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Review

Medicinal properties and conservation of *Pelargonium sidoides* DC.

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ABSTRACT

Ethnopharmacological relevance: Pelargonium sidoides DC. (Geraniaceae), a popular medicinal plant used in traditional medicine in the treatment of gastrointestinal ailments has been transformed into a phytopharmaceutical (EPs® 7360) for treating respiratory tract infections. The increasing international demand for *Pelargonium sidoides* has led to localised overexploitation of its wild populations in southern Africa. The aim of the review is to provide a synthesis of the current state of scientific knowledge on the phytochemical, pharmacological and toxicological properties of *Pelargonium sidoides* as well as the potential role of plant biotechnology in its conservation. The review highlights knowledge gaps in these research areas.

Materials and Methods: A comprehensive literature search involving mainly electronic and library sources of information were used to collate and synthesise published data.

Results: Experimental results from *in vitro* studies indicate that bioactive phytochemical constituents of *Pelargonium sidoides* may not possess a direct antimicrobial effect, but instead act by interfering with microbial binding to host cell receptors, inhibition of key enzymes and the production of antimicrobial effector molecules such as nitric oxide and interferons (IFNs) by the host cells. Furthermore, clinical evaluations in randomised, double-blind, placebo-controlled trials have demonstrated the beneficial effect of *Pelargonium sidoides* in the treatment of respiratory tract infections with few side effects. However, there is lack of adequate information on the safety evaluation of the plant. On the other hand, the increasing demand for *Pelargonium sidoides* has led to localised illegal harvesting of wild plants.

Conclusions: Pharmacological data reported in literature suggest that *Pelargonium sidoides* shows a beneficial effect in the treatment of respiratory tract infections. However, more studies are required to elucidate the mode of action of the active constituents exhibited in the treatment of respiratory tract infections and other health conditions caused by microbial attack. Furthermore, the pharmacological usefulness of *Pelargonium sidoides* must take cognisance of the broader context involving the need for conservation-friendly approaches in its utilisation. In this regard, plant biotechnology applications can play a meaningful role in a holistic conservation strategy.

Contents

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1. Introduction

Pelargonium sidoides DC. (Synonym: *Pelargonium sidaefolium* (Thunb.) R. Knuth; Common names: Umckaloabo, Uvendle, Kalwerbossie, Khoaara e nyenyane; Family: Geraniaceae) is a perennial geophyte predominantly found in the Eastern Cape Province of South Africa and the Lesotho highlands. The plant is adapted to a wide altitudinal range, spanning from near sea level in the Eastern Cape to 2746 m in the Lesotho highlands (Newton et al., 2013). The plant is widely used by local communities as a traditional medicine for curing various ailments, including diarrhoea, colic, gastritis, tuberculosis, cough, hepatic disorders, menstrual complaints and gonorrhoea (Brendler and van Wyk, 2008; Colling et al., 2010). The roots are also the main ingredient in a remedy used to treat a stomach ailment known as *instila* in infants (Hutchings et al., 1996). Powdered plant materials which are soaked in water are used as a facial cream in the treatment of skin pimples (Lewu et al., 2007). This indicates that *Pelargonium sidoides* may exhibit antibacterial properties. Probably the most compelling ethnobotanical use of *Pelargonium sidoides* has been in the treatment of tuberculosis, which subsequently led to its introduction in Europe in the late 1890s (Bladt and Wagner, 2007; Brendler and van Wyk, 2008). The traditional ethno-veterinary applications of *Pelargonium sidoides* include the use of root decoctions as an anthelmintic remedy in calves (Hutchings et al., 1996); boiled leaves to protect wounds against maggots; and the prevention of purging in horses (Brendler and van Wyk, 2008). An extract obtained by soaking the roots in water is administered orally in the treatment of dysentery in cattle (Lewu et al., 2007). Based on its medicinal properties in the treatment of respiratory-related ailments, *Pelargonium sidoides* has been formulated into phytopharmaceuticals, namely EPs® 7630 (Umckaloabo®, Dr. Willmar Schwabe GmbH & Co. KG Pharmaceuticals, Germany) and Linctagon® (Nativa, South Africa). The commercial success of Umckaloabo® is attributed to numerous factors, including > 15 years of extensive scientific and clinical research (Gericke, 2011). On the other hand, only negligible research (Bourdette, 2012; Motsamai, 2012) has been done to evaluate the efficacy and safety of Linctagon® (which mainly contains 350 mg *Pelargonium sidoides*, 500 mg Vitamin C, 50 mg bromelain, 80 mg quercetin, 5 mg zinc). This review provides a synthesis of the state of scientific knowledge on the pharmacology and safety evaluation of *Pelargonium sidoides* as well as exploring the potential of plant biotechnology applications in its conservation.

2. Phytochemistry

The extensive use of *Pelargonium sidoides* in traditional medicine coupled with its popularisation in modern medical systems in Europe have led to an upsurge in scientific exploration of its chemical composition in an effort to identify the active principles. This has resulted in a considerable body of literature exploring the phytochemical properties of *Pelargonium sidoides* (Gödecke et al., 2003, 2005; Hauer et al., 2010; Kayser and Kolodziej, 1995; Latté et al., 2000; Schoetz et al., 2008). Details of the phytochemistry of the plant were comprehensively summarised in an excellent review by Kolodziej (2007). However, identification of individual chemical constituents responsible for specific pharmacological activities has remained largely elusive. The chemical constituents of the root ethanolic extract of the plant consist largely of oligo- and polymeric proanthocyanidins, which are based on gallocatechin and epigallocatechin moieties (Theisen and Muller, 2012). The pharmacological efficacy of *Pelargonium sidoides* has been partly attributed to the biological activity of highly oxygenated coumarins (7-hydroxy-5, 6-di-methoxycoumarin; 6,8-dihydroxy-5,7-dimethoxycoumarin), gallic acid-derivatives, flavonoids, phenolic and hydroxycinnamic acid-derivatives (Kayser and Kolodziej, 1995; Kolodziej, 2007; Colling et al., 2010). Recently, 6-Methoxy-7-(sulfooxy)-2H-1-benzopyran-2-one and 6,8-Bis(sulfooxy)-7-methoxy-2H-1-benzopyran-2-one were identified in *Pelargonium sidoides* for the first time (Hauer et al., 2010). Most significantly, Hauer et al. (2010) characterised two novel compounds, 7-Hydroxy-6-methoxy-8-(sulfooxy)-2H-1-benzopyran-2-one and 8-Hydroxy-7-methoxy-6-(sulfooxy)-2H-1-benzopyran-2-one (Fig. 2). These novel compounds have to be screened for pharmacological activity as they may represent the individual active constituents that have so far remained elusive.

3. Pharmacological properties

It is interesting to note that the repertoire of health conditions for which *Pelargonium sidoides* is used has expanded beyond the original traditional uses against gastrointestinal disorders to include respiratory tract infections such as acute bronchitis, asthma, sinusitis and tonsilllopharyngitis. Accordingly, most of the experimental and clinical research has focused on the treatment of respiratory tract infections in line with the development of the phytopharmaceutical, EPs® 7630 (Umckaloabo®). In this regard, a wide array of pharmacological studies involving *in vitro* (Table 1), *in vivo* (Table 2) and randomised, double-blind, placebo-controlled clinical trials (Table 3) has been conducted. Concomitantly, a diverse range of test systems has been used to evaluate the pharmacological properties of *Pelargonium sidoides*. The approaches, which are characterised by different levels of complexity, including the antimicrobial microdilution assay, anti-adhesion assay using HEp-2 cells, penicillin/gentamicin-protection assay, neuraminidase inhibition assay, fibroblast-virus protection assay and reverse transcription-polymerase chain reaction (RT-PCR) assay have helped in deciphering the pharmacological efficacies as well as the possible modes of action involved in the healing processes.

3.1. In vitro studies

Pelargonium sidoides extracts have been tested and exhibited good activity against a number of viruses, including influenza A viruses (H1N1, H3N2), coxsackie A9 virus, human coronavirus, respiratory syncytial virus (RSV), parainfluenza virus 3 and herpes simplex viruses (HSV-1, HSV-2) (Table 1). However, the EPs® 7630 phytopharmaceutical had poor activity ($IC_{50} = > 100\ \mu g/ml$) against the highly pathogenic avian influenza A virus (H5N1) (Michaelis et al., 2011). Notably, the EPs® 7630 extract had high *in vitro* activity against H1N1 ($IC_{50} = 5.4\ \mu g/ml$) (Theisen and Muller, 2012) and H3N2 ($IC_{50} = 8.66\ \mu g/ml$) (Michaelis et al., 2011) attesting to its use in respiratory health conditions. The authors noted that the EPs® 7630 extract was more active against enveloped viruses compared to non-enveloped viruses (adenovirus 3, adenovirus 7 and human rhinovirus). Interestingly, antiviral bioactivity (EC_{50}) of isolated phenolic constituents against H1N1 increased in the order of complexity of their chemical chain structure as follows: epigallocatechin ($42.5\ \mu g/ml$) > gallocatechin ($28.4\ \mu g/ml$) > gallocatechin-(4β→8)-gallocatechin ($7.3\ \mu g/ml$) > epigallocatechin-(4β→8)-gallocatechin ($6.3\ \mu g/ml$) > oligo-/polymeric fraction ($2.8\ \mu g/ml$) (Theisen and Muller, 2012). The study reported a poor direct virucidal activity of EPs® 7630 ($250\ \mu g/ml$). Instead the authors discovered that the extract and its phenolic constituents imparted anti-viral activity by interfering with virus binding to host cell receptors and through inhibition of the neuraminidase enzyme. Furthermore, the control of viral infections may occur through the production of interferons (IFNs) by the host cells, suggesting that therapy by *Pelargonium sidoides* may be through the stimulation of the innate immune system (Kolodziej, 2007). Based on the current state of research using

Table 1
Evaluation of the pharmacological activities of *Pelargonium sidoides* DC. using *in vitro* methods.

Biological activity (Test assay)	Extract/ formulation	Test organism	Observed activity and effective concentration	Possible mode of action	Control	Reference
Antiviral activity (Neuraminidase inhibition assay)	[a]EPs® 7630	H1N1 A/Puerto Rico/8/34	EPs® 7630 = 5.4 μg/ml (EC_{50}); EPs® 7630 (121.7 μg/ml) reduced neuraminidase activity by 50% Oligo-/polymeric fraction = 2.8 μg/ml (EC_{50}) Gallocatechin-(4β→8)_gallocatechin = 7.3 μg/ml (EC_{50}) Epigallocatechin-(4β→8)_gallocatechin = 6.3 μg/ml (EC_{50}) Epigallocatechin = 42.5 μg/ml (EC_{50}) Gallocatechin = 28.4 μg/ml (EC_{50})	Interference with virus binding to host cell receptors	–	Theisen and Muller (2012)
		H3N2 A/Luxembourg/ 01/2005	EPs® 7630 = 50 μg/ml (EC_{50})			
Anti-viral activity (Cytopathogenic effect reduction assay)	EPs® 7630	H1N1 A/New Caledonia/20/99	IC_{50} = 9.45 μg/ml	Inhibition of viral replication	–	Michaelis et al. (2011)
		H3N2 A/California/7/2004	IC_{50} = 8.66 μg/ml	Inhibition of viral replication		
		RSV ATCC-No. VR-1540	IC_{50} = 19.65 μg/ml	Inhibition of viral replication		
		Human coronavirus HCo-229E	IC_{50} = 44.50 μg/ml	Inhibition of viral replication		
		Parainfluenza virus 3 ATCC-No. VR-93	IC_{50} = 74.35 μg/ml	Inhibition of viral replication		
		Coxsackie A9 virus (isolated from a patient)	IC_{50} = 14.80 μg/ml	Inhibition of viral replication		
		H5N1 A/Thailand/1(Kan-)/04	IC_{50} > 100 μg/ml	–		
		Adenovirus 3 GB ATCC-No. VR-3	IC_{50} > 100 μg/ml	–		
		Adenovirus 7 strain Gomen ATCC-No. VR-7	IC_{50} > 100 μg/ml	–		
		Human rhinovirus	IC_{50} > 100 μg/ml	–		
Antiviral activity (Fibroblast/EMCV protection assay)	EPs® 7630	Encephalomyocarditis virus (EMCV)	EPs® 7630 (10 μg/ml) = 80 U/ml	Inhibition of Cytopathic effect	LPS (1.0 ng/ml) = 80 U/ml	Thäle et al. (2011)
Anti-viral activity (Plaque reduction assay)	Aqueous root extract	HSV-1 strain KOS	IC_{50} = 0.00006%	Inhibition of virus replication	Acyclovir	Schnitzler et al. (2008)
		HSV-2 strain HG52	IC_{50} = 0.000005%	Inhibition of virus replication	Acyclovir	
Anti-viral activity (Fibroblast/EMCV protection assay)	Crude root extract	Encephalomyocarditis virus (EMCV)	EPs® 7630 (1.6 μg/ml) = 100% inhibition	Inhibition of cytopathic effect; Modulation of IFN system	IFN-γ (100 U/ml)	Kolodziej et al. (2003)
Antibacterial activity (Microdilution assay)	80% methanol tuber extract	*Staphylococcus aureus* ATCC 12600	MIC = 0.683 mg/ml	Inhibition of bacterial growth	Neomycin (MIC = 1.302 mg/ml)	Moyo et al. (2013)
	80% methanol leaf extract	*Enterococcus faecalis* ATCC 19433	MIC = 0.097 mg/ml	Inhibition of bacterial growth	Neomycin (MIC = 625 mg/ml)	
Antibacterial activity (Microdilution assay)	Hairy root clones	*Bacillus subtilis* ATCC 6051	Hairy root clone A4T-C (MIC = 390 μg/ml)	Inhibition of bacterial growth	Streptomycin (MIC = 1.56 μg/ml)	Colling et al. (2010)
		Staphylococus aureus ATCC 12600	A4T-C (MIC = 780 μg/ml)			
		Escherichia coli ATCC 11775	A4T-C (MIC = 780 μg/ml)			
Antibacterial activity (microdilution assay)	Root extract	*Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Streptococcus viridians*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Neisseriae* spp.; *Haemophilus influenza* (Clinical strains)	MIC = 200–1600 μg/ml	Inhibition of bacterial growth	–	Uslu et al. (2009)
Antibacterial activity (Microdilution broth method)	Aqueous acetone root extract	*Staphylococcus aureus* ATCC 25923 incl. multi-resistant strains (1150.93; 1583.93; 999.93; 134.93; 1000.93)	MIC = 3.3 mg/ml	Inhibition of bacterial growth	Bacterial starins resistant to antibiotics *e.g.* ciprofloxacin, erythromycin	Kolodziej et al. (2003)
		Proteus mirabilis ATCC 14153	MIC = 3.3 mg/ml			
Antibacterial activity (Anti-adhesion	EPs® 7630 (aqueous	*Streptococcus pyogenes* DSM 2071 (serogroup A)	45% inhibition (@ 30 μg/ml)	Interaction with binding sites	Untreated group	Janecki et al. (2011)

Table 1 (*continued*)

Biological activity (Test assay)	Extract/ formulation	Test organism	Observed activity and effective concentration	Possible mode of action	Control	Reference
assay using human HEp-2 cells)	ethanolic extract)					
Antibacterial activity (Anti-adhesive assay; substrate – gastric epithelial (AGS) cells)	EPs® 7630	*Helicobacter pylori* (Clinical strains)	EPs® 7630 (50; 100 μg/ml) reduced bacterial attachment to AGS cells by 77% and 91%, respectively	Prevention of bacterial adhesion to AGS-cell membranes	Amoxicillin = no activity	Beil and Kilian (2007)
Antibacterial activity (Anti-adhesive assay; substrate human stomach epithelial tissue)	EPs® 7630	*Helicobacter pylori* type I, strain G27	Dose-dependent reduction in *Helicobacter pylori* adhesion to human gastric AGS-cell membranes (100% inhibition @ 10 mg/ml)	Inactivation of host (mucosal glycoproteins and epithelial mucins) bacterial adhesins interaction	3′-/6′-Sialyllactose = 100% inhibition; *Abelmoschus Esculentus* > 90% inhibition	Wittschier et al. (2007)
Antibacterial activity (Flow cytometric adhesion assay with human HEp-2 cells and as substrate)	EPs® 7630	*Streptococcus pyogenes* (Group A streptococci-GAS) DSM 2071 (serogroup A)	GAS adhesion to HEp-2 cells reduced by 46% @ 30 μg/ml	EPs® 7630 reduces bacterial adhesion to HEp-2 cells by targeting adhesion factors of GAS	Control (Samples without EPs® 7630): adhesion = 80% HEp-2 cells	Conrad et al. (2007b)
Antibacterial activity (Flow cytometric adhesion assay with BEC as substrate)	EPs® 7630	*Streptococcus pyogenes* (GAS) DSM 2071 (serogroup A)	EPs® 7630 (30 μg/ml): bacterial attachment was7-folder > control	Enhances attachment of bacteria to decaying BEC	Control: bacterial attachment to BEC = 12.8%	Conrad et al. (2007b)
Antimycobacterial activity (Microdilution assay)	Butanol root extract	*Mycobacterium smegmatis* MC^2 155	MIC 0.156 μg/ml	Inhibition of bacterial growth	Ciprofloxacin (MIC = 0.125 μg/ml)	Mativandlela et al.(2007)
	Scopoletin	*Mycobacterium smegmatis* MC^2 155	MIC = 7.81 μg/ml	Inhibition of bacterial growth		
	Umckalin	*Mycobacterium smegmatis* MC^2 155	MIC = 62.5 μg/ml	Inhibition of bacterial growth		
	Catechin	*Mycobacterium smegmatis* MC^2 155	MIC = 31.25 μg/ml	Inhibition of bacterial growth		
	Epigallocatechin	*Mycobacterium smegmatis* MC^2 155	MIC = 7.81 μg/ml	Inhibition of bacterial growth		
Antimycobacterial activity (BACTEC 460-radiometric assay)	Scopoletin; Umckalin; Catechin; Epigallocatechin	*Mycobacterium tuberculosis* ATCC 27294	No activity @ 200 μg/ml	N/A	Isoniazid (@ 0.02 μg/ml	Mativandlela et al. (2007)
Antimycobacterial activity (Microdilution susceptibility assay)	EPs® 7630 fatty acids	*Mycobacterium aurum* A+	Oleic acid (MIC-4 μg/ml); linoleic acid (MIC-2 μg/ml)	Inhibition of mycobacterial growth	Isoniazid (MIC = 0.06 μg/ml)	Seidel and Taylor (2004)
		Mycobacterium smegmatis ATCC 14468	Linoleic acid (MIC-4 mg/ml)	Inhibition of mycobacterial growth	Isoniazid (MIC = 1.0 mg/ml)	
Antimycobacterial activity (Microdilution assay)	EPs® 7630 fatty acids (*n*-hexane extract)	*Mycobacterium Aurum* (Clinical strain)	Oleic acid (MIC-2 μg/ml); linoleic acid (MIC-2 μg/ml)	Inhibition of mycobacterial growth	–	Taylor (2003)
Antimycobacterial activity (Microplate Alamar blue assay)	Crude root extract	*Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294	Extract = 100 μg/ml (MIC)	Inhibition of mycobacterial growth	Rifampicin (MIC = 0.06 μg/ml)	Kolodziej et al. (2003)
Antimycobacterial activity (BACTEC 460-radiometric assay)	Crude root extract	*Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294	96% Inhibition (@12.5 μg/ml)	Inhibition of mycobacterial growth		Kolodziej et al. (2003)
Antifungal activity	80% methanol leaf extract	*Candida albicans* ATCC 10231	0.781 mg/ml (MIC)	Inhibition of fungal growth	Amphotericin B (MIC = 0.488 mg/ml)	Moyo et al. (2013)
Antifungal activity (Microbiological killing assay)	EPs® 7630 (aqueous ethanolic extract)	*Candida albicans* DSM 1386 ATCC 10231	EPs® 7630 (30 μg/ml) = 31% reduction in viable *C. albicans* cells	Intracellular killing is caused by a positive effect EPs® 7630 on the PBP killing activity	–	Conrad et al. (2007a)
Antifungal activity (Flow cytometry-based, whole blood method)	EPs® 7630 (aqueous ethanolic extract)	*Candida albicans* DSM 1386 ATCC 10231	EPs® 7630 (30 μg/ml) = increased burst-active PBP to 120% after 4 min	Quick release of PBP to fight pathogens	–	Conrad et al. (2007a)
Antiparasitic activity (Intracellular	EPs® 7630	*Leishmania major* strain LT 52, clone CC-1pXG-GFP		Stimulation of NO release	Amphotericin B = 1.0 μM	Thäle et al. (2011)

Table 1 (*continued*)

Biological activity (Test assay)	Extract/ formulation	Test organism	Observed activity and effective concentration	Possible mode of action	Control	Reference
leishmanicidal activity)			EPs® 7630 (0.1–10 μg/ml) Dose-dependent antileishmanial activity			
Antiparasitic activity (Intracellular leishmanicidal activity)	Methanol extract	*Leishmania donovani*	EC_{50}=2.7 μg/ml	Activation of leishmanicidal macrophage functions	Pentostam (EC_{50}=7.9 μg/ml)	Kayser et al. (2001)
	Petroleum ether extract	*Leishmania donovani*	EC_{50}= < 0.1 μg/ml			
	Ethyl acetate	*Leishmania donovani*	EC_{50}= < 0.1 μg/ml			
	n-Butanol	*Leishmania donovani*	EC_{50}= < 3.3 μg/ml			
	Gallic acid	*Leishmania donovani*	EC_{50}= < 4.4 μg/ml			
	Gallic acid methyl ester	*Leishmania donovani*	EC_{50}= < 12.5 μg/ml			
	Coumarims	*Leishmania donovani*	EC_{50}= > 25 μg/ml			
Immunomodulatory activity (Griess assay)	EPs® 7630 (1–30 μg/ml)	*Listeria monocytogenes* strain EGD serotype 1/2b	EPs®7630 (1–30 μg/mL) increased levels of NO; production of IL-1, IL-12, TNF-α	Bacterial inhibition through the antimicrobial effector molecule – NO	LPS (10 ng/ml)/IFN-γ (100 U/ml)	Thäle et al. (2008)
Immunomodulatory activity (Fluorescence-activated cell sorter analysis - FACS)	EPs® 7630 (30 μg/ml)	*Listeria monocytogenes* strain EGD serotype 1/2b	Concentration dependent increase in IL-1, IL-12 and TNF-α	Cytokine-induced macrophage activation	LPS (10 ng/ml)/IFN-γ (100 U/ml)	Thäle et al. (2008)
Immunomodulatory activity (Enzyme-linked immunosorbent assay - ELISA)	EPs® 7630 (1–30 μg/ml)	*Listeria monocytogenes* strain EGD serotype 1/2b	TNF-α (*Listeria*-infected BMMΦ)=(11.4–16.0 ng/mL)	Cytokine-induced macrophage activation	LPS (10 ng/ml)/IFN-γ (100 U/ml)= (9.8 ± 1.1 ng/mL)	Thäle et al. (2008)
Immunomodulatory activity (Fibroblast-lysis assay-TNF activity)	EPs® 7630	*Leishmania donovani*	Ethyl acetate fraction (25 μg/ml)=20.2 U/ml	Cytokine-induced macrophage activation	LPS (10 ng/ml)= 184 U/ml	Kayser et al. (2001)
			n-Butanol fraction (25 μg/ml)= 18.9 U/ml	Cytokine-induced macrophage activation		
			Gallic acid (25 μg/ml)= 39 U/ml	Cytokine-induced macrophage activation		
			Gallic acid methyl ester (25 μg/ml)=25.1 U/ml	Cytokine-induced macrophage activation		
Immunomodulatory activity (Fibroblast-virus protection assay-IFN activity)	EPs® 7630	EMCV	Gallic acid: 12.5 μg/ml= 0.4 U/ml; 25 μg/ml=3.7 U/ml; 50 μg/ml=17.9 U/ml	Inhibition of cytopathic effect; Modulation of IFN system	LPS (10 ng/ml)	Kayser et al. (2001)
Immunomodulatory activity (Greiss assay)	EPs® 7630	*Leishmania donovani*	Gallic acid=54 μM (nitric oxide)	Induction of anti-infective effector molecule (=NO)	LPS 10 ng/ml)= 119 μM	Kayser et al. (2001)
			7-Hydroxy-5,6-dimethoxycoumarin=40.8 μM (nitric oxide)	Induction of anti-infective effector molecule (=NO)		
			6,8-Dihydroxy-5,7-dimethoxycoumarin=46 μM (nitric oxide)	Induction of anti-infective effector molecule (=NO)		
Immunomodulatory activity (Reverse transcription-polymerase chain reaction: RT-PCR)	EPs® 7630	*Leishmania major* LV9	EPs® 7630 (50 μg/ml) up-regulation of iNOS, IL-12 and IL-18 mRNA levels	Molecular activation of cytokine gene expression	IFN-γ (100 U/ml)/ LPS (10 ng/ml)	Trun et al. (2006)
Immunomodulatory activity (RT-PCR)	EPs® 7630	*Leishmania major* LV9	EPs® 7630 (50 μg/ml) up-regulation of iNOS and cytokine mRNA levels	Molecular activation of cytokine gene expression	IFN-γ (100 U/ml)/ LPS (10 ng/ml)	Kolodziej et al. (2005)

AGS=gastric epithelial cells; BEC=buccal epithelial cells; BMMΦ=Murine bone marrow-derived macrophages; ELISA=enzyme-linked immunosorbent assay; EMCV=Encephalomyocarditis virus; FACS=fluorescence-activated cell sorter analysis; GAS=Group A-streptococci; GFP=green fluorescent protein; Hep=Human epithelial cells; H1N1, H3N2=Influenza A virus strains; HSV=Herpes simplex virus type; IFN=interferon; IL=interleukin; LPS=Lipopolysaccharides; MIC=minimum inhibitory concentration; NO=inorganic nitric oxide; iNOS=inducible nitric oxide synthase; RSV=Respiratory syncytial virus; PBP=human peripheral blood phagocytes; RT-PCR=reverse transcription-polymerase chain reaction; TNF=tumour necrosis factor.

[a] EPs® 7630=liquid herbal drug preparation of the root of *Pelargonium sidoides* (drug/extract ratio of 1:8–10) using aqueous ethanol (11% (m/m)) as extraction solvent (Conrad et al., 2007a,b).

Table 2
In vivo studies in the pharmacological evaluation of *Pelargonium sidoides* DC.

Biological activity	Extract/ formulation	Experimental organism	Test organism	Administration	Dose range	Active concentration	Response	Control	Reference
Antiviral activity	EPs® 7630	Female BALB/c mice	A/Puerto Rico/ 8/34H1N1 virus	Inhalation	5 mg/ kg	5 mg/kg	Increased survival of virus-infected mice	Mice treated with vehicle	Theisen and Muller (2012)
Anticoagulant activity	EPs® 7630	Male Sprague–Dawley rats	N/A	Oral	10, 75, 500 mg/kg	500 mg/kg p.o.	No effect on blood coagulation	Warfarin (0.05 mg/kg p.o.)	Koch and Biber (2007)
Central nervous system activity	Epigallo- and gallocatechin based oligomers of EPs® 7630	Male NMRI mice	N/A	–	200 mg/kg	200 mg/kg	None to moderate effect on behavioural activity	Mice treated with vehicle	Schötz and Nöldner (2007)
Lipopolysaccharide (LPS) induced sickness behaviour	EPs® 7630	Male NMRI-mice	N/A	Oral	100, 200, 400 mg/kg	400 mg/kg	Complete counteraction of LPS-induced sickness-behaviour	Mice treated with vehicle	Nöldner and Schötz (2007)

EMCV=encephalomyocarditis virus; LPS=lipopolysaccarides; N/A=not applicable; NO=nitric oxide; Warfarin=3-(a-acetonylbenzyl)-4-hydroxy-coumarin.

in vitro models it is clear that the mode of action of *Pelargonium sidoides* in antiviral activity may involve several mechanisms. The mode of action of the antiviral constituents in *Pelargonium sidoides* has also not been explained using *in vivo* models, for example Theisen and Muller (2012) only showed that EPs® 7630 increased survival of H1N1 virus-infected mice, but did not explain the underlying mechanisms involved (Table 2). Further studies incorporating molecular approaches may help to understand the mechanism of action of the antiviral active constituents.

In traditional medicine, extracts of *Pelargonium sidoides* are used to treat bacterial-related conditions such as diarrhoea, dysentery and tuberculosis (Hutchings et al., 1996). In South Africa, many plant species are used to alleviate symptoms of tuberculosis caused by *Mycobacterium tuberculosis*, the fifth largest cause of mortality in the country (McGaw et al., 2008). *Pelargonium sidoides* plant extracts have been tested against *Mycobacterium* species as well as several Gram-positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Helicobacter pylori*) bacteria. Most notably, EPs® 7630 had moderate *in vitro* activity against multi-resistant *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 3.3 mg/ml (Kolodziej et al., 2003). The same bacterial strains were resistant to common antibiotics such as ciprofloxacin and erythromycin. This study provided a significant finding and highlighted the importance of *Pelargonium sidoides* as a potential alternative antibacterial remedy at a time when resistance to antibiotics is fast becoming a medical challenge. However, there is need of a paradigm shift towards experimentation that provides more biological insights on the molecular mechanisms involved in the observed *in vitro* activity. A *Pelargonium sidoides* butanol extract had higher activity (MIC=0.156 μg/ml) compared to that of its isolated chemical entities such as umckalin (MIC=62.5 μg/ml) against *Mycobacterium smegmatis* possibly due to synergistic effects (Mativandlela et al., 2007). The efficacy of a natural product may be due to two or more constituents acting synergistically, whereby their respective bioactivities often diminish or disappear upon separation into individual chemical entities (Li and Vederas, 2009). Synergism of bioactive compounds in phytopharmaceuticals is normally claimed to account for their therapeutic effectiveness (Wagner and Ulrich-Merzenich, 2009). In particular, polyphenolic compounds in plant extracts are known to increase the pharmacokinetic rate of anti-infective constituents in phytopharmaceuticals thereby enhancing their bioavailability (Wagner, 2011). In addition, these natural products can also work synergistically with antibiotics thereby improving their overall pharmacokinetic properties against resistant bacterial strains (Schmidt et al., 2007). However, the moderate MICs suggest that *Pelargonium sidoides* constituents have no direct effect on the bacteria. Thus, further studies have attempted to elucidate the modes of action of its active constituents. Beil and Kilian (2007) and Conrad et al. (2007b) working on *Helicobacter pylori* and *Streptococcus pyogenes*, demonstrated that EPs® 7630 prevents the bacteria from adhering to gastric AGS-cell membranes and HEp-2 cells, respectively. These studies showed that the possible mode of action of the bioactive constituents is through modulation of host-bacteria interactions and phagocytosis, which prevent bacterial attachment to the substrate (gastric and human epithelial cells) thereby rendering the pathogens ineffective. The bioactive constituents of *Pelargonium sidoides* have been shown to specifically target adhesion factors of the bacteria rather than those of the epithelial cell membranes (Conrad and Frank, 2008).

Immunomodulatory activities of *Pelargonium sidoides* and its related phytopharmaceutical medicine may also account for the observed moderate bioactivity against bacteria associated with respiratory diseases through activation of the host cells macrophage machinery. Kayser et al. (2001) demonstrated that murine macrophages infected with *Leishmania* parasites induced the production of the reactive nitrogen intermediary, inorganic nitric oxide (NO), a known antimicrobial effector molecule. The study showed that gallic acid and the highly oxygenated coumarins, 7-hydroxy-5,6-dimethoxycoumarin (umckalin) and 6,8-dihydroxy-5,7-dimethoxycoumarin were the most potent NO-inducers. Thäle et al. (2008) further proved that activated macrophages obtained from C57BL/6 WT mice were key components of the antimicrobial immune response against *Listeria monocytogenes* through the production of NO. Compared to the control (2.2 μM), infected cells treated with EPs® 7630 significantly increased release of NO in a dose-dependent response from 1.0 μg/ml (2.8 μM) to 30 μg/ml (5.5 μM). In both models, infected macrophages had a higher NO-inducing effect compared to non-infected macrophages indicating that the nonspecific immune response was triggered under disease attack (Kayser et al., 2001; Thäle et al., 2008). In *Leishmania major*-infected macrophage-like RAW 264.7 cells exposed to EPs® 7630, there was a marked up-regulation of inducible nitric oxide synthase (iNOS) gene expression evaluated by reverse transcription-polymerase chain reaction (RT-PCR) (Kolodziej et al., 2005; Trun et al., 2006). In diseased cells, the molecular expression of iNOS in activated macrophages stimulates the production of high levels of NO, which is an antimicrobial effector molecule (Nathan and Hibbs, 1991). In particular, gallic acid, one of the constituents of the EPs® 7630 (Kayser et al., 2001) has been shown to induce prolonged iNOS mRNA expression in

Table 3
Clinical studies on *Pelargonium sidoides* DC. and its related pharmaceutical products.

Disease	Study design	No. of patients	Age (years)	Group size	Dosage /day	Duration	Outcomes measures	Result	Observed adverse events	Safety	Reference
Acute bronchitis	Double-blind, placebo-controlled clinical trial with one adaptive interim analysis	220	1–18	EPs® 7630=111 vPlacebo=109	3 × 10 Drops (1–6 years) 3 × 20 drops (6–12 years) 3 × 30 drops (12–18 years)	7 Days	Change in BBS score	EPs® 7630 (4.4); Placebo (2.9) ($p < 0.0001$)	Gastrointestinal	All adverse events were non-serious	Kamin et al. (2012)
Acute bronchitis	Randomized, double-blind, placebo-controlled clinical dose-finding study with 4 parallel treatment groups	400	6–18	EPs® 7630 (30 mg/day)=100 EPs® 7630 (60 mg/day)=99 EPs® 7630 (90 mg/day)=99 Placebo=101	30, 60, 90 mg EPs® 7630	7 Days	Change in BBS score	EPs® 7630 (30 mg/day)=3.6 EPs® 7630 (60 mg/day)=4.4 EPs® 7630 (90 mg day)=5.5 Placebo=3.3 ($p < 0.0001$)	Gastrointestinal	All adverse events were non-serious	Kamin et al. (2010)
Acute bronchitis	Randomised, double-blind, placebo-controlled, multicentre trial	217	≥ 18	EPs® 7630=108 Placebo=109	3 × 30 Drops	7 Days	Change in BBS score	EPs® 7630 (7.6); Placebo (5.3) ($p < 0.0001$) EPs® 7630-strong antitussive and "anti-fatigue" effects	Increased erythrocyte sedimentation rate	No serious adverse events recorded	Matthys and Funk (2008)
Acute bronchitis	Randomised, double-blind, placebo-controlled, multicentre study	205	18–66	EPs® 7630=108 Placebo=109 Adults: 18–66 years	3 × 30 Drops	7 Days	Change in BBS score	Decrease in BBS by: EPs® 7630 (7.6) Placebo (5.3); $p < 0.0001$	Gastrointestinal	No serious adverse events occurred during the study	Matthys and Heger (2007a)
Acute bronchitis	Prospective, open, multicentre outcomes study			*N*=205			Mean total score of 5 typical bronchitis symptoms	Score decrease 6.1 (baseline)-2.8 (day 7)	Not specified	No serious adverse effects	Matthys and Heger (2007b)
Acute bronchitis	multi-centre, prospective, open observational study	2099	0–93	All patients (*n*=2099); Children (3–18 years, *n*=498); infants (< 2 years, N=78)	3 × 10 Drops (< 6 years) 3 × 20 drops (6–12 years) 3 × 30 drops (> 12 years)	14 Days	Change in BBS score	BSS decreased from a median of 7.1 at baseline to 1.0 at the 3rd follow-up	Gastrointestinal	All adverse events were non-serious	Matthys et al. (2007)
Acute bronchitis	Open and uncontrolled study	742	0–12	Children (upto 12 years old, N=742)	3 × 5 Drops (0–2 years) 3 × 10 drops (2–6 years) 3 × 20 drops (> 6 years)	14 Days	Change in BBS score	Decrease in BBS score from 6.0 (baseline) to 1.4 (end of study) ($p < 0.001$)	Exanthema; dyspnoea; diarrhoea	Adverse events-minor and transitory; disorders disappeared in 2 days	Haidvogl and Heger (2007)
Acute bronchitis	Randomised, Double-blind, placebo-controlled trial	124	≥ 18	EPs® 7630=64 Placebo=60	3 × 30 Drops	7 Days	Change in BBS score	EPs® 7630 (7.2); Placebo (4.9) ($p < 0.0001$)	Not specified	All adverse events were non-serious	Chuchalin et al. (2005)
Acute bronchitis	Double-blind, placebo-controlled trial	468	≥ 18	EPs® 7630=233 Placebo=235	3 × 30 Drops	7 Days	Change in BBS score	EPs® 7630 (5.9); Placebo (3.2) ($p < 0.0001$)	Gastrointenstinal; nervous system; respiratory and mediastinal; ear and labyrinth compliants	All adverse events were non-serious	Matthys et al. (2003)
Transient hypogammaglobuline-mia of infancy (THI)	Double-blind, placebo controlled, prospective, monocentric pilot study	28	1–5	EPs® 7630=14Placebo=14	3 × 30 Drops	7 Days	Symptom scoring	EPs® 7630 and placebo-no significant difference in symptom scores	Not specified	No adverse events	Patiroglu et al. (2012)
Immunomodulatory effect	Double-blind; Placebo-controlled trial	25	26–56	*Pelargonium sidoides* extract=11Placebo=14	3 × 30 Drops	28 Days	Level of increase in salivary sIgA concentra-tion	Herbal extract-213 μg/ml; Placebo-41.16 μg/ml	Not specified	No safety evaluation done	Luna et al. (2011)
Common cold	Randomised, double-blind, parallel group, placebo-controlled trial	103	18–55	EPs® 7630=52 Placebo=51	3 × 30 drops	10 Days	Change in SSID	SSID values: EPs® 7630 (14.6); Placebo (7.6) ($p < 0.0001$)	Tracheitis;epistaxis	All adverse events were non-serious	Lizogub et al. (2007)

Table 3 (*continued*)

Disease	Study design	No. of patients	Age (years)	Group size	Dosage /day	Duration	Outcomes measures	Result	Observed adverse events	Safety	Reference
Acute rhinosinusitis (of presumably bacterial origin)	Randomised; Double-blind, parallel group, placebo-controlled trial	103	18–60	EPs® 7630 = 51 Placebo = 52	3 × 60 Drops	22 Days	Change in SSS	SSS mean decrease: EPs® 7630 (5.5); Placebo (2.5) ($p < 0.00001$)	Gastrointestinal; allergic skin reaction	All adverse events were non-serious	Bachert et al. (2009)
Asthma attacks during viral infections	Asthmatic children in randomised study	61	1–14	EPs® 7630 = 30 Control = 31	3 × 10 drops (1–5 years) 3 × 20 drops (6–12 years) 3 × 30 drops (> 12 years)	5 Days	Symptom score	EPs® 7630 group had less frequency of asthma attacks ($p < 0.05$)	Not specified	No safety evaluation done	Tahan and Yaman (2013)
Chronic obstructive pulmonary disease (COPD)	Double-blind, parallel group, placebo-controlled clinical trial	200	≥ 18	EPs® 7630 = 99 Placebo = 101	3 × 30 Drops	24 Weeks	Time to first exacerbation of COPD	EPs® 7630 = 57 days; Placebo = 43 days ($p = 0.005$) EPs® 7630: 37.8% needed antibiotic treatment *vs* 73.3% for placebo ($p < 0.0001$)	Gastrointestinal	All adverse events were non-serious	Matthys et al. (2013)
Acute non-group A beta-hemolytic *streptococcus* tonsillopharyngitis	Randomized, double-blind, placebo-controlled trial	143	6–10	EPs® 7630 = 73 Placebo = 70; Children (6–10 years)	3 × 20 Drops	6 Days	Change in TSS score from baseline	TSS mean decrease: EPs® 7630 (7.1 points); Placebo (2.5 points); ($p < 0.0001$)	Not specified	Adverse events were independent of investigational medication	Bereznoy et al. (2003)

BBS = Bronchitis Symptom Score; COPD = Chronic obstructive pulmonary disease; sIgA = secretory immunoglobulin A; SSID = Symptom Intensity Differences; SSS = Sinusitis Severity Score; THI = Transient hypogammaglobulinemia of infancy; TSS = Tonsillopharyngitis Severity Score.

Leishmania-infected cells. In addition, release of tumour necrosis factor (TNF-α) and interferons (IFN) provided further evidence for cytokine-induced macrophage activation and plays a crucial role in immune defence mechanisms in infected cells (Reiner and Locksley, 1995). Experimental data has shown that the TNF-inducing potential of *Pelargonium sidoides* is strongly associated with its phenolic constituents (Kolodziej and Kiderlen, 2007). The methanol extract of *Pelargonium sidoides* (25 μg/ml) exhibited negligible TNF-inducing potential (< 0.1 U/ml) compared to the moderate activity of the ethyl acetate (20.2 U/ml) and *n*-butanol (18.9 U/ml) fractions (Kayser et al., 2001). Most significantly, gallic acid (6.25–50 μg/ml) led to a high dose-dependent increase in TNF-inducing potency (21–43.7 U/ml) of infected cells. Radtke et al. (2004) also demonstrated the concomitant TNF-α mRNA gene expression in infected cells. On the other hand, gallic acid was shown to increase IFN-α level in *Leishmania*-infected cells (Kolodziej et al., 2005) coupled with the up-regulation of IFN-γ mRNA transcripts (Radtke et al., 2004; Kolodziej et al., 2005). Most notably, umckalin, the signature chemical entity in *Pelargonium sidoides* exhibited poor cytokine gene expression profiles.

3.2. *In vivo studies*

Table 2 presents a summary of pharmacological evaluations using *in vivo* animal models. Despite the importance of animal models in the evaluation of pharmacological efficacy, only a few studies have utilised this system for *Pelargonium sidoides* and/or its related formulations. Good research practice for plant-derived medicines dictates that *in vitro* tests should be backed up with *in vivo* and ultimately clinical studies (Houghton et al., 2007). More studies on *Pelargonium sidoides* should adopt this approach so as to avoid the pitfalls associated with extrapolating *in vitro* test results to claim *in vivo* activity and efficacy. Cos et al. (2006) argued that animal models are indispensable in validating *in vitro* activity because they take into account pharmacokinetic, metabolic and toxicological phenomena. Based on this approach, Yu et al. (2010), Tian et al. (2011) and Li et al. (2013) used *in vitro* and *in vivo* antiviral models thereby obtaining comprehensive pharmacological data including efficacy and toxicology of several Chinese herbal medicines. Recently, Theisen and Muller (2012) confirmed *in vitro* antiviral activity of EPs® 7630 using an *in vivo* animal model system. Therefore, both *in vitro* and *in vivo* models should be considered in future *Pelargonium sidoides* anti-infective research. In addition, the research can also focus on developing better *in vivo* model systems to elucidate the actual mechanisms involved in the therapeutic processes of *Pelargonium sidoides*, which have so far remained unclear.

3.3. *Clinical studies*

The phytopharmaceutical, EPs® 7630 has undergone numerous clinical evaluations in randomised, double-blind, placebo-controlled trials. Incidentally, it has been clinically evaluated against respiratory-related conditions, namely acute bronchitis, acute rhinosinusitis, common cold, chronic obstructive pulmonary disease and asthma (Table 3). The clinical trials conducted for EPs® 7630 have included patients of all ages. The reported results suggest that the *Pelargonium sidoides* herbal medicine may be effective in the treatment of respiratory infections caused by bacteria and viruses. Among the common respiratory tract infections, most of the clinical studies have evaluated the phytopharmaceutical against acute bronchitis in placebo-controlled studies. Acute bronchitis is characterised by acute inflammation of the respiratory tract and is caused by viral infections in 95% of the cases (Matthys and Heger, 2007b). However, more clinical studies have to be done to determine its efficacy in the

treatment of other common respiratory diseases. Despite the limited benefit of antibiotic therapy for acute bronchitis, they are prescribed in 60–80% of the cases (Matthys and Heger, 2007a). Against this background, more appropriate treatment options are required so as to prevent the overuse and/or abuse of antibiotics. In this regard, phytopharmaceuticals such as *Pelargonium sidoides*-based products may provide an alternative therapeutic option in the treatment of respiratory tract infections and related ailments.

3.4. Safety evaluation

It is of paramount importance that medicinal plants and phytopharmaceuticals are safe and do not cause undesirable side effects in patients. However, despite its long history of use in traditional medicine, there is a dearth of scientific information pertaining to the safety evaluation of *Pelargonium sidoides*. The *Pelargonium sidoides* extract, EPs® 7630, did not cause obvious toxic effects in mice as there were no significant differences in body weight, body temperature as well as organ weight (lungs, liver, spleen, kidneys) between the treatment and control groups (Theisen and Muller, 2012). Based on clinical data, the tolerability of treatment with EPs® 7630 has been shown to be good in both adults and children (Table 3). Nevertheless, some of the commonly reported adverse events included gastrointestinal complaints such as diarrhoea (Matthys et al., 2003, 2007, 2013; Haidvogl and Heger, 2007; Matthys and Heger, 2007a; Bachert et al., 2009; Kamin et al., 2010, 2012), nervous system complaints (Matthys et al., 2003), respiratory and mediastinal ailments (Matthys et al., 2003), ear and labyrinth complaints (Matthys et al., 2003), exanthema (Haidvogl and Heger, 2007), tracheitis and epistaxis (Lizogub et al., 2007) and allergic skin reactions (Bachert et al., 2009). Recently, a clinical study by Teschke et al. (2012) concluded that *Pelargonium sidoides* did not reveal evidence of hepatotoxicity. However, even though the adverse events in most clinical studies have been reported as non-serious, more rigorous studies are still required to ascertain the safety of *Pelargonium sidoides* and its related formulations. In addition, further toxicological studies involving toxicity and mutagenic tests need to be done to evaluate the safety of this plant. Although the benefits of medicinal plants are widely acknowledged, the need for safety evaluation remains critical so as to distinguish between toxic effects and pharmacological efficacy of plant extracts (Aremu and van Staden, 2013). Based on the numerous studies reported in the current review, there is compelling experimental and clinical evidence to suggest that *Pelargonium sidoides* may be efficacious against respiratory tract infections and may be safe for both adults and children, but more stringent toxicological studies are required. Verschaeve and Van Staden (2008) have comprehensively discussed the various methods that are used in evaluating the safety of medicinal plants.

4. Commercial potential and world marketing

Besides its local use as a multipurpose traditional remedy, *Pelargonium sidoides* has evolved into an international phytopharmaceutical. The commercialisation of *Pelargonium sidoides* dates back to the early 1900s when the plant was introduced in Europe as a tuberculosis remedy by Charles Henry Stevens after being reportedly cured by a traditional healer in Lesotho (Bladt and Wagner, 2007; Brendler and van Wyk, 2008; Wynberg et al., 2012). The growing international demand for the *Pelargonium sidoides* root extract has led to an increase in the number of gatherers as well as the volume of harvested plant materials. Its evolution from a traditional medicine exclusive to southern Africa, mainly South Africa and Lesotho, into a successful phytopharmaceutical (Umckaloabo®) has created 'the *Pelargonium* industry' involving a network of harvesters, local buyers and processors and international pharmaceutical manufacturers, such as Dr. Willmar Schwabe GmbH & Co., KG Pharmaceuticals (Karlsruhe, Germany). *Pelargonium* trade has evolved from a largely unregulated to a more formalised industry over the past 20 years (Wynberg et al., 2012). An unknown number of harvesters in the Eastern Cape (South Africa) and Lesotho collect and sell the plant materials to local intermediary buyers (Van Niekerk and Wynberg, 2012; Newton et al., 2013). The organisation of trade in medicinal plants normally involves a chain of local dealers buying plant materials from local collectors and eventually selling to larger trading and export companies (Lubbe and Verpoorte, 2011). In the Eastern Cape, Gowar Enterprises collects and supplies the *Pelargonium sidoides* tuberous roots to another intermediary, BZH Import and Export, responsible for drying, shredding, packaging and onward routing to Parceval Pharmaceuticals, which exports the materials to the end-product manufacturer in Germany (Van Niekerk and Wynberg, 2012). In Lesotho, Bophelo Processing is responsible for collecting, processing and supplying of the tubers to Parceval Pharmaceuticals (Wynberg et al., 2012). The processing of the raw materials obtained from developing economies involved in the medicinal plant trade is still predominantly undertaken in European countries by end-product manufacturers (Lubbe and Verpoorte, 2011) and this also applies to *Pelargonium sidoides*.

The commercial success of Umckaloabo® provides a good example of what can be achieved with a well-directed research and development initiative and astute marketing (Gericke, 2011). Most of the harvesters mainly rely on wild collection of *Pelargonium sidoides* for their livelihoods due to limited alternative economic opportunities in these communities. Amidst the overall commercial success of the *Pelargonium* trade, questions have been asked about the possible vulnerability of the harvesters and their lack of bargaining power arising from the monopolistic marketing chain (Van Niekerk and Wynberg, 2012). Large trading companies have significant control over the pricing system due to their central role in the medicinal plant trade coupled to large scale purchases and ownership of critical infrastructure to perform several functions including quality control (Lubbe and Verpoorte, 2011). However, despite these challenges related to beneficiation equity from medicinal resources, lessons arising from the *Pelargonium sidoides* case study are clearly invaluable for future natural product research and development programmes in developing countries. An increasing number of medicinal plant suppliers in China, India, Mexico and Malaysia are now exporting processed products to end-product manufacturers in Western countries (Lubbe and Verpoorte, 2011). The '*Pelargonium* industry' may have to evolve in tandem to this growing trend in the medicinal plant trade.

5. Biotechnology applications in *Pelargonium sidoides* conservation

The increasing commercial demand for *Pelargonium sidoides* on the international market has led to localised uncontrolled, indiscriminate and sometimes illegal harvesting of wild plants (Lewu et al., 2006, 2007; Wynberg et al., 2012). The escalation in demand can cause irreparable reductions to wild populations (Colling et al., 2010), which may result in a biodiversity threat to *Pelargonium sidoides*. However, the conservation status of *Pelargonium sidoides* was recently revised from 'declining' (Red Data List of South African Plants, 2009) to 'least concern' (Red Data List of South African Plants, 2013). In Lesotho, the conservation status of *Pelargonium sidoides* has not been evaluated. In addition, the plant is not listed on either the International IUCN Red List of Threatened Species or Convention on International Trade in Endangered Species (CITES) database (Newton et al., 2013).

There is a conspicuous lack of comprehensive data on annual harvested and traded volumes of *Pelargonium sidoides*. Varying estimates derived from data collected through interviews have been reported in literature. The estimates of annual harvested fresh material range from 9 to 45 t (Newton et al., 2013) and 26–440 t (Van Niekerk and Wynberg, 2012). Despite this lack of accurate data, tight conservation regulations have been formulated to curb uncontrolled harvesting in South Africa (Newton et al., 2013). Notwithstanding the occasional financial returns obtained by the local communities, the overexploitation of the resource may affect its availability in the future. In the Eastern Cape, the plants had a slow regeneration rate, which was significantly lower than the initial harvest after the second year (Lewu et al., 2007). To meet the ever-expanding demand in international trade of raw materials, *Pelargonium sidoides* may have to be cultivated on a large scale. Currently, the plant is only cultivated on a small, negligible scale (Colling et al., 2010). There are also limitations in the use of seed propagation, due to their low viability coupled with low germination (Lewu et al., 2006).

Alternatively, biotechnology tools such as plant tissue culture (Fig. 1D) may play a critical role in the provision of genetically uniform clones for the *Pelargonium* industry (Moyo et al., 2013). Recently, Kotzé (2011), Moyo et al. (2012) and Moyo et al. (in press) reported on micropropagation and acclimatisation procedures with the capacity to produce millions of uniform *Pelargonium sidoides* clones throughout the year. There is a long history of the commercial production of high-value phytochemicals using tissue culture systems (Canter et al., 2005). However, a major challenge with the cultivation of medicinal plants is the perception that such plants do not produce a similar quality of bioactive compounds as wild plants. White et al. (2008) and Moyo et al. (2013) showed that greenhouse produced plants had similar bioactive compounds as wild plants in terms of the umckalin (6-hydroxy-5,7-dimethoxycoumarin) and phenolic compounds, respectively. In addition, extracts of greenhouse and wild plants exhibited comparable antimicrobial and antioxidant properties (Moyo et al., 2013). In the long term, the production of Umckaloabo® may have to be based on plant material produced under consistent cultivation practices. Such cultivated material produced under controlled environmental conditions is preferred for the phytopharmaceutical industry as it minimises qualitative and quantitative variations in the composition of natural products (Lubbe and Verpoorte, 2011). In addition, the quality of phytopharmaceuticals can be improved by the use of genetically uniform clones, which eliminates problems associated with product adulteration (Schmidt et al., 2007).

On the other hand, the future pharmaceutical production of Umckaloabo® may also eventually depend on the application of bioreactor technology in the synthesis of *Pelargonium sidoides* bioactive compounds. Colling et al. (2010) demonstrated the ability of transgenic *Pelargonium sidoides* hairy root cultures, transformed using *Agrobacterium rhizogenes*, to produce the desired bioactive pharmaceutical compounds. The authors concluded that the system has immense potential as a conservation strategy for this medicinally important plant. However, there is a possibility that phytotherapeutics produced using biotechnology may not be readily acceptable by consumers of 'natural' medicines. Notwithstanding these concerns, it may be inevitable that biotechnology will play a key role in the production of plant-derived pharmaceutical compounds in the future. Hairy root cultures which are transformed using *Agrobacterium rhizogenes* often sustain stable and high productivity in plant growth regulator-free medium (Canter et al., 2005). Thus, the use of biotechnology approaches can allow for ease of standardisation of the chemical constituents of the phytopharmaceutical thereby guaranteeing consistent quality of the end-product. Overall, this will help to avert the current plant biodiversity conservation dilemma arising from overharvesting practices of wild populations.

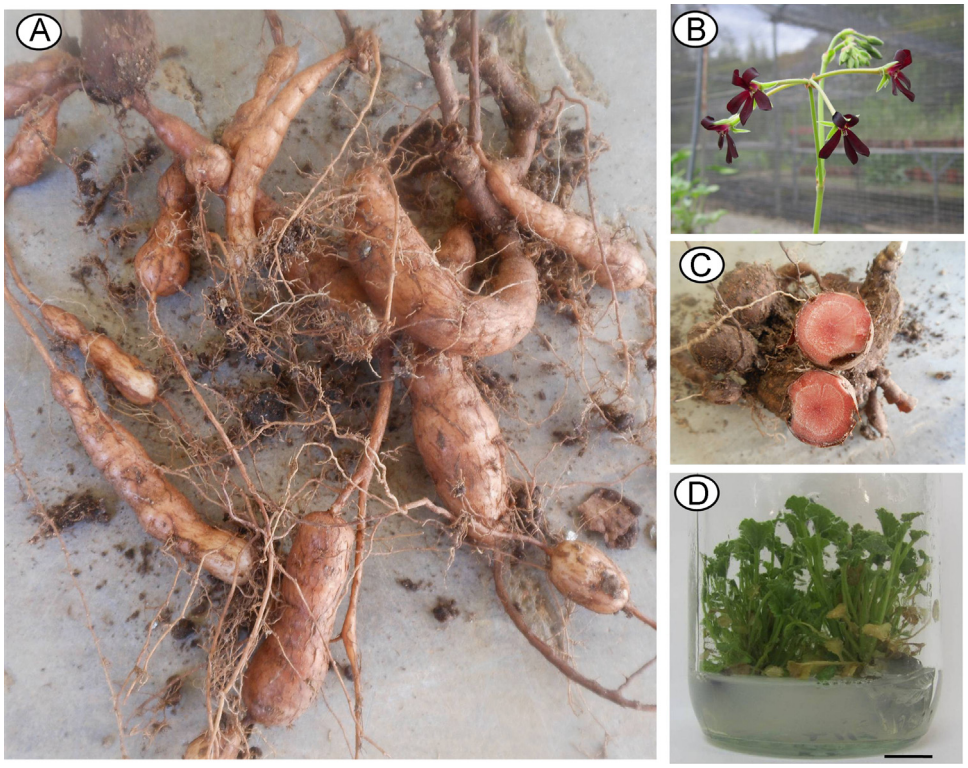

Fig. 1. *Pelargonium sidoides*. (a) Young tuberous roots; (b) a flowering plant; (c) cross section of a tuberous root; and (d) high shoot multiplication in a plant tissue culture system.

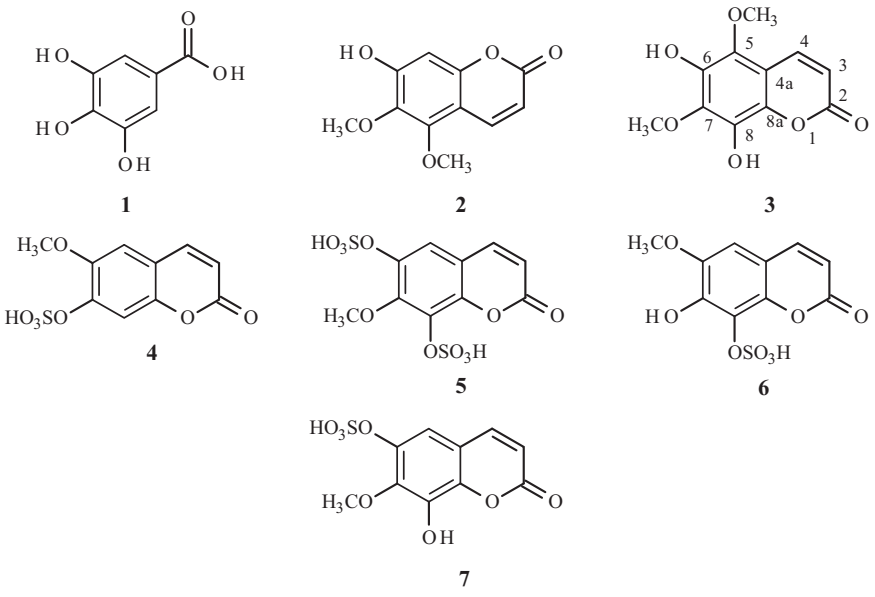

Fig. 2. Chemical structures of signature constituents found in *Pelargonium sidoides* DC.: Gallic acid (1); 7-hydroxy-5,6-dimethoxycoumarin (2); and 6,8-dihydroxy-5,7-dimethoxycoumarin (3); 6-Methoxy-7-(sulfooxy)-2H-1-benzopyran-2-one (4); 6,8-Bis(sulfooxy)-7-methoxy-2H-1-benzopyran-2-one (5); 7-Hydroxy-6-methoxy-8-(sulfooxy)-2H-1-benzopyran-2-one (6); 8-Hydroxy-7-methoxy-6-(sulfooxy)-2H-1-benzopyran-2-one (7).

6. Conclusions

Despite the emergence, in recent times, of high-throughput screening of synthetic chemical libraries as an alternative technology for the pharmaceutical industry, natural products remain a vital component in drug discovery. In particular, medicinal plants offer several advantages as potential sources of both novel chemical entities and phytopharmaceuticals. The pharmacological activities that have been demonstrated in various *in vitro*, *in vivo* and clinical studies indicate that *Pelargonium sidoides* possesses moderate direct anti-infective properties but highly notable immunomodulatory activity. In addition, *Pelargonium sidoides* root extract is effective and well tolerated in the treatment of respiratory-related infections. Based on both experimental and clinical pharmacological evidence, the transformation of *Pelargonium sidoides* from a traditional medicine to a successful phytopharmaceutical provides a compelling argument for the continued exploration of medicinal plants and indigenous medical systems for the next generation of phytopharmaceuticals. Even though significant milestones have been achieved in *Pelargonium sidoides* pharmacological research, further studies are still needed to fully elucidate the mechanisms of action and the biological principles underlying its therapeutic capacity. Most notably, the bulk of the pharmacological research on *Pelargonium sidoides* has been skewed towards respiratory tract infections at the expense of ailments for which the plant is used in Traditional African Medicine. Clinical research should also focus on other respiratory tract infections besides acute bronchitis, which has so far attracted the most attention. Furthermore, there is lack of conclusive data on the toxicological properties of *Pelargonium sidoides* despite having been used in traditional medicine for a long time. Stringent evaluation of the toxicological properties of *Pelargonium sidoides* is of paramount importance given its extensive use in medicinal formulations.

In the long term, the successful commercialisation of this southern African medicinal plant must be viewed in a much wider and holistic context that includes conservation-friendly practices and the sustainable supply of raw materials. In that context, the sustenance of the *Pelargonium sidoides* phytopharmaceutical industry will require innovative approaches, which utilise biotechnology tools such as plant tissue culture and bioreactors for the production of genetically uniform clones and therapeutic secondary metabolites, respectively.

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