

The potential role of antibacterial, antioxidant and antiparasitic activity of *Peltophorum africanum* Sond. (Fabaceae) extracts in ethnoveterinary medicine

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*Peltophorum africanum* (From Venter & Venter (2002), Making the most of Indigenous Trees)



## Declaration

The experimental material and results described in this thesis is my original work (except where the input of others is acknowledged), conducted in the Phytomedicine Programme , Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, and has not been submitted in any other form to any other University or academic institution. I declare the above statement to be true.

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## List of abbreviations

AOX	Antioxidants
TAA	Total antibacterial activity
MIC	Minimum inhibitory concentration
DPPH	1,1-diphenyl-2-picryl hydrazyl
ANOVA	Analysis of variance
WAAVP	World Association for the Advancement of Veterinary Parasitology
TLC	Thin layer chromatography
FAWE	Formic acid: acetic acid: water: ethyl acetate (3:2:30:70)
BEA	Benzene: ethanol: ammonium hydroxide (18:2:0.2)
CEF	Chloroform: ethyl acetate: formic acid (18:8:2)
EMW	Ethyl acetate: methanol : water (10:1.35:1)
INT	p-iodonitrotetrazolium
NCCLS	National Committee for Clinical Laboratory Standards
DMSO	Dimethyl sulfoxide
UPBRC	University of Pretoria Biomedical Research Centre
MTT	3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide
TEAC	Trolox equivalent antioxidant capacity
SEM	Standard error of mean
EPA	Environment Protection Agency
ABTS	2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

## Publications

### *Full articles prepared from the thesis:*

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Bizimenyera, E. S., Swan, G. E., Chikoto, H., Eloff, J. N., 2005. There is a rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. *Journal of South African Veterinary Association*, **76**: 54-58

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Bizimenyera, E. S., Githiori, J. B., Swan, G. E., Eloff, J. N., 2006. *In vitro* ovicidal and larvicidal activity of the leaf, bark and root extracts of *Peltophorum africanum* Sond (Fabaceae) on *Haemonchus contortus*. *Journal of Animal and Veterinary Advances*, **5**: 606-614.

Bizimenyera, E. S., Aderogba, M.A ., Eloff, J. N., Swan, G. E., 2007. Potential of neuroprotective antioxidant-based therapeutics from *Peltophorum africanum* Sond. (Fabaceae) . *African Journal of Traditional, Complementary and Alternative Medicines*, **4**: 99-106

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a) Local

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Bizimenyera, E. S., Githiori, J. B., Eloff, J. N., Swan, G. E., 2005. Anthelmintic activity of *Peltophorum africanum* (Fabaceae) extracts against parasitic gastrointestinal nematodes of livestock. Faculty Day, Faculty of Veterinary Sciences, University of Pretoria, pg.31. **Onderstepoort** (14<sup>th</sup> Sept 2005), South Africa.

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Bizimenyera, E. S., Swan, G. E., Eloff, J. N., 2004. Justification for use of *Peltophorum africanum* for animal infections. Indigenous Plant Use Forum, pg 20. **Clanwilliam** (5-8<sup>th</sup> July 2004), South Africa.

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nematodes of ruminants. 34<sup>th</sup> Congress of the Parasitological Society of Southern Africa, pg.16. **Magoebaskloof Hotel** (25-28<sup>th</sup> Sept 2005), Limpopo, South Africa.

Bizimenyera, E. S., Eloff, J. N., Swan, G. E., 2006. Prospects for use of *Peltophorum africanum* Sond. (Fabaceae) extracts in neurodegenerative diseases. Indigenous Plant Use Forum, pg 26. **University of Botswana** (3-6<sup>th</sup> July 2006), Gaborone, Botswana.

Bizimenyera, E.S., Swan, G.E., Oguttu, J.W., Eloff, J.N, 2007.*Peltophorum africanum* Sond. (Fabaceae) extracts have potential role in medicine. Pharmatox 2007 Conference. **Buffellspoort** (2-5<sup>th</sup> Oct. 2007), South Africa.

c) International

Bizimenyera, E. S., Swan, G. E., Eloff, J. N., 2003. Antibacterial compounds in *Peltophorum africanum*. Antimicrobial Resistance Congress. **Durban** (27-29<sup>th</sup> Oct 2003), South Africa.

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Bizimenyera, E. S., Swan, G. E., Githiori, J .B., Eloff, J. N., 2005. Ethnomedical leads as tools in neuroscience. Psychiatry and Neuroscience in Africa (SONA/IBRO), pg 27. **Cape Town** (18-22 April 2005), South Africa.

Bizimenyera, E. S., Githiori, J. B., Eloff, J. N., Swan, G. E., 2005. Potential sustainable use of *Peltophorum africanum* as anthelmintic by rural communities. 6<sup>th</sup> Global Conference on conservation of Domestic Animal Genetic Resources, pg.59. **Magalies Park Resort** (9-13<sup>th</sup> Oct 2005), South Africa.

Bizimenyera, E. S., Aderogba, M. A., Eloff, J. N., Swan, G. E., 2005. Plants rich in antioxidants warrant study for neurodegenerative diseases. International Neuroscience Conference, pg. 69. **Al Ain** (26-29<sup>th</sup> Nov. 2005), United Arab Emirates.

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neurodegenerative diseases. 1<sup>st</sup> International Neuroscience Conference, pg 20. **Imo Concorde Hotel** (9-13<sup>th</sup> July 2006), Owerri, Nigeria.

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Bizimenyera, E. S., Eloff, J. N., Swan, G. E., 2007. Prospects of antioxidant-based therapeutics from *Peltophorum africanum* Sond. (Fabaceae) against human immunodeficiency virus (HIV). The 5<sup>th</sup> African conference on child abuse and neglect: HIV-AIDS and children, pg . **Kampala** (27-29<sup>th</sup> March 2007), Uganda.

## Summary

There is an increasing interest in ethnomedical and ethnoveterinary practices, especially as it relates to the use of medicinal plants for treating various ailments. As a result, the current trend in government health authorities is to integrate herbal medicine with primary health care. This arises because nearly 80% of people in the developing world, particularly those from rural communities where modern drugs are unaffordable, inaccessible or, unavailable, depend on phytomedicine for primary healthcare. Despite this, however, most medical and veterinary professionals distrust herbal medicines due to concerns of scientific evidence of efficacy and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Traditional healers and rural farmers use extracts of *Peltophorum africanum* (a medicinal plant wide-spread in southern Africa and other tropical regions), to treat diarrhoea, helminths and abdominal parasites, dysentery, HIV-AIDS, acute and chronic pain, anxiety and depression, infertility, and to promote well-being and resistance to diseases.

To evaluate these ethnobotanical leads, dried leaves, bark and root from mature *P. africanum* (Fabaceae) trees were extracted with acetone, ethanol, dichloromethane and hexane. Chromatograms were made on silica gel plates. Thin layer chromatograms (TLC) were sprayed with 0.2% 2, 2-diphenyl-1-picryl hydrazyl (DPPH) for qualitative screening for antioxidants. Quantification of antioxidant activity was done in comparison with L-ascorbic acid and Trolox (6-hydroxy-2, 5, 7, 8-tetranethylchromane-2-carboxylic acid). With regard to the extracts, minimum inhibitory concentrations (MIC) were determined for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The total antibacterial activity (TAA), signifying the volume to which active compounds present in 1 g of plant material can be diluted and still inhibit bacterial growth, was also determined. *In vitro* anthelmintic activity was evaluated by effects of acetone extracts on the egg hatching and larval development of parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*. The eggs and larvae of the two parasites were incubated in various concentrations of the leaf, bark and root extracts for two and five days respectively. Furthermore the efficacies of the acetone extracts were tested on lambs artificially induced with *H. contortus* and *T. colubriformis* infections. Toxicity was performed in brine shrimp and MTT assay on Vero monkey kidney cells.

The extracts had substantial activity against both Gram-positive and Gram-negative bacteria, with MIC values of 0.08 mg ml<sup>-1</sup> for *Staphylococcus aureus* and 0.16 mg ml<sup>-1</sup> for *Pseudomonas aeruginosa*; the corresponding TAA values were 1263 and 631 ml g<sup>-1</sup>. The acetone extracts of

the bark, and root of *P. africanum* had higher antioxidant activity than L-ascorbic acid (Vitamin-C) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid), a synthetic vitamin-E analogue, and much higher than *Ginkgo biloba* extract (EGb 761). The standardized extract of *Ginkgo biloba* (EGb 761) is widely employed for its significant benefit in neurological disorders. The respective EC<sub>50</sub> for the *P. africanum* root, bark and leaf extracts, L-ascorbic acid, and EGb761 were 3.82 µg ml<sup>-1</sup>, 4.37 µg ml<sup>-1</sup>, 6.54 µg ml<sup>-1</sup>, 5.04 µg ml<sup>-1</sup>, and 40.72 µg ml<sup>-1</sup>.

The extracts inhibited egg hatchability and larval development (from L<sub>1</sub> to infective stage L<sub>3</sub>) of both *H. contortus* and *T. colubriformis* (both parasitic nematodes of ruminants) at concentrations of 0.2-1.0 mg ml<sup>-1</sup>. The plant extracts, at concentrations of 5-25 mg ml<sup>-1</sup> completely lysed larval forms (L<sub>1</sub>) and eggs of the nematodes. In all assays, the root extracts had higher antibacterial, antioxidant and anthelmintic activity than the bark and leaf. Although the extracts were safe and non-toxic, the reduction in faecal egg and adult worm counts in lambs infected with *H. contortus* and *T. colubriformis* was not statistically significant (P=0.073).

From the acetone extracts of the root, a brownish crystalline compound, bergenin was isolated. Bergenin was also assayed for toxicity with brine shrimp and Vero monkey kidney cells like the extracts, where the compound was found to be not toxic. In a disc diffusion test, the inhibitory activation of bergenin was determined for the bacteria *E. coli*, *P. aeruginosa*, *Mycobacterium vaccae*, and the fungi *Sporobolomyces salmonicolor* and *Penicillium notatum*. Bergenin had reasonable antimicrobial activity against *S. salmonicolor*, moderate activity against *M. vaccae*, *E. coli* and *P. aeruginosa*, but non inhibitory against *P. notatum*.

*P. africanum* extracts have therefore, potential for treatment of infection-related diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. The traditional use of *P. africanum* concoctions against diarrhoea, dysentery and unthriftiness, may be also due to anthelmintic activity as these signs are consistent with parasitic gastroenteritis.

Antioxidants are also important in boosting the immunity, critical in the management of helminthosis. There is ample scientific and empirical evidence supporting the use of plant-derived antioxidants in the control of human immunodeficiency virus (HIV) and neurological diseases. Synergistic activity of plant antioxidants has been proposed as a mechanism by which viral replication and immune cell killing in HIV infection can be inhibited. Antioxidants may have neuro-protective (preventing apoptosis), as well as neuro-regenerative roles. Due to the high antioxidant activity of its extracts, *P. africanum* has prospects in the chemotherapy of

HIV and management or control of neurodegenerative diseases. Thus there is great potential of *P. africanum* extracts in medicine.

Further isolation and bioassay characterization of bioactive compounds from *P. africanum* is recommended as well as refinement of *in vivo* tests in target livestock, or clinical trials. Better methods of plant extraction easily adaptable to rural communities for sustainable exploitation of the tree, may have to be devised especially those using the leaves instead of bark or root.



## Table of contents

Title page.....	i
Photograph of <i>Peltophorum africanum</i> plant.....	ii
Declaration.....	ii
Acknowledgements.....	iv
List of abbreviations.....	vi
Publications .....	vii
Conference presentations.....	vii
Summary .....	xi
<b>CHAPTER 1 Introduction .....</b>	<b>1</b>
1.1 Background.....	1
1.2 Hypothesis.....	3
1.3 Aim .....	3
1.4 Objectives .....	3
<b>CHAPTER 2 Literature Review.....</b>	<b>5</b>
2.1 Antibacterials.....	5
2.2 Antioxidants.....	5
2.3 Anthelmintics.....	7
2.4 <i>Peltophorum africanum</i> .....	8
2.4.1 General aspects.....	8
2.4.2 Ethnomedical and ethnoveterinary use .....	8
2.4.3 Phytochemistry.....	9
2.4.4 Biological activity.....	9
<b>CHAPTER 3 Rationale for using <i>P. africanum</i> extracts in veterinary medicine.....</b>	<b>10</b>
3.1 Introduction.....	10
3.2 Materials and Methods.....	12
3.2.1 Collection, preparation and storage of materials.....	12
3.2.2 Extraction.....	12
3.2.3 Chromatography.....	12
3.2.4 Polyphenols.....	12
3.2.5 Antioxidant screening.....	13





3.2.6 Antibacterial screening.....	13
3.3 Results.....	14
3.4 Discussion.....	19
3.5 References.....	21
<b>CHAPTER 4 <i>In vitro</i> ovicidal and larvicidal activity of the leaf, bark and root extracts of <i>P. africanum</i> on <i>H. contortus</i>.....</b>	<b>24</b>
4.1 Introduction.....	24
4.2 Materials and methods.....	26
4.2.1 Collection, storage and preparation of plant material.....	26
4.2.2 Preparation of plant extracts.....	26
4.2.3 Egg recovery and preparation.....	27
4.2.4 Egg hatch inhibitory (EH) assay.....	27
4.2.5 Larval development (LD) inhibition assay.....	28
4.2.6 Calculation and statistical analysis.....	28
4.3 Results.....	29
4.4 Discussion and conclusion.....	31
4.5 References.....	33
<b>CHAPTER 5 <i>In vitro</i> activity of <i>P. africanum</i> extracts on egg hatching and larval development of the parasitic nematode <i>T. colubriformis</i>.....</b>	<b>37</b>
5.1 Introduction.....	37
5.2 Materials and methods.....	39
5.2.1 Collection, storage and preparation of plant material.....	39
5.2.2 Plant extract preparation.....	39
5.2.3 Recovery and preparation of eggs .....	40
5.2.4 Egg hatch inhibition assay (EH).....	40
5.2.5 Larval development assay (LD).....	41
5.2.6 Calculations and statistical analysis.....	41
5.3 Results.....	42
5.4 Discussion and conclusion.....	45
5.5 References.....	47
<b>CHAPTER 6 Efficacy of <i>P. africanum</i> extracts on <i>H. contortus</i> and <i>T. colubriformis</i> in sheep.....</b>	<b>51</b>
6.1 Introduction .....	51



6.2 Materials and Methods.....	53
6.2.1 Collection, storage and preparation of plant material.....	53
6.2.2 Plant extraction.....	53
6.2.3 Experimental animals.....	53
6.2.4 Experimental design.....	54
6.2.4.1 Preparation and administration of infective larvae.....	54
6.2.4.2 Treatment procedures.....	54
6.2.4.3 Full haematology and liver enzymes analysis.....	55
6.2.5 Evaluation.....	5
6.2.5.1 Faecal egg counts.....	55
6.2.5.2 Larval cultures and identification.....	56
6.2.5.3 Adult worm counts.....	56
6.2.6 Calculations and statistical analysis.....	56
6.3 Results.....	56
6.4 Discussion.....	61
6.5 References.....	62
<b>CHAPTER 7 Safety profiles of <i>P. africanum</i> extracts.....</b>	<b>67</b>
7.1 Introduction.....	67
7.2 Materials and methods.....	69
7.2.1 Plant material.....	69
7.2.2 Extraction.....	69
7.2.3 Toxicity tests.....	69
7.2.3.1 Brine shrimp lethality.....	69
7.2.3.2 MTT assay.....	70
7.2.4 Safety of extracts in sheep.....	71
7.2.5 Statistical analysis.....	71
7.3 Results.....	71
7.4 Discussion.....	73
7.5 References.....	75
<b>CHAPTER 8 Isolation and bioassay characterization of bergenin from the root extract of <i>P. africanum</i>.....</b>	<b>79</b>
.....	
8.1 Introduction.....	79
8.2 Methodology.....	80



8.2.1 Plant material.....	80
8.2.2 Isolation of compounds.....	80
8.2.3 Toxicity assays with bergenin.....	81
8.2.3.1 Brie shrimp lethality.....	81
8.2.3.2 Cytotoxicity, MTT assay.....	81
8.2.3.3 Antiviral assay.....	82
8.2.4 Bioactive assays.....	83
8.2.4.1 Antioxidant.....	83
8.2.4.2 Inhibition of microbial growth.....	84
8.2.5 Statistical analysis.....	84
8.3 Results.....	84
8.4 Discussion.....	88
8.5 References.....	89
<b>CHAPTER 9 Potential of neuroprotective antioxidant-based therapeutics from <i>P. africanum</i>.....</b>	<b>92</b>
.....	
9.1 Introduction.....	93
9.2 Methodology.....	96
9.2.1 Collection, storage and preparation of plant material.....	96
9.2.2 Chemicals.....	96
9.2.3 Evaluation of antioxidant activity.....	96
9.3 Results.....	98
9.4 Discussion and conclusion.....	99
9.5 References.....	101
<b>CHAPTER 10 General discussions and conclusion.....</b>	<b>105</b>
10.1 <i>P. africanum</i> in traditional medicine.....	105
10.2 Extraction.....	105
10.3 Antibacterial activity.....	106
10.4 Antioxidant activity.....	106
10.5 Anthelmintic activity.....	107
10.6 Safety of <i>P. africanum</i> .....	108
10.7 Conclusions.....	108
<b>CHAPTER 11 References.....</b>	<b>110</b>



<b>APPENDICES</b> .....	<b>125</b>
Annexure 1 Journal of South African Veterinary Association.....	125
Annexure 2 Journal of Animal and Veterinary Advances.....	126
Annexure 3 Veterinary Parasitology.....	127
Annexure 4 African Journal of Traditional, Complementary and Alternative Medicines.....	128

## List of Tables

Table 3.1 Minimum inhibitory concentration (MIC) values of bark, root and leaf extracts.....	17
Table 3.2 Total antibacterial activity values of bark, root and leaf extracts.....	18
Table 4.1 Percent mean inhibition of egg hatch and larval development of <i>H. contortus</i> by <i>P. africanum</i> extracts.....	29
Table 4.2 Kruskal-Wallis and ED <sub>50</sub> values of extracts of <i>P. africanum</i> against <i>H. contortus</i> ..	29
Table 4.3 Larvicidal activity of acetone extracts of <i>P. africanum</i> against <i>H. contortus</i> .....	31
Table 5.1 p-values (Kruskal-Wallis) and ED <sub>50</sub> values of <i>P. africanum</i> extracts against <i>T. colubriformis</i> .....	42
Table 6.1 Treatment groups and individual doses.....	55
Table 7.1 Cytotoxicity of <i>P. africanum</i> extracts.....	72
Table 8.1 Cytotoxicity and antioxidant activities of bergenin.....	86
Table 8.2 Antimicrobial activity of bergenin against five microbial species. ....	87
Table 9.1 Commercial plants effective in control of nervous or chronic conditions.....	95
Table 9.2 TEAC and Vit.C equivalent values acetone extracts of leaf, bark and root of <i>P. africanum</i> .....	99

## List of Figures

Figure 3.1 Extraction efficiency of ethanol, acetone, dichloromethane and hexane on <i>P. africanum</i> leaf, bark and root.....	15
Figure 3.2 Percentage of polyphenols in bark, root and leaf extracts of <i>P. africanum</i> .....	15
Figure 3.3 Chromatograms of root, bark and leaf extracts of <i>P. africanum</i> sprayed with DPPH .....	16
Figure 3.4 TEAC values of bark, root and leaf extracts of <i>P. africanum</i> by various extracts .....	17
Figure 4.1 Dose-response egg hatch inhibition of <i>H. contortus</i> by <i>P. africanum</i> leaf, bark and root extracts.....	30
Figure 4.2 Dose-response larval development inhibition of <i>H. contortus</i> by <i>P. africanum</i> .....	30
Figure 5.1 Percent mean inhibition of egg hatch of <i>T. colubriformis</i> by <i>P. africanum</i> leaf, bark and root extracts .....	43
Figure 5.2 Percent mean inhibition of larval development of <i>T. colubriformis</i> by extracts of <i>P. africanum</i> .....	43
Figure 5.3 Dose-response profile for egg hatch inhibition of <i>T. colubriformis</i> by <i>P. africanum</i> extracts.....	44
Figure 5.4 Dose response profile for inhibition of larval development of <i>T. colubriformis</i> by <i>P. africanum</i> extracts.....	44
Figure 6.1 Faecal egg counts.....	57
Figure 6.2 Mean egg per gram (EPG) per day of trial.....	58
Figure 6.3 <i>H. contortus</i> adult worm counts.....	58
Figure 6.4 <i>T. colubriformis</i> adult worm counts.....	59
Figure 6.5 Daily hay consumption (kg) per group post treatment.....	60
Figure 7.1 Haemoglobin and liver enzyme analysis.....	73
Figure 8.1 NMR of bergenin.....	85
Figure 8.2 MS of bergenin.....	86
Figure 9.1 Chromatogram of 200 µg acetone extracts of leaf, bark and root extracts of <i>P. africanum</i> separated by EMW and sprayed with DPPH.....	98