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Investigation of Medicinal Plants for the Treatment of Neglected Diseases

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Abstract: Higher plants represent a rich source of new molecules with pharmacological properties, which could become lead compounds for the development of new drug products. During the last decades, the renewed interest in investigating natural substances has led to the introduction of several important drugs, such as the anticancer substances vinblastine, vincristine, taxol and camptothecine derivatives, or the antimalarial agent artemisinin. Despite recent scientific and technological advances, parasitic diseases continue to affect millions of people in both tropical and subtropical zones of the world. On the other hand the drugs used for the treatment of most parasitic diseases are extremely limited. In this field, natural products constitute a reservoir of new molecules with potential therapeutic interest. An outline is presented here of some important results obtained by the Laboratory of Pharmacognosy and Phytochemistry of the University of Geneva on plants used to treat some of the neglected diseases. The strategy employed for the study of these plants is outlined, covering all aspects from the selection of plant material to the isolation of the active substances. Different bioactivities have been investigated such as the search for new molluscicidal and larvicidal agents. Results are also included for antileishimanial and antimalarial compounds.

Keywords: Bioactive-guided isolation · Natural products · Parasitic diseases · Plant metabolites

1. Introduction

For a long time, plants have been the almost exclusive therapy available to humans. The plant kingdom is still an untapped reservoir of new molecules with potential therapeutic interest and only a relatively small percentage of the 350,000 known plant species have been studied from a phytochemical and a pharmacological viewpoint. Research in pharmacognosy has demonstrated that potent bioactive products can be obtained from plants. In the present drug discovery programs, natural products or compounds derived from natural products account for more than 40% of new registered drugs. A recent statistical investigation into the structural complementarity of natural and synthetic compounds also proved that the potential for new natural products is not exhausted and they still represent an important source for the lead finding process. There is an increasing medical need for new drugs to cover a range of 'neglected' infectious diseases. Malaria, leishmaniasis and schistosomiasis continue to be the cause of suffering for many millions of people in both tropical and subtropical zones of the world in the last 30 years [1]. The available therapeutic tools for the treatment of most parasitic diseases are extremely limited. Moreover, the development of parasites resistant to many of the available drugs is also responsible for disease persistence and death [1]. New drugs are not being developed quickly enough and potential vaccines have so far not fulfilled expectations in field trials. The molecular diversity and efficacy of antiparasitic plants, extracts and herbal preparations have been discussed extensively in reviews [2–6]. Natural products provide the chance to discover new molecules of unique structure with high activity and selectivity which can be further optimized by semi- or fully synthetic procedures [7].

This article will attempt to show some important results after more than 20 years' work on investigation of medicinal plants for the treatment of neglected diseases by the Laboratory of Pharmacognosy and Phytochemistry.

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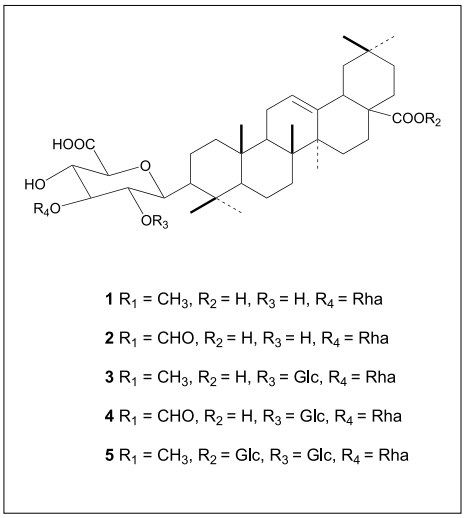
2. Plants Used Against Parasitic Diseases

2.1. *Plants with Molluscicidal Activities*

Schistosomiasis, commonly known as bilharzia, is caused by thread worms of the genus *Schistosoma* and is endemic throughout Africa. It occurs also in Asia and in Central and South America. 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*. 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'-dichloro-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 [8].

CHIMIA **2005**, *59*, No. 6



1 $R_1 = CH_3$, R_2 = H, R_3 = H, R_4 = Rha

2 R_1 = CHO, R_2 = H, R_3 = H, R_4 = Rha

3 $R_1 = CH_3$, R_2 = H, R_3 = Glc, R_4 = Rha

4 R_1 = CHO, R_2 = H, R_3 = Glc, R_4 = Rha

5 $R_1 = CH_3$, R_2 = Glc, R_3 = Glc, R_4 = Rha

Table 1. Molluscicidal activities of saponins from *Bobgunnia madagascariensis* (Leguminosae)

Saponin	R_1	R_2	R_3	R_4	Molluscicidal activity[a]
1	CH_3	H	H	Rha	3
2	CHO	H	H	Rha	25
3	CH_3	H	Glc	Rha	25
4	CHO	H	Glc	Rha	>50
5	CH_3	Glc	Glc	Rha	No activity

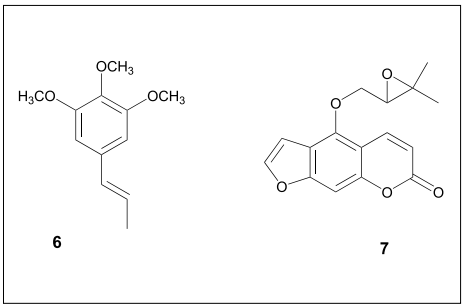
[a] Molluscicidal activity against *Biomphalaria glabrata* [mg/l].

Bobgunnia madagascariensis (Desv.) J.H. Kirkbr. & Wiersama (formerly known as *Swartzia madagascariensis* (Desv.) Leguminosae) 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 [9]. Phytochemical investigation has enabled the identification of the saponins responsible for the molluscicidal activity of an aqueous extract of the dried fruits [10]. 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 **1**–**5** (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 **1** has also been isolated from the root bark of *Diospyros zombensis* (B.L. Burtt) F. White (Ebenaceae), a tree found in Malawi [11]. The results of biological testing showed that saponin **1** 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 (**2** and **4**) had a lower activity (>25 mg/1). The bidesmosidic saponin **5**, carrying an additional sugar moiety at position C-(28) of oleanolic acid, had no activity.

2.2. *Plants with Larvicidal Activities*

Mosquitoes, in particular species of *Anopheles*, *Aedes* and *Culex*, are important vectors of tropical diseases. *Aedes* species, and most notably *Aedes 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 WHO for the control of these tropical diseases is to destroy their vectors. 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 [12]. 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 h.

In the course of this screening, a few useful leads have been picked up. The dichloromethane leaf extract of *Diplolophium buchanani* (Benth. ex Oliv.) Norman, 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 (**6**), elemicin, *trans*-isoelemicin, together with the furanocoumarin oxypeucedanin (**7**). 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) [13].



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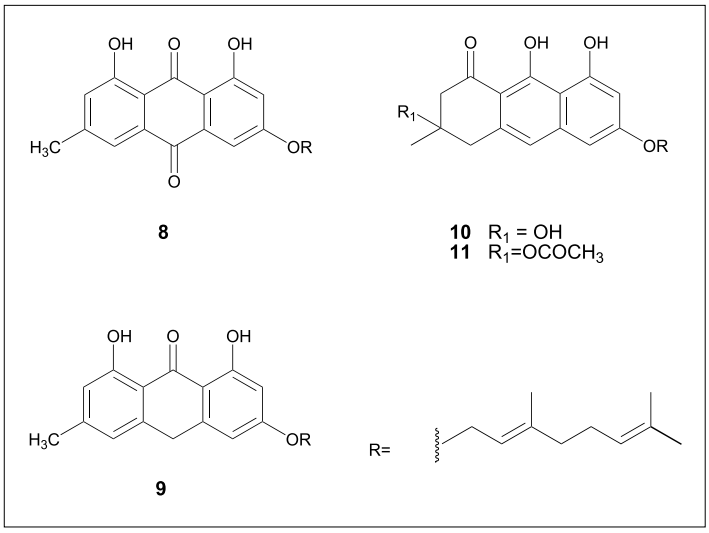
2.3. *Plants with Antimalarial Activities*

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 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* Spach. (Guttiferae) root bark displayed appreciable activity (Table 2) and was around four times more active than an ethanolic extract of *Artemisia annua* (Asteraceae), one of the plants presently exciting much hope for the future treatment of malaria [14]. *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 [15]. The tetrahydroanthracene vismione D (**10**) was the most active

CHIMIA **2005**, *59*, No. 6

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.



2.4. *Plants with Antileishmanial Activities*

Leishmaniases are parasitic diseases with a wide range of clinical symptoms: cutaneous, mucocutaneous and visceral. They are caused by different species of protozoan parasites belonging to the genus *Leishmania* transmitted by the bite of a tiny 2- to 3-mm-long insect vector, the phlebotomine sandfly. This disease affects about 12 million people around the world and the incidence of leishmaniasis is currently increasing [16]. Leishmaniasis is spreading in several areas of the world as a result of epidemiological changes which sharply increase the overlapping of AIDS and visceral leishmaniasis [1]. Since vaccines against leishmaniasis are still under development (see [17]), the control of this disease relies on prompt diagnosis and chemotherapy in infected humans as well as in dogs, which are the main reservoir of *Leishmania infantum* in Mediterranean countries [18]. On the basis of these considerations, the study of new molecules for leishmaniasis treatment is strictly necessary.

In the course of our search for new bioactive lead compounds, the dichloromethane extract of the roots of *Thamnosma rhodesica* (Baker f.) Mendonça (Rutaceae) was found to show a marked activity against the intracellular protozoan parasite *Leishmania major*. This is one of the agents of cutaneous leishmaniasis, a disease of man and other species of mammals in which the pathogen invades skin macrophages, leading to characteristic disfiguring lesions. An activity-guided fractionation led to the isolation of nine compounds. All of them were tested against *Leishmania major* [19] (Table 3): rutacridone (**12**) and gravacrinedol (**13**) were found to be slightly active at a 10 μmolar dilution against *L. major* promastigotes without being toxic on macrophages at the same concentration. These compounds did not show any activity against the intracellular parasites. Furthermore, rhodesiacridone (**14**) showed a marked activity at the same concentration against the two stages of parasite. All other compounds were inactive. It is interesting to relate the structure of rutacridone (**12**) with those of skimmianine or of 2-substituted quinoline alkaloids such as chimanines A-D isolated from *Angostura longiflora* (K. Krause) Kallunki, a South American species from the Rutaceae family used by the Chimane Indians in Bolivia in the treatment of cutaneous leishmaniasis [20]. These compounds showed *in vivo* leishmanicidal properties in mice and are among the most promising natural products ever found to treat leishmaniasis.

Table 2. *In vitro* antimalarial activities of extracts of *Psorospermum febrifugum* and *Artemisia annua* and their constituents.

Sample	**Antimalarial activity/IC50 [μg/ml] (*Plasmodium falciparum*)**
P. febrifugum petroleum ether extract	0.82
Compound **9**	50
Compound **9**	5.6
Vismione D (**10**)	0.095
Acetylvismione D (**11**)	0.383
Artemisia annua ethanol extract	3.9
Artemisinin	0.0028
Quinine 2.HCl	0.038

3. Conclusion

Based on the knowledge that traditional medicine has historically furnished a wide variety of therapeutically active and food plants, the Laboratory of Pharmacognosy and Phytochemistry has been involved for over 20 years in research into the phytochemistry and bioactivity of plant constituents. The approach employed for this on-going investigation is briefly outlined in this article. Molluscicidal, larvicidal, antimalarial and antileishmanial tests have been performed with a great number of plant extracts. 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 an important number of active compounds. Many of the plant extracts have not shown activity 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 therapeutics. 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.

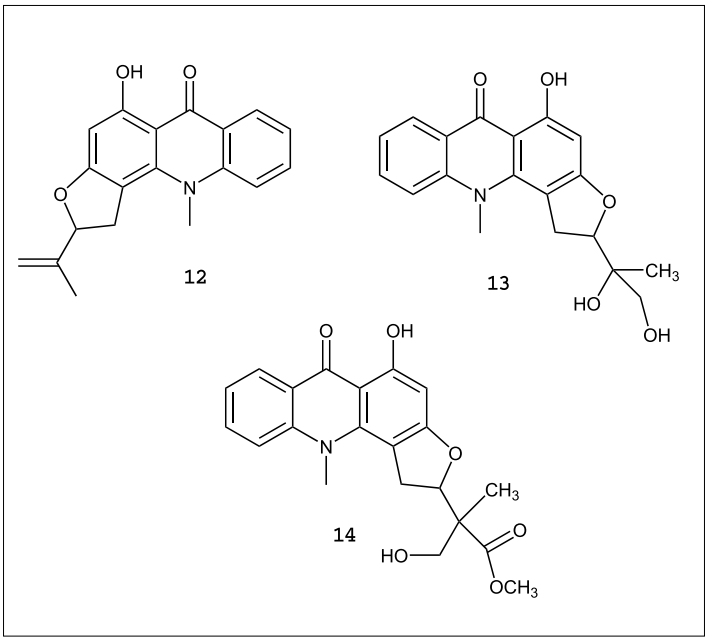


Table 3. Antileishmanial and antifungal activities of the isolated compounds from the heartwood of *Thamnosma africana*

Compounds	*Leishmania major promastigote*[a]		*Leishmania major amastigote*[b]	
12	34.9 ± 1.5	69.9 ± 2.8	88.0 ± 5.1	82.0 ± 4.0
13	54.0 ± 1.1	97.2 ± 2.2	9.5 ± 1.0	58.0 ± 3.1
14	30.7 ± 3.2	96.0 ±1.8	6.2 ± 0.7	48.6 ± 2.7
Amphotericin B	0.2 ± 0.04	71.9 ± 4.4	0.4 ± 0.02	0.5 ± 0.03
Nystatine	n.t.	n.t.	n.t.	n.t.

[a]Extracellular survival (%) of *L. major* at 10 μM (left hand-side column) and 1 μM (right hand-side column); [b]Intracellular survival (%) of *L. major* at 10 μM (left hand-side column) and 1 μM (right hand-side column); n.t. not tested

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[1] 'Drugs Against Parasitic Diseases: R&D Methodologies and Issues', Eds. A.H. Fairlamb, R.G. Ridley, H.J. Vial. Discoveries and Drug Development, World Health Organization, Geneva, Switzerland, **2001**.
[2] J.D. Phillipson, C.W. Wright, *J. Ethnopharmacol.* **1991**, *32*, 155–165.
[3] J. Phillipson, C.W. Wright, G.C. Kirby, D.C. Warhust, in 'Tropical Plants as Source of Antiprotozoal Agents'. Eds. K.R. Downum, J.R. Romeo, H.A. Staord, 'Phytochemical Potential of Tropical Plants. Recent Advances in Phytochemistry', Plenum Press, New York, **1993**, vol 27, pp 1–40.
[4] U. Holzgrabe, A. Bechthold, *Chemother. J.* **1999**, *8*, 69–78.
[5] M.R.C. Corona, S.L. Croft, J.D. Phillipson, *Curr. Opin. Anti-Infect. Investig. Drugs* **2000**, *2*, 47–62.
[6] O. Kayser, A. Kiderlen, S.L. Croft, *Parasitology Research* **2003**, *90*, 55–62.
[7] K. Hostettmann, 'Tout Savoir sur le Pouvoir des Plantes, Source des Médicaments', Favre SA, Lausanne, Switzerland, **1997**.
[8] A. Marston, M. Maillard, K. Hostettmann, *J. Ethnopharmacol.* **1993**, *38*, 215–223.
[9] A. Mozley, *Trans. Roy. Soc. Edin.* **1939**, *59*, 687.
[10] C. Borel, K. Hostettmann, *Helv. Chim. Acta* **1987**, *70*, 570–576.
[11] F. Gafner, J.C. Chapuis, J.D. Msonthi, K. Hostettmann, *Phytochemistry* **1987**, *26*, 2501–2503.
[12] F. Cepleanu, M. Hamburger, B. Sordat, J.D. Msonthi, M.P. Gupta, M. Saadou, K. Hostettmann, *Int. J. Pharmacog.* **1994**, *32*, 294.
[13] A. Marston, K. Hostettmann, J.D. Msonthi, *J. Nat. Prod.* **1995**, *58*, 128–130.
[14] J.M. Watt, M.G. Breyer-Brandwijk, 'Medicinal and Poisonous Plants of Southern and Eastern Africa', Eds. E. Livingston, S. Livingstone, Edinburgh, **1962**.
[15] K. Hostettmann, A. Marston, in 'Studies in Natural Products Chemistry', Ed. Atta-ur-Rahman, Elsevier, Amsterdam, **1990**, vol. 7, pp. 405.
[16] L. Fumarola, R. Spinelli, O. Brandonisio, *Research in Microbiology* **2004**, *155*, 224–230.
[17] O. Brandonisio, G. Carelli, L. Ceci, B. Consenti, A. Fasanella, V. Puccini, *Eur. J. Epidemiol.* **1992**, *8*, 273–276.
[18] O. Brandonisio, R. Spinelli, *Curr. Drug Targets Immune Endocr. Metabol. Disord.* **2002**, *2*, 193–199.
[19] K.M. Ahua, J.-R. Ioset, A. Ransijn, J. Mauël, S. Mavi, K. Hostettmann, *Phytochemistry* **2004**, *65*, 963–968.
[20] A. Fournet, A.A. Barrios, V. Munòz, R. Hocquemiller, A. Cavé, J. Bruneton, *Antimicrobial Agents and Chemotherapy* **1993**, *37*, 859–863.