CHAPTER 11

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APPENDICES/ANNEXURES

Article — Artikel

Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine

S E Bizimenyera[a], G E Swan[a], H Chikoto[a] and J N Eloff[a*]

ABSTRACT

Peltophorum africanum (Fabaceae) is a deciduous tree widespread in southern Africa. The plant has many ethnomedical and ethnoveterinary uses. Root and bark decoctions are used to treat diarrhoea, dysentery, sore throat, wounds, back and joint pains, HIV-AIDS, venereal diseases and infertility. Pastoralists and rural farmers use the root and bark extracts to treat diarrhoea, dysentery, infertility, and to promote well-being and resistance to diseases in cattle. To evaluate these ethnobotanical leads, dried leaves, stem bark and root bark were extracted with ethanol, acetone, dichloromethane and hexane. Polyphenols in the extract were determined by the Folin-Ciocalteu method with gallic acid as standard. Qualitative antioxidant activity was screened by spraying thin layer chromatograms (TLC) of the extracts with 0.2 % 1,1-diphenyl-2-picryl hydrazyl (DPPH), and quantified with Trolox equivalent antioxidant capacity (TEAC) assay. Minimum inhibitory concentration (MIC) and total antibacterial activity (TAA) were determined by serial microplate dilution for *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis*, with gentamicin as standard and tetrazolium violet as growth indicator. Acetone and ethanol extracted the largest quantity of material. Polyphenols concentration was 49.2 % in acetone extract of the root and 3.8 % in dichloromethane extract of the leaf. Antioxidant activity of at least 5 antioxidant compounds as measured by TEAC ranged from 1.34 (ethanol extract of the root) to 0.01 (hexane extract of the leaf). The total antibacterial activity (volume to which active compounds present in 1 g plant material can be diluted and still inhibit bacterial growth) was 1263 mℓ/g for ethanol extract of the root against *S. aureus*, and 800 mℓ/g for acetone extract of the root against *P. aeruginosa*. There was substantial activity against both Gram-positive and Gram-negative bacteria, with MIC values of 0.08 mg/mℓ for *S. aureus* and 0.16 mg/mℓ for *P. aeruginosa*. There is therefore a rationale for the traditional use of root and bark of *P. africanum* in treating bacterial infection related diseases.

Key words: antioxidant, antibacterial, ethnoveterinary, extracts, *Peltophorum africanum*.

Bizimenyera S E, Swan G E, Chikoto H, Eloff J N **Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine**. *Journal of the South African Veterinary Association* (2005) 76(2): 54–58 (En.). Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Phytotherapy, the treatment and prevention of disease using medicinal plants, is an ancient tradition that has existed with human habitations. About 80 % of the world's people still depend on the traditional healthcare practices using herbs.[11] This is so mainly in rural communities in the developing world where modern drugs may be unaffordable or inaccessible. Disease concepts are largely similar in humans and animals; in many traditional systems, healers of people are often called upon to treat animals and *vice versa*[28]. Healers frequently use the same herbs, compounds or techniques.

Many pharmacologically active compounds have been discovered following ethnobotanical leads[4,11]. As tropical forests still present a great storehouse of medicinal genetic resources, the search for compounds with novel bioactivity from plants continues[32].

Peltophorum africanum (Sond), commonly called 'weeping wattle' or 'huilboom', is a member of the Fabaceae. It is a deciduous tree growing up to 15 m high with a wide canopy that occurs widely in medium to low altitudes in wooded grassland areas of southern Africa[24]. Whereas the genus is found throughout the tropics, *P. africanum* is the only member of the genus in southern Africa. The plant has many traditional medicinal uses in humans and animals. The roots and bark are used to treat sore throat, wounds, diarrhoea, dysentery, helminthosis, abdominal pains, ascites, back and joint pains, HIV-AIDS, venereal diseases, infertility, colic and eye infections[12,31,34]. Pastoralists and rural farmers use the root and bark to treat diarrhoea, dysentery and infertility in cattle and to promote well-being and resistance to disease[30,34]. Bark from *P. africanum* was identified as one of the most important products sold in informal medicinal plant markets in Pretoria[17].

The phytochemistry of *P. africanum* has been studied by several authors. A sulphate ester of trans-4-hydroxypipecolic acid has been isolated from the seed[10]. Several condensed flavonoids, a novel cyanomaclurin analogue[1], profisetinidin-type 4-arylflavan-3-ols and related δ-lactones[2] were found in the heartwood. Mebe and Makuhunga[19] isolated new compounds (bergenin, norbergenin and 11-0(E)-*p*-coumaroylbergenin from ethanol extracts of the bark. Khattab and Nassar[14] isolated coumarins from the leaves. The chemical structures of the novel compounds isolated were elucidated but the biological activity of isolated compounds has hardly been investigated. Leaf extracts have beta-adrenergic activity on the rabbit jejunum, an effect that was blocked by propranolol[20], and anticestodal activity[21]. Bark acetone extracts of *P. africanum* had MIC values of 0.02 to 0.08 mg/mℓ towards *Staphylococcus aureus*[17] in an unpublished M.Sc. thesis of a member of our group. These results further motivated this study.

Use of the bark and root are destructive practices that may lead to destruction of resources and even to plant extinction. Qualitative and quantitative investigation of the bioactive compounds present in the leaves, bark and root merits further study, to determine if there is a rationale in the traditional use of the plant by rural farmers and whether leaves may not be used. Suitable methods of extraction of bioactive compounds, adapted to resources available in rural communities, could be developed for sustainable use of *P. africanum* extracts in primary health care practices.

MATERIALS AND METHODS

Collection, preparation and storage of plant material

Leaves, stem bark and root bark (referred to as leaf, bark and root in this article) were

[a]Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail : kobus.eloff@up.ac.za; fax: +27 12 5298304.

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In vitro Ovicidal and Larvicidal Activity of the Leaf, Bark and Root Extracts of *Peltophorum africanum* Sond. (Fabaceae) on *Haemonchus contortus*

[1,2]E.S. Bizimenyera, [1]J.B. Githiori, [1]G.E. Swan and [1]J.N. Eloff
[1]Programme for Phytomedicine, Department of Paraclinical Sciences,
University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa
[2]Department of Veterinary Medicine, Makerere University, P.O. Box 7062, Uganda

Abstract: The *In vitro* efficacy of the extracts of *Peltophorum africanum* Sond. (Fabaceae), was determined against *Haemonchus contortus*. Acetone extracts of the leaf, bark and root, at concentrations of 0.008 to 25 mg mL^{-1} were incubated at 23°C with the eggs and larval stage (L_1) of the parasite for two and five days, respectively. Thiabendazole and water were positive and negative controls, respectively. Increasing the concentration of extracts caused a significant ($p<0.05$) increase in inhibition of egg hatching, and larval development. At concentrations of 0.2 and 1.0 mg mL^{-1} the extracts inhibited egg hatching and development of L_1 to the infective stage (L_3). No eggs and larvae (L_1) of *H. contortus* were detected at concentrations of 5 and 25 mg mL^{-1}. The *in vitro* model may provide support of the traditional use of *P. africanum* extracts against helminthosis. Suitable methods of plant extraction, adaptable to rural use may help rural communities control helminthosis.

Key words: Ovicidal, larvicidal, extracts, *Haemonchus contortus*, *Peltophorum africanum*

INTRODUCTION

In the tropics and sub-tropics, helminthosis remains one of the most prevalent and economically important parasitoses of domesticated animals[1,2]. Gastrointestinal nematodes are the chief parasitoses responsible for disease-related production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants[3]. Haemonchosis (caused by *Haemonchus contortus*) has been listed among the top 10 most important conditions hampering production of sheep and goats in tropical countries[1,4]. The disease is characterised by anaemia, haemorrhagic gastroenteritis, hypoproteinaemia (manifested by oedema or 'bottle jaw'), sudden death or chronic emaciation[5,6]. Adult *H. contortus* females have high egg-producing capacity, of 5000-15000 eggs per day[7]. The high fecundity combined with the high rainfall and temperatures, favour permanent larval development in the environment leading to heavy contamination of pastures with the infective larval (L_3) forms.

Use of synthetic and semi-synthetically produced anthelmintic drugs has for long been considered the only effective method of control of gastrointestinal nematode infections of small ruminants. However, most of the proprietary drugs are expensive and unavailable to rural subsistence livestock keepers, who are tempted to use substandard doses. Conversely, in more developed farming systems, the massive use of the drugs has created multiple anthelmintic resistance against all of the major families of broad spectrum anthelmintics[8-10], that may lead to failure of control of worm parasites in ruminants. Surveys in South Africa, indicate anthelmintic resistance to be serious on sheep and goat farms[11]. The foregoing has created delicate situations, where at one extreme there are heavy mortalities of young stock, while at the other the economic control of helminth parasites is difficult. These constraints indicate that entire reliance on synthetic anthelmintics may present difficulties in the management of gastrointestinal parasitic infections in livestock, necessitating novel alternative methods of helminth control[12-14].

Use of indigenous plant preparations as livestock dewormers is gaining ground as one of the alternative and sustainable methods readily adaptable to rural farming communities[15,13]. About 80% of people in the developing world rely on phytomedicine for primary healthcare[16,17]. Ethnomedicine often does not follow the western paradigms of scientific proof of efficacy; hence the medical and veterinary professionals distrust herbal remedies[18,19]. There is need therefore, for scientific validation of efficacy of herbal medicines before their acceptance and use.

Corresponding Author: E.S. Bizimenyera, Programme for Phytomedicine, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

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In vitro activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*

E.S. Bizimenyera [a,b,*], J.B. Githiori [a], J.N. Eloff [a], G.E. Swan [a]

[a] *Programme for Phytomedicine, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa*
[b] *Department of Veterinary Medicine, Makerere University, PO Box 7062, Kampala, Uganda*

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Abstract

Trichostrongylus colubriformis is an important cause of parasitic gastroenteritis in ruminants, where it causes protracted diarrhoea, rapid loss of weight, loss of production and death. The *in vitro* efficacy of extracts of *Peltophorum africanum* was determined against this parasitic nematode. Eggs and larvae of *T. colubriformis* were incubated at 23 °C in the extracts of the leaf, bark and root of *P. africanum* at concentrations of 0.008–25 mg ml^{-1} for 2 and 5 days, respectively. Thiabendazole and water were used as positive and negative controls, respectively. Inhibition of egg hatching and larval development increased significantly ($P < 0.05$) with increasing concentrations of the extracts. Concentrations of 0.2–1.0 mg ml^{-1} of the extracts of leaf, stem bark, and root bark of *P. africanum* completely inhibited the hatching of eggs and development of larvae. No eggs and larvae of *T. colubriformis* could be observed in wells incubated with all the three extracts at concentrations of 5 and 25 mg ml^{-1}. The *in vitro* model results support the traditional use of *P. africanum* against nematode parasites. Further research is required to isolate and structurally identify the active anthelmintic compounds, and to improve methods of plant extraction of the effective anthelmintic components that will be readily adaptable for use by rural communities against helminthosis.

Keywords: Ovicidal; Larvicidal; Extracts; *Trichostrongylus colubriformis*; *Peltophorum africanum*

1. Introduction

Gastrointestinal nematodes remain a major constraint to economic productivity of livestock throughout the world, being the chief parasitoses responsible for disease-related production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants (Perry and Randolph, 1999; Chiejina, 2001). *Trichostrongylus colubriformis*, an intestinal nematode, is one of the most important causes of parasitic enteritis causing protracted diarrhoea, weakness, loss of production and death. Infestation of sheep with *T. colubriformis* causes a severely infected animal to pass dark diarrhoea that has earned the parasite the name of "black scours worm" (Soulsby, 1982). The parasite is frequently identified in large numbers in infected sheep and cattle in South Africa (Horak, 2003; Horak et al., 2004). The infective larvae (L_3) of *T. colubriformis* have a high capacity to survive even in adverse weather conditions (Urquhart

* Corresponding author. Tel.: +27 781677665; fax: +27 125298304.
E-mail address: ebizimenyera@yahoo.co.uk (E.S. Bizimenyera).

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POTENTIAL OF NEUROPROTECTIVE ANTIOXIDANT-BASED THERAPEUTICS FROM *PELTOPHORUM AFRICANUM* SOND.(FABACEAE)

E. S. Bizimenyera*[a,b], M. A. Aderogba [a], J. N. Eloff [a], and G. E. Swan [a]

[a] Programme for Phytomedicine, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.
[b] Department of Veterinary Medicine, Makerere University, P O Box 7062, Kampala, Uganda.
* Corresponding author: **Tel.** +27781677665; **Fax:** +2712-529-8304; **E-mail:** ebizimenyera@yahoo.co.uk

Abstract

There is ample scientific and empirical evidence supporting the use of plant-derived antioxidants for the control of neurodegenerative disorders. Antioxidants may have neuroprotective (preventing apoptosis) and neuroregenerative roles, by reducing or reversing cellular damage and by slowing progression of neuronal cell loss. Although demand for phytotherapeutic agents is growing, there is need for their scientific validation before plant–derived extracts gain wider acceptance and use. We have evaluated antioxidant potential of *Peltophorum africanum* (weeping wattle), a plant widespread in the tropics and traditionally used, *inter alia*, for the relief of acute and chronic pain, anxiety and depression. The dried leaves, bark and root of *P. africanum* were extracted with acetone. Thin layer chromatograms were sprayed with 0.2% 2,2-diphenyl-1-picryl hydrazyl (DPPH) in methanol for screening for antioxidants. Quantification of antioxidant activity was assessed against 6-hydroxy-2, 5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and L-ascorbic acid (both standard antioxidants), using two free radicals, 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and DPPH, respectively. Results of our study show that the bark and root extracts had higher antioxidant activity than L-ascorbic acid and Trolox, a synthetic vitamin-E analogue. The respective TEAC (Trolox Equivalent Antioxidant Capacity) values for the bark and root extracts, and Trolox were 1.08, 1.28 and 1.0. EC_{50} values for L-ascorbic acid (5.04 µg/mL) was more active than the leaf 6.54 (µg/mL), but much less active than the bark (4.37 µg/mL) and root (3.82 µg/mL) extracts. Continued work on *P. africanum*, and other plants rich in antioxidants, may avail neuroscientists with potent neuroprotective antioxidant therapeutics.

Key words: Antioxidant; Extracts; Neurodegeneration; Neuroprotection; Oxidative stress; *Peltophorum africanum*

Introduction

Oxidative stress is the result of an imbalance in the pro-oxidant/antioxidant homeostasis leading to the generation of excess reactive oxygen species (ROS), implicated *inter alia* in the cause of carcinogenetic, inflammatory, infectious, cardiovascular and neurological diseases in man and animals (Nair et al., 2003). Under normal conditions, the body is equipped with defense mechanisms that scavenge ROS and protect the cells from oxidative damage. However, the detoxifying enzyme processes get overwhelmed, saturated, and faulty under conditions of low dietary antioxidant intake, inflammation, aging or exposure to environmental factors such as irradiation or tobacco smoke, inducing some enzymes like cyclooxygenase-2 (COX-2), lipoxygenase (LOX) and inducible nitric acid synthase (iNOS) that generate intermediaries that damage cellular macromolecules including DNA (Floyd, 1999; Rao and Balachandran, 2000; Nair et al., 2003). The damage is made on proteins, lipids, and nucleic acids signaling cascades leading to disruption of ion homeostasis and modification of the genetic apparatus, with consequence of apoptotic cell death (Sun and Chen, 1998; Sing et