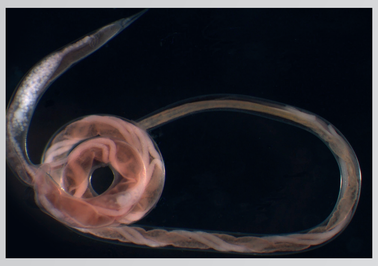
Pharmacological investigation of medicinal plants used traditionally in southern Africa to treat **gastrointestinal nematode infections** of small ruminants

Introduction



Haemonchus contortus is a major gastrointestinal nematode parasite causing haemonchiasis in production animals. It causes fever, anaemia, weight loss, poor reproductive efficiency, increased susceptibility to microbial infections and ultimately death. Development of resistance against all major groups of synthetic anthelmintic drugs necessitates the search for alternative treatment options. Medicinal plants have been widely used in ethnoveterinary medicine to treat nematode infections and in the present study some of these were investigated for pharmacological activity.

Materials and Methods

Acetone and water leaf extracts of six plants (Fig 1) were tested for *Haemonchus contortus* egg hatch inhibition using WAAVP guidelines [1]. Cytotoxicity against Vero monkey kidney cells was investigated using the colorimetric MTT assay [2]. Antibacterial and antifungal activity was determined using a serial microdilution method against ATCC strains and laboratory isolates [3,4]. Test bacteria were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Salmonella typhimurium* and *Bowkeria* for antibacterial activity and fungi included *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, A. niger, Penicillium italicum* and *Fusarium oxysporum*. Selectivity index (SI) values (LC_{50} / MIC) for each crude extract against each organism were calculated.

Fig 1: Pictorial representation of plant species

Fig 2: TLC fingerprints, anticryptococcal bioautography and DPPH radical scavenging potential of *Diospyros whyteana* crude extract, fractions and isolated compounds

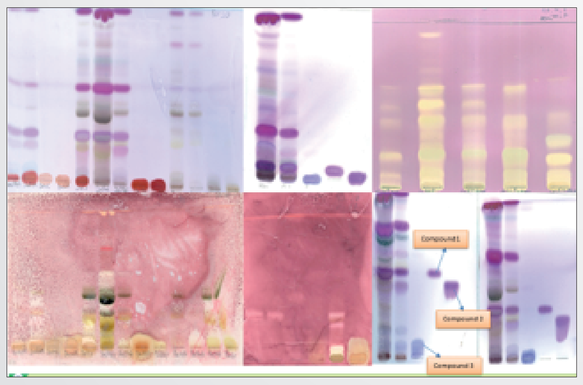


Table 1: Cytotoxicity (LC_{50}), and antibacterial (MIC) values (mg/ml) of six South African anthelmintic plants

Bac	Ext	PAF	DWH	CGM	SBR	BCT	SPT	Gent
Ec	A	0.16	0.08	0.16	0.32	0.16	2.5	0.015
	W	2.50	0.32	1.25	0.32	2.50	2.5	
Pa	A	0.02	0.02	0.04	0.04	0.16	2.5	0.250
	W	0.63	0.63	1.25	0.04	0.63	2.5	
St	A	0.08	0.08	0.16	0.32	0.16	2.5	0.500
	W	1.25	0.32	1.25	0.32	1.25	2.5	
Ef	A	0.04	0.04	0.32	0.08	2.50	2.5	0.063
	W	0.63	0.08	1.25	0.16	1.25	2.5	
Bc	A	0.04	0.02	0.16	320	0.04	2.5	0.031
	W	2.50	0.32	2.50	160	2.50	2.5	
Sa	A	0.08	0.04	0.16	0.63	0.08	2.5	0.500
	W	2.50	0.32	2.50	0.32	2.50	2.5	
Cyto	A	0.04	>1	>1	0.11	0.063	0.25	Dox
	W	>1	>1	0.182	>1	>1	-	0.04

Table 2: Egg hatch inhibition (IC_{50}), and antifungal (MIC) values (mg/ml) of six South African anthelmintic plants

Fun	Ext	PAF	DWH	CGM	SBR	BCT	SPT	Amp
Ca	A	-	0.16	0.16	0.32	0.63	0.02	0.08
	W	-	0.08	0.02	0.32	-	1.25	
Af	A	-	0.16	0.32	1.25	2.50	0.08	0.06
	W	-	2.50	2.50	1.25	-	2.50	
An	A	-	0.16	0.08	0.02	-	0.02	0.25
	W	-	2.50	2.50	2.50	2.50	2.50	
Cn	A	-	0.08	0.08	2.50	2.50	0.04	0.25
	W	-	0.32	0.16	2.50	-	0.63	
Pi	A	-	0.04	0.02	0.02	-	0.02	0.20
	W	-	2.50	0.02	0.02	2.50	0.63	
Fo	A	-	0.16	0.63	2.50	-	0.08	0.31
	W	-	0.63	0.04	2.50	0.63	2.50	
EHIC	A	1.08	0.73	1.69	1.41	1.17	1.50	Alb
	W	1.75	1.51	1.32	1.67	1.77	1.76	0.163

NB: EHIC: Effective egg hatch inhibitory concentration, DMSO: *Dimethyl- sulfoxide,* Alb: *Albendazole,* Dox: *Doxorubicin*, Gent: *Gentamicin,* Amph B: *Amphotericin B*, PAF: *P. africana*, DWH: *D. whyteana*, CGM: *C. gummiflua*, SBR: *S. brachypetala*, BCT: *B. citina*, SPT: *S. petersiana*

Results and Discussion

The acetone extracts of *D. whyteana* and *P. africana* had good activity in the egg hatch assay (EC_{50} values of 0.73 and 1.08 mg/ml), good antimicrobial activity (MIC = 0.02 – 2.5 mg/ml) and little toxicity to Vero cells (Tables 1 and 2). The antimicrobial activity against some selected pathogens is of pharmacological significance. The other extracts tested had varying degrees of egg hatch inhibition. Most extracts had little to no cytotoxicity at the highest concentration tested (1 mg/ml) except for *P. africana* and *B. citrina* where LC_{50} values were below 0.1 mg/ml. *D. whyteana* and *P. africana* were selected for further investigation and three anticryptococcal compounds have been isolated to date.

Conclusion

The six plant species selected on the basis of ethnoveterinary use had activity in the *Haemonchus contortus* egg hatch inhibition assay, and several had promising antibacterial and antifungal efficacy with little to no cytotoxicity. This study therefore supports the ethnoveterinary use of these medicinal plants as anti-endoparasitic agents. *D. whyteana* had good antifungal and anthelmintic activity. Isolation of further active compounds from selected species is the focus of current work.

References

1. Coles, G.C. Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J. 1992. *Vet. Parasitol*. 44, 35–44.
2. Mosmann T. 1983. J. *Immunol. Methods.* 65, 55–63.
3. Eloff, J.N. 1998. *Planta Med.* 60, 1–8.
4. Masoko, P. Picard, J., Eloff, J.N. 2005. *J. Ethnopharmacol.* 99, 301–308.

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