "kola-red". The catechin derivatives have a tendency to associate with the caffeine and thus the amount of available caffeine varies with the state of the kola seeds – that is, whether they are fresh or dried. The effects due to caffeine are thus more gradual than in the case of coffee.

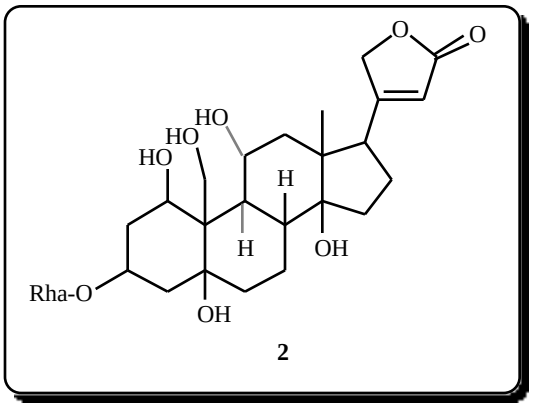
Kola nuts are employed in the preparation of non-alcoholic beverages, in particular Coca-Cola. Coca leaves were removed from the original formulation of Coca-Cola in 1904. This soft drink thus no longer contains cocaine but it does have non-negligible quantities of caffeine which originate from extracts of cola nuts.

Strophanthus spp. (Apocynaceae)

There are about 30 species of *Strophanthus* found in Africa. Their seeds have a long history of use as arrow poisons in East and West Africa. *Strophanthus kombé* (known to the local population of southern Malawi as "kombi") grows in the lake region of East Africa. It is a liane with seeds which are 12-18 mm long. Seeds of the kombé drug contain a mixture of glycosides (4-5%) called k-strophanthin. There are at least 12 different glycosides in this mixture, several with strophanthidin as aglycone. The major glycoside, k-strophanthoside, is a triglycoside of strophanthidin.

Strophanthus gratus is found in tropical West Africa and has bitter-tasting seeds which contain 4-5% of cardenolides. The major component (**2**, 90%) of this mixture is the crystalline glycoside g-strophanthin (or ouabain, isolated also from another member of the Apocynaceae called

Acokanthera ouabaio by the French scientist Arnaud). Ouabain is extremely polar and is hardly resorbed after oral administration. Its action in the treatment of heart problems is rapid and brief. This cardiac glycoside is used by *i.v.* injection in cases of acute cardiac insufficiency. The mixture k-strophanthin from *S. kombé* is used for the same indications as ouabain.



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Adansonia digitata (Bombacaceae)

The baobab is a well-known tree of immense girth found over large areas of Africa and often referred to as "Africa's upside-down tree". The fruits contain tartaric acid and the fruits and seeds are used as a remedy for dysentery in Central Africa [1]. In Nigeria and Senegal, the fruits are reputed to be effective against microbial diseases. These observations have been confirmed in tests against both bacteria and fungi, although the active constituents have yet to be isolated [9].

Prunus africana (Rosaceae)

Prunus africana (syn. *Pygeum africanum*) is an evergreen tree which grows in the mountain forests of West Africa, East Africa, Southern Africa and Madagascar. The bark, bruised leaves and fruits have been used in witchcraft and as arrow poisons. Tribes on the slopes of Mount Cameroun revealed to colonists that they had used the bark to treat "old man's disease" for centuries [10]. Bark extracts have stimulated considerable interest in Europe since 1969 for the symptomatic treatment of mild and moderate benign prostatic hyperplasia (BPH). A suitable solvent is chloroform. These bark extracts contain as putative active ingredients phytosterols in free and glucosylated form (phytosterols have anti-inflammatory effects by interfering with the accumulation of pro-inflammatory prostaglandins in the prostate), pentacyclic triterpenes (anti-edema or decongesting action), ferulic esters of long chain fatty alcohols (reduce prolactin levels and block accumulation of cholesterol in the prostate – this is significant because prolactin increases uptake of testosterone by the prostate and cholesterol increases binding sites for dihydroxytestosterone) and esters of fatty acids [11]. Pharmacological studies have proved that the extract has some other important properties that may account for its efficacy in the therapy of BPH: inhibition of the proliferation of prostatic fibroblasts and of the production of chemotactic leukotrienes, modulating activity on age-related hypercontractility of the bladder and improvement in the secretory activity of the prostate.

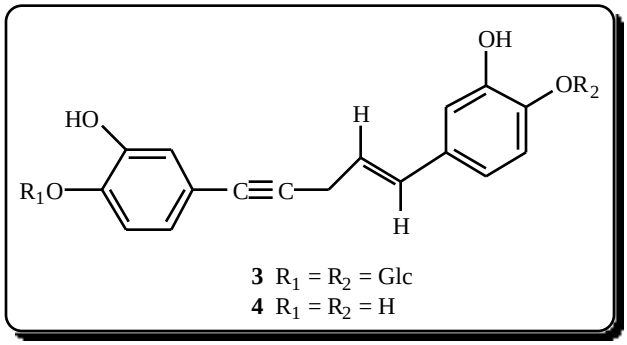
A review of the published clinical data from 2262 patients during the last 25 years has shown that the extract of *P. africana* bark is an effective and well-tolerated drug for the treatment of BPH [12].

There are certain problems at the moment associated with the supply of *P. africana* bark. So much has been collected in the wild (the annual harvest is now estimated at 3,500 tons) that in certain areas (notably Cameroun), the tree is threatened with extinction. Cultivation is being attempted and sustainable collections are necessary [10].

Hypoxis hemerocallidea (Hypoxidaceae)

The rhizomes of *Hypoxis* species are used in African traditional medicine for the treatment of urinary infections, prostate hypertrophy and internal cancer [1]. *Hypoxis hemerocallidea* (formerly *H. rooperi*) from southern Africa contains a norlignan diglucoside called hypoxoside (**3**) [13,14]. Enzymatic hydrolysis of this compound gives the aglycone, rooperol (**4**), which inhibits the growth of certain cancer cells. Rooperol is also a lipoxygenase inhibitor and effective against mutagenesis in the Ames test. Since hypoxoside acts as a prodrug in humans, clinical trials on cancer patients are underway in South Africa with dried methanol extracts of the rhizomes [15]. Patients suffering from AIDS are

also being treated with the methanol extract of *Hypoxis* species.



3 $R_1 = R_2 = Glc$
4 $R_1 = R_2 = H$

Lipophilic extracts of *H. hemerocallidea* bulbs are used for treatment of prostate problems in Europe. They have anti-inflammatory activity and relieve the symptoms of prostate adenoma. As in the case of *P. africana*, these extracts are mainly mixtures of phytosterols and in Germany it is claimed that β-sitosterol is responsible for the activity. However, this is open to speculation, in view of the daily intake of the same sterol from other sources. In the prostate, the extracts are inhibitors of prostaglandin synthesis, lower cholesterol levels and stabilise membranes.

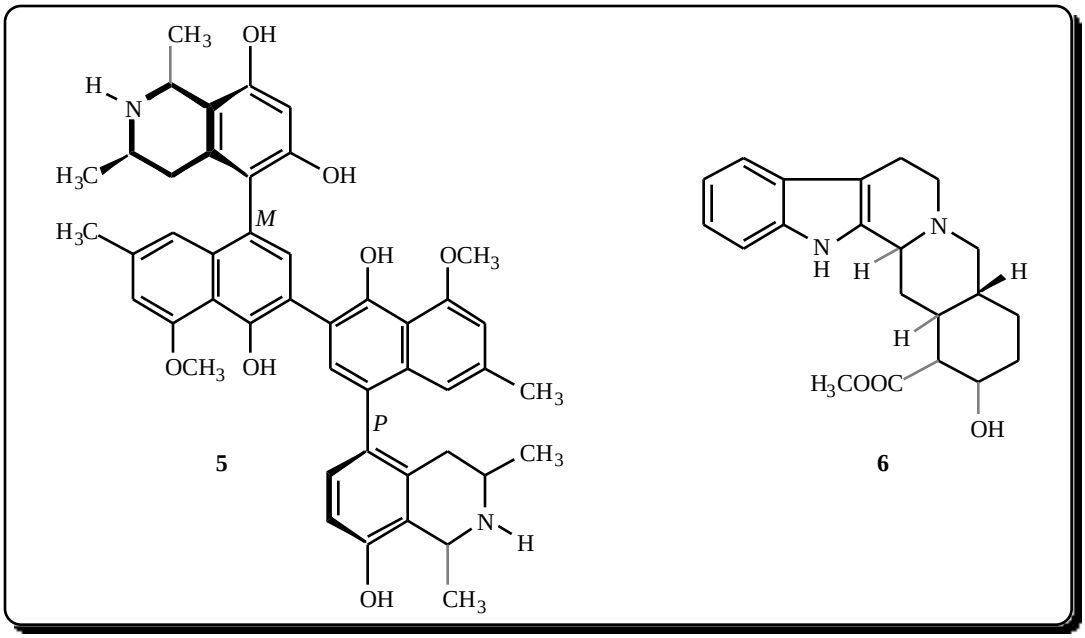
Ancistrocladus korupensis (Ancistrocladaceae)

A naphthylisoquinoline dimeric alkaloid, michellamine B (**5**), has recently been isolated from the leaves of the liana *A. korupensis*, found in the Korup National Park in Cameroun. Bioactivity-guided isolation of this compound was performed after it was discovered that an extract of the plant was active in the National Cancer Institute (NCI, USA) anti-HIV screening programme [16]. Michellamine B shows *in vitro* activity against a broad range of strains of both HIV-1 and HIV-2, including several resistant strains of HIV-1 [16]. Preclinical development is underway, despite the narrow therapeutic index of the drug. And in order to assure sufficient quantities of material, attempts are being made at the synthesis and semi-synthesis of the compound.

Pausinystalia yohimbe (Rubiaceae)

The stem bark of yohimbe (*P. yohimbe*, formerly known as *Corynanthe yohimbe*) contains 1-6% of indole alkaloids. Most of these are yohimbane-type alkaloids, the major one being yohimbine (**6**), which is structurally related to reserpine. Yohimbe trees grow in the forests of Cameroun, Gabon and Congo.

Yohimbine is a selective inhibitor of α-2-adrenergic receptors and, while at low dose it has hypertensive activity, at high dose it is hypotensive (vasodilation of peripheral vessels). It is this latter effect, and especially vasodilation of the corpus cavernosum, which is at the origin of the reputation of yohimbine as an aphrodisiac. Tests have shown, indeed, that increased libido and easier ejaculation result from treatment with yohimbine. It is used with success in the treatment of erectile dysfunction [17].



The action of yohimbine on smooth muscle favours tonus and movement of the intestine. Yohimbine also acts on α-2-adrenergic receptors of adipocytes, leading to increased lipolysis.

Vuka vuka

With the introduction of Viagra for the treatment of erectile dysfunction, a sudden of interest in the use of "natural" alternatives has been registered, especially with the advent of the Worldwide Web and the ease with which products can be ordered via this medium. One of these preparations, called "Vuka-vuka", originates from Zimbabwe, where it has been used for many years – long before Viagra! Originally distributed by traditional healers, it is now sold as a dietary supplement by firms originating in Zimbabwe, South Africa and the USA.

An investigation of preparations prescribed by traditional healers and sold in markets in Zimbabwe has shown the frequent presence of the following herbs: root bark of *Mondia whiteii* (Asclepiadaceae), roots of *Albizzia antunesiana* (Leguminosae) and stem bark of *Ozoroa insignis* (Anacardiaceae). Other constituents may include: *Pouzolzia hypolenca* (Urticaceae) stem bark or root, *Elephantorrhiza goetzei* (Leguminosae) roots and *Cassia singueana* (Leguminosae) stem bark. Phytochemical and pharmacological analysis of these preparations is underway at the Institute of Pharmacognosy and Phytochemistry, University of Lausanne to provide a scientific basis for the observations made with Vuka-vuka. It is known, however, that in certain cases there is addition of powdered blister beetles and/or yohimbine (see above), both of which are known to be efficient provokers of erections [18].

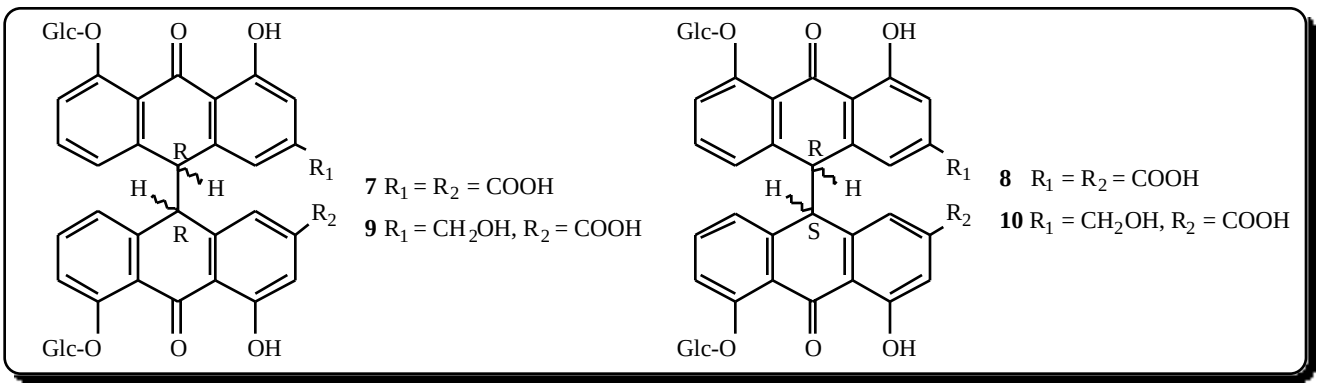
Cassia senna (Leguminosae)

The well-known laxative senna consists of either the dried leaflets or fruits of *Cassia senna* and is known in commerce as Alexandrian senna. Alexandrian senna plants are small shrubs indigenous to tropical Africa (Tinnevelly senna, *C. angustifolia*, in contrast, is indigenous to both Africa and India) and are mainly cultivated in Sudan. Senna has been used since the ninth or tenth century (introduced by Arab doctors), and derives its name from the export of the Sudanese drug through Alexandria. World production of leaves and pods of senna was more than 5,000 tons in 1986.

The active constituents of the leaves and fruits are essentially the same and consist of glycosides of 1,8-dihydroxyanthracene (2-5%). In the fruits, these are concentrated in the pericarp of the pods. The dried drug contains sennosides (**7**-**10**, with sennosides A (**7**) and B (**8**) as the major constituents – isolated by Stoll and co-workers in 1941), together with small quantities of free anthraquinones, glycosides of anthraquinones and glycosides of anthrones [19]. The dianthrone glycosides **7**-**10** are little resorbed in the small intestine but once in the colon, they are hydrolysed by the bacterial flora and the anthraquinones formed are reduced to form the active anthrones, responsible for the laxative activity.

Harpagophytum procumbens (Pedaliaceae)

The perennial herbaceous plant *H. procumbens* grows in semi-desertic savannah of Namibia, Botswana and South Africa. It is also known as "devil's claw", due to the form of the dry fruits, which have claw-like protrusions. The secondary roots, in the form of tubers, have a multitude of



traditional uses, such as treatment of indigestion, purgative, bitter tonic, febrifuge, treatment of sores and skin lesions [1]. After reports of use for various therapeutic purposes by German soldiers stationed in South-West Africa (now Namibia) at the beginning of the century, information about the plant reached Europe. First studies were performed by Zorn and co-workers at the University of Jena in 1958; extracts of the roots were shown to have anti-inflammatory properties. Extracts, teas and decoctions of the drug are effective in the treatment of degenerative rheumatism, arthritis and tendinitis. More than 15 clinical studies have confirmed the beneficial effects of these preparations and pharmacological testing has shown analgesic and anti-inflammatory actions [20].

While extracts have confirmed activity, the identity of the active principles is still uncertain. The main constituents of the tubers are iridoids (harpagoside, harpagide, procumbide etc.). It has been postulated that the very bitter derivative harpagoside (**11**) is responsible for the activity and while this glycoside is probably at least in part responsible for the therapeutic effects, its contribution to the overall picture is still very much debated [21].

Catharanthus roseus (Apocynaceae)

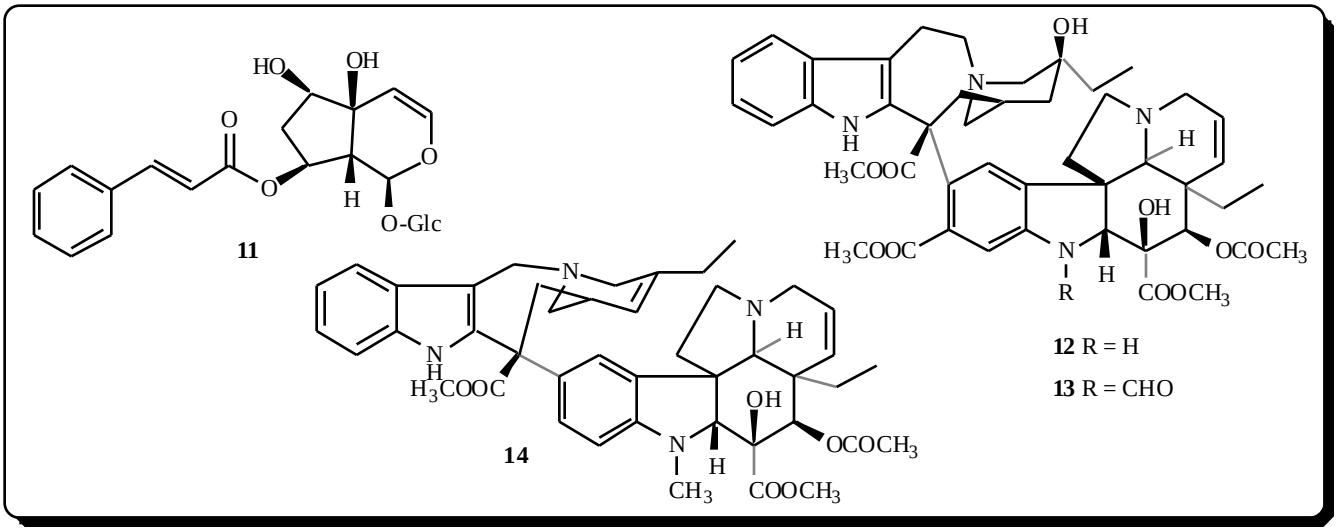
Perhaps one of the best-known and most quoted examples of an African medicinal plant is the Madagascar periwinkle, *Catharanthus roseus*. Not only is this an attractive ornamental shrub but it can now be found in most tropical regions of the world. Strangely enough, the story of the plant started in the West Indies, where it had aroused interest for its anorexigenic effect. During a screening for plants with antidiabetic activities, it was found by scientists from the University of Western Ontario in Canada that extracts of *C. roseus* from the West Indies had leukopenic activity, while a random screening programme at the Eli Lilly Laboratories in Indianapolis, USA, showed the plant to have anticancer properties. Combined efforts led to the isolation of the active bis-indole alkaloids vinblastine (**12**) and vincristine (**13**), which were developed as commercial drugs [22,23]. More than 80 monomeric and dimeric indole alkaloids have now been identified from *C. roseus*.

The major use of vinblastine is in the treatment of patients with Hodgkin's disease, non-Hodgkin's lymphomas and renal, testicular, head and neck cancer. Vincristine is widely used, in combination with other anticancer agents, in the treatment of acute lymphocytic leukaemia in children, and for certain lymphomas and sarcomas, small cell lung cancer, and cervical and breast cancer.

A semi-synthetic derivative, 5'-nor-anhydrovinblastine (vinorelbine, **14**) has been developed as an anticancer drug in France. It has lower neurotoxicity and broader anticancer activity than vinblastine or vincristine [24].

RECENT RESULTS IN THE INVESTIGATION OF AFRICAN MEDICINAL PLANTS

Having now given an impression of what is already available from African plants, an outline of the various axes of research in this field by the



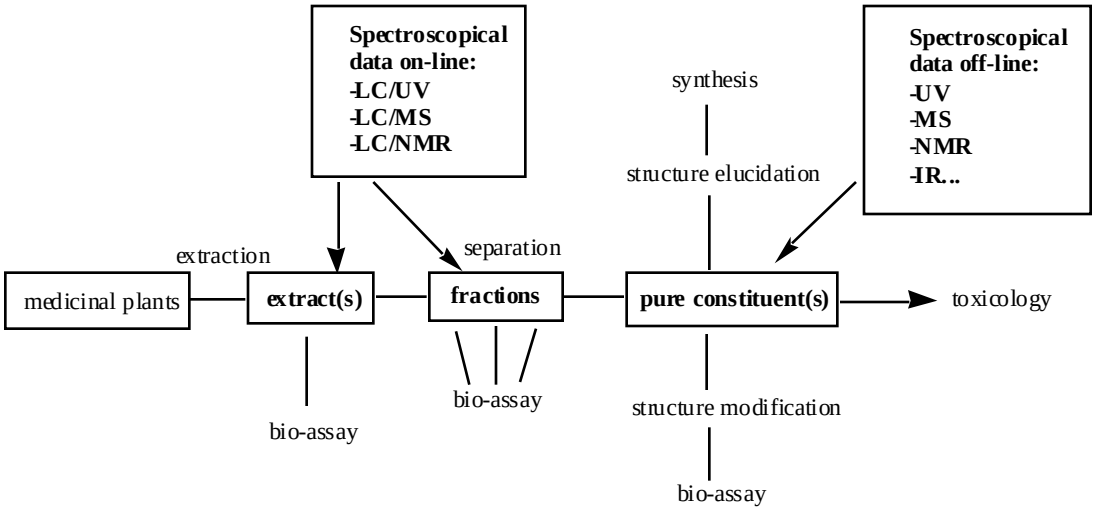


Fig. (1). Procedure for obtaining the active principles from plants and use of LC-hyphenated techniques as strategic analytical screening tools during the isolation process.

Institute of Pharmacognosy and Phytochemistry will be presented.

In order to find new drugs in plants, it is necessary to screen extracts for the presence of novel compounds and to investigate their biological activities. Once novel compounds are suspected, they are generally isolated in order to have material available for further biological and toxicological testing (Fig. **1**). The path which leads from the intact plant to its pure constituents is long. It involves work which might last anything from weeks to years and includes the following steps:

- correct identification of the plant with the aid of specialists (botanists)
- collection and drying of the vegetable material; precautions need to be taken to avoid the formation of artefacts.
- preparation of extracts using different solvents; analysis of these extracts by different chromatographic methods.
- fractionation of the extracts by different preparative chromatographic techniques (column chromatography, centrifugal partition chromatography etc.)
- purity control of the isolated products.
- structure elucidation of the constituents by combinations of diverse spectroscopic techniques (UV/VIS, IR spectrophotometry, carbon and proton nuclear magnetic resonance, mass spectrometry, X-ray diffraction) and chemical techniques (hydrolyses, formation of derivatives, degradation reactions etc.).
- synthesis or semi-synthesis of the natural product.
- modification of structure with a view to establishing structure-activity relationships.
- pharmacological and toxicological testing.

Selection of Medicinal Plants

When the researcher undertakes a phytochemical investigation of medicinal plants with the aim of isolating and identifying the active substances, the correct choice of vegetable material has to be made. Considering the number of plants which have not yet been studied from both a phytochemical and pharmacological point of view, this choice is difficult. The factors to be considered are as follows:

- chemotaxonomic criteria.
- information from traditional medicine.
- field observations.
- random collection.

Chemotaxonomy, or the science of classification of plants as a function of the structures of their chemical constituents, can introduce useful elements. Constituents are often

specific to a given botanical family, to a genus or to a species. If a natural product has interesting therapeutical properties, it may be possible to find analogous substances in species of the same genus or the same family. For example, the gentian family (Gentianaceae), which consists of approximately 1100 different species, is characterised by the presence in the roots and the leaves of bitter principles which stimulate digestion, and of xanthones with antidepressive properties. If an investigator is interested in the latter substance class, he can select gentians from different localities in order to increase his chances of discovering new molecules with therapeutical potential. Before beginning a phytochemical and pharmacological investigation, a literature search is performed on the species in question. Easily accessible data banks facilitate this task. In a short time, it is possible to discover all the previous research which has been performed on the plant selected for study.

Selection of plants based on **data from traditional medicine** can also lead to the discovery of promising new molecules. Plants from tropical and subtropical regions occur in abundance and, furthermore they have been little studied. They represent an enormous reservoir of new molecules with potential therapeutic activity which are waiting to be discovered. Certain representatives of the pharmaceutical industry are aware of this potential and have introduced screening programmes for plants from tropical regions. International conventions have been drawn up in order to prevent a drain of these natural resources from Third World countries to industries of the northern hemisphere. Within these conventions, a special place has been reserved for threatened species, to ensure their protection. Meetings between traditional healers and the research team, in the presence of local representatives (university personnel, botanists etc.) are arranged to discuss medicinal plants, their identification and use. Preference is of course given to endemic plants which have not been investigated. Is the information gathered from these healers reliable? A root decoction which is claimed to be laxative can easily be checked. Plants used for the treatment of sores or wounds are of great interest because wound healing is easy to verify. However, diagnosis by the healers of internal problems is much more difficult to rely on. Moreover, traditional healers often call upon supernatural forces during their treatments. And the placebo effect is often involved. Therefore, a discerned evaluation of this information needs to be made before choosing the plants for study.

During plant collecting expeditions, **field observations** are obviously very important. A species which grows in a hostile environment, such as tropical forests, in which there is danger of attack from insects, fungi, bacteria or viruses, will attempt to protect itself by synthesising insecticidal, fungicidal, antibacterial or virucidal constituents. If one observes, for example, that leaves of a plant in such an environment show no signs of attack, they may contain defensive compounds against insects or microorganisms. Roots often biosynthesise antifungal substances because soil is rich in pathogenic fungi. These compounds may also have an antifungal effect against human pathogenic fungi. A yellow layer under the bark of a tree can indicate the presence of antifungal polyphenols, as found, for example, in the case of the African species *Brackenridgea zanguebarica* (Ochnaceae).

Random collection is also indispensable. Given the potential of plants which have not yet been investigated and the combat which needs to be fought against disease, many different avenues need to be taken for the discovery of novel therapeutic agents. Action needs to be taken quickly, notably against diseases for which there is not yet an effective remedy: AIDS, multiple sclerosis, Parkinson's disease, Alzheimer disease and certain cancers.

Biological and Pharmacological Targets

Obviously when undertaking an investigation of a plant to identify the active principles, it is impossible to isolate all the constituents. Among the hundreds or thousands of different substances, one or only a few are responsible for the therapeutic action (or the toxic activity, if this is relevant). It is necessary, therefore, to have relatively simple biological or pharmacological tests available in order to localise the chosen activity in the plant extracts or in the numerous fractions resulting from the different purification steps which lead from the plant to the pure active constituents. These tests have to be very sensitive because the active substances may be present in the plant in very low concentrations. They are also

required to be specific for the target involved. The principal targets for biological tests can be divided into 6 groups:

- lower organisms: microorganisms (bacteria, fungi, viruses)
- invertebrates: insects, crustaceans, molluscs
- isolated subcellular systems: enzymes, receptors
- animal or human cell cultures
- isolated organs of vertebrates
- whole animals

If an antifungal or antibacterial activity needs to be investigated, the process is relatively simple. For example, a plant extract or an isolated substance is placed in contact with human pathogenic fungi. It is then straightforward to observe inhibition of spore growth or their death. There is presently a great deal of research underway to develop new antimycotics. This is because of the increased prevalence of systemic mycoses associated with AIDS infections. It is obvious that the fight against viral diseases such as AIDS or herpes is of high priority for numerous research laboratories. Plants are of great potential for their antiviral and antifungal properties. On the other hand, their antibacterial activity, although known for essential oils and in plants such as bearberry, is relatively weak when compared with antibiotics of microbiological origin.

Certain plants have insect repellent or insecticidal properties, while others are active against insect larvae or molluscs (molluscicides). Screening tests for these activities on invertebrates are simple to perform. Insecticidal or larvicidal plants can play an important rôle in the prevention of tropical parasitic diseases, like malaria or yellow fever, transmitted by mosquitoes. As for molluscicidal plants, they can stop the propagation of schistosomiasis (bilharzia), a parasitic disease with a mollusc (freshwater snail) as intermediate host, which affects over 250 million people in Third World countries.

The spectacular progress made during the last few years in cellular biology and molecular pharmacology is of particular importance for biological and pharmacological tests based on mechanisms of action. When the causes of a disease are known, it is possible to act directly on the receptors or enzymes implicated in the etiology of the complication. For example, substances which inhibit cyclooxygenase or 5-lipoxygenase, enzymes involved in the process of inflammation, are of great utility in the search for new anti-inflammatory agents. In the war against cancer, inhibitors of the enzymes topoisomerase I and II and protein kinase C, as well as substances which act on the polymerisation of tubulin, are targets for these tests. In the case of benign hyperplasia of the prostate (BPH), very frequent in elderly males, inhibitors of enzymes which modify testosterone levels (5α-reductase, aromatase) are of value. For the treatment of depression, efforts are made to find selective inhibitors of monoamine oxidase, which transforms certain neurotransmitters in the brain. The tests mentioned above are *in vitro* tests, with enzymes of human or animal origin. The substance under test is placed directly in contact with the target, which is not necessarily the case when taking a medicine orally. The active principle has to be transported in the organism to the target, where it is supposed to exert its effects. Enzymatic tests are generally very specific and very sensitive. They are of special value during the screening of large numbers of samples. The experiments are often relatively easy and require only small amounts of material.

Other *in vitro* tests are made on cell cultures. These are of great importance in cancer research. One of the basic tests is to find cytotoxic molecules or growth inhibitors for tumour cells of human origin. While numerous substances are active *in vitro* on isolated cancer cells, these unfortunately often do not produce useful chemotherapeutic agents. They are toxic to normal cells or do not reach the target tumour.

Sometimes, tests on cell cultures are replaced by investigations on isolated organs of animals. Pharmacological models such as the perfused frog heart have been used for the study of cardiac glycosides. Other tests are carried out on the perfused liver, guinea pig heart, isolated chicken veins etc. The information provided by these tests is often useful but hardly gives any indication of the mode of action of the sample and cannot be extrapolated to the human situation.

Finally, testing on live animals still takes up a large place in the development of new therapeutic agents, even though enormous efforts are being made to find substitutes for this procedure.

Chemical Screening

When searching for active plant metabolites, biological screening followed by activity-guided fractionation is the standard procedure. Bioassays also serve as a guide during the isolation process. However, the number of available targets is limited. Moreover, bioassays are not always predictive for clinical efficacy. And the bioassay-guided fractionation strategy frequently leads to the isolation of known metabolites. Chemical screening of crude plant extracts therefore constitutes an efficient complementary approach, allowing localisation and targeted isolation of new types of constituents with potential activities. This procedure also enables recognition of known metabolites at the earliest stage of separation, thus avoiding costly and time-consuming isolation of common constituents. The potential of the chemical screening strategy has been considerably increased by the recent development of hyphenated techniques, which are able to provide efficient separation of metabolites and, at the same time, valuable structural information on-line.

High performance liquid chromatography (HPLC) is used routinely in phytochemistry to "pilot" the preparative isolation of natural products (optimisation of the experimental conditions, checking of the different fractions throughout the separation) and to control the final purity of the isolated compounds. For chemotaxonomic purposes, the botanical relationships between different species can be shown by chromatographic comparison of their chemical composition. Comparison of chromatograms, used as fingerprints, between authentic samples and unknowns permits identification of drugs and/or search for adulteration. HPLC is thus the best suited technique for an efficient separation of crude plant extracts and can be coupled with different spectroscopic detection methods [25]. By this means, an enormous quantity of structural information can be obtained about the constituents of a vegetable extract with only several micrograms of sample.

HPLC coupled with UV photodiode array detection (LC/UV) has been used for more than a decade by phytochemists in the screening of plant extracts and is now widely employed in many laboratories. The UV spectra of natural products give useful information on the type of constituents and also, as is the case for polyphenols, information on the oxidation pattern. New instruments allow the recording of UV spectra of reference compounds in databases and computer matching can be realised automatically when screening for known constituents.

HPLC coupled to mass spectrometry (LC/MS) has been introduced recently and is still not very common in the phytochemical community. At present, MS is one of the most sensitive methods of molecular analysis. Moreover, it has the potential to yield information on the molecular weight as well as on the structure of the analytes. Due to its high power of mass separation, very good selectivities can be obtained. The coupling between LC and MS has not been straightforward since the normal operating conditions of a mass spectrometer (high vacuum, high temperatures, gas-phase operation, and low flow rates) are diametrically opposed to those used in high performance liquid chromatography (HPLC), namely liquid-phase operation, high pressures, high flow rates, and relatively low temperatures. Because of the basic incompatibilities between HPLC and mass spectrometry (MS), on-line coupling of these instrumental techniques has been difficult to achieve and to cope with these different problems, different LC/MS interfaces have been conceived. Each of these interfaces has its own characteristics and range of applications and several of them are suitable for the analysis of plant secondary metabolites. For the HPLC screening of crude plant extracts, three interfaces, thermospray (TSP), continuous flow FAB (CF-FAB) and electrospray (ES), have been investigated. They cover the ionisation of relatively small non-polar products (aglycones, 200 Da) to highly polar molecules (glycosides, 2000 Da).

HPLC coupled with nuclear magnetic resonance (LC/NMR), despite being known for over twenty years, is not yet a widely accepted technique, mainly because of its lack of sensitivity. However, the recent progress in pulse field gradients and solvent suppression, the improvement in probe

technology and the construction of high field magnets have given a new impulse to this technique. LC/NMR has an important potential for on-line structure identification of natural products in plant extracts i.e. without their isolation. Indeed, nuclear magnetic resonance (NMR) spectroscopy is by far the most powerful spectroscopic technique for obtaining detailed structural information about organic compounds in solution.

Once a target compound has been decided upon, the pure active substance has to be separated from the hundreds of other components of the plant extract matrix. Some of the bioactive compounds isolated from African plants by the Insitute of Pharmacognosy and Phytochemistry will be described below.

PLANTS USED AGAINST PARASITIC DISEASES

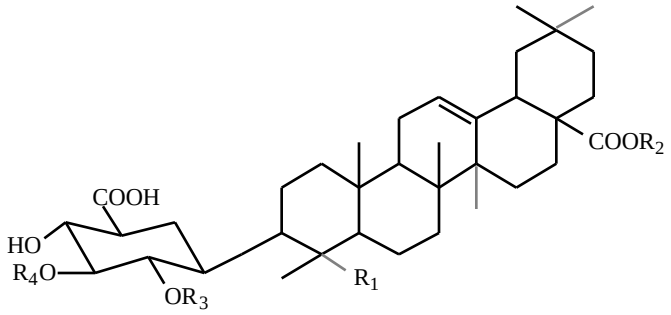
Plants with Molluscicidal Activities

Schistosomiasis, commonly known as bilharzia, is caused by thread worms of the genus *Schistosoma* and is endemic throughout Africa. It affects more than 250 million people in over 76 countries worldwide. The reproductive cycle of schistosomes involves a stage implicating aquatic snails of the genera *Biomphalaria* and *Bulinus*, in which the parasite multiplies into cercariae. These cercariae, after leaving the snails, can penetrate the skin of humans who come into contact with contaminated water. Once through the skin, they change gradually into the mature trematodes known as schistosomes. The schistosomes mate and lay eggs which are carried away with faeces or urine. As eggs reach water, they produce miracidia, which locate snails of the appropriate species and the cycle begins again. One way to attack the problem of schistosomiasis is to destroy the carrier snails ("molluscicide") and thus remove a link in the life cycle. This may be achieved with the aid of synthetic products such as Bayluscide (2,5'-di-chloro-4'-nitrosalicylanilide) or, alternatively, with molluscicides from plant sources. The use of molluscicidal plants growing abundantly in areas where schistosomiasis is endemic is a simple, inexpensive and appropriate technology for local control of the snail vector and may become in the near future a useful complement for the control of this disease [26]. Among the plants which are of greatest interest are those which contain large quantities of saponins. Saponins possess high toxicity towards cold blooded organisms including snails; they are often present in large amounts in plants; and owing to sufficient water solubility, their extraction and application is simple and does not require any sophisticated apparatus or specially trained people.

'Endod' (*Phytolacca dodecandra*, Phytolaccaceae) was one of the first plants to have been extensively studied [27]. The berries contain triterpenoid saponins with high molluscicidal activities, and promising field trials have been undertaken in Ethiopia. When the berries were extracted with solvents of increasing polarity (petroleum ether, chloroform and methanol), the saponins were found in the methanol extract. However, the methanol extract was not molluscicidal and, on further investigation, it was discovered that almost exclusively bidesmosidic saponins (two glycoside chains) were present. Apparently, extraction with methanol inactivates hydrolytic enzymes which cleave the ester-linked sugar chains during treatment of the powdered berries with water [28].

Endod has, however, a limited geographical distribution and has to be cultivated if required for use in other countries. *Bobgunnia madagascariensis* (formerly known as *Swartzia madagascariensis*, Leguminosae), on the other hand, is a very common tree in many regions of Africa. It bears large fruits which were already shown to be toxic to snails in 1939 [29]. Phytochemical investigation has enabled the identification of the saponins responsible for the molluscicidal activity of an aqueous extract of the dried fruits [30]. The fruits, collected in Tanzania, were extracted with distilled water. The aqueous extract was partitioned between n-butanol and water. Separation of the butanol extract by different chromatographic techniques afforded the saponins **15**-**19** (Table 1), with final purification achieved by MPLC and LPLC on reversed-phase supports. The isolated saponins were shown to be glucuronides of oleanolic acid and of gypsogenin by chemical and spectral means (FAB-MS, ^{13}C-NMR, GC-MS of methylated alditol acetates). It is interesting to note that all the saponins carry a rhamnopyranosyl unit at position C-3 of the glucuronic acid moiety. Saponin **15** has also been

Table 1. Molluscicidal Activities of Saponins from *Bobgunnnia madagascariensis* (Leguminosae)



Saponin	R_1	R_2	R_3	R_4	Molluscicidal activity[a]
15	CH_3	H	H	Rha	3
16	CHO	H	H	Rha	25
17	CH_3	H	Glc	Rha	25
18	CHO	H	Glc	Rha	>50
19	CH_3	Glc	Glc	Rha	no activity

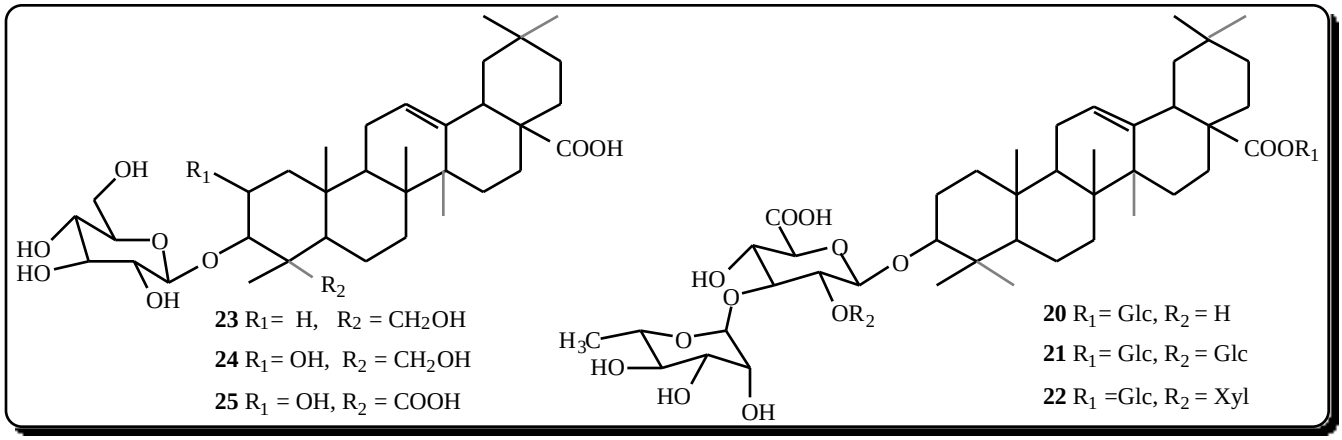
[a] Molluscicidal activity against *Biomphalaria glabrata* [mg/1]

isolated from the root bark of *Diospyros zombensis* (Ebenaceae), a tree found in Malawi [31]. The results of biological testing showed that saponin **15** presented the highest molluscicidal activity (3 mg/1) against *Biomphalaria glabrata* snails. Saponins with disubstituted glucuronic acid, as well as those with gypsogenin as aglycone (**16** and **18**) had a lower activity (⩾25 mg/1). The bidesmosidic saponin **19**, carrying an additional sugar moiety at position C-28 of oleanolic acid, had no activity.

Bobgunnia madagascariensis appears to be one of the most promising plants for the control of schistosomiasis and as each tree can carry up to 30-40 kg of pods (which are not eaten by the local population), there is no shortage of vegetable material. Water extracts exerted significant molluscicidal activity against *Biomphalaria glabrata* and *Bulinus globosus* snails up to dilutions of 100 mg of ground pods per liter. Thus it was decided to test the efficiency of water extracts of *B. madagascariensis* fruits in natural habitats harbouring *Bulinus globosus* populations. The field trials were carried out at Ifakara, Tanzania, in collaboration with the Swiss Tropical Institute (Basle) and its Field Laboratory in South-Eastern Tanzania [32]. Two field trials with mature pods were undertaken in October 1984, at the end of the dry season, when the water level in the ponds was very low. Extraction by water and application of extracts was straightforward. The molluscicidal activity could be monitored by a simple, semi-quantative haemolysis test, which paralleled the concentration of saponins as determined by TLC. The short half-life (12-24 h) of the saponins under field conditions reduced the risk of toxicity to humans; rapid biodegradability is important since this implies that the plant molluscicide is appropriate for community-based actions. However, the results indicated that the extracts were probably not active against *Bulinus globosus* egg masses and at least two applications of pod extracts were needed in order to achieve low snail densities.

Sesbania sesban (Leguminosae) is a fast-growing tree, distributed throughout large areas of Africa. Following reports of the molluscicidal activity of the leaves, a phytochemical investigation of the aerial parts revealed the presence of four saponins (**15**, **20**-**22**) [33]. These were glucuronides of oleanolic acid. The monodesmosidic saponin **15**, previously isolated from *Bobgunnia madagascariensis*, was responsible for the molluscicidal activity, while the bidesmosidic saponins **20**-**22** were inactive.

Roots of *Dolichos kilimandscharicus* (Leguminosae) from Kenya, after extraction with dichloromethane and methanol gave a methanol extract which was both molluscicidal and fungicidal. TLC analysis, indicated the presence of saponins and these were subsequently isolated by



a combination of flash chromatography, droplet countercurrent chromatography (DCCC), low pressure LC and Sephadex LH-20 gel filtration. They were found to be monosaccharides of hederagenin (**23**), bayogenin (**24**) and medicagenic acid (**25**) [34]. All three were molluscicidal against *Biomphalaria glabrata* snails (15 mg/l of **23**, 7.5 mg/l of **24** and 25 mg/l of **25** required to give 100% snail kill after 24 h).

Tetrapleura tetraptera (Leguminosae) is a wellknown Nigerian medicinal plant, referred to locally as "aridan". Extracts of the fruits of this plant have molluscicidal properties comparable to *Phytolacca dodecandra* [35]. A field control project in Nigeria produced encouraging results, with a large decrease in the number of *Bulinus globosus* snails at sites treated with concentrations of 10 mg/l of methanol extract [36]. The water extract gave 100% mortality at a concentration of 50-100 mg/l over a period of 24 h in the field. There was also an effect on cercarial transmission. In order to isolate the saponins responsible for the activity, dried fruit pulp from Nigeria was extracted successively with dichloromethane, methanol and water. The methanol extract was partitioned between n-butanol and water; the butanol extract was fractionated by column chromatography on silica gel, MPLC on RP-8, DCCC and Sephadex LH-20 gel filtration to give four saponins **26**-**29** [37]. These were novel *N*-acetylglycosides, with either oleanolic acid (**26**, **28**, **29**) or echinocystic acid (**27**) as aglycone. At position C-3 they all had a 2-acetamido-2-deoxy-β-D-glucopyranose moiety, with **28** and **29** being further substituted by galactose and glucose moieties, respectively. Saponins **26** [38], **27**, **28** and **29** were all molluscicidal; their toxicity to snails increased with increasing glycosylation (20 mg/l of **26** and 2.5 mg/l of **28** required for 100% toxicity to snails).

Cussonia spicata (Araliaceae) is another African medicinal plant with interesting molluscicidal properties. An active water extract of the stem bark from Malawi was fractionated by MPLC on silica gel, after preliminary partitioning with n-butanol. Further MPLC on reversed-phase supports afforded two pure saponins. They were both arabinoglucuronopyranosides of oleanolic acid and while the disaccharide **30** was active against *Biomphalaria glabrata* snails at 12.5 mg/l,

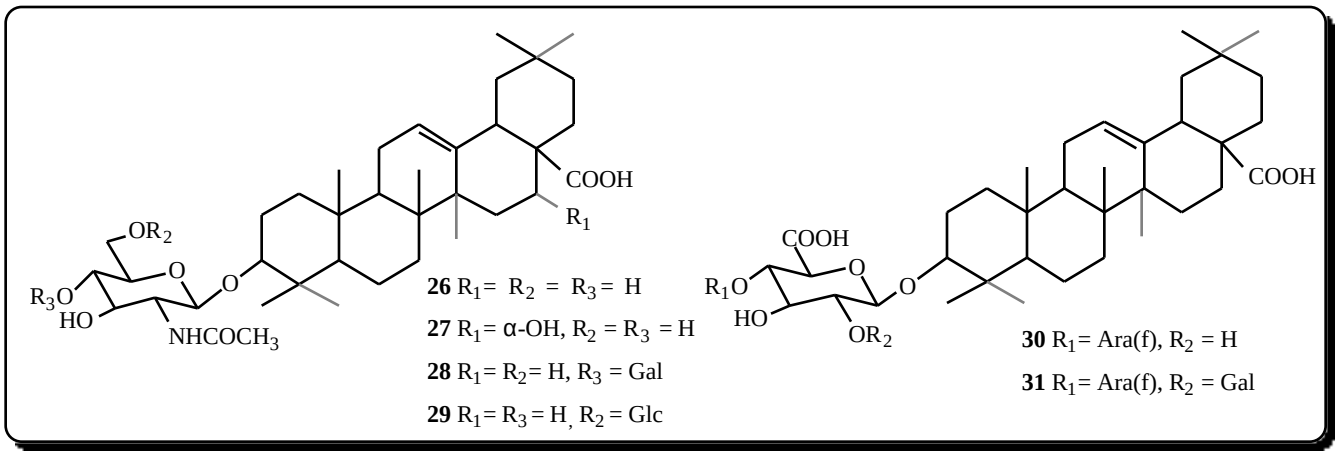
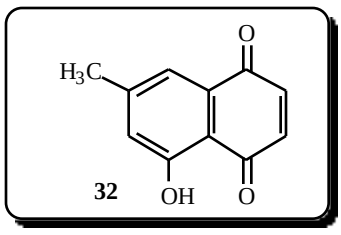


Table 2. African Plants with Molluscicidal Activities

Class of compounds	Plants	Country	Reference
Naphthoquinones	*Diospyros usambarensis* (Ebenaceae)	Malawi	[40]
Naphthylisoquinoline alkaloids	*Triphyophyllum peltatum* (Dioncophyllaceae)	Ivory Coast	[101,102]
Naphthylisoquinoline alkaloids	*Ancistrocladus abbreviatus* (Ancistrocladaceae)	Ivory Coast	[101]
Oleanane saponins	*Cussonia spicata* (Araliaceae)	Malawi	[39]
Oleanane saponins	*Polyscias dichroostachya* (Araliaceae)	Mauritius	[103]
Oleanane saponins	*Diospyros zombensis* (Ebenaceae)	Malawi	[31]
Ursane saponins	*Aphloia theiformis* (Flacourtiaceae)	Mauritius	[104]
Oleanane saponins	*Bobgunnia madagascariensis* (Leguminosae)	Tanzania	[30]
Oleanane saponins	*Dolichos kilimandscharicus* (Leguminosae)	Kenya	[34]
Oleanane saponins	*Sesbania sesban* (Leguminosae)	Tanzania	[33]
Oleanane saponins	*Tetrapleura tetraptera* (Leguminosae)	Nigeria	[37]
Epoxyoleanane saponins	*Rapanea melanophloeos* (Myrsinaceae)	Zimbabwe	[59]
Oleanane saponins	*Phytolacca dodecandra* (Phytolaccaceae)	Ethiopia	[28]
Oleanane saponins	*Talinum tenuissimum* (Portulacaceae)	Malawi	[105]
Oleanane saponins	*Clerodendrum wildii* (Verbenaceae)	Malawi	[60]

the trisaccharide **31** was only active at 100 mg/l [39]. A preliminary screening for spermicidal activity against human spermatozoids showed **30** to be active at 1 mg/l and **31** at 3 mg/l, within 3 minutes.

Other African medicinal plants have been investigated phytochemically for their molluscicidal constituents (Table 2): some contain saponins, some contain naphthylisoquinoline alkaloids and some contain naphthoquinones. In the latter case, the simple naphthoquinone 7-methyljuglone (**32**) from *Diospyros usambarensis* (Ebenaceae) had strong molluscicidal activity (5 mg/l required to kill 100% of snails after 24 h) [40]. The search is continuing because it is necessary to find even more active and selective plant extracts and constituents.



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Plants with Larvicidal Activities

Mosquitoes, in particular species of *Anopheles*, *Aedes* and *Culex*, are important vectors of tropical diseases. *Aedes* species, and most notably *A. aegypti*, transmit diseases caused by arboviruses (*ar*thropod *bo*rne *virus*) such as yellow fever and dengue fever. While yellow fever has been reasonably brought under control with the development of a vaccine, there is no vaccine available yet against dengue fever. The current strategy postulated by the W.H.O. for the control of these tropical diseases is to destroy their vectors. The majority of mosquitoes are active at night and/or live near and in settlements, where they cannot be reached by sprayed insecticides. The ideal control method is thus the systematic treatment of their breeding places with larvicidal agents. Plants can provide lead compounds for the development of new larvicidal agents. At the same time, plant-derived preparations can represent an alternative to the use of synthetic pesticides, cheap and readily available to the populations concerned.

A simple bench-top assay has been recently included in our screening assays and crude plant extracts are now systematically tested for larvicidal properties [41]. The testing procedure involves second instar larvae of *A. aegypti*. The eggs of *A. aegypti* are easy to handle and can be stored in a controlled atmosphere (26-28°C, 70-80% rel. humidity) for up to six months. Larvae hatch readily when put into tap water and incubated for 24 h. The assay consists of exposing

approximately 20 larvae to various dilutions of the extracts, previously solubilized in DMSO. Mortality is evaluated with the naked eye after 30 min. and 24 h. A sample is considered active when all larvae have been killed after 24 hours.

In the course of this screening, a few useful leads have been picked up. The dichloromethane leaf extract of *Diplolophium buchanani*, a shrub of the family Apiaceae from the Zomba Plateau in Malawi, showed potent larvicidal and fungicidal properties. Activity guided fractionation carried out mostly by centrifugal partition chromatography (CPC) resulted in the isolation of the phenylpropanoids myristicin (**33**), elemicin, trans-isoelemicin, together with the furanocoumarin oxypeucedanin (**34**). Myristicin and oxypeucedanin (LC_{100} 25 mg/l) were larvicidal at concentrations similar to that of the reference compound β-asarone (LC_{100} 16 mg/l) [42].

Another interesting species is *Melantheria albinervia* (Asteraceae) collected in Zimbabwe, the lipophilic root extract of which exhibited significant larvicidal activity. Bioassay-guided fractionation carried out by a combination of CC on silicagel and low pressure liquid chromatography afforded the larvicidal diterpenes entkaur-16-en-19-oic acid (**35**) and 9(11),16-kauradien-19-oic acid (**36**), which had LC_{100} of 62.5 and 250 mg/l, respectively [43].

Plants with Antimalarial Activities

Different species of *Anopheles* mosquitoes are responsible for the transmission of malaria which still remains endemic in more than 100 countries and affects 250 million people in the world. In view of the widespread development of resistant strains of *Plasmodium*, enormous efforts are being made to find alternative antimalarial drugs, other than the classical drugs such as quinine and synthetic antimalarials.

With the aim of finding plant extracts with antimalarial properties, a small amount of screening work has been performed in an *in vitro* test which determines the inhibition of incorporation of 3H-hypoxanthine by malaria parasites, using a multidrug resistant KI strain of *Plasmodium falciparum*. In this bioassay, the petroleum ether extract of *Psorospermum febrifugum* (Guttiferae) root bark displayed appreciable activity (Table 3) and was around four times more active than an ethanolic extract of

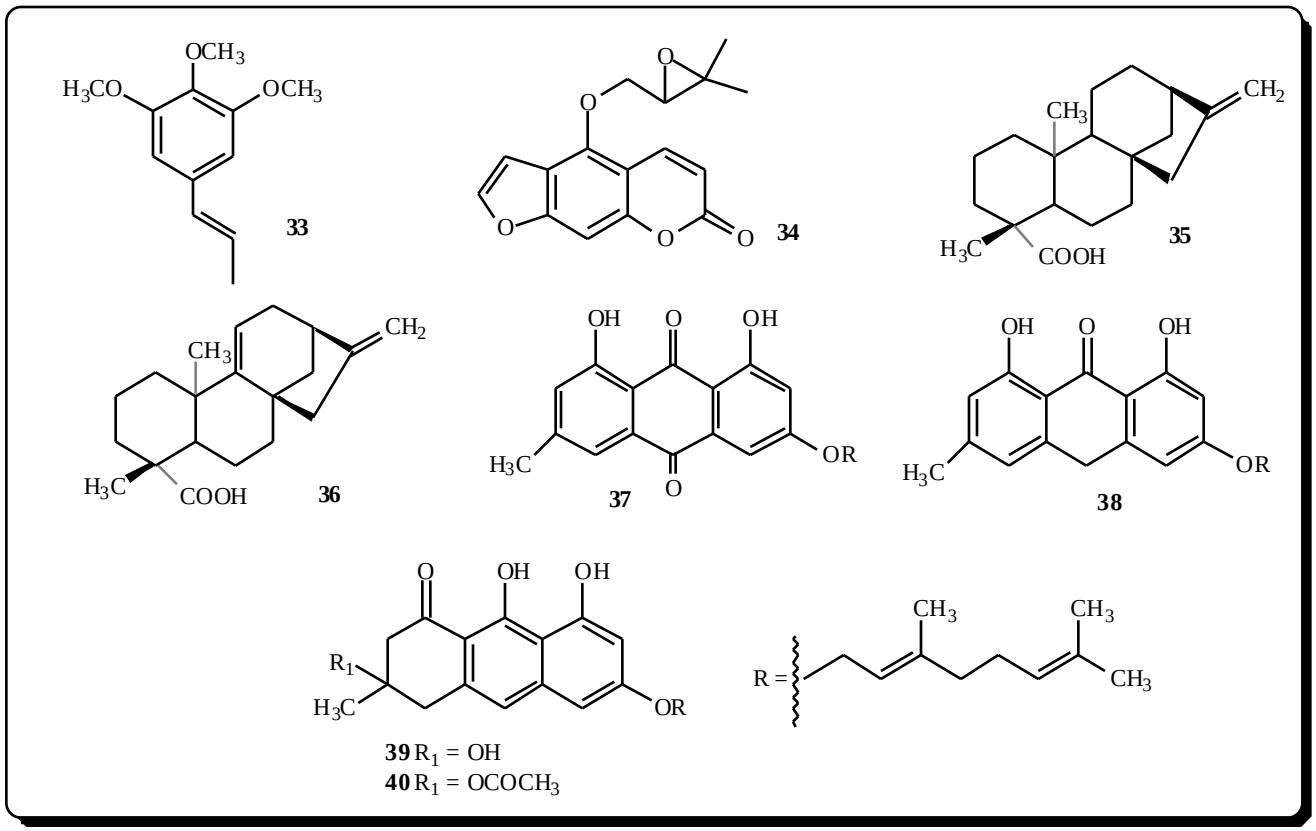
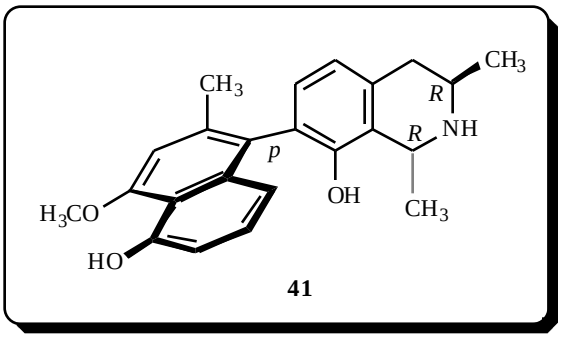


Table 3. *In vitro* Antimalarial Activities of Extracts of *Psorospermum febrifugum* and *Artemisia annua* and their Constituents

Sample	Antimalarial activity (*Plasmodium falciparum*) IC_{50} (μg/ml)
P. febrifugum petroleum ether extract	0.82
3-Geranyloxy-6-methyl-1,8-dihydroxy-anthraquinone (**37**)	50
3-Geranyloxy-6-methyl-1,8-dihydroxy-anthrone (**38**)	5.6
Vismione D (**39**)	0.095
Acetylvismione D (**40**)	0.383
Artemisia annua ethanol extract	3.9
Artemisinin	0.0028
Quinine.2HCl	0.038

Artemisia annua (Asteraceae), one of the plants presently exciting much hope for the future treatment of malaria [7]. *P. febrifugum* is a shrub with a wide distribution over southern and central Africa. It finds use in African traditional medicine for the treatment of malaria, leprosy, wounds, skin diseases and fever [1]. The tetrahydroanthracene vismione D (**39**) was the most active pure compound of *P. febrifugum*, with an activity comparable to that of quinine. Artemisinin, from *A. annua* was, however, considerably more inhibitory. Unfortunately, *in vivo* testing of the lipophilic extract of *P. febrifugum* and the pure compounds has shown their unsuitability for future development because of their toxicity to mice.

In addition to the molluscicidal activity shown by naphthylisoquinoline alkaloids (see above), some of these also have interesting antimalarial activity. For example, 5'-*O*-demethyldioncophylline A (**41**), from stem bark of the Ivory Coast liana *Triphyophyllum peltatum* (Dioncophyllaceae) shows in vitro antimalarial activity (IC_{50} = 0.34 μg/ml) against erythrocytic forms of *P. falciparum* [44].



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PLANTS WITH ANTIFUNGAL ACTIVITIES

The increasing incidence of mycoses associated with AIDS and also those arising after treatment by immunosuppressive drugs has given fresh impetus to the search for novel antifungal agents. There are few really effective antifungal preparations currently indicated for the treatment of systemic mycoses and their efficacy is rather limited. Another area which is badly in need of new lead compounds is agrochemicals. Consequently, the investigation of higher plants for antifungal properties is of great importance at the moment.

For the isolation of active compounds by activity-guided fractionation, bioautography is the method of choice. This technique combines TLC with a bioassay *in situ* and allows localisation of active constituents in a plant extract. Spore-producing fungi, such as *Aspergillus*, *Penicillium* and *Cladosporium* spp. can all be employed as target organisms in direct bioautographic procedures. After migration and drying of the TLC solvent, the plates are sprayed with a mixture of the microorganism and the nutrition medium. They are then incubated in a humid atmosphere [45]. Zones of inhibition appear where fungal growth is prevented by the active components of the plant extract. Bioautography with *Cladosporium cucumerinum* has been used successfully in our laboratory for several years now, and a large number of fungicidal natural products of different origins and chemical structures have been isolated [46]. In this bioautography assay, 0.1 μg of the reference compound propiconazole and 1 μg of amphotericin B are the minimum amounts required

to inhibit growth of the fungus. A minimum inhibitory concentration (MIC) of 10 μg/ml is obtained for amphotericin B in solution assays with *C. cucumerinum.*

Since direct bioautography is not possible with yeasts such as *Candida albicans*, a simple and rapid agar overlay assay has been developed [47]. This contact bioautography technique relies on the transfer of active compounds from the stationary phase into the agar layer (which contains the microorganism) by a diffusion process. After incubation, the plate is sprayed with methylthiazolyltetrazolium chloride (MTT) which is converted into a MTT formazan dye by the fungus. Inhibition zones are observed as clear spots against a purple background. The reference substance amphotericin B inhibits fungal growth down to 1 μg in the TLC assay and has a MIC

Table 4. African Plants with Antifungal Activities

Class of compounds	Plants	Country	Ref.
Monoterpenes	*Valeriana capense* (Valerianaceae)	Malawi	[49]
	Ocotea usambarensis (Lauraceae)	Rwanda	[65]
Diterpenes	*Parinari capensis* (Chrysobalanaceae)	Zimbabwe	[50]
	Clerodendrum uncinatum (Verbenaceae)	Malawi	[51]
	Bobgunnia madagascariensis (Leguminosae)	Zimbabwe	[52]
Triterpene aglycones	*Clerodendrum wildii* (Verbenaceae)	Malawi	[60]
Triterpene glycosides	*Rapanea melanophloeos* (Myrsinaceae)	Zimbabwe	[59]
	Dolichos kilimandscharicus (Leguminosae)	Kenya	[34]
	Clerodendrum wildii (Verbenaceae)	Malawi	[60]
Naphthoquinones	*Swertia calycina* (Gentianaceae)	Rwanda	[54]
	Newbouldia laevis (Bignoniaceae)	Guinea-Conakry	[55]
	Diospyros usambarensis (Ebenaceae)	Malawi	[40]
Naphthoxirenes	*Sesamum angolense* (Pedaliaceae)	Malawi	[53]
Pyrones	*Ravensara anisata* (Lauraceae)	Madagascar	[58]
Anthraquinones	*Morinda lucida* (Rubiaceae)	Congo	[57]
Chromenes	*Hypericum revolutum* (Guttiferae)	Malawi	[62]
Chromonocoumarins	*Polygala fructicosa* (Polygalaceae)	South Africa	[63,64]
Furanocoumarins	*Diplolophium buchanani* (Umbelliferae)	Malawi	[42]
Lignans	*Ocotea usambarensis* (Lauraceae)	Rwanda	[65]
Acetylene derivatives	*Inulanthera nuda* (Asteraceae)	Zimbabwe	[61]
Chalcones	*Myrica serrata* (Myricaceae)	Zimbabwe	[66]
Flavones	*Helichrysum nitens* (Asteraceae)	Malawi	[68]
Flavonols	*Psiadia trinerva* (Compositae)	Mauritius	[67]
Flavans	*Mariscus psilostachys* (Cyperaceae)	Zimbabwe	[70]
Flavanones	*Chenopodium procerum* (Chenopodiaceae)	Rwanda	[71]
Isoflavones	*Chenopodium procerum* (Chenopodiaceae)	Rwanda	[71]
Pterocarpans	*Dolichos marginata* (Leguminosae)	Malawi	[72]
Xanthones	*Hypericum roeperanum* (Guttiferae)	Zimbabwe	[77]
	Polygala nyikensis (Polygalaceae)	Malawi	[78]
Pyranoxanthones	*Garcinia gerrardii* (Guttiferae)	South Africa	[75]
Prenylated xanthones	*Garcinia livingstonei* (Guttiferae)	South Africa	[76]
Benzofuran derivatives	*Brackenridgea zanguebarica* (Ochnaceae)	Malawi	[73]

value of 1μg/ml when the test is performed in solution.

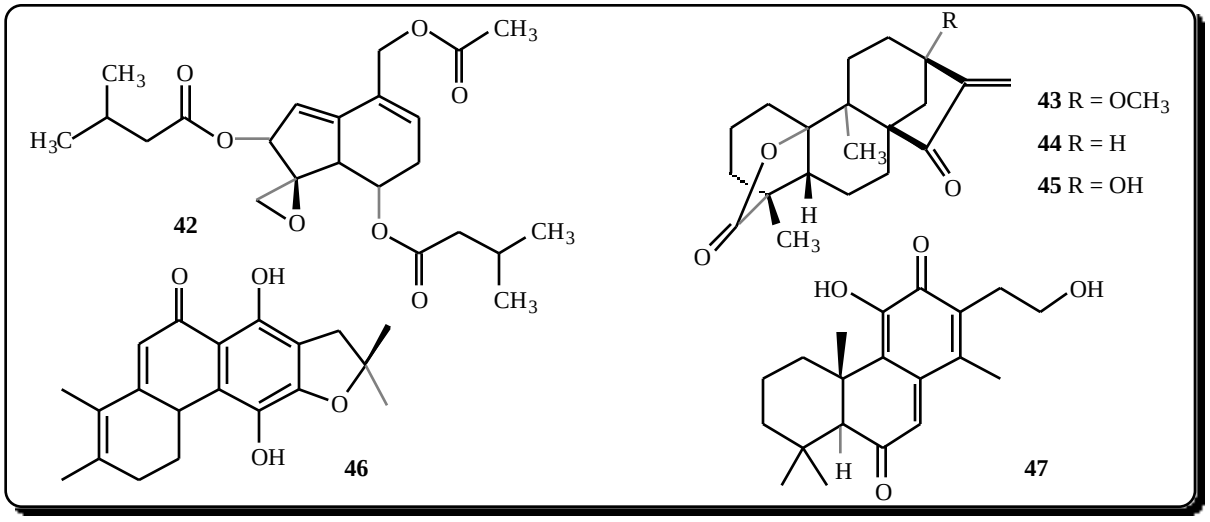
African plants are good candidates in the search for fungicidal compounds because they have to resist difficult conditions and attack by all manners of parasites. Often antifungal constituents are found in root bark because this organ is in direct contact with the fungi found in the soil.

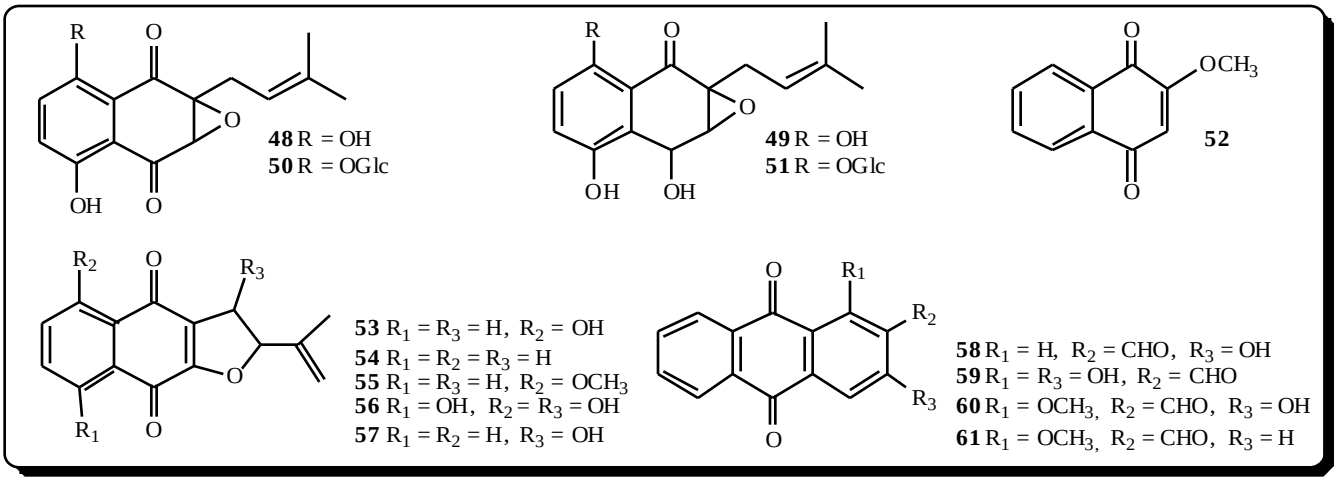
The results of screening conducted on 28 African plants representing 15 families showed, for example, that 13 extracts out of a 100 tested displayed interesting fungicidal activities against *C. cucumerinum* [41]. A more recent survey performed on 20 Malian plants used in traditional medicine demonstrated that 15 extracts out of 78 were active against *C. cucumerinum* or *C. albicans* [48].

In our laboratory more than thirty African plants presenting antifungal activities have been studied following a bioactivity guided isolation procedure and many natural products with interesting antifungal properties have been isolated (Table 4). The antifungal agents include terpenoid, quinonoid, phenylpropanoid and polyphenolic types of constituents.

Concerning the **monoterpenes**, for example, a lipophilic crude extract of *Valeriana capense* (Valerianaceae) whole plant collected in Malawi exhibited activity against *C. cucumerinum*. Fractionation by a combination of CC on silicagel, liquid-solid extraction and semipreparative HPLC on RP-18 provided the active compound valtrate (**42**), together with several inactive valepotriates. Valepotriates are well known from *Valeriana* species, such as *V. officinalis*, but the antifungal properties of valtrate had not been previously noticed. Valtrate inhibited the growth of *C. cucumerinum* at 1 μg in the bioautography assays. High structural specificity was observed, since the isomer isovaltrate, as well as dihydrovaltrate were completely inactive. In a dilution assay using solid media, the minimal inhibitory concentrations (MIC) of **42** were 10 μg/ml against *C. albicans* and *Aspergillus fumigatus*, and 20 μg/ml against *Trichophyton mentagrophytes*. As valtrate exhibited noteworthy *in vitro* activity against *C. cucumerinum*, it was tested *in vivo* against other plant pathogenic fungi. Valtrate was active against *Cercospora arachiodicola*, a pathogenic fungus for the peanut plant, *Erysiphe graminis*, a fungus which infests barley plants and *Venturia inequalis*. Since the activity of **42** against *E. graminis* was comparable to that of the commercial product Calixin, valtrate could be of interest as an agricultural fungicide [49].

In the case of **diterpenes**, the dichloromethane extract of *Parinari capensis*, a Chrysobalanaceae from Zimbabwe, displayed interesting activity against *C. cucumerinum*. An activity-guided fractionation afforded three diterpene lactones. Two of them **43** and **44** displayed a MIC value of 20 μg/ml while the third compound **45** was the least active with a limit of activity at 100 μg/ml. **43** was found to be a new natural product and its absolute configuration was proved after X-ray analysis of its brominated derivative [50]. Other antifungal diterpenes were of the **quinonoid** type. The hydroquinone diterpene uncinatone **46**, isolated from a petroleum ether extract of *Clerodendrum uncinatum* (Verbenaceae) root bark,





exhibited strong antifungal activity. The roots of this plant are supposed to have contraceptive activity and a decoction is also used as a gargle for sore throats. The study of *C. uncinatum* was provoked by reports (unfounded) that the roots are reputed by traditional healers to cure schistosomiasis. Uncinatone (**46**) was isolated as red crystals; it was found to be a new natural product and was active against C. *cucumerinum* at 0.5 µg on TLC [51]. A very potent antifungal agent of the 'quinone methide' diterpene (**47**) type was isolated from the root bark of *Bobgunnia madagascariensis*. This compound showed strong antifungal properties towards human pathogenic fungi, in particular the yeast *C. albicans* (MIC: 0.19 µg/ml) as well as against other *Candida* spp. The activity of this new natural product was found to be more potent than that of reference compounds amphotericin B and fluconazole (MIC: 0.5 µg/ml) and it represents a promising lead for the development of novel antimycotic drugs [52]. The 'quinone methide' diterpene **47** is currently in preclinical trials and the path to its discovery is related in a following section.

Other quinonoids such as the two novel **naphthoxirene** derivatives **48** and **49** and their glucosides **50** and **51** isolated from *Sesamum angolense* (Pedaliaceae), found in Malawi, exhibited antifungal activity as well as growth-inhibitory activity against a human colon carcinoma cell line. The structure of **48** was established by X-ray diffraction analysis, while the identities of the other naphthoxirenes were deduced by spectroscopic and chemical methods. In this series of compounds, the fungicidal activity seemed to require the presence of a hydroxyl group in the *peri* position to a carbonyl function. Naphthoxirene **48**, in which two such functional groups are present, exhibited the strongest activity; compounds **49** and **50**, which contain only one hydroxyl group *peri* to a carbonyl**,** were about 10 times less active. Glucoside **51**, which does not possess this arrangement, was inactive [53]. A **naphthoquinone**, 2-methoxy-1,4-naphthoquinone (**52**, see Fig. **2**), was also found to be the compound responsible for the antifungal activity of the dichloromethane extract of a Gentianaceae from Rwanda, *Swertia calycina*. This compound, which strongly inhibited the growth of *C. cucumerinum* and *C. albicans* in TLC bioautography, was found to be a widespread constituent which had already been reported for this type of activity. However this was the first time a naphthoquinone had been found in the Gentianaceae family and the way in which this simple natural product was successfully dereplicated by chemical screening with LC/UV, LC/MS and LC/NMR will be discussed later [54]. Numerous other naphthoquinones, such as those isolated from *Newbouldia laevis*, a Bignoniaceae from Guinea-Conakry have also displayed interesting antifungal activities against *C. cucumerinum* and *C. albicans* in both bioautography and dilution assays. This was the case in particular for various dehydroiso-α-lapachone derivatives (**53-57**) (Table 5) [55]. In addition, *Diospyros usambarensis* (Ebenaceae) root bark has furnished a number of simple naphthoquinones (e.g. **32**, TLC *C. cucumerinum*: 0.025 µg) which have both antifungal and molluscicidal activities [40]. In spite of strong bioactivities, the medical use of naphthoquinones is limited. Owing to their mechanism of action, these compounds are likely to be toxic for all living organisms [56].

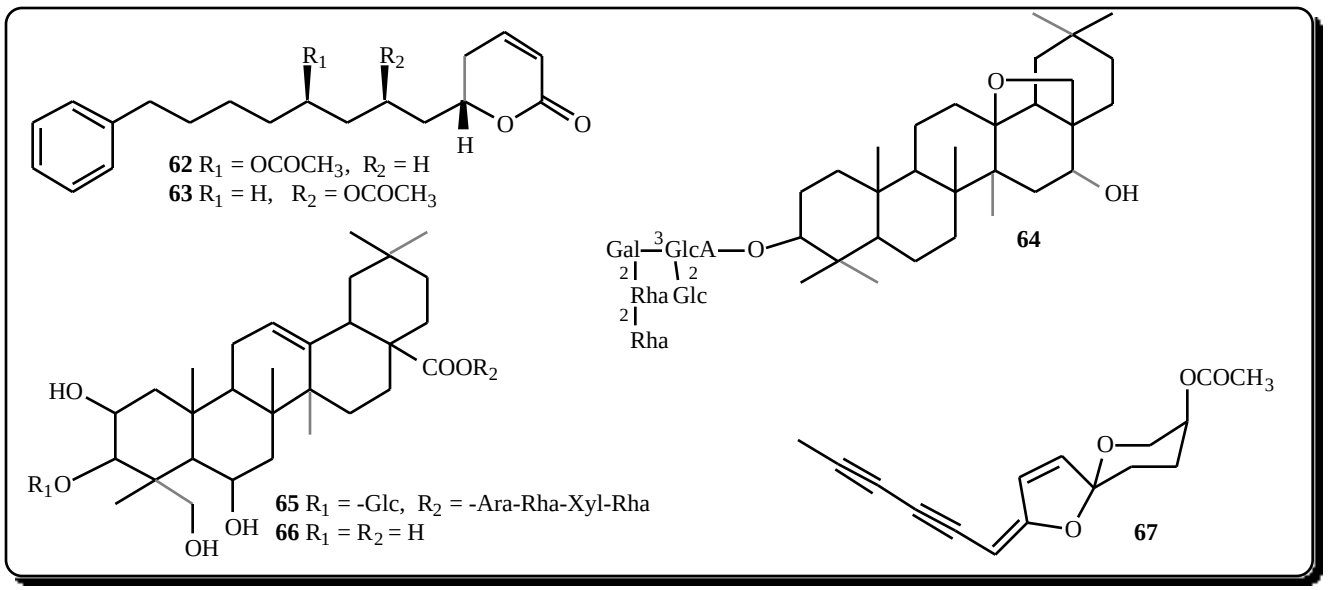
Anthraquinones also exhibited antifungal activities. Out of ten of these constituents isolated from *Morinda lucida*, a Rubiaceae from Zaire, four were active against both *C. cucumerinum* and *C. albicans*. The antifungal anthraquinones possessed an aldehyde group which was not present in the inactive derivatives. In the TLC assay, 3-hydroxy-2-carbaldehyde-anthraquinone (**58**) inhibited *C. cucumerinum* at 2 µg and *C. albicans* at 5 µg; nordamnacanthal (**59**) and damnacanthal (**60**) showed growth inhibition against both microorganisms at 1 µg. Alizarin-1-methyl ether (**61**) had the lowest detection limits (0.5 µg - *C. cucumerinum* and 1 µg - *C. albicans*). On further investigation of the antifungal activity in dilution assays against human pathogenic fungi, **61** was weakly inhibitory against *C. albicans*, *A. fumigatus* and *T. mentagrophytes* (MIC: 10, 100, 50 µg/ml respectively). Surprisingly the three aldehyde derivatives (**58-60**) were found to be inactive in the dilution assays [57].

Two new **pyrones 62** and **63** isolated from *Ravensara anisata* (Lauraceae), an endemic plant from Madagascar, displayed also a moderate activity against both *C. cucumerinum* and *C. albicans* (MIC 100 µg/ml). It was the first time that such an activity has been reported for this class of natural product [58].

In the terpene type of fungicidal agents, **triterpene glycosides** (saponins) may also exhibit interesting activities. This was the case, for example, for a sakurasaponin **64** isolated from the methanolic leaf extract of *Rapanea melanophloeos* (Myrsinaceae) from Zimbabwe. Four saponins were isolated from this plant but only **64** was found to be active against *C. cucumerinum* (1 µg on TLC). This fact suggested that the 13β,28-epoxy moiety of **64** plays an important role for the activity since it is absent in the other saponins. For further structure-activity investigations, the monomethyl ester of saponin **64** was tested but it was found to be inactive [59]. Other saponins such as the 3-O-β-D-glucopyranoside derivatives of hederagenin **23**, bayogenin **24** and medicagenic acid **25** isolated from a Leguminosae from Kenya, *Dolichos kilimandscharicus*, possessed notable activity against *C. cucumerinum* (TLC: 5, 2.5 and 5 µg respectively) and displayed also molluscicidal activity against *B. glabrata* [34]. Mi-saponin A (**65**) and its aglycone **66** isolated from *Clerodendron wildii*, a Verbenaceae from Malawi, also inhibited significantly the growth of *C. cucumerinum* on TLC (1.5 and 3 µg respectively) [60].

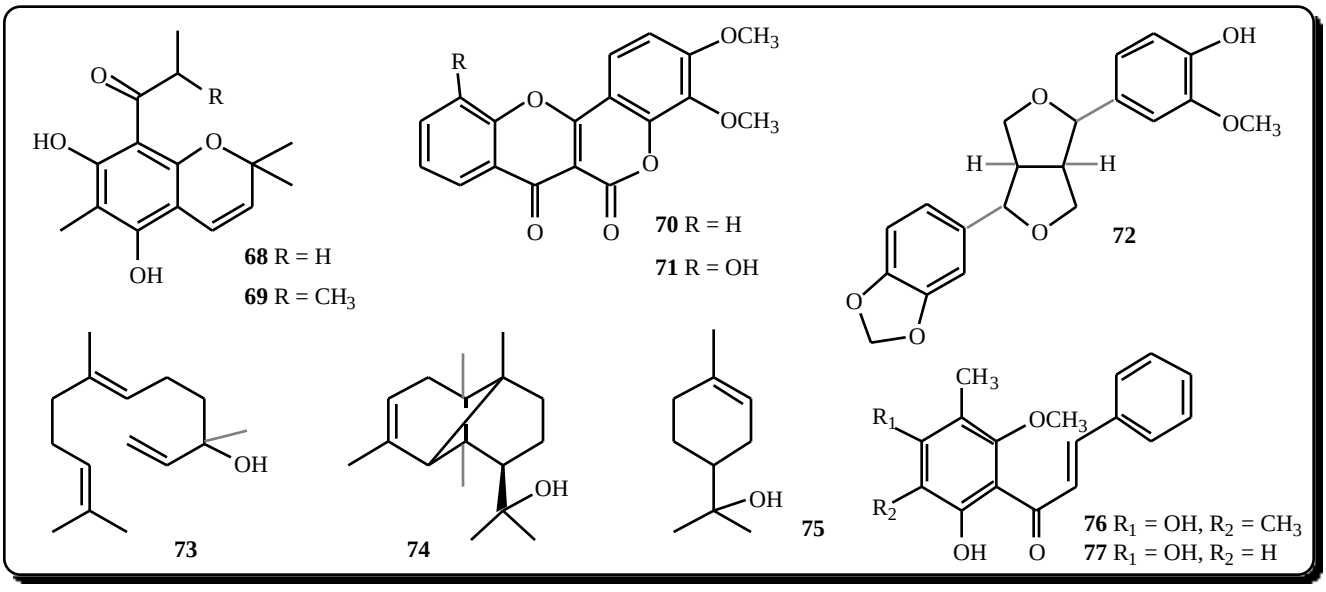
Acetylenic compounds such the spiroacetal enol ether (*E*)-*O*-acetyldendranthemenol (**67**) isolated from an Asteraceae from Zimbabwe, *Inulanthera nuda*, displayed activity against *C. cucumerinum* in both TLC and dilution assays (TLC: 5 µg, MIC: 80 µg/ml). This compound was however found to be inactive against *C. albicans* [61].

Numerous phenolic derivatives from African plants such as **phenylpropanoids** or **polyphenols** also possess interesting fungicidal activities. Species of the genus *Hypericum* (Guttiferae) are



known to contain antibacterial and antifungal constituents. During an expedition to Malawi, leaves and twigs of *Hypericum revolutum,* a shrub native to south-east Africa growing at high altitude in open mountain grassland and at the margins of evergreen forest, were collected. The light petrol ether extract showed antifungal properties. Subsequently, it was subjected to fractionation by various chromatographic techniques to afford an antifungal oil. Analytical HPLC on RP-18, using a photodiode array detector, showed the oil to be a mixture of two compounds with identical UV. These compounds, isolated by semi-preparative HPLC, were found to be two chromenes **68** and **69**. The separation was performed in the absence of acid because the chromenes **68** and **69** form the corresponding dichromenes in acidic media. The minimum quantity of both compounds required to show activity against *C. cucumerinum* on the plate was 5 µg. The acetylation product of **68**, was inactive [62].

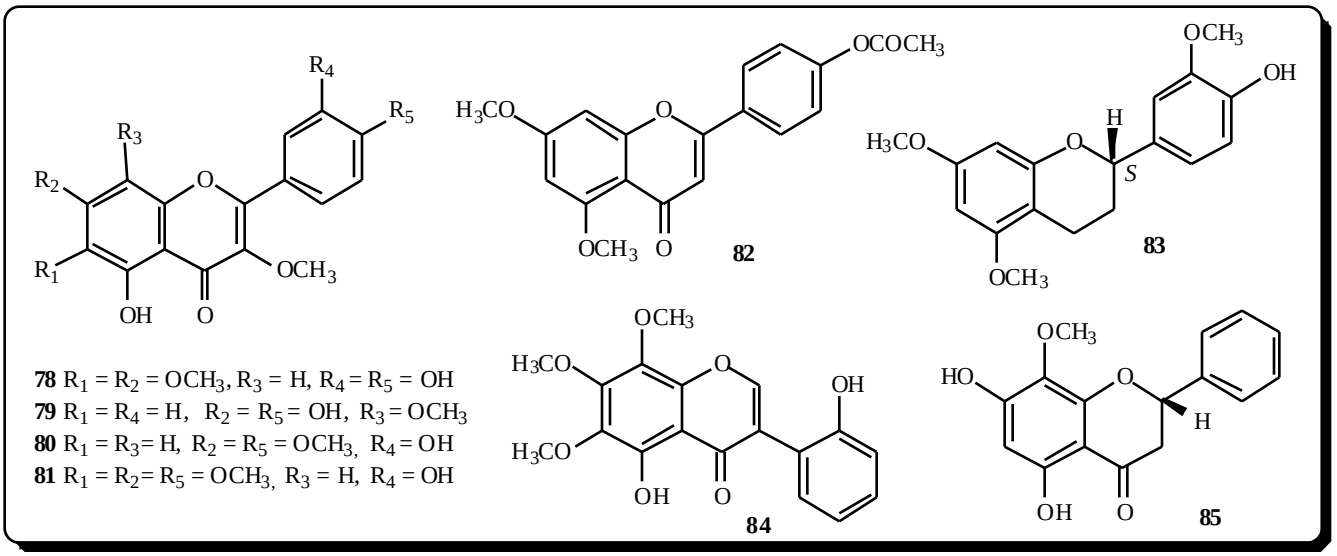
Coumarins have also been demonstrated as powerful antifungal agents. Three chromonocoumarins have been isolated from the leaves and root bark of *Polygala fruticosa* (Polygalaceae), a shrub growing in the Cape Province of South Africa and in Natal. The roots have been used by the Zulus in the treatment of dropsy, scrofula and tuberculosis. Separation of the fungicidal dichloromethane extract of the leaves yielded two constituents, frutinones A (**70**) and B. Frutinone C (**71**) was purified from a methanolic extract of the roots. Frutinone A (**70**) is most unusual in that it is an aromatic, flavone-like compound. When chromonocoumarins **70**-**71** were tested against *C. cucumerinum*, only frutinone A (**70**) was found to be active - but the activity was high (0.25 µg). Substitution by methoxyl or hydroxyl groups, therefore, rendered the compounds inactive [63]. Frutinone A was also recently isolated from *P. gazensis*, a plant from Zimbabwe. Its activity was measured against both *C. cucumerinum* and *C. albicans* in a dilution assay (MIC: 20 µg/ml). In the same plant, lignans such as eudesmin and kobusin were antifungal on TLC plates but were not active in the dilution test at a concentration of 100 µg/ml [64]. Oxypeucedanin (**34**) a **furanocoumarin** isolated from a Malawian Umbelliferae, *Diplolophium buchanani,* was found to be very active in the TLC assay against *C. cucumerinum* (1 µg); its hydrate form was less active (10 µg). Two other phenylpropanoids isolated from the same plant, elemicin and trans-isoelemicin, obtained as a inseparable mixture, were also active (8 µg) [42]. The **lignan** (+)-piperitol (**72**) isolated from *Ocotea usambarensis*, (Lauraceae) from Rwanda, prevented growth of *C. cucumerinum* on TLC (5 µg) while another derivative of the same type, (+)-sesamin did not show any activity against this fungus. The other constituent compounds of *O. usambarensis* were volatile and could not be isolated. In order to investigate these compounds, the **essential oil** was extracted by water distillation from the fresh bark and tested against *C. cucumerinum,* showing several antifungal spots when a minimum amount of 25 µg was deposited. GC/MS analysis of the essential oil allowed the identification of nearly 70 constituents. However



this analysis did not give any information on the active constituents. Isolation of the active compounds was performed by over-pressure layer chromatography (OPLC). The fractions obtained were tested by direct bioautographic assays separated again by automated multiple development (AMD) bidimensional TLC. As the biological assay was feasible on this type of TLC plate, the active spots were extracted from another plate developed under the same conditions and analysed again by GC/MS. This procedure allowed the identification of three main active principles α-terpineol (**73**), nerolidol (**74**) and α-copaene-11-ol (**75**). The antifungal activity of α-terpineol (**73**) (15 µg) and nerolidol (**74**) (12 µg) against *C. cucumerinum* was determined on the plate using commercially available standards [65].

Various **flavonoids** and even **chalcones** were found to be antifungal. An investigation of the dichloromethane leaf extract of *Myrica serrata* (Myricaceae) from Zimbabwe, yielded five different chalcones. Two of them, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (**76**) and 2',4'-dihydroxy-6'-methoxy-5'-methylchalcone (**77**), were found to be active against *C. cucumerinum* (TLC: 6 and 4 µg, MIC: >160, 80 µg/ml, respectively). These two compounds exhibited also interesting antibacterial activities against *Bacillus subtilis* and *Escherichia coli* [66].

Species of the genus *Psiadia* (Asteraceae) are used in African traditional medicine for the treatment of bronchitis and asthma and have analgesic properties. The shrub *Psiadia trinervia* is an endemic species of the Mascarene Islands and both a dichloromethane extract and a hydrolysed methanol extract of the leaves were active against *C. cucumerinum*. Fractionation of these extracts yielded thirteen 3-*O*-methoxylated flavonols. Based on these 13 flavonols, 29 derivatives were prepared by permethylation and selective methylation of the free hydroxyl group at C-5. Among all these flavonols, the genuine compounds chrysosplenol-D (**78**) and 5,7,4'-trihydroxy-3,8-dimethoxyflavone (**79**) were the most active (TLC: 5 µg each), while ayanin (**80**) and casticin (**81**) had borderline activity (TLC: 20 µg each). Concerning the derivatives, some acylated reaction intermediates were active; the most interesting one was 4'-acetyloxy-3,5,7-trimethoxyflavone (**82**) (TLC: 1 µg). The other tested flavonols did not display any noticeable activity [67]. Similar methylated flavonoids deposited on the leaf and stem surfaces of *Helichrysum nitens* (Asteraceae) are also fungicidal and have a defensive role, preventing the invasion of microorganisms. Thus, by soaking the aerial parts of *H. nitens* in dichloromethane, an extract containing the following antifungal flavones was obtained: 5,7-dimethoxyflavone, 5,6,7-trimethoxyflavone, 3,5,7-trimethoxyflavone, 5,6,7,8-tetramethoxyflavone, 3,5,6,7-tetramethoxyflavone and 3,5,6,7,8-pentamethoxyflavone. The minimum amounts of methylated flavonoids required for growth inhibition of *C. cucumerinum* spores varied between 5 µg and 1 µg [68,69]. This compared well with the value (2 µg) obtained for the known antifungal flavone tangeretin (5,6,7,8,4'-pentamethoxyflavone). By contrast alnetin (5-hydroxy-6,7,8-trimethoxyflavone) or 5-hydroxy-6,7-dimethoxyflavone were inactive [68]. More

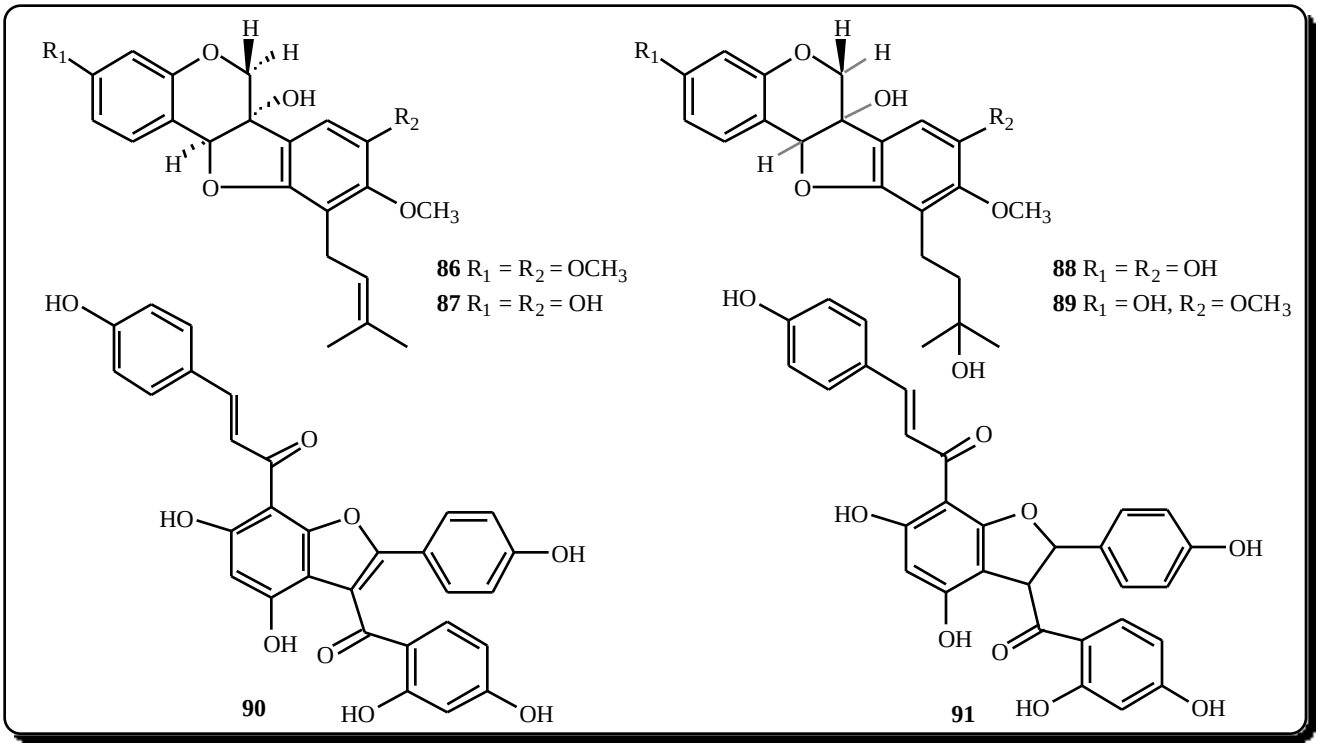


78 $R_1 = R_2 = OCH_3, R_3 = H, R_4 = R_5 = OH$
79 $R_1 = R_4 = H, R_2 = R_5 = OH, R_3 = OCH_3$
80 $R_1 = R_3 = H, R_2 = R_5 = OCH_3, R_4 = OH$
81 $R_1 = R_2 = R_5 = OCH_3, R_3 = H, R_4 = OH$

recently, the investigation of **flavans** from *Mariscus psilostachys*, a Zimbabwean Cyperaceae, revealed that the new (2S)-4'-hydroxy-5,7,3'-trimethoxyflavan (**83**) was active in the TLC assay, but its activity in the dilution assay against *C. albicans* was rather weak (MIC 50 µg/ml). The absolute configuration of this compound was based on its CD spectrum [70]. **Isoflavones**, such as irilin A (**84**), or the **flavanone** dihydrowogonin (**85**) isolated from a Rwandan Chenopodiaceae, *Chenopodium procerum*, have also displayed antifungal activities on TLC (5 µg). These compounds however were found to be inactive against *B. subtilis* [71].

Other polyphenols, such as the new **pterocarpinoids** isolated from *Dolichos marginata* ssp. *erecta* (Leguminosae), were also moderate fungicidal agents. The quantities of the 6α-hydroxypterocarpans sphenostylins A (**86**), B (**87**), C (**88**) and D (**89**) required for *C. cucumerinum* spore growth inhibition were respectively 6 µg (**86**), 10 µg (**87**), 20 µg (**88**) and 50 µg (**89**) [72]. The polyphenols responsible for the orange coloration of the inner bark of *Brackenridgea zanguebarica* (Ochnaceae, from Malawi) also inhibited the growth of *C. cucumerinum*. The benzofuran **90** and the dihydrobenzofuran **91** were active at 10 and 50 µg, respectively. The other polyphenols did not display any noticeable activities. These compounds could be directly obtained in a pure form after CPC separation of the crude methanolic of the stem bark of this plant [73]. The different polyphenolic constituents of *B. zanguebarica* were previously attributed to *Cordia goetzei* (Boraginaceae), but the plant was incorrectly named [74].

Plants from the Gentianaceae, Polygalaceae and Guttiferae are an important source of **xanthones**. In view of their interesting pharmacological properties (monoamine oxidase inhibition, antitumour activity etc.) and their use in chemotaxonomy as systematic markers, the xanthones are currently attracting a good deal of attention. During our investigation of different species of the Guttiferae, it was found that a dichloromethane extract of the root bark of a South African species, *Garcinia gerrardii*, exhibited fungicidal activity. Rare pyranoxanthones were isolated from this plant and a dihydropyranoxanthone was isolated for the first time. Xanthones **92-94** were tested for activity against *C. cucumerinum* in our TLC bioassay. Compounds **92** and **93** were inactive at 50 µg, whereas xanthone **94** prevented growth of the fungus at 0.2 µg [75]. Of the five prenylated xanthones isolated from another plant from the same genus *G. livingstonei*, two of these **95** and



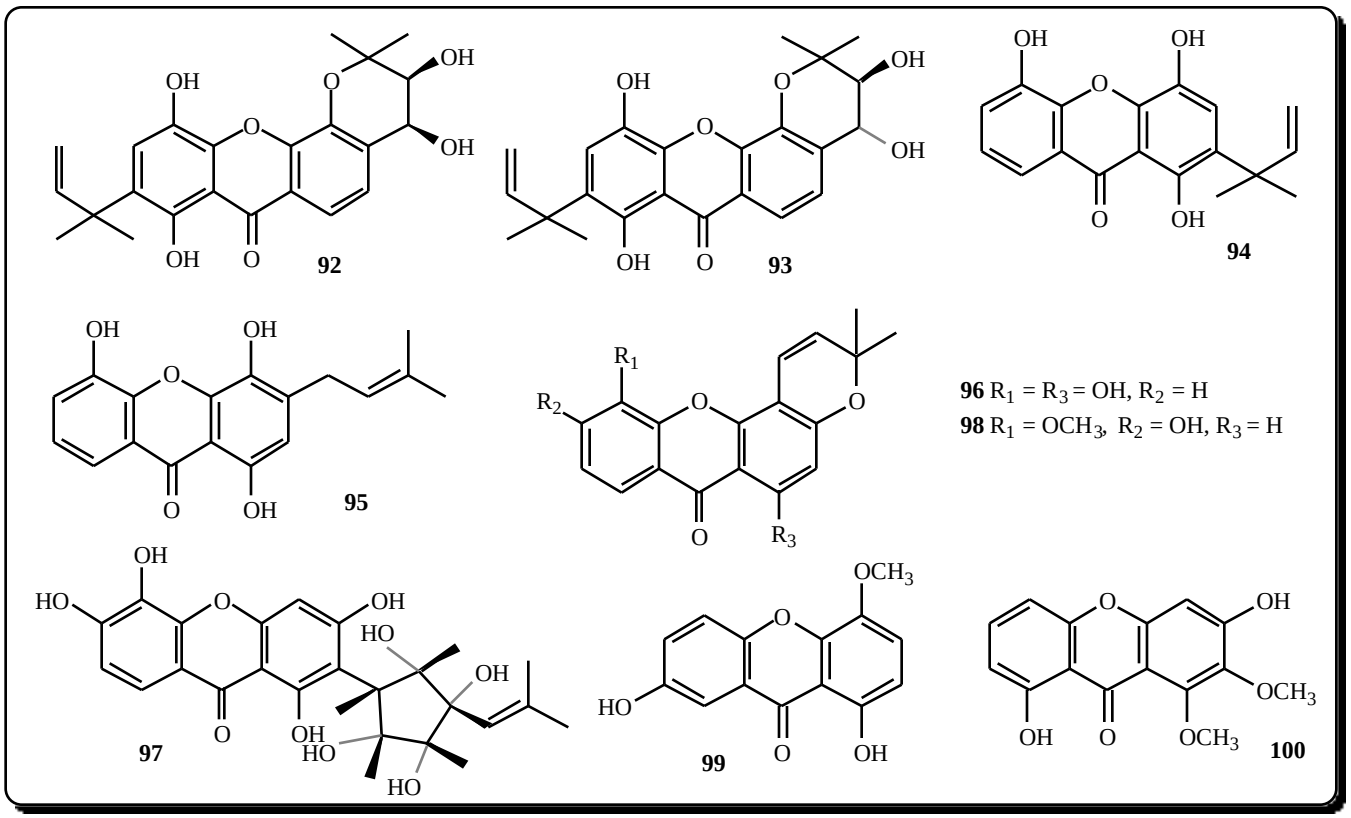
96, displayed significant activity in the TLC assay (0.5 and 0.2 µg respectively), while the others were inactive [76]. Other antifungal xanthones were found in the roots of *Hypericum roeperanum*, a Guttiferae from Zimbabwe. The minimum amount of xanthone **97** required to inhibit the growth of *C. albicans* was 1 µg whereas xanthone **98** showed antifungal activity at 5 µg in the TLC bioassay. All the other xanthones from this plant were inactive at a level of 10 µg. It must be noted, in this case, that the crude dichloromethane extract did not show any activity against *C. albicans* at the usual test level of 100 µg, owing to the low concentrations of xanthones. None of the xanthones of this plant inhibited the growth of *C. cucumerinum* at a level of 10 µg [77]. In a Polygalaceae from Malawi, *Polygala nyikensis*, two simple xanthones **99** and **100**, which were found to be the first representatives of 1,4,7- (**99**) and 2,3,4,5-oxygenated xanthones (**100**) found in this family, exhibited interesting activities against *C. cucumerinum* (0.6 and 0.4 µg respectively by TLC) [78].

Thus, as shown, natural products of very different types may present interesting fungicidal properties. African plants in particular have demonstrated to be a rich source of this type of bioactive constituents. In the two following sections, selected examples of the strategy followed for the efficient dereplication of known antifungal compounds or for the targeted isolation of promising leads are described.

New Strategies for the Rapid Dereplication of Antifungal Constituents

As has been mentioned, African plants contain numerous interesting fungicidal agents but some of them are known natural products with known types of biological activities. In order to avoid the time-consuming isolation of these products, a very valuable approach is to perform LC chemical screening of the extracts by LC/UV LC/MS and LC/NMR, combined with LC microfraction and testing of the fractions by bioautography.

In our on-going studies on plants belonging to the Gentianaceae family, numerous extracts were screened chemically by LC/UV and LC/MS in order to detect the presence of novel xanthones and to target their isolation [79,80]. As a part of this broad screening, the dichloromethane extract of *Swertia calycina*, from Rwanda, was analysed. Surprisingly, the dichloromethane extract of *S. calycina* presented a strong activity against the fungi *C. cucumerinum* and *C. albicans* [54]. In



order to dereplicate the compounds responsible for this activity, an LC/UV/MS analysis of the extract was undertaken together with a LC microfractionation for the localisation of the active constituents. The LC/UV chromatogram of *S. calycina* was rather simple and three main peaks (**52**, **101**, **102**) were detected. The peaks collected after the LC/UV separation were spotted on TLC and, after migration, bioautography with *C. cucumerinum* was performed. By this means the antifungal activity was clearly assigned to compound **52** (Fig. **2**).

The LC/UV/MS analysis revealed that compound **102** presented a UV spectrum with four absorption bands characteristic of a xanthone.

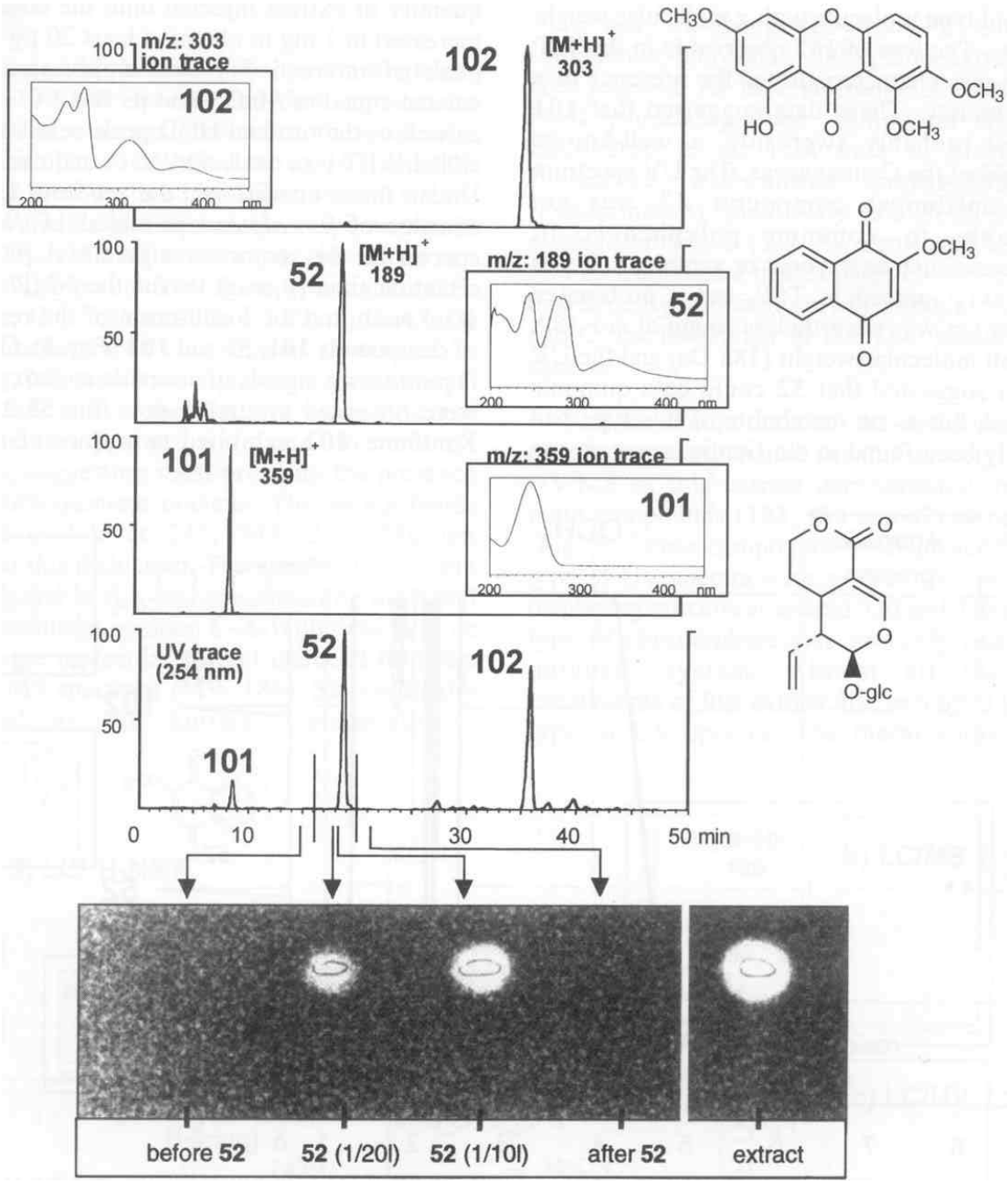


Fig. (2). LC/UV and LC/TSP-MS analysis of the crude CH_2Cl_2 extract of *Swertia calycina* (Gentianaceae) [81]. The UV trace was recorded at 254 nm and UV spectra from 200-500 nm. HPLC: Column, RP-18 NovaPak (4µm, 150 x 3.9 mm i.d.); gradient, MeCN-H_2O (0.1% TFA) 5:95 to 65:35 in 50 min (1ml/min), NH_4OAc 0.5 M (0.2 ml/min post-column). TSP: positive ion mode; filament off; vaporiser 100 °C; source 280 °C.

Its TSP-MS spectrum exhibited a strong protonated ion $[M+H]^+$ at *m/z* 303, indicating a xanthone with a molecular weight of 302 substituted by one hydroxyl and three methoxyl groups (Fig. **2**). This information, together with the comparison with a in-house UV spectral library, led to the identification of **102** as decussatin, a widespread xanthone from the Gentianaceae family. The on-line data obtained for compound **101** indicated the presence of a secoiridoid type molecule with a molecular weight of 358 Da. The loss of 162 observable in the TSP spectrum was characteristic for the presence of a hexosyl moiety. These data suggested that **101** was most probably sweroside, a well-known secoiridoid of the Gentianaceae. The UV spectrum of the antifungal compound **52** was not attributable to common polyphenols of Gentianaceae such as flavones or xanthones. It was very weakly ionised in TSP, but a protonated molecular ion was nevertheless found at *m/z* 189. This small molecular weight (188 Da) and the UV spectrum suggested that **52** could be a quinonic compound, but as no metabolite of this type had previously been found in the Gentianaceae, it was not possible to identify it on-line only based on UV, MS and chemotaxonomical information.

In order to confirm these attributions and to obtain more structure information on-line, the extract was submitted to an on-line LC/^{1}H-NMR analysis on a 500 MHz instrument [81]. The same LC conditions as for the LC/UV/MS analysis were used except that the water of the LC gradient system was replaced by D_2O. However, the quantity of extract injected onto the column was increased to 1 mg to obtain at least 20 µg for each peak of interest. For the suppression of the solvent signal of MeCN and its two ^{13}C satellites, as well as the residual HOD peak, a fast sequence called WET was run before each acquisition [82]. Under these conditions, the on-flow LC/NMR analysis of *S. calycina* provided LC/^{1}H-NMR spectra for the major constituents. A plot of the retention time (y axis) versus the NMR shifts (x axis) permitted the localisation of the resonances of compounds **101**, **52** and **102** (Fig. **3**). On this 2-D plot, strong signals of aromatic methoxyl groups were observed around 4 ppm for **52** and **102**. Xanthone **102** exhibited two pairs of aromatic

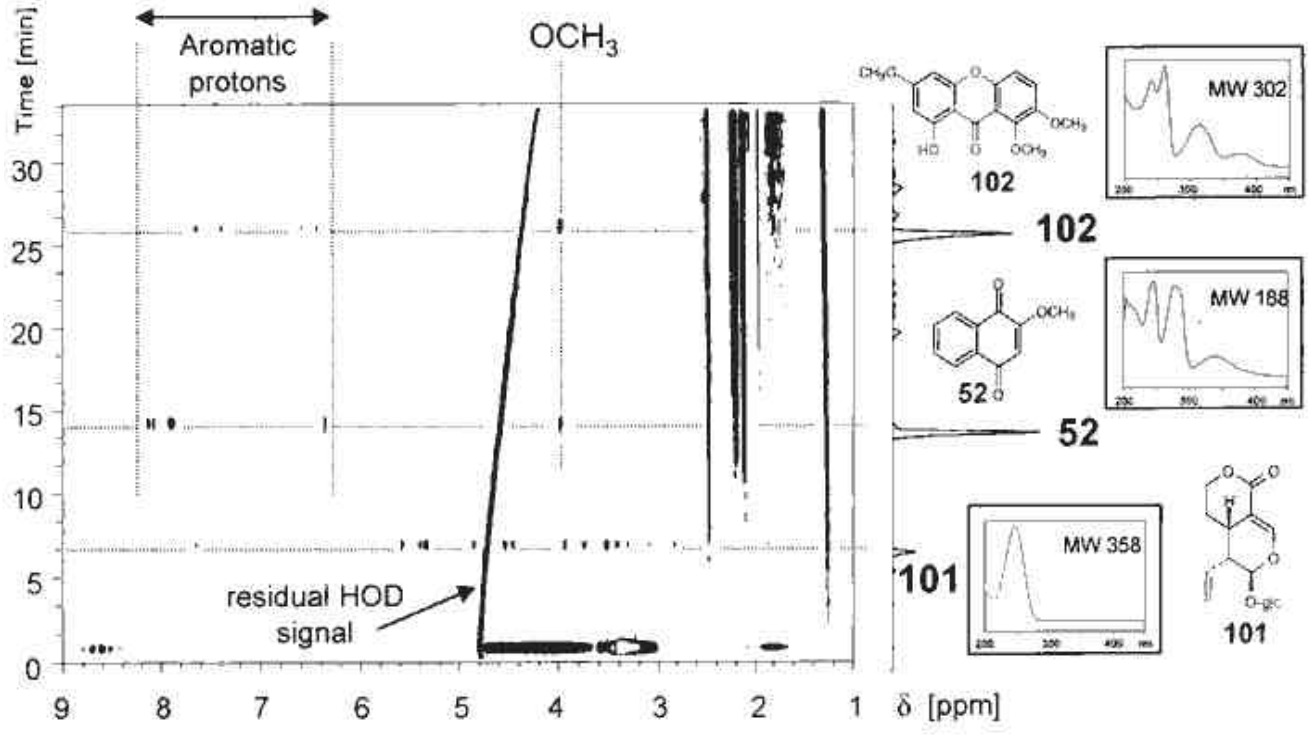


Fig. (3). Bidimensional LC/^{1}H-NMR chromatogram of the crude CH_2Cl_2 extract of *Swertia calycina* (Gentianaceae) [81]. Methoxyl groups and aromatic proton signals of **52** and **102** are clearly visible together with all the resonances of the monoterpene glycoside **101**. The signal of HOD is negative and was continually shifted during the LC gradient. The same HPLC conditions as in Fig. **2** were used except that H_2O was replaced by D_2O and the injection amount was increased to 400 µg. LC/NMR: 24 scans/increment, flow cell (60 µl, 3 mm i.d.), 500 MHz.

protons, while the quinonic compound **2** presented five other low field protons. The more polar secoiridoid **101** showed different signals between 3 and 6 ppm. The important trace starting from 4.8 ppm (at 0 min) and ending to 4 ppm (at 30 min) was due to the change of the chemical shift of the residual negative water (HOD) signal during the LC gradient. The traces between 1 and 2.6 ppm were due to residual MeCN signals and solvent impurities.

A slicing of this bidimensional plot in single on-line LC/^{1}H-NMR spectra for each constituent allowed a precise assignment of their specific resonances. The ^{1}H-NMR data of **101** and **102** confirmed their on-line identification by LC/UV/MS as sweroside and decussatin, respectively. In the LC/^{1}H-NMR spectrum of the unknown antifungal compound **52** (Fig. **4**), two signals (2H, δ 8.11, m, H-(C5,8) and 2H, δ 7.89, m, H-(C6,7)) were characteristic of four adjacent protons of an aromatic ring with two equivalent substituents. The low field shift of the H-(C5,8) signal indicated that these two protons were *peri* to carbonyl functions, suggesting most probably the presence of a naphthoquinone nucleus. The strong bands recorded in UV at 243, 248, 277, 330 nm confirmed this deduction. The singlet at 6.35 ppm was attributed to H-3 and the remaining methoxyl group was thus at position C-2. With these on-line data and the molecular weight deduced from the LC/TSP-MS spectrum (MW 188), **52** was finally identified as the known 2-methoxy-1,4-naphthoquinone, which was responsible for the antifungal activity of the extract and was reported for the first time in the Gentianaceae family [81]. By the use of this efficient dereplication strategy, its isolation was avoided.

Investigation of the Antifungal Constituents of *Bobgunnia madagascariensis*

In our on-going research on antifungal compounds, more than 2000 plants extracts have been screened for their antifungal properties. The most promising lead compound has been discovered in the root bark of a tree from Zimbabwe, *Bobgunnia madagascariensis* (Leguminosae), previously known as *Swartzia madagascariensis* [52]. As mentioned before, its root bark contained indeed pale yellow metabolites that strongly inhibited growth of both *C. albicans* and *C. cucumerinum* in the TLC bioautography assays.

Various compounds of the dichloromethane extract were responsible for this activity. An LC/UV/MS of this extract demonstrated that three main compounds (**103**, **104** and **47**) were present (Fig. **5**). These compounds all displayed the same type of UV spectra with a main absorption band displaying maxima at around 320 and 330 nm. This type of chromophore was probably related to a quinonic system. Almost all the minor constituents of this extract also exhibited the same type of UV spectra. The thermospray LC/MS

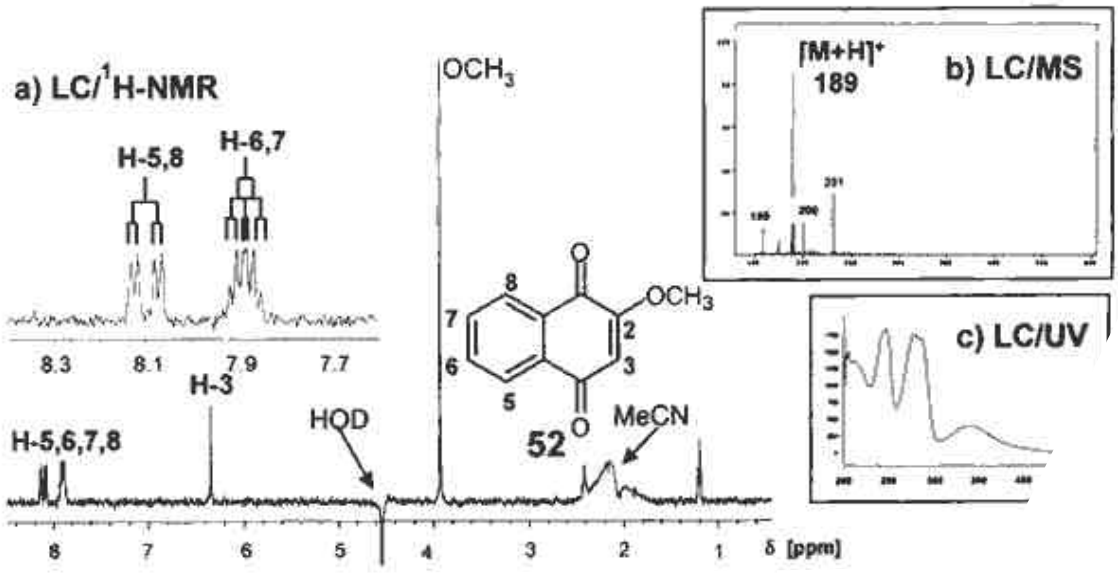


Fig. (4). Summary of all the information obtained on-line for the antifungal naphthoquinone **52**.

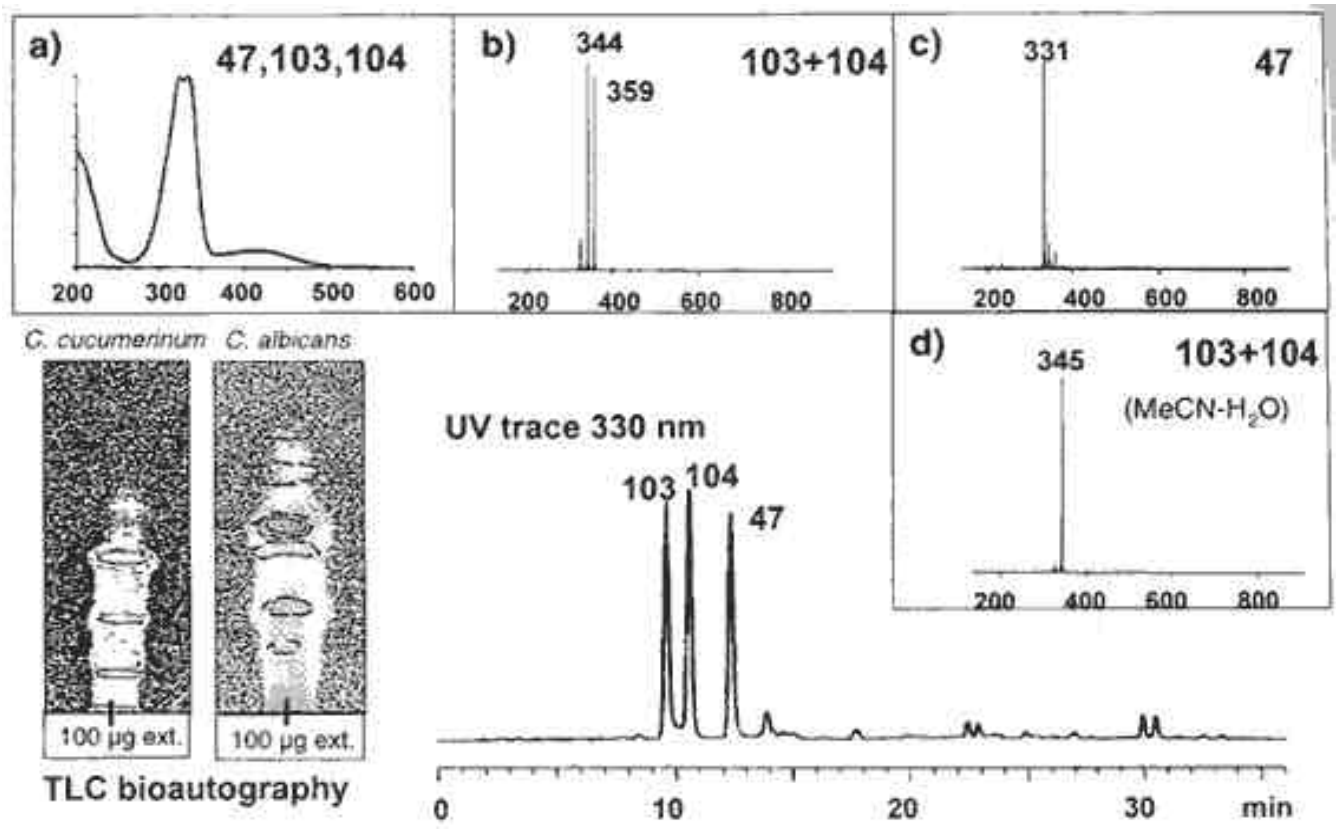


Fig. (5). LC/UV and LC/TSP-MS analysis of the crude root bark CH_2Cl_2 extract of *Bobgunnia madagascariensis* (Leguminosae) [84]. UV trace was recorded at 330 nm and UV spectra from 200-600 nm. HPLC: Column, RP-18 NovaPak (4µm, 150 x 3.9 mm i.d.); gradient, MeOH-H_2O (0.1% TFA) 60:40 -> 100:0 in 35 min (1ml/min), NH_4OAc 0.5 M (0.2 ml/min post-column). TSP: positive ion mode; filament off; vaporiser 95 °C; source 200 °C. a) UV spectra for **47**,**103**,**104** were similar. b) TSP-MS spectra of **103** and **104** recorded in a MeOH-H_2O solvent system. c) TSP-MS spectrum of **47**. d) TSP-MS spectra of **103** and **104** recorded in a MeCN-H_2O solvent system.

analysis revealed a protonated molecule $[M+H]^+$ at *m/z* 331 Da for **47**, while **103** and **104** presented both ions at *m/z* 344 and 359 when the extract was analysed in a methanol and water gradient. Curiously, **103** and **104** displayed a single ion at 345 Da when the separation was carried out with an acetonitrile and water system. These different measurements indicated a molecular weight 330 Da for **47**. For the isomers **103** and **104** the molecular weight was 344 Da and the ion observed at *m/z* 359 was probably an artefact due to a methylation in the source with methanol.

These first on-line data were compared to those of compounds already reported for *B. madagascariensis*. This plant was indeed known to contain molluscicidal saponins [30], as well as flavonoids and pterocarpans. The results proved that the compounds screened were not yet reported in *B. madagascariensis*. Moreover no natural product with these molecular weights and this type of UV spectra could be found in databases of natural products. All constituents of the dichloromethane extract were thus potential new bioactive natural products and their targeted isolation was undertaken.

The dichloromethane extract of *B. madagascariensis* was fractionated by column chromatography (silica gel), yielding 8 fractions. Compound **47** was purified by medium-pressure liquid chromatography (MPLC; Diol material) from fraction F. The structure of **47** was established from spectroscopic data. Definitive evidence and relative configuration were obtained from a single-crystal X-ray analysis (Fig. **6**). As suspected from the on-line data, **47** represents a new type of natural product, a 'quinone methide' diterpene with a cassane skeleton. For the determination of its absolute configuration, an X-ray analysis of a 16-*O*-(4-bromobenzoyl) derivative of **47** was performed. The absolute configuration at C(5) (S) and C(10) (S) was based on the anomalous dispersion effect of the Br-atom.

Compounds **103** and **104**, found in fraction D, could not be however isolated on the preparative scale. Their structure could be finally assigned in the mixture with the aid of LC/NMR analysis of their respective LC peaks recorded on-line and with extensive 2D NMR correlation experiments performed on the mixture. **103** and **104** were found to be hemiacteals derived from **47**. The presence of this hemiacetal group also explained the equilibrium which was observed between **103** and **104** (Fig. **6**). Indeed, a new asymmetric centre was present at C(17) and the instability of **103** and **104** in solution was due to the epimerisation of this chiral position. **103** and **104** were found to be new natural products. The IC_{50} of the mixture of the hemiacetals **103/104** towards *C. albicans* in the dilution assay (7.0 µg/ml) was significantly less active than that of the other 'quinone methide' **47** (IC_{50} 0.2 µg/ml) [83,84]. The complete phytochemical investigation of the extract led finally to the isolation of 14 new diterpene quinones [84].

The antifungal activity of the major compound **47** was compared with that of commercial antifungal agents towards a panel of commercial and clinical fungi, such as several *Candida* species and pathogenic fungi of the type *Aspergillus.* Activities considerably superior to those of amphotericin B and fluconazole were obtained against *Candida* species (*C. albicans* TLC: 0.1 µg, MIC: 0.19 µg/ml). This prompted the filing of a patent (US Patent No. 5.929.124) [85] and incorporation of seven of the isolated diterpene quinones in preclinical testing. *In vitro* assays determining the susceptibility of several fungal organisms to the compounds are underway. Cytotoxicity tests and first experiments on animals are also in progress. An agreement has been made with a pharmaceutical company to investigate the utilisation of the diterpene quinones in humans. If these reach the stage of commercialisation, benefit sharing will involve the Institute of Pharmacognosy and Phytochemistry of the University of Lausanne and also the country of origin (Zimbabwe) of the plant material, in

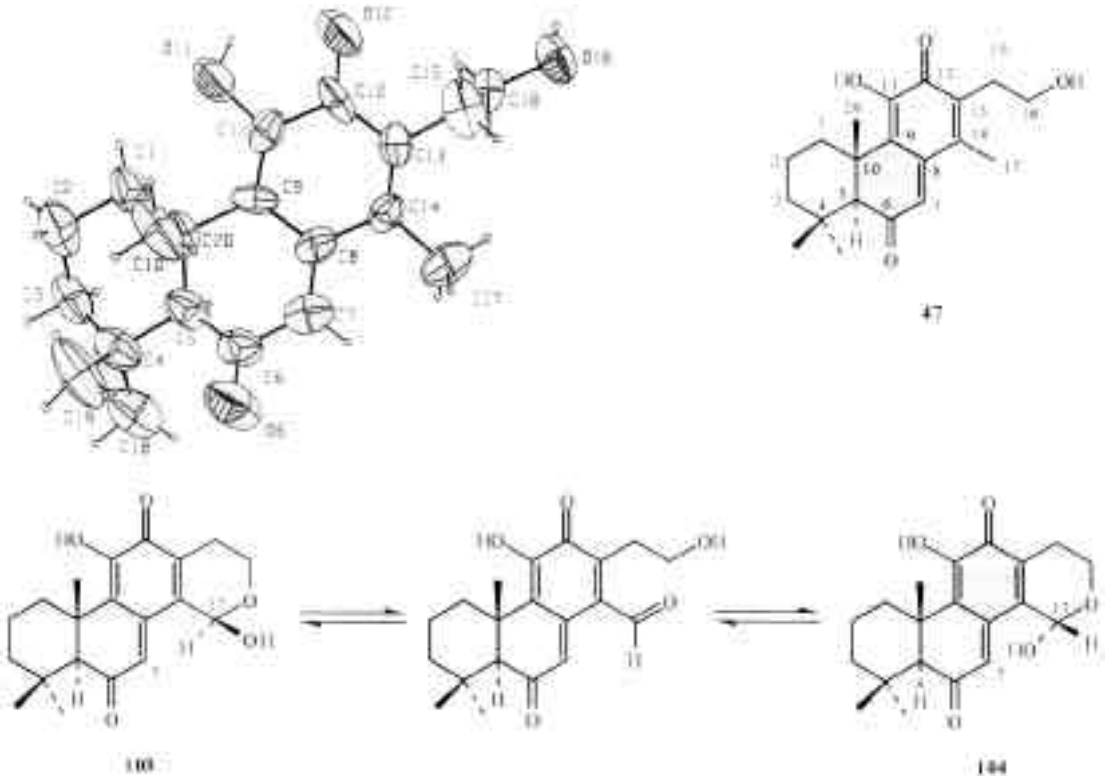


Fig. (6). X-ray perspective view of the antifungal 'quinone methide' diterpene **47**. The absolute configuration at C(5) (S) and C(10) (S) was determined on a halogenated derivative of **47** (16-*O*-(4-bromobenzoyl)) and based on the anomalous dispersion effect of the Br-atom [52]. Structures of the hemiacetals **103** and **104** are given. The structures interconvert in solution.

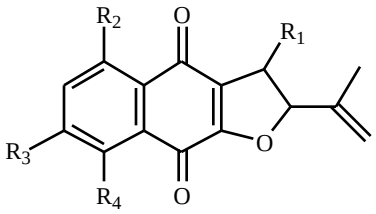
accordance with the Rio Convention on Biological Diversity.

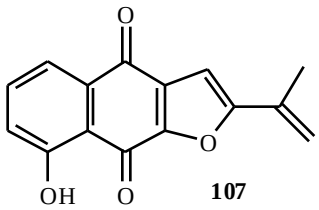
The ultimate aim is to introduce new oral formulations of antifungal drugs which can be used for the treatment of systemic mycoses associated with HIV infections. Furthermore, candidate antifungal preparations are needed which may be of importance in agrochemistry. Thus, the value of the dual screening approach has been amply demonstrated for the discovery of important plant-derived bioactive compounds and work is continuing on various other leads from extracts of material collected in Africa.

Plants with Antibacterial Activities

The number of people with new tuberculosis infections in 1995 was estimated to be 8.9 million, increasing the total of infected people worldwide to about 22 million. This disease is transmitted by a bacterium (*Mycobacterium tuberculosis*), which is transmitted by the coughing or sneezing of infected persons. Tuberculosis is an example of a disease which was thought to be under control but turned out to cause the third largest number of deaths worldwide in 1995 after acute infections of the respiratory system and diarrhoea [86]. One cause for this is the resistance of *M. tuberculosis* to medical treatment. This is not a new problem, but the prevalence of resistance has taken on spectacular dimensions while the development of new antibacterial drugs has slowed down. Another alarming case is the infections by a new strain of *Escherichia coli* [86]. This strain has stricken several countries. It has been responsible for different outbreaks of diseases associated with bad diarrhoea and an insufficiency of the kidney, which - in some cases - proved to be lethal.

Table 5. Antifungal and Antibacterial Activities of Naphthoquinones from *Newbouldia laevis* (Bignoniaceae)





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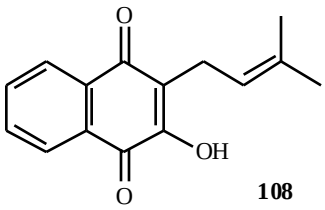

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Compound	R_1	R_2	R_3	R_4	*Cladosporium cucumerinum*		*Candida albicans*		*Bacillus subtilis*		*Escherichia coli*	
53	H	OH	H	H	0.02[a]	5[b]	0.1[a]	10[b]	0.02[a]	1.25[b]	0.06[a]	1.25[b]
54	H	H	H	H	0.06[a]	n.d.	0.4[a]	20[b]	0.2[a]	40[b]	0.2[a]	5[b]
55	H	OCH_3	H	H	0.2[a]	n.d.	4[a]	80[b]	0.2[a]	40[b]	0.6[a]	n.d
56	OH	H	H	OH	0.01[a]	5[b]	0.1[a]	10[b]	0.02[a]	1.25[b]	0.1[a]	10[b]
57	OH	H	H	H	0.1[a]	n.d.	1[a]	40[b]	0.2[a]	20[b]	2[a]	n.d
105	H	H	OH	H	4[a]	n.d.	0	0.1[b]	0.1[a]	10[b]	0.2[a]	2.5[b]
106	OH	OCH_3	H	H	2[a]	n.d.	0	n.d.	2[a]	n.d.	2[a]	n.d
107					0.2[a]	n.d.	4[a]	80[b]	0.04[a]	1.25[b]	0.1[a]	n.d
108					0.6[a]	n.d.	10[a]	n.d.	1[a]	n.d.	2[a]	n.d
Propiconazole					0.1[a]							
Amphotericin B					1[a]	10[b]	1[a]	1[b]				
Chloramphenicol									0.01[a]	1[b]	0.1[a]	

[a] Minimum amount (µg) of compound to inhibit growth on a silica gel TLC plate..[b] Minimum inhibition concentration MIC (µg/ ml) of compound in an agar-dilution assay.. n.d. = MIC of compound not determined.

The above-mentioned examples give evidence for the urgent need for efficacious treatments for bacterial infections. The screening for antibacterial compounds in our Institute has been limited to two species of bacteria*: E. coli* (gram-negative)and *Bacillus subtilis* (gram-positive). Both species are easy to manipulate and with the choice of a gram-negative and a gram-positive bacterium, the activity against two different types of bacteria could be evaluated. Furthermore, infections with *B. subtilis* are extremely rare, which means the bacterium is not very dangerous, but there are other *Bacillus* species which are well known as sources of infectious diseases (e.g. *B. anthracis*, *B. cereus*). The antibacterials assay were performed by following the same methodology mentioned above for the antifungal assays [47]: both dilution methods or bioautographic assays can be performed with these bacterial species.

In our assays, a plant widely used in African traditional medicine, *Newbouldia laevis* (Bignoniaceae), was studied for an evaluation of its antibacterial constituents. The root dichloromethane extract displayed interesting antibacterial activity in the assays and, as mentioned above, the naphthoquinone constitutents of this plant also exhibited a noticeable antifungal activity. These derivatives of dehydoisolapachone (**53-57** and **105-108**) were particularly active against both *B. subtillis* and *E. coli* in the bioautography assays with minimal inhibiting amounts varying between 0.02 and 2 µg [55] (Table 5). As the structures of these compounds were similar, preliminary structure-activity relationships could be established from the results of the bioassays. Thus a substitution of the quinonoid moiety with a 2-isopropenyl furan ring resulted in an increased activity. A further enhancement of the activity was observed with 5 or 8-hydroxyl substitued compounds. Increased activity of compounds with a chelated hydroxyl group has also been observed [87]. A hydroxyl substitution in other positions (position 3 or 7) as well as a methoxyl substitution did not have a large impact on the activity compared to dehydroiso-α-lapachone [55].

Another plant which displayed interesting antibacterial activity is *Myrica serrata*, a Myricaceae from Zimbabwe. In this case, the chalcones (**76** and **77**) which were responsible for the antifungal activity of the dichloromethane leaf extract also displayed strong antibacterial activities (*B. subtilis* TLC: **76** 2 µg, **77** 1 µg, MIC: **76** 20 µg/ml, **77** 40 µg/ml) (*E. coli* TLC: **76** 0.6 µg, **77** 0.8µg, MIC: **76** 5 µg/ml, **77** 20 µg/ml) [66].

Plants with Cytotoxic Activities

Numerous research groups are actively involved in the search for new antitumour agents. Although thousands of plant extracts have already been submitted to various screening methods, only a very few species have produced valuable drugs for the chemotherapy of cancer. Notable examples are *Catharanthus roseus* (Apocynaceae) alkaloids, *Podophyllum peltatum* (Berberidaceae) podophyllotoxin derivatives and, more recently, *Taxus brevifolia* (Taxaceae) diterpenes. Murine *in vivo* models such as the P-388 and L-1210 leukaemias are not the best indicators for drug activity in human tumours, particularly of epithelial origin and *in vitro* protocols using representative human tumour cell lines have been proposed as alternatives. In our laboratory, a rapid test has been used for the screening of cytotoxic activities of plant extracts and plant-derived natural products, using a human tumour cell line (Co-115) derived originally from an adenocarcinoma of the ascending colon. In our opinion, this assay is more representative of the vast majority of human neoplasms which are of epithelial origin than the various assays which generally involve leukaemic cell lines. Cells are incubated with an ethanolic solution of a plant extract or of a pure substance [88]. Following this treatment, the number of living cells is measured by the method of Landegren [89], in which the cells are treated with a chromogenic substrate, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (NAG). The substrate is cleaved by *N*-acetyl-β-D-hexosaminidase, a lysosomal enzyme ubiquitous to the cells. The *p*-nitrophenol liberated from the substrate is determined spectrophotometrically at 405 nm. Since there is a direct relationship between the absorbance and the number of cells, the ED_{50} (concentration of substance or extract which kills 50% of the cells) can be determined from a plot of cell number against concentration of substance.

A first study of 260 extracts from 75 different plant species gave a total of 29 species with ED_{50} values lower than 10 µg/ml [90]. The most active

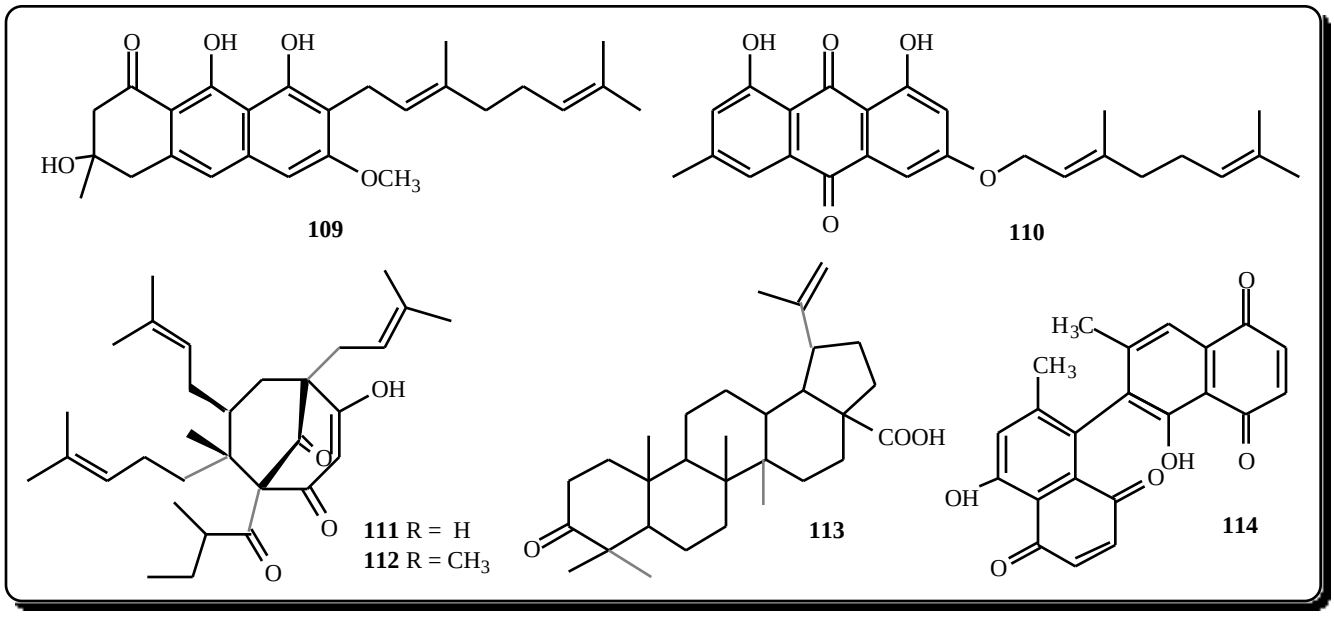
plants comprised the following species: *Diospyros abyssinica, D. zombensis* (Ebenaceae), *Stillingia lineata* (Euphorbiaceae), *Hypericum annulatum, H. revolutum*, *Mammea africana, Psorospermum febrifugum* (Guttiferae) *Indigofera swaziensis, Neorautanenia pseudopachyrrhiza, Bobgunnia madagascariensis* (Leguminosae), *Sesamum angolense* (Pedaliaceae), *Crossopteryx febrifuga* (Rubiaceae) and *Clerodendrum uncinatum* (Verbenaceae). Petroleum ether and dichloromethane extracts produced the highest activities.

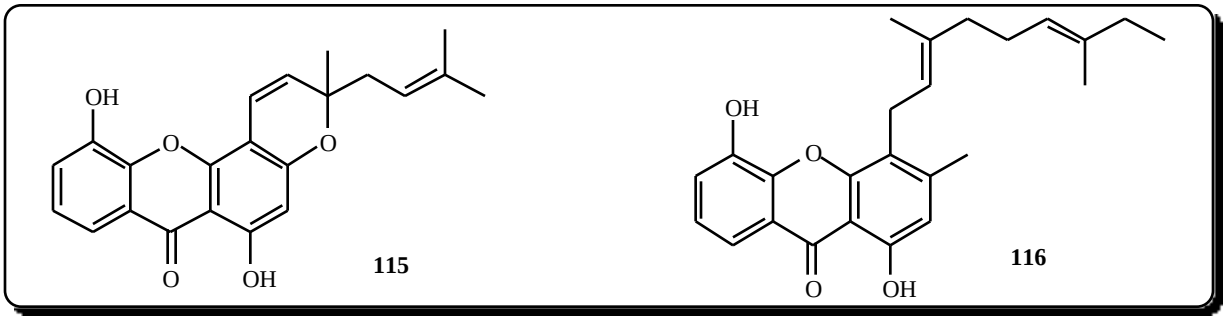
Species of the Guttiferae family are of particular interest and one of these, *Psorospermum febrifugum*, was examined in detail. Petroleum ether (ED_{50} 0.48 µg/ml) and chloroform (ED_{50} 0.35 µg/ml) extracts of the root bark were growth inhibitory in the Co- 115 colon carcinoma cell line. A bioactivity guided frationation of the petroleum ether extract led to the isolation of tetrahydroanthracene derivatives **40**, **39** (vismione D) and **109** which exhibited marked antiproliferative activities, while the new anthraquinone derivative **110** was inactive. The cell growth inhibition of vismione D (**39**) approached that of the cancer chemotherapeutic agent 5-fluorouracil (ED_{50} 6.5 x 10^{-2} µg/ml) but was less than vincristine (**13**) (ED_{50} 7.0 x 10^{-3} µg/ml) one of the antileukaemic alkaloids of *Catharanthus roseus* (Apocynaceae) [88].

Hypericum revolutum is another member of the Guttiferae which has given positive results in the cell growth inhibition bioassay. The isolation of antifungal compounds from a petroleum ether extract of the aerial parts of this plant has already been mentioned. Activity-guided fractionation of the petroleum ether extract of the root bark by droplet countercurrent chromatography (DCCC) resulted in the isolation of two new hyperforin derivatives **111** and **112** [91]. The structure of **111** was established by X-ray analysis. That of the homologue **112** was deduced by comparison of its UV, 'H- and ^{13}C-NMR spectra with those of **111.** Compounds **111** and **112** were obtained as a crystalline mixture which showed tumour cell growth inhibitory activity in the Co-115 cell line (ED_{50} 0.7 µg/ml). They were finally separated by semipreparative HPLC on RP18.

Another plant found to be positive in the initial Co-115 cell line screening was *Crossopteryx febrifuga* (Rubiaceae). An active dichloromethane extract of the stem bark yielded a large amount of betulic acid after column chromatography and crystallisation. Betulic acid (**113**) was growth inhibitory in the Co-115 colon carcinoma cell line (ED_{50} 0.37 µg/ml) [92] but was inactive in a KB cell line, derived from a human epidermoid carcinoma of the nasopharynx [93].

Other classes of compounds have also shown interesting activities in the Co-115 bioassay. These include the naphthoquinones 7-methyljuglone (**32**) and isodiospyrin (**114**) from *Diospyros* species (Ebenaceae) [31] and the dihydroquinone uncinatone (**46**) from *Clerodendrum uncinatum* (Verbenaceae), which has ED_{50} 0.75 µg/ml [51].





In addition, the antifungal naphthoxirene **48** from *Sesamum angolense* (Pedaliaceae) (ED_{50} 0.87 µg/ml) [53] and the antifungal prenylated xanthone, garcigerrin A (**13**) from *Garcinia gerrardii* (Guttiferae) (ED_{50} 3 µg/ml) [75] exhibited growth-inhibitory activity in the Co-115 human colon carcinoma cell line.

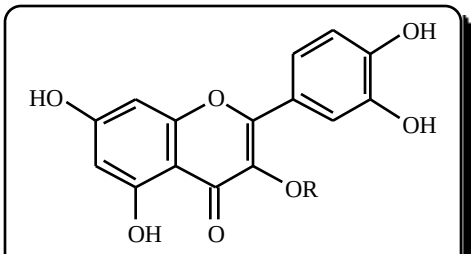
Prenylated xanthones from another *Garcinia* species, *Garcinia livingstonei*, from South Africa also exhibited interesting cytotoxic activities *in vitro*. SW 480 colon carcinoma cells and to a lesser extent Co-115 cells proved to be quite sensitive to the new xanthones **115** and **116** *in vitro*. Furthermore, the growth of SW 480 cells could be inhibited by comparable concentrations of xanthone **116** and 5-fluorouracil, a synthetic drug used in the chemotherapy of colon cancer (ED_{50} 0.6 vs 0.4 µg/ml respectively). Xanthone **95** isolated from the same plant, which displayed a strong antifungal activity, was not found to be cytotoxic and no correlation between the cytotoxic and antifungal activities measured could be noticed [76].

PLANTS WITH ANTIINFLAMMATORY ACTIVITIES

Inflammation is a relatively non-specific response which may be initiated by a variety of physical, mechanical, chemical or immunological means. Certain chronic diseases such as rheumatoid arthritis have an inflammatory background and still do not have a cure. Furthermore, drugs which are employed for the treatment of inflammatory diseases have side effects. For example, corticosteroids can only be used for short periods of time because of their toxicity, and non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid show gastrointestinal side effects. For these reasons, a search for other sources of anti-inflammatory drugs is being pursued. It is not always easy to choose a suitable target for these investigations because the mechanisms involved in the inflammatory process are manifold.

In collaboration with the University of Valencia in Spain, a number of African plants have been screened for anti-inflammatory activity. Two representative tests were chosen: 12-*O*-tetradecanoylphorbol acetate (TPA)-induced mouse ear oedema and carrageenan-induced mouse paw oedema. The former measured topical anti-inflammatory activity and the latter oral activity. In an initial screening, 15 plants were tested and extracts of the following were potent enough to warrant further investigation: *Diospyros leucomelas* (Ebenaceae), *Erythrospermum monticolum* (Flacourtiaceae), *Ficus polita* (Moraceae), *Heteromorpha trifoliata* (Umbelliferae), *Hypericum lalandii* (Guttiferae), *Pterocarpus angolensis* (Leguminosae) and *Zanha africana* (Sapindaceae) [94].

Further study on the methanol extract of the leaves of *Erythrospermum monticolum* (Flacourtiaceae) from Mauritius led to the isolation of two anti-inflammatory flavonol glycosides (**117**, **118**), with quercetin as the aglycone. The glycosides were active against acute inflammation in mice induced by TPA, producing significant reductions in oedema (71% for **118** and 62% for **117**) at a dose of 0.5 mg/ear. Their activity was



117 R = -Rha

118 R = -Rha^2-Xyl

within the range of the reference drug indomethacin [95].

Investigation of another plant from Mauritius, *Diospyros leucomelas* (Ebenaceae), gave three triterpenes (betulin, betulinic acid and ursolic acid) from a dichloromethane extract of the leaves. These were all active in two ear oedema tests. In the TPA assay, the reduction in oedema at a dose of 0.5 mg/ear was 81% for betulin, 86% for betulinic acid and 73% for ursolic acid. In the ethyl phenylpropiolate (EPP) model, the reduction in ear oedema was 46% for betulin, 30% for betulic acid and ursolic acid (dexamethasone gave 61% reduction). In carrageenan-induced mouse paw oedema, swelling reduction for all three triterpenes was of the same order of magnitude as phenylbutazone. Further tests indicated that betulinic acid probably exerts its effects by means of a mechanism strongly related to that of glucocorticoids [96].

Activity-guided fractionation of a methanol extract of *Heteromorpha trifoliata* (Umbelliferae) leaves from Malawi led to the isolation of two saikosaponins (**119**, **120**). Both saponins showed marked topical activity against TPA-induced ear oedema (89% reduction for **119** and 87% reduction for **120**, as compared to 89% for indomethacin) at a dose of 0.5 mg/ear. The saponins had a slight effect against carrageenan paw oedema, while only **119** was active in the EPP mouse ear oedema model [97].

Saponins (**121-123**) were also isolated from the root bark of *Zanha africana* (Sapindaceae) from Malawi, together with the cyclitol pinitol (**124**). These were all very effective inhibitors of phospholipase A_2 from *Naja naja* snake venom [98].

OTHER ACTIVITIES

In the future, there will be an increasing need for therapeutics with **antiviral** activity. The treatment of viral infections is often unsatisfactory and new viral pathogens are likely to be discovered. The example provided by infection with HIV provides a timely warning. A number of compilations of plants used in African traditional medicine (e.g. [1], [4]) cite antiviral properties and for this reason, some screening of antiviral activity of African plants has been undertaken. From a total of 56 extracts from 18 plants, 27 exhibited strong activity against poliovirus, 18 against rhinovirus type 2, and 6 against HSV-1 [99]. Out of the extracts tested in the survey, 9 showed an indirect antiviral activity via induction of interferon (IFN)-α,β. Most of these were negative in the investigation of direct antiviral activity, indicating that in addition to a strong antiviral potential, medicinal plants may possess interesting **immunomodulatory** activities. For this reason, a short study was instigated into the enhancement of *in vitro* non-specific immune functions by extracts of various African medicinal plants. The tests were

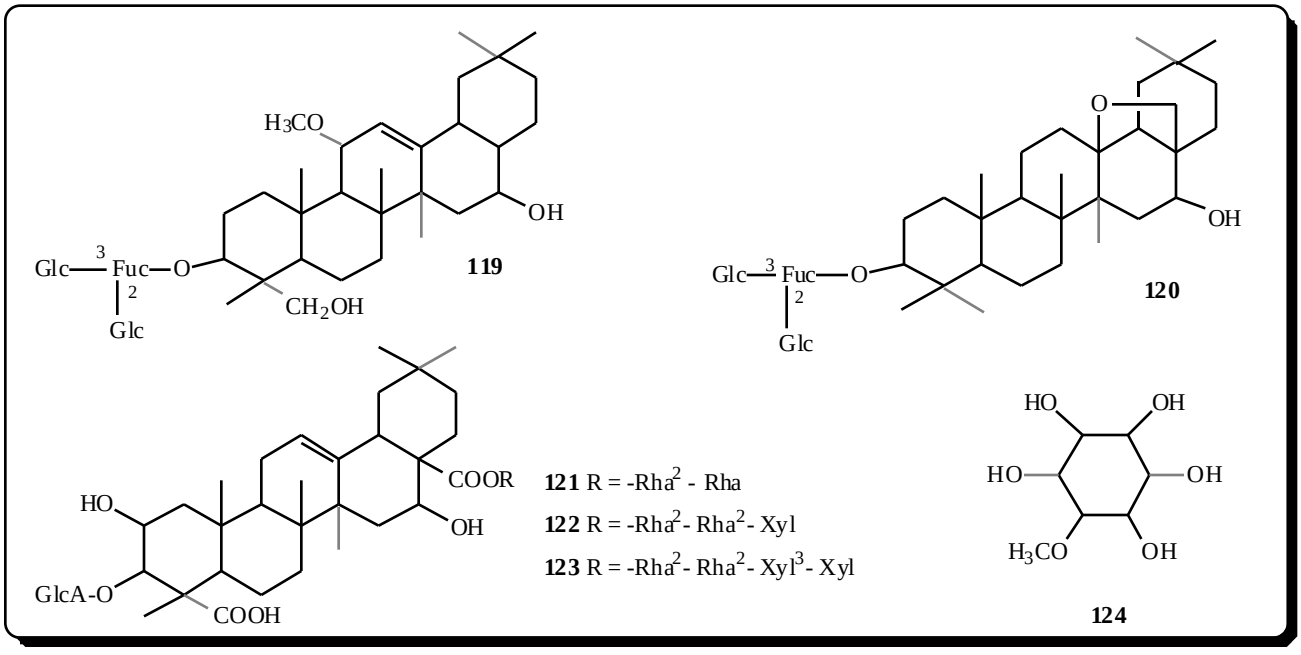


Table 6. African Plants Investigated at the IPP from 1983 to 2000

Plant species collected in Africa	1337
Extracts screened	4679
Plants studied phytochemically	47
Compounds isolated	263
New compounds	75
Scientific publications with African partners	63
Participating countries	18

carried out on immune cells from 3 different strains of mice, and included enhancement of phagocytic activity of peritoneal macrophages and tumoricidal potential of macrophages, stimulation of the oxidative burst of macrophages and granulocytes, and enhancement of the proliferative capacity of lymphocytes [100].

CONCLUSION

Based on the knowledge that the African continent has historically furnished a wide variety of therapeutically-active and food plants, the Institute of Pharmacognosy and Phytochemistry of the University of Lausanne has been involved for over 15 years in research into the phytochemistry and bioactivity of African plant constituents. The approach employed for this on-going investigation is briefly outlined in this article. A combination of chemical and biological screening of plant extracts, together with the application of state-of-the-art chromatographic procedures has allowed the isolation of over 260 natural products from African plants (Table 6). Of these, 75 were new compounds. The major axis of research has been the search for new antifungal agents. The most interesting candidate compound, found in the root bark of *Bobgunnia madagascariensis* (Leguminosae) from Zimbabwe, has prompted deposition of a patent and tests are presently underway to discover whether an oral antimycotic can be developed. Other bioassays in the battery at hand include those for molluscicidal, larvicidal, antibacterial and tumour growth inhibitory activities. In collaboration with other groups, anti-inflammatory and antimalarial tests have been performed. The range of bioassays needs to be as large as possible in order to make the most of the vegetable material available.

When one looks at Table 6, it is evident that only a small fraction of the plant species collected in collaboration with the different participating countries has undergone thorough phytochemical analysis. Many of the plant extracts have not tested positive in the available bioassays and for this reason have not been further studied. However, the fact that a biological activity has not been detected does not mean that the plant is uninteresting. It may contain natural products with other activities or useful lead compounds which can be modified to provide interesting therapeuticals. Thus, there is still a tremendous amount of work to be done and this needs to be accomplished rapidly, before the natural habitats of the plants are destroyed.

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