MEDICINAL PLANTS AS A SOURCE OF ANTIPLASMODIAL COMPOUNDS

C. Ramalhete[1], D. Lopes[2], S. Mulhovo [3], V. Rosário[2], M. J. U. Ferreira[1]

iMed.UL
Institute for Medicines and Pharmaceutical Sciences

[1]iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600-083 Lisbon, Portugal;
[2]CMDT, Institute of Hygiene and Tropical Medicine, UNL, R. da Junqueira 96, 1349-008 Lisbon, Portugal;
[3] Polytechnic Institute of Gaza (ISPG), Chokwe, Mozambique.

INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to public health, despite great progress in therapy. The impact of infectious diseases is especially important in developing countries, where drugs are limited and the emergence of widespread drug resistance is a reality.[1]
Malaria is a major parasitic disease in the tropical and subtropical regions of the world. It is responsible for over 1 million deaths per year and approximately 3.2 billion people are presently at risk in several countries.[2] An example is Mozambique, one of the ten most affected nations. In this country, malaria is the most common disease and the primary cause of morbidity and mortality. To a large majority of people in Mozambique, the treatment is mainly based on traditional medicine. In fact, from Mozambique's approximately 5500 plant species, up to 10 percent have been identified as being used to treat parasitic diseases such as malaria or its symptoms.[3] However, few phytochemical studies have yet been published.

The aim of this study was to carry out a scientific evaluation of the claimed antimalarial properties of plants used in traditional medicine against malaria and fever, in order to validate their use and to determine their potential as new sources of antimalarial drugs.

Figure 1. Some of the most active plants.

RESULTS AND DISCUSSION

The antimalarial activity of fifty seven extracts, prepared by sequential extraction with solvents of increased polarity (hexane, dichloromethane, ethyl acetate and methanol) was evaluated against 3D7 *P. falciparum* strain.

As illustrated in Table 1 and figure 2, from the fifty seven extracts tested, two of them (4 %) showed a significant activity (IC_{50} < 5 µg/ml), 14 extracts (25%) showed moderate activity (10 <IC_{50} < 50 µg/ml), 20 (35 %) weak activity (50 <IC_{50} < 100 µg/ml) and 21 extracts (36%) were inactive (IC_{50} > 100 µg/ml).

The bioguided-fractionation of the most active extract of *Momordica balsamina* L., by chromatographic techniques, has afforded three major compounds (figure 3), which share the cucurbitane skeleton: 7-methoxycucurbita-5,24-diene-3β,23β-diol (1), 3β,23β-dihydroxycucurbita-5,24-dien-7-*O*-*β*-D-allopyranoside (2) and 3β,23β-dihydroxycucurbita-5,24-diene-7-*O*-*β*-D-glucoside (3). Their structures were established by physical and spectroscopic data (IR, MS, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR – ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY), (Figure 4). The evaluation of the antiplasmodial activity of the compounds, against sensitive and resistant strains *P. falciparum,* is ongoing.

The preliminary results showed by the plant extracts and fractions are promising and seem to validate the traditional use of *Momordica balsamina* L. in the treatment of malaria by local people.

Table 1. Antimalarial activity of crude extracts against *P. falciparum* 3D7.

Species	Family	IC_{50} (µg/ml) Hexane Extract	CH_2Cl_2 Extract	AcOEt Extract	MeOH Ectract
***Acacia Karroo* Hayne**	Fabaceae	99	60	52	>100
***Aloe parvibracteata* Schonland**	Aloaceae	>100	>100	>100	-
***Bridelia cathartica* Bertol. f.**	Euphorbiaceae	99	>100	44	>100
***Cassia abbreviata* Oliv.**	Fabaceae	>100	40	>100	>100
***Cassia occidentalis* L.**	Fabaceae	**36**	60	48	88
***Crossopteryx febriguga* Benth**	Rubiaceae	-	59	-	>100
***Leonotis leonurus* R. Br.**	Lamiaceae	>100	61	**31**	>100
***Momordica balsamina* L.**	Cucurbitaceae	>100	**22**	**1**	75±
***Parkinsonia aculeata* L.**	Caesalpiniaceae	32	66	**35**	80
***Pittosporum tobira* W. T. Aiton**	Pittosporaceae	**28**	57	**5**	>100
***Plumbago auriculata* Lam.**	Plumbaginaceae	67	46	35	80
***Schlefflera actinophylla* Harms**	Araliaceae	**22**	34	41	>100
***Senna didymobotrya* Fresen.**	Fabaceae	64	92	99	75
Tabernaemontana elegans Strapt.	Apocynaceae	59	**27**	99	>100
***Trichilia emetica* Vahl**	Meliaceae	>100	>100	>100	>100

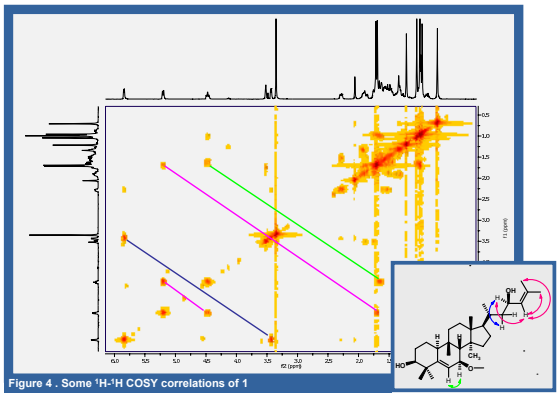


Figure 4 . Some ^{1}H-^{1}H COSY correlations of 1

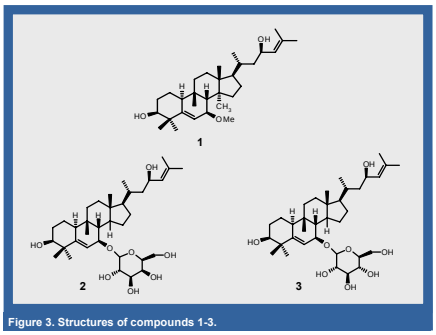


Figure 3. Structures of compounds 1-3.

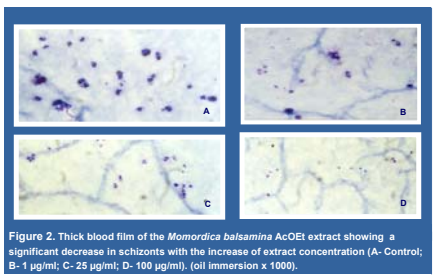
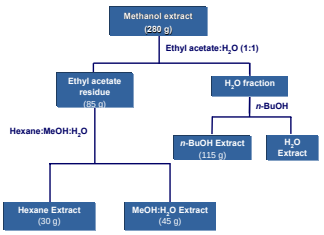


Figure 2. Thick blood film of the *Momordica balsamina* AcOEt extract showing a significant decrease in schizonts with the increase of extract concentration (A- Control; B- 1 µg/ml; C- 25 µg/ml; D- 100 µg/ml). (oil immersion x 1000).

Scheme 1- Methodology of the liquid-liquid extraction.



MATERIALS AND METHODS

Preparation of plant extracts: The air-dried powdered plant parts (roots, leaves, seeds, and bark) or whole plant were sequentially extracted, with hexane, dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt), and methanol (MeOH), at room temperature.

Bioguided-frationation of *Momordica balsamina* L.: The powdered aerial parts of Momordica balsamina were extracted at room temperature with methanol. The methanol extract was further fractionated, by liquid-liquid extraction, according to Scheme 1. The resulting methanol:H_2O extract was then studied, by using chromatographic methods, until the isolation of pure compounds.

Antimalarial test: The *in vitro* antimalarial activity of crude extracts was evaluated by means of the Mark III test, as developed by the WHO. *Plasmodium falciparum* strain 3D7, sensitive to chloroquine was cultured in RPMI 1640 medium containing 25 mm HEPES and 6.8 m hipoxantin supplemented with 0.5% AlbuMax II at 37 ºC and under atmosphere of 5% O2, 3-5% CO2, and N2. The crude extracts solutions, fractions (5mg/ml) and pure compounds (1mg/ml) were prepared by dissolution in DMSO or Ethanol followed by dilution in RPMI.

Acknowledgement: FCT, Portugal (PhD grant SFRH/BD/22321/2005).

References: [1] Mf Dolabela, SG Oliveira, JM Nascimento, JM Peres, H Wagner, MM Póvoa, AB de Oliveira, *Phytomedicine*, 2008, 15, 367-372; [2] WHO, World Malaria Report 2008, Geneva. [3]"WHO/HTM/GMP/2008.1, 1-215. [3] *Community-Based Solutions for Effective Malaria Control: Lessons from Mozambique*, United States Agency for International Development (USAID), March 2005.