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South African Journal of Botany 74 (2008) 260–267

SOUTH AFRICAN
JOURNAL OF BOTANYwww.elsevier.com/locate/sajb

Measuring and optimising umckalin concentration in wild-harvested and cultivated *Pelargonium sidoides* (Geraniaceae)

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Received 24 July 2007; received in revised form 18 November 2007; accepted 29 November 2007

Abstract

Pelargonium sidoides DC. (Geraniaceae) root extracts are used locally and globally as herbal medicines. Subsequently, high levels of wild root harvest in the years preceding this study, to supply international demand for raw materials, prompted this investigation of the prospects for sustainable root harvest through wild collection and greenhouse cultivation. A novel method was developed for the purification of umckalin, a bioactive constituent in root extracts, such that the root umckalin concentrations of wild and cultivated plants could be quantified by HPLC. A geographical survey of wild plants revealed that root umckalin concentrations were inversely related to the average annual rainfall of the collection site ($r^2=0.43$, $p<0.0001$) and directly related to soil pH ($r^2=0.46$, $p<0.0001$). Thus, the possibility of inducing high umckalin concentrations in greenhouse-cultivated plants was investigated by subjecting plants to water stress. This treatment, and those using leaf applied hormones (cytokinin and gibberellin) and root competition with a fast growing annual (*Conyza albida*), did not significantly affect root umckalin concentrations compared to well-watered controls. However, greenhouse-cultivated control plants showed wild equivalent umckalin concentrations and circa six times greater growth rates than plants in a wild harvest experiment. These results support the ex situ cultivation of roots to supply future market demand.

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Keywords: Conservation; Coumarin; Cultivation; EPs 7630; *Pelargonium sidoides*; Umckalin; Umckaloabo; Wild harvest

1. Introduction

Many South African medicinal plants, including *Pelargonium sidoides*, *Harpagophytum procumbens* and *H. zerheri* (devil's claw), *Aloe ferox* (Cape aloe) and *Agathosma betulina* (buchu), are used in the production of internationally marketed herbal remedies for the treatment of various ailments (Van Wyk et al., 2000). As a result, the wild stocks of these plants are susceptible to over-exploitation.

Traditionally, infusions made from the tuberous, woody roots of *P. reniforme* and *P. sidoides* are used for the treatment of diarrhoea, dysentery, colds and lung infections, including tuberculosis (Bladt, 1977; Van Wyk and Gericke, 2000). This usage is particularly prevalent amongst the Xhosa people of the Eastern Cape Province of South Africa. In addition to these traditional

uses, commercial *P. sidoides* root extracts have become popular (e.g. *Pelargonium*, Medicherb UK; *Pelargonium* Syrup, Bioharmony Africa and Umckaloabo[®], Spitzner) for the treatment of upper-respiratory tract infections. These are available internationally in countries including Germany, Mexico, Turkey, Brazil (e.g. Matthys et al., 2003; Chuchalin et al., 2005) and South Africa (White, 2007). The bioactive constituents isolated from *P. sidoides* root extracts include: the coumarins umckalin and 6,8-dihydroxy-5,7-dimethoxycoumarin (Kayser and Kolodziej, 1997), gallic acid and its methyl ester (Kayser and Kolodziej, 1997), (+)-catechin (Kolodziej et al., 2003), and certain fatty acids (Seidel and Taylor, 2004) and tannins (Kolodziej et al., 2005).

This combined traditional and commercial use has led to the extensive harvest of wild plants in the Eastern Cape. Harvest is not restricted to *P. sidoides*, the species preferred by the international market, as *P. reniforme* plants are mistakenly harvested due to their very similar appearance to those of *P. sidoides*, except when in flower. Despite the demand for harvested roots, speculation still

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surrounds the quantity of these species harvested and the sustainability of such harvesting. Popular articles in two newspapers and one online publication reported the uncontrolled harvest of at least 20 tonnes of *P. reniforme* and *P. sidoides* roots in the Eastern Cape in 2002 (Bisseker, 2002; Gerardy, 2002; Limson, 2002).

This large and potentially damaging harvest of wild *Pelargoniums* has caused alarm amongst conservationists and raised the need for research addressing the development and implementation of sustainable harvesting practices for wild populations; and methods for the effective cultivation of this species (the World Health Organization, WHO et al., 1993). The potentially sustainable methods that have been investigated are the substitution of leaf for root harvests (Lewu et al., 2006a), and the replanting of vegetative aerial plant parts after root harvests (Lewu et al., 2006b). Lewu et al. (2006a) compared the antibacterial activity of *P. sidoides* leaf and root extracts and found no significant differences. However, the overall effectiveness of *P. sidoides* extracts in clinical trials (Matthys et al., 2003; Chuchalin et al., 2005) appears to be due to a combination of immunological and antimicrobial activities, shown in vitro (Kolodziej et al., 2003; Kolodziej et al., 2005; Mativandlela et al., 2006; Trun et al., 2006). As the immunological activity of leaf extracts has not yet been determined, further investigations are required before this leaf substitute can be used commercially. Additionally, Lewu et al. (2006b) found that plants can be easily propagated by planting excised petioles, which would otherwise be discarded following root harvest. Such practice is applicable to the ex situ cultivation of plants, but not for the conservation of wild populations in arid environments where supplementary watering would not be feasible.

In medicinal plant cultivation, the use of plant breeding techniques and selecting stock with high bioactivity can provide plants that are genetically uniform, high-yielding, and that produce a less variable final product (WHO et al., 1993). Selecting high-yielding stock is possible as the yield and composition of secondary metabolites of certain medicinally important plants has been found to vary within and between populations (Van Wyk et al., 1995; Wills and Stuart, 1999; Homer et al., 2000; Binns et al., 2002; Kim et al., 2006). Such variation may be influenced by geographic and environmental factors. Such factors may be manipulated in cultivation to optimise the yield of bioactive compounds. Coumarins, the class of compounds to which umckalin belongs, have been hypothesised to be produced in response to water stress (Ojala, 2001) and have an allelopathic function (Uritani, 1999; Urdangarin et al., 2004). In addition to responding to environmental factors, the production of certain coumarins can be increased by the application of plant hormones (Pastřová et al., 2004; Kai et al., 2006).

Commercial *Pelargonium* cultivation has several distinct advantages not least of which would be ensuring a supply of roots of the correct species that have documented medicinal efficacy. The use of irrigation can increase the biomass yield and decrease chemical variability of plants (WHO et al., 1993), but due to the removal of natural environmental factors may reduce secondary metabolite production. The genetic improvement of the stock, finding optimal cultivation conditions and the best

form of propagation; and finding ways to protect plants from pests and diseases and control weeds are also important considerations (WHO et al., 1993).

This investigation compared the variation in the concentration of umckalin (6-hydroxy-5,7-dimethoxycoumarin) within and between plant populations collected from different geographical locations and monitored the affect of greenhouse cultivation on plants from one of these locations. Various cultivation techniques including the manipulation of soil water content, the introduction of root competition in an attempt to elicit an allelopathic response and the application of hormonal treatments were investigated.

The analysis of root umckalin levels required the purification of an umckalin standard, the development of a viable High Performance Liquid Chromatography (HPLC) protocol and the optimisation of a technique for extracting umckalin from dried roots. The in situ growth rates of harvested, treated and replanted *P. sidoides* shoots in an existing wild population were also determined.

2. Materials and methods

2.1. Root extraction

P. sidoides roots were collected from the Greater Fish River Reserve (GFRR, 33° 07' 13" S, 26° 43' 29" E) in the Eastern Cape, washed with tap-water, sliced with a stainless steel knife, oven dried at ca. 40 °C and powered in a hammer-mill. This powder was either extracted with acetone for the purpose of standard preparation, or extracted with ethanol for umckalin quantification by HPLC (see below). The choice of acetone and ethanol here followed the methods used in the literature and for commercial extract production, respectively (Kayser and Kolodziej, 1995, acetone extraction; and Matthys et al., 2003, aqueous ethanolic extraction).

The duration required to optimise the ethanol extraction of umckalin was determined as follows. One gram of dry powdered root was combined with 100 mL of absolute ethanol in sealed conical flasks and extracted for 1, 2 and 6 days at 25 °C in a shaking water bath ($n=3$). The extracts were then centrifuged at 17400 g for 20 min, with the temperature maintained between 0 and 20 °C. Supernatants were filtered through 0.45 µm nylon filters (Cameo) and analysed by HPLC. After removal of the supernatant, the pellets from the 1 and 6-day samples were re-extracted with a further 100 mL of ethanol and the supernatants analysed. All subsequent HPLC analyses were performed on 1-day ethanol extractions using the method described above.

2.2. Umckalin purification and analysis

Umckalin was purified chromatographically using a combination of HP-20 polystyrene resin, silica gel and normal phase semi preparative HPLC; guided by diagnostic TLC and NMR spectroscopy. ¹³C and ¹H and two dimensional NMR analyses, combined with UV absorbance and mass spectrometer data, confirmed the compound's identity (as first described by Wagner et al., 1974).

An HPLC based assay was developed for the quantification of umckalin concentrations in ethanolic *P. sidoides* root extracts following the guidelines of Snyder et al. (1997). This included the use of a C-18 column (125 × 4 mm; Nucleosil 100-5 C-18, particle size 125 µm with a CC 8/4 Nucleosil 100-5 C-18 guard column, Macherey-Nagel), a mobile linear gradient of water:methanol, 7:3 to 3:7, over an 8 min period at a flow rate of 1.0 mL/min, and yielded an umckalin retention time of 7.6 min. Standard curves using at least three concentrations of pure umckalin were used for calibrations.

2.3. Geographical and age variation in root umckalin concentration

In order to identify the most suitable plant material for propagation, the geographical variation of *P. sidoides* root umckalin concentrations was measured for ten plants from each of five sites representing three different regions of the Eastern Cape Province, South Africa. Two sites each were chosen at the GFRR and Killaloe farm (Kei Road district, KR; 32° 43' 03" S, 27° 39' 30" E) and one site was chosen in the Peddie district (33° 13' 17.7" S, 27° 03' 55.5" E). The three regions differ greatly in average annual rainfall (values calculated for at least 14 years of data): KR = 760 mm/yr; Peddie = 513 mm/yr and the GFRR = 397 mm/yr. Plant voucher specimens were collected from each site and are housed in the Selmar Schonland Herbarium (GRA), Grahamstown.

Ten plants were collected from each site in April 2005 for chemical analysis and their roots separated into old and new growth based on cortical tissue colour and bark appearance. Newly produced roots had creamy white, as opposed to red, cortices and their bark was light brown and not brittle. Old roots had brittle bark and were dark in colour. Roots were dried and weighed. The umckalin concentration in new and old roots was analysed by HPLC. Soil samples ($n=3$) were collected from each site. The soil pH, carbon, nitrogen, potassium and phosphorus concentrations; percent coarse, medium and fine sand and clay contents were determined by Matrocast Laboratories, Cape Town, South Africa.

2.4. Root umckalin concentration in cultivated plants

Individual plants were collected from one of the KR sites in April and August 2004 taking care to excavate as much of the intact roots as possible. Leaves were removed before plants were transported back to the Rhodes University Botany Department, Grahamstown. The average mass of each transported plant was 64.8 g and 205.1 g for the April and August collections, respectively. Additionally, the total mass of leafless plants collected from that site was 7.84 kg and 20.51 kg for the April and August collections, respectively. Each leafless plant was weighted before 80% of this leafless plant mass was removed as root. The remaining 20% fractions (termed 20% replants) were then planted in locally sourced topsoil in 8 L black plastic nursery bags and grown in a plastic greenhouse tunnel. The arrangement of plants in the greenhouse was randomly changed occasionally to reduce the effects of spatial differences in light intensity and temperature. Plants were transplanted from the black bags to hard 8 L plastic

pots in December 2004. In January 2005 all plants were moved to a second, cooler, tunnel as the temperature in the first tunnel reached 50 °C on some days causing many of the plants to wilt. Once in the second tunnel all plants produced new leaves, did not wilt again and were nearly continuously in flower till the end of the study. All plants were well-watered once every 3 or 4 days, and were grown for at least 18 months before treatment implementation. Plants from this stock were randomly drawn for experiments to determine the effects of water stress, hormone application and root competition on root umckalin concentrations.

2.4.1. Water stress treatment

Water was withheld from 10 plants for 1 month, while 10 controls were well-watered every 3 or 4 days. A pressure chamber (Scholander et al., 1965) was used to measure the pre-dawn leaf water potential (Ψ_{leaf}) of single leaves from each plant before the treatment began and then at intervals during the course of the experiment. Additionally, a Theta probe (ML2x, Delta-T Devices, UK) was used to measure percent soil moisture content in each pot at the same time intervals. After 1 month, plants were harvested, separated into inflorescence, leaf, stem, and roots, dried and weighed. Roots were divided into new and old growth as described above and umckalin concentrations determined by HPLC.

2.4.2. Hormone treatments

Eight plants were supplied with a foliar application of either cytokinin or gibberellic acid. 250 mg of benzyladenine (cytokinin) and gibberellic acid (Sigma-Aldrich) were dissolved in small amounts of 5% aqueous NaOH and diluted with water to yield a final concentration of 0.11 mM cytokinin and 0.072 mM gibberellic acid. These concentrations were selected according to Ranwala et al. (2003) and Liu et al. (2001) for gibberellic acid and cytokinin, respectively. 500 mL of the hormone solution was applied per plant, evenly spraying both axial and abaxial surfaces. Following the initial application in December 2005, a further two applications were made at 2-week intervals. Eight untreated plants were selected as controls, protected from hormone contamination and well-watered for the duration of the experiment. After approximately 1 month, plants were harvested and separated into inflorescence, leaf, stem, and roots, dried and weighed. Root umckalin concentrations were determined by HPLC.

2.4.3. Root competition treatment

Two or three individuals of an annual common to disturbed fields (*Conyza albida*—fleabane) were transplanted into each of a further eight pots, each containing a single established *P. sidoides* plant. Controls were as for the hormone experiment and were regularly weeded. After 1 month, plants were harvested, separated into inflorescence, leaf, stem, and roots, dried and weighed. Root umckalin concentrations were determined by HPLC.

2.5. Wild harvest and estimation of root regeneration

Fifty plants were harvested at the beginning of the 2004 dry season (autumn) from the xeric site at the GFRR, weighed and

had their leaves and petioles removed by cutting the petioles approximately 2 cm from the basal attachment to the stem. Each leafless plant was then weighted before 80% of this leafless plant mass was removed as root (as for the plants prepared for cultivation, Section 2.4). These 20% replants were then returned to the ground and marked with labelled stainless steel tags secured into the ground with six inch steel nails. The site was revisited 12 months after the harvest and all surviving plants were dug up and fresh plant and root masses determined. Old root was separated from new root growth as described above.

These data were used to estimate the time that it would take 20% replants to regrow to the average pre-harvest mass under field conditions. The fresh weight of 20% replants was recorded at the time of planting and the subsequent mass of roots produced by surviving plants was determined, after 12-months, with the above destructive harvest. The relationship between replant mass and root mass produced was plotted and the exponential relationship between these variables gave an indication of how root regrowth may change with increasing accumulated root mass. The equation of the trendline fitted to this data was then used to extrapolate the growth of 20% replants from the average 20% replant mass to the average pre-treatment whole replant mass. The average replant mass was obtained from 100 plants harvested from the same site as the 50 mentioned above.

2.6. Statistics

Levene's test was used to test the normality of all the datasets generated and homogeneity of variances was tested using

Shapiro-Wilk's test. Data that were not homogeneous or normally distributed were transformed to their natural logarithm or square root. One-way or factorial analysis of variance (ANOVA) was used for testing variance within continuous data. Non-parametric analyses of variance were used for non-normal and non-homogenous continuous data that could not be corrected by transformation. Relationships between root umckalin concentration and soil pH or rainfall and this compound were analysed using simple linear regression. Unless otherwise stated, values in text and figures are averages \pm one standard error.

3. Results

3.1. Extraction, purification and quantification of umckalin

The identity of the purified umckalin was confirmed by NMR analyses combined with UV absorbance and mass spectrometer data and was then used to calibrate the HPLC.

Powered root collected from the GFRR yielded 163 ± 2 μg umckalin/g dry root when extracted in ethanol for 24-h. When the duration of the extraction was increased from 1 to 6 days the yield of umckalin increased by 41%, and a re-extraction of the 6-day root material yielded a further 13% of umckalin (total yield = 251 $\mu\text{g}/\text{g}$). Hence, the 24-h single extraction yield of 163 ± 2 μg umckalin/g dry root represented 65% of the total yield of 251 $\mu\text{g}/\text{g}$. For practical purposes a 1-day extraction was chosen for all further analyses but the extracted yields were increased by 35% in order to represent a complete yield, the combined yield of 6-day extraction and re-extractions.

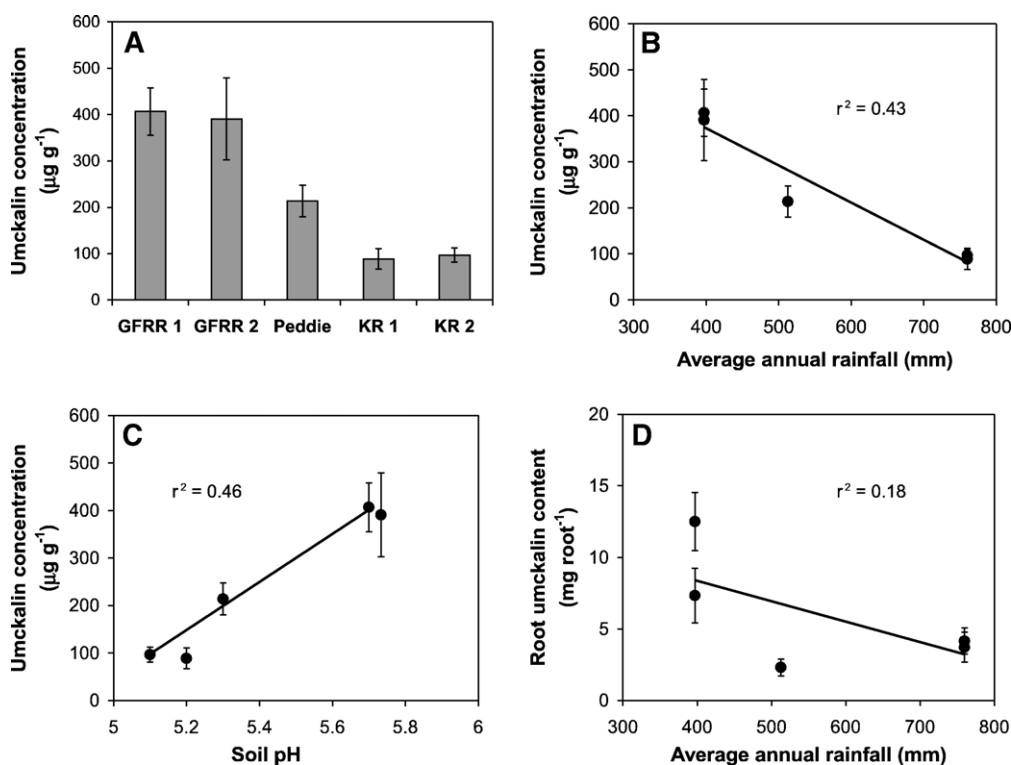


Fig. 1. (A) Root umckalin concentrations of plants collected at the Great Fish River Reserve (GFRR), Peddie and Kei Road (KR); at the GFRR and KR plants were collected from two geographically separate populations of plants. (B) Response of root umckalin concentration to rainfall or; (C) soil pH. (D) Response of root umckalin yield per plant to rainfall. $n=10$ for each site, the linear regressions in B, C and D are to all data and not to the average values shown in the figure.

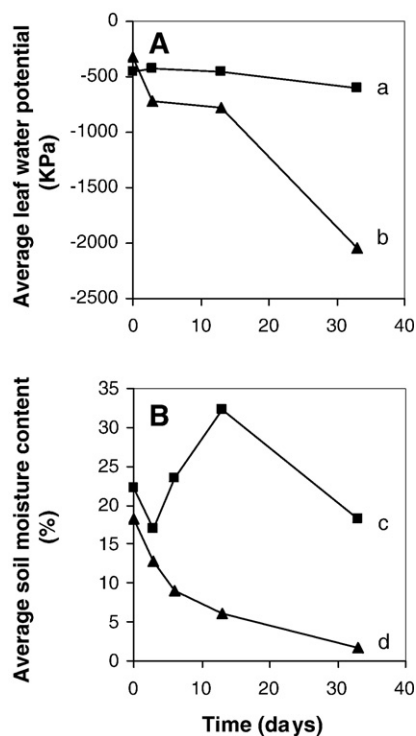


Fig. 2. (A) Change in average pre-dawn leaf water potential and (B) percent soil moisture content for well-watered controls (squares) and plants from which water was withheld (triangles). $n=5$, statistically homogenous groups at the 95% confidence level (Tukey) for each measurement on day 33 are identified by letters a to d.

The average umckalin concentrations in old ($94 \pm 13 \mu\text{g/g}$) and new ($67 \pm 18 \mu\text{g/g}$) wild-harvested roots from plants originating from the KR region were not significantly different, nor was root umckalin concentration ($73 \pm 9 \mu\text{g/g}$) significantly altered when these plants were grown under greenhouse conditions. Hence, cultivation appears not to compromise root bioactivity attributed to umckalin and meant that in this study all further analyses could be performed only on new roots.

3.2. Geographical variation of root umckalin concentration

The umckalin concentration of harvested roots differed significantly between geographical locations ($p < 0.0001$, $F=34.0$, Fig. 1A) and were strongly correlated to both mean annual precipitation ($p < 0.0001$, $F=36.5$, Fig. 1B) and soil pH ($p < 0.0001$, $F=40.6$, Fig. 1C). pH was the only measured soil parameter to which root umckalin concentration showed a strong correlation.

The highest average umckalin concentrations were found in roots from the area of lowest rainfall and vice versa. However, as rainfall affected root size in a reciprocal fashion to root umckalin concentration (data not shown), the final umckalin yield per plant (concentration multiplied by root mass) showed a weaker relationship to rainfall ($p=0.002$, $F=10.26$, Fig. 1D).

3.3. Optimising root umckalin concentrations in cultivated plants

The relationship between mean annual precipitation and root umckalin concentration provided the rationale for attempting to

optimise root umckalin concentrations by imposing water stress on cultivated plants. The 1-month water stress treatment resulted in progressively decreasing soil water content (SWC) that was paralleled by decreasing pre-dawn Ψ_{leaf} (Fig. 2A and B), such that by the end to the period, both SWC and Ψ_{leaf} were significantly different between treated and control plants ($p < 0.001$, $U=0.00$, $Z=-2.61$ and $p < 0.001$, $U=0.00$, $Z=-2.61$ for the SWC and Ψ_{leaf} data, respectively).

The water stress treatment, however, had no significant affect on root umckalin concentrations (Fig. 3) despite reducing soil water contents. The geographical rainfall relationship observed for wild-harvested roots either cannot be replicated by short-term drought treatments, or is the result of some other undetermined factor which, like soil pH, is a co-variant of rainfall.

Like the water stress treatment, neither the foliar application of cytokinin or gibberellin, nor the root competition treatment, significantly increased root umckalin concentrations. A factorial ANOVA showed no treatment effects ($p=0.55$, $F=0.77$, Fig. 3).

3.4. Wild harvest

The regrowth of roots over 12 months was exponentially related to the mass of the “leafless” 20% replants (Fig. 4A), the smaller replants producing relatively more mass than the larger replants. This relationship was used to calculate yearly increments in plant mass from the average fresh weight of the 20% replants (13.4 g), to a final mass representing the average plant weight prior to root excision (61.1 g; Fig. 4B, line D). At each increment the calculated mass was added to the plant mass and the next year’s increment calculated, this produced an exponential growth curve (Fig. 4B, line C). The average time estimated for 20% replants to regrow to the average pre-harvest mass was ca. 56 years under field conditions (Fig. 4B; intercept of lines C and D). This procedure was repeated using the equations for the upper and lower confidence limits in Fig. 4A, suggesting that regeneration times could range from ca. 11.5 to 410 years. The ca. six times greater wet root yield observed for well-watered greenhouse-cultivated replants compared to wild-grown replants, lends

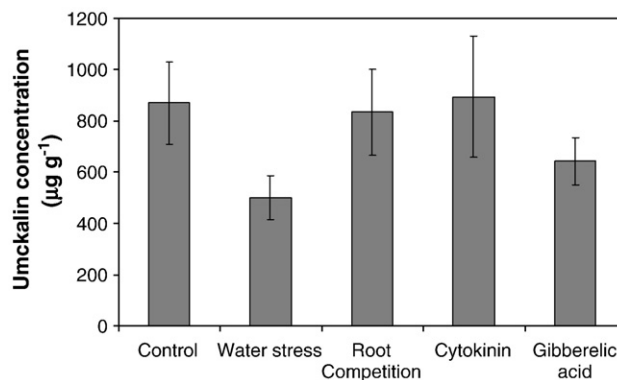


Fig. 3. Average root umckalin concentrations of either well-watered controls, or plants subjected to the indicated stress, root competition or plant hormone treatments. $n \geq 5$.

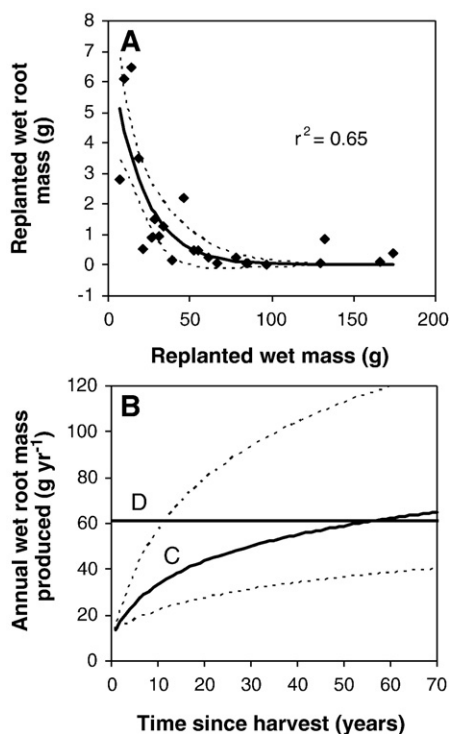


Fig. 4. (A) Relationship between replant mass and subsequent annual mass of root produced by plants following the GFRR autumn harvest. The solid line represents the regression fit $y = 7.37^{-0.0516x}$ and the dashed lines its 95% confidence intervals. (B) The modeled incremental regrowth of plants (line C) starting with the average 20% replant mass subsequent to 80% root harvests (13.4 g), and continuing to intersect with the average pre-harvest plant mass (61.1 g, line D). The model uses the regression fit from Fig. 4A, the upper and lower estimations (dashed lines) were created by substituting the upper and lower 95% confidence values of the fitted parameters a and b into the equation used to fit line C, where $y = a^{-bx}$.

support to the lower estimated regeneration time of 11.5 years under favourable rainfall conditions.

4. Discussion

Identifying and sourcing the most suitable propagation material for medicinal plant cultivation is recommended by the World Health Organization (WHO et al., 1993). Accordingly, the phytochemical survey of *P. sidoides* roots across three regions of the Eastern Cape was used here. This survey required the development of a, previously unpublished, rapid HPLC method for the detection of umckalin in ethanolic root extracts. Umckalin was used as a bioactive marker for the purposes of this study, however, there is a need to confirm its reported pharmacological activity and to correlate its efficacy against that of crude *Pelargonium* extracts such that it can be used confidently as a marker in optimizing plants with high pharmacological activity.

The analysis of root extracts from the three regions revealed that increasing root umckalin concentrations were strongly related to both increasing soil pH and decreasing annual rainfall of the collection sites. Rainfall and soil pH are correlated as high rainfall tends to leach elements such as calcium, resulting in more acidic soils (Waugh, 1995). Average root umckalin concentration did not vary significantly between plant populations from the same region and the umckalin concentrations of wild-harvested roots

were maintained when plants were cultivated under well-watered greenhouse conditions. This suggests that it may be possible to use climatic data across the *P. sidoides* distributional range to identify populations with high root umckalin concentrations and hence bioactivity. This would be valuable for sourcing the best propagation material for cultivation studies and commercial operations.

The imposed water stress treatment did not significantly alter the root umckalin concentrations of cultivated plants, despite the relationship described. This, and because wild root concentrations were unaltered by cultivation, suggests genotypic differences between populations from different geographic regions and detracts from the potential of producing large roots under cultivation which can be subsequently induced to produce high levels of umckalin via water stress. However, it does mean that once high-yielding genotypes are selected their umckalin concentrations will be maintained under cultivation. It is possible that some other abiotic factor that is a covariate of rainfall may be required to induce higher levels of umckalin and soil pH is one such potential factor worth investigating with future studies.

The foliar application of the plant hormone salicylic acid has been demonstrated to increase coumarin production in *Matricaria chamomilla* leaves (Pastírová et al., 2004), showing that hormone treatments may be used to manipulate plant bioactive contents. However, in this study, applications of gibberellin and cytokinin had no effect on the content of the root coumarin, umckalin. Furthermore, the suggestion that umckalin is produced as an allelopathic compound in response to root competition was not supported and the physiological and metabolic function of umckalin in *P. sidoides* remains unresolved.

The *Pelargonium* plants from low rainfall regions are potentially the most economical for harvesting owing to their high umckalin yield. However, the long root-regeneration time under conditions of low rainfall raises questions about the sustainability of long-term harvests from these sites. Additional studies on root production under conditions of different water supply and across multiple years are required. The potentially slow root regeneration highlights the need to know the size of the wild *Pelargonium* resource and together with regeneration studies would inform decision-makers as to whether harvesting roots from the wild would be sustainable in the long-term. A similar rationale was presented by Rock et al. (2004) following their study on the sustainability of harvesting whole wild *Allium tricoccum* plants for their bulbs. These authors assessed the effects of removing between 25 and 100% of the total number of *A. tricoccum* plants from different sites within protected areas in the Appalachian region, USA. Using a population growth model they found that population recovery could be affected by harvests of, as low as, 5% of the available resource. As a result, the authors recommended further research in other areas of the species' distribution range, improved monitoring of private party harvests and greater enforcement of harvest limits by way of a permit system.

The widespread occurrence of *P. sidoides* across a variety of climate and vegetation types (Dreyer and Marais, 2000) necessitates the gathering of harvest response data as well as population dynamics information for each proposed commercial

harvest site. The distribution of *P. sidoides* populations and harvest within several South African provinces and Lesotho (Smith, 2006) will also require streamlining provincial and international legislation concerning indigenous plant conservation and harvest. This will help ensure that over-exploitation is stopped completely and not only moved from one province or country to another. This principle was highlighted by Mayer et al. (2006) in their study of tree harvest, for timber, in the boreal forests of south-eastern Finland and just over the border in north-western Russia. Additionally; on the socio-economic level, it may be important for a governmental or non-governmental organisation to investigate the fairness of the trade between harvesters and middlemen (Bisseker, 2002).

The results presented here offer promising prospects for the commercial cultivation of *P. sidoides* roots to supply the international and local markets. Firstly, plants with high root umckalin concentrations can be selected from regions of low rainfall and their growth markedly increased by cultivation under irrigation. Secondly, the concentration of umckalin of field selected plants appears not to be significantly reduced by greenhouse cultivation, suggesting the maintenance of medicinal value. Hence, in the best interests of the conservation of the species and the sustainable supply of roots for the local and international markets, the results of this study strongly recommend that cultivation be further researched and pursued as a promising alternative to wild harvest.

Acknowledgements

The South African National Research Foundation and the Rhodes University Joint Research Council for financial support. To all those who contributed to and supported this work at Killaloe Farm and the Greater Fish River Reserve.

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