Isolation of compounds from *Sceletium tortuosum* and the detection of antimalarial activity of the isolates and extracts

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INTRODUCTION

The genus *Sceletium* is classified as part of the Family Mesembryanthemaceae and belongs to the sub-family Mesembryanthemoideae. Some of the names used when referring to this genus are 'living skeletons' or 'skeleton plants' (http://ujdigispace.uj.ac.za), and popularly known *kanna* (Khoi) and *kougoed* (Afrikaans), the latter referring to the use of the plant material by chewing. The genus *Sceletium* occurs in the south-western parts of South Africa and these plants have an affinity for arid environments; they are also reported to occur in the Namaqualand Rocky Hills, Knersvlakte and Ceres Karoo (Gerbaulet, 1996). The genus is derived from 'sceletus' meaning skeleton which refers to the prominent leaf veins visible in the dry and withered leaves. The species are distinguished on the basis of vegetative, flower, fruit and seed characteristics (Gericke and Viljoen, 2008).

Figure 1.1: *Sceletium* species are characterised by their decumbent habit, succulent leaves and flowers ranging from white, to light yellow or pink (Gericke & Viljoen, 2008)

Figure 1.2: Geographical distribution of *Sceletium* in South Africa (redrawn from Smith et al., 1998)

Traditionally, plants of the genus Sceletium (Mesembryanthemaceae) have been used to relief thirst and hunger, to combat fatigue, as medicines and for social and spiritual purposes by San hunter-gatherers and Khoi pastoralists (Gericke and Viljoen, 2008).

OBJECTIVES

The study focused on the phytochemical isolation of compounds and biological screening of isolated constituents together with crude extracts for various diseases with particular reference to malaria

METHODOLOGY

Plant collection

Aerial plant material was collected at Kamieskroon (Northern Cape, South Africa). Storage, transportation and handling were done according to standard operating procedures.

EXTRACTION

The extraction process followed is summarised in the flow diagram below (**Figure 2.1**)

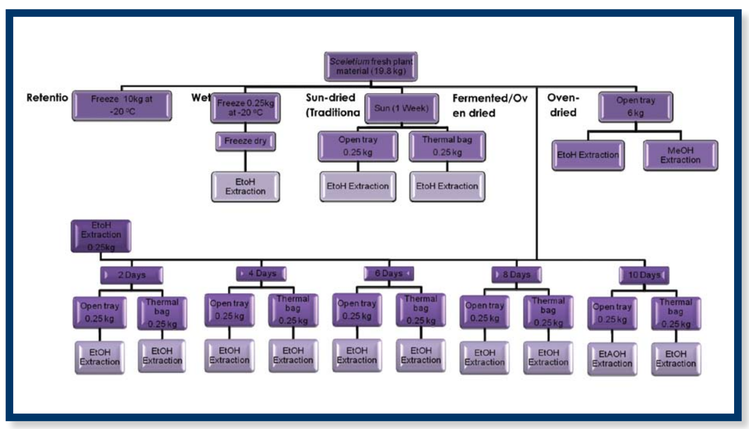


Figure 2.1: Extraction process followed for *Sceletium tortuosum*

Phytochemistry

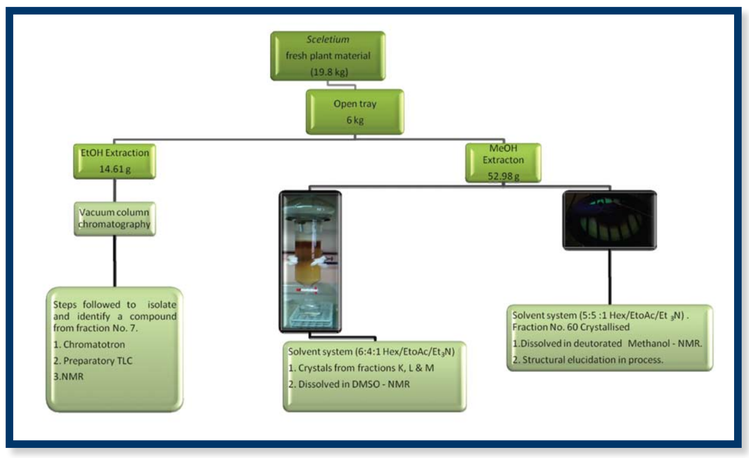


Figure 2.2: Summary of phytochemical process

pLDH SCREENING

An *in vitro* diagnostic assay based on the specific detection of *Plasmodium* lactate dehydrogenase (pLDH) activity was used. This assay exploits a panel of monoclonal antibodies that capture the parasite enzyme and allow for the quantitation and speciation of human malaria infection (Piper *et al*., 1999).

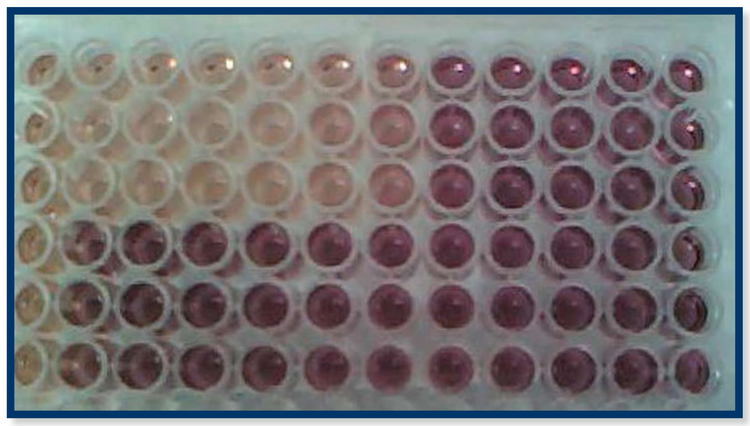


Figure 2.3: Plasmodium lactate dehydrogenase (pLDH) assay plate

RESULTS AND DISCUSSION

The major compound from the ethanol extract was isolated and identified as mesembrine. This was determined by comparing chemical shifts of ^{1}H and ^{13}C NMR spectra to those found in the literature. Outstanding features of the proton NMR spectrum are the aromatic, N-methoxy and N-methyl groups. The ^{13}C spectra showed a carbonyl carbon peak at 211.5 ppm and six carbons signals in the aromatic region between 108.9 ppm and 148.8 ppm.

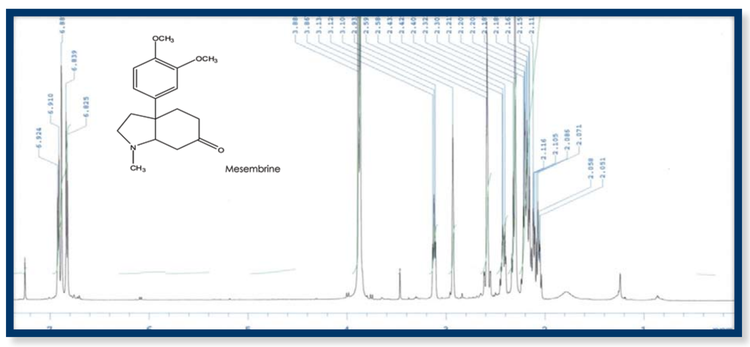


Figure 3.1: 600 MHz 1H NMR spectrum and structure of mesembrine

A second isolated compound was suspected to be pinitol. this was based on the characteristics presented on TLC before and after being sprayed with ninihydrin and vanilin. The chemical shifts were compared to those published by Misra and Siddiqi (2004).

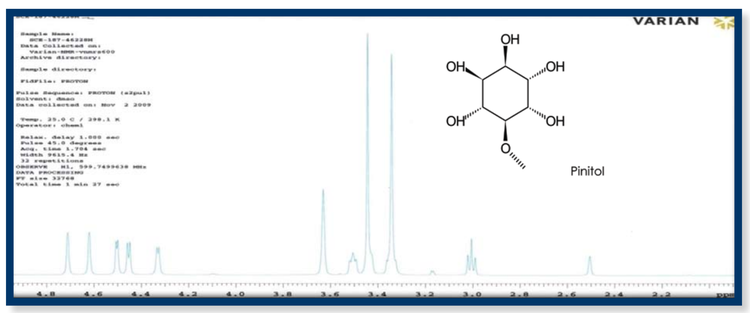


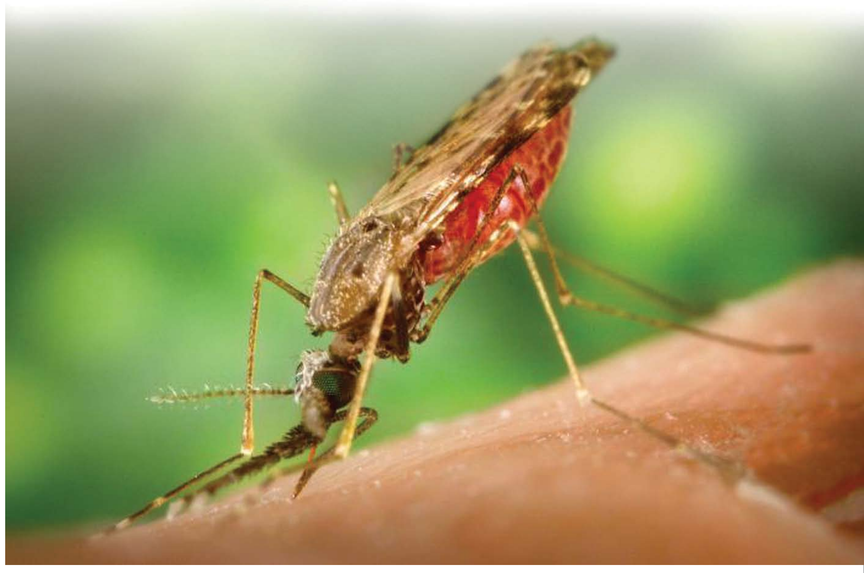
Figure 3.2: 600 MHz 1H NMR spectrum and structure of pinitol

Table 3.1: IC50 and Z'-factors of test samples from a single screening assay

	Test sample	IC50 (µg/ml)	Z' factor
1	SCE187-46201A	37.96	0.59
2	SCE187-46201B	>100	0.59
3	SCE187-46202A	12.10	0.58
4	SCE187-46202B	32.74	0.85
5	SCE187-46204A	>100	0.85
6	SCE187-46204B	31.07	0.57
7	SCE187-46205A	1.5	0.71
8	SCE187-46205B	1.47	0.71
9	SCE187-46205FD	18.14	0.57
10	SCE187-46206A	19.94	0.66
11	SCE187-46206B	15.65	0.66
12	SCE187-46207A	7.32	0.73
13	SCE187-46207B	2.68	0.73
14	SCE187-46208	>100	0.85
15	SCE187-46208A	33.38	0.63
16	SCE187-46208B	32.28	0.63
17	SCE187-46222	69.18	0.85
18	SCE187-46229	>100	0.76
19	SCE187-46245	>100	0.73
20	Control- Chloroquine	11.79	0.70

- The tests were performed in triplicates; those with good activity are shown in red

With malaria being one of the world's deadliest diseases, research combines science and indigenous knowledge to find a solution for all.



Crude extracts SCE187-46205A (1.5 ug/ml), SCE187-46205B (1.47 ug/ml), and SCE187-46207B (2.68 ug/ml) showed potent *in vitro* antimalarial activity against the chloroquine-sensitive strain, 3D7 of *P. falciparum* while SCE187-46207A (7.32 ug/ml) showed moderate activity. The Z' factors for these crude extracts were 0.71 for SCE187-46205A and SCE187-46205B and 0.73 for SCE187-46207A and SCE187-46207B. The two compounds SCE187-46229 (>100 ug/ml) and SCE187-46245 (>100 ug/ml) did not show any activity in the assay

CONCLUSION

Two compounds, namely an alkaloid, mesembrine, and pinitol were isolated and identified from the ethanol extract of the aerial parts of *Sceletium tortuosum*. Different ethanolic extracts of the plants were evaluated for their anti-malarial activity and three extracts exhibited potent *in vitro* anti-malarial activity against the chloroquine-sensitive strain, 3D7 of *P. falciparum*. Bioassay-guided fractionation will be conducted on these extracts to isolate and identify the active constituents

PRESENT AND FUTURE WORK

- LC-MS and HPLC profiling of all crude extracts was successfully completed; analysis in process
- Bioassay-guided fractionation of crude extracts showing good anti-malarial activity
- Further phytochemical isolation and purification of compounds.

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