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

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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. EC₅₀ values for L-ascorbic acid (5.04 µg/mL) was more active than the leaf 6.54 (µg/mL), but much less active than the bark (4.37 µg/mL) and root (3.82 µg/mL) extracts. Continued work on *P. africanum*, and other plants rich in antioxidants, may avail neuroscientists with potent neuroprotective antioxidant therapeutics.

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

Introduction

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

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 chemicals 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 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 Geriche, 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. Most 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*.

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.

Methodology

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 Medicinal 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 °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.

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).

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 the Trolox 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: -

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

$\text{Abs}_{\text{sample}}$ is the absorbance of the sample, $\text{Abs}_{\text{blank}}$ is the absorbance of the blank and $\text{Abs}_{\text{control}}$ 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 Science) showed a good coefficient of determination, with most values being $r^2 \geq 0.910$.

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 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 2). 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 $\mu\text{g/mL}$) was more active than the leaf 6.54 ($\mu\text{g/mL}$), but much less active than the bark (4.37 $\mu\text{g/mL}$) and root (3.82 $\mu\text{g/mL}$) extracts (the higher the $\mu\text{g/mL}$ value, the less AOX activity).

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

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

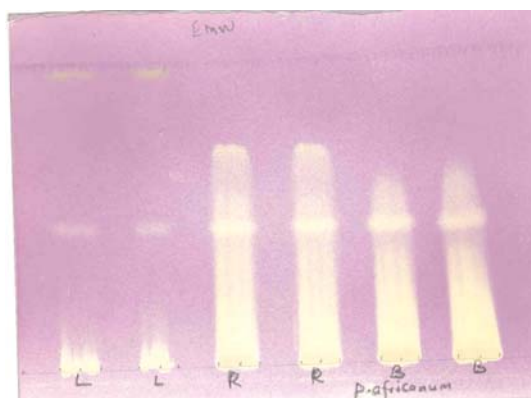


Figure 1 Chromatogram of 200 μg acetone extracts of leaf (L), bark (B) and root (R) of *P. africanum*, separated with EMW and sprayed with DPPH. Note the high antioxidant activity, indicated by yellow areas

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 EC₅₀ 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 EC₅₀ 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.

Table 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 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 Geriche, 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 suggest *P. africanum*, due to its with 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; 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. 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.

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