

RESEARCH ARTICLE

Open Access

# In vitro antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity

Jean P Dzoyem<sup>1,2</sup>, Lyndy J McGaw<sup>1</sup> and Jacobus N Eloff<sup>1\*</sup>

## Abstract

**Background:** The Fabaceae family is the second largest family of medicinal plants, containing more than 490 species which are being used as traditional medicine. The aim of this study was to determine the antioxidant and antibacterial activity as well as the cytotoxicity of acetone leaf extracts of nine tree species from the Fabaceae family that have not been investigated well previously for possible use in animal health and production.

**Methods:** The antibacterial activity was determined by a serial microdilution method against three Gram-positive and three Gram-negative bacteria. Antioxidant activity was determined using free-radical scavenging assays. The safety of the extracts was ascertained using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Vero African green monkey kidney cells.

**Results:** Six of the nine acetone extracts had significant antibacterial activity against at least one of the six bacterial species with (MIC 20–80 µg/mL). The *Crotalaria capensis* extract had the highest activity against *Salmonella typhimurium*, followed by *Indigofera cylindrica* with MICs of 20 µg/mL and 40 µg/mL respectively. The *Dalbergia nitidula* extract had free radical scavenging capacity (IC<sub>50</sub> of 9.31 ± 2.14 µg/mL) close to that of the positive control Trolox in the DPPH assay. The *Xylocarpus torreana* extract also had high activity (IC<sub>50</sub> of 14.56 ± 3.96 µg/mL) in the ABTS assay. There was a good correlation between antioxidant activity and total phenolic content (R<sup>2</sup> values > 0.8). The extracts had weak or no toxicity to Vero cells, compared to the positive control doxorubicin with the LC<sub>50</sub> varying from 10.70 ± 3.47 to 131.98 ± 24.87 µg/mL at the concentrations tested.

**Conclusion:** Extracts of *D. nitidula*, *X. torreana*, *C. capensis* and *I. cylindrica* had a low cytotoxicity and high antimicrobial and/or antioxidant activity. These species are therefore promising candidates for the development of useful antimicrobial/antioxidant preparations with a low cytotoxicity that may be useful in promoting animal health and productivity.

**Keywords:** Antioxidant, Antimicrobial, Cytotoxicity, Efficacy, Fabaceae, Safety

\* Correspondence: kobus.elloff@up.ac.za

<sup>1</sup>Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa

Full list of author information is available at the end of the article

## Background

Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds known as reactive oxygen species. Many such reactive species are free radicals derived either from normal metabolic processes in the human body or from external sources [1]. Free radicals and peroxidative damage have been implicated in several human and animal pathological disorders, including inflammatory ailments and microbial infectious diseases [2]. The deleterious effects of the free radicals can be diminished by natural antioxidants available in plants. Among the various natural compounds extracted from plants that have demonstrated biological activities, many are receiving particular attention as radical scavengers [3]. Therefore, the discovery of novel antimicrobial agents from plants that also target free radicals with a low toxicity may be useful in the field of phytotherapy. Natural products present in higher plants are an important source of therapeutic agents and many research groups are currently screening different biological activities of plants [4]. Several studies have proven the antioxidant or radical scavenging properties of medicinal plant extract [5]. The Phytomedicine Programme of the University of Pretoria ([www.up.ac.za/phyto](http://www.up.ac.za/phyto)) has contributed to this area by determining the antibacterial and antifungal activity of acetone leaf extracts of more than 500 tree species against 8 very important bacterial and fungal pathogens. Nine tree species belonging to the Fabaceae family, with very few or no previous pharmacological investigations reported were selected from the Phytomedicine plant database for in depth investigation.

The Fabaceae is the third largest family among the angiosperms after the Orchidaceae and Asteraceae, consisting of more than 700 genera and about 20 000 species of trees, shrubs, vines, and herbs worldwide [6]. It is the second largest family of medicinal plants, containing over 490 species used as traditional medicine [7]. To the best of our knowledge, the plant species involved in this study are being screened for the first time, except *Erythrina caffra* Thunb., which has been reported to have antimicrobial activity [8]. Some of these species are used traditionally to combat infections (Table 1). In this study, *in vitro* antibacterial and antioxidant activities of the acetone leaf extracts of nine South African medicinal plants belonging to the Fabaceae family were investigated. Because plant material from nearly 290 species belonging to 100 genera of the Fabaceae has been reported to be toxic [7], the cytotoxicity was determined to ascertain the safety of the studied extracts.

Extracts with a good antimicrobial activity or good antioxidant activity and low toxicity may be useful in addressing animal health and productivity in aspects such as

microbial infections, replacing antibiotic feed additives and treating infectious diarrhoea, a focus area of the Phytomedicine Programme.

## Methods

### Plant material and extraction

The leaves of plants were collected in the Pretoria National Botanical Garden in South Africa. The identity of the plant material was confirmed by the curator and voucher specimens were placed in the HGWJ Schweickerdt Herbarium of the University of Pretoria (PRU) (Table 1). Collected tree leaves were dried at room temperature in a well-ventilated room and ground to a fine powder in a Macsalab Mill (Model 2000 LAB Eriez). One gram of each plant was extracted in 10 mL of acetone, (technical grade, Merck) in a polyester centrifuge tube. The tube was vigorously shaken for 30 min on an orbital shaker, then centrifuged at  $4000 \times g$  for 10 min and the supernatant was filtered using Whatman No.1 filter paper before being transferred into pre-weighed glass containers. This was repeated thrice on the same plant material and the solvent was removed by evaporation under a stream of air in a fume hood at room temperature to produce the dried extract.

### Chemicals

Sodium carbonate was obtained from Holpro Analytic, South Africa. Gentamicin was purchased from Virbac, South Africa. Fetal calf serum (FCS) and minimum essential medium (MEM with L-glutamine) was provided by Highveld Biological, Johannesburg, South Africa. Phosphate buffered saline (PBS) and trypsin were purchased from Whitehead Scientific, South Africa. Doxorubicin was obtained from Pfizer, South Africa. Dimethyl sulphoxide (DMSO), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), p-iodonitrotetrazolium violet (INT), Folin-Ciocalteu reagent, gallic acid, 2,5,7,8-tetramethylchroman carboxylic acid (Trolox) and potassium persulfate were purchased from Sigma-Aldrich St. Louis, MO, USA. Müller Hinton agar and broth were from Sigma-Aldrich, India.

### Antibacterial assay

The antimicrobial activity of extracts dissolved in acetone was determined against six bacterial strains: three Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 14579) and three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028). This activity was evaluated by the determining the minimal inhibitory concentration (MIC) using a rapid broth

**Table 1 Characteristics of the nine plants of Fabaceae family investigated**

Name of plant	Common names	Synonyms	Parts used	Traditional or other uses	Voucher number
<i>Baphia racemosa</i> (Hochst) Baker.	Violet pea, Natal camwood (Eng.); boskammhout (Afr.); Tshuphu (isiXhosa); Fithi (Zulu)	<i>Bracteolaria racemosa</i> Hochst.	Leaves	Source of food for larvae of the blue-spotted charaxes. Parrots feed on young seeds [9].	PMDN 397
<i>Crotalaria capensis</i> Jacq.	Cape rattle-pod, Cape laburnum (Eng.); Kaapse klapperpeul (Afr.); Bukheshezane (Zulu)	-	Flowers	Decorative horticultural value [10].	PMDN 318
<i>Dalbergia nitidula</i> Baker	Glossy flat-bean (English) Mudima (Shona) Murima (Shona) Purple-wood dalbergia (English)	<i>Dalbergia dekindtiana</i> Harms	Roots	For some species in this genus, a paste of charred and powdered stems mixed with water is used for sore mouths in infants [11].	PMDN 367
<i>Erythrina caffra</i> Thunb.	Coast coral tree (Eng.); kuskoraalboom (Afr.); umsinsi (Zulu); umsintsi (Xhosa)	<i>Erythrina viarium</i> Tod.	Bark, leaves, roots	Sores, tuberculosis, respiratory infections, wounds, eardrops for earache, sprains [11].	PMDN 280
<i>Indigofera cylindrica</i>	Natal Camwood (Eng.) Natal Camwood	<i>Indigofera frutescens</i> <i>Indigofera jucunda</i> Schrire	-	Plants of the genus are used for infertility, menstrual cramps, toothbrushes, mouthwash [12].	PMDN 178
<i>Lonchocarpus nelsii</i> (Schinz) Heering & Grimme	Apple-leaf lance-pod, Kalahari apple-leaf, Kalahari lance-pod (Eng.)	<i>Dalbergia nelsii</i> , <i>Philenoptera nelsii</i> (Schinz) Schrire	-	Plants of the genus are used as a laxative, for treating convulsion in infants, and for minor skin trouble [13].	PMDN 612
<i>Podalyria calyprata</i> (Retz.) Willd.	Sweetpea bush, large pink keurtjie, water blossom-pea (Eng.); keur, keurtjie, keurbloem, ertjiebos, waterkeurtjie (Afr.)	<i>Podalyria kunthii</i> Walp., <i>Podalyria lanceolata</i> (E. Mey.) Benth.	-	Purely decorative as no records of other uses could be traced.	PMDN 759
<i>Virgilia divaricata</i> Adamson.	Blossom tree (Eng.); keurboom (Afr.)	-	-	Transparent gum exudate from the bark is used as a substitute for starch [14].	PMDN 192
<i>Xylia torreana</i> Brenan	Hairy sand ash, Zambezi ash (Eng.)	<i>Xylia africana</i> sensu Torre	-	No information on tradition use found	PMDN159

:- not reported, Eng.: English, Afr.: Afrikaans.

microdilution technique with p-iodonitrotetrazolium violet (INT) as growth indicator as developed by Eloff [15]. The samples were serially diluted to provide a final concentration range of 2.5 to 0.02 mg/mL. In addition to MIC, the total activity was calculated as the total mass in mg extracted from 1 g of plant material divided by the MIC value (mg/mL). The total activity in ml/g indicates the volume to which the extract derived from 1 g of plant material can be diluted and still inhibits the growth of the microorganism [16].

#### Antioxidant assays

##### Total phenolic content determination

The total phenolic content was determined colorimetrically using a Folin-Ciocalteu 96-well microplate assay developed by Zhang et al [17]. The total phenolic content was calculated from the linear equation of a standard curve prepared with gallic acid, and expressed as gallic acid equivalent (GAE) per g of extract.

##### ABTS radical assay

The ABTS radical scavenging capacity of the samples was measured with modifications of the 96-well microtitre plate method described by Re et al. [18]. Trolox and ascorbic acid were used as positive controls, methanol as negative control and extract without ABTS as blank. The percentage of ABTS<sup>•+</sup> inhibition was calculated using the formula: Scavenging capacity (%) = 100 - [(absorbance of sample - absorbance of sample blank) × 100 / (absorbance of control) - (absorbance of control blank)]. The IC<sub>50</sub> values were calculated from the graph plotted as inhibition percentage against the concentration.

##### DPPH assay

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams et al. [19]. Ascorbic acid and Trolox were used as positive controls, methanol as negative control and extract without DPPH as blank. Results were expressed as percentage reduction of the initial DPPH absorption in relation to the control. The concentration of extract that reduced the DPPH colour by 50% (IC<sub>50</sub>) was determined as for ABTS<sup>•+</sup>.

##### Cytotoxic activity

The cytotoxicity of the acetone extracts against Vero monkey kidney cells was assessed by the MTT reduction assay as previously described [20] with slight modifications. Cells were seeded at a density of 1 × 10<sup>5</sup> cells/ml (100 µl) in 96-well microtitre plates and incubated at 37°C and 5% CO<sub>2</sub> in a humidified environment. After 24 h incubation, extracts (100 µl) at varying final concentrations were added to the wells containing cells. Doxorubicin was used as a positive reference. A suitable

blank control with equivalent concentrations of acetone was also included and the plates were further incubated for 48 h in a CO<sub>2</sub> incubator. Thereafter, the medium in each well was aspirated from the cells, which were then washed with PBS, and finally fresh medium (200 µl) was added to each well. Then, 30 µl of MTT (5 mg/ml in PBS) was added to each well and the plates were incubated at 37°C for 4 h. The medium was aspirated from the wells and DMSO was added to solubilize the formed formazan crystals. The absorbance was measured on a BioTek Synergy microplate reader at 570 nm. Cell growth inhibition for each extract was expressed in terms of LC<sub>50</sub> values, defined as the concentration that caused 50% of inhibition of cell viability. The selectivity index (SI) values were calculated by dividing cytotoxicity LC<sub>50</sub> values by the MIC values (SI = LC<sub>50</sub>/MIC). Tests were carried out in quadruplicate and each experiment was repeated thrice.

#### Statistical analysis

All experiments were conducted in triplicate and values expressed as mean ± standard deviation. For the MIC values, re-evaluation of the growth inhibition was conducted where variation within the three experiments was noticed. Statistical analysis was performed using one way ANOVA and results were compared using the Fisher's least significant difference (LSD) at a 5% significance level.

## Results and discussion

### Antimicrobial activity

The MIC values of the leaf extracts of 9 species of the Fabaceae family are presented in Table 2. Extracts had a broad spectrum of activity against all bacterial strains. Crude extracts with an MIC < 100 µg/mL are usually considered as significantly active [21] and therefore could be regarded as promising candidates for further studies. In this study, six of the nine extracts investigated had significant activity, including extracts of *C. capensis* against *S. typhimurium* followed by the extract of *I. cylindrica* with respective MICs of 20 and 40 µg/mL. Significant activities were also recorded (MIC of 80 µg/ml) with extracts of *C. capensis*, *E. caffra* and *L. nelsii* against *E. faecalis*. *D. nitidula* and *V. divaricata* had similar activity against *B. cereus*. Olajuyigbe and Afolayan previously reported moderate antibacterial activity of the ethanolic extract from *E. caffra* bark against two Gram positive (*E. faecalis*, *S. aureus*) and two Gram negative (*E. coli*, *P. aeruginosa*) bacteria (MICs ranged from 156.6 to 625 µg/mL) [8].

A significant antimicrobial activity of an extract is a highly promising result, since an extract consists of a mixture of compounds. A bioassay-guided fractionation might lead to the isolation of active ingredients with an

**Table 2 Minimal inhibitory concentration (MIC in µg/mL) and total activity (TA in mL/g) of acetone leaf extracts from nine plants of the Fabaceae family against bacterial strains**

Plant name	Extraction yield (%)	ME (mg)	Microorganisms											
			<i>S. aureus</i>		<i>E. faecalis</i>		<i>B. cereus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. typhimurium</i>	
			MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA
<i>Baphia racemosa</i>	3.7	37	310	119.35	160	231.25	625	59.20	625	59.20	>1000	<37	625	59.20
<i>Crotalaria capensis</i>	4.6	46	625	73.60	<b>80</b>	575	310	148.39	625	73.60	625	73.60	<b>20</b>	<b>2300</b>
<i>Dalbergia nitidula</i>	4.2	42	160	262.5	160	262.5	<b>80</b>	525	160	262.5	310	135.48	160	262.5
<i>Erythrina caffra</i>	3.9	39	>1000	<39	<b>80</b>	487.5	310	125.81	160	243.75	160	243.75	310	125.81
<i>Indigofera cylindrica</i>	1.9	19	625	30.40	310	61.29	160	118.75	310	61.29	310	61.29	<b>40</b>	475
<i>Lonchocarpus nelsii</i>	0.9	9	>1000	<9	<b>80</b>	112.5	625	14.40	625	14.40	>1000	<9	<b>80</b>	112.5
<i>Podalyria calyprata</i>	3.4	34	>1000	<34	625	54.40	>1000	<34	310	109.68	>1000	<34	160	212.5
<i>Virgilia divaricata</i>	8.9	89	310	287.1	625	142.40	<b>80</b>	<b>1112.5</b>	310	287.1	>1000	<89	<b>80</b>	<b>1112.5</b>
<i>Xylia torreana</i>	1.2	12	310	38.71	160	75	160	75	310	38.71	310	38.71	160	75
Gentamicin	nd	nd	0.2	nd	0.2	nd	0.8	nd	0.4	nd	0.2	nd	1.56	nd

Mass in mg extracted from 1 g (ME).

Values in bold indicate MICs lower than 100 µg/mL and total activity higher than 1000 ml/g, nd: not determined.

activity better than that of standard antibiotics. However, low activity was noticed with all the extracts against the Gram-positive *S. aureus* and two Gram-negative bacteria, namely *E. coli* and *P. aeruginosa*. The significant activity of extracts against Gram-negative bacteria (including *S. typhimurium*) which are often resistant to antimicrobial agents compared to Gram-positive [22], could be explained by the possibility that the active compounds may act by inhibiting bacterial growth without necessarily penetrating into the bacterial cell itself. Although an interesting activity was found with the extracts of *C. capensis* against *S. typhimurium*, the calculated total activity was lower (475 mL/g) compared to that of the *I. cylindrica* extract (2 300 mL/g). Therefore, the extract of *I. cylindrica* appears to be the most promising antimicrobial candidate for isolation of biologically active compounds as well as for the rational use of the plant extract in primary health care by rural communities for human or animal health.

#### Antioxidant activity and total phenolic content

The antioxidant capacity expressed as IC<sub>50</sub> value and total phenolic content of all extracts are shown in Table 3. All the extracts had moderate to potent antioxidant activity. The IC<sub>50</sub> values occurred in the range of 9.31 ± 2.14 to 271.58 ± 51.96 µg/mL in the DPPH assay, and 14.56 ± 3.96 to 207.09 ± 70.75 µg/mL in the ABTS radical assay. The use of at least two different assays in evaluating antioxidant activity of plant extracts has been recommended by Moon and Shibamoto [23]. Significant variations (p < 0.05) were observed between the extracts tested. With IC<sub>50</sub> of 9.31 ± 2.14 µg/mL, extracts from *D. nitidula* had free radical scavenging capacity close to that of the positive control Trolox (IC<sub>50</sub> of 9.71 ± 2.23 µg/mL) in the DPPH assay. A similar

result was obtained with the *Xylia torreana* (IC<sub>50</sub> of 14.56 ± 3.96 µg/mL) extract in the ABTS assay where Trolox had an IC<sub>50</sub> of 12.48 ± 3.7 µg/mL. These results indicate that these two extracts could be a potential source of natural antioxidants.

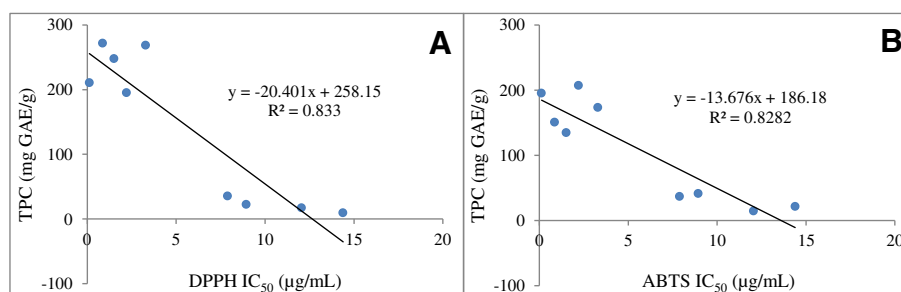
The total phenolic contents (TPC) of the extracts were in the range of 0.12 ± 0.1 to 14.39 ± 0.62 mg GAE/g. According to Makkar [24] the TPC of an extract amounting

**Table 3 Antioxidant activity and total phenolic content (TPC) of acetone extracts from nine plants of the Fabaceae family**

Plant name	DPPH IC <sub>50</sub> (µg/mL)	ABTS IC <sub>50</sub> (µg/mL)	TPC (mg GAE/g)
<i>Baphia racemosa</i>	210.69 ± 65.48 <sup>a</sup>	195.10 ± 54.68 <sup>a</sup>	0.12 ± 0.1 <sup>a</sup>
<i>Crotalaria capensis</i>	195.26 ± 30.64 <sup>a</sup>	207.09 ± 70.75 <sup>a</sup>	2.21 ± 0.95 <sup>b</sup>
<i>Erythrina caffra</i>	268.6 ± 29.69 <sup>a</sup>	173.28 ± 43.04 <sup>a</sup>	3.29 ± 1.38 <sup>c</sup>
<i>Lonchocarpus nelsii</i>	247.70 ± 66.47 <sup>a</sup>	134.64 ± 16.49 <sup>a</sup>	1.51 ± 0.74 <sup>b</sup>
<i>Virgilia divaricata</i>	271.58 ± 51.96 <sup>a</sup>	150.57 ± 19.23 <sup>a</sup>	0.86 ± 0.53 <sup>d</sup>
<i>Indigofera cylindrica</i>	22.31 ± 9.92 <sup>b</sup>	41.39 ± 15.74 <sup>b</sup>	8.94 ± 1.52 <sup>e</sup>
<i>Xylia torreana</i>	16.90 ± 5.45 <sup>b</sup>	<b>14.56 ± 3.96<sup>c</sup></b>	12.05 ± 1.23 <sup>f</sup>
<i>Podalyria calyprata</i>	35.21 ± 3.46 <sup>c</sup>	36.66 ± 3.59 <sup>b</sup>	7.89 ± 1.48 <sup>e</sup>
<i>Dalbergia nitidula</i>	<b>9.31 ± 2.14<sup>d</sup></b>	21.30 ± 5.07 <sup>d</sup>	14.39 ± 0.62 <sup>g</sup>
Trolox	9.71 ± 2.23 <sup>d</sup>	12.48 ± 3.7 <sup>c</sup>	nd
Ascorbic acid	2.44 ± 0.9 <sup>e</sup>	3.14 ± 1.07 <sup>e</sup>	nd

Values in bold indicate MICs lower than 100 µg/mL and total activity higher than 1000 ml/g.

n = 3, means values within a column with different superscript letters are significantly different at p < 0.05; mg GAE/g: mg of gallic acid equivalent per g of dry plant material.



**Figure 1** Correlation between antioxidant capacity and total phenolic content of nine species from the Fabaceae family. Antioxidant capacity was measured by DPPH assay (A) and ABTS radical assay (B). GAE: gallic acid equivalent.

to 5 mg GAE/g is considered to be significant and could have a beneficial antioxidant efficacy. Taking into account this cut-off, extracts of *D. nitidula*, *I. cylindrica*, *P. calyprata* and *X. torreana* contain significant amounts of phenols. The TPC correlated well with the respective antioxidative activity of extracts (Figure 1). Good correlation was found between the TPC and IC<sub>50</sub> values for both DPPH and ABTS ( $R^2 > 0.833$  and  $R^2 > 0.828$  respectively). Polyphenols have been reported to be responsible for the antioxidant activity in plant extracts [25].

The high levels of TPC observed with the extract of *D. nitidula* and *I. cylindrica* could be related to their good antimicrobial activity because many phenolic compounds have antimicrobial activity and antioxidant activity [26]. Although no *in vivo* experiments have been done, the good antioxidant activity of acetone extracts combined with good antibacterial activity indicates that these extracts may fulfill a dual role if used therapeutically.

In the case of *C. capensis*, *E. caffra*, *L. nelsii* and *V. divaricata* the good antibacterial activity was apparently not due to the presence of phenolics. Many secondary

metabolites other than phenolic compounds are well known for their antimicrobial activities [27].

#### Cytotoxic activity

The cytotoxicity was determined using an *in vitro* assay with Vero monkey kidney cells. The LC<sub>50</sub> and the selectivity index (SI) values were calculated (Table 4). The LC<sub>50</sub> values ranged from  $10.70 \pm 3.47$  to  $131.98 \pm 24.87$  µg/mL while the SI ranged from 0.003 to 8.25. Extracts of *P. calyprata* had the highest LC<sub>50</sub> (lowest toxicity) of  $131.98 \pm 24.87$  µg/mL. *B. racemosa* had an LC<sub>50</sub> of  $10.70 \pm 3.47$  µg/mL, which is relatively cytotoxic. Our results indicated that all the plant extracts investigated were less toxic to Vero cells than the positive control, doxorubicin. Apart from *B. racemosa* and *E. caffra*, all extracts had LC<sub>50</sub> values greater than 30 µg/mL.

In addition to ascertaining the likely safety of plant extracts for their potential use, standard cell-based toxicity assays are also performed *in vitro* at an early stage of the drug development process in order to remove high-risk materials [28]. Moreover, this helps to ensure that the biological activity of the plant extract is not due to a general

**Table 4** Cytotoxicity of acetone leaf extracts from nine species of the Fabaceae family on Vero monkey kidney cells and their selectivity index (SI) against bacterial strains

Plant name	IC <sub>50</sub> (µg/ml)	Selectivity index (LC <sub>50</sub> /MIC)					
		SA	EF	EC	PA	BC	ST
<i>Podalyria calyprata</i>	$131.98 \pm 24.87^a$	<0.1	0.21	0.43	<0.3	<0.1	0.82
<i>Xylia torreana</i>	$82.51 \pm 9.46^b$	0.27	0.52	0.27	0.27	0.52	0.52
<i>Lonchocarpus nelsii</i>	$81.09 \pm 7.18^b$	<0.1	<b>1.01</b>	0.13	<0.1	0.13	<b>1.01</b>
<i>Indigofera cylindrica</i>	$77.59 \pm 6.10^b$	0.13	0.25	0.25	0.25	0.48	<b>1.94</b>
<i>Dalbergia nitidula</i>	$51.28 \pm 11.47^c$	0.32	0.32	0.32	0.17	0.64	0.32
<i>Crotalaria capensis</i>	$45.47 \pm 10.48^c$	0.07	0.57	0.07	0.07	0.15	<b>2.27</b>
<i>Virgilia divaricata</i>	$30.08 \pm 4.96^c$	0.1	0.05	0.1	<0.1	0.38	0.38
<i>Erythrina caffra</i>	$19.93 \pm 2.20^d$	<0.1	0.25	0.12	0.12	0.06	0.06
<i>Baphia racemosa</i>	$10.7 \pm 3.47^e$	0.03	0.07	0.02	<0.1	0.02	0.02
Doxorubicin	$2.29 \pm 1.04^f$	nd	nd	nd	nd	nd	nd

For IC<sub>50</sub>, n = 3, means values with different superscript letters are significantly different at  $p < 0.05$ ; nd: not determined. In bold are interesting results with SI value >1. SA: *Staphylococcus aureus*, EF: *Enterococcus faecalis*, BC: *Bacillus cereus*, EC: *Escherichia coli*, PA: *Pseudomonas aeruginosa*, ST: *Salmonella typhimurium*.

metabolic toxic effect. Extracts of *C. capensis* and *L. nelsii* had SI values of 2.27 and 1.01 respectively. The higher the selectivity index is for a crude extract the more likely it is that the activity is not due to a general metabolic toxin [29]. Although elimination of toxic components by manipulation of the extract may yield more suitable antibacterial extracts [30] selection of an extract with a high selectivity index increases the potential that a useful herbal medicine can be produced.

## Conclusion

Acetone leaf extracts of *D. nitidula*, *X. torreana*, *C. capensis* and *I. cylindrica* had a relatively low cytotoxicity and a high antibacterial and/or antioxidant activity. Thus, these plant species could be considered as potential candidates for the development of novel antimicrobial formulations with reduced toxicity that can be used to promote animal health and productivity [31], as well as for further studies to isolate the active compounds [32]. These aspects are being followed up with some plant species in the Phytomedicine Programme.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

JPD performed experiments and wrote the first draft of manuscript. LJM supervised the work and revised the manuscript; JNE initiated the project and revised the final manuscript. All authors read and approved the final manuscript.

## Acknowledgments

The University of Pretoria provided a postdoctoral fellowship to JPD. The National Research Foundation (NRF) and Medical Research Council (MRC) provided funding to support this study.

## Author details

<sup>1</sup>Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa. <sup>2</sup>Present address: Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon.

Received: 28 October 2013 Accepted: 29 April 2014

Published: 5 May 2014

## References

1. Lobo V, Patil A, Phatak A, Chandra N: Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev* 2010, **4**(8):118–126.
2. Alviano DS, Alviano CS: Plant extracts: Search for new alternatives to treat microbial diseases. *Curr Pharm Biotechnol* 2009, **10**(1):106–121.
3. Baratta MT, Dorman HJ, Deans SG, Biondi DM, Ruberto G: Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *J Essent Oil Res* 1998, **10**(6):618–627.
4. Kuethe V, Efferth T: Cameroonian medicinal plants: pharmacology and derived natural products. *Front Pharmacol* 2010, **1**:123–123.
5. Atawodi SE: Antioxidant potential of African medicinal plants. *Afr J Biotechnol* 2005, **4**(2):128–133.
6. Stevens PF: Onwards Angiosperm Phylogeny Website 2001. Version 12 2012, <http://www.mobot.org/MOBOT/research/APweb/>.
7. Gao T, Yao H, Song J, Liu C, Zhu Y, Ma X, Pang X, Xu H, Chen S: Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. *J Ethnopharmacol* 2010, **130**(1):116–121.

8. Olajuyigbe OO, Afolayan AJ: *In vitro* antibacterial and time-kill evaluation of the *Erythrina caffra* Thunb. extract against bacteria associated with diarrhoea. *Sci World J* 2012, **2012**:738314.
9. Van Wyk AE, Van Wyk P: *Field Guide to Trees of Southern Africa*. Cape Town: Random House Struik (Pty.); 1997.
10. Pooley E: *The Complete Field Guide to Trees of Natal, Zululand and Transkei*. Durban, South Africa: Natal Flora Publications Trust; 1993.
11. Hutchings A, Scott AH, Lewis G, Cunningham AB: *Zulu Medicinal Plants: An Inventory*. Pietermaritzburg, South Africa: University of Natal Press; 1996.
12. Kirtikar KR: *Indian Medicinal Plants*. Allahabad: Leader Press; 1993.
13. Ogbuide ON, Parvez M: A simple colorimetric quantification of flavonoids in the flowers of *Lonchocarpus cyanescens* genus - *Lonchocarpus*. *Plant Foods Hum Nutr* 1991, **41**(3):233–239.
14. Goldblatt P, Manning J: *Cape plants. A conspectus of the Cape flora of South Africa*, National Botanical Institute, Pretoria & Missouri Botanical Garden. 2000.
15. Eloff JN: A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med* 1998, **64**(8):711–713.
16. Eloff JN: On expressing the antibacterial activity of plant extracts - A small first step in applying scientific knowledge to rural primary health care. *S Afr J Sci* 2000, **96**(3):116–118.
17. Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ: A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *J Appl Phycol* 2006, **18**(3–5):445–450.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999, **26**(9–10):1231–1237.
19. Brand-Williams W, Cuveleir ME, Berset C: Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* 1995, **28**(1):25–30.
20. Mosmann T: Rapid colorimetric assay for cellular growth and survival - application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**(1–2):55–63.
21. Eloff JN: Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine* 2004, **11**(4):370–371.
22. Nikaido H: Antibiotic resistance caused by Gram-negative multidrug efflux pumps. *Clin Infect Dis* 1998, **27**(Suppl 1):S32–S41.
23. Moon JK, Shibamoto T: Antioxidant assays for plant and food components. *J Agric Food Chem* 2009, **57**:1655–1666.
24. Makkar HPS: *Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual*. Dordrecht: Kluwer Academic Publishers; 2003.
25. Piluzza G, Bullitta S: Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharm Biol* 2011, **49**(3):240–247.
26. Mulaudzi RB, Ndhala AR, Kulkarni MG, Finnie JF, Van Staden J: Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. *J Ethnopharmacol* 2011, **135**(2):330–337.
27. Cowan MM: Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999, **12**(4):564–582.
28. Omar NS, Kannan TP, Ismail AR, Abdullah SF, Samsudin AR, Hamid SSA: *In vitro* cytotoxic evaluation of processed natural coral in human osteoblasts. *Int J Toxicol* 2011, **30**(4):443–451.
29. Cho-Ngwa F, Abongwa M, Ngemenya MN, Nyongbela KD: Selective activity of extracts of *Margaritaria discoidea* and *Homalium africanum* on *Onchocerca ochengi*. *BMC Complement Altern Med* 2010, **10**:62.
30. Eloff JN, Famakin JO, Katerere DRP: Combretum woodii (Combretaceae) leaf extracts have high activity against Gram-negative and Gram-positive bacteria. *Afr J Biotechnol* 2005, **4**:1161–1166.
31. Suleiman MM, Duncan JN, Eloff JN, Naidoo V: A controlled study to determine the efficacy of *Loxostylis alata* (Anacardiaceae) in the treatment of aspergillosis in a chicken (*Gallus domesticus*) model in comparison to ketoconazole. *BMC Vet Res* 2012, **8**:210.
32. Eloff JN, Katerere DR, McGaw LJ: The biological activity and chemistry of the southern African Combretaceae. *J Ethnopharmacol* 2008, **119**:686–699.

doi:10.1186/1472-6882-14-147

**Cite this article as:** Dzoyem et al.: *In vitro* antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity. *BMC Complementary and Alternative Medicine* 2014 **14**:147.