our future through science

Antimalarial properties of South African medicinal plants

[1]C CLARKSON, [2]VJ MAHARAJ, [3]NR CROUCH, [2]P PILLAY, [4]N BHAGWANDIN, [1]PJ SMITH, [4]PI FOLB, [5]R VLEGGAAR, [1]K CHIBALE

[1]Pharmacology Division/Department of Chemistry, University of Cape Town,K-45 OMB GSH, Observatory, South Africa, 7925
[2]Biosciences, CSIR, P.O. Box 395, Pretoria, South Africa, 0002
[3]Ethnobotany Unit, South African National Biodiversity Institute, P.O. Box 52099, Berea Road, Durban, South Africa, 4007
[4]Medical Research Council, P.O. Box 19070, Tygerberg, South Africa, 7505
[5]Department of Chemistry, University of Pretoria, Pretoria, South Africa, 0002.
[2]Email: ppillay@csir.co.za

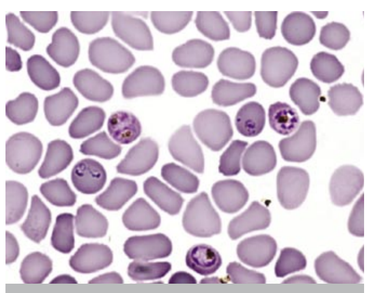
INTRODUCTION

Malaria continues to be one of the major health problems facing Sub-Saharan Africa. One of the contributing factors to the increased prevalence and distribution of the disease is the emergence and spread of drug resistant malaria parasites, highlighting the need for new chemically diverse, effective drugs. Historically, one of the major sources of antimalarial agents and novel template compounds has been higher order plants. South Africa boasts remarkable biodiversity, with over 24 000 indigenous plant species, as well as a long tradition of medicinal plant use. A national multidisciplinary-consortium was established to scientifically investigate South African medicinal plants for the treatment of malaria. The results arising from the study of one of the plants, *Vernonia staehelinoides Harv*. (*Asteraceae*), are discussed.

METHODOLOGY

- Plant taxa, native to or naturalised in South Africa with reported medicinal use related to malaria and/or fever, were selected semi-quantitatively using weighted criteria. Plant material, collected from various locations around the country, was dried, ground and extracted sequentially with dichloromethane, dichloromethane/methanol (1:1), methanol and water.
- Extracts of 134 taxa, representing 54 families, were tested for *in vitro* activity against a *Plasmodium falciparum* strain D10 (chloroquine-sensitive) using the parasite lactate dehydrogenase (pLDH) assay. Active extracts ($IC_{50} \leq 10$ µg/ml) were evaluated against the chloroquine-resistant K1 *P. falciparum* strain. Plants were prioritised for further study based on *in vitro* efficacy and literature precedent.
- The organic extracts of *V. staehelinoides*, collected in the Magaliesburg region of the Gauteng province in South Africa, were subjected to bioassay-guided fractionation using silica gel chromatography and in vitro antiplasmodial activity against the D10 *P. falciparum* strain as the biological indicator.
- In order to determine the specificity of the antiplasmodial activity, the active compounds were tested for cytotoxicity against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
- Compounds were characterised by NMR spectroscopy and mass spectrometry.

Vernonia staehelinoides



Plasmodium falciparum

RESULTS AND DISCUSSION

Of the 134 plant taxa assayed, 66 species (49%) showed promising antiplasmodial activity with IC_{50} values of ≤ 10 µg/ml and 23 species (17%) were found to be highly active with IC_{50} values of ≤ 5 µg/ml (Clarkson et al, 2004). Several plant species were shown for the first time to possess in vitro antiplasmodial activity, of which *V. staehelinoides* was one (**Table 1**).

Extract type	D10 (Exp. 1) IC_{50} (µg/ml)	D10 (Exp. 2) IC_{50} (µg/ml)	K1 IC_{50} (µg/ml)
Dichloromethane	2.0	4.0	2.8
Dichloromethane/methanol (1:1)	3.0	9.0	4.5
Water	>10	>10	-
D10 = *P. falciparum* chloroquine sensitive strain KI = *P. falciparum* chloroquine resistant strain			

Table 1: *In vitro* antiplasmodial activity of *V. staehelinoides* extracts

Bioassay-guided fractionation of the active organic extracts identified hirsutinolides **1**, 8α-(2-methylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-O-acetate, and **2**, 8α-(5′-acetoxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-O-acetate, as the active components of *V. staehelinoides* (**Figure 1**). Overall, compounds **1** and **2** may not be considered as viable antimalarial drug leads as their activity and selectivity does not compare to that of standard clinically used drugs such as chloroquine (**Table 2**). The compounds, however, proved to be attractive scaffolds for structure-activity relationship studies.

Compound	D10 IC_{50} (ng/ml)	K1 IC_{50} (ng/ml)	CHO IC_{50} (µg/ml)	SI*
Chloroquine	12	182	18.5	1542
1	260	1800	2.9	11
2	240	2600	0.9	4
Mucochloric acid	152	137	4.8	32
Mucobromic acid	422	359	6.3	15
D10 = Antiplasmodial activity against *P. falciparum* chloroquine-sensitive strain KI = Antiplasmodial activity against *P. falciparum* chloroquine-resistant strain CHO = Cytotoxicity against Chinese hamster ovarian cells SI (selectivity index) = cytotoxicity CHO IC_{50} / antiplasmodial D10 IC_{50}				

Table **2:** *In vitro* antiplasmodial activity, cytotoxicity and SI values for chloroquine, compounds **1** and **2**, mucochloric acid and mucobromic acid

Two main privileged substructures, a 2(*5H*)-furanone unit and a dihydrofuran-4-one unit, were identified as potential pharmacophores (**Figure 1**). In order to verify this, mucochloric and mucobromic acids were selected as appropriate 2(*5H*)-furanone substructures and were found to display superior activity against the K1 strains and improved selectivity to the malaria parasites relative to the hirsutinolide natural product (**Table 2**). The antiplasmodial data obtained in respect of mucochloric and mucobromic suggests that the 2(*5H*)-furanone is at least one of the key pharmacophores responsible for the observed antiplasmodial activity and the synthesis of structure-activity derivatives around these simplified structures is currently under way.

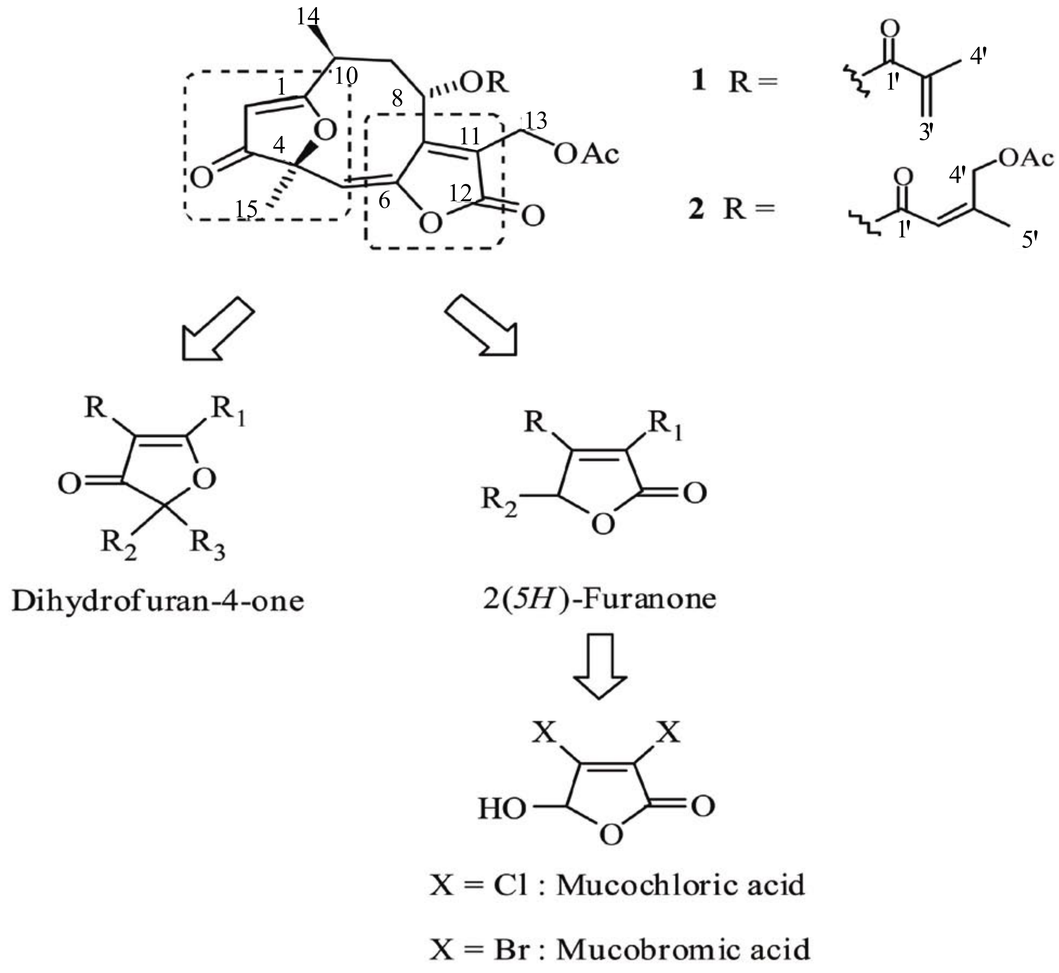


Figure 1: Structures of hirsutinolides **1** and **2**, and the identified pharmacophores

CONCLUSIONS

The study identified a number of promising South African medicinal plants for further investigation as plant-based antimalarial agents. The overall screening results, coupled with the identification of potential pharmacophores from the further investigation of the antiplasmodial properties of *V. staehelinoides*, support a rational rather than random approach to the selection of screening candidates as potential sources of antimalarial lead compounds.

REFERENCES

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., Folb, P.I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J. Ethnopharmacol.* 92, pp. 177-191

Pillay, P., 2005. Structure elucidation of antiplasmodial sesquiterpene lactones from *Vernonia Staehelinoides* and *Oncosiphon piluliferum*. MSc thesis, University of Pretoria, Pretoria, pp. 33-52.