Antimalarial Sesquiterpene Lactones from *Oncosiphon piluliferum*

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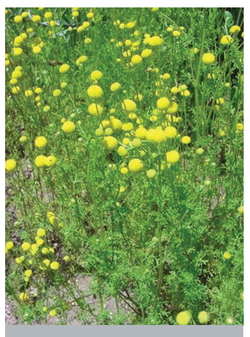
INTRODUCTION

Malaria continues to be a major cause of mortality and morbidity, especially in Sub-Saharan Africa. The emergence and spread of drug resistant parasites has highlighted 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. A national multidisciplinary-consortium was established to scientifically investigate South African medicinal plants for the treatment of malaria. Over 134 plant taxa native to or naturalised in South Africa were selected and plant extracts thereof tested for *in vitro* activity against the D10 *P. falciparum* strain of which 17% were found to be highly active ($IC_{50} \leq 5\mu g/ml$). The work presented reports on the active ingredients isolated from the indigenous plant, *Oncosiphon piluliferum*, their antiplasmodial activity against the D10 *P. falciparum* strain and cytotoxicity against Chinese Hamster Ovarian (CHO) cells.



Oncosiphon piluliferum growing in Graaf-Reinet

METHODOLOGY

Plant material was collected from Middleburg in the Eastern Cape and extracted with dichloromethane. The crude extract was subjected to bio-assay guided fractionation using *in vitro* antiplasmodial activity against the D10 *P. falciparum* strain as the biological indicator. Parasite viability was measured using the parasite lactate dehydrogenase (pLDH) assay. Compounds were tested for *in vitro* cytotoxicity against a CHO cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Compounds were characterised by NMR spectroscopy, mass spectrometry, X-ray crystallography and selected derivatisations.

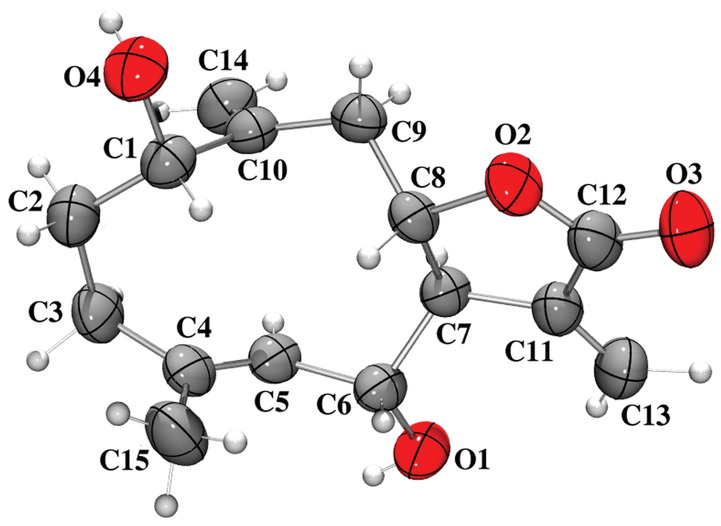


Figure 1: X-ray structure of compound (**5**)

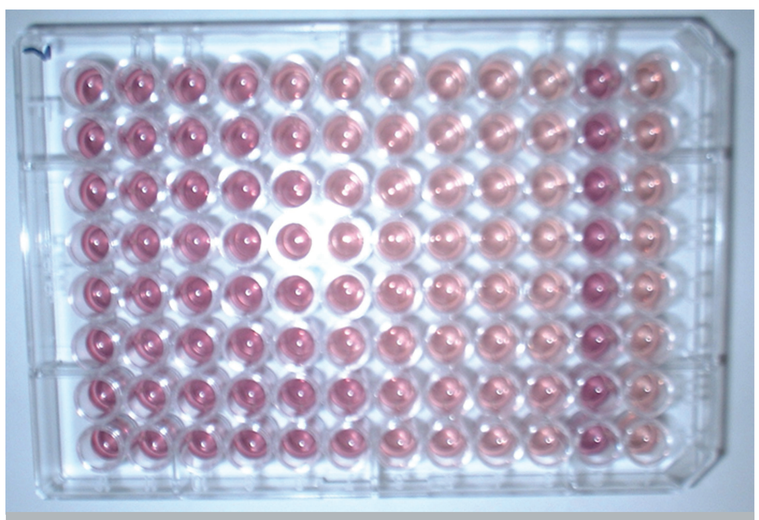
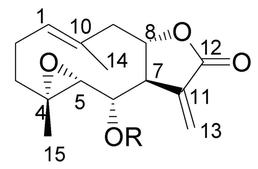
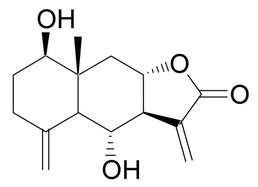
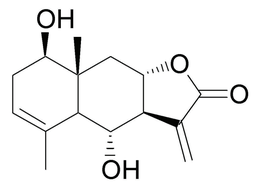
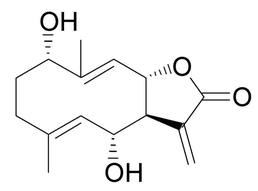
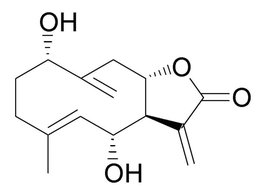
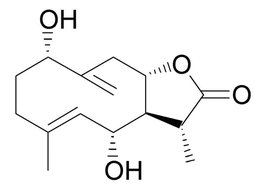


Figure 2: pLDH *in vitro* assay

RESULTS AND DISCUSSION

Five sesquiterpene lactones of the germacranolide and eudesmanolide type were isolated and identified, (**Table 1**, Compounds (**1**) – (**5**)). Due to the marked instability of the germacranolide (**1**), the structural elucidation and bioassaying was conducted on the acetylated derivative (**1a**). Mosher's method was applied to determine the absolute stereochemistry of the major compound (**5**), identifying it as (1*S*,6*R*,7*S*,8*S*)-1,6-dihydroxy-4*E*,10(14),11(13)-germacratrien-12,8-olide.The reduction of the α-methylene group of compound (**5**) using $NaBH_4$/MeOH yielded compound (**6**).

Compound	D10 IC_{50} ($\mu g/ml$)	CHO IC_{50} ($\mu g/ml$)	SI*
Chloroquine	11.1×10^{-3}	18.5	1666.7
 (**1**) R = H (**1a**) R = Ac	0.5 #	2.2 #	4.4 #
 (**2**) desacetyl-β-cyclopyrethrosin	4.4	10.1	2.3
 (**3**) Sivasinolide	2.6	4.0	1.5
 (**4**) tatridin A / tavulin	0.4	6.0	15.0
 (**5**) Tanachin	0.5	6.4	12.8
 (**6**)	70.0	>100	>1.4

* SI (selectivity index) = cytotoxicity CHO IC_{50}/antiplasmodial D10 IC_{50}

\# Results for (**1a**)

Table 1: *In vitro* antiplasmodial activity, cytotoxicity and SI values for chloroquine and compounds (**1a**) - (**6**)

The germacranolides (**1a**), (**4**) and (**5**) showed equipotent antiplasmodial activity and were found to be significantly more active than the eudesmanolides (**2**) and (**3**). The antiplasmodial and cytotoxicity assay results of compound (**6**) clearly show that the C(11)-C(13) exocyclic double bond of compound (**5**) is primarily responsible for both the antiplasmodial activity and toxicity to CHO cells, as both are significantly decreased when this double bond is reduced.

CONCLUSION

None of the compounds was sufficiently active or selective to be a viable drug candidate but the potential for further structure-activity relationship (SAR) studies exists. The compounds could be used as scaffolds to generate lead molecules with enhanced antiplasmodial activity and reduced cytotoxicity. Further SAR work will also provide more insight into whether the observed antiplasmodial activity is due to biological activity or general cytotoxicity.

REFERENCES

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2. Pillay P, 2005. Structure elucidation of antiplasmodial sesquiterpene lactones from *Vernonia Staehelinoides* and *Oncosiphon piluliferum*. MSc thesis, submitted to University of Pretoria, Pretoria, pp. 53-97.