CHAPTER 8

ISOLATION AND BIOASSAY CHARACTERISATION OF BERGENIN FROM THE ROOT EXTRACT OF *PELTOPHORUM AFRICANUM* SOND. (FABACEAE)

Edmund S Bizimenyera, Gerald E Swan , Faga B Samdumu,
Irene B Kamara , and Jacobus N Eloff

(As submitted to South African Journal of Science, 2007)

Abstract

South Africa is home to many medicinal plants. Phytochemists have taken interest in analyzing many of these plants for possible isolation of bioactive compounds. *Peltophorum africanum* Sond (Fabaceae), commonly called 'African wattle', is an outstanding plant traditionally used for treatment of more less similar disease conditions in man and livestock. Concoctions of the plant are used in the treatment of abdominal problems, pain, wounds and depression. Polyphenols, flavonoids, appear to be the most common compounds isolated from the plant by phytochemists, but many of these compounds have yet to undergo bioassay characterizations. Bergenin, the main compound obtained from the root extract, showed antibacterial and antioxidant activities and was not toxic in the cell line assay. This is the first report of bergenin being isolated from the root of *Peltophorum africanum*. Bergenin had antioxidant activity comparable to L-ascorbic acid, with the respective EC_{50} values of 5.86 and 5.03 µg ml-1. The compound exhibited antimicrobial activity, with good action against *Sporobolomyces salmonicolor*, moderate activity against *Mycobacterium vaccae*, *Pseudomonas aeruginosa* and *Escherichia coli*, but no activity against *Penicillium notatum*. Bergenin had no effect on the feline herpesvirus *in vitro*. The apparent lack of toxicity of bergenin, and the extracts of *P. africanum* leads support to the promotion of the use of the plant in traditional medicine. As bergenin has been shown to have neuroprotective effect, *P. africanum* extracts have great potential in treatment of neurodegenerative diseases.

Key words: -Herbal extracts; Antimicrobial; Antioxidant; Bergenin; *Peltophorum africanu*

8.1. Introduction

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 [1] . There is a renaissance of phytotherapy, with phytochemists, pharmacologists and clinicians alike turning to searching for bioactive compounds from plants. South Africa is home to many medicinal plants [2]. One of such medicinal plants is *Peltophorum africanum* Sond (Fabaceae), a deciduous tree widely distributed in southern Africa and other tropical regions.

Despite being a unique plant that is traditionally used to treat more or less similar disease conditions in man and animals, and despite its phytochemistry having been studied by several authors [3, 4, 5, 6, 7, 8], the compounds from *P. africanum* have not received much attention in terms of testing for biological activity. Most of these compounds are flavonoids and other polyphenols. However, antibacterial [9, 10], antioxidant [10, 11], and anthelmintic [12, 13] activities of the extracts have been reported. Bessong *et al*.[14] reported inhibitory activities of the extracts against HIV-AIDS type 1 reverse transcriptase and integrase; the anti-HIV activity was shown both by the extracts and bergenin isolated from the bark.

Since the previous investigation had shown the root had showed most activity (antibacterial, antioxidant and anthelmintic), and acetone appeared to extract more compounds from *P. africanum*, the current work was an attempt to isolate compounds from the root using acetone extracts.

8.2 Methodology

8.2.1 Plant material

The root bark collected from mature *Peltophorum africanum* Sond. (Fabaceae) trees naturally growing at Onderstepoort, Pretoria, South Africa (bearing label S.A. Tree No. 215), was 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.

8.2.2 Isolation of compounds

The acetone extract of the root was used for compound isolation. When an attempt was made to re-dissolve the dry extract back into acetone, a compound crystallized. The extract was further run in a Sephadex LH 20 (Sigma) column eluted with ethylacetate: hexane, in series of 1:1, 5:1, 9:1 from which eight compounds crystallized. Next was elution with chloroform: methanol, in a series of 9:1, 7:1, 5:1, 3:1, 1:1 and 1:3 where a further three compounds crystallized. The crystallized compounds were washed up, and subjected to NMR and mass spectrometer tests, and finally identified.

8.2.3 Toxicity assays with bergenin , the isolated compound

8.2.3.1 Brine shrimp lethality

A modification of the technique of Solis *et al*. [15] was used to test the bergenin in a range of concentrations to obtain an LD_{50} value. Briefly, brine shrimp (*Artemia salina*) eggs, obtained from a local pet shop, were hatched in artificial sea water (3.8 g NaCl + 100 ml distilled H_2O). After 48 h, the phototrophic nauplii (larvae) were collected using a Pasteur pipette and transferred to a fresh beaker.

Bergenin solutions in dimethyl sulphoxide 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. A solvent blank control was used to test the effect of the final concentration used on the larvae. Podophyllotoxin (Sigma) was the positive control while distilled water acted as negative control.

Bergenin solution (100 µl) was put in each well of a 96-well microtitre plate in 4 replicates per dilution. Nauplii suspension (100 µl, containing 10-15 nauplii) was put in each well. The microtitre plates were covered and incubated in a humidified chamber for 24 h at room temperature (23°C). The numbers of dead and live nauplii were counted using a stereomicroscope. If deaths occurred in the solvent controls at the end of the treatment, corrected percent death values in treated plates were calculated using Abbott's formula described by Rasoanaivo and Ratsimamanga-Urverg [16].

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

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})

8.2.3.2 Determination of cytotoxicity (MTT assay)

The cytotoxicity assay used 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, as described by Moosmann [17]. The viable cell growth after incubation with bergenin 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. MTT is a yellow water-soluble tetrazolium dye that is reduced by live, but not dead cells, to a purple formazan product that is insoluble in aqueous solutions.

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 $ml^{-1.}$ 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). 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 (bergenin) solution 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 solution resulting in a 50% reduction of absorbance compared to untreated cells.

8.2.3.3 Antiviral assay

Crandell feline kidney (CRFK) cells were obtained from the Department of Veterinary Tropical Diseases, University of Pretoria. Cultures were grown in Eagle's essential medium (MEM) containing 10% fetal calf serum (FCS) and 50 µl/ml gentamicin. Confluent cultures were maintained at 37°C in humidified 5% CO_2 atmosphere.

Virus for use in the assay was produced in 75 cm^2 flasks of confluent CRFK cells. Flasks were inoculated with virus stock and then incubated until approximately 90% of the monolayer showed cytopathic effect (CPE). This suspension was centrifuged at 1000 X g for 10 minutes and the supernatant stored at -70^{0}C. The virus used in this case was feline herpesvirus (FHV-1).

Bergenin was diluted in sterile de-ionized water to final dilutions of 1: 50. Virus stock (0.5 ml) was then mixed with 0.5 ml of bergenin (contact time 20 minutes). A serial 10 fold dilution was performed by taking 0.5 ml from the mixture of virus and bergenin and placing in tubes containing 4.5 ml MEM. The above mentioned 10 fold dilutions was performed in 8 different tubes and 200 µ l of each dilution placed in a 96-well flat bottom microtitre plate. Eighty microliter of CRFK cells (480,000 cells/ ml) was added to each well (with 200 µl) in 5 replicates. Each extract test included a virus control and a toxicity test.

The plates were incubated for 5 days at 37^{0}C in a 5% CO_2 atmosphere.

The CPE was observed by the use of an inverted light microscope. One hundred percent cell damage was scored with a 4 while 75% cell damage was scored a 3 and so on. A zero indicated that the cells were viable. The tissue culture infectious dose 50 was calculated using the Karber method [18].

The Karber method was used for calculating the $TCID_{50}$.

Negative log of the ID_{50} end point titer=

[Negative log of the highest virus concentration used] – [{sum of % mortality at each dilution- 0.5} x (log of dilution)]

8.2.4 Bioactive assays with bergenin

8.2.4.1 Antioxidant

The antioxidant assay was done following a previous method [11], comparing the antioxidant activity of bergenin to that of ascorbic acid (Vitamin C).

8.2.4.2 Inhibition of microbial growth

The antimicrobial activity of bergenin was determined by the "hole-plate diffusion method" [19]. The test organisms were *Escherichia coli* (SG 458), *Pseudomonas aeruginosa* (K 799/61), *Mycobacterium vaccae* (IMET 10670), *Sporobolomyces salmonicolor* (SBUG 549), and *Penicillium notatum* (JP 36).The tested microbial suspension was homogeneously seeded onto petri dishes containing 15 ml of the MH agar medium. Holes were aseptically bored into the agar with a hollow punch and 25 µl aliquots of the bergenin solution were placed into wells with a sterile pipette. The plate was kept for 1 h at room temperature for the diffusion of the bergenin into the agar. Subsequently, the plate was incubated at 37°C for 18 h. Chloramphenicol was used as positive control and 70% ethanol was used as negative control. The microorganism control consisted of a seeded petri dish with no plant material, solvent (70% ethanol) or chloramphenicol. Results were recorded as the mean of triplicate experiments.

Microbial growth inhibition was determined as the diameter of the inhibition zones around the holes. The inhibition diameter was the average of four measurements per hole.

8.2.5 Statistical analysis

The Excel package was used in data analysis.

8.3 Results

Bergenin (molecular formula $C_{14}H_{16}O_9.H_2O$),chemically called **3,4,8,10-tetrahydroxymethyl-9-methoxy-3,4,4a, 10b-tetrahydro-2H-pyranol[3,2-c]isochromen-6-one**, molecular weight 328 was found to be a major component of the root extract of *P. africanum*, as more than 5. 641 g were isolated from 36.06 g of the acetone extract of the root material. Out of this amount, 1.554 g of pure bergenin crystallized following attempts to re-dissolve the dry acetone extract residue in acetone. All the nine compounds isolated from the root extract turned to be identical by the nuclear magnetic resonance (NMR) amd mass spectroscopy (MS) analysis. The NMR and MS spectra of bergenin are displayed in Figure 8.1 and Figure 8.2 respectively.

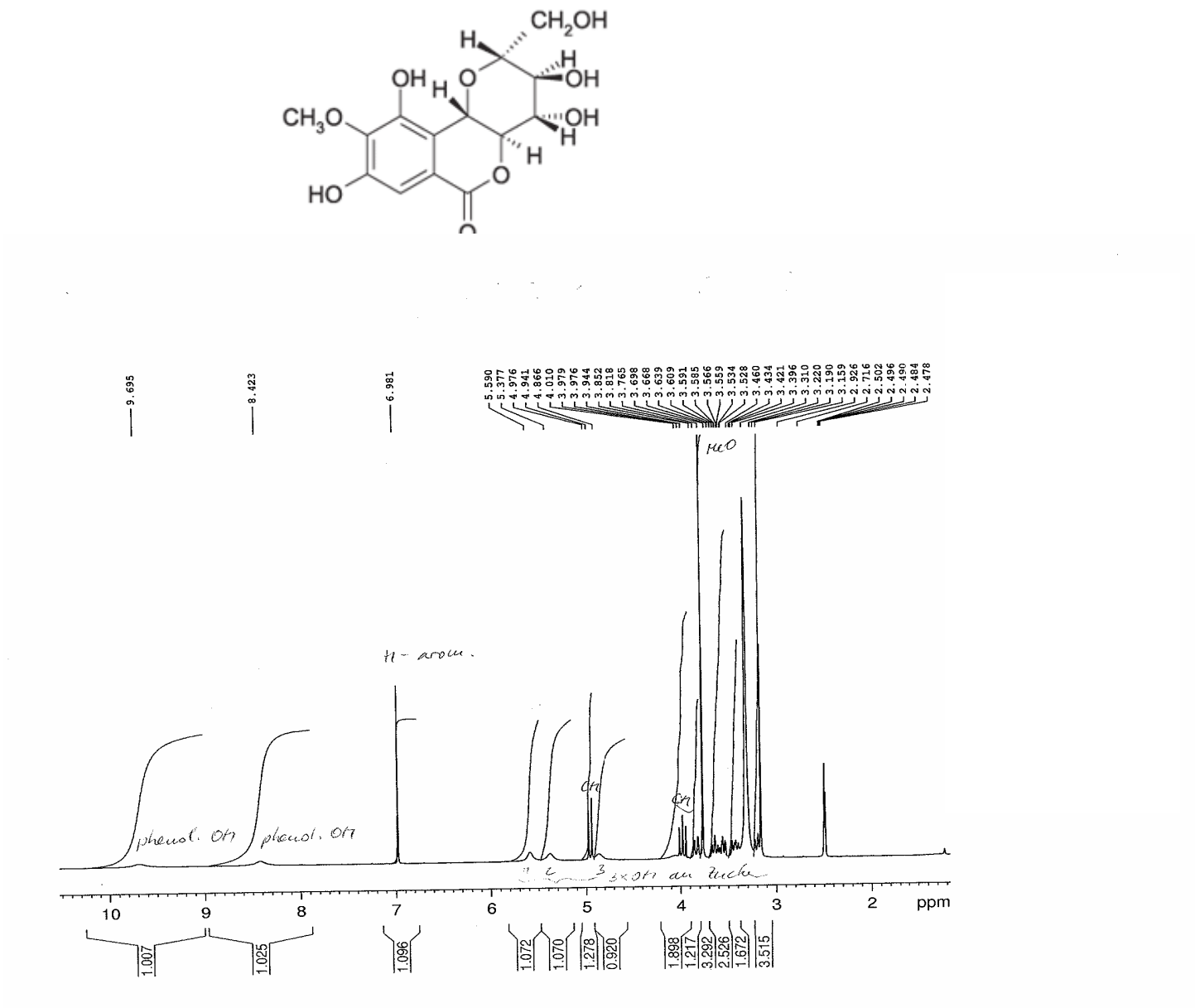

Figure 8.1: Nuclear magnetic resonance (NMR) of bergenin

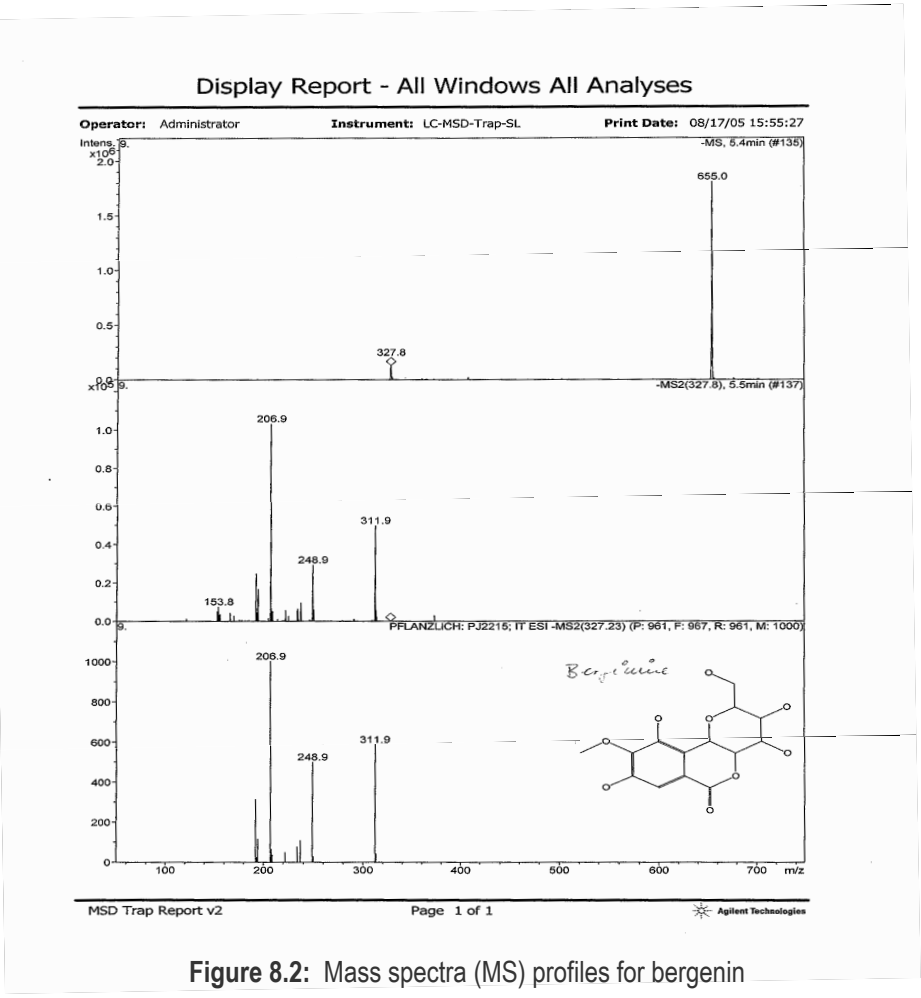

Figure 8.2: Mass spectra (MS) profiles for bergenin

Table 8.1: Cytotoxicity and antioxidant activities of bergenin

Item	Brine shrimp[a] LC_{50} (µg ml^{-1})	MTT[b] LC_{50} (µg ml^{-1})	Antioxidant EC_{50} (µg ml^{-1})
Bergenin	>1000	>1000	5.86± 0.41
Podophytotoxin	7.01		
Berberine		9.82	
Ascorbic acid			5.03± 0.5

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

Bergenin was obtained as a brown powder with a molecular formula of $C_{14}H_{16}O_9.H_2O$. From ESI-MS, the mass was found to be 328. Proton NMR, ^{13}C NMR and DEPT analyses were performed. The ^{13}C together with DEPT spectrum revealed 14 carbons with carbon signals: δ 118.03 (s), 115.93 (s), 148.04 (s), 140.59 (s), 150.92 (s), 109.50 (d), 163.34 (s), 72.17 (d), 73.73 (d), 79.82 (d), 70.71 (d), 81.77 (d), 61.16 (t), 59.84 (q). After comparing the spectra with literature [20], it deduced as bergenin.

At the maximum concentration used (5 mg ml^{-1}) bergenin did not show toxicity in both brine shrimp and Vero monkey kidney cell line (Table 8.1).

Bergenin had more antioxidant activity than L-Ascorbic acid (Vitamin C), the respective EC_{50} values being 5.86 and 5.03 µg ml^{-1}(Table 8.1).

Bergenin showed good antibacterial activity against *S. salmonicolor*, moderate action against *M. vaccae*, *P. aeruginosa* , and *E.coli*, and no activity against *P. notatum* (Table 8. 2).

Table 8.2: Antimicrobial activity of bergenin against five microbial species

Microorganism	Zone of inhibition	
	(mm)	Colonies
E.coli (SG 458)	15	p
P. aeruginosa (K 799/61)	17	p
Myco. vaccae (IMET 10670)	19	p
S. salmonicolor (SBUG 549)	26	
P. notatum (JP 36)	13	p

Key:
0-15 mm no activity
16-20 mm moderate activity
21-25 mm good activity
> 25 mm strong activity
p= few colonies in the inhibition zone (moderate activity)

There was no activity by bergenin against feline herpesvirus (results not shown). The tissue culture infectious dose 50 ($TCID_{50}$) was used to calculate the antiviral activity of bergenin. Antiviral activity is the difference between the virus titre in the absence and in the presence of the compound (δ $\log_{10}$ $TCID_{50}$/ml). Cytopathic effect (CPE) was determined by microscopic examination of the cells, and the results used to calculate the $TCID_{50}$. In the case of bergenin, there was no difference between the virus titre in the presence of the compound and in its absence.

8. 4 Discussion

Bergenin (molecular formula $C_{14}H_{16}O_9.H_2O$), technically called **3,4,8,10-tetrahydroxymethyl-9-methoxy-3,4,4a, 10b-tetrahydro-2H-pyranol[3,2-c]isochromen-6-one** appears to be a major constituent of the root extract of *P. africanum* since more than 5.641 g of pure bergenin was isolated from 36.06 g of the acetone extract of the plant. It was surprising that all the crystalline compounds that were isolated at different processes turned out to be pure bergenin. Indeed bergenin is a constituent of many medicinal plants, having first been isolated from *Saxifraga stolomifera* by Masotoshi *et al*. [20]. However, it has since then been isolated from other medical plant. Bergenin was first isolated from *P. africanum* ethanolic extract of heartwood by Bam *et al*. [5] and of the bark by Mebe and Makuhunga [7]. This is the first time it is reported isolated from the acetone extract of the root of the plant.

Bergenin was found to have antioxidant activity comparable to L-ascorbic acid (Vitamin C), with their respective EC50 values being 5.86 and 5.03 µg ml^{-1} . Though the antioxidant activity of bergenin was lower than that of the bark and root extract , their respective EC_{50} values being 4.37 and 3.82 µg ml^{-1} (as reported by Bizimenyera *et al*. [11]), bergenin nevertheless might be contributing significantly to the antioxidant activity of *P. africanum*. Antioxidant activity of higher plants is largely due to phenols, however, and various phenolic compounds have been reported in the plant [3, 4, 5, 6, 8]. Various studies of natural and synthetic bergenin, have shown the compound to protect against hepatic toxicity [21, 22], treatment of peptic ulcers [23, 24], anti-HIV/AIDS activity [25, 14] and neuroprotective and antioxidant activity [26]. The present work found bergenin to have strong antioxidant activity, comparable to ascorbic acid (Vitamin C). The abundance of bergenin in *P. africanum*, offers prospects for use of the plant extracts in control of neurodegenerative diseases [11].

The brine shrimp and Vero monkey kidney cell line cytotoxicity assay results have showed that bergenin is not toxic. Brine shrimp assay has been frequently used in *in vitro* cytotoxicity tests [15]. The test is routinely used in the toxicity tests of plant extracts [27, 28, 29] in South Africa. However, some plants known to be toxic to livestock have displayed non-toxicity to brine shrimp [30], prompting skepticism that the brine shrimp assay is capable of detecting toxic effects of plant extracts. That is why cell-line cytotoxicity was applied alongside the brine shrimp assay for the compound in the present work; mammalian cell line gives better correlation.

Antimicrobial (against Gram-positive and Gram-negative bacteria and fungi) activities were displayed by bergenin. Bergenin may be acting in synergy with other compounds, since its activity is less than the crude extract in both cases. This would further appear to validate the traditional use of the plant against infection related diseases [10].

In conclusion, there is a great potential for *P. africanum* Sond. (Fabaceae) in promotion of health in both man and animals. This may in part be because of containing phenolic compounds like bergenin that have antibacterial and antioxidant activity. Synergistic activity of plant-derived antioxidants has been proposed as a mechanism by which HIV replication and immune cell killing (apoptosis) in HIV infected people can be reversed. However suitable laboratory and clinical studies are called for, in addition to further search for effective compounds from plants such as *P. africanum*. Furthermore, if the active compounds were isolated, their synthetic varieties could be made available. This would reduce on the demand on the plant material, with a view to conserving the environment.

Acknowledgement

Makerere University Staff Development Programme, Uganda, the National Research Foundation, South Africa and the University of Pretoria funded the work. Technical assistance was provided by Dr. L McGaw in the brine shrimp and MTT assays, as well as valuable suggestions and editions /corrections in the manuscript. Drs. J. Angeh and M. Aderogba assisted in the technical aspects of the extraction and separations.

8. 5. References

1. Houghton, P.J., Raman, A. (1998). Laboratory handbook for the fractionation of natural extracts. Chapman & Hall.London, pg 1-7.
2. Van Wyk, B. E., Wink, M. (2004). *Medicinal Plants of the world*. Briza Publications. Pretoria.
3. El Sherbeiny, A.E.A., El Ansari, Nawwer, M. A. M., El Sayed, N. H.(1977). Flavonol glycosides and flavonol glucoside gallates from *Peltophorum africanum*. *Plant Medica*, **32**: 165-170
4. 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.
5. Bam, M., Ferreira, D., Brandit, E. (1988). Novel cyanomaclurin analogue from *Peltophorum africanum*. *Phytochemistry*, **27**: 3704-3705
6. 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.
7. Mebe, P. P., Makuhunga, P. (1992). 11-(E)-*p*-coumaric acid ester of bergenin from *Peltophorum africanum. Phytochemistry,* **31**: 3286-3287.

8. 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
9. 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
10. 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.
11. Bizimenyera, E.S., Aderogba, M.A., Eloff, J. N., Swan, G.E. (2007). Potential of europrotective antioxidant-based therapeutics form *Peltophorum africanum* Sond. (Fabaceae). *Afr.J.Trad.CAM*, **4**:99-106
12. 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
13. 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.
14. 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.
15. 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
16. Rasoanaivo, P., Ratsmamanga-Urverg, S. (1993). Biological evaluation of plants with reference to the Malagasy flora. NAPRECA, Madagascar, pp.9-43, 72-83.
17. Moosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth*, **65**: 55-63.
18. Karber, G. (1931). 50% end point calculation. *Arch. Exp. Pathol. Pharmak*., **162**: 480- 483
19. Bennet, J. V., Brodie, J. L., Benner, J. L., Kirby, W. M. M.(1996). Simplified accurate method for antibiotic assays of clinical specimens. *Applied Microbiol*. ,**14**:170-177
20. Masotoshi, T., Seichi, Y., Michio, K., Masao, H. (1983). Isolation of norbergenin from *Saxifraga stolomifera, Phytochemistry.,* **22**: 1053-1054
21. Kim, H. S., Lim, H. K., Chung, M. W., Kim, Y. C. (2000). Antihepatotoxic activity of bergenin, the major constituent of *Mallotus japonicus*, on carbon tetrachrolide intoxicated hepatocytes. *J. Ethnopharmacol*., **69**: 79-83

22. Lim, H. K., Kim, H. S., Choi, H. S., Choi, J., Kim, S. H., Chang, M. J. (2001). Effects of bergenin, a major constituent of *Mallotus japonicus*, against D- galactosamine-induced hepatotoxicity in rats. *Pharmacology*, **63**: 71-75

23. Goel, R. K., Maiti, R. N., Manickam, M., Ray, A. B. (1997). Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa. *Indian J. Exp. Biol*., **35**: 1080-1083

24. Zhang, Y. H., Fang, L.H., Lee, M. K., Ku, B. S. (2003). In vitro inhibitory effects of bergenin and norbegenin on bovine adrenal tyrosine hydroxylase. *Phytother. Res*., **17**: 967-969

25. Piacente, S., Pizza, C., Tommasi, N. D. (1996). Constituents of *Ardisia japonica* and their in vitro anti-HIV activity. *J. Nat. Prod*., **59**: 565-569

26. Takahashi, H., Kosaka, M., Watanabe, Y., Nakade, K., Fukuyama, Y. (2003). Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. *Bioorg. Med. Chem*., **11**: 1781-1788

27. Fennell, C. W., Lindsey, K. L., McGaw, L. J., Sparg, S. G., Stafford, G. I., Elgorashi, 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

28. 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-VCH Verlag GmbH & Co. Weinheim, pp.

29. McGaw, L. J., Steenkamp, V., Elff, J. N. (2007). Evaluation of *Arthrixia* tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. *J. Ethnopharmacol*.,**110**: 16-22.

30. 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