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

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Co-promoter: Prof Jacobus N Eloff

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

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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.

Signed: ……

Edmund S Bizimenyera

Date: ……..

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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:

a) Published: -

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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b) Submitted: -

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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 (14th Sept 2005), South Africa.

b) National and regional

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c) International

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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 heath 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^{-1} for *Staphylococcus aureus* and 0.16 mg ml^{-1} for *Pseudomonas aeruginosa* ; the corresponding TAA values were 1263 and 631 ml g^{-1}. 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_{50} for the *P. africanum* root , bark and leaf extracts, L-ascorbic acid, and EGb761 were 3.82 µg ml^{-1}, 4.37 µg ml^{-1}, 6.54 µg ml^{-1}, 5.04 µg ml^{-1}, and 40.72 µg ml^{-1}.

The extracts inhibited egg hatchability and larval development (from L_1 to infective stage L_3) of both *H. contortus* and *T. colubriformis* (both parasitic nematodes of ruminants) at concentrations of 0.2-1.0 mg ml^{-1}. The plant extracts, at concentrations of 5-25 mg ml^{-1} completely lysed larval forms (L_1) 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 activition 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 unthriftness, 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

List of Tables

List of Figures