CHAPTER 7

SAFETY PROFILES OF *PELTOPHORUM AFRICANUM* SOND. (FABACEAE) EXTRACTS

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Abstract

Peltophorum africanum Sond (Fabaceae), commonly called 'weeping wattle', is a plant widely used traditionally for medicinal purposes in both man and animals. Traditionally the extracts have been used against diarrhoea, dysentery, helminthosis, acute and chronic pains, resistance to infection and depression. Antibacterial, anthelmintic, and antioxidant activities have been demonstrated in its extracts *in vitro*. The safety and toxicity of the extracts has received little attention. From *in vitro* toxicity tests, the leaf, bark and root extracts were not toxic to brine shrimp and Vero monkey kidney cells. The apparent lack of toxicity of the extracts of *P. africanum* leads to support the promotion of its use in traditional medicine.

Key words: -Herbal extracts; Toxicity; Safety; Bergenin; *Peltophorum africanum*

7.1. Introduction

The use of medicinal plants in treating diseases is an ancient tradition that has co-existed with human habitation. Herbal medicines form a significant part of culture and traditions of rural people in developing countries. As a result there is an increasing trend to integrate traditional medicine with primary health care. This arises because about 80% of people in the developing world today, especially where modern drugs are not affordable, or are inaccessible or unacceptable, depend on traditional herbal remedies [1, 2]. Disease concepts are largely similar in humans and animals. Traditional healers of people are often called to treat animals (and vice versa), often employing the same herbs, compounds or manipulative techniques [3, 4]. In developed Western countries half of all prescriptions dispensed contain substances of natural origin, 50% of which have plant derived active principles [5].The green movement in Western society has changed attitudes in the general population, who now conceive naturally derived substances and plant extracts as being inherently safer and more desirable than synthetic chemical products [6]. Hence there is renewed interest in traditional pharmacopoeias, with researchers determining the scientific rationale of plant usage, discovery of

new compounds, or using plant-derived compounds as models for chemical syntheses of novel pharmaceuticals.

Over 122 drugs from 94 plants, covering a wide range of activity such as antibacterial, anti-inflammatory, antioxidant, anthelmintic, anti-amoebic, antischistosomal, antimalarial, as well as psychotropic and neurotropic activities have been discovered following botanical leads [7]. The trend towards phytotherapy notwithstanding, many medical and veterinary professionals do not trust the use of herbal medicines [3, 8]. Despite extensive use of plants as medicines, herbal remedies are not as safe as frequently claimed. Plants contain substances (such as saponins, viscotoxins, tannins, cyanogenetic glycosides, furanocoumarins, pyrrolizidine alkaloids, sesquiterpenes, etc), possibly naturally produced for defense against pathogens and for discouragement of ingestion by man and animals, which render many herbal medicines poisonous [9]. South Africa has made a world contribution with herbal teas and plant remedies such as Cape aloes, rooibos, buchu, honeybush and devil's claw [10]. Nevertheless surveys have indicated many of the medicinal plants to be toxic or poisonous [11, 12] and many people have died from medicinal plant poisoning [13]. There are similar trends in other parts of the world. Hence the need for their scientific validation (for efficacy and safety) before plant–derived extracts gain wider acceptance and use.

Peltophorum africanum Sond (Fabaceae), is a deciduous tree widely distributed in southern Africa and other tropics. It is a unique plant in that it is traditionally used to treat more or less similar disease conditions in man and animals. The bark and root extracts are traditionally used to treat diarrhea, dysentery, acute and chronic pains, wounds, internal parasites, for boosting resistance to disease, and to treat infertility and depression [14,15, 16]. Traditional healers have used the root extract as a component in the '*Kgatla doctors*' mixture to promote wellbeing, fertility and resistance to disease. Livestock farmers use the root and bark extracts against diarrhoea, dysentery and colic and as a general tonic. In southern Africa, women who lose their spouses take the bark or root decoctions for up to a year, possibly for relief of post-traumatic stress and depression.

The phytochemistry of this species has been investigated by many authors who reported mainly flavonoids, and other phenolic compounds [17, 18, 19, 20, 21]. Reports of testing for biological activity of extracts or isolated compounds are scanty. However, antibacterial [22, 23] , antioxidant and antibacterial [24, 25], antihelmintic [26, 27] and inhibitory properties against HIV- AIDS type 1 reverse transcriptase and integrase [28] have been reported.

P. africanum is one of the dominant plants found in the Pretoria medicinal plant market[16] and very popular among the rural Madikwe community where it is used for livestock treatments [29]. Based on the traditional usage and results of *in vitro* work, the extracts and compounds of *P. africanum* have potential for health of both man and animals. The present study aimed at establishing the safety of the plant extracts.

7. 2. Materials and methods

7. 2.1 Plant material

The leaves, stem bark and root bark collected from mature *Peltophorum africanum* Sond. (Fabaceae) trees naturally growing at Onderstepoort, Pretoria, South Africa (bearing label S.A. Tree No. 215), were dried in the shade at ambient temperature. A voucher specimen (PM 001) is stored in the Medicinal Plant Herbarium, Department of Paraclinical Sciences, University of Pretoria. The dried material was ground to powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley.

7. 2.2 Extraction

A previous study showed that acetone was the best extractant for *P. africanum* compared to ethanol, hexane and dichloromethane [24]. Therefore, acetone was the solvent selected for the bioassays, in the ratio of plant material to acetone of 1:10 (weight to volume) in an overnight extraction. As the dried extracts extracted by acetone do not fully dissolve back in acetone, the extracts were not concentrated to dryness [30] and made to a stock concentration of 100 mg ml^{-1}.

7.2.3. Toxicity assays

7.2.3.1 Brine shrimp lethality

The brine shrimp lethality test is fully described by Solis *et al*. [31] who used the test for plant extracts in a range of concentrations to obtain an LD_{50} value. The brine shrimp (*Artemia salina*) eggs were obtained from a local pet shop, and hatched in artificial sea water (3.8 g NaCl + 100 ml distilled H_2O), yielding phototrophic nauplii (larvae). The acetone extracts of the leaf, bark and root were tested at concentrations of 0.1, 1, 2, and 5 mg ml^{-1}, in 4 (four) replicates, the test solution made to required volumes with distilled water. The final acetone concentration acted as solvent blank control for the nauplii, whereas Podophyllotoxin (Sigma) was the positive control, and distilled water acted as negative control. Four (4) 96-well microtitre plate replicates, with each well having 100 µl, of plant extract solution and 100 of larvae suspension containing

10-15 nauplii were incubated for 24 h at room temperature (23°C). A stereomicroscope was used in observing and counting the larvae, and any deaths in controls were adjusted for in the treated plates by using Abbott's formula described by Rasoanaivo and Ratsimamanga-Urverg [32].

$$\text{Corrected mortality percent} = \frac{m-M}{S} \times 100$$

m= mean of dead larvae in treated plates %

M= mean of dead larvae in controls %

S= mean of living larvae in controls %

(The reference compound Podophyllotoxin®, LC_{50}= 5 g ml^{-1})

7.2.3.2 MTT assay (cell line cytotoxicity) of extracts

Moosmann [33] fully describes the assay, that essentially is based on the reduction of the yellow coloured 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT), by mitochondrial dehydrogenases of metabolically active cells (live cells) to a purple formazan. The viable cell growth after incubation with extracts was determined using MTT (Sigma), for measuring cell proliferation and cytotoxicity. The intensity of colour (measured spectrophotometrically) of the formazan produced by living, metabolically active cells is proportional to the number of live cells present. Formazan is an insoluble purple substance, a result of reduction of the yellow water soluble tetrazolium dye (MTT) by the live and not dead cells.

The growth medium used was Minimum Essential Medium (MEM, Highveld Biological, Johannesburg), supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Adcock-Ingram). Cells of a subconfluent culture of Vero monkey kidney cells , obtained from the Department of Veterinary Tropical Diseases, University of Pretoria, were harvested and centrifuged at 200xg for 5 minutes, and re-suspended in growth medium to 2.4 x 10^3 cells ml^{-1}. A total of 200 µl of the cell suspension was pipetted into each well of columns 2 to 11 of a sterile 96-well microtitre plate. Growth medium (200 µl) was added to wells of column 1 and 12 to minimize the "edge effect" and maintain humidity. The plates were incubated for 24 h at 37°C in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. Then the MEM was aspirated from the wells using a fine tube attached to a hypodermic needle, and replaced with 200 µl of test extract at different concentrations prepared in growth medium. The cells were disturbed as little as possible during the aspiration of the medium and addition of the test extracts. Each dilution was tested in quadruplicate. The microplates were further incubated for 5 days at 37°C in a 5% CO_2 incubator with the test material. Untreated cells and positive control, Berberine Chloride (Sigma) was included.

After incubation, 30 µl MTT (a stock solution of 5 mg ml^{-1} in PBS) was added to each well and the plates incubated for a further 4 h at 37°C. After incubation with MTT the medium in each well was carefully removed, without disturbing the MTT crystals in the wells. The MTT formazan crystals were dissolved by addition of 50 µl DMSO to each well. The plates were shaken gently until the crystals were dissolved. The amount of MTT reduction was measured immediately by detecting the absorbance in a microplate spectrophotometer reader (Versamax®) at wavelength of 570 nm. The wells in column 1, containing the medium and MTT but no cells, were used to blank the plate reader. The LC_{50} values were calculated as the concentration of test extract resulting in a 50% reduction of absorbance compared to untreated cells.

7.2.4 Safety of extracts in sheep

In a related work reported elsewhere (Chapter 6), acetone extracts were administered by stomach tube to sheep artificially infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*.

7.2.5 Statistical analysis

The Excel package was used in data analysis.

7.3 Results

The leaf, bark and root extracts (at the maximum concentration of 5 mg ml^{-1} employed) did not show toxicity in the brine shrimp or Vero monkey kidney cell line assays, Table 7.1.

Table 7.1: Cytotoxicity of *P. africanum* root, bark and leaf extracts

Item	Brine shrimp[a] LC_{50} (µg ml^{-1})	MTT[b] LC_{50} (µg ml^{-1})
Root*	>1000	>1000
Bark*	>1000	>1000
Leaf*	>1000	>1000
Podophytotoxin	7.01	
Berberine		9.82

Key:
[a]Brine shrimp larval mortality assay
[b]Cytotoxicity assay aginst Vero Monkey kidney cell line
*Extracts at 5 mg ml^{-1} , the highest concentration used

The administration of extracts did not affect the sheep in any manner, whether by way of feed consumption, or any deleterious effect or expression.The haematological parameters and liver enzymes of the sheep that received treatment with *P. africanum* extracts for parasitic nematodes were not affected, Figure 7.1.

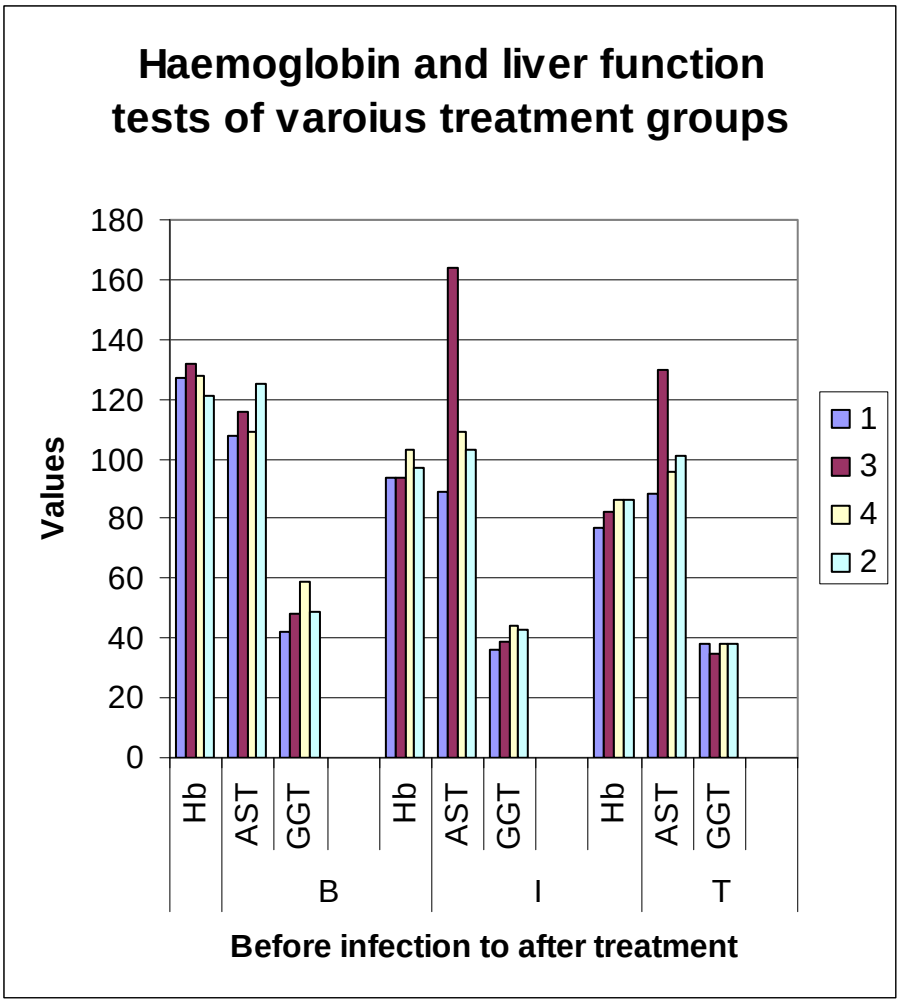

Figure 7.1: Haemoglobin and liver function tests of four treatment groups

Notes: i) Units of values, Hb=g/l and IU/l for AST & GGT

ii) 1-4 are extract treatment groups; 1 being control with no treatment

iii) B=before infection with parasitic nematodes, I= during the course of infection and T= after treatment with extracts.

7. 4 Discussion

P. africanum has been traditionally used in the treatment of a wide range of conditions including diarrheoa, dysentery, acute and chronic pain, wounds, anxiety and depression, and as a tonic for fertility and resistance to diseases[14, 15, 16]. The root and bark from *P. africanum* are important components of the products sold in informal medicinal plant markets in Pretoria [16], cattle markets of Setswana-speaking people

of Madikwe area [29], traditional healers gatherings of Botswana [34], and other regions in southern Africa. This has stimulated scientific research studies on the medicinal plant. In addition to several phytochemists who have isolated various compounds, bioassay characterization studies have shown that extracts of *P. africanum* have antibacterial [22, 23] , antioxidant and anthelmintic [26, 27, 25], as well as anti-HIV/AIDS [28] activities.

P. africanum is not toxic, as the brine shrimp and Vero monkey kidney cell line cytotoxicity assay results have showed. Bessong *et al*. [28] found no toxicity when they tested *P. africanum* extracts in a HeLaP4 cell line. Brine shrimp assay has been used in *in vitro* cytotoxicity screening tests [31] , and the test is also routinely used in the plant extracts [12, 10, 35] in South Africa. However, some plants known to be toxic to livestock have displayed non-toxicity to brine shrimp [35] , casting a doubt whether the brine shrimp assay is capable of detecting toxic effects of plant extracts. Therefore cell-line cytotoxicity was applied alongside the brine shrimp assay for *P. africanum* extracts in the present work, as mammalian cell line gives better correlation.

In another related work pending publishing, *P. africanum* extracts were administered (by stomach tube) to lambs artificially induced with *Haemochus contortus* and *Trichostrongylus colubriformis* infections. There were no abnormal behaviors, toxicity signs or any other abnormality in the lambs attributable to the extracts that were given up to a maximum dosage rate of 750 mg kg^{-1}. Setswana-speaking pastoralists of the Madikwe area of the North West Province, South Africa, who give the extracts to cattle for diarrhea and as a general tonic for resistance to disease, reported no side effects or toxicity in treated animals [29]. To paraphrase Weiss and Fintelmann [36], "if a plant extract has been used for ages, repeatedly asked for by patients and prescribed by doctors, one must assume that it is effective and safe, even without double-blind studies". By the same token, from the traditional use, and from research tests carried out to date, *P. africanum* extracts may be assumed safe. This, nevertheless, gives no room for complacency as many herbal medicines are toxic [9, 11, 12], requiring more defined laboratory and clinical tests. Though Joubert [37] purified a proteinase inhibitor (a potent poison like snake venom) from the seeds of *P. africanum*, animals routinely browse the leaves and young stock [38, 39]. Nevertheless, mature trees are unpalatable and shunned by browsing wild ruminants [40].

In conclusion, the extracts of *P. africanum* Sond. (Fabaceae) appear to be safe. But further laboratory and clinical work is called for. There is a great potential for the ubiquitous plant (*P. africanum*) in the promotion of health, in both man and animals. The traditional use of the bark and root for medication is a practice that may not be sustainable, as in some parts of South Africa the tree has been stripped bare (D. E. N. Mabogo, personal communication). More research is required to innovate better extraction methods that would utilize

the leaves. Furthermore, if the active compounds were isolated, their synthetic varieties could be made available. This would reduce the demand of the plant material, with a view to conserving the environment.

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7. 5. References

1. Cunningham, A. B. (1991). Development of a conservation policy on commercially exploited medicinal plants. A case study from Southern Africa. In: *The conservation of medicinal plants (*Akarele O, Heywood V and Synge H (Eds.). Cambridge University Press.Cambridge
2. McCorkle, C. M., Mathias-Mundy, E., Schillhorn van Veen, T. W. (1996). In: Ethnoveterinary Research and Development. Intermediate Technology Publications. London
3. Sofowora, A. (1982). *Medicinal Plants and traditional medicine in Africa*. John Wiley Sons. Chichester,UK.
4. Schwabe, C. W. (1996). Ancient and modern veterinary beliefs, practices and practitioners among the Nile valley peoples. In: *Ethnoveterinary research and development* (Eds. C M McCorkle, E Mathias and Schillhorn van Veen T W) , pg 37-45. Intermediate Technology Publications. London
5. Farnsworth, N. R. (1977). Foreword. In: *Major medicinal plants*, by J Morton. Charles C Thomas. Springfield.
6. Houghton, P.J., Raman, A. (1998). Laboratory handbook for the fractionation of natural extracts. Chapman & Hall.London, pg 1-7.
7. Fabricant, D. S., Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect*., **109**: 69-75
8. Thompson, A. (1997). As patients embrace herbal remedies, dearth of scientific evidence frustrates clinicians. *Am. J. Health Syst. Pharm*, **54**: 2656-2664.
9. Capasso, R., Izzo, A. A., Pinto, L., Bifulco, T., Vitobello, C., Mascolo, N. (2000). Phytotherapy and quality of herbal medicines. *Fitoterapia*, **71**: S58-S65.

10. Eloff, J. N., McGaw, L. J. (2006). Plant extracts used to manage bacterial, fungal and parasitic infections in southern Africa. In: *Modern Phytomedicine. Turning Medicinal Plants into Drugs*. Eds.I. Ahmad, F. Aqil and M. Owais. Wiley-VCHVerlag GmbH & Co. Weinheim, pp.

11. Taylor,J.L S., Elgorashi, E.E., Maes, A., van Gorp, U., De Kimpe, N., van Staden, J., Verschaeve, L. (2003). Investigating the safety of plants used in South Africa traditional medicine: testing for genotoxicity in the micronucleus and alkaline comet assays. *Environ. Molec. Mutagen*., **42**: 145-154

12. Fennell, C. W., Lindsey, K. L., McGaw, L. J., Sparg, S. G., Stafford, G. I., Elgorashi, E. E., Grace, O. M., van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *J. Ethnopharmacol*., **94**: 205-217

13. Stewart, M. J., Moar, J. J., Steenkamp, V. P., Kokot, M. (1999). Findings in fatal cases of poisonings attributed to traditional medicine in South Africa. *Forensic Sci. Interv.*, **101**: 77-183

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

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

16. 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.

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

18. Bam, M., Ferreira, D., Brandit, E. (1988). Novel cyanomaclurin analogue from *Peltophorum africanum*. *Phytochemistry*, **27**: 3704-3705

19. Bam M, Malan J. C. S, Young D A, Brandt E V, Ferreira D (1990). Profisetinidin- type 4— arylflavin-3-ols and related δ-lactones. *Phytochemistry,* **29**: 283-287.

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

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

22.Obi C. L., Potgieter N., Bessong P. O., Masebe T., Mathebula H., Molobela P. (2003). *In vitro* antibacterial activity of Venda medicinal plants. *S. Afr. J. Botany*, **69**: 199-203

23.Samie ,A., Obi, C. L., Bessong, P. O., Namrita, L. (2005). Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacteria species. *Afr. J. Biotechnol*.,**4**: 1443-1451

24.Bizimenyera, E.S., Swan, G.E., Chikoto, H., Eloff, J.N. (2005). Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. *J. S. Afri. Vet. Assoc*., **76**: 54-58.

25.Bizimenyera, E.S., Aderogba, M.A., Eloff, J. N., Swan, G.E. (2007). Potential of neuroprotective antioxidant-based therapeutics form *Peltophorum africanum* Sond. (Fabaceae). *Afr.J.Trad.CAM*, **4**: 99-106

26.Bizimenyera E. S., Githiori J.B., Swan G.E., Eloff J.N. (2006, a). *In vitro* ovicidal and larvicidal activity of the leaf, bark and root extracts of *Peltophorum africanum* Sond. (Fabaceae) on *Haemochus contortus*. *J. Anim. Vet. Adv.*, **5**: 608-614

27.Bizimenyera E. S., Githiori J.B., Swan G.E., Eloff J.N. (2006, b). *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Vet. Parasitol.*, **142**: 336-343.

28.Bessong, P. O., Obi, C. L., Andréola, M., Rojas, L. B., Pouységu, L., Igumbor, E., Meyer, J. J. M, Quideau, S., 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.

29.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.

30.Eloff, J. N. (2004). Quantification of the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine*, **11**: 370-371

31.Solis, P.N., Wright, C. W., Anderson, M.M., Gupta, M.P., Phillipson, J.D. (1993). A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica*, **59**: 250-252

32.Rasoanaivo, P., Ratsmamanga-Urverg, S. (1993). Biological evaluation of plants with reference to the Malagasy flora. NAPRECA, Madagascar, pp.9-43, 72-83.

33.Moosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth*, **65**: 55-63.

34.Hargreaves, B. J., Mosesane, G. (2003). AIDS and traditional medicine in Botswana. Presentation at the Indigenous plant use Forum Congress, Rustenburg,7-10 July, pg10.

35.McGaw , L. J., Eloff, J. N. (2005). Screening of 16 poisonous plants for antibacterial, anthelmintic and cytotoxicity activity in vitro. *S.Afr J. Botany*, **71**: 302-306

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

37.Joubert, F. J. (1981). Purification and some properties of a proteinase inhibitor (DE-1) from *Peltophorum africanum* (weeping wattle) seeds. *Hoppe Seylers Z. Physiol. Chem*., **362**: 1515-1521

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

39. Van Wyk, B., Van Wyk, P., Van Wyk, B. E. (2000). In: Photographic guide to plants of Southern Africa. 1st Ed., Briza Publications. Pretoria, pg. 229

40. Cooper, S. M., Owen-Smith, N., Bryant, J. P. (1988). Foliage acceptability to browsing ruminants in relation to seasonal changes in the leaf chemistry of woody plants in a South African savanna. *Oecologia* (Berlin), **75**: 336-342