

**DEVELOPMENT OF CONSERVATION AND PROPAGATION TECHNIQUES
FOR *PELARGONIUM SIDOIDES* DC: AN OVEREXPLOITED MEDICINAL
PLANT IN THE EASTERN CAPE**

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GENERAL ABSTRACT



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GENERAL ABSTRACT

The collection of medicinal plants from the wild for international market and local uses is a very common activity in the Eastern Cape Province of South Africa. *Pelargonium sidoides* is an indigenous medicinal plant of South Africa harvested for local uses and international trade. Due to its high economic importance, the rate and the number of its harvesters have increased in recent time. This has led to a drastic drop in the population of the species in the wild. This is a report on the conservation initiative for *Pelargonium sidoides*; a South African herb widely used for the treatment of many respiratory and bacterial diseases in humans especially in children and livestock. The aims of the study were to validate the medicinal efficacy of the species and document various conservation and propagation techniques for the plant.

In order to develop sustainable conservation program for the species, various studies were conducted on its general growth and propagation techniques. These include the collection of ethnobotanical information, validation of medicinal efficacy of the species, vegetative and micropropagation methods, germination study of its seeds and its morphological characterization.

The results revealed that the roots of the species are used mainly for the treatment of cold related diseases in both humans and livestock, and that the rate and methods of harvesting are not sustainable. A comparative study of the roots and shoots of the species revealed that the shoot could as well substitute for the roots in the treatment of bacterial infections. Vegetative propagation of *P. sidoides* was achieved using the vine and the petiole. Three vine tip cuttings (2, 4 and 6 cm) of the species were rooted in a potted experiment under controlled environment. 6 cm vine length exhibited the best result for different

parameters measured. However, petioles gave a significant yield difference over 6 cm vine length and generally showed high potential for vegetative propagation in terms of rooting ability and survival rate. Seed germination was affected by the age, temperature, pre-chilling condition and source of seeds. The result of the antimicrobial properties of the species revealed a broad spectrum activity against ten bacterial and five fungal species which further validates its uses for the treatment of various infectious diseases by the people of the Eastern Cape. In the micropropagation study of the plant, callus induction was achieved with the application of two separate hormones (IBA and 2,4-D) under different light conditions. The 3 mg.l⁻¹ IBA under continuous darkness gave the optimal response in the callus induction experiment. Shoot differentiation was achieved from stem derived callus after one week in hormone-free MS medium. The development of micropropagation technique for the species may open a way for rapid propagation of the herb.

Currently, there exist accessions of *P. sidoides* in the wild populations within the Eastern Cape. Using morphological fingerprinting, experimental findings revealed 29 morphotypes out of the 30 accessions studied, suggesting high genetic variability in the natural habitat. Further exploration of its natural habitat may help capture genetically desirable traits of *P. sidoides* for the purpose of its genetic improvement and conservation.

CHAPTER 1

Introduction



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CHAPTER 1

INTRODUCTION

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GENERAL INTRODUCTION

In Africa, the majority of the population still relies on indigenous herbal remedies for health care delivery. Today, more than 80% of South Africans make use of indigenous medicinal plants for the treatment of various ailments. Most of these plant species are collected in the wild for local uses (Cunningham, 1988) and more recently, for international trade (Dold and Cocks, 2002). In Kwazulu-Natal for example, the bark and roots (54%), bulb and whole plant (28%) constitute the most important ingredients of Zulu herbal medicine which are sold on the street markets (Mander, 1998). Due to the rapid increase of informal trade in medicinal plants, the current demand has exceeded the supply with traders reporting acute shortages and price increases. Yet, South Africa has one of the highest concentration of threatened plant species in the world (Cowling and Hilton-Taylor, 1994) with over 3400 species facing the danger of extinction (Hilton-Taylor, 1996) and over 700 species intensively harvested for medicinal trade (Mander et al., 1997). Among these species is *Pelargonium sidoides* DC, which is collected in the wild for local and international trade.

Due to the increase in the rate of exploitation and lack of organized cultivation, the natural regeneration pattern of *P. sidoides* has been adversely affected. Most gatherers of this species collect both young and adult plants in a process that kills the entire plant. As a measure to controlling overexploitation of this species, the South African Department of Agriculture recently introduced the use of collection permits in order to reduce the rate of *Pelargonium* harvesting. However, this measure, like other wild species (O'Brien and Kinnaid, 1996; McKean, 2003), has not stopped the illegal removal of the plant from unprotected community lands. Since the control of wild harvesting has failed, a more realistic measure will be to develop different conservation programs for the species. Currently, there is a dearth of information on the propagation and

cultivation requirement for *P. sidoides* in the literature and no effort has been made to conserve the species in its natural habitat. It has become important, therefore, to document propagation and conservation techniques for the herb.

Pelargonium sidoides belongs to the family Geraniaceae. It is a rosette-like herb with thickened underground roots and sparsely branched stems from the base (Figure 1). At maturity, the species is about 20-50 cm high, forming a whorl of heart shaped leaves around its base. The plant is widely distributed in South Africa. It is found in the Free State and Gauteng Provinces and very prominent in the Eastern Cape. It grows naturally in short grasslands and sometimes on stony soils that vary from sand to clay-loam, shale or basalt. *P. sidoides* is found at altitudes ranging from near the sea level to 2300 m in Lesotho (Van der Walt and Vorster, 1977; Ebrahim, 2001). When cut, the inside of the underground parts (the tuberous root) appears bright red, a property commonly associated with *Pelargonium* species used for folk medicine (van Wyk and Wink, 2004). *P. sidoides* occurs naturally with a close relative, *Pelargonium reniforme* Curt (Figure 2). The main distinguishing feature between the two taxa is the flower. While the flower of *P. sidoides* is characterized by black red colour, *P. reniforme* has pink colour flowers (van Wyk and Wink, 2004).

Medicinal use of P. sidoides

P. sidoides is commonly used in traditional medicine for the treatment of different infections including cough, fever, tuberculosis, sore throat, fatigue and weakness of the body. The recorded use of this herb for the treatment of respiratory diseases dates back to 1897 when Charles Stevens, an Englishman, was cured of tuberculosis by a Basuto tribal healer using a decoction prepared



Figure 1: Leaves and flowers of *Pelargonium sidoides*. Note the characteristic black red colours of the flowers.

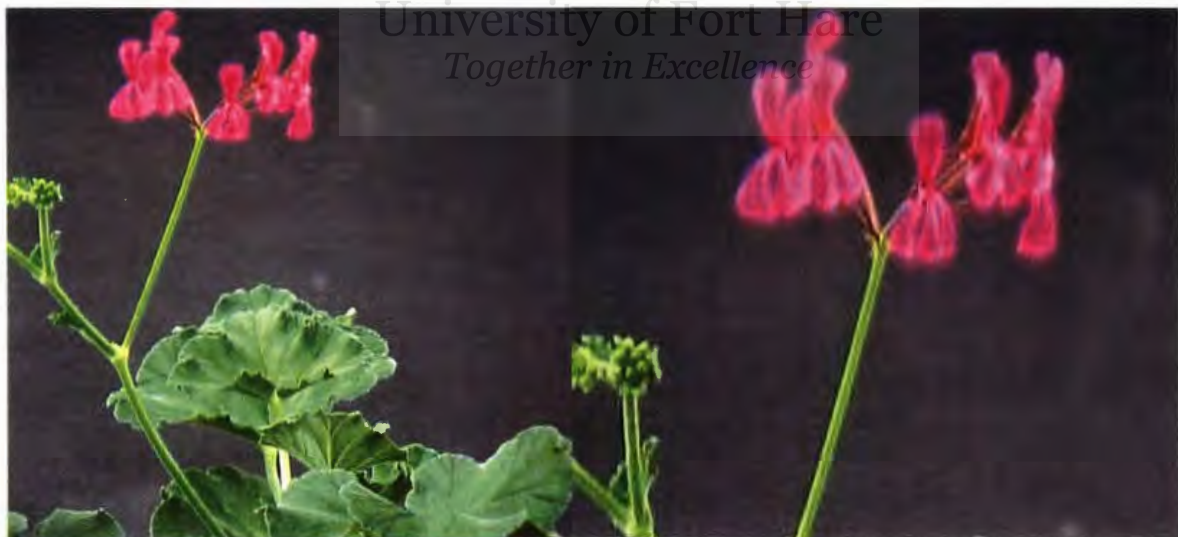
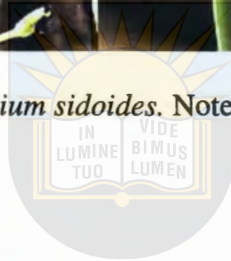
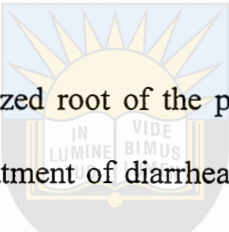


Figure 2: Leaves and flowers of *Pelargonium reniforme* which is usually confused with *P. sidoides*. Note the characteristic bright pink colours of the flowers.

from the root of a plant that was later known as *P. sidoides* (Thompson, 2004). When fully recovered, Charles Stevens returned to England with his 'mysterious' remedy which became popular throughout Europe as Steven's Consumption Cure. Adrien Sechehay, then, a missionary doctor, learnt of Steven's cure in 1920 and treated over 800 patients during the next nine years in Switzerland with a homeopathic preparation of the medicine. In 1929, he published the medical case studies. However, with the introduction of synthetic tuberculosis drugs, Steven's remedy became largely forgotten in Western medicine until its recent rediscovery by European scientists in the early 1980s (Thompson, 2004).



In traditional Xhosa medicine, the pulverized root of the plant is mixed in fresh cow milk and administered orally to children for the treatment of diarrhea and gastritis while freshly harvested leaves are ground and placed on wounds in humans and livestock to heal wounds. The pulverized root is also mixed with other herbs to treat dysentery in livestock (Batten & Bokelmann, 1966). In Germany, the root of *P. sidoides* is used by Schwabe Pharmaceutical Company in the preparation and formulation of *Umckaloabo*, which is used for the treatment of acute bronchitis, the common cold, sinusitis, pharyngitis (sore throat), and tonsillitis (Van Wyk et al., 1997; Van Wyk and Gericke, 2003). In South Africa, NATURA Pharmaceutical Company uses *P. sidoides* to produce *Linctus* which is used to treat tuberculosis. Clinical reports confirmed the efficacy of the drug against acute bronchitis, tonsillopharyngitis and sinusitis (Matthys et al., 2003, Donald and Brown, 2004). This was a follow-up of earlier studies on the antimicrobial activity of the crude extract of the root and essential oils of the plant (Kayser and Kolodziej, 1995, 1997; Kayser et al., 2003). Due to the high medicinal value of the plant, the species is in high demand both in local and international markets.

Motivation for this study

Herbal medicine is widely used in South Africa and most of the plants used for this purpose are harvested from the wild populations. This has led to a boom in medicinal plants trade. The plant trade is believed to generate a large volume of economic activity with an estimated value of R 500 millions per year (Mander, 1998). Currently, several plant species, such as wild ginger (*Siphonochilus aethiopicus*) and the pepper-bark tree (*Warburgia salutaris*) have been exploited to such an extent that they are seldom found in unprotected areas (Cunningham, 1988). While some of these exploited species may have certain traits that enable them to recover quickly and are able to adapt to continuous harvesting (Siebert, 2004), others exhibit characteristics that make them very sensitive to uncontrolled harvesting and may not recover for a long time (Nault and Gagnon, 1993; Olmsted and Alvarez-Buylla, 1995; Pfab and Scholes, 2004). Most affected of the latter group are species harvested for their underground bulbs or roots (Cunningham, 1991; Rock et al., 2004) among which is *Pelargonium sidoides*.

Apart from the local uses of the herb for the treatment of different respiratory diseases, there is a high demand for the plant for export, therefore, the rate of harvesting and number of plant gatherers have currently increased and its exploitation is becoming unsustainable. Unfortunately, most of the gatherers in the Eastern Cape are from the rural communities without defined source of livelihood and have no access to paid job; hence, they rely on plant natural resources for subsistence. Although, the business of harvesting and sale of medicinal plants from the wild do make a positive impact on the financial status of the rural communities, the current rate of harvesting of *P. sidoides* is unsustainable and could affect its availability for future generations (Toman, 1992, McKean, 2003). Considering the current rate of over-exploitation and the

unsustainable harvesting of the herb, there is an urgent need for scientific study and documentation of conservation alternatives for *P. sidoides*.

The scope of the thesis

This thesis is a documentation of the results of my findings on the survey carried out to assess the method of collection and the rejuvenation potential of *P. sidoides* within its natural habitat. It also documents the antimicrobial activities of the different parts of the plant, its population genetics and different propagation techniques for *P. sidoides*. The specific aims and objective of this study are detailed under the following headings.

1. Survey on the harvesting methods and use of *P. sidoides*.

The current increase in the commercialization of medicinal plants has resulted in the over-harvesting and, in some cases, near extinction of some valued indigenous plant species (Williams et al., 2000). *P. sidoides* is used locally to treat respiratory diseases. Commercially, the roots of the herb are used in the production of *Umckaloabo*, which is used for the treatment of acute bronchitis, common cold, sinusitis, pharyngitis (sore throat), and tonsillitis in Germany and other parts of Europe (Van Wyk et al., 1997; Van Wyk and Gericke, 2003). Most of these plant materials are harvested in South Africa from the wild. Due to the high demand for local and export trade, many communities in the Eastern Cape harvest this plant and sell to medicinal plant traders for subsistence. However, the rate of harvesting appears to exceed rate of natural rejuvenation thereby negatively affecting its demography in its natural habitat. A study was therefore conducted to investigate the impact of the rate and methods of harvesting of the herb on its availability in the wild. Specifically, the study was designed to assess the intensity and frequency of collecting the species, the ecological impact of the harvesting method, the plant's natural regeneration potential

as well as the socioeconomic impact of the commercial exploitation of the plant in the study area using rapid rural appraisal technique.

2. Antimicrobial activity of *P. sidoides*:

Verbal ethnobotanical information has revealed that the root of this species is used for the treatment of several respiratory diseases in livestock and humans especially in children. Early investigations also documented the antimicrobial properties of the plant on some bacterial species (Kayser and Kolodziej, 1995, 1997; Kayser et al., 2003). However, these reports were based on the studies carried out on the European ecotype of *P. sidoides*. Mativandlela et al., 2006 recently reported the antituberculosis activity of *P. sidoides* and *P. reniforme* in South Africa. *Bacillus cereus* and *Klebsiella pneumoniae* are respiratory bacterial pathogens commonly associated with infectious diseases (Viljoen et al., 2004). This research included these important pathogens in the antimicrobial study of the plant species in order to link its activity to the reported local uses of the plant by the people of South Africa. One of the objectives of this study was to test crude extracts of this plant against 10 bacteria and five fungal species. According to Mathekaga and Meyer (1998), *in vitro* antimicrobial screening have provided the needed preliminary observations necessary to select among crude plant extracts, those with potentially useful properties for further chemical and pharmacological investigations.

A separate antimicrobial experiment was conducted to determining the differences and similarities in the pharmacological actions of the aerial and underground parts of the plant. The purpose of the experiment was to encourage the use of an alternative (aerial) part of the herb rather than the roots. The overall objective was to suggest the harvesting of the shoots rather than the roots for medicinal use thus encouraging *in situ* conservation technique.

3. Conservation of *P. sidoides* through cultivation.

Plant conservation could be defined as the protection, management, sustainable use, restoration and enhancement of plant natural resources. Plants could be conserved either *in situ* or *ex situ*. While *in situ* conservation is the maintenance of plants in the habitat where they exhibit their natural properties, *ex situ* refers to the maintenance of plants outside their natural habitats. Some researchers have suggested the use of alternative parts of plants such as leaves and stems in traditional medicine of rare and endangered species (Zschocke et. al., 2000; Lewu et al., 2006). However, in our preliminary survey, regeneration of *P. sidoides* following harvesting, even after one year, was as low as 10% in some areas. This has limited the process of natural regeneration thereby negatively affecting its demography in the natural habitat. Another objective of the study was to develop propagation techniques that would encourage its cultivation. Seed germination being an important determinant in the distribution of a species, it is critical to study and understand the various factors that could affect the germinability of the seeds of any plant species before embarking on its propagation and conservation. In this study, *ex situ* conservation approaches were undertaken using different propagation methods. One method was to determine the appropriate plant part that could serve as alternative propagules for germination and development of *P. sidoides* on the field in addition to seeds, while another methods was to determine minimum vine length suitable for the vegetative propagation of the plant. Again the suitable conditions necessary for seed germination in *P. sidoides*, was determines, all for the purpose of encouraging *ex- situ* propagation and conservation of the herb.

4. Micropropagation of *P. sidoides*

Tissue culture technology is a useful tool for the conservation and rapid propagation of endangered

medicinal species (Chukwujekwu et al., 2002; McCartan and van Staden, 2002; Liu and Li, 2002). The development of methodologies for *in-vitro* multiplication and regeneration of *P. sidoides* could go a long way towards its propagation and conservation. It could help to reduce the pressure on the wild population of the species. Tissue culture technology could also solve the problem of low natural cultivation of the species which is hindered by high collection rate and low viability of seeds in the wild. Another objective of the study was to initiate tissue culture protocol for *in vitro* propagation of *P. sidoides* as *ex situ* propagation initiative.

5. Collection and Characterization of Germplasm.

The collection and exchange of genetic materials amongst researchers have helped genetic conservationists to preserve whole range of variabilities in plant species (Marshall, 1990). In our survey; it was observed that there exist some variations among germplasms of *P. sidoides* found growing in different natural habitats. As a baseline study and contribution to developing propagation and conservation strategies for the herb, germplasms of *P. sidoides* were collected from three populations within the Eastern Cape and morphologically characterized. The main objective this study was to contribute to future breeding programmes and other possible utilization of naturally evolving genotypes of the species.

The structure of the thesis

This thesis consists of published articles, manuscripts accepted for publication in various international journals and those ready for submission for publication. The impact of harvesting on the rate of rejuvenation of *P. sidoides* is presented in chapter 2. Chapter 3 is the reprint of the *in situ* conservation study on the plant. Chapter 4 is a reprint of the vegetative propagation study for *P. sidoides* suggesting *ex situ* conservation alternative. The validation of the medicinal efficacy of

the plant is presented as a reprint in chapter 5. The morphological characterization of the species within the Eastern Cape is reported in chapter 6. Chapter 7 is the report on the seed germination study for *P. sidoides* and chapter 8 is the report on the tissue culture propagation of the species. Chapter 9 is the general discussion and conclusion of the study.



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CHAPTER 2

**Commercial harvesting of *Pelargonium*
sidoides in the Eastern Cape: striking a
balance between resource conservation and
sustainable rural livelihood**

CHAPTER 2

Commercial harvesting of *Pelargonium sidoides* in the Eastern Cape: striking a balance between resource conservation and sustainable rural livelihood.



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Commercial harvesting of *Pelargonium sidoides* DC in the Eastern Cape: striking a balance between resource conservation and sustainable rural livelihood.

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Abstract

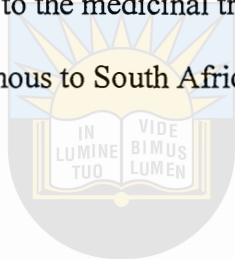
Pelargonium sidoides is used locally in the Eastern Cape for the treatment of several cold related ailments in man and livestock. There has been a great increase in demand for the plant for both local uses and international pharmaceutical producers, therefore the number of the collectors and its rate of harvesting have increased in recent years. The socioeconomic influence of the commercial exploitation of the herb was assessed by means of RRA. The rate of harvesting is intense and appears to exceed its potential for natural regeneration. The socioeconomic implication of uncontrolled harvesting of *P. sidoides* as a medicinal plant is discussed.

Key words: *Pelargonium sidoides*, over-harvesting, conservation, regeneration, socioeconomic impact.

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Introduction

There are up to 100 million herbal remedy consumers in southern Africa with as many as 500 000 traditional healers in the region (Mander and Le Breton, 2005). The same study also revealed that up to 700 000 tonnes of plant materials is consumed annually with an estimated value of as much as 150 million US dollars per annum. Most of these plant species are collected in the wild for local uses (Cunningham, 1988) and international trade (Lange, 1997). In South Africa, the informal trade in medicinal plant has increased in recent years with demand exceeding supply (Dold & Cocks 2002). *Pelargonium sidoides* DC has recently been added to the medicinal trade market both locally and internationally. The species is indigenous to South Africa and it belongs to the family Geraniaceae.



P. sidoides is an aromatic plant with crowded, velvety, heart-shaped, long-stalked leaves. It has a system of thickened underground root-like branches with sparsely branched aerial parts from the base. The species is evergreen in cultivation but probably dying back to varying degrees during winter in the wild. At flowering, the velvety stem is about 20-50 cm high carrying the earth-shaped leaves tangentially to its length. The inflorescence is a branched system of two (rarely up to four or more) pseudo-umbels, each with three to seven (occasionally up to 14) flowers. The flowers are 15 to 17 mm in diameter. The pedicel is usually very short compared to the well-developed hypanthium, and the petals are very dark reddish purple. Morphologically it is very similar to its close relative; *Pelargonium reniforme* but it is readily distinguished by its blackish rather than pink petals, and by its much wider geographical distribution. *P. sidoides* is widely distributed in South Africa but very prominent in the Eastern Cape Province. It is also found in Free

State and Gauteng Provinces of South Africa and Lesotho (Van der Walt and Vorster, 1988; Lawrence, 2001).

The plant is commonly used by the traditional medical practitioners for the treatment of different cold related ailments (Thompson 2004). Apart from the local uses, the plant is used to prepare Umckaloabo®, a medicine produced by a German company, Schwabe Pharmaceuticals, which is said to be effective against bronchitis in children (Van Wyk et al., 1997; Matthys et al., 2003; Van Wyk and Gericke, 2003; Donald and Brown, 2004). This has lead to a high demand for the plant for export and the rate of its harvesting and number of plant gatherers have recently increased.

Most of the gatherers in the Eastern Cape are from the rural communities without defined source of income and have little access to formal employment; hence, they rely on natural resources for subsistence through direct use or resale. Although, harvesting this species from the wild and its sale may bring occasional financial returns to the people, the consequence of uncontrolled harvesting may affect its availability for future generation (Toman, 1992). This study discusses the use and value of the plant, the collectors and examines its regeneration potential after a two-year period.

2. Methodology

2.1. Study area

The research was carried out amongst the Xhosa speaking people living in the Amatola region of South Africa. This area belongs to one of the former homelands in the present

Eastern Cape Province. The province is home to 14.4% of South Africa's total population of 44.8 million in 2001. Over eighty-six percent of the Eastern Cape population is African, 48% is urbanized, 38% of the households live in traditional dwellings and 49% is unemployed (Statistics South Africa, 2001). The province incorporates two of the former 'homelands' of the apartheid era, viz., Ciskei and Transkei. Many aspects of traditional culture are still preserved in these former homeland areas and many of the inhabitants are now dependent on natural resources and pension payments for subsistence (Cocks and Wiersum 2003; Shackleton et al. 2002). The survey and regeneration studies were confined to the Ciskei homeland area and they include: Dyamala, Grahamstown, Gqumahasha, Khayamnandi, Krwakrwa, Maskahane, Peddie, Pumlani, Sheshegu, Thembisa, Tukululu, Tyali and Wesley. The whole area falls within latitude 32°09' - 33°58'S and longitude 26°34' - 27°61'E at altitudes varying from 304 m to 693 m above sea level. The area is bounded by the sea in the east and the drier Karoo, a semi-arid region, in the west.

2.2. The use, value and collectors of *P. sidoides*

The study involved the use of Rapid Rural Appraisal technique based on semi-structured interviews (Marshall and Newton, 2003). The interviews were conducted in English and isiXhosa. At the start of each interview, we identified ourselves as non governmental but academic group interested in the continuous availability of this plant in the wild in order to ensure their ability for sustainable harvest. This information was necessary in order to obtain the most objective response at every instance. A total of 42 respondents were interviewed individually over a period of three months (February-April, 2005). Scheduled visits were made to selected individuals who were chosen at random within the village.

The interviews were conducted in the early hours of the day to evening. Thirty six of the 42 respondents were plant gatherers from 10 peri-urban settlements (Dyamala, Gqumahasha, Khayamnandi, Krwakrwa, Maskahane, Pumlani, Sheshegu, Thembisa, Tukulu, Tyali). Prices and quantities of plant materials sold at each collection centre were recorded for a period of one month. Two visits were made at two weeks interval and data on sales from each collection centre were obtained as pre arranged with designated respondents. Some of the respondents were representatives of the plant collectors at the village level. Among the interviewees were traditional medical practitioners (AmaGqhira or Sangomas) who provided information on the traditional uses of the plant. Information on the employment status of every respondent was obtained during the interview. The settlements included in the survey were selected at random located around the collection centers identified in the study.



2.3. Regeneration Study

In order to determine the regeneration rate, three plots each of 20 m² were marked out in four sites located in Khayamnandi, Peddie, Tukulu and Wesley. Plots were selected based on the availability of *P. sidoides* in those areas and the assurance that the experimental sites would be secured till the end of the study. All the plots were permanently marked out and monitored for growth over a period of 2 years. During the study period, each experimental site was protected from harvesting either by the communities in Khayamnandi, Peddie and Tukulu or by private land owner in Wesley. All the sites were also secured from grazing animals by using 2m long metal rods and strings to demarcate each plot. Rectangular metal plates of equal length were constructed and used at the major ends of each plot to reinforce the fence against animals' encroachment. The rate of

regeneration of *P. sidoides* was determined by counting and recording the number of shoots per plot before the first harvest. This activity was repeated in the first and second year after the first harvest. Fresh biomass (total weight in kg of aerial parts and roots) of every plot was obtained after the first harvest. The first harvest was carried out in March, 2004 and a second harvest was made during the month of May 2006. *P. sidoides* was distinguished from the close taxa; *P. reniforme* (pink flower) by the black red flower. Juvenile members of the populations have thin and limited number of petioles. They mostly sprout by producing a single cluster of petioles unlike those that grow from previous harvest which could have one or more sprout points. However, after separating *P. reniforme* from the harvest, each *P. sidoides* was checked for points or scars of the previous harvests and the presence of old or dead roots attached to the growing plants. These are important features used to distinguish sprout by seeding or otherwise. Only regeneration by re-sprout was recorded and the study did not consider regeneration by seeding in the data analysis. Data were collected over a period of 2 years and were subjected to statistical analysis using Proc GLM method of the SAS package. Each of the three plots in every site was treated as replicate of the site. Where significant differences were observed, means were separated by LSD at 5% level of probability (SAS, 1999).

Results and Discussion

3.1. The use, value and collectors of P. sidoides

Pelargonium sidoides is commonly known in the study area as Uvendle or Ikhubalo. This study revealed that the plant is used by the people of the Eastern Cape for the treatment of dysentery in cattle. At the beginning of spring, sharp changes in the diet of ruminants

(from previously dry and high fibred meal, to succulent and low fibred grasses) induce dysentery in the livestock. The roots of *P. sidoides* is chopped and soaked for three days in water and the extract is administered orally to the animals. A closely related species, *P. reniforme*, was also reported to have similar uses (Batten and Bokelmann, 1966).

Pulverized roots of *P. sidoides* are mixed with fresh cow milk by the traditional healers commonly known as Sangomas (AmaGqhira in isiXhosa) to treat diarrhea and gastritis in children and occasionally in adults. Our study on the antimicrobial activities of *P. sidoides* confirms the use of this species by the people of the Eastern Cape, for the treatment of various bacterial infections (Lewu et al., 2006a; Lewu et al., 2006b). The powdered plant materials could be soaked in water, rubbed on a stone to make a brown paste and used as facial rub for protection from sun or as a cream to treat pimples. Most plant gatherers do not know the difference between *P. sidoides* and its close relative *P. reniforme*. Due to the striking resemblance of the two species, both are often mistakenly combined by plant gatherers during harvest. Although, this report emphasizes on the harvest and sales of *P. sidoides* in the study area, there is great chances that *P. reniforme* was also included in the collection.

There were two groups of collectors identified in the study. The first are those that knew this plant only in the last few years and constitute about 86% of the respondents. They collect the plant mainly for sales within the limited time of demand. This is usually during the months of February and April before the winter season. The second group constitutes the remaining 14% who collect the species for local traditional medicinal uses (AmaGqhira or Sangomas) and local or national market. The second group tends to stay

longer in the business than the first, as local trade network on the species as been reported (Dold and Cocks 2002). All the respondents confirmed that the tubers alone are collected for medicinal use or sold to plant exporters. The findings also revealed that more than 26 tonnes of the species was collected from 10 settlements over a period of one month (Table 1). The buyers usually comb the villages at two weeks intervals to buy collected roots and an estimated \$14, 000 worth of the species were sold within one month in the 10 settlements. All respondents, who were mostly women without formal basic education, agreed that the species was becoming more difficult to get in the wild. Over 72% of them were aware of the negative impact of the current harvesting method on the landscape of the area, yet this did not deter them from exploiting the plant. According to the respondents, they have to harvest to make some income. Most are female dependants and a few live on social grants and pension. Only 14% of the interviewees have other sources of income other than medicinal plant collection. Their vocations include: inter village transporter, peasant farmers, traditional healers and their apprentice.

Two hundred and fifty two plant gatherers were enlisted in the 10 settlements. Although, the age and gender distribution of the population was not studied in detail, most of the gatherers were middle aged women. The number of collectors ranged from 4 in Tukulu to 49 in Sheshegu (Figure 1). Gatherers, mostly women living in rural communities, rely on the sales of many natural resources for subsistence. Among these resources is *P. sidoides*. Although, the collection and sale of *P. sidoides* may contribute little financial income to the people of the Eastern Cape, a sustainable wild harvesting program is important; in order to guarantee regeneration potential for the species (Ghimire et. al., 2004).

3.2. Regeneration of *P. sidoides* two years after harvesting

The mean population of *P. sidoides* recorded per plot in the initial count was about 23.6 followed by 12.3 and 13.5 after the first and second years respectively (Figure 2). These values represent 52.3 and 57.3% rate of regeneration after the first and second year of harvest. Initial population was statistically different ($p < 0.05$) from the subsequent counts recorded during the first and second year. The initial mean of fresh biomass was about 5.7 kg per plot (Figure 3). After two years, the same population yielded 2.5 kg fresh weight per plot, representing 43.9% rate of biomass yield. Although, percentage plant count was higher than 57% during the same period, the biomass yield had dropped comparatively by 14% suggesting low rate of biomass regeneration. The research could not ascertain how long it took the species to grow to the initial population before the study, but our results indicated that, yield after two years was significantly lower (> 0.01) than the initial harvest. The method of harvesting which involves the complete removal of the roots from the soil leaves most of the harvesting sites with open soil fragments (Figures 4a and b). This further exposes the soil to agents of erosion.

The results of this study revealed that the current rate of harvesting may not support annual collection as the second year of regeneration study gave less than 50% biomass yield compared to the initial weight. If left for some years after the first harvesting, the population of *P. sidoides* is able to recover slowly. This recovery may depend on several environmental and soil factors in each micro population. It is advisable to harvest only matured plants (Lama et al., 2001) while younger plants are left to grow to maturity and probably produce seeds that could increase natural regeneration in the wild.

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Table 1. Quantity of harvest sold and price per Kg over 4 weeks of gathering *Pelargonium sidoides* in the surveyed settlements.

Location	Quantity of plant harvested (Kg)	Price of plant per Kg (US \$)	Estimated returns from harvest (US \$)
*Khayamnandi	3545	.653	2314.89
Thembisa	4054	.653	2647.26
Masakhane	1120	.653	731.36
Sheshegu	1550	.653	1012.15
*Dyamala	4200	.392	1646.4
Gqumahasha	2900	.392	1136.8
Krwakrwa	1720	.392	674.24
*Tukulu	725	.653	473.425
*Tyali	3800	.392	1489.6
Totals	26354		13915.35

*Sale centers of *P. sidoides* in the study area.

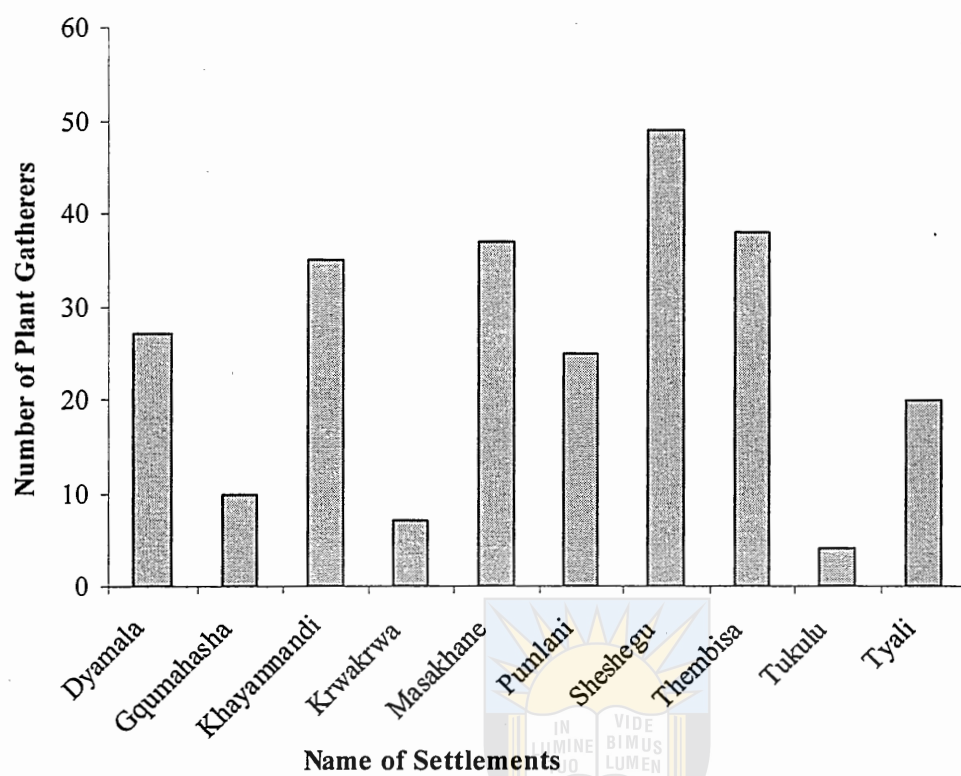


Figure 1. Number of Plant Collectors per Settlement

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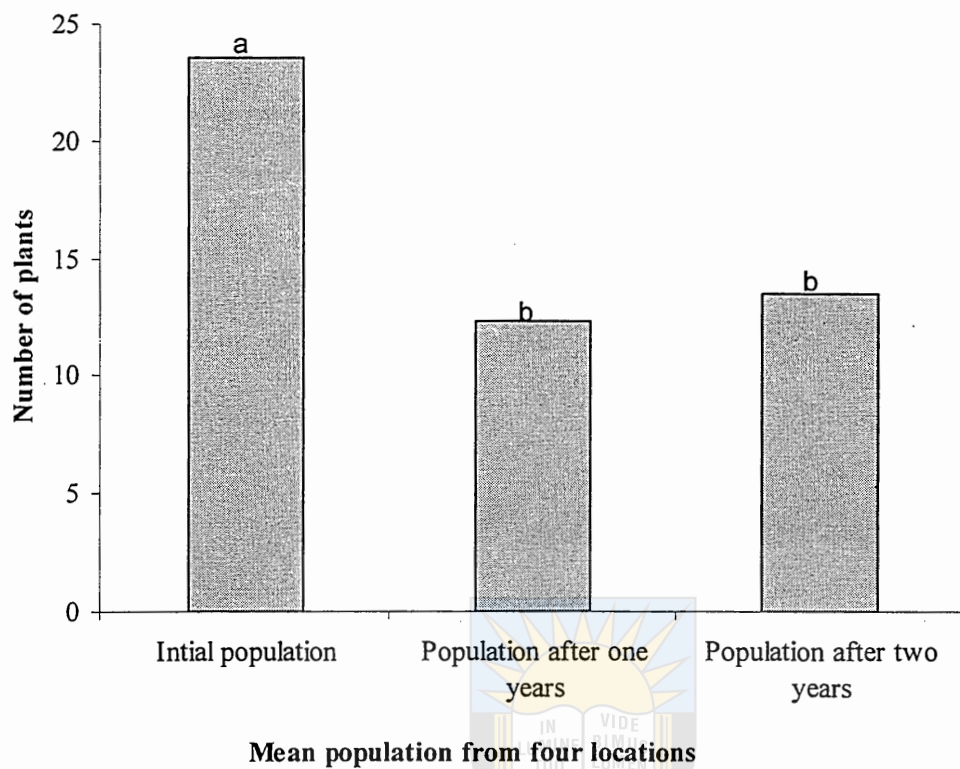


Figure 2. Initial and final population of *P. sidoides* after two years of harvests

Bars with different letters indicates significant difference at $p < 0.01$, $LSD = 1.688$ and n value = 12.

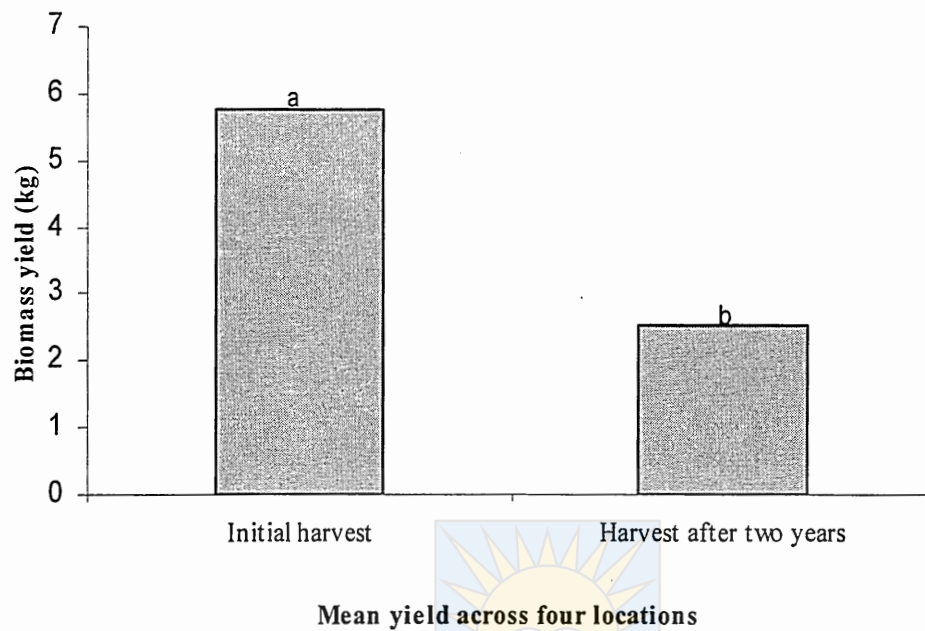


Figure 3. Fresh biomass yield of *P. sidoides* two years after harvesting

Bars with different letters indicates significant difference at $p < 0.01$, $LSD = 0.404$ and n value =12.



Figures 4 A and B: The destruction of the soil surface following the harvest of *P. sidoides*

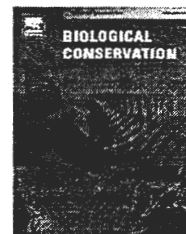
CHAPTER 3

The leaves of *Pelargonium sidoides* may
substitute for its roots in the treatment of
bacterial infections

CHAPTER 3

**The leaves of *Pelargonium sidoides* may substitute for its roots
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Short communication

The leaves of *Pelargonium sidoides* may substitute for its roots in the treatment of bacterial infections

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ABSTRACT

Pelargonium sidoides (Geraniaceae) is a medicinal plant used for the treatment of various infections including tuberculosis and bronchitis. Traditionally, only the roots of the plant are used for medicinal formulations in the Eastern Cape. In addition to traditional uses, there is a great demand for the plant for export, therefore, the rate of its harvesting and the number of its collector has increased in recent years. This has led to a drastic drop in the population of the species in the wild. A method that could achieve the sustainable harvesting of *P. sidoides* would be to substitute its shoot for the roots in medicinal formulations. A comparative study of the antibacterial activities of the leaves and the roots of the plant was conducted on the materials collected from three different populations. Generally, both the shoot and the roots of *P. sidoides* have antimicrobial properties. There was no significant observable difference between the MIC of extracts from both parts. The choice of the root by the traditional healers over the leaves may be arbitrary or due to easy collection. The leaves of *P. sidoides* may, as well, substitute for its roots in medicinal formulations especially in the treatment of bacterial diseases like tuberculosis and bronchitis.

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1. Introduction

In South Africa, medicinal plants are threatened by large scale exploitation for local use and recently for export trade (Lange, 1997). While some of these species may have certain traits that enable them to recover quickly and are able to adapt to continuous harvesting (Siebert, 2004), others exhibit traits that make them very sensitive to uncontrolled harvesting and these species may not recover for a long time (Nault and Gagnon, 1993; Olmsted and Alvarez-Buylla, 1995; Pfab and Scholes, 2004). Most affected of the latter group are species harvested for their underground bulbs or roots (Cunningham, 1991; Rock et al., 2004) among which is *Pelargonium sidoides* DC.

Pelargonium sidoides (Geraniaceae) is indigenous to South Africa and more predominant within the Eastern Cape Province. It is a rosette-like plant with a system of thickened underground roots which is commonly used by the traditional medical practitioners for the treatment of different infections including cough, tuberculosis, bronchitis, sore throat, fatigue and weakness of the body. Tuberculosis, sore throat and bronchitis are caused by bacterial infection. Apart from the local uses, there is a high demand for the plant for export. In Germany, *P. sidoides* imported from South Africa is used in the production of syrup which is widely used for the treatment of bronchitis in children. Due to the high demand for this plant, the rate of harvesting and number of its collectors

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have currently increased and its exploitation is becoming unsustainable.

In our preliminary study, it was discovered that only the roots of the species are being collected for local uses and export trade. This is a destructive method of plant harvesting. It reduces the opportunity for natural rejuvenation and could negatively affect plant demography. A number of strategies to solve the problem of overharvesting have been suggested among which is the use of leaves and stems as alternatives to tubers and roots for medicinal purposes (Zschocke et al., 2000).

A prerequisite for achieving this objective is to assess the differences and similarities between the various parts of the same plant with respect to their chemical and pharmacological properties. In this paper, we present the results of the comparative studies of the antibacterial activities of the shoots and roots of *P. sidoides* collected from three different populations. The studies aimed at determining the differences and similarities in the pharmacological actions of the aerial and underground parts of the plant. This may encourage the use of an alternative (aerial) part of the herb rather than the roots. The overall objective is to suggest the harvesting of the leaves rather than the roots for medicinal use.

2. Materials and methods

2.1. Plant collection

Plant materials were collected in February 2005 from Grahamstown, Peddie and Wesley, all in the Eastern Cape Province, South Africa. The vegetation is rich in diverse flora and the area has a mean annual rainfall of about 700 mm and average temperature range of 18–25 °C. The species was authenticated by Dr. D. Grierson of the Department of Botany, University of Fort Hare. Voucher specimens were prepared and deposited in the Herbarium of the Department of Botany (Lewu Med. 2005/1, Lewu Med. 2005/2 and Lewu Med. 2005/3).

2.2. Preparation of extracts

Shoot and root samples were chopped, air dried and pulverized before extraction. Portions (100 g each) of the pulverized materials were extracted separately in acetone and methanol. The extracts were filtered through Watman No. 1 filter paper, evaporated to dryness under reduced pressure at a maximum of 40 °C using a rotating evaporator and re-dissolved in their respective solvents of extraction to the required concentrations. The choice of acetone and methanol and the exclusion of water was based on our previous observation and other reports that water extracts of plants generally showed little or no antimicrobial activities (Meyer and Afolayan, 1995; Masika and Afolayan, 2002). In addition, our preliminary screening of the aqueous treatment did not show any antibacterial activity at 10 mg ml⁻¹ which was the highest concentration used in this test.

2.3. Preparation of agar-extract plates

Nutrient agar (Biolab) was prepared in the usual fashion by autoclaving, and allowed to cool to about 60 °C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 mg ml⁻¹ were poured into Petri dishes, swirled carefully until the agar began to set and left overnight for solvents to evaporate (Afolayan and Meyer, 1997). Plates containing 1.0 ml of acetone or methanol were used as controls.

2.4. Bacterial strains and bioassay

Bacterial species used in the study were laboratory isolates obtained from the Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa. Five of the bacteria were Gram-positive while five were Gram-negative. The organisms were maintained on nutrient agar plates and were revived for bioassay by culturing them in fresh nutrient broth (Biolab) for 24 h.

Table 1 – Comparative antibacterial activity of the root and shoot of *Pelargonium sidoides* from three populations within the Eastern Cape Province, South Africa

Bacterial species	Gram ±	Minimum inhibition concentration (mg ml ⁻¹)											
		Acetone						Methanol					
		RG	SG	RW	SW	RP	SP	RG	SG	RW	SW	RP	SP
<i>Bacillus cereus</i>	+	2.5	5.0	5.0	7.5	2.5	5.0	5.0	2.5	5.0	5.0	5.0	5.0
<i>Staphylococcus epidermidis</i>	+	na ^a	na	5.0	na	5.0	na	1.0	7.5	na	10.0	2.5	5.0
<i>Staphylococcus aureus</i>	+	1.0	2.5	2.5	7.5	2.5	5.0	2.5	2.5	7.5	7.5	5.0	5.0
<i>Micrococcus kristinae</i>	+	2.5	5.0	5.0	na	2.5	5.0	2.5	1.0	5.0	5.0	1.0	2.5
<i>Streptococcus pyogenes</i>	+	2.5	2.5	5.0	5.0	2.5	5.0	5.0	2.5	5.0	5.0	5.0	2.5
<i>Escherichia coli</i>	–	na	na	na	na	na	na	na	na	na	na	na	na
<i>Salmonella pooni</i>	–	2.5	2.5	5.0	7.5	2.5	5.0	2.5	2.5	5.0	5.0	5.0	2.5
<i>Serratia marcescens</i>	–	na	na	na	na	na	na	na	na	na	na	na	na
<i>Pseudomonas aeruginosa</i>	–	na	na	na	na	na	na	na	na	na	7.5	na	na
<i>Klebsiella pneumoniae</i>	–	na	na	na	na	10.0	na	na	na	na	na	na	na

RG: root from Grahamstown, SG: shoot from Grahamstown, RW: root from Wesley, SW: shoot from Wesley, RP: root from Peddie, SP: shoot from Peddie.

a No activity.

Organisms were streaked in radial patterns, and the plates were incubated at 37 °C for 24–48 h. The minimum inhibitory concentration (MIC) values were recorded, where no bacterial growth was observed. Experiments were replicated thrice.

3. Results and discussion

The results of the bioassay are presented in Table 1. (Since no activity was demonstrated by the water extracts, the results from the aqueous treatment were omitted.) Generally, there was no difference in the bioactivity of plants collected from different populations. This suggests that plant materials collected from these populations are essentially of same accession and have similar bioactivity potential.

With the exception of *Staphylococcus epidermidis* and *Micrococcus kristinae* the extracts from both the roots and the leaves showed activity against all the Gram-positive bacteria tested with MIC ranging from 1.0 to 7.5 mg mol⁻¹. However, these values were slightly higher in the shoot than in the root especially in the acetone extracts. The result suggests that compounds identified in the roots of the plant may be similarly present in the leaves but with different concentrations of the active compounds. Zschocke et al. (2000) reported similar result on the roots and leaves of *Siphonochilus aethiopicus*. In this study, both the root and shoot extracts did not inhibit the growth of the Gram-negative bacteria with the exception of *Salmonella pooni*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* which showed slight inhibition. Generally, both the shoot and the roots of *P. sidoides* have antimicrobial properties. There was no significant observable difference between the MIC of extracts from the two plant parts. This may also be true with their medicinal values, yet our survey has revealed that only pulverized root is administered orally with fresh milk to treat cough and tuberculosis in traditional Xhosa medicine. The choice of the root by the traditional healers over the leaves may be arbitrary or due to easy collection. The leaves of *P. sidoides* may, as well, substitute for its roots in medicinal formulations especially in the treatment of bacterial diseases like tuberculosis and bronchitis.

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CHAPTER 4

Clonal propagation of *Pelargonium sidoides*: A threatened medicinal plant of South Africa

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Short Communication

Clonal propagation of *Pelargonium sidoides*: A threatened medicinal plant of South Africa

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Pelargonium sidoides is used in herbal medicine for the treatment of various infections. Apart from the local uses, the roots are being indiscriminately harvested for local and export trade. A study was conducted to determine the appropriate plant part and the minimum vine length suitable for the clonal propagation of *P. sidoides* in two separate experiments. In the first experiment, three vine tip cuttings (2, 4 and 6 cm) of the species were rooted in potted experiment in a green house. In the second experiment, ten days old petioles were excised from their mother plants and rooted along side 6 cm vines length to assess suitable plant part for vegetative propagation of the species. Data were collected and analyzed on various growth and development indices. In the first experiment, the 6 cm vine length exhibited best result for different parameters measured. Petioles gave significant yield difference over 6 cm vine length and generally showed high potential for vegetative propagation in terms of rooting ability and survival rate. The importance of these findings on the propagation and conservation of *P. sidoides* is discussed.

Key words: Clonal propagation, *ex situ* conservation, medicinal plant, *Pelargonium sidoides*, stem cutting.

INTRODUCTION

The current trade in medicinal plants in Africa has negatively affected the demography of most plant species harvested from the wild (Cunningham, 1988; Lange, 1997) and many workers have reported unsustainable harvesting of some of these species (Ake-Assi, 1988; Kokwaro, 1991; Cunningham and Mbenkum, 1993; Gu, 1998; Ghimire et al., 2005; Tang et al., 2005). Amongst these is *Pelargonium sidoides* DC. It is a rosette-like plant with thickened underground roots and sparsely branched stems from the base. At flowering, the velvety stem is about 20-50 cm high carrying the heart-shaped leaves tangentially to its length. The species is indigenous and widely distributed in South Africa. It is found in several provinces including Free State and Gauteng Provinces but very prominent in the Eastern Cape. It occurs naturally in short grasslands and sometimes on stony soils that vary from sand to clay-loam, shale or basalt. It is found at altitudes ranging from near the sea level to 2300 m in Lesotho.

P. sidoides is used in traditional medicine for the treatment of different infections including cough, fever, tuberculosis, sore throat, fatigue and weakness of the body. Apart from the local uses, the root is being indiscriminately collected in large quantity to meet the current demand for local and export trade. The increased rate of harvesting and number of plant gatherers has made its exploitation unsustainable. Our preliminary survey revealed a negative trend in the demography of the species. With the current increase in the number of plant gatherers and lack of organized cultivation, the species appears threatened in its natural habit. Although, the South African Department of Agriculture recently introduced the use of permit as a control measure against the unsustainable harvesting of *P. sidoides* from the wild, this measure, like other medicinal plants (O'Brien and Kinnaird, 1996; M^cKean, 2003), has not stopped the illegal removal of the species especially from unprotected lands.

Naturally, regeneration of the species is by seed and from the development of perennates through the underground root system. However, the root is removed during harvesting which threatens the opportunity for perennial rejuvenation. Our ongoing studies indicate that

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Table 1. Means of various growth parameters of *Pelargonium sidoides* raised from different vine lengths.

Length of vines (cm)	Plant height (cm)	Root length (cm)	Number of roots	Number of leaves	Survival rate (%)
2	4.572b	5.40b	1.00b	8.00b	15.56c
4	7.35a	5.39b	1.25b	10.00a	31.67b
6	8.63a	8.56a	4.25a	11.50a	56.89a

Means with the same letter in a column are not significantly different at $p < 0.05$.

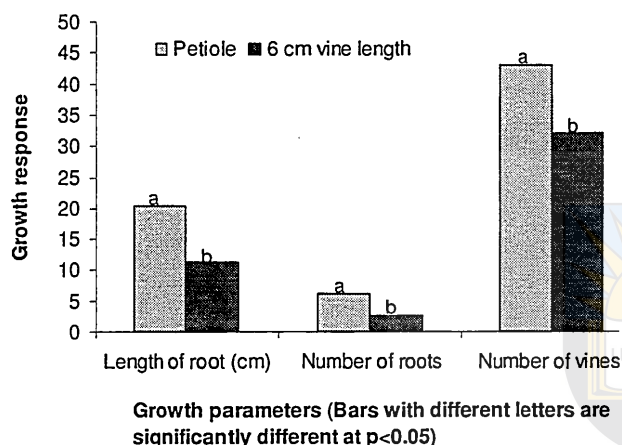


Figure 1. Growth parameters of *Pelargonium sidoides* raised from petioles and 6 cm vine length.

the viability of seeds collected from the wild is low coupled with very low seed germination. One of the most pragmatic measures against over exploitation of plant natural resources is to improve propagation and encourage cultivation, especially at the village level. This in turn, helps in the reduction of poverty related pressure on plant natural resources. The objective of this study was to determine the appropriate plant part and the minimum vine length suitable for the clonal propagation of *P. sidoides*, for the purpose of encouraging *ex situ* conservation of the species.

MATERIALS AND METHODS

Plant collection and preparation of growth pots

Matured plants were collected from Khayamnandi (32°09'S and 26°60'E; altitude 624 m) during the month of March 2005 from their natural habit. Plant materials were transported in polyethylene bags to the green house of the Faculty of Science and Agriculture, University of Fort Hare where the experiment was conducted. The green house was maintained at 18 - 26°C and at 60% relative humidity. The soil used in this experiment was obtained from the University of Fort Hare experimental farm. Topsoil (0-15 cm) were collected in polythene bags and spread in the drying room for 48 h (Anderson and Ingram, 1993). This was later sieved through a 2 mm wire mesh, thoroughly homogenized and 8 kg of each soil samples was potted in polythene bags.

Determination of the minimum length of sproutable vines

Healthy vines of *P. sidoides* were cut into sections of 2, 4 and 6 cm rooted in potted soils. The experiment was laid out in a randomized complete design with three treatments replicated four times. Each treatment (2, 4, and 6 cm long vines) comprised 60 cuttings. All cultural practices were the same during the entire period of the experiment. Data on survival rate, number of roots, length of longest root, number of leaves and plant height were recorded after sixty 60 days by adopting standard procedures.

Determination of best plant part for clonal propagation

In another experiment, 10 days old petioles (excised from their mother vines) and 6 cm long vines were rooted directly in potted soils prepared as above. This experiment was aimed at assessing the best plant part for clonal propagation of *P. sidoides*. Both experiments were repeated twice.

Data were subjected to statistical analysis using the SAS package. Where significant difference was observed, means were separated by LSD at 5% level of probability (SAS, 1999).

RESULTS

Generally, the length of the shoot planted had significant effect on the growth parameters of the plant. The 6 cm shoot length exhibited the highest growth performance compared to the other shoot lengths planted. In addition, it showed significant yield differences, both in the number and length of roots, than the other vine lengths. In the case of seedling establishment, the 6 cm vine length also demonstrated significantly higher rate of survival than the other treatments. Although, there was no significant difference in biomass yield between the 6 and 4 cm shoot length, both were higher than the 2 cm vine length in terms of number of leaves and plant height (Table 1).

In the second experiment, the petioles excised from their mother plants 10 days after sprouting rooted successfully. Generally, the petioles showed high potential for vegetative propagation in terms of rooting ability. They showed significant higher yields over the 6 cm vine length in terms of length and number of roots. In fact, the petioles yielded twice the number of roots than the 6 cm vine length. The survival rate of the treatment propagated by petioles showed remarkable results much higher than the 6 cm vine length (Figure 1).

DISCUSSION

Recent reports on the clonal propagation of medicinal plants have exploited the use of chemicals, like the hormones, to improve rooting (Nadeem et al, 2000; Tchoundjeu et al., 2004; Isutsa, 2004) or increase the rate of production (Aslam Khan et al., 2004). However, resource poor farmers usually practice marginal input farming which results into low yields ((Sanchez et al., 1997; FAO, 1999). Yields from poor farmers' fields are largely at variance with scientific publications or with wealthy farmers' fields, mainly due to resource related factors (Crowley and Carter, 2000). The development of adaptable approach and low input propagation technique would better serve the need of the rural poor farmers.

This study has demonstrated the possibility of propagating *P. sidoides* through the use of its vegetative aerial parts. These parts are conventionally thrown away and wasted during the collection of the roots for medicinal trade. Although, 6 cm vine length showed good yield success and survivability, the result demonstrated a high potential for clonal propagation via the petioles both in terms of rooting ability and survival rate. In addition, the petioles of vigorously growing vines, with well established roots could be excised once in every 10 days and rooted directly in the nursery to produce healthy plants if properly managed.

This is the first report on the propagation of *P. sidoides* under marginal input. The results obtained from this work indicated that 6 cm vine length is the minimum length that could give substantial survivability and that the use of petioles, which are largely wasted during harvesting, has the best potential for rooting in the clonal propagation of *P. sidoides*. The encouragement of vegetative propagation of *P. sidoides* could serve as a viable option for the *ex situ* conservation of this plant in the Eastern Cape. Cultivation of the plant could be the alternative source of supply in order to take pressure off the wild stock.

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CHAPTER 5

Extracts from *Pelargonium sidoides* inhibit the growth of bacteria and fungi



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Extracts from *Pelargonium sidoides* Inhibit the Growth of Bacteria and Fungi

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Abstract

Pelargonium sidoides DC (Geraniaceae) is a medicinal plant used by the people of the Eastern Cape, South Africa, for the treatment of various diseases in man and livestock. Acetone and methanol extracts of the shoot and root of the herb were investigated for antimicrobial activity against 10 bacterial and 5 fungal species by the dilution method on solid agar medium. With the exception of *Staphylococcus epidermidis*, extracts obtained from both solvents demonstrated significant activity against all the Gram-positive and two of the Gram-negative bacteria tested in this study. The extracts also showed appreciable inhibitory activity against all the fungal species tested with inhibition ranging from 52.5% on *Aspergillus flavus* to 82.5% on *Mucor hiemalis* at 5 mg ml⁻¹, the highest concentration tested in the study. The ability of the extracts of this plant to inhibit the growth of several bacteria and fungi is an indication of the broad-spectrum antimicrobial potential of *P. sidoides* that further validates the use of this plant for the treatment of various ailments by the people of the Eastern Cape.

Keywords: Antimicrobial activity, ethnobotany, medicinal plants, *Pelargonium sidoides*.

Introduction

Today, an estimated 80% of the black population of South Africa makes use of indigenous medicinal plants for the treatment of various ailments in man and animals (Kelmanson et al., 2000). This practice may be due to easy access to traditional medication, especially in rural areas, where the majority of people cannot afford prescribed medicines.

Pelargonium sidoides DC (Geraniaceae) is indigenous to South Africa. It grows widely in the Eastern Cape Province where it is used for the treatment of human and livestock diseases. It is a rosette-like plant with a system of thickened underground roots that are commonly used by the traditional medical practitioners. Many of its uses relate to the treatment of microbial infections, which includes coughs, tuberculosis, and wounds in man and livestock (Van Wyk et al., 1997). Information gathered during our preliminary investigation on the local uses of the species also revealed its medicinal importance for the treatment of stomachache, dysentery, and diarrhea, especially in children. The pulverized root of the plant is mixed in fresh cow's milk and administered orally to children, and freshly harvested leaves are ground and placed on wounds in man and livestock. The pulverized root is also mixed with other herbs to treat dysentery in livestock (Batten & Bokelmann, 1966). *Umckaloabo*, a medicine prepared from this plant by a German company (Schwabe), is said to be effective against bronchitis in children (Van Wyk et al., 1997; Van Wyk & Gericke, 2003).

Despite the documented information on the medicinal use of the species, there is little or no information on its antibacterial property, especially in this part of the world. The work of Kayser and Kolodziej (1997) on the antibacterial activity of this plant was devoted to the European ecotype. In this paper, we report the antimicrobial activity of the acetone and methanol extracts of *P. sidoides* growing in South Africa with 10 bacteria and 5 fungal species. According to Mathekaga and Meyer (1998), *in vitro* antimicrobial screening has provided the preliminary observations necessary to select among crude plant extracts, those with potentially useful

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properties for further chemical and pharmacological investigations.

Materials and Methods

Plant collection and preparation of extracts

Shoot and root samples of *P. sidoides* were collected in February 2005 from Peddie (33°11.10'S and 7°10.60'E; altitude 695 m), Eastern Cape Province, South Africa. The vegetation is rich in diverse flora, and the area has a mean annual rainfall of about 700 mm and annual temperature range of 18–25°C. The species was authenticated by Dr. D. Grierson of the Department of Botany, University of Fort Hare. A voucher specimen was prepared and deposited in the herbarium of the Department of Botany (Lewu Med. 2005/2).

Samples were chopped, air-dried, and pulverized before extraction. Portions (100 g each) of the pulverized materials were extracted separately for 24 h in acetone and methanol. The extracts were filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40°C using a rotary evaporator. The extracts were re-dissolved in their respective solvents to the required concentrations for the bioassay analysis.

Antibacterial assay

Bacterial species used in the study were laboratory isolates obtained from the Department of Biochemistry and Microbiology, Rhodes University, South Africa. Five Gram-positive (*Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus pyogenes*) and five Gram-negative (*Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) species were used in this study. The organisms were maintained on nutrient agar plates and were revived for bioassay by culturing them in fresh nutrient broth (Biolab, South Africa) for 24 h before use.

Nutrient agar (Biolab, South Africa) was prepared by autoclaving and allowing to cool to about 60°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 1.0, 2.5, 5.0, 7.5, and 10.0 mg ml⁻¹ were poured into Petri dishes, swirled carefully until the agar began to set, and left overnight for the solvents to evaporate (Afolayan & Meyer, 1997). Plates containing 1% of acetone or methanol were used as controls (Dulger & Ugurlu, 2005). Organisms were streaked in radial patterns, and the plates were incubated at 37°C for 24 to 48 h. The minimum inhibitory concentration (MIC) values where no bacterial growth was observed were recorded. Experiments were replicated three-times.

Antifungal assay

Five species of fungi, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium*, *Mucor hiemalis*, and *Penicillium notatum*, were used for the antimycotic investigation. The fungal cultures were maintained on potato dextrose agar (PDA) (Biolab) and were recovered for testing by subculturing on fresh PDA for 3 days prior to bioassay.

PDA plates were prepared by autoclaving before the addition of the extracts. Each extract was mixed with the molten agar (at 45°C) to final extract concentrations of 0.1, 0.5, 1.0, and 5.0 mg ml⁻¹ of molten agar and poured into Petri dishes. Blank plates containing only PDA or PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs obtained from actively growing portions of the mother fungal plates and incubated at 25°C for 5 days. Diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates (Afolayan & Mayer, 1997; Barreto et al., 1997; Quiroga et al., 2001). Significant differences within the means of treatments and controls were calculated using the LSD statistical test as described by Steel and Torrie (1960). LC₅₀ (the concentration at which 50% of the growth was obtained) was calculated by extrapolation.

Results and Discussion

Antibacterial activity

The results of the acetone and methanol extracts are presented in Table 1. With the exception of *Staphylococcus epidermidis*, all the extracts demonstrated activity against the Gram-positive bacteria tested in this study. The MIC ranged from 1 to 5 mg ml⁻¹ except for the acetone root extract against *Klebsiella pneumoniae* where the value was 10 mg ml⁻¹. The extracts were more active against the Gram-positive bacteria than the Gram-negative strains. Three Gram-negative bacteria, *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, were not inhibited by any of the extracts at the highest concentration (10 mg ml⁻¹) tested. Observations on very weak/no activity of aqueous plant extracts on Gram-negative bacteria are well documented (Eloff, 1998; Madamombe & Afolayan, 2003; Afolayan, 2003).

P. sidoides has been used traditionally for the treatment of various respiratory diseases by the people of the Eastern Cape for a long time. *Bacillus cereus* and *Klebsiella pneumoniae* are respiratory pathogens commonly associated with colds and flu (Viljoen et al., 2004). All the extracts showed significant activity against *Bacillus cereus* while acetone root extracts was active on *Klebsiella pneumoniae* at 10 mg ml⁻¹. The inhibitory property of extracts from *P. sidoides* against pathogens of respiratory infections might have justified the use of this plant for the treatment of cough and influenza fever by

Table 1. Antibacterial activity of the acetone and methanol extracts of the shoots and roots of *Pelargonium sidoides*.

Bacterial species	Gram +/–	Minimum inhibitory concentration (mg ml ⁻¹)			
		Acetone		Methanol	
		AR	AS	MR	MS
<i>Bacillus cereus</i>	+	2.5	5	5	5
<i>Staphylococcus epidermidis</i>	+	5	na	2.5	5
<i>Staphylococcus aureus</i>	+	2.5	5	5	5
<i>Micrococcus kristinae</i>	+	2.5	5	1	2.5
<i>Streptococcus pyogenes</i>	+	2.5	5	5	2.5
<i>Escherichia coli</i>	–	na	na	na	na
<i>Salmonella pooni</i>	–	2.5	5	5	2.5
<i>Serratia marcescens</i>	–	na	na	na	na
<i>Pseudomonas aeruginosa</i>	–	na	na	na	na
<i>Klebsiella pneumoniae</i>	–	10	na	na	na

AR, acetone root extract; AS, acetone shoot extract; MR, methanol root extract; MS, methanol shoot extract; na, no activity.

the people of the Eastern Cape, South Africa. Kayser and Kolodziej (1997) reported similar antibacterial effect of this herb against *K. pneumoniae* in Europe.

The choice of acetone and methanol as solvents of extraction and the exclusion of water extracts in this analysis was based on our previous observation and other reports that water extracts of plants generally showed little or no antimicrobial activities (Meyer & Afolayan, 1995; Masika & Afolayan, 2002).

Generally, Gram-negative bacteria have been reported to be more resistant to plant extracts than the

Gram-positive strains (Rabe & Van Staden, 1997; Grierson & Afolayan, 1999; Afolayan, 2003). This is also confirmed in this study.

Antifungal activity

The acetone and methanol extracts showed significant inhibition against most of the fungi tested in this study (Table 2). Except for the acetone extracts (shoots and roots) against *A. niger* and the methanol extract of the roots against *M. hiemalis*, all the extracts showed more

Table 2. Antifungal activity of the acetone and methanol extracts of the shoots and roots of *Pelargonium sidoides*.

Concentration (mg ml ⁻¹)	Growth inhibition (%)									
	Shoot extract					Root extract				
	Af	An	Fo	Mh	Pn	Af	An	Fo	Mh	Pn
Acetone										
5	60.83 ^d	45.73 ^c	65.83 ^d	71.11 ^d	75.56 ^c	52.50 ^c	49.44 ^d	71.39 ^c	68.06 ^d	69.72 ^d
1	45.00 ^c	32.22 ^b	42.78 ^c	27.50 ^c	63.89 ^d	47.78 ^d	43.33 ^c	62.78 ^d	28.06 ^c	60.00 ^c
0.5	43.74 ^c	26.95 ^b	43.89 ^c	16.39 ^b	47.50 ^c	42.78 ^c	27.78 ^b	56.67 ^c	13.89 ^b	56.94 ^c
0.1	33.34 ^b	26.67 ^b	40.00 ^b	0.00 ^a	31.11 ^b	31.39 ^b	24.45 ^b	31.67 ^b	0.00 ^a	46.95 ^b
Acetone	0.00 ^a	0.833 ^a	0.00 ^a	0.00 ^a	1.94 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.55 ^a
PDA	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	2.26	>5	2.25	3.06	0.58	2.88	>5	0.39	3.19	0.22
Methanol										
5	74.72 ^d	59.17 ^d	65.83 ^d	82.50 ^c	71.09 ^d	56.95 ^d	55.28 ^c	66.35 ^c	29.72 ^c	75.56 ^d
1	58.61 ^c	41.11 ^c	42.78 ^c	6.67 ^b	64.72 ^d	50.28 ^c	46.11 ^d	59.17 ^d	11.11 ^b	69.7 ^{c,d}
0.5	45.28 ^b	31.1 ^b	43.89 ^c	0.00 ^a	56.66 ^c	38.34 ^b	38.61 ^c	49.72 ^c	0.00 ^a	63.89 ^c
0.1	41.11 ^b	30.28 ^b	40.00 ^b	0.00 ^a	42.22 ^b	0.00 ^a	30.28 ^b	34.72 ^b	0.00 ^a	40.48 ^b
Methanol	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
PDA	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.68	2.97	1.84	3.29	0.32	0.99	2.70	0.51	<5	0.26

Values are means of percentage growth inhibition of three replicates. Values within a column of a treatment followed by the same superscript are not significantly different at $p < 0.05$ according to the LSD test. Af, *Aspergillus flavus*; An, *Aspergillus niger*; Fo, *Fusarium oxysporium*; Mh, *Mucor hiemalis*; Pn, *Penicillium notatum*. LC₅₀: values in mg ml⁻¹ were calculated by extrapolation.

than 50% inhibition with activity ranging from 52.5% on *A. flavus* to 82.5% on *M. hiemalis* at 5 mg ml⁻¹ (the highest concentration tested in the study).

The ability of the extracts of this plant to inhibit the growth of several bacteria and fungi is an indication of the broad-spectrum antimicrobial potential of *P. sidoides* that further validates the use of this plant for the treatment of various ailments by the people of the Eastern Cape.

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CHAPTER 6

Morphological diversity among accessions of *Pelargonium sidoides* in the Eastern Cape, South Africa

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Morphological diversity among accessions of *Pelargonium sidoides* in the Eastern Cape, South Africa

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Abstract *Pelargonium sidoides* is an indigenous medicinal plant of South Africa harvested for local use and international trade. Due to its high economic and medicinal importance, the rate and the number of its harvesters have increased in recent years. This has led to a drastic drop in the population of the species in the wild. In order to develop breeding and propagation program for this species, a baseline study was conducted to determine the morphological variability exhibited by 30 accessions of *P. sidoides* collected from three locations within the Eastern Cape. A list of descriptors developed by IPGRI/ITA/BAMNET for Bambara groundnut was adopted and a total of 16 traits (2 qualitative and 14 quantitative) were determined during the vegetative and reproductive stages. Data were subjected to univariate and multivariate statistical analyses. Ample variability was found for most traits measured. Cluster analysis showed unique clustering of accessions forming 29 morphotypes (clusters) out of the 30 accessions studied. At the core level, plant materials collected from the same location were found to cluster in pair of accessions thus implying similarity of adaptation. The reproductive characters which include number

of flowers per inflorescence, length and width of inflorescence and number of inflorescence per vine showed the highest variation among the quantitative characters measured. The importance of this work on the propagation and of *Pelargonium sidoides* is discussed.

Keywords Medicinal plant · Morphological characterization · *Pelargonium sidoides* · Plant propagation

Introduction

Several medicinal plant species have been harvested unsustainably in Africa (Ake-Assi 1988; Kokwaro 1991; Cunningham and Mbenkum 1993). Cunningham (1993) identified unemployment and low level of formal education as a major reason for the current increase in medicinal plant gatherers in the continent. Most of these plant materials are collected in the wild for local uses (Cunningham 1988) and more recently for international (Lange 1997) trade. Among plant species exploited unsustainably in recent time is *Pelargonium sidoides* DC. The plant belongs to the family Geraniaceae and is indigenous to South Africa.

It is commonly used in traditional medicine for the treatment of different infections including cough, fever, tuberculosis, sore throat, fatigue and

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weakness of the body. The use of *P. sidoides* for the treatment of respiratory diseases dates back to 1897 when Charles Stevens, an Englishman, was cured of tuberculosis by a Basuto tribal healer using a decoction prepared from the root of a plant that was later known as *P. sidoides* (Thompson 2004). When fully recovered, Charles Stevens returned to England with his mysterious remedy which became popular throughout Europe as "Steven's Consumption Cure". Adrien Sechehayé (a former missionary doctor) learned of Steven's cure in 1920 and treated over 800 patients during the next 9 years in Switzerland with a homeopathic preparation of the medicine. In 1929, he published the medical case studies. However, with the introduction of synthetic tuberculosis drugs, Steven's remedy became largely forgotten in Western medicine until its recent rediscovery by European scientists in the early 1980s.

In traditional Xhosa medicine, the pulverized root of the plant is mixed with fresh cow milk and administered orally to children while freshly harvested leaves are ground and placed on wounds in human and livestock. The pulverized root is also mixed with other herbs to treat dysentery in livestock (Batten and Bokelmann 1966). *Umckaloabo*, a medicine prepared from this plant by a German company (Schwabe) is said to be effective against bronchitis in children (Van Wyk et al. 1997; Van Wyk and Gericke 2003).

P. sidoides is a rosette-like plant with a system of thickened underground roots. The stem is sparsely branched from the base. At flowering, the velvety stem is about 20–50 cm high carrying the heart-shaped leaves tangentially to its length. The species is widely distributed in South Africa and found in several provinces among which include Free State and Gauteng Provinces but very prominent in the Eastern Cape. It grows naturally in short grasslands and sometimes on stony soils that vary from sand to clay-loam, shale or basalt. It is found at altitudes ranging from near the sea level to 2300 m in Lesotho (Van der Walt and Vorster 1977; Ebrahim 2001). When cut, the inside of the underground parts (the tuberous root) appears bright red; a property commonly associated with *Pelargonium* species used for folk medicine. *P. sidoides* occurs in the same habitat

with a close relative; *Pelargonium reniforme* Curt. The main distinguishing feature between the two taxa is the flower. While the flower of *P. sidoides* is characterized by black red colour, *P. reniforme* has pink colour flowers (Van Wyk and Wink 2004). However, our observation has shown some likely variations among germplasms collected from different natural habitats of *P. sidoides* within the Eastern Cape.

Apart from local uses, there is a high demand for the plant for export, therefore, the rate of harvesting and number of plant gatherers have increased in recent time and its exploitation is becoming unsustainable. With both species currently under intense collection for local and international uses; accessions of the species evolve, thereby developing local adaptations (Omolaja et al. 2000; Vilaró et al. 2004). Meanwhile, meaningful crop improvement in the short and long terms is achieved only with germplasms of broad genetic variability (Allard 1960). However, this variability will be of great use for propagation and breeding development programme if accompanied by characterization data. The objectives of this study were to collect and characterize populations of *Pelargonium sidoides* in order to contribute to the breeding programmes and other possible utilization of naturally evolving genotypes of the species.

Materials and methods

Seeds were collected from the wild in February 2005 at Dyamala, Peddie and Wesley, all in the Eastern Cape Province, South Africa. Data on the coordinates and altitudes of the collection centres are presented in Table 1. The vegetation of the Eastern Cape is rich in diverse flora and the region has a mean annual rainfall of about 700 mm and average temperature range of 18–25°C. The experiment was conducted in the green house of the Faculty of Science and Agriculture, University of Fort Hare. The green house was maintained at 18–26°C and at 60% relative humidity. The soil used in this experiment was obtained from the University of Fort Hare experimental farm. Topsoil (0–15 cm) was collected in polythene bags and spread in the drying

Table 1 Provenances of the accessions included in the study

Accession number	Municipality	Location name	Altitude (m)	Latitude	Longitude
*UFPs 1	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 2	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 3	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 4	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 5	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 6	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 7	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 8	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 9	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 10	Nkonkobe	Dyamala	538	32°34'S	26°60'E
UFPs 11	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 12	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 13	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 14	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 15	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 16	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 17	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 18	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 19	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 20	Ngqushwa	Peddie	695	33°10'S	27°60'E
UFPs 21	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 22	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 23	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 24	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 25	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 26	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 27	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 28	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 29	Amatole	Wesley	304	33°58'S	27°61'E
UFPs 30	Amatole	Wesley	304	33°58'S	27°61'E

*UFPs University of Fort
Hare *Pelargonium*
sidoides

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room four 48 h (Anderson and Ingram 1993). This was later sieved through a 2 mm wire mess, thoroughly homogenized and 8 kg of each soil samples was potted in polythene bags.

Seeds were assigned with accession numbers and samples were planted in tray and placed in the nursery until they germinated. After 2 weeks, young plants were transferred to the plastic pots in a row of 10 plants per accession. For all traits, 5 healthy plants were selected per plot and assessed for 16 characters using the plant prescription segment of the preliminary descriptors for Bambara groundnut (IPGRI et al. 2000). A total of 30 accessions were characterized. Two qualitative characters and 14 quantitative characters (9 vegetative and 7 reproductive) were recorded from each plant and data were statistically analyzed using the SAS package (SAS 1999). Both univariate and multivariate analyses were performed on the data. The univariate statistics calculated

were: coefficient of variation, mean, standard deviation, range and the multivariate statistics were cluster analysis and Principal Component Analysis (PCA).

Results and discussion

Qualitative characters

Table 2 shows the coefficient of variation, standard deviation, mean, maximum and minimum values obtained from both qualitative and quantitative characters measured in the accessions evaluated. *Pelargonium sidoides* forms a whorl of petioles carrying one leaf each around a single vine. With regards to the size of the leaf, this varied considerably among the accessions. Most of the accessions studied have a peltate leaf shape while some are peltately-digitate. Some of

Table 2 Descriptive statistics of the 16 traits measured on the 30 accessions collected from the three locations

Traits measured	Standard variation	Mean	Minimum	Maximum	CV
Plant spread	5.90	35.83	27.00	48.00	16.47
Leaf length	1.16	5.59	3.46	7.80	20.70
Leaf breadth	1.69	7.40	4.63	11.10	22.88
Leaf petiole length	3.21	14.13	10.00	20.30	22.72
Number of flowers per inflorescence	20.92	62.47	42.00	131.00	33.48
Length of inflorescence	9.36	46.62	23.60	62.20	20.07
Petiole length	1.44	12.76	9.35	15.50	11.31
Sepal length	0.80	7.43	6.25	9.40	10.79
Internodes length of inflorescence	4.80	28.46	18.73	35.95	16.85
Width of inflorescence	0.59	2.05	1.25	3.45	28.99
Petiole width	0.46	2.48	1.40	3.47	18.65
Number of inflorescence per vine	2.45	7.17	3.00	13.00	34.20
Number of petiole per vine	19.49	56.00	26.00	97.00	34.80
Number of leaves per plant	8.00	30.47	18.00	47.00	26.25
Flower colour	0.50	0.50	0.00	1.00	101.71
Leaf shape	0.50	0.50	0.00	1.00	83.05

the accessions produced large leaves but limited number of leaves per plant while some produced smaller leaves but many leaves per plant. A few of the accessions produced many large leaves showing advantage of producing high foliage than most of the accessions within the populations. Our preliminary study revealed that clonal propagation of the species could be achieved via the vines and petioles. Accessions exhibiting high production of foliage could be cultivated by clonal propagation to meet the increasing demand for the species in order to take pressure off wild stock. The reproductive characters showed wide variability. Three distinctive flower colours (petal) ranging from black red to deep red and bright red colours were found within the populations studied. The occurrence of different flower colours among the studied accessions may be due to association of *Pelargonium sidoides* with its close relative; *Pelargonium reniforme* (with pink colour) found growing in the same habitat. Attributes that did not exhibit variation are the leaf and shoot pubescence (data not shown). These characters were present in all the accessions studied. Accessions showing promising attributes could be rapidly multiplied through clonal propagation to maintain desirable traits. Conservation and propagation priorities could be given to accessions that present distinctive characters not found in the remaining

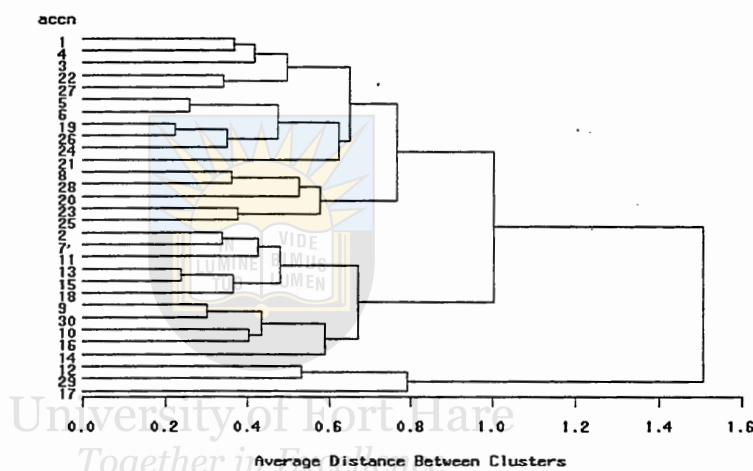
accessions. 'However, such priorities will be based on breeders and other researchers' traits of preference.

Quantitative characters

The mean, minimum, maximum, standard deviation and coefficient of variation (CV) for various morphological traits are shown in Table 2. All quantitative characters exhibited variability evident by high CV observed in most of the traits studied. The high CV observed among reproductive characters which include length and breadth of the flower petals, the number of flowers per inflorescence, and the width of inflorescence is an indication of some level of variability within the populations of *P. sidoides* found growing in these habitats. These characters showed the highest variation among quantitative characters measured. Moderately high variation was also observed in vegetative characters like leaf length and breadth, plant spread, petiole width and number of leaves per vine. The principal component analysis revealed that the first six principal components exhibited eigenvalues greater than 1 and explained 82% of the total variability, contributing all the variables to the morphological diversity of the collection (Table 3). The quantitative variability exhibited by the studied germplasms indicated that, a good possibility

Principal component	Eigenvalue	Proportion	Cumulative
1	4.9	0.29	0.29
2	2.75	0.16	0.45
3	1.78	0.11	0.55
4	1.71	0.1	0.65
5	1.44	0.09	0.74
6	1.32	0.08	0.82

Fig. 1 Dendrogram of cluster analysis



Omolaja et al. 2000; Vilaró et al. 2004) or may
be due to genetic exchange among germplasms
of *P. sidoides* and its closely related taxa, *Pelargonium*
reniforme found growing in the same
habitat.

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CHAPTER 7

Suitable conditions for the seed germination of *Pelargonium sidoides*: an overexploited herb of South Africa



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Suitable conditions for the seed germination of *Pelargonium sidoides*: an overexploited herb of South Africa.

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Suitable conditions for the seed germination of *Pelargonium sidoides*: an overexploited herb of South Africa.

Abstract

Pelargonium sidoides DC is used in herbal medicine for the treatment of various infections. The roots are being indiscriminately harvested for local and export trade and the collection rate is becoming unsustainable. Seed germination dynamics of this species was studied with the aim of adopting the appropriate germination conditions for use in ex-situ propagation and conservation program. Germination was affected by age of seeds, temperature and pre-chilling conditions. Temperature higher than 25°C reduced germination by 60%. When seeds of *P. sidoides* were subjected to pre-chilling treatment for seven days, there was a significant drop in final germination by 29% compared to the other treatments. Although, final germination was not affected by different light treatments, continuous light significantly promoted germination velocity. Seeds harvested from the plants grown in the green house performed better in germination than those collected from the field. The ecological implications of these observations on the propagation and conservation of *P. sidoides* are discussed.

Keywords: seed germination; *Pelargonium sidoides*; ex-situ conservation; medicinal plant.

Introduction

Cultivation of wild plant species through *ex-situ* propagation is considered a good means of reducing the risk of their extinction (Bowes, 1999). The genetic diversity of local ecotypes is maximized when plants are multiplied from seeds (Fay & Muir, 1990). However, different species have peculiar requirements for seed germination as a means of adapting to changing environmental conditions (Meyer & Monsen, 1991; Schütz & Milberg, 1997). Although, propagation from seed is usually effective and inexpensive, germination requirements for most indigenous species are often unknown. Most affected are the species threatened by high collection pressure, which shortens their lifespan, thereby making it difficult to obtain propagules for further natural regeneration.

Amongst the species affected by high collection pressure in the Eastern Cape, South Africa, is *Pelargonium sidoides* DC (Geraniaceae). It is a rosette-like herb with thickened underground roots and sparsely branched stems from the base. At full bloom, the species is about 20-50 cm high forming a whorl of heart shaped leaves around its base. The species is indigenous to South Africa growing in short grass lands in the Eastern Cape.

P. sidoides is used in traditional medicine for the treatment of different infections including cough, fever, tuberculosis, sore throat, fatigue and weakness of the body. The root is being indiscriminately collected in large quantities to meet the current demand for local and export trade. The increased rate of harvesting and number of plant gatherers have made its exploitation unsustainable. As a measure to controlling overexploitation of this species, the South African Department of Agriculture recently introduced the use of collection permits in order to reduce the rate of *Pelargonium* harvesting. However, this measure, like other medicinal plants (O'Brien & Kinnaird, 1996; McKean, 2003), has not stopped the illegal removal of the species from unprotected community lands. Due to the

increase in the rate of exploitation and lack of organized cultivation, the natural regeneration pattern of the species has been affected. Most gatherers of this species collect both young and adult plants in a process that kills the entire plant. In our preliminary survey (un published), regeneration of *P. sidoides* following harvesting even after one year was as low as 10% in some areas. This practice has limited the process of natural regeneration thereby negatively affecting its demography in the natural habitat. A practical means of addressing over exploitation of this plant is to develop propagation techniques and encourage its cultivation. Seed germination is an important determinant in the distribution of a species. It is therefore, critical to study and understand the various factors that could affect the germinability of the seeds of any plant species before embarking on its propagation and conservation. Although natural regeneration of this plant is by seed and from the development of perennates through the underground root system, no information is available on the factors that could affect the germination of this species in the natural habitat. The objective of this study was to determine the suitable conditions of germination of *P. sidoides*, for the overall purpose of encouraging *ex-situ* propagation and conservation of the species.

Materials and methods

Seed collections

Seeds for the first germination experiment was obtained from matured plants collected during the summer of 2004 from plants growing in three natural populations, Grahamstown, Khayamnandi and Wesley, all in the Eastern Cape Province, South Africa (latitude 32° 09'-33° 58'S, longitude 26° 48'-27° 61'E and altitude 304-695m above sea level). The vegetation of the area is rich in diverse flora with a mean annual rainfall of

about 700 mm and average temperature range of 18 to 25°C. After initial viability test, one half of the seeds collected were stored in opened glass bottle at room temperature (18-25°C) for one year. Another set of seeds were collected from plant materials raised in the green house of the Faculty of Science and Agriculture, University of Fort Hare, for the second germination experiment. The green house was maintained at 18 - 26°C and at 60% relative humidity. All seeds collected from plants growing in the green house were used within one month after harvesting.

Viability test

To determine the viability of the seeds, samples from each location were separately subjected to viability tests using the tetrazolium technique (Grabe, 1970). Seeds were imbibed for 24 h in water, cut along the margin without damaging the embryo, and soaked in colorless 2,3,5-tetraphenyltetrazolium chloride (TTC) solution for 16 h at 25°C in the dark. The seeds were then removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol to permit direct observation of the embryo. The embryos of viable seeds appeared reddish in colour. Three replicates of 50 seeds each were examined.

Germination Tests

Germination trials were conducted in 9-cm sterile Petri dishes lined with two Whatman No. 1 filter papers and moistened with sterile double-distilled water to ensure adequate moisture for the seeds. Treatments were arranged in a randomized complete block design with three replicates of 60 seeds each. Seed treatments included continuous light, continuous darkness and 16/8 hours light/dark photoperiod. Other treatments were; one, three and seven days pre-chilling at 4°C. The effect of temperature was determined by

placing the Petri dishes in germination chamber (incubator) for 35 days at constant temperatures of 25°C, 30°C, 35° and under alternating day/night temperature (18-25°C) and light (16 hours daylight/8 hours night) as outlined by the Association of Official Seed Analyst (AOSA, 1993). Seeds were surface sterilized in 0.1 mercuric chloride solution for 60 sec to prevent fungal attack and rinsed in several changes of sterile water. For treatment subjected to continuous darkness, the Petri dishes were covered with aluminum foil and daily observations were carried out under green safe light. Light treatments were exposed to continuous illumination produced by white fluorescent tubes with a mean photon flux density of 24 $\mu\text{mol}^{-2} \text{s}^{-1}$. The fluorescent tubes also provided the source of red light. The seeds were examined daily. A seed is considered germinated when the radicle was visible. At the end of the trials, final percentage values of 0% were replaced with 0.0125% (Gomez & Gomez, 1984) before arc-sine transformation for linearity. Data were subjected to statistical analysis using the SAS package. Where significant differences were observed, means were separated by LSD at 5% level of probability (SAS, 1999). Germination velocity was evaluated through the Kotowski Coefficient of velocity (González-Zertuche and Orozco-Segovia, 1996):

$$CV = (\sum n_i / \sum (n_i t_i)) * 100,$$

where n_i is the number of seeds germinated on day i , and t_i is the number of days after sowing. A high CV represents high germination velocity.

Results and Discussion

Viability test

The viability of *P. sidoides* collected from different populations dropped by over 20% after one year of storage (Table 1). The viability of several wild species have been

reported to decrease with time (Vakeswaran & Krishnasamy, 2003a). The germination percentage of seeds collected from the wild was subsequently low. This is in agreement with early study on over 600 species documented in literature (Leist & Krämer, 2003). There were slight differences in the viability of seeds collect from different locations and this may be due to factors induced by micro climates.

Source of seeds and Rate of Germination

Seeds collected from the wild and stored for one year had less than 10% percentage germination; hence, results are not presented. Seeds raised in the green house under a relatively stable environment (with 94% viability) gave better germination responses in terms of dynamics and total germination percentage. For instance, seeds harvested from the green house (within one month) had higher coefficients of velocity (Table 2) compared to one month old seeds collected from the wild (high CV represents high germination velocity) and the final germination percentages were significantly higher at $p < 0.01$. Seeds pre-chilled for 7 days and those incubated at temperatures higher than 25°C also showed similar effects (Table 2). Continuous light significantly ($p > 0.05$) promoted germination velocity (Table 2); a condition typical of small-seeded plant species (Pons, 1992; Clarke et al., 2000).

Seeds collected from the green house and germinated under continuous light had 100% germination while the highest germination percentage recorded from seeds collected from the wild was 21% (Figure 1). The maximum percentage germination of 21% obtained from seeds harvested from the wild is very low. This result is in agreement with viability test result. *P. sidoides* grows naturally in the wild where temperature ranges from 31°C in summer to 2°C in the winter. Viability and subsequently the germination of seeds

collected from the wild could have been affected by fluctuation in different elements of climate. This combined with the practice of over exploitation explain why there is low natural regeneration with consequent low plant populations of *P. sidoides* in its natural habitat.

Cold Treatment

Subjecting seeds of *P. sidoides* to lower temperatures (4°C) prior to germination for seven days resulted in a significant negative effect on the percentage germination when compared to the control (Figure 2). Viable seeds of *P. sidoides* germinate *in-situ* after sufficient rains are received in the months of October to March when the temperature are relatively cool. The average temperatures at this time of the year range between 18 and 25°C. Thus, this provides the ideal conditions for germination to take place. Although cold stratification, has been reported to break dormancy of viable seeds and enhance germination in some species (Baskin et al., 2001), it is also documented to cause lethal effect on viable seeds (Ren & Tao, 2004). This study agreed with the latter report. When seeds of *P. sidoides* were subjected to pre-chilling treatment for seven days, there was a significant ($p < 0.01$) drop in germination by 29% compared to the other treatments.

Temperature

Figure 3 shows the time course of germination of *P. sidoides* under different incubation temperature conditions. Although, initial germinations were observed within the first two days of incubation, high temperature (30 and 35°C) negatively affected final germination percentage. There was a progressive drop in germination percentage (by 30%) at every 5°C rise in temperature (above 25°C) resulting to a 60% loss in germination probably due to loss of viability induced by increased temperature. Although germination is usually

controlled by a combination of factors which include moisture, light, temperature and photoperiod (Bell, 1999; Kambizi et al, 2005), temperature as been implicated as a primary environmental factor regulating seed germination (Mott, 1972; Baskin & Baskin, 1988).

From this study, it is evident that germination of seeds of this species is dependent in part, on temperature condition, pre-chilling and sources of seed. Thus, it is suggested that seeds be raised from controlled environment and planted within short time (when viability is relatively high) in the nursery for subsequent transfer to the field as an ex- situ conservation initiative and as buffer to supplement wild collection. A method for the optimum temperature condition for the seed bank conservation of the seeds of this species is currently in progress in our institute.



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Table 1. Effect of source of seed and duration of storage on the viability of *P. sidoides*.

Duration	Seed viability (%)		
	Khayamnandi	Grahamstown	Wesley
One month	51.67 (2.01) ^a	62.0 (2.59) ^{a*}	45.67 (2.01) ^a
One year	36.0 (2.59) ^b	36.67 (2.60) ^b	35.33 (2.59) ^b

Note: Values represent percentage mean viability of seeds of *P. sidoides*, one month and one year after harvesting (replicated three times) with standard error in parenthesis. Means with different letters in a column are significantly different at $p < 0.01$. *Indicates statistical significance within the row for seeds harvested within one month at $p < 0.01$.

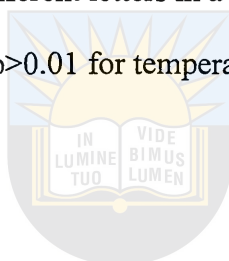


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Table 2. Mean Kotowski's Coefficient of Velocity for all the experiments performed on the seeds of *P. sidoides*

Pre-chilling		Temperature		Light			
				<u>Screen house</u>		<u>Wild</u>	
One day	11.07 ^a	25 ⁰ C	9.14 ^{ab}	Alternate	8.43 ^{ab}	Alternate	4.46 ^a
Three days	10.54 ^a	30 ⁰ C	7.46 ^b	Continuous light	9.74 ^a	Continuous light	4.60 ^a
Seven days	7.21 ^b	35 ⁰ C	1.43 ^c	Continuous dark	7.75 ^b	Continuous dark	4.31 ^a
Control	10.67 ^a	Control	10.33 ^a				

Note: Means (n = 3 replicates) with different letters in a column are significantly different at $p < 0.05$ (pre-chilling and light) and $p > 0.01$ for temperature.



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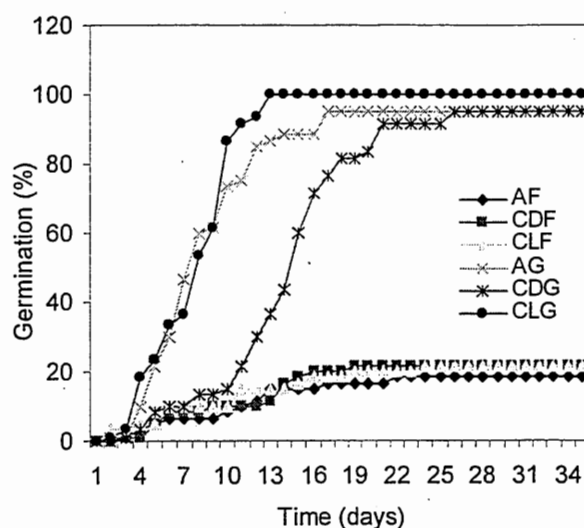


Figure 1. Cumulative germination percentage of *P. sidoides* collected from the wild (homogenized from three populations) and green house tested under the three light conditions. AF, CDF and CLF: alternating light and dark, continuous dark and continuous light collected from the field respectively; AG, CDG and CLG: alternating light and dark, continuous dark and continuous light collected from the glass house respectively.

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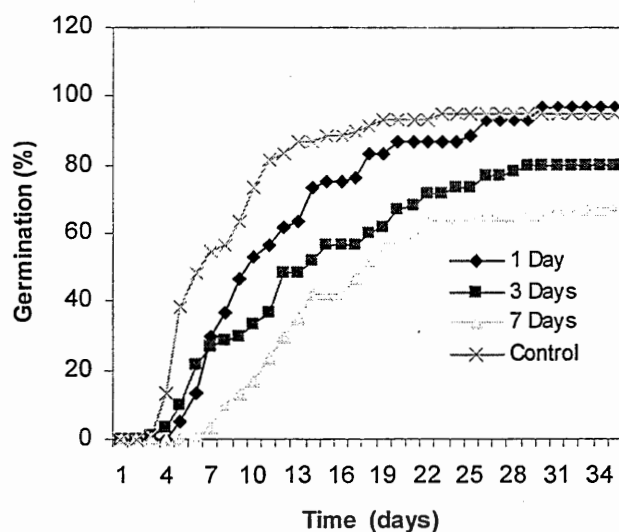


Figure 2. Time course of germination of *P. sidoides* in response to pre-chilling treatment (4°C) for 1, 3 and 7 days incubated under alternating light and darkness conditions and at $18\text{-}25^{\circ}\text{C}$. Results are expressed as means of three replicates.

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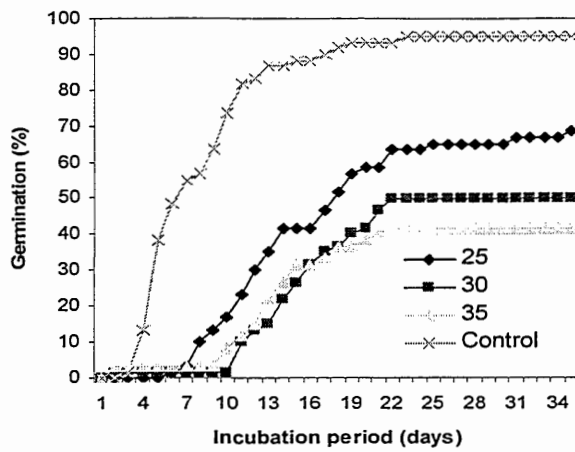


Figure 3. Effects of temperature on the cumulative germination of *P. sidoides*. All seeds used in these experiments were harvested from the green house within one month before the study.



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CHAPTER 8

Micropropagation of *Pelargonium sidoides*: a

South African herb



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CHAPTER 8

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*This chapter is being considered for publication in this format in the '**Journal of plant cell, tissue and organ culture**'

Micropropagation of *Pelargonium sidoides*: a South African herb.

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Abstract

Pelargonium sidoides is a medicinal plant harvested for local and export trades in South Africa. In order to contribute to the propagation initiative of the herb, a preliminary study on the micropropagation of the plant was conducted. Leaf, seed and stem explants were used to initiate callus formation with two separate hormones. Our findings revealed that Callus induction was achieved by using different concentrations of IBA and 2,4-D. Optimal response was obtained at 3 mg l⁻¹ IBA under continuous dark condition. The study also showed that shoot differentiation is achievable from stem derived callus after one week in hormone free media under continuous light. The percentage of callus formation ranged from 0 to 88.9% depending on the type of hormones used, the concentration and photogenic conditions. Transferred plantlets by simple acclimation procedure survived and produced healthy plants. The significance of these on the propagation and conservation of *P. sidoides* is highlighted in the discussion.

Key words: *Pelargonium sidoides*; hormones; callus induction; medicinal plants.

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Introduction

Herbal medicine is widely used in South Africa and most of the plants used for this purpose are harvested from the wild populations. Apart from local uses of medicinal plants, there is a current upsurge in their harvesting by the rural communities mainly for local and international trades (Cunningham, 1988; Lange, 1997). This practice has negative affect on plant demography especially in unprotected lands (Ake-Assi, 1988; Kokwaro, 1991; Cunningham and Mbenkum, 1993; O'Brien and Kinnaid, 1996; Gu, 1998; M^cKean, 2003; Ghimire et al., 2005; Tang et al., 2005). Among such plant species facing the danger of over-harvesting is *Pelargonium sidoides* DC.

Pelargonium sidoides is used by the people of Eastern Cape for the treatment of various diseases in human and livestock (Batten and Bokelmann, 1966). The use of this plant for the treatment of various diseases by the people of Eastern Cape, has been validated through microbial bioassay (Lewu et al., 2006c). In Germany, Schwabe Pharmaceutical produces *Umckaloabo* from the roots of *P. sidoides* imported from South Africa. The product is said to be effective against bronchitis in children (Van Wyk et al., 1997; Matthys et al., 2003; Van Wyk and Gericke, 2003; Donald and Brown, 2004). Due to the high demand for the species, the rate of harvesting has exceeded the rate of natural regeneration. Our preliminary study indicated that the viability of seeds collected from the natural population is very low followed by low seed germination. In continuation of our effort to promote the conservation and propagation of this plant both in situ and ex

situ (Lewu et al., 2006a, b) we report our findings in this paper, the initiation of tissue culture protocol for the clonal propagation of the species.

Materials and methods

Plant materials

The experiments were carried out in the phytomedicine laboratory of the Department of Botany, University of Fort Hare, South Africa. For stem and leaf cultures, plants raised in the greenhouse were used as the source of primary explants. Seeds were surface sterilized with 70% ethanol for two minutes, and 0.1% mercuric chloride for 15 minutes. The sterilized seeds were rinsed several times with double distilled sterile water and soaked for 24 h. The swollen mature embryos were removed from seeds with a scalpel and were then plated cut-side down on induction media. The leaf and stem explants were sterilized by immersing in 70% ethanol for 30 sec, followed by 0.1% mercuric chloride for 5 minutes. The explants were rinsed in several exchanges of double distilled water before dissecting into sections and culturing as described below.

Callus induction

The callus induction medium contained Murashige and Skoog's (MS) basal salts, supplemented with 1.0 - 4.0 mg l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) or indole-3-butyric acid (IBA), Na₂EDTA (7.4g.l⁻¹), myo-inositol (20 g l⁻¹), thiamine-HCl (0.1 g l⁻¹), 2.0 mg l⁻¹ glycine, 690 mg l⁻¹ proline, sucrose (30 g l⁻¹) and was solidified with 5 g l⁻¹ Difco bacto-agar. The pH was adjusted to 5.8 and the media were sterilized by

autoclaving at 121°C for 20 min. The embryos were incubated for callus induction on the media at 25 ±3°C under continuous illumination with a photosynthetic photon flux density of 184.8 (±5) $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by cool-white fluorescent lamps. The same experiment was duplicated under continuous dark condition in three replicates. Twenty four mature embryos were inoculated per treatment making a total of 48 samples for both light and dark experiments. After three weeks of culture on induction medium, the percentage of mature embryos producing primary calli were determined, and the calli were then cut into smaller sizes and transferred to the same medium for another two weeks.



Callus subculture and shoot differentiation.

The basal composition of the subculture medium was the same as that of the induction medium except for the removal of plant growth regulators and the reduction of agar to 4 mg l⁻¹. The cultures were transferred onto fresh subculture medium every two weeks and were maintained at 25 ±3°C under continuous illumination. Each callus was cut into smaller pieces (approximately 0.5 g fresh weight) during transfer and subcultured three times. After three weeks, the percentage of calli forming shoots were recorded.

Regenerated shoots rooted in the same medium without growth hormones. Rooting percentage, number and length of roots were recorded after three weeks on the rooting media. At about 4 cm height and with four visible leaves, the plantlets with healthy-looking roots were removed from culture, rinsed in water (to remove media) and transplanted into a mixture of equal parts (v/v) of sterilized soil and vermiculite. They

were watered with half-strength MS salts solution and acclimatized under humid conditions in plastic pots.

Data analysis

The callus induction experiment was analyzed in a factorial pattern with hormones and light being the main factors. Two hormones (2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-butyric acid (IBA) at four levels each were tested under continuous light and darkness conditions. The first data was analyzed using a proc GLM model of the SAS package in a factorial arrangement. Duncan Multiple Range test ($P < 0.05$) was used for multiple mean comparisons of the interactions between the different levels of hormones and light. In the second experiment, only callus derived from stem source differentiated to shoot after two subcultures in hormone-free media in the continuous light condition. The analysis of the data was performed using proc GLM analysis and means were separated by LSD (SAS, 1999).

Results and Discussion

Effect of hormones on callus formation

Table 1 shows the effect of hormones on the formation of callus from seeds and stems of *Pelargonium sidoides*. The percentage of callus formation ranged between 0 to 63.9% with 3 mg l⁻¹ of IBA being the optimum concentration in the study. IBA generally showed good potential for callus initiation in *P. sidoides*. With the result obtained from this experiment, it appears that callus formation in this plant could be impaired from any

concentration above 3 mg l⁻¹ as the explants did not produce any callus above this concentration in both hormones used. Unlike other reports on many species (Chang et al., 2000; Cucco and Rossi, 2000 Wang et al., 2003 Feeney and Punja, 2003), 2,4-D generally showed limited influence on callus formation in *P. sidoides* with the highest percentage being 13.9% at 2 mg l⁻¹ (Figure 1). This concentration is however, consistent with the general range of concentration (1.0-3.0 mg l⁻¹) reported in the literature. Only about three calli were formed from leaf culture and did not survive during the process of the first subculture. Therefore, the result of the leaf culture is not included in the study.

Influence of hormones and light on callus formation

Table 2 shows the morphogenic response of seed and stem explants of *Pelargonium sidoides* to the two hormones under two photogenic conditions in a factorial arrangement. The percentage of callus formation ranged between 0 to 88.9% depending on the type of hormone, the concentration, part of explant used, and photogenic conditions. The interesting part of the results is the interaction between the different levels of hormones and the two levels of light conditions considered in the study. Apart from cases where no callus were formed, statistical analysis showed significant (<0.01) interactions between all levels of hormones and the two light conditions tested in the study. Callogenesis was generally influenced by hormones and photogenic conditions. However, IBA at 3 mg l⁻¹ and under continuous dark condition demonstrated the optimum conditions for callus formation especially in seed explants (88.9%) and the best yield obtained from 2,4-D (19.5%) was under continuous light condition. Seed callus had the highest weight of 1.6 g and about 2 cm radius after 21 days in initiation media (Figure 1a).

Shoot differentiation, root formation and acclimatation

Two initial subcultures of callus from the two explants (seed and stem) sources in their respective hormones did not produce any shoot. After two repeated cultures in hormone free media, the callus formed from seed source did not differentiate to shoot either. However, stem derived calli produced shoots (average of two shoots per callus) in hormone free full MS medium in the first subculture (Table 3 and Figure 1b). In this study, the rate of stem formation from callus was very low with an optimum of 27.8% in 3 mg l⁻¹ IBA derived callus followed by 2 mg l⁻¹ 2,4-D. Explants derived from 3 mg l⁻¹ IBA demonstrated significant (<0.01) yield advantage over others thereby producing more healthy plantlets. Rooting was interestingly achieved in the hormone free media with an average of 1 to 2.7 roots per plant. Conventionally, this species perennates itself in the wild through the under ground root systems. The ability to produce roots without rooting hormone may be connected to this innate quality. All the plantlets IBA derived explants produced a single long and thin root while plantlets derived from 2,4-D produced between two and three but shorter root systems. Plantlets transferred by simple acclimatation procedure involving the use of equal volume (v/v) of sterilized soil and vermiculite produced healthy plants (Figure 1c).

Micropropagation techniques have been one of the recently used tools for the propagation and conservation of threatened and endangered plant species (Castillo and Jordan, 1997; Saxena et al., 1997; Murch et al., 2000). With increasing demand for herbal based medicines in the local and international market (Cunningham, 1988; Lange, 1997), micropropagation technique has become a necessary tool for the conservation and

multiplication of several plant species. Our findings revealed that Callus induction was achieved by using different concentrations of IBA and 2,4-D. Optimal response was obtained by the use of IBA at 3 mg l⁻¹ under continuous dark conditions. The study also showed that shoot differentiation is achievable from stem derived callus after one week in hormone free media under continuous light condition.

Acknowledgement

We thank the National Research Foundation of South Africa for financial support.



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Table 1. Influence of hormones on the formation of callus from the seeds and stems of *Pelargonium sidoides* after three weeks in culture

Hormones (mg l ⁻¹)	Mean value of callus from seed and stem (%)
indole-3-butyric acid (IBA)	
1	*18.1 ^b
2	4.2 ^d
3	63.9 ^a
4	0 ^e
2,4-dichlorophenoxyacetic acid (2,4-D)	
1	
2	11.1 ^c
3	13.9 ^c
4	0 ^e
	0 ^e

Values followed by the same letter in the column are not significantly different at

$P < 0.01$. *The result shown above is a statistically mean value of the percentage yield of *P. sidoides* as influenced by hormones at different concentrations.

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Table 2. Effects of the factorial interaction of hormones and light on the formation of callus from the seeds and stems of *Pelargonium sidoides* after three weeks in culture

Hormones (mg l ⁻¹)	Callus formed from seed explant (%)	Callus formed from stem explant (%)	Light condition ^a
indole-3-butyric acid (IBA)			
1	13.9*	0	+
2	8.3	0	+
3	38.9	44.5	+
4	0	0	+
1	22.2	8.3	-
2	0	0	-
3	88.9	44.5	-
4	0	0	-
2,4-dichlorophenoxyacetic acid (2,4-D)			
1	8.3	8.3	+
2	19.5	8.3	+
3	0	0	+
4	0	0	+
1	13.9	0	-
2	8.3	11.1	-
3	0	0	-
4	0	0	-

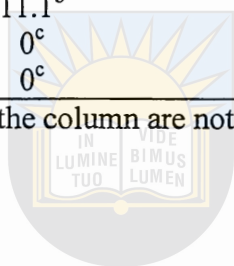
^a+ indicates continuous light and – indicates continuous darkness.

*There was significant ($P<0.01$) interaction between the two levels of light and both hormones levels tested except for values with zero. All values are presented as means of three replicates.

Table 3. Initiation of shoots and roots from stem derived callus after three weeks in culture

Sources of callus (mg l ⁻¹)	Callus differentiating to stem (%)	Average length of roots (cm)	Average number of roots
indole-3-butyric acid (IBA)			
1	8.3 ^b	6.6 ^b	1 ^b
2	0 ^c		
3	27.8 ^a	7.4 ^a	1 ^b
4	0 ^c		
2,4-dichlorophenoxyacetic acid (2,4-D)			
1	0 ^c		
2	11.1 ^b	3.2 ^c	2.7 ^a
3	0 ^c		
4	0 ^c		

Values followed by the same letter in the column are not significantly different at $P < 0.01$



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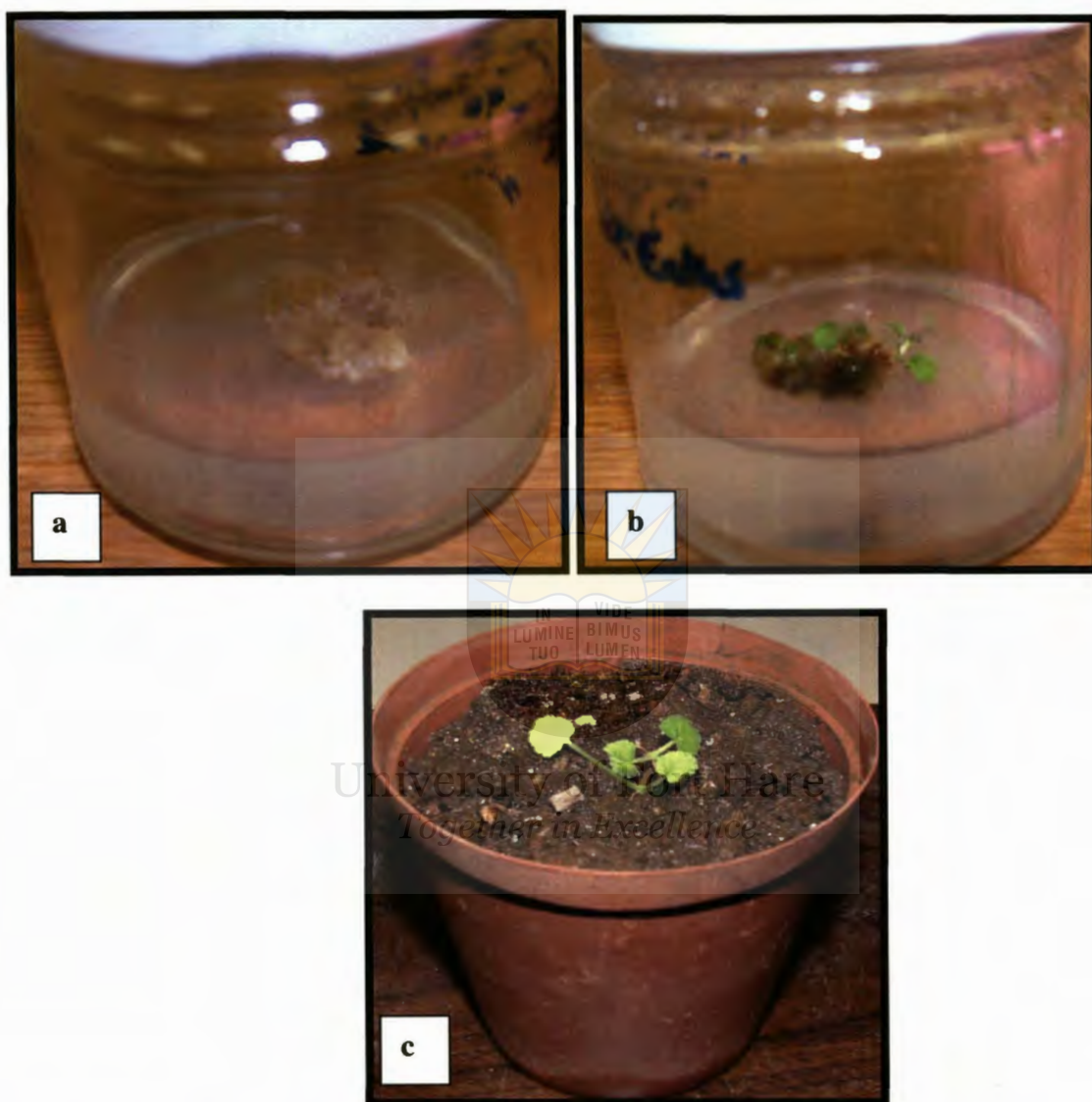


Figure 1a. Callus formed from the seed of *Pelargonium sidoides*, b; shoot formed from stem derived callus two weeks after subculture, c; transplanted explant of *Pelargonium sidoides* after five weeks in media.

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CHAPTER 9

General Discussion and Conclusion



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CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

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GENERAL DISCUSSION

Today, an estimated 80% of the population of South Africa makes use of indigenous medicinal plants for the treatment of various ailments in man and livestock (Kelmanson *et al.*, 2000). Unfortunately however, most of the plants are harvested from wild populations and are not cultivated (Mander, 1997). In addition to the local trades of about 700 species of plants in South Africa (Mander *et al.*, 1997), some other valuable species are also traded in the international market (Lange, 1997). Most of these herbs are over-exploited in their natural habitats thereby driving them to near extinction (Williams *et al.*, 2000). The most affected of these are species harvested for their underground bulbs or roots (Cunningham, 1991; Rock *et al.*, 2004) among which is *Pelargonium sidoides*. Considering the rapid rate of harvesting of this species from the wild, the need to study and document the propagation and conservation techniques for the herb cannot be over emphasized.



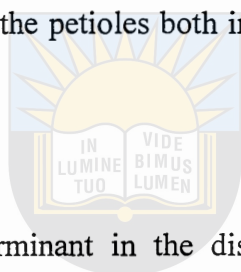
The field survey carried out as part of this study has revealed that over-harvesting has become a threat to the survival of *P. sidoides* in the wild. Due to its medicinal tuberous root, the plant is completely removed from the soil by plant harvesters. This activity is negatively affecting its demography. The low rate of rejuvenation coupled with the destructive method of harvesting has led to the unsustainability of the exploitation of the plant. The harvesting method also exposes the soils to agents of erosion. Although the collection and sale of *P. sidoides* currently plays a major economic role in the livelihood of the people of the Eastern Cape, however, for sustainable harvesting of the plant from the wild, it is important that the collectors consider its ecology and regeneration potential.

Pelargonium sidoides has been used traditionally for the treatment of various respiratory diseases by the people of the Eastern Cape for a long time. *Bacillus cereus* and *Klebsiella pneumoniae* are respiratory bacterial pathogens commonly associated with infectious diseases (Viljoen *et al.*, 2004). All the extracts showed significant activity against *Bacillus cereus* while acetone root extracts was active on *Klebsiella pneumoniae* at 10 mg ml⁻¹. The inhibitory property of extracts from *P. sidoides* against pathogens of respiratory infections might have justified the use of this plant for the treatment of these diseases by the people of the province. Kayser and Kolodziej (1997) reported similar antibacterial effect of this herb against *K. pneumoniae* in Europe. Although, the MIC values obtained in the antimicrobial studies were higher than results of other plant species (Feresin *et al.*, 2001, Eldeen *et al.*, 2005), this report compared favorably with early report on *P. sidoides* (Veronique and Taylor, 2004). The acetone and methanol extracts showed significant inhibition against most of the fungi tested in this study. The ability of the extracts of this plant to inhibit the growth of several bacteria and fungi is an indication of its broad spectrum antimicrobial potential which further validates the use of this plant for the treatment of various ailments by the people of the Eastern Cape.

Considering the results of the comparative antibacterial experiments carried out on the aerial and subterranean parts of *P. sidoides*, it was interesting to note that, generally, both the shoots and the roots of the plant have similar antimicrobial properties. There was no significant observable difference between the MICs of extracts from the two plant parts. This may also be true with their medicinal values. The choice of the root by the traditional healers over the leaves may therefore, be arbitrary or due to easy collection. The leaves of *P. sidoides* may, as well, substitute for its roots in medicinal formulations especially in the treatment of bacterial diseases like tuberculosis. Scientific information such as this could enlighten the collectors of this species and encourage them to

collect only the shoots rather than the underground parts.

Conservation of *P. sidoides* through cultivation may be the plausible way to save the species from extinction in the Eastern Cape. However, considering the low germination rates of its seeds, it may be necessary to use other parts of the plant as propagules. Usually, the vegetative aerial parts of the herb are conventionally thrown away and wasted during the collection of the roots for medicinal trade. As part of this study, both the petioles and vines (different lengths) were used as propagules. Although, 6 cm vine length showed good yield success and survivability, the result demonstrated a high potential for clonal propagation via the petioles both in terms of rooting ability and survival rate.



Seed germination is an important determinant in the distribution and survival of a species. Considering the low rate of germination of the seeds of *P. sidoides* in the wild, it became essential to investigate the germination requirements of its seeds. The effects of pre-chilling, source of seeds, temperature and light in the germination of its seeds were investigated. Statistically, germination dropped significantly ($p < 0.01$) by 29% after pre-chilling for seven days. Seed germination was also affected by temperature. There was a progressive drop in germination percentage at every 5°C rise in temperature resulting in a 60% loss in germination. The optimum temperature for germination in the study was 25°C. Exposure to continuous light promoted seed germination velocity which is characteristic of small-seeded species (Pons, 1992; Clarke et al., 2000).

Seeds harvested from *P. sidoides* cultivated under a controlled environment had significantly higher germination than seeds collected from the wild. Also it was discovered that the seeds raised

in the greenhouse under a relatively stable environment gave better germination responses in terms of dynamics and total germination percentage. From this study, it was evident that germination of seeds of this species is dependent in part, on light, temperature condition, pre-chilling and sources of seed. Thus, it is suggested that seeds be raised from controlled environment and planted within short time (when viability is relatively high) in the nursery before subsequent transfer to the field as an *ex situ* conservation initiative and as buffer to supplement wild collection.

In our survey, it was observed that there exist some variations among germplasms of *P. sidoides* found growing in different natural habitats. Plant materials were collected from three different populations and morphologically characterized in order to delineate the accessions of the species found growing within the Eastern Cape Province. Qualitative and quantitative characters measured revealed high variability between and within populations of the species. Cluster analysis showed unique clustering of accessions forming 29 morphotypes (clusters) out of the 30 accessions studied. Accessions exhibiting high production of foliage could be cultivated by clonal propagation to meet the increasing demand for the species in order to take pressure off wild stock. The quantitative variability exhibited by the studied germplasms indicated that a good possibility exists for developing promising cultivars of *P. sidoides* from desirable traits.

Conclusions and Recommendations

- In South Africa, herbal medicine still plays a vital role in the health delivery system of the people, especially in the rural communities where there is little or no access to western medicine.
- This study provides the baseline information on the local medicinal use, propagation and conservation techniques for *P. sidoides*.

- Over harvesting of *P. sidoides* for traditional use and medicinal trade (both local and international) have resulted in its low population in its natural habitat.
- Our findings showed the possibility of using the aerial parts of the plant for antimicrobial formulation instead of the root suggesting *in situ* conservation and sustainable use of the herb.
- Vegetative propagation of the herb was achieved through the vines (6 cm) and petioles.
- Successful seed production for the species will require raising seeds from a controlled environment as germination was affected by light, source of seeds, temperature and pre chilling-conditions.
- Currently, there exist accessions of *P. sidoides* in the wild populations of the species within the Eastern Cape. Using morphological finger print, our findings revealed 29 morphotypes out of the 30 accessions studied suggesting high genetic variability in its natural habitat.
- Further exploration of its natural habitat may help capture genetically desirable traits for the purpose of genetic improvement and conservation.
- The initiation of tissue culture protocols for the plant may open way for rapid propagation of the herb.
- It is recommended that the report of this study be made available to the communities in the Eastern Cape where high level of genetic erosion of the species is in rapid progress.
- The potential for accelerated expansion by micropropagation of selected plant materials of high concentration of antimicrobial properties with promising potential for yield could be further exploited in future research.

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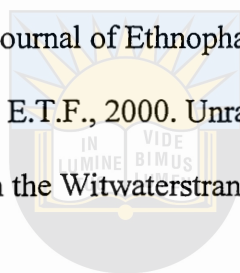
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Department of Botany
Impact assessment of *Pelargonium sidoides* on Users in the Eastern Cape Province.

Questionnaire

Questionnaire no.....

Date

Sex

Age

Village

Location

Pelargonium sidoides

1. What is the common name of *P. sidoides* in your area?
2. How long have you known *P. sidoides*?
3. Do you use this plant?.....
4. For what?
5. Where do you get the plant market or bush?
6. What diseases do you use this plant to cure?

A. Users

1. Place of res.....
2. Tribe
3. Family representation (father, mother, etc)
4. Highest western education obtained
5. Any formal training on the use of *P. sidoides*?
6. Any gender restriction in the trade on *P. sidoides*?.....
7. List reasons for 6 above
8. What is the average income from *P. sidoides* per month?
9. Any known alternative to *P. sidoides* in terms of medicinal use?.....
10. What part of the plant do you usually collect for sale?.....
11. Who are the major buyers of this plant?.....
12. What part of plant do they buy?.....
13. Can you give an estimate of the quantity of plant part (kg) collected per month?.....

Appendix A2
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Department of Botany

Exploration Form for *Pelargonium sidoides*

1. Collection no.		2. Accession no.	
3. Collector		4. Day/month/year	
5. Latin name			
6. Cultivar name		7. Ethnic group	
8. country		9. Province/state	
10. Precise location Km. N/S/E/W of to			
11. Latitude N/S	12. Longitude E/W	13. Altitude M	
14 Vegetation			
15. Status: 1. Wild 2. weed 3. cultivated		16. Frequency: 1. Abundant 3. Occasional 2. Frequent 4. Rare	
17. Source: 1. Crop 3. Wild vegetation 2. Market 4. Farm store 5. others		18. Material: 1. seed 2. vegetative parts	
19. Other data.			

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