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Soil Parameters Affecting the Antioxidant Activity of *Hypoxis hemerocallidea* Corm Extracts in Different Areas of South Africa

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Hypoxis hemerocallidea is wild harvested and widely used due *inter alia* to its strong antioxidant activity. Antioxidant activity is linked to plant stressors like soil heavy metals concentrations and pH. If high antioxidant activity is caused by heavy metals stressing the plant, the plant may not be completely safe. Soils and *H. hemerocallidea* corms were collected from five different geographical regions of South Africa. The highest corm and soil heavy metals concentration were Fe, Mn and Cr, with Fe having the highest, particularly for corms collected from Ga-rankuwa (83.7 ± 0.03 µg/g). The soil and corm samples from Ga-rankuwa with high levels of metals (Fe, Cr, Ni, Pb) had greater antioxidant activity (EC_{50} of 1.68 ± 0.49 µg/mL). Despite corms showing ability to bio-accumulate heavy metals, the antioxidant activity could not be linked to environmental conditions. The results highlight potential danger of using naturally harvested bulbs growing in unidentified soils.

Keywords: Antioxidant activity, Heavy metals, *Hypoxis hemerocallidea* corm, Polyphenolic content.

INTRODUCTION

Throughout the ages, humans have relied on nature for their basic needs such as the production of food stuffs, shelter, flavours, fragrances and not the least medicine, with as many as 80% of the world's population relying on herbal remedies [1]. With plants forming the basis of traditional medicine system for thousands of years, it is not surprising that they continue to provide mankind with new remedies more so together with substantial research in the field and with some communities still being wholly reliant [1,2]. While numerous reasons are contributory to the prominence of herbal remedies in an age of "modern medicine", one area of interest come from the belief that plant-based medicines may have fewer side effects when compared with orthodox drugs, which may be valid in chronic diseases management. Another concern is the cost of treatment, for which the herbal remedies may serve as a low-cost alternative.

Hypoxis hemerocallidea Fisch. & C.A. Mey (Hypoxidaceae), a herbaceous perennial plant also known as *Hypoxis rooperi*, grows in meanders grassland and mountainous areas of South Africa, South America, Australia and coastal regions of Asia [3]. In Southern African traditional medicine, the plant is collected from the wild and the extracts of the corm have been ingested for a diversity of ailments [4] including tuberculosis, cancer and diabetes. It has been speculated that the plant may be active due to its high phenolic content, which from other studies are compound known to have multiple biological effects including antioxidant activity [5-7]. Non-enzymatic antioxidants as well as antioxidant enzymes are known to counteract the effect of reactive oxygen species and reactive nitrogen species. Aside from the enzymatic antioxidants such as superoxide dismutase, peroxidase and catalase, the formation of reactive oxygen species is also prevented by a non-enzymatic antioxidant system (the low molecular mass compounds such as glutathione, ascorbic acid, α-tocopherol, carotenoids and phenolic compounds) [8,9]. Moreover, in the presence of the metal ion cofactors such as Cu, Zn or Mn, superoxide dismutase located in the cytosol and mitochondria, can catalytically convert the $O_2^{\bullet-}$ into oxygen and H_2O_2 [9].

In the plant, the polyphenolic compounds are important for normal growth development and defence against infection and injury [10-12]. They also have a metal chelation potential [8]. With the phenolic compounds being protective to the plant, there is evidence that the induction of phenolic metabolism in plants in general may be a response to heavy metal stress and organic matter [13,14]. While the plant is used medicinally for its high phenolic content, if heavy metals stimulate the high antioxidant activity, there is concern that the plant may pose a threat to human health due to heavy metals accumulation. Previous studies by Chang [15] and Steenkamp *et al.* [16] have also raised concerns that the heavy metals present in plants used medicinally in South Africa could pose a risk to human health. For this study we investigated if the antioxidant and polyphenol content of the African potato could be due to the heavy metal concentration within the corm and the soil it grows in, in an attempt to evaluate the safety of this commonly used herbal plant.

EXPERIMENTAL

Site description: *H. hemerocallidea* corms and soil, were collected in January 2017, from five sites in different South African geographical locations of Eastern cape province 31.5667°S, 28.7667°E (Mthatha); KwaZulu-Natal province 29.5310°S, 30.9340°E (Ndwedwe); Gauteng province 25.6200°S, 27.9800°E (Ga-rankuwa); Mpumalanga province 27.3667°S, 29.8833°E (Volkstrust) and Limpopo province 23.9000°S, 29.4500°E (Polokwane). Sites were selected from the reported previous sites for the natural occurrence of the plants.

Plant material collection: Five mature corm of approximately 10-15 cm in diameter to the total of 0.5 kg in wet weight were harvested. *H. hemerocallidea* were verified by SANBI (South African National Biodiversity Institute) at the National Herbarium in Pretoria. The voucher specimens were deposited and conserved in the Herbarium of the department of Biology, Sefako Makgatho Health Science University, South Africa. All the plant samples were washed in running water to remove soil particles and dried at room temperature. The plant corm samples were grinded into fine powder (to a size of less than 1 mm) for extraction (Jankel and Kunkel grinder).

Soil samples collection: At each location of sampling, five soil samples were also collected from each of the above mentioned five locations from 0 to 30 cm depth with the aid of an auger of 7.0 cm in diameter. After sampling at each site, the auger was thoroughly washed using deionized water so as to avoid mixing of soils. The soil sample from Volkstrust was collected from a mountainous area and was best described as dark vertisols. The sample from Ga-Rankuwa collected from a hill slope region was described as rusty-red plinthosols. The sample from Polokwane collected from a level terrain was described as yellowish-brown cambisols. While the samples from Mthatha and Ndwedwe were both on a hill side described as dark brown leptosols.

Heavy metal analysis of the soil and plant samples: In order to determine trace metals content in soil, the ground soil samples were further sieved to pass through a mesh < 60 µm. The total metal content of the 0.5 g plant and 5 g soil samples were determined by digesting the samples with a mixture of HNO_3 (10 and 12 mL for plant and soil samples, respectively) and HCl (3 and 5 mL for plant and soil samples, respectively) (65% Merck supra pure). The resulting solutions were analyzed for trace element content of copper, zinc, manganese, iron, chromium, lead, nickel using inductive couple plasma mass spectrophotometer (ICP-MS) high resolution.

Organic matter content of soil samples: Organic matter contents of the soil samples were determined on loss-on-ignition at 550 °C for at least 30 min. After cooling in the desiccator to ambient temperature, weighing to the nearest 1 mg, (*ma)* was done. Weighing into the crucible 0.5 to 5 g of the dried sludge to the nearest 1 mg, (*mb)* was also done, which then was heated in the furnace at 550 °C for at least 60 min. The mass of the residue on ignition and thus the loss on ignition was regarded as constant when the mass obtained after a further 0.5 h of ignition at 550 °C in the preheated furnace, (*mc-ma),* differs max. 0.5% of the previous value or 2 mg, whichever was the greater. For the drive of quality reassurance this procedure was repeated twice and blanks were prepared separately for the soil materials.

pH of soil samples: The soil pH was determined in 0.01M $CaCl_2$ (1:2 soil solution ratio) and in distilled water using a pH meter fitted with glass electrode (Jen Wal Model 3015 digital). It was of importance to make soil slurry of both solutions since they may provide different pH values. For most acidic soils, a buffer of pH 7 and another of pH 4 were used to calibrate the pH meter. For alkaline soils pH 10 and pH 6 buffers were required.

Preparation of *H. hemerocallidea* corm extracts: 5 g of each grounded sample of *H. hemerocallidea* corms were extracted with 50 mL of methanol and were placed on a labotec shaker for 0.5 h and then centrifuged at 3000 rpm for 10 min. The resulting extracts were filtered using Whatman No. 1 filter paper to remove plant debris and the filtrates were allowed to dry under a stream of air at room temperature. The extracts were subsequently used for *in vitro* experiments.

Qualitative and quantitative antioxidant activity of *H. hemerocallidea* corm extracts: The extracts prepared above were dissolved in methanol and were visualized on Merck TLC E_{254} 10 × 20 cm after elution in four solvent systems: ethyl-acetate:methanol:water (E:M:W) (10:1,35:1 v/v), benzene: ethanol:ammonia hydroxide (B:E:A) (18:2:0.2), chloroform: ethyl acetate:formic acid (C:E:F) (10:8:2) and ethyl acetate: butanone:water:formic acid (BUTANONE) (5:3:1:1). Plates were visualized under a UV fluorescent light or after reaction with vanillin. Free radical scavenging bands were visualized by spraying plates with 0.2% of 1,1-diphenyl-2-picrylhydroazyl (DPPH) in methanol as descried by Parejo *et al.* [17]. The free radical scavenging potential of the extracts was also quantified using the DPPH method. Briefly, the methods relied on a potential antioxidants ability to react with DPPH and convert it to 1,1-diphenyl-2-picryl hydrazine, as measured by change in the absorbance produced at 517 nm, after 1 h at room temperature. The extent of DPPH radical scavenging (mechanism) at serial concentrations (1000-0.49 µg/mL) of *H. hemerocallidea* extracts was measured with EC_{50} calculated. Trolox and L-ascorbic acid were used as positive controls.

Determination of total polyphenolic content: Total polyphenolic content of the corm extracts from each site were determined according to the method of Ragazzi and Veronese [18]. A 20 µL of each extract (125 µg/mL) was added to 200 µL distilled water and 40 µL of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min and then 40 µL of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Gallic acid was used as a standard for the calibration curve. Total polyphenol content was calibrated using the linear equation based on the calibration curve. Total polyphenolic content was expressed as mg gallic acid equivalents (GAE)/g.

Data analysis: All experiments were performed in triplicate and repeated three times. Data are presented as the mean ± standard error of mean. Statistical analyses were performed by one-way analysis of variance (ANOVA) with SPSS 20 (IBM) software and significantly different at $p < 0.05$. The concentration of heavy metals per site in the soil and corm were analyzed through descriptive statistics. The differences between locations were compared by either the student t-test (for dichotomous variables), while the association between the soil and corm concentrations were evaluated by linear regression. Correlation coefficient was used to rule out any interactions on corm and heavy metal concentrations.

RESULTS AND DISCUSSION

Heavy trace metal analysis of the soil and corm samples: The concentration of metals measured in the soil and corm of the various areas of collection are presented in Table-1. In general, the soil and corm metal concentrations between the elements varied by site. Trends were however present, with the highest concentrations for all the elements being Fe, Mn and Cr. Fe concentrations were in general high for all sites, with the exception samples from Polokwane which had a low mean value for soil sample (0.09 ± 0.03 µg/g) and for corm sample (33.8 ± 0.17 µg/g). The Ga-rankuwa corm samples had the highest concentration of Fe. The ratio of Mn to Fe was highest for collections in Mthatha, Volksrust and Ndwedwe corm samples *versus* in Ga-rankuwa and Polokwane corm samples. While the ratio of Mn to Fe was low for all sites soil samples. No relationship was present between a particular heavy metals concentration in the soil *versus* the concentrations within the corm heavy trace metal, irrespective of the site of sampling.

Organic matter content and pH of the soil samples: The mean concentration for soil organic matter content from all the study areas ranged from 0.04 to 0.67%. The lowest concentration of organic matter content was observed from Ga-rankuwa soil sample (Table-2). Moreover, there seemed to be an increase

TABLE-1
MEAN CONCENTRATION OF HEAVY METALS FROM THE SOIL AND CORM SAMPLES FROM THE DIFFERENT STUDY AREAS

Metals (µg/g)	Mthatha			Volksrust			Ndwedwe		
	Soil	Corm	Ratio	Soil	Corm	Ratio	Soil	Corm	Ratio
Cu	0.18 ± 0.01	0.32 ± 0.07	1:2	6.13 ± 0.02	0.49 ± 0.02	13:1	2.07 ± 0.02	0.16 ± 0.02	1:13
Zn	88.46 ± 0.13	25.4 ± 0.35	5:1	17.29 ± 0.02	13.2 ± 0.07	1.3:1	33.73 ± 0.02	0.33 ± 0.01	51:0.5
Mn	89.08 ± 0.55	86.1 ± 0.01	1.04:1	88.98 ± 0.12	67.3 ± 0.02	1.3:1	104.6 ± 0.55	80.2 ± 0.01	1.3:1
Fe	130.1 ± 0.03	34.1 ± 0.02	4:1	293.9 ± 7.81	63.5 ± 0.02	5:1	284 ± 0.01	57.7 ± 0.15	5:1
Cr	0.02 ± 0.01	0.11 ± 0.02	1:6	0.08 ± 0.01	0.12 ± 0.01	1:2	0.04 ± 0.01	0.1 ± 0.02	3:1
Ni	1.85 ± 0.10	0.15 ± 0.03	12:1	0.91 ± 0.04	0.15 ± 0.01	6:1	0.82 ± 0.03	0.16 ± 0.02	5:1
Pb	23.7 ± 0.57	2.98 ± 0.01*	8:1	27.33 ± 0.01	2.75 ± 0.08*	10:1	28.23 ± 0.06	5.21 ± 0.02*	5:1
Metals (µg/g)	Ga-rankuwa			Polokwane					
	Soil	Corm	Ratio	Soil	Corm	Ratio			
Cu	42.06 ± 0.50	4.49 ± 0.01	9:1	0.64 ± 0.04	6.7 ± 0.01	11:1			
Zn	20.56 ± 0.06	0.47 ± 0.02	44:1	4.86 ± 0.02	32.7 ± 0.06	1:7			
Mn	115.3 ± 0.12	19.05 ± 0.02	6:1	0.252 ± 0.01	0.8 ± 0.10	3.2:1			
Fe	255.4 ± 0.36	83.7 ± 0.03	3:1	0.09 ± 0.03	33.8 ± 0.17	0.01:4			
Cr	41.71 ± 0.01	0.13 ± 0.01	16:0.05	239.7 ± 0.10*	0.13 ± 0.01	92:0.05			
Ni	40.12 ± 0.02	0.17 ± 0.03	12:0.05	22.15 ± 0.01	0.15 ± 0.01	73:0.5			
Pb	30.5 ± 0.20	5.01 ± 0.01*	6:1	20.91 ± 0.08	3.01 ± 0.01*	7:1			

Heavy metals mean concentration ± SD. *Maximum not allowable limits of heavy metal.

TABLE-2
ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLIC CONTENT OF CORM CRUDE EXTRACTS AND, pH VALUES AND ORGANIC MATTER CONTENT OF THE SOIL FROM DIFFERENT SITES

Collection sites	Corm extract		Soil pH $CaCl_2$:H_2O	Soil organic matter (%)
	DPPH Scavenging ability (EC_{50} µg/mL)	Total phenolic content (mg GAE/g crude)		
Polokwane	5.95 ± 1.53	49.78 ± 6.04	6.51:6.60	0.49
Mthatha	8.65 ± 0.44	42.12 ± 7.53	5.82:5.98	0.58
Volksrust	7.64 ± 1.46	53.08 ± 7.98	5.79:6.01	0.60
Ga-rankuwa	1.68 ± 0.46	56.53 ± 8.94	4.95:5.79	0.04
Ndwedwe	9.70 ± 2.68	42.01 ± 6.81	5.96:6.26	0.67
L-ascorbic acid	1.57 ± 0.93	Not determined	Not applicable	Not applicable
Trolox	3.59 ± 1.70	Not determined	Not applicable	Not applicable

Results expressed as mean ± SD (standard deviation)

in DPPH activities as organic matter content decreases. Also the pH of the soils from the study sites differed from 4.95 to 6.51 ($CaCl_2$) and 5.79 to 6.60 (H_2O) (Table-2).

Qualitative and quantitative evaluation of antioxidant activity of corm extracts: There was variation in antioxidant activities for the *H. hemerocallidea* corm extracts collected from different sites, with the Ga-rankuwa corm extract showing more bands with free radical scavenging (Fig. 1). The radical scavenging effect was found to generally increase for both the tested samples and standards in a dose dependent manner. Table-2 shows the scavenging effect of crude extract from all the five mentioned different sites on DPPH radicals. EC_{50} analysis showed that the DPPH radical scavenging activity of the Ga-rankuwa corm crude extract (1.68 ± 0.49 µg/mL) was found to be significantly higher ($p < 0.05$) followed by Polokwane corm crude extract (5.95 ± 1.53 µg/mL) when compared to other crude extract of different sites. The EC_{50} value for Ga-rankuwa corm crude extract was not significantly different ($p < 0.05$) to that for L-ascorbic acid (2.14 ± 0.86 µg/mL) and Trolox (3.04 ± 0.44 µg/mL), which were the positive controls (Table-2). In general, soil and corm crude samples (*e.g.* from Ga-rankuwa) with high levels of metals (Fe, Cr, Ni, Pb) yielded greater antioxidant activity (Table-2). The correlation coefficient between individual soil and corm heavy metal concentration and corm DPPH antioxidant activity varied widely R^2 was from 0.020-0.962. This indicates that the soil and corm heavy metals concentrations are mostly not responsible for the antioxidant activity although there may be some of overlap (Table-3).

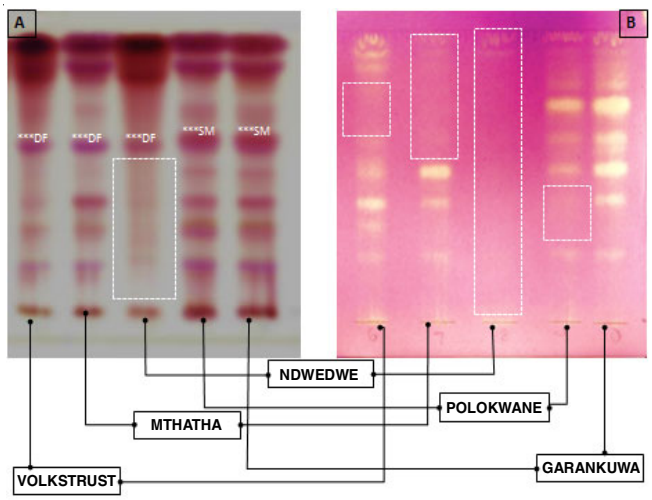

Fig. 1. Butanone eluent (a) TLC chromatogram for methanol extracts from different areas in South Africa ***DF indicates different compounds ***SM indicates same compounds. (b) Antioxidant TLC chromatogram for methanol extracts from different sites in South Africa. Rectangular dotted lines: indication of not a better resolution of compounds

Determination of total phenolic contents: Total phenolic content of the extracts was calculated from the regression equation of calibration curve ($y = 26.093x$; $R^2 = 0.998$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The absorbance values obtained at different concentrations of gallic acid were used for the construction of calibration curve. The total phenolic contents (determined as gallic acid equivalents or GAE) of the corm crude extract from different sites are shown in Table-2. The Ga-rankuwa corm extract showed the highest total phenolic content (56.53 ± 8.94 mg GAE/g crude), whereas the phenolic content of Ndwedwe corm extract was lower (42.01 ± 6.81mg GAE/g crude). Through correlation analysis for phytochemical contents with EC_{50} values of radical scavenging activities of various corm extracts from different sites, the content of phenolic and EC_{50} of scavenging ability on DPPH radicals was found to be significant with positive correlation.

The use of herbal remedies is an important practice in South Africa, to such an extent the Government of South Africa has recently brought control measures to protect consumers. This control unfortunately does not include herbal remedy practitioners who prepare more patient specific medicines. This raises concerns as some plants may be dangerous due to factors such as their heavy metal concentrations of wild harvested plant material. Cases of heavy metal poisoning from exposure to high levels of arsenic, chromium and magnesium have reported from the use of *Bulbine natalensis* and *Alepidea amatymbica* [16,19,20]. For this study we investigated if a commonly used herbal medicine could pose health risks, from the accumulation of selected heavy metals. We also investigated if selecting crops based on efficacy does not lead to higher potential heavy metal exposure [15]. We found that plants accumulated high concentrations of Fe, particular for the corms collected from Ga-rankuwa and Mthatha. This corroborates previous findings that *H. hemerocallidea* has the capacity to take up and accumulate high amounts of Fe [21].

Another important finding was that the high antioxidant activity, free radical scavenging activity and the high phenolic content from the Ga-rankuwa corms was associated with the plant growing in soils with the lowest concentration of organic matter and lowest pH as compared to corms collected from other sites. From a physiological point, the antioxidant and free radical scavenging activity can be linked to the plants polyphenolic content. Within the plant, these polyphenols play a vital role as an antioxidant in living systems due to the presence of hydroxyl groups in *ortho* and *para*-positions [22-24]. Moreover, some of the elements, for example, Zn, Mn, Cu and Fe determined in this study are known also to play beneficial role as co-enzymes in antioxidant processes and deficiency in any of these essential elements may impair the overall function of the oxidation systems [25-28]. However,

TABLE-3
CORRELATION COEFFICIENT (R^2) BETWEEN MEAN CONCENTRATIONS OF HEAVY METALS OF THE SOIL OR CORM SAMPLES FROM DIFFERENT SITES AND CORM DPPH ANTIOXIDANT ACTIVITY (EC_{50})

Samples	Relationship between corm antioxidant activity and heavy metal						
	Cu	Zn	Ni	Mn	Fe	Pb	Cr
Corm	0.699	0.188	0.634	0.768	0.576	0.253	0.836
Soil	0.889	0.437	0.962	0.020	0.048	0.321	0.302

extremely high levels of these essential elements can be toxic [29]. This illustrates the dangers of selecting plants by their beneficial activity without taking into consideration other factors such as potential toxicity. Fe, Ni and Cr measured concentrations did not exceed the maximum not allowable limits of heavy metal in soil and plant established by standard regulatory bodies such as World Health Organization (WHO), Food and Agricultural Organization (FAO) and E U, Standard Guidelines in Europe (Fe soil 50000 µg/g and plant 425.00 µg/g; Ni soil 50 µg/g and plant 67.00 µg/g; Cr soil 100 µg/g; Pb soil 100 µg/g and plant 0.30 µg/g). From a pathological aspect, high intake of Fe, Ni and Cr may be toxic, causing severe damage in the stomach or haematemesis leading to gastric discomfort, nausea, vomiting and diarrhoea. It may also lead to necrosis of mucosal cells and perforation of the gut wall. Nonetheless while the levels were not at toxic levels, this does not preclude a more insidious chronic effect, as these metals can accumulate in the body and food chain [27]. When trying to ascertain the link between plant and soil concentrations, Olowoyo *et al.* [30] indicated that while Fe, Ni and Cr levels in the plant may also be reliant on the concentrations of Fe, Ni and Cr in soil. Which may not be the case in present study, since no relationship was present between a particular heavy metals concentration in the soil *versus* the concentration within the corm heavy trace metal, irrespective of the site of sampling when multiple regression model was used to rule out any interactions on corm and heavy metal concentrations. One reason for this may be the link between metal uptake by plants being dependent on the bioavailability of the metal in the water phase, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. In addition, many researchers [31-34] have demonstrated that the uptake of Fe, Ni and Cr in the plant can be governed by factors such as soil pH and organic matter content of the soil. Moreover, according to Lee *et al.* [35] the common micronutrients such as Fe, Zn and Ni are more accessible within a soil pH range of 5 to 7 which was in line with the soil pH range and the organic matter levels obtained in this study.

Conclusion

The study was unable to show a clear link between the corm's antioxidant activity and environmental conditions. The latter was a general concern, that the selection of plants based on only their activity could be inadvertently result in exposure of people to heavy metals. Nonetheless the high levels of metal concentrations found in some of the samples do support general concerns that the safety and quality assurance in South African herbal medicines wild harvested requires screening for heavy metals. This may also indicate that the cultivation of valued medicinal plants may be necessary to avoid heavy metal accumulation as well as to enable reliability in terms of quality and efficacy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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