

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

S E Bizimenyera<sup>a</sup>, G E Swan<sup>a</sup>, H Chikoto<sup>a</sup> and J N Eloff<sup>a\*</sup>

### ABSTRACT

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

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

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### INTRODUCTION

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

Many pharmacologically active com-

pounds have been discovered following ethnobotanical leads<sup>4,11</sup>. As tropical forests still present a great storehouse of medicinal genetic resources, the search for compounds with novel bioactivity from plants continues<sup>32</sup>.

*Peltophorum africanum* (Sond), commonly called 'weeping wattle' or 'huilboom', is a member of the Fabaceae. It is a deciduous tree growing up to 15 m high with a wide canopy that occurs widely in medium to low altitudes in wooded grassland areas of southern Africa<sup>24</sup>. Whereas the genus is found throughout the tropics, *P. africanum* is the only member of the genus in southern Africa. The plant has many traditional medicinal uses in humans and animals. The roots and bark are used to treat sore throat, wounds, diarrhoea, dysentery, helminthosis, abdominal pains, ascites, back and joint pains, HIV-AIDS, venereal diseases, infertility, colic and eye

infections<sup>12,31,34</sup>. Pastoralists and rural farmers use the root and bark to treat diarrhoea, dysentery and infertility in cattle and to promote well-being and resistance to disease<sup>30,34</sup>. Bark from *P. africanum* was identified as one of the most important products sold in informal medicinal plant markets in Pretoria<sup>17</sup>.

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

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

### MATERIALS AND METHODS

#### *Collection, preparation and storage of plant material*

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

<sup>a</sup>Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

\*Author for correspondence.

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

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collected in May 2003 from a mature tree growing naturally on the campus of the Faculty of Veterinary Sciences at Onderstepoort. A voucher specimen (PM 001) is stored in the Medicinal Plant Herbarium, Department of Paraclinical Sciences, University of Pretoria. The plant material was dried in the shade at ambient temperature and ground to fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley. The powdered material was stored separately in the dark in tightly closed glass bottles before analysis.

#### Extraction

Four solvents with varying polarities<sup>13</sup> were selected to extract a diversity of compounds from the plant material *i.e.* technical grade ethanol, acetone, dichloromethane and hexane (Merck®). One gram of the leaf, bark and root was extracted in 10 ml of the respective solvent in a centrifuge tube by vigorously shaking on an orbital shaker (Labotec®, model 202, South Africa) for 10 minutes. After centrifugation for 10 minutes, the extract was decanted into pre-weighed glass vials. The process was repeated 3 times. The solvent was evaporated in a stream of air at room temperature overnight to determine the mass extracted<sup>5</sup>.

#### Chromatography

Dried extracts were re-dissolved in the corresponding extraction solvent to yield a 10 mg/ml solution. An aliquot of 10 µl of each solution (*i.e.* 100 µg) was applied on thin layer chromatography (TLC) plates (Merck®, silica gel 60). Four solvent systems were used as eluents: benzene/ethanol/ammonium hydroxide (18/2/0.2) (BEA); chloroform/ethyl acetate/formic acid (10/8/2) (CEF); formic acid/acetic acid/water/ethyl acetate (3/2/30/70) (FAWE) and ethyl acetate/methanol/water (10/1.35/1) (EMW).

Separated compounds were inspected and marked under visible and ultraviolet light (255 and 360 nm Camac Universal UV lamp). The TLC plates were subsequently sprayed with vanillin spray reagent [0.1 g vanillin (Sigma) in 28 ml methanol to which 1 ml of concentrated sulphuric acid was carefully added]. Corresponding plates were sprayed with *p*-anisaldehyde spraying agent [1 ml *p*-anisaldehyde (Sigma) in 18 ml ethanol to which 1 ml concentrated sulphuric acid was carefully added]. Thereafter, the plates were heated at 100 °C for a few minutes for optimal colour development.

#### Polyphenols

Polyphenols were quantified by the Folin-Ciocalteu method, with gallic acid as a standard<sup>27</sup>.

#### Antioxidant screening

Reaction of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a purple stable free radical with an antioxidant results in decolouration of the free radical. Chromatograms were uniformly 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<sup>29</sup>.

Quantification of antioxidant activity was by the Trolox equivalent antioxidant capacity (TEAC) assay. The decolourising assay method<sup>26</sup> was used with minor modifications. Free radical was preformed by reacting potassium persulphate (Sigma) with 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma) and was stored at 4 °C before use. The free radical was diluted with absolute ethanol (Merck) to an absorbance of  $0.7 \pm 0.02$  at 734 nm (Beckman). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Fluka) is a synthetic water soluble vitamin E analogue. It was prepared fresh in ethanol solution and used as the antioxidant standard. Initially, 4 different concentrations of the Trolox and extracts were made. One ml of ABTS was added to 10 µl (in quadruplicate) of each concentration of Trolox and extracts. Only the solutions that gave absorbency of between 20 % and 80 % of the initial absorbency were considered. The TEAC values were measured after 6 minutes. If an extract had equivalent antioxidant activity to Trolox, its TEAC value would be 1 and if the extract was more active its TEAC value would be greater than 1. Re *et al.*<sup>26</sup> used equivalent mM concentrations of different standards to obtain their results. With unknown compounds in a plant extract this is not possible and we used equivalent mg/ml concentrations. By bringing the molecular mass into consideration, we can convert the published data<sup>26</sup> to data comparable with our results.

#### Antibacterial screening

Acetone was used to dissolve dried extracts to 10 mg/ml because of the low toxicity of acetone for the test organisms<sup>5</sup>. A modification of the bioautography procedure described by Begue and Kline<sup>3</sup> was used. TLC plates were sprayed with concentrated broth culture suspensions of actively growing cells of *S. aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29219). These are the strains recommended by the National Committee for Clinical Laboratory Standards<sup>22</sup>. Cultures grown overnight were centrifuged and the pellet was resuspended with fresh broth in

about 50 % of the original volume to yield a high concentration of the bacteria. The number of bacterial cells in the concentrated bacterial suspensions used for spraying chromatograms cultures was determined by a modified serial dilution technique<sup>16</sup>. The values ranged from  $28\text{--}50 \times 10^{16}$  colony-forming units per ml (cfu/ml). The plates were incubated at 38 °C and 100 % relative humidity overnight. The plates were then sprayed with 2 mg/ml *p*-iodonitrotetrazolium (INT). Clear zones on the chromatogram indicated inhibition of bacterial growth after further incubation at 37 °C for 30–120 minutes.

A microplate serial dilution method<sup>6</sup> was used to determine the minimum inhibitory concentrations (MIC) for the test bacteria, *i.e.* the lowest concentrations of the extract that inhibit bacterial growth. INT was used as growth indicator dye and gentamicin (50 µg/ml) was the positive control.

Total antibacterial activity (TAA) equivalent (ml/g) of the plant parts was determined by dividing the quantity of material (mg) extracted from 1 g of dried plant material by the MIC value (mg/ml)<sup>8</sup>. The TAA value is the volume in ml to which the extract obtained from 1 g of plant material can be diluted and still inhibits bacterial growth<sup>8</sup>.

## RESULTS

Whereas the non-polar extractants extracted a small quantity of compounds from root and bark, ethanol and acetone extracted the largest quantity in total from all plant parts (Fig. 1). There was hardly any extract obtained at the 3rd extraction with hexane and dichloromethane, and only a small quantity with acetone and ethanol, indicating that extraction was exhaustive.

Polyphenol concentrations were high (more than 20 %) in the acetone and ethanol extracts of the root, bark and leaves (Fig. 2).

There were 5–6 antioxidant compounds present in acetone and ethanol extracts of especially root and bark, and the highest antioxidant activity was in the ethanol and acetone extracts of the root and the ethanol extract of the bark (Figs 3, 4). In all the acetone and ethanol extracts, a compound with an  $R_f$  value of *c.* 0.48 (similar to that of catechin) in the EMW TLC system was present.

The antibacterial compounds in root and bark were highly polar and hardly moved from the origin when EMW was used with *S. aureus* as test organism (results not shown). In all leaf extracts there were 2 or 3 non-polar antibacterial compounds present with  $R_f$  values of 0.74,

0.85 and 0.92 (EMW system). These values corresponded to some of the  $R_f$  values of antioxidant compounds present in root, bark and leaves (Fig. 3).

The minimum inhibitory concentration (MIC) values of the extracts varied from 0.08 to 2.5 mg/ml (Table 1). There was substantial activity against both Gram-positive and Gram-negative bacteria, with an MIC value 0.08 mg/ml for *S. aureus* and 0.16 mg/ml for *P. aeruginosa*. The ethanol extract of the root had the same MIC value (0.08 mg/ml) as the dichloromethane extract of the leaf against *S. aureus*. *E. coli* was the most resistant against the extracts.

The total antibacterial activity values obtained for the ethanol and acetone extracts of the root and barks were high, varying from 79 to 1263 ml/g (Table 2).

## DISCUSSION

Pharmacologically active plant-derived compounds have been discovered by following the ethnobotanical use of plants<sup>11</sup>. The ability to isolate compounds depends on the ability to screen using the 'seek and ye shall find' as the operative principle<sup>4</sup>. Use of organic solvents is the most popular method for obtaining plant extracts<sup>15</sup>. There was hardly any extract obtained at the 3rd extraction with hexane and dichloromethane, and only a small quantity with acetone and ethanol. This would imply that the ground particles were small and that the shaking and separation of phases by centrifugation was efficient. Acetone and ethanol extracted the largest quantity in total, from all plant parts. Acetone has already been shown to be a good extractant for a diversity of plant compounds<sup>5</sup>. Extractable compounds from the root and bark were mainly polar, as few compounds were extracted from the root and bark by non-polar solvents. This indicates that *P. africanum* does not have a high concentration of non-polar compounds in the bark and root. The TLC and bioautography results support this conclusion, because root and bark extracts had low  $R_f$  values even in polar solvent systems.

Our results show that *P. africanum* had several antioxidant compounds especially in the root and bark (Fig. 3). Antioxidants in plants prevent damage caused by free radicals to DNA and other molecules, reduce inflammation, and promote good health and resistance to disease<sup>15,18,25</sup>. In the qualitative antioxidant analysis most of the compounds had a high polarity as would be expected of polyphenols or tannins. The antioxidant activity in *P. africanum* may be due to polyphenols or tannins as these compounds have been found in its heartwood<sup>1,2,14,19</sup>. The Folin-

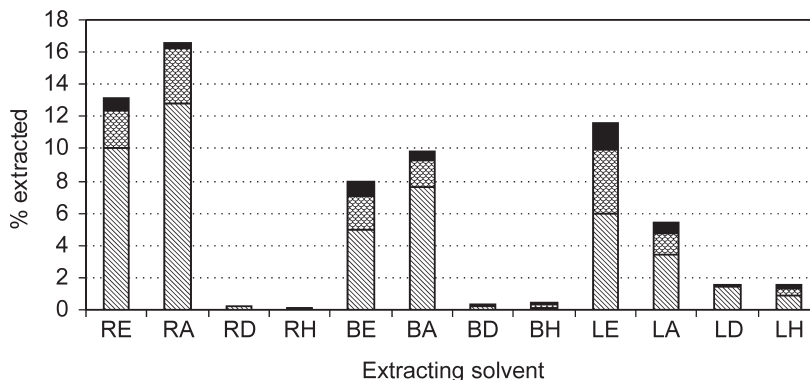


Fig. 1: Extraction efficiency based on original dry mass of ethanol (E), acetone (A), hexane (H) and dichloromethane (D) from root (R), bark (B) and leaf (L) of *Peltophorum africanum*. First extracted at bottom, 2nd in middle, 3rd at top.

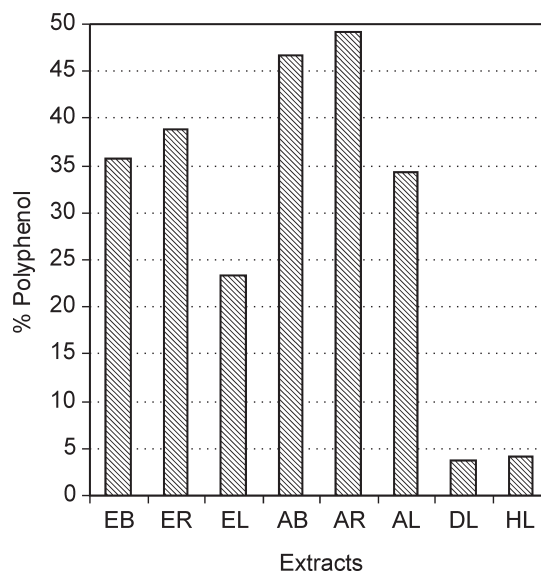


Fig. 2: Percentage polyphenols in bark (B) root (R) and leaf (L) extracts of ethanol (E), acetone (A), dichloromethane (D) and hexane (H).

Cioalciu method does not differentiate between tannins and many other phenolics that are not tannins<sup>27</sup>.

The TEAC values of 1.32, 0.95 and 0.83 for the ethanol and acetone extracts of the root and acetone extracts of the bark

respectively, compare favourably with the value of 1.49 for pure Vitamin C found by Re *et al.*<sup>26</sup>.

Manana<sup>17</sup> found that the acetone extracts of *P. africanum* bark had MIC values of 0.02–0.08 mg/ml against *S. aureus*. Obi



Fig. 3: Chromatogram of 100 µg of bark (B), root (R) and leaf (L) extracted by ethanol (Eth), acetone (Ac), hexane (Hx) and dichloromethane (Dc) separated by EMW and sprayed with DPPH reagent. Light areas indicate anti-oxidant activity. Lanes from left to right R-Eth, B-Eth, L-Eth, L-Ac, B-Ac, R-Ac, L-Hx and L-Dc.

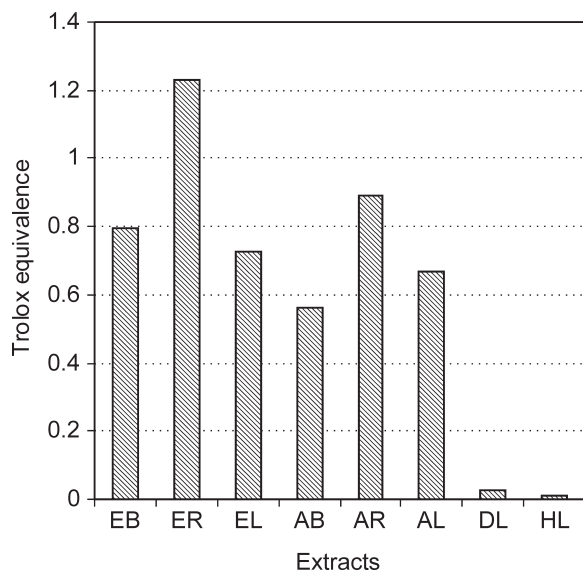


Fig. 4: Trolox equivalent antioxidant capacity (TEAC) values of bark (B), root (R) and leaf (L) extracted with ethanol (E), acetone (A), dichloromethane (D) and hexane (H).

*et al.*<sup>23</sup> using disc diffusion method demonstrated antibacterial action of *P. africanum* extracts against some Gram-negative bacteria (not done for Gram-positive). In the present work, we found the best MIC values of 0.08 mg/ml were obtained for ethanol extracts of the root and dichloromethane extracts of the leaf against *S. aureus*. The best values for Gram-negative bacteria were 0.16 mg/ml for acetone and ethanol extracts of the bark and root against *P. aeruginosa*.

As Eloff<sup>8</sup> pointed out, when selecting plants for investigation in rural areas, not

only MIC values but also the quantity extracted should be incorporated to calculate the total antibacterial activity. Whereas the MIC values for ethanol extract of the root and dichloromethane extract of the leaf against *S. aureus* were the same (0.08 mg/ml), extracts from 1 g of plant material could have been diluted to 1263 ml in the former and 188 ml in the latter and still inhibit growth of *S. aureus*. This in effect would imply that much less plant material would have to be extracted by acetone compared with the amount of plant material that would be required by

Table 1: Minimum inhibitory concentration (MIC) values of the bark (B), root (R) and leaf (L) extracts of ethanol (E), acetone (A), dichloromethane (D) and hexane (H) against 4 bacteria.

Extractant	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
EB	0.32	0.32	0.63	0.63
AB	0.16	0.16	0.16	0.63
ER	0.08	0.32	0.16	0.63
AR	0.16	0.16	0.16	0.63
EL	0.32	0.32	0.16	0.63
AL	0.16	0.16	0.16	1.25
DL	0.08	2.5	2.5	2.5
HL	2.5	2.5	2.5	2.5

Table 2: Total antibacterial activity (volume to which active compounds present in 1 g of plant material can be diluted and still inhibit growth) values (ml/g) of ethanol (E), acetone (A), dichloromethane (D) and hexane (H) extracts of the bark (B), root (R) and leaf (L) tested on 4 bacteria.

Extractant	Mass (mg) extracted from 1 g	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
EB	50	156	156	79	79
AB	76	475	475	475	121
ER	101	1263	316	631	160
AR	128	800	800	800	203
EL	60	188	188	375	95
AL	34	213	213	213	27
DL	15	188	6	6	6
HL	15	6	6	6	6

extraction with dichloromethane to achieve the same antibacterial activity. If 2 different plant species have different TAA values, this information helps to select the best plant for isolating active compounds and for application in rural areas. The TAA values of 1263 and 800 ml/g for ethanol and acetone extracts of the root, respectively, are much higher than those obtained to date from the members of Combretaceae and Celastraceae<sup>7,9</sup>.

The high level of antibacterial and antioxidant activity in acetone and ethanol extracts validates some of the traditional uses of *P. africanum* in people and animals<sup>12,17,30,34</sup>. The results provide a rationale for the use of the plant in treating bacterial infection-related diseases in cattle by rural farmers in the Madikwe area of South Africa<sup>30</sup>.

The antibacterial activity in the roots and bark are probably largely due to tannins<sup>33</sup>, based on the high polyphenol content of these extracts and the high polarity of the active compounds based on bioautography results. The effect on animal infections may either be directly by inhibiting growth of pathogens or indirectly by stimulating the immune system of the host. The high polarity of the bioactive compounds in the root and bark also means that these compounds may be extractable by the polar solvents available to rural users.

We are in the process of isolating and characterizing the antioxidant and antibacterial compounds from roots and leaves and then testing extracts of crude extracts and isolated compounds on animals to confirm the *in vitro* results.

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