CHAPTER 9

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

E S Bizimenyera, M A Aderogba , J N Eloff , and G E Swan

(As published by *African Journal of Traditional, Complementary and Altrnative Medicines*, 2007, **4**: 99-10
Annexure 4)

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. 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.

Keywords: Antioxidant; Extracts; Neurodegeneration; Neuroprotection; Oxidative stress; *Peltophorum africanum*

9.1 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; Singh *et al*., 2004). The brain is in particular very sensitive to oxidation stress possibly because of its high lipid content, high aerobic metabolic activity and low catalase activity (Halliwell and Gutteridge, 1985; Cao *et al*., 1988; Floyd and Carney, 1992; Gilgun-Sherki *et al*., 2001.

Antioxidants (AOX) are considered a promising therapeutic approach as they may be playing neuroprotective (preventing apoptosis) and neuroregenerative roles (Moosmann and Behl, 2002). Plant-derived antioxidants offer prospects in this regard. In nature, AOX are grouped as endogenous or exogenous. The endogenous group includes enzymes (and trace elements part-of) like superoxidase dismutase (zinc, manganese, and copper), glutathione peroxide (selenium) and catalase, and proteins like albumin, transferrin, ceruloplasmin, metallothionein and haptoglobin. The most important exogenous AOX are dietary phytochemicals (such as polyphenols, quinones, flavonoids, catechins, coumarins, terpenoids) and the smaller molecules like ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E) and beta-carotene vitamin-E, and supplements. The antioxidant processes occur in cytosol, mitochondria or in plasma (Larson 1988; Namiki *et al*., 1993; Berger, 2005). Though their mode of action is not yet completely elucidated, and clinical trials involving them are still relatively scarce, AOX offer a promising approach in the control or slowing down progression of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and ischaemic and haemorrhagic stroke (Maxwell, 1995; Floyd, 1999; Mattson, 2000; Moosmann and Behl, 2002; Nair *et al*., 2003; Berger, 2005). To write off antioxidants as potentially harmful, is ultimately keeping a potential weapon out of the therapeutic arsenal.
Strategies aimed at limiting ROS oxidative stress damage, may slow the progression of neurodegenerative diseases (Halliwell, 2001; Singh *et al*., 2004). Since endogenous AOX defences are not always completely

effective, and since exposure to damaging environmental factors is increasing, exogenous AOX will find more roles in diminishing the cumulative effects of oxidative damage (Gilgun-Sherki *et al*., 2001). Plant derived AOX are regarded as effective in controlling the effects of oxidative damage, and hence have had influence in what people eat and drink (Viana *et al*., 1996; Sun *et al*., 2002; Pinder and Sandler, 2004). As the focus of medicine shifts from treatment of manifest disease to prevention, herbal medicine (with its four pillars of phytochemistry, phytopharmacy, phytopharmacology and phytotherapy) is coming into consideration, being a renaissance of age-old human tradition (Weiss and Fintelmann, 2000). The 'Green' movement in Western society has changed attitudes in the general population who now conceive naturally derived substances and extracts as being inherently safer and more desirable than synthetic chemical products, with the net effect of increase in sales of herbal preparations (Houghton and Raman, 1998; Capasso *et al*., 2000). About 80% of people in the developing world rely on phytomedicine for primary healthcare for man and livestock (Plotkin, 1992; McCorkle *et al*., 1996).

However, despite the demand of phytotherapeutic agents growing (Capasso et al., 2000), most medical and veterinary professionals still distrust the use of herbal medicines, due to lack of scientific evidence of efficacy and safety (Sofowora, 1982; Thompson, 1997). Hence the need for their scientific validation before plant-derived extracts gain wider acceptance and use. In this regard, many plants nevertheless have been scientifically proved to be effective in control of acute and chronic nervous disorders (Table 9.1). As herbal extracts are a complex mixture of compounds, the active molecules, mode of action, bioavailability and pharmacokinetics, and toxicity issues become difficult to evaluate.

Peltophorum africanum (weeping wattle), a plant widespread in southern Africa and most tropical areas, is unique in that it is traditionally used to treat more less similar disease conditions in man and domesticated animals. The root and bark decoctions are used to treat wounds, colic (acute pains), joint and back pain (chronic pains), ascites and abdominal disorders, diarrhoea and dysentery, infertility, and depression (Watt and Breyer-Brandwijk, 1962; Venter and Venter, 1996; Van Wyk and Gericke, 2000; Manana, 2003). In southern Africa, women who lose their spouses take the bark/root decoctions for up to a year, possibly for relief of post-traumatic stress. In livestock, the plant is used against diarrhoea, dysentery, colic and as a general tonic. Pastoralists use root as a component in the '*Kgatla doctors'* mixture to promote well-being, resistance to diseases and fertility (Watt and Breyer-Brandwijk, 1962; Cunningham and Zondi, 1991; Van der Merwe, 2000). From the foregoing, the traditional use point to *P. africanum* as having antibacterial, anthelmintic, anti-inflammatory and antioxidant activities. Many of the compounds isolated by phytochemists are polyphenols, typical of antioxidants of higher plants (Larson, 1988; Paya *et al*., 1992; Braca *et al*., 2002).

It is not surprising therefore, that Bizimenyera *et al*. (2005), reported high concentrations (20-50%) of polyphenols in the extracts of *P. africanum* .

Table 9. 1 Commercialised medicinal plants proven effective in control of nervous/chronic conditions (Van Wyk and Wink, 2004)

Plant	Common name	Active ingredients	Activity / action
Harpagophytum procumbens	Devil's claw	Coumarins; phenolic glycosides	Anti-inflammatory; ant-rheumatic
Hypericum perforatum	St. John's wort	Phenolic compounds; hyperforin	Analgesic; psychomotor disturbances; anti-depressant
Withania somnifera	Winter cherry	Steroids; witherferin	Ant-inflammatory; sedative
Ginkgo biloba	Ginkgo	Flavonoids; proanthocyanidins	Enhances memory & learning; dementia; insomnia
Valeriana officinalis	Valerian	Valeranone; sesquiterpenoids	Epilepsy & insomnia; tranquilizer
Rouvolfia serpentium	Indian snake root	Indole alkaloids	Psychomotor disturbances; tranquilizer
Sutherlandia frutescens	Cancer bush	Flavonoids; triterpenoids	Cancer; general tonic
Vitis vinifera	Grape vine	Proanthocyanidins; flavonoids	Circulatory disturbance; antioxidants
Humulus lupulus	Hop plant	Phenolics; proanthoyanidins	Sedative; mood disorders
Papaver somniferum	Opium poppy	Alkaloids	Narcotic; analgesic

Hence, *P. africanum* could have potential for neuroprotective antioxidant –based therapeutics.

In the study described hereafter, the comparative antioxidant activity potential of *P. africanum* extracts 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 2, 2-diphenyl-1-picryl hydrazyl (DPPH) , respectively.

9.2 Methodology

9.2.1 Collection, storage and preparation of plant material

Leaves, stem bark and root bark were collected from mature *Peltophorum africanum* Sond. (Fabaceae) trees, growing naturally at Onderstepoort, South Africa. A voucher specimen (PM 001) is stored in the Medical Plant Herbarium, Department of Paraclinical Sciences, University of Pretoria, South Africa. The collected plant material was dried in the shade at ambient temperature, and ground to powder before extraction. A known mass of each of the powdered material was then percolated with ten volumes of acetone at room temperature for 24 hours and filtered. Acetone was used as extractant, as it has been found to extract large quantities of bioactive plant material (Eloff, 1998). The extracts obtained were concentrated under vacuum at 40 ^{o}C using a rotary evaporator (Buchi®, Switzerland) to give the crude extracts of each plant material. The dry extracts were stored in sealed vials in the refrigerator prior to further processes.

9.2.2 Chemicals

All chemicals used were of analytical grade. L-ascorbic acid (Merck), potassium persulphate (Sigma), 2,2´-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) (Sigma), 6-hydroxy-2, 5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®) (Fluka), 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma) and absolute ethanol (Merck).

9.2.3 Evaluation of antioxidant activity

Qualitative screening for antioxidant activity was done using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) according to Takao *et al*. (1994). Thin layer chromatograms (TLC) of extracts developed in EMW (ethyl acetate / methanol / water (10 / 1.35 / 1) solvent system were sprayed with 0.2% DPPH in methanol. Antioxidant activity is detected on the chromatogram when the initially purple DPPH background turns yellow in bands where an antioxidant is present (Bors *et al*., 1992).

Quantification of AOX activity was determined spectrophotometrically using two radicals, ABTS and DPPH and the Versa-max® microplate reader (Labotec). In one method, use was made of theTrolox equivalent antioxidant capacity (TEAC) assay (Re *et al*., 1999) based on the scavenging of the ABTS radical into a colourless product. The absorbance was read at 734 nm. This method was also used in a previous analysis

(Bizimenyera *et al*., 2005). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) is a Vitamin-E analogue. If an extract had antioxidant activity equivalent to Trolox, its TEAC value would be 1 and if the extract were more active its TEAC would be greater than 1.

The second method described by Mensor et al. (2001), employed the DPPH free radical assay. Different concentrations of the extracts were prepared between 20.0 and 1.0 µg/ml. Ten µL of 0.4 mM DPPH in ethanol was added to 25 µL of each concentration of extract tested and allowed to react at room temperature in the dark for 30 minutes. Blank solutions were prepared with each test sample solution (25 µL) and 10 µL ethanol only while the negative control was DPPH solution, 10 µL plus 25 µL ethanol. L-ascorbic acid was the positive control. The decrease in absorbance was measured at 518 nm. Values obtained were converted to percentage antioxidant activity (AOXA%) using the formula: -

$$AOXA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$$

Abs_{sample} is the absorbance of the sample, Abs_{blank} is the absorbance of the blank and $Abs_{control}$ is the absorbance of the control.

L-ascorbic acid (vitamin C) was used as a positive control (antioxidant agent). The antioxidant activity is expressed as effective concentration (EC_{50}) values. The lower the EC_{50} value, the more effective antioxidant activity. The EC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts (µg/mL) against the mean percentage of the antioxidant activity obtained from three replicate assays. For statistical analysis, the results were expressed as mean ± SEM (standard error of mean) and the EC_{50} values obtained from the regression plots (SigmaPlots^R 2001, SPSS) showed a good coefficient of determination, with most values being $r^2 \geq 0.910$.

9.3 Results

All the extracts (leaf, bark and root), from qualitative screening, contained compounds that exhibited considerable free radical scavenging activity, as shown by the yellow bands (of antioxidant activity) on DPPH chromatograms, Figure 9.1. The root and bark had more antioxidant activity compared to the leaf. The antioxidant activity of the root and bark extracts was higher than both L-ascorbic acid and Trolox (Table 9. 2).

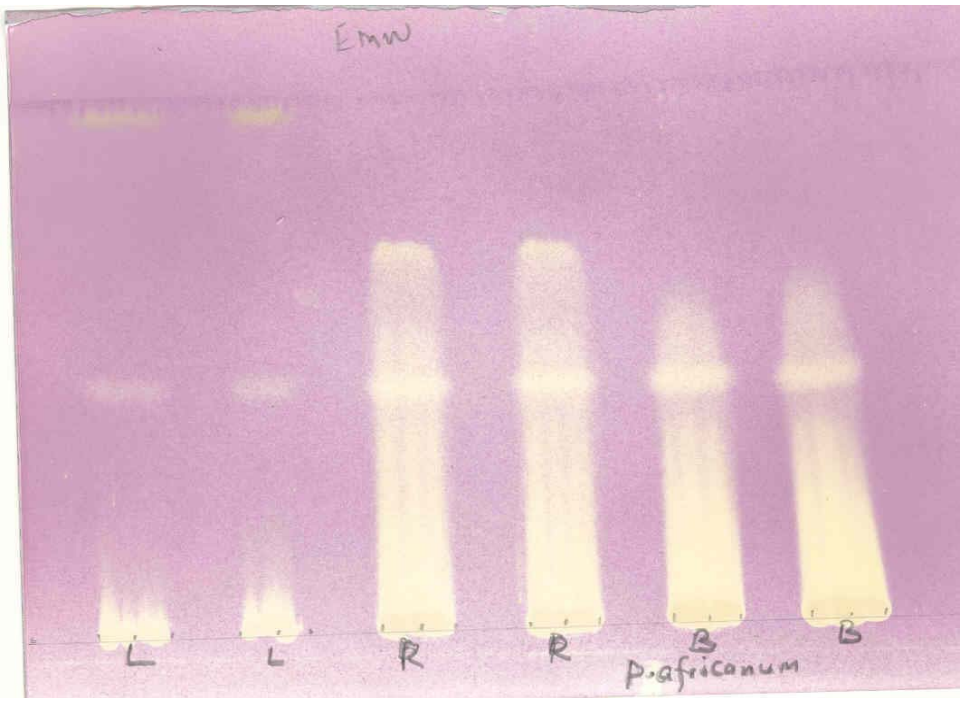

Figure 9. 1 Chromatogram of 200 µg acetone extracts of leaf (L), bark (B) and root (R) of *P. africanum* , separated with EMW and sprayed with DPPH. Note the high antioxidant activity, indicated by yellow areas

Table 9.2 Trolox (TEAC) and Vitamin-C equivalent[#] values of acetone extracts of leaf, bark and root of *P. africanum*

Plant part		**AOX Values***	
		TEAC	**DPPH(EC_{50}±SEM (µg/ml)**
Leaf		0.57	6.54± 0.49
Bark		1.08	4.37± 0.41
Root		1.28	3.82± 0.58
Standards	Trolox	1.0	N/A
	L-ascorbic acid	N/A	5.04± 0.65

{Note: - [#] If the extract had antioxidant activity equivalent to Trolox, its TEAC value would be 1 and if the extract was more active its TEAC value would be greater than. The reverse is true with DPPH.; the values above 5 mean less antioxidant activity and those lower than 5 mean higher activity}

The bark and root extracts had higher TEAC values than Trolox (Vitamin-E analogue), with respective values of 1.08, 1.28 and 1.0. 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 (the higher the µg/mL value, the less AOX activity).

9.4. Discussion and conclusion

Laboratory results showed the extracts had high levels of antioxidant compounds, especially the root and bark extracts. Both Trolox and L-ascorbic acid have been used as standards in quantifications of antioxidant activity (Van den Berg *et al*., 1999; Fukomoto and Mazza, 2000). The EC_{50} of the root (3.82 µg/ml) and bark (4.37 µg/ml) extracts indicated higher antioxidant activity than L-ascorbic acid (5.04 µg/ml), and more effective than *Ginkgo biloba* extract (EGb 761) whose EC_{50} is 40.72 µg/mL (Mensor *et al*., 2001; Bridi *et al*., 2001; Aderogba *et al*., 2004). The standardised extract of *Ginkgo biloba* (EGb 761) has been widely employed for its significant benefit in neurodegenerative disorders (Bridi *et al*., 2001).

The antioxidant activity in plants may largely be due to polyphenols (Thabrew *et al*., 1998), and in particular polyphenols make up nearly 50 % of *P. africanum* root and bark extracts (Bizimenyera *et al*., 2005). The presence of antioxidant compounds also agrees with the phytochemical analyses by other investigators (El Sherbeiny *et al*., 1977; Evans *et al*., 1985; Bam *et al*., 1988 and 1990; Khattab and Nasser, 1998; Mebe and Makuhunga, 1992) who isolated flavonoids, coumarins, gallic acid and other polyphenols from *P. africanum* extracts.

The root and bark appear to have the highest concentrations of the AOX. Coincidentally, traditional healers also use the root and bark concoctions (Watt and Breyer-Brandwijk, 1962; Cunningham and Zondi , 1991; Venter and Venter, 1996; Van der Merwe, 2000; Van Wyk and Gericke, 2000; Manana, 2003). In all the traditional treatments, decoctions and infusions are made from the root or bark. That is why the present authors believe the rationale for traditional healers using *P. africanum* in treating inflammation, pain and depression may be due to high AOX compounds present in the plant.

Various chronic degenerative diseases with different clinical appearances appear to share common biochemical, genetic and cellular alterations that are related to disease pathogenesis (Nair *et al*., 2003; Barnharm *et al*., 2004). Empirical evidence suggests *P. africanum*, due to its high AOX activity, could be influential in the control of these conditions. Mixtures of dietary antioxidants or foods rich in antioxidants have been shown to increase the ability of lymphocytes to withstand DNA oxidation (Duthie *et al*., 1996; Pool-Zobel *et al*., 1997; Porrini and Riso, 2000). Diets rich in flavonoids appear to be protective against ischemic heart disease (Hertog *et al*., 1993; Viana *et al*., 1996; Berger, 2005) and supplementing diets with antioxidants generally appears preventing occurrence and progression of many neurodegenerative diseases (Rice-Evans and Diplock, 1993; Rice-Evans *et al*., 1995; Prasad *et al*., 1999; Singh *et al*., 2004).

The safety of P. *africanum* extracts still has to be confirmed. From inquiries from pastoralists (Van der Merwe, 2000) and Pretoria market vendors of traditional herbs, *P. africanum* extracts were reported safe. Tests in our laboratory involving acetone extracts on monkey kidney cells and brine shrimp (awaiting publication) showed no toxicity effects at the highest dose of 5 mg ml^{-1}used. Bessong *et al*. (2005) reported no toxicity of the extracts. This, however, is no cause for complacency, as toxicity of herbal medicines has been reported (Capasso *et al*., 2000).

This paper reports *P. africanum* leaf, bark and root extracts as showing considerable antioxidant activity. The root and bark had more antioxidants than the leaf. The antioxidant activity of the root and bark extracts, was higher than L-ascorbic acid (Vitamin-C) and Vitamin-E equivalent (Trolox), both standard antioxidants. The results of these *in vitro* tests would appear to justify the traditional use of the plant in treatment of acute and chronic nervous disorders. Antioxidants appear to have neuroprotective and neurodegenerative roles, reducing or slowing down neuronal cell death, and have an important role to play in diminishing the cumulative effects of oxidative damage. Further *in vivo* research and clinical trials are required for generation of conclusive data for use of the plant in treating neurodegenerative diseases. Continued work on plants like *P. africanum*, rich in antioxidants, may avail medicine with antioxidant-based neuroprotective therapeutics.

Acknowledgements

Staff Development Programme Makerere University, Uganda, the National Research Foundation, South Africa, and the University of Pretoria funded this work

9.5 References

1. Aderogba, M .A., Okoh, E. K., Adelanwa, T. A. and Obuotor, E. M. (2004). Antioxidant properties of the Nigerian *Piliostigma species*. *J. Biol. Sci*., **4**: 501-503
2. Bam M., Ferreira D. and Brandt E. (1988). Novel cyanomaclurin analogue from *Peltophorum africanum Phytochemistry,* **27**: 3704-3705.
3. Bam M, Malan J. C. S, Young D. A., Brandt E. V. and Ferreira . (1990). Profisetinidin-type 4-arylflavin-3-ols and related δ-lactones. *Phytochemistry,* **29**: 283-287.
4. Barnham K. J., Masters . L. and Bush A. I. (2004). Neurodegenerative diseases and oxidative stress *Nature Rev. Drug Disc*., **3**:205-214
5. Berger M. M. (2005). Can oxidative damage be treated nutritionally? *Clinical Nutrition*, **24**: 172-183.
6. Bessong P. O., Obi C. L., Andréola M., Rojas B., Pouységu L., Igumbor E., Meyer J. J. M., Quideau S. and Litvak S. (2005). Evaluation of selected South African medicinal plants for inhibitory properties against human immunodeficiency virus type 1 reverse transcriptase and integrase. *J. Ethnopharmacol*, **99**: 83-91.
7. Bizimenyera E. S., Swan G. E., Chikoto H and Eloff J.N. (2005). Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. *J. S. Afri. Vet. Assoc*., **76**: 54-58.
8. Bors W., Saran .and Eltsner E. .F (1992). Screening of plant antioxidants. *Modern Methods of Plant Analysis*, **13**: 277-295
9. Braca A. , Sortino C., Politi M., Morelli. and Mendez J. (2002). Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J Ethnopharmacol*, **79**: 379-381.
10. Bridi R., Crossetti F. P., Steffen V. M. and Henriques A. T. (2001). The antioxidant activity of standardised extracts of *Ginkgo biloba* (EGb 761) in rats. *Phytother. Res*., **15**: 449-451
11. Cao W., Carney J. M., Duchon A., Floyd R. A. and Chevion M (1988). Oxygen free radicals involvement in ischemia and repurfusion injury to brain. *Neurosci Lett*, **88**: 233-238.
12. Capasso R., Izzo A. A., Pinto L., Bifulco T., Vitobello C. and Mascolo N. (2000) . Phytotherapy and quality of herbal medicines. *Fitoterapia*, **71**: S58-S65.

13. Cunningham A. B. and Zondi A. S. (1991). Cattle owners and traditional medicines used for livestock. *Investigational Report No 69*, pg 35. Institute of Natural Resourses, University of Natal. Petermaritzburg.

14. Duthie S. J, Ma A., Ross M. A. and Collins A. R. (1996). Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res*, **56**:1291-1295

15. Eloff J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol,* **60**: 1-8.

16. El Sherbeiny A. E. A., El Ansari, Nawwar M. A. M and El Sayed N. H. (1977). Flavonol glycosides and flavonol glucoside gallates from *Peltophorum africanum*. *Planta Medica*, **32**:165-170

17. Evans S. V., Shing T. K. M., Aplin R. T., Fellows L. E. and Fleet G. W .J. (1985). Sulphate ester of trans-4-hydroxypipecolic acid in seeds of *Peltophorum. Phytochemistry,* **24**: 2593-2596.

18. Floyd R. A. (1999). Antioxidants, oxidative stress, and degenerative neurological disorders. *Proceedings of the Society for Experimental Biology and Medicine*, **222**: 236-245.

19. Floyd R. A. and Carney J. M. (1992). Free radical damage to protein and DNA: Mechanisms involved and relevant observations on brain undergoing oxidative stress. *Ann. Neurol*, **32**: S22-S27.

20. Fukumoto L. R., Mazza G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem*., **48**: 3597-3604

21. Gilgun-Sherki Y., Melamed E. and Offen D. (2001). Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacology,* **40**: 959-975

22. Halliwell B. and Gutteridge J. M. C. (1985). Oxygen radicals and the nervous system. *Trends Neurosci*, **8**: 22-26.

23. Halliwell B. (2001). Role of free radicals in neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs & Aging*, **18**: 685-716.

24. Hertog M. G. L., Feskens E. J. M. and Hollmann .P C. H. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zuphen Elderly Study. *Lancet*, **342**:1007-1011

25. Houghton P.J. and Raman A. (1998) . Laboratory handbook for the fractionation of natural extracts. Chapman & Hall.London, pg 1-7.

26. Khattab A. M. and Nassar M. I. (1998) . Phytochemical and molluscidal studies on *Peltophorum africanum* and *Sesbania sesbani*. *Bull. Natl.Res.Cent*, Egypt, **23**: 401-407

27. Larson R. A. (1988). The antioxidants of higher plants. *Phytochemistry*, **27**: 969-978.

28. Manana J. V. (2003). Identification of commonly used traditional medicines by planar chromatography for quality control purpose. *M.Sc. Dissertation*. University of Pretoria, South Africa.

29. Mattson M. P. (2000). Apoptosis in neurodegenerative disorders. *Nature Rev. Mol. Cell Biol*., **1**:120-130

30. Maxwell S. R. J (1995). Prospects for the use of antioxidant therapies. *Drugs*, **49**: 345-361.

31. McCorkle C. M., Mathias-Mundy E. and Schillhorn van Veen T. W. (1996). In: *Ethnoveterinary Research and Development*. Intermediate technology publications.London.

32. Mebe P. P. and Makuhunga P. (1992). 11-(E)-*p*-coumaric acid ester of bergenin from *Peltophorum africanum. Phytochemistry,* **31**: 3286-3287.

33. Mensor L. L., Menezes F. S., Leitao G. G., Reis A. S., Santos T. C., Coube C. S. and Leitao S. G. (2001). Screening of Brazilian plants for antioxidant activity by use of DPPH free radical method. *Phytother. Res*., **15**: 127-130

34. Moosmann B. and Behl C. (2002). Antioxidants as treatment for neurodegenerative disorders. *Expert Opinion on Investigational Drugs*, **11**:1407-1435.

35. Nair U., Bartsch H. and Nair J. (2003). Prevention of degehnerative diseases; clues from studies investigating oxidative stress, Brussels,13 November 2002. *Mutagenesis*, **18**: 477-483.

36. Namiki M., Yamashita K. and Osawa . (!993). Food-related antioxidants and their activities *in vivo*. In: *Active oxygen, lipid peroxidases and antioxidants*. Japan Science Society Press, pg. 319-332. Tokyo.

37. Paya M., Halliwell B. and Hoult J. R. S. (1992). Interaction of a series of coumarins with reactive oxygen species. *Biochem. Pharmacol,* **44**: 205 – 214.

38. Plotkin M. J. (1992). Ethnomedicine: past, present and future. In: *Natural resources and human health: plants of medicinal and nutritional value*. Proceedings, pg 79-86. Elsevier, Amsterdam.

39. Pinder R. M. and Sandler M. (2004). Alcohol, wine and mental health: focus on dementia and stroke. *J. Psychopharm.*, **18**: 449-456.

40. Pool-Zobel B. L., Bub A., Muller H., Wollowski I. and Rechkemmer G (1997). Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis*,**18**: 1847-1850.

41. Porrini M. and Riso P. (2000). Lymphocyte lycopene concentration and DNA protection from oxidative damage is is increased in women after a short period of tomato consumption. *J Nutri*, 130:189-192.

42. Prasad K. N., Cole W. C., Hovland A. R., Prasad K. C., Nahreini .P, Kumar B., Edwards-Prasad J. and Andreatta C. P. (1999). Multiple antioxidants in the prevention and treatment of neurodegenerative disease: analysis of biological rationale. *Curr. Opin. Neurol*., **12**: 761-770.

43. Rao A. V.and Balachandran B. (2002). Role of oxidative stress and antioxidants in neurodegenerative diseases. *Nutri. Neurosci*., **5**: 291-309

44. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolourising assay. *Free Radic. Biol. Med,* **26**: 1231-1237.

45. Rice-Evans C. A, Diplock A. T. (1993). Current status of antioxidant therapy. *Free Rad. Biol. Med*, **15**: 77-96.

46. Rice-Evans C. A., Miller N. J., Bolwell G. P., Bramley P. M. and Pridham J. B. (1995). The relative antioxidant activities of plat-derived polyphenolic favonoids. *Free Radic. Res*., **22**: 375-383

48. Singh R. P., Sharad S. and Kapur S. (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. J. *Ind. Acad. Clin. Med*., **5**: 218-225

49. Sofowora A. (1982). In: *Medicinal Plants and traditional medicine in Africa*. John Wiley and Sons. Chichester,UK.

50. Sun A. Y., Chen Y. (1998). Oxidative stress and neurodegenerative disorders. *J. Biomed. Sci*, **5**:401-414.

51. Sun A. Y., Simonyi A., Sun G. Y. (2002). The "French Paradox" and beyond: neuroprotective effects of polyphenols. *Free Radic. Biol. Med*., **32**: 314-318

52. Takao T., Kitatani .F, Watanabe N., Yagi A. and Sakata K. (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Biosci. Biotech. Biochem,*. **58***:* 1780-1783.

53. Thabrew M. I., Hughes R. D. and McFarlane I. G. (1998). Antioxidant activity of *Osbeckia aspera. Phytother. Res*., **12**: 288-290

54. Thompson A. (1997). As patients embrace herbal remedies, dearth of scientific evidence frustrates clinicians. *Am. J. Health Syst. Pharm*, **54**: 2656-2664.

55. Van der Merwe D. (2000). Use of ethnoveterinary medicine plants in cattle by Setwana-speaking people in the Madikwe area of the North West Province. *M.Sc Dissertation*. University of Pretoria. South Africa.

56. Van den Berg R., Haenen G. R. M. M., Van der Berg H. and Bast A. (1999). Application of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity of measurement of mixtures. *Food Chem*, **66**:511-517.

57. Van Wyk B. E. and Gericke N. (2000). In: *People's plants*. Briza Publications, Pretoria, pp. 130.

58. Van Wyk B E., Wink M (2004). In*: Medicinal plants of the world*. 1st Ed ,2nd Revised impression. Briza. Pretoria.

59. Venter F. and Venter J. A. (1996). In: *Making the most of Indigenous Trees*. Briza publications.Pretoria, pg 20-21.

60. Viana M., Barbas C., Banet B., Bonet M. V., Castro M., Fraile M. U. and Herrela . (1996). *In vitro* effect of a flavonoid-rich extract on LDL oxidation. *Atheloscleros*is , **123**: 83-91

61. Watt J. M. and Breyer-Brandwijk M. G. (1962) . The medicinal and poisonous plants of Southern and Eastern Africa. 2nd Ed. E& S Livingstone. London , pp. 638.

62. Weiss R. F. and Fintelmann V. (2000). In: *Herbal Medicine*. 2nd edition. Thieme. Stuttgart,pg. 3-20.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSION

10.1 *Peltophorum africanum* in traditional medicine

This work was stimulated by the reports by van der Merwe (2000) and Manana (2003). The former reported the plant being used by pastoralists of Madikwe area for diarrhoea and dysentery in cattle as well as tonic for well being and general resistance to disease in cattle. The latter found *P. africanum* to be a common medicinal plant in Pretoria medicinal plant market, where the healers claimed the bark had anti-inflammatory activity and was also used for diarrhoea, dysentery, abdominal pains, and helminthosis in people. Earlier, Mabogo (1990) had reported the plant to be used for stomach complaints, intestinal parasites and pain in people. Another dimension of use by traditional health workers has been in relief of anxiety and depression. The bark or root extract is taken by women who lose their spouses for up to a year (Watt and Breyer-Brandwijk, 1962), possibly for relief of post-traumatic stress. From the foregoing, .*P. africanum* was considered to have antimicrobial, anthelmintic and immune boosting activities. There is so much utilisation of *P. africanum* by traditional healers that in some parts of South Africa the plant has been stripped bare through removal of the bark (Mabogo, personal communication).

10.2 Extraction

Extractable compounds from the bark and root were mainly polar, as non polar solvents (hexane and dichloromethane) extracted very little (quantity) material from *P. africanum*, (Chapter 3). Most of the compounds isolated from the plant by many chemists or phytochemists (El sherbeiny *et al*., 1977; Evans *et al*., 1985; Bam *et al*., 1988 and 1990; Khattab and Nasser, 1998; Mebe and Makuhunga, 1992; Bessong *et al*., 2005) have been polyphenols (flavonoids, coumarins, catechins, gallic acid). Acetone was selected as a suitable solvent for the extracts used in bioassays as it extracts compounds of wide polarity range, is miscible with organic and aqueous solvents, and is non-toxic to test organisms. Bergenin in the present work was isolated from the acetone extracts of the root.

10.3 Antibacterial activity

The acetone and ethanol extracts showed considerable antibacterial activity, against both Gram-positive and Gram-negative bacteria (Chapter 3). Using the microplate dilution method of Eloff (1998 a), the minimum inhibitory concentration (MIC) values of 0.08 mg ml^{-1} were obtained for ethanol extracts of the root and dichloromethane extracts of the leaf against *Staphylococcus aureus*. The MIC value for acetone and ethanol extracts of the bark and root against *Pseudomonas aer*uginosa were 0.16 mg ml^{-1}. Bergenin isolated from the acetone extracts of the root had moderate inhibitory action against both *P. aeruginosa* and *E. coli*, by the disc diffusion method of Bennet *et al*. (1996). Bergenin had good antimicrobial activity against *Sporobolomyces salmonicolor* though no activity against *Penicillium notatum* (Chapter 8).

The results of the current work confirmed reports of previous investigators of antimicrobial action of *P. africanum* extracts (Manana, 2003; Obi *et al*., 2003; Samie *et al*., 2003). Manana (2003), using the microdilution technique, found acetone extracts of the bark to have MIC values of 0.08 against *S. aureus*. Samie *et al*. (2003) and Obi *et al*. (2003) employing the disc diffusion method demonstrated good antibacterial activity of the methanol and ethanol extracts of the bark and root. Although to date there are no reports of *in vivo* antibacterial trials with *P. africanum* extracts, the results of *in vitro* work reported would appear to validate the traditional use of the plant extracts for treatment of bacterial diseases of the gastrointestinal tract that cause diarrhoea. The traditional use of *P. africanum* extracts against diarrhoea, dysentery and unthriftness could as well be treating gastrointestinal nematodes, as they share similar clinical symptoms.

10.4 Antioxidant activity

P. africanum is one of the plants rich in antioxidants, plants that have found much use in traditional medicine. The antioxidants appear to be concentrated more in the root and bark than the leaf (Chapters 3 and 9). Antioxidant activity in plants is largely attributable to polyphenols (Thabrew *et al*., 1998). Other investigators have isolated flavonoids, coumarins, gallic acid, catechin, and other polyphenols from *P. africanum* (El Sherbeiny *et al*., 1977; Evans *et al*., 1985, Bam *et al*., 1088, 1990; Mebe and Makuhunga, 1992). The antioxidant activity of the root and bark extracts was higher than L-ascorbic acid (Vitamin C) and Vitamin–E equivalent (Trolox). Traditional healers also use the root and bark concoctions (Watt and Breyer-Brandwijk, 1962; Cunningham and Zondi, 1991; van der Merwe, 2000; Manana, 2003).

Antioxidants are a promising approach in the fight against human immunodeficiency virus (HIV), cardiovascular and neurodegenerative diseases, conditions associated with pro-oxidative conditions in the

body with overabundance of reactive oxygen species (Greenspan *et al*., 1994; Maxwell, 1995; Floyd, 1999; Mattson, 2000; Moosmann and Behl, 2002; Nair *et al*., 2003; Berger, 2005). Compounds of plant origin have been identified that inhibit replication of human immunodeficiency virus (HIV), slowing progression and limiting apoptosis of immune cells (Greenspan *et al*., 1994; Pengsuparp *et al*., 1995; Vlietinck *et al*., 1998; Asres *et al*., 2001). The extracts of *P. africanum* (Bessong *et al*., 2005) and bergenin (Piacente *et al*., 1996) which we have also isolated from the *P. africanum* root extracts has inhibitory effect against HIV. A synergistic action of plant oxidants has been proposed as one of the mechanisms by which replication and cell killing (apoptosis) in HIV infection is inhibited (Greenspan *et al*., 1994). It is not surprising that the extracts of *P. africanum* are used against HIV infections in Botswana (Hargreaves and Mosesane, 2003). The traditional use of the extracts of *P. africanum* by women after losing their spouses (Watt and Breyer-Brandwijk, 1962), could imply therapeutic effects of its compounds on depression and post-traumatic stress, and therefore give the plant a role in neuroscience.

10.5 Anthelmintic activity

The extracts of *P. africanum* had significant inhibitory effect on the egg hatching and larval development of the parasitic nematodes, *Haemonchus contortus* and *Trichostrongylus colubriformis in vitro* (Chapters 4 and 5) at a concentration of 0.2-1 mg ml^{-1}. Though tannins in plant extracts have anthelmintic activity on their own, some anthelmintic activity was retained in tannin-free extract and in the bergenin isolated from the root extract. Traditional healers use extracts of *P. africanum* for gastrointestinal helminths and other abdominal disorders (Watt and Breyer-Brandwijk, 1962; Venter and Venter, 2002). The traditional use of the plant extracts against diarrhoea and dysentery may in fact be due to anthelmintic activity, as these clinical symptoms are consistent with parasitic gastroenteritis. *In vitro* anthelmintic activity of *P. africanum* has been shown against cestodes (Mølgaard *et al*., 2001).

The translation of the *in vitro* anhelmintic activity did not produce significant action in lambs artificially infected with *H. contortus* and *T. colubriformis,* when the extracts were administered at 50, 500 and 750 mg kg^{-1} (Chapter 6). However, this does not nullify traditional usage of the tree, or *in vitro* results reported as higher doses of the extracts may have been more effective. Nevertheless, the extracts did not show toxicity to the lambs even at the highest dose (750 mg kg^{-1}) administered.

Parasitic gastrointestinal nematodes rank as number one cause of production losses in small ruminants (sheep and goat) livestock systems in the tropical and subtropical regions of the world (Perry *et al*., 2002). Due to anthelmintic resistance, and exorbitant cost of pharmaceutically derived drugs (Prichard, 1990; Wolstenholme

et al., 2004; Jabbar *et al*., 2006) there is a trend to seek plant anthelmintics as one of the alternative and cost benefit ways of controlling helminth infections (Hammond *et al*., 1997; Danø and Bøgh, 1999).

10.6 Safety of *Peltophorum africanum*

P. africanum extracts were not found toxic in both brine shrimp and Vero monkey kidney cell line assays (Chapter 7). Bergenin, a major compound of its root was not toxic either (Chapter 8). In a parallel work in lambs infected with nematode parasites, the extracts at 750 mg kg^{-1} did not show any toxic or abnormal effects in the lambs. Toxicity of *P. africanum* has not been reported, by both traditional human and veterinary practitioners. Bessong *et al*. (2005) reported no toxic effect on the HeLaP4 (human epitheloid carcinoma cell line) by the aqueous and methanol extracts of *P. africanum*.

P. africanum is a valuable plant in the veld as its leaves and twigs are eaten by the elephant, rhino, giraffe, kudu, impala and grey duicker, and also browsed by the goats and sheep (Venter and Venter, 2002; van Wyk and Gericke, 2000). It is a tree that grows up to 15 metres tall, with a wide canopy. The common name 'weeping wattle' is due to sap-sucking insects (*Ptyelus grossus*) that attach to the branches, and some of the sap drips to the ground. The larvae of the Van Son's charaxes (*Charaxes vansoni*), Satyr charaxes (*C. ethalion*) and and Braines's charaxes (*C. brainei*) feed on the leaves (Venter and Venter, 2002).

Other than the bitter taste, possibly attributed to the tannin content, *P. africanum* extracts appear to be safe. The apparent lack of toxicity of *P. africanum* leads support to the promotion of its use in traditional medicine. However, more controlled laboratory and clinical tests are required to ascertain the full details its efficacy and safety.

10.7 Conclusions

P. africanum is a widely distributed plant in southern Africa and other tropical regions. In Botswana, because of its ubiquity, and wide availability, *P. africanum* has earned the name '*Lejaja*'. Because of its antibacterial and antioxidant activities, it has good potential in treatment of infection-related diseases either by inhibiting growth of pathogens or indirectly by stimulating the immune system of the host. The use against diarrhoea may be due to its effect on enteric microbial or parasitic agents. *In vitro* activity of *P. africanum* extracts against HIV, and the rich antioxidant content may imply the plant has potential in the management of HIV-AIDS. The rich antioxidant content further puts the plant in line for treatment of neurodegenerative and cardiovascular diseases. There is thus good potential of *P. africanum* in medicine.

However the use of the bark and root by traditional healers, may ultimately lead to exhaustion of the plant resources.

As a result of this study on *P. africanum* a number of recommendations are outlined:-

1. Study of the activity of extracts to determine their stability and seasonal or regional variation of action,
2. Isolation of potent anthelmintic ,antioxidant and antibacterial compounds,
3. Devising better extraction methods and potentiating procedures would yield extracts with a higher activity,
4. More clinical efficacy and safety trials using extracts or isolated compounds, and
5. Preparation of suitable dosages (under low level technology adaptable to rural conditions) for use by resource-poor rural communities.