Investigation of medicinal plants for the treatment of neglected diseases

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Investigation of Medicinal Plants for the Treatment of Neglected Diseases

Emerson F. Queiroz, Kouassi Maximin Ahaa, and Kurt Hostettmann*

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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 therapeut-ic interest and only a relatively small per-cent age of the 350,000 known plant species have been studied from a phytochemical and a pharmacological viewpoint. Research in pharmacognosy has demonstrated that potential 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 im-portant 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 deve-loped quickly enough and potential vac-cines 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 fur-ther 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 Phy-tochemistry.

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 through-out 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 prob-lem of schistosomiasis is to destroy the carrier snails (mollusciciding) and thus remove a link in the life cycle. This may be achieved with the aid of synthetic products such as Bayluscide (2,2-dichloro-4-nitro-salicylanilide) or, alternatively, with molluscicides from plant sources. The use of molluscsidal plants growing abundantly in areas where schistosomiasis is endemic is a simple, inexpensive and appropriate tech-nology for local control of the snail vector and may become in the near future a useful complement for the control of this disease [8].
Bohugmia madagascariensis (Desv.) J.H. Kirkbr. & Werners (formerly known as Swartzia madagascariensis (Desv.) Lecomminea) 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]. Physichochemical 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 saponin 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 oleandrin acid and of gypsogenin by chemical and spectral means (FAB-MS, 1H-NMR, GC-MS of methylated alditol acetates). It is interesting to note that all the saponins carry a hexa- or penta-pyranosyl unit at position C-3 of the glucuronic acid moiety. Saponin 1 has also been isolated from the root bark of Diospyros zanzibarica (J.L. Burm.) 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/l) against Biomphalaria glabrata snails. Saponins with disubstituted glucuronic acid, as well as those with gypsogenin aglycones (2 and 4) had a lower activity (25 mg/l). The bidesmosidic saponin 5 carrying an additional sugar moiety at position C-26 of oleandrin acid, had no activity.

2.2. Plants with Larvicidal Activities

Mosquito, 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 (arbovirus or virus) such as yellow fever and dengue fever. White 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 may represent an alternative to the use of synthetic pesticides, cheap and readily available so 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 each day when put into tap water and incubated for 24 h. The assay consists of exposing approximately 20 larvae to various dilutions of the extract, previously submitted to DMSC. Mortality is evaluated with the naked eye after 30 min. and 24 h. A sample is considered active when all larvae are killed after 24 h.

In the coarse of this screening, a few useful leads have been picked up. The dihydrochloride leaf extract of Diplophili- um buchmannii (Benth. ex Olivi) Norman, a shrub of the family Apocynaceae 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 phenolpropanoids myristicin (6), elemi-in-trans-isoelemo, together with the furanocoumarin oxypseudocumatin (7). Myristicin and oxypseudocumatin (LC50 25 mg/l) were larvicidal at concentrations similar to that of the reference compound β-asarone (LC50 16 mg/l) [13].

2.3. Plants with Antimalarial Activities

Different species of Anopheles mosquitoes are responsible for the transmission of malaria which still remain 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 drug, 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 concludes the inhibition of incorporation of 3H-hypoxanthine by malaria parasites, using a multidrug resistant K1 strain of Plasmodium falciparum. In this bioassay, the petroleum ether extract of Pescospermum fenugricum Spach. (Gut- tiferae) 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 existing much hope for the future treatment of malaria [14]. P. fenugricum 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 intrahod dentro- cene viscometer D (8) was the most active.
compound of *P. falciparum*, 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. falciparum* and the pure compounds has shown their unsuitability for future development because of their toxicity to mice.

### 2.4. Plants with Antileishmanial Activities

Leishmaniasis 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); ruticidone (12) and grava-
crinedol (13) were found to be slightly active at 18 μMolar dilution against *L. major* promastigotes without being toxic on macrophages at the same concentration. These compounds did not show any activity against far intracellular parasites. Furthermore, rhodesicaridine (14) showed a marked activity at the same concentration against the two stages of parasite. All other compounds were inactive.

### Table 2. In vitro antimalarial activities of extracts of *Porospermum falciparum* and *Artemisia annua* and their constituents.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antimalarial activity/IC50 (μg/ml) (Plasmodium falciparum)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> petroleum ether extract</td>
<td>0.82</td>
</tr>
<tr>
<td>Compound 9</td>
<td>50</td>
</tr>
<tr>
<td>Compound 9</td>
<td>5.6</td>
</tr>
<tr>
<td>Vamorone D (16)</td>
<td>0.095</td>
</tr>
<tr>
<td>Acetylvamorone D (11)</td>
<td>0.383</td>
</tr>
<tr>
<td>Artemisia annua ethanol extract</td>
<td>3.9</td>
</tr>
<tr>
<td>Artemisin</td>
<td>0.0028</td>
</tr>
<tr>
<td>Quinine 2HCl</td>
<td>0.038</td>
</tr>
</tbody>
</table>

American species from the Rutaceae family used by the Chimane Indians in Bolivia in the treatment of cutaneous leishmaniasis [20]. These compounds showed in vitro leishmanicidal properties in mice and are among the most promising natural products ever found to treat leishmaniasis.

### 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. Molluscidal, 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 need to develop these compounds and this needs to be accomplished rapidly, before the natural habitats of the plants are destroyed.
Table 5. Antileishmanial and antifungal activities of the isolated compounds from the heartwood of *Tetradendron strigosum*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Leishmania major promastigotea</th>
<th>Leishmania major amastigotea</th>
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</thead>
<tbody>
<tr>
<td>12</td>
<td>34.9 ± 1.5</td>
<td>68.0 ± 5.1</td>
</tr>
<tr>
<td>13</td>
<td>54.0 ± 1.1</td>
<td>58.0 ± 3.1</td>
</tr>
<tr>
<td>14</td>
<td>30.7 ± 3.2</td>
<td>48.6 ± 2.7</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.2 ± 0.04</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td>Nystatine</td>
<td>n.t.</td>
<td>n.t.</td>
</tr>
</tbody>
</table>

aExtracellular survival (%) of *L. major* at 10 μM (left hand-side column) and 1 μM (right hand-side column); bIntracellular survival (%) of *L. major* at 10 μM (left hand-side column) and 1 μM (right hand-side column); n.t. not tested.

Acknowledgements

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