Bio-evaluation of South African Plants for Insecticidal Properties

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INTRODUCTION

Malaria remains a serious public health problem in South Africa affecting 4 million people in the low altitude areas of the north-eastern parts of the country. The principal vector of malaria in this country is *Anopheles arabiensis*. In South Africa, resistance to pyrethroids and DDT have been reported and the potential for carbamate resistance has been detected in *An. arabiensis*. This resistance detected in the vector populations in South Africa has initiated a search for new insecticides which will be effective, safe and easily accessible at low cost. Larvicides are used as a complementary strategy to indoor residual spraying and have been suggested as a strategy for eliminating over-wintering larval populations.

In the present study, 381 crude plant extracts were investigated for their adulticidal and larvicidal effect on larvae of *An. arabiensis*. These extracts were prepared from plants found in the southern African floral region. The goal of this study was to identify the most promising plant extracts for further research aimed at the discovery of new biologically active products that could be used as insecticides.

MATERIALS AND METHODS

Plant material

Figure 1: Some of the plants tested for bio-activity

Plants were selected for evaluation based on their traditional use, which was obtained from literature sources. Samples consisting of roots, stems, leaves and fruit were collected separately from different localities throughout South Africa.

Extract preparation

Dried, ground material of the different plant components was extracted using organic solvents (dichloromethane, a 1:1 dichloromethane/methanol mixture, methanol) and water.

Mosquito rearing

Anopheles arabiensis maintained in a colony at the Medical Research Council were used to evaluate these plant extracts.

Adulticidal test

The extracts were prepared as a 10 µg/ml solution in acetone and sprayed onto porcelain tiles. Blood fed 3 day old female *An. arabiensis* were exposed to the extracts to determine activity following the methods of WHO (1963). Deltamethrin was used as a positive control in the assays.

Figure 2: Larvae in test containers

Larvicidal test

Thirty, third stage *Anopheles arabiensis* larvae were placed in a beaker containing 0.25 litres of distilled water. 1 ml of a 10 mg/ml acetone solution of plant extract was added to the container. Mortality was recorded after 24 hours exposure and at 24 hour intervals over a period of 7 days. The commercial compound, Temephos was used as a positive control in the assays. During the test, the larvae were maintained in the insectary under the conditions used for rearing mosquitoes.

RESULTS

The 381 plant extracts evaluated represented 79 taxa and 37 plant families. The extracts were obtained from different parts of the plant, namely roots, leaves, stems, fruit, flower, seeds, twigs and bark. The extracts comprised 94 dichloromethane, 119 dichloromethane/methanol (1:1), 38 methanol, 128 water, 1 diethyl ether and 1 essential oil.

For the adulticidal assays an 80% mortality of mosquitoes was regarded as potent activity. The results indicated that 5 extracts exhibited mortality between 40 and 59 per cent, demonstrating that only limited toxicity against the target species exists. One of these taxa has been selected for further development work. 334 extracts exhibited little or no activity with mortality between 0 and 19 per cent. Further work into the adulticidal effects of the remaining extracts was abandoned.

The susceptibility level of *An. arabiensis* larvae to the extracts of different plants was determined. The results indicated that most of the taxa demonstrated varying degrees of larvicidal activity (**Table 1**). These were prioritised based on the percentage mortality, with 80 to 100% regarded as potent and 60 to 79% regarded as moderate activity.

Primary screening larvicidal results % Mortality				
1-19	**20-39**	**40-59**	**60-79**	**80-99**
Agathosma puberula	Aloe ferox	Aloe ferox	Aloe marlothii	Cappris tomentosa
Aloe ferox	Aloe marlothii	Aloe greatheadii davyana	Annona senegalensis	Catha edulis
Aloe marlothii	Annona senegalensis	Aloe marlothii	Carissa edulis	Crotalaria burkeana
Artemisia afra	Artabotrys brachypetalus	Anthocleista grandiflora	Catha edulis	Euphorbia tirucalli
Cappris tomentosa	Cappris tomentosa	Carissa edulis	Crotalaria burkeana	Flueggea virosa
Carissa edulis	Carissa edulis	Catha edulis	Ekebergia capensis	Gnidia cuneata
Catha edulis	Clausena anisata	Chenopodium ambrosioides	Kigela africana	Pentzia globosa
Ceratotheca triloba	Crotalaria burkeana	Crotalaria burkeana	Leucas martinicensis	Plumbago zeylanica
Chromolaena odorata	Croton gratissimus subgratissimus	Cussonia spicata	Lippia javanica	Psiadia punctulata
Cissus cornifolia	Cussonia spicata	Dichrostachys cinera africana	Maytenus senegalensis	Ptaeroxylon obliquum
Crotalaria burkeana	Diplorhynchus condylocarpon	Euphorbia tirucalli	Parinari curatellifolia	Schefflera umbellifera
Datura stramonium	Ekebergia capensis	Flacourtia indica	Pittosporum viridiflorum	Siphonochilus aethiopicus
Dichrostachys cinera africana	Euclea natalensis	Helichrysum species	Plumbago zeylanica	Toddalia asiatica
Diplorhynchus condylocarpon	Flacourtia indica	Maytenus senegalensis	Psiadia punctulata	Ximenia caffra
Dodonaea viscosa	Helichrysum species	Nuxia floribunda	Pterocarpus angolensis	
Euphorbia tirucalli	Lippia javanica	Parinari curatellifolia	Rauvolfia caffra	
Hyptis pectinata	Parinari curatellifolia	Pittosporum viridiflorum	Ricinus communis	
Kigela africana	Pittosporum viridiflorum	Plantago major	Schefflera umbellifera	
Litogyne gariepina	Plantago major	Plectranthus laxiflorus	Strychnos pungens	
Lonchocarpus capassa	Psiadia punctulata	Plumbago zeylanica	Vernonia fastigata	
Oncosiphon piluliferum	Ptaeroxylon obliquum	Psiadia punctulata	Ziziphus mucronata	
Parinari curatellifolia	Pterocarpus angolensis	Ptaeroxylon obliquum		
Pentzia globosa	Ricinus communis	Rumex crispus		
Pterocarpus angolensis	Rumex crispus	Schefflera umbellifera		
Rauvolfia caffra	Schefflera umbellifera	Strychnos madagascariensis		
Ricinus communis	Spirostachys africana	Vangueria infausta		
Rumex crispus	Strychnos potatorum	Warburgia salutaris		
Schefflera umbellifera	Vernonia myriantha			
Strychnos pungens	Ximenia caffra			
Vangueria infausta				
Vernonia fastigata				
Vernonia staehelinoides				
Ximenia caffra				
Ziziphus mucronata				

Table 1: Resultant mortality after exposure to various plant extracts tested at 40 µg/ml.

Four taxa were selected for further development and dose response studies were conducted at concentrations ranging from 0.004 to 40 µg/ml on these crude extracts (Figure 3). The resultant mortality at the various concentrations was found to be greatest in *Aloe spp*. Of significance, activity was seen at the lowest concentration of 0.004 µg/ml demonstrating that the *Aloe spp* extract has highly effective larvicidal properties at low concentrations.

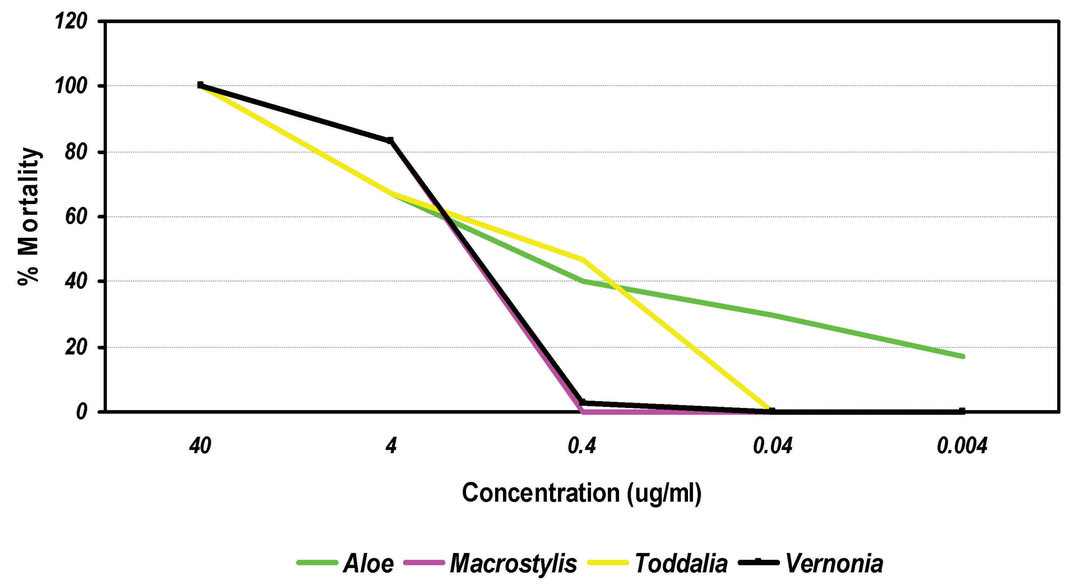

Figure 3: Dose-response curves of four promising plant candidates.

CONCLUSION

The results show that a proportion of South African traditional medicinal plants do posses larvicidal activity however only limited adulticidal activity was demonstrated. Fourteen taxa demonstrated potent larvicidal activity with at least four species viz., *Aloe, Macrostylus, Todallia* and *Vernonia* demonstrating good dose response curves. These plants are the subject of further evaluation to elucidate the constituents responsible for observed activities.

REFERENCE

1. WHO (1963) Insecticide resistance and vector control. 13th Report of the Expert committee on insecticides. WHO Technical Report Series No, 265.