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Short communication

In vitro biological activities of South African *Pelargonium* (Geraniaceae) species

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Abstract

Despite commercial interest and ethnobotanical data, the pharmacological activities of a number of indigenous *Pelargonium* species hitherto remain poorly explored. The acetone extracts of twenty-one *Pelargonium* species (section *Pelargonium*) were included in this study. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, potent anti-oxidant activity was observed for the crude extracts of *P. betulinum* and *P. crispum* (IC_{50} values of 4.13 ± 0.14 µg/ml and 4.49 ± 0.18 µg/ml, respectively compared to ascorbic acid, IC_{50} = 4.72 ± 0.14 µg/ml). The antimicrobial (both bacterial and fungal) potential of the extracts was evaluated by using the minimum inhibitory assay. The crude extracts of *P. glutinosum* (SBG), *P. pseudoglutinosum*, *P. scabrum* and *P. sublignosum* exhibited considerable antimicrobial activity against the Gram-positive test bacteria, with *P. pseudoglutinosum* demonstrating the highest inhibitory activity (MIC = 39 µg/ml); however the aforementioned species were also relatively cytotoxic. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cellular viability assay was used to determine the toxicity of the acetone extracts against transformed human kidney epithelial (Graham) cells. The acetone extracts of *P. sublignosum* and *P. citronellum* (NBG) displayed the highest toxicities (IC_{50} = 11.89 ± 1.54 µg/ml and 19.14 ± 0.98 µg/ml, respectively). The results from the toxicity assay suggested that the antimicrobial activity of the extracts may be ascribed to general cytotoxic effects. Considering all the results collectively, *Pelargonium cordifolium* appears to be the most promising species requiring further investigation.
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Keywords: Acetone extracts; Antimicrobial activity; Anti-oxidant activity; *Pelargonium*; Toxicity

1. Introduction

Of the over 250 *Pelargonium* species (Geraniaceae), approximately 80% occur in South Africa with the highest concentration of species growing in the winter rainfall region of the south-western Cape (Van der Walt and Vorster, 1988; Lis-Balchin, 1996). The common name geranium has been erroneously used to refer to a plant that should correctly be called a pelargonium (Van der Walt, 1977). The *Pelargonium* species are divided into 16 recognised sections (Bakker et al., 2004) that are phylogenetically arranged. Van der Walt (1985) taxonomically revised the section *Pelargonium*, which contains a total of twenty-four species. Many of the species are aromatic and in particular, *P. capitatum*, *P. graveolens* and *P. radens* are used in cultivation programmes for the ennoblement of 'geranium' oil. Studies on *Pelargonium* have intensively focused on the chemical composition of the essential oils (Williams and Harborne, 2002).

In South Africa, many scented and unscented *Pelargonium* species are used as traditional remedies by the Sotho, Xhosa, Khoi-San and Zulus (Watt and Breyer-Brandwijk, 1962) for wounds, abscesses, fever, colic, nephritis and suppression of urine, colds and sore throats, haemorrhoids, gonorrhoea, and are also used for stimulating milk-production, for anti-helminthic infections and as an insecticide (Lis-Balchin, 1996).

Pelargoniums were also used in the past to provide relief in cases of diarrhoea and dysentery; this suggested a potential antispasmodic mechanism of action on smooth muscle (Lis-Balchin, 1996) which was experimentally verified in some of the fractions of the many different scented and unscented species studied (Lis-Balchin et al., 1996). The profound antimicrobial activity of Pelargoniums may partly explain their wound-healing properties (Lis-Balchin, 1996). Previous reports have documented the antimicrobial activity of the extracts of Pelargoniums and

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their constituents against several bacterial pathogens such as *Staphylococcus aureus*, *S. pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* (Mativandlela et al., 2006). The methanolic extracts of representative species and cultivars of *Pelargonium* (Geraniaceae) were found to exert pronounced antibacterial and anti-oxidant activities (Lis-Balchin and Deans, 1996). A study carried out by Lis-Balchin et al. (1998) showed that the methanolic and petroleum spirit extracts of a number of *Pelargonium* species and cultivars have substantial antibacterial activity. The latter two studies suggest that *Pelargonium* extracts can potentially be used as novel anti-oxidant and antibacterial agents in the food and cosmetic industries.

Regardless of the proclaimed uses of various *Pelargonium* species, there has been very little commercial exploitation. *Pelargonium reniforme* and the closely related *P. sidoides* are used as ingredients in a German remedy marketed as "Umckaloabo", which is used to treat bronchitis in children. The presence of umckalin and structurally related coumarins may be partly responsible for the activity of the medicine prepared from *P. reniforme* (Van Wyk et al., 2002).

The utilisation of natural products in all industrial sectors is increasing and studies of the various biotherapeutic properties of the extracts of several unexplored Pelargoniums will better equip us to further develop the therapeutic application and commercialisation of *Pelargonium* species. To provide scientific evidence for their reported uses, the aims of the present study were to investigate the *in vitro* antimicrobial and anti-oxidant properties as well as the *in vitro* toxicity of the acetone extracts of selected *Pelargonium* species (section *Pelargonium*).

2. Materials and methods

2.1. Collection of plant material

The aerial parts (stems, stalks and leaves) of 21 *Pelargonium* species were harvested from the National Botanical Garden (NBG), Stellenbosch Botanical Garden (SBG) and the Walter Sisulu Botanical Garden (WSBG). Voucher specimens of all the material collected were deposited in the herbarium of the Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg.

2.2. Preparation of plant extracts

Plant material was dried at 30 °C. The dried, ground material was extracted at 40 °C for 3 h in acetone. The extracts were filtered through Whatman® No. 41 filter paper (pore size 20–25 µm) and dried under vacuum using a rotary evaporator. The extracts were further reduced in the fumehood and once concentrated to dryness were kept at 4 °C until required.

2.3. Anti-oxidant activity assay

The radical scavenging activities of the acetone extracts were determined using a modified version of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Mambro et al., 2003). Briefly, the stable DPPH radical has strong absorption at 550 nm, but upon reduction by an anti-oxidant or radical species bleaching of the purple coloured DPPH occurs, with a decrease in absorption at 550 nm.

Fifty microlitres of extract (100 µg/ml) dissolved in dimethyl sulfoxide (DMSO; Saarchem) and 200 µl DPPH (Fluka) dissolved in analytical grade methanol (Ultrafine Limited) to yield a concentration of 96 µM constituted the reaction mixture, which was added to alternating columns of the plate with the reaction mixtures for negative controls containing extracts and methanol. Analytical grade ascorbic acid was used as a positive control. The plates were shaken for 2 min using an automated microtitre plate reader (Labysystems Multiskan RC). After allowing the plates to stand for 30 min at room temperature in the dark, the absorbance at 550 nm was measured using an UV/VIS spectrophotometer linked to a computer equipped with GENESIS® software. The percentage decolourisation as a measure of the free radical scavenging activity of the sample was calculated using the following equation:

% Decolourisation = ([Av controls − (Av sample$_{DPPH}$ − Av sample$_{methanol}$)] × 100)/Av controls. Where: − Av controls = average absorbance of all DPPH control wells − average absorbance of all methanol control wells; Av sample$_{DPPH}$ = average absorbance of sample wells with DPPH and Av sample$_{methanol}$ = average absorbance of sample wells with methanol.

The IC_{50} values (concentration of extract producing 50% decolourisation) were determined using Enzfitter® version 1.05 software. Each experiment was carried out in triplicate.

2.4. Minimum inhibitory concentration (MIC) assay

The antimicrobial activity of each non-volatile extract was evaluated using the microtitre plate dilution method (Eloff, 1998). The minimum inhibitory concentration values were determined against four micro-organism strains which included two Gram-positive bacteria *S. aureus* (ATCC 12600) and *Bacillus cereus* (ATCC 11778), one Gram-negative bacterium *K. pneumoniae* (NCTC 1633) and one fungal strain *Candida albicans* (ATCC 10231). The reference stock cultures were obtained from the National Health Laboratory Services (NHLS), Johannesburg and were maintained in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

Stock solutions of the plant extracts were prepared in acetone which did not inhibit the bacterial or fungal growth at the concentrations tested. Serial 1:1 dilutions of the plant extracts (100 µl) were prepared in sterile water (100 µl) before culture (100 µl) containing a final density of 1×10^6 CFU's/ml was added to each well. For the observation of normal bacterial and fungal growth, extract-free solutions were included as blank controls. The antimicrobial agents, ciprofloxacin (Oxoid) or amphotericin B (Oxoid), were used as positive controls for bacteria and fungi, respectively. The plates were incubated under normal atmospheric conditions, at 37 °C for 24 h (bacteria) and 48 h (yeast). After the incubation period, 131.8 µM *p*-iodo-nitrotetrazolium violet (INT; Sigma) solution

Table 1
In vitro anti-oxidant, antimicrobial and toxicity properties of selected *Pelargonium* acetone extracts

Species	Locality (and voucher no.)	Anti-oxidant activity IC_{50} (μg ml^{-1})	Antibacterial activity MIC (μg ml^{-1})			Antifungal activity MIC (μg ml^{-1})	Toxicity IC_{50} (μg ml^{-1})
			K. p. NCTC 1633	*B. c.* ATCC 11778	*S. a.* ATCC 12600	*C. a.* ATCC 10231	
P. betulinum	NBG (AV 941)	4.13±0.14	1000	330	1130	2000	88.55±1.51
P. capitatum	Strand (AV 918)	Nd	1000	1000	2000	1500	101.59±1.75
P. citronellum	NBG (AV 943)	23.70±3.68	3000	250	160	1000	19.14±0.98
P. citronellum	SBG (AV 1172)	84.01±16.08	3000	410	160	500	59.94±2.33
P. cordifolium	NBG (AV 1173)	5.01±0.55	1500	250	750	750	74.70±2.17
P. crispum	NBG (AV 1175)	4.49±0.18	2000	380	560	1330	74.02±2.30
P. cucullatum	SBG (AV 1176)	40.18±5.65	1500	250	1000	1000	73.81±2.62
P. cucullatum	WSBG (AV 1177)	10.91±0.54	3330	500	2000	2000	118.89±5.32
P. glutinosum	SBG (AV 939)	16.41±0.33	2000	78	78	1000	31.44±1.27
P. glutinosum	WSBG (AV 1178)	29.17±0.78	2000	250	500	2000	46.29±0.81
P. graveolens	SBG (AV 947)	14.49±0.46	2000	2000	4000	3330	83.31±2.56
P. greytonense	SBG (AV 920)	Nd	3200	250	500	1500	39.65±1.18
P. hermanniifolium	SBG (AV 1181)	13.50±0.73	1500	3000	1500	1500	46.47±1.38
P. hispidum	SBG (AV 946)	12.78±0.45	2000	4000	4000	3000	84.30±1.16
P. panduriforme	SBG (AV 949)	Nd	2000	2000	2000	2000	32.93±3.75
P. panduriforme	WSBG (AV 926)	91.58±4.45	2000	500	500	1000	42.65±0.90
P. papilionaceum	NBG (AV 944)	81.24±13.44	4000	8000	2000	1190	53.76±1.60
P. pseudoglutinosum	NBG (AV 1182)	52.38±0.67	2000	39	39	540	30.54±5.46
P. quercifolium	SBG (AV 940)	17.15±0.58	8000	330	160	2000	85.61±1.93
P. quercifolium	WSBG (AV 921)	61.87±3.19	2000	1000	1000	1000	48.69±1.04
P. radens	SBG (AV 942)	Nd	2500	500	2000	2000	30.81±1.23
P. scabroide	SBG (AV 1183)	Nd	2000	250	1000	1000	53.76±1.57
P. scabrum	SBG (AV 948)	7.15±0.11	2000	59	78	380	37.78±1.72
P. sublignosum	SBG (AV 1184)	17.61±3.18	2000	130	78	500	11.89±1.54
P. tomentosum	SBG (AV 950)	28.16±2.65	2000	2000	2000	2330	195.13±7.90
P. vitifolium	SBG (AV 945)	Nd	4000	1000	2000	2000	178.48±5.44
Controls	–	4.72±0.14	2.5 (a)	0.31 (a)	2.5 (a)	1.25 (b)	125.56±5.04 (c)

Abbreviations: NBG=National Botanical Garden (Kirstenbosch), SBG=Stellenbosch Botanical Garden, WSBG=Walter Sisulu Botanical Garden (Johannesburg); *K.p.*=*K. pneumoniae*, *B.c.*=*B. cereus*, *S.a.*=*S. aureus*, *C.a.*=*C. albicans*; Microbial strains: ATCC=American Type Culture Collection, NCTC=National Collection of Type Cultures; Nd=not determined due to insufficient extract for testing; (a): ciprofloxacin, (b): amphotericin B, (c) quinine; IC_{50} values for anti-oxidant activity and toxicity are mean±standard deviation (n=3); minimum inhibitory concentration (MIC) determination experiments for the antimicrobial activity were carried out in trplicate (n=3).

was added to all the wells and left to stand for 6 h (bacteria) and 24 h (yeast). Thereafter, the plates were visually assessed for colour change, since metabolically active micro-organisms reduce the colourless tetrazolium salt to an intensely red-coloured formazan salt. Minimum inhibitory concentration values were recorded as the lowest concentration resulting in complete inhibition of micro-organism growth (the first clear well). The MIC results were recorded in triplicate.

2.5. Toxicity assay

The toxicity profiles of the extracts were assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) microculture tetrazolium viability assay as described by Mosmann (1983). Transformed human kidney epithelial (Graham) cells were maintained continuously in culture at 37 °C in 5% CO_2. The trypsinised cell suspension (0.25 million cells/ml) was plated out into 96-well microtitre plates and incubated under humidified conditions at 37 °C in 5% CO_2 for 6 h to allow the cells to adhere to the bottom of the wells (Van Zyl and Viljoen, 2002). Thereafter, the various concentrations of plant samples were plated out in triplicate. Each plate included untreated cell controls and a blank cell-free control. Quinine was used as the positive control. After 44 h of incubation, MTT (12 mM) was added to each well and the plates incubated for a further 4 h. Thereafter, DMSO was added to each well to stop the reaction and to solubilise the formazan crystals. The absorbance was read at a test wavelength of 540 nm and a reference wavelength of 690 nm using a microtitre plate reader (Labsystems iEMS Reader MF) connected to a computer equipped with Ascent® software. The percentage cellular viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50% of cellular growth (IC_{50} value) was determined from the log sigmoid dose–response curves generated by the Enzfitter® software. Experiments for each extract were carried out in triplicate.

3. Results and discussion

3.1. Anti-oxidant activity

Pelargonium betulinum (IC_{50}=4.13±0.14 μg/ml) and *P. crispum* (IC_{50}=4.49±0.18 μg/ml) displayed greater anti-oxidant activity than the positive control ascorbic acid (IC_{50}=4.72±0.14 μg/ml). Other species which showed potent radical

scavenging activity include *P. cordifolium* and *P. scabrum* (Table 1). These results are in accordance with a study conducted by Lis-Balchin and Deans (1996), where pronounced anti-oxidant activity was displayed by a number of methanolic extracts of representative species and cultivars of *Pelargonium.* Marked differences were noted in the anti-oxidant activities of the duplicate samples of *P. citronellum, P. cucullatum* and *P. quercifolium*.

Latté and Kolodziej (2004) established that the flavonoids and hydrolyzable tannins isolated from *P. reniforme* produced higher anti-oxidant activity than ascorbic acid. The flavonoid derivatives detected in the tested *Pelargonium* extracts (Lalli, 2006) may contribute to their observed *in vitro* anti-oxidant activities.

The *Pelargonium* species, in particular *P. betulinum, P. crispum* and *P. cordifolium* are possible sources of anti-oxidant compounds since these extracts were relatively non-toxic (Table 1). These observations prompt the necessity for further studies of the abovementioned species, focusing on the isolation and structure elucidation of their anti-oxidant compound/s, since they have potential use as therapeutic agents in managing diseases associated with free radicals and also have the potential to be employed as additives in the food or cosmetic industries.

3.2. Antimicrobial activity

Overall, the extracts showed very promising antimicrobial activity (Table 1). The extracts were more selective for the Gram-positive test pathogens (in particular, *B. cereus*) than for the Gram-negative bacterium which is in accordance with studies reporting that Gram-positive bacteria are generally more sensitive than Gram-negative bacteria to solvent extracts (Cosentino et al., 1999; Karaman et al., 2003).

The *P. pseudoglutinosum* extract exerted the greatest antimicrobial activity against *B. cereus* and *S. aureus* (MIC = 39 µg/ml). The acetone extracts of *P. glutinosum* (SBG), *P. scabrum* and *P. sublignosum* also produced considerable antimicrobial activity against the Gram-positive bacteria. *Candida albicans* was found to be least resistant to *P. scabrum* (MIC = 380 µg/ml) which was the second most active extract against *B. cereus* (MIC = 59 µg/ml). Other extracts with substantial activity against *C. albicans* include *P. citronellum* (SBG), *P. pseudoglutinosum* and *P. sublignosum*; while the extracts of *P. betulinum* and *P. capitatum* were most active against *K. pneumoniae* (MIC = 1000 µg/ml). These favourable antibacterial and antifungal activities have been reported for species belonging to Geraniaceae (Lis-Balchin, 1990). The *Pelargonium* species tested by Lis-Balchin and Deans (1996) showed pronounced antibacterial activity, but weak antifungal activity. However, *P. capitatum* and *P. cucullatum* in particular showed no activity against *K. pneumoniae* and *S. aureus*, respectively. In the study conducted by Lis-Balchin and Deans (1996) the hydrophilic (methanolic) fractions were tested, whereas in this study the acetone extracts were tested.

When comparing the antimicrobial activities of the duplicate samples it was noted that *P. cucullatum* (SBG) is more active than *P. cucullatum* (WSBG). Similarly, *P. glutinosum* (SBG) exerted greater antimicrobial activity than *P. glutinosum* (WSBG). Distinct differences in activity against *S. aureus* were observed for the duplicate samples of *P. glutinosum* and *P. quercifolium*. In addition, the duplicate samples of *P. quercifolium* displayed prominent variation in activity against *K. pneumoniae*.

The extracts of *P. glutinosum* (SBG), *P. pseudoglutinosum, P. scabrum* and *P. sublignosum* displayed the greatest activity against *S. aureus* which is one of the most persistent infectious micro-organisms and has been associated with primary skin infections and is commonly found in nosocomial infections (Steenkamp et al., 2004). An infection can hamper the process of healing (Priya et al., 2002) thus topical antimicrobial therapy should form an important part of wound management. The extracts of the abovementioned species may be potential sources of topical antimicrobial agents for the treatment of wound infections and may be sources of effective agents for nosocomial infections. The traditional use of Pelargoniums to treat various ailments, such as wounds, abscesses, colds, sore throats, diarrhoea, dysentery and gonorrhoea may be related to their antimicrobial properties as illustrated for *K. pneumoniae* and *S. aureus*.

3.3. Toxicity

The acetone extracts of *P. sublignosum* and *P. citronellum* (NBG) displayed the highest toxicities ($IC_{50} = 11.89 \pm 1.54$ µg/ml and $IC_{50} = 19.14 \pm 0.98$ µg/ml, respectively). Species exhibiting very low toxic profiles included *P. capitatum* and *P. cucullatum* (WSBG). *Pelargonium tomentosum* and *P. vitifolium* were not only non-toxic, but showed promising antimicrobial activities (Table 1). *Pelargonium tomentosum* also displayed considerable anti-oxidant activity. The extracts of *P. glutinosum* (SBG), *P. pseudoglutinosum, P. scabrum* and *P. sublignosum* exhibited considerable antimicrobial activity against the test Gram-positive bacteria and also produced relatively high levels of cytotoxicity.

The results from the MTT assay suggest that the antimicrobial action of the extracts may be ascribed to general cytotoxic effects. Extracts from plant material of the same species collected from different localities that showed reasonably similar toxicity profiles include the *P. glutinosum* and *P. panduriforme* samples (Table 1). The other three duplicate samples showed considerable differences in their toxicity profiles. It is well-known that the toxicity can vary considerably for members of the same species of a plant growing in different areas; since the geographical variation influences the composition and ratio of constituents (Steyn, 1934). Variations in growing conditions such as solar radiation and water content can contribute to the synthesis of different related compounds in aromatic plants (Nuńez and De Castro, 1992).

The *in vitro* pharmacological properties displayed by the extracts in this study substantiate their use in traditional medicines and furthermore, promote their potential commercial utilisation within the realm of the food, cosmetic and pharmaceutical industries; however, their toxicity profiles must be

considered. In particular, *P. betulinum*, *P. cordifolium*, *P. crispum*, *P. hispidum*, *P. quercifolium* (SBG) and *P. tomentosum* have a potential niche in therapeutics due to their promising *in vitro* biological activities and their low toxicity profiles. Further studies involving biological systems are necessary to provide a rational basis for validation of their potential therapeutic applications.

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