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Phytochemical and antioxidant analysis of wild and *ex situ* cultivated shoots and tubers of *Harpagophytum procumbens* (Burch)DC ex. Meisn from Botswana

Motlhanka D.M.T.
Botswana College of Agriculture, Medicinal Plants Research Laboratories, Department of Basic Sciences, Private Bag 0027, Gaborone, Botswana

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ABSTRACT

Comparative phytochemical analysis [TLC method] and antioxidant activity of wild and *ex situ* cultivated shoots and tubers of *Harpagophytum procumbens* were done. Total phenolic content [Folin-Ciocalteu method] and free radical scavenging activity [1,1-diphenyl-2-picryl-hydrazyl assay] of both chloroform and methanol extracts were determined. Analysis of *ex situ* cultivated plant material showed presence of phytochemicals comparable with those found in the wild plants. The total phenolic contents (mg GAE/L) of methanolic tuber extracts from wild plants (3366±22.68) were comparable to the methanolic tuber extracts (3297.00±54.56) from *ex situ* cultivated plants. The total phenolic contents of methanolic leaf extracts from wild (2562±158.77) plants were also not significantly different from the *ex situ* (2686.00±10.49) cultivated *H. procumbens*. Similar trends were observed in the chloroform extracts of both wild and *ex situ* cultivated *H. procumbens*. The free radical scavenging activities also correlated well with the total phenolic contents of both wild and *ex situ* cultivated plants. At all tested concentrations, the methanolic extracts of both wild and ex situ cultivated plants were ≥80%. The scavenging potencies of chloroform extracts were consistently lower than the methanol extracts in both *ex situ* cultivated plants and wild species. The consistency in presence of phenolic compounds in these plant materials is of interest from both the pharmacological and conservation point of view given the role played by these compounds in oxidative stress. These results indicate that *ex situ* cultivation can be both a conservation strategy and can provide an alternative and sustainable source of therapeutically active compounds.

Key words: *Harpagophytum procumbens*; Phenolic content; free radical scavenging activity; wild shoots and tubers; *ex situ* cultivation

BACKGROUND

Harpagophytum procumbens (Burch.) DC. Ex Meisn (Pedaliaceae) commonly known as Kalahari Devil's claw is one of the most harvested plants native to Southern Africa. In Botswana the plant is overharvested for its clinical role in the treatment of heart disease, degenerative rheumatoid arthritis, kidney inflammation and tendonitis (Motlhanka and Makhabu, 2011). Globally, *H. procumbens* can bring annual economic returns in magnitudes of 30 million Euro. Despite these extensive encouraging therapeutic and economic benefits, not a lot has been done in Botswana to conserve this near threatened species in *ex situ* botanical gardens. The IUCN* red data list criteria is South Africa assessed the conservation status of *H. procumbens* as the least concern even though this plant is rated by the department of Forestry and range resources in Botswana, as near threatened. In this study, *H. procumbens* was removed from the wild and cultivated ex situ in a newly established botanical garden. In the present study, phytochemical and antioxidant analysis was performed on the tubers and shoots of *ex situ* cultivated and wild plants.

INTRODUCTION

Medicinal plants are at an increasing risk from destruction of their habitats, overharvesting and prospecting for new sources of drugs. These activities often lead to a rapid decline and risk of extinction of the most after sought medicinal plants from unsustainable harvesting. While the collection and sale of plant products provide additional income for rural poor, it also raises concerns about overharvesting of endangered species (Wynberg, 2002). Harvesting of wild plants is often destructive. For example, Augustino and Gillah, 2005, reported the frequent use of roots for medicines among African herbalists and suggested these practitioners be trained in sustainable harvesting as a priority. There an urgent need to come up with sustainable management strategies to conserve wild populations and ensure sustainable, optimized sources of plant derived natural products. As conservation approach, *ex situ* cultivation is becoming a key element of modern day conservation strategies (Pfab and Scholes, 2004). The removal of plants from their native wild habitats to botanical gardens for domestication should be conducted with extreme care as some plants may fail to synthesize the bioactive constituents following relocation. Existing literature indicates that the majority of pharmacologically important compounds of plant origin are products of defense and secondary metabolism (Andrew *et al*., 2007; Kuzel *et al*., 2009;Sudha and Ravishankar, 2002). This ability of plants to respond to physical and/or chemical stimuli can be used for elicitation of pharmacologically active substances by subjecting an intact plant to stress

**Corresponding Author's E-mail: motlhankadan@yahoo.com*
*IUCN: International Union for Conservation of Nature

plant origin are products of defense and secondary metabolism (Andrew *et al*., 2007; Kuzel *et al*., 2009;Sudha and Ravishankar, 2002). This ability of plants to respond to physical and/or chemical stimuli can be used for elicitation of pharmacologically active substances by subjecting an intact plant to stress factor (s) (Kuzel *et al*., 2009). It is important to recognize that growing a plant outside its environment under ideal conditions may result in it being unable to produce the desired bioactive substance, hence need for prior evaluation. In addition, propagation techniques are often accompanied by limitations such as low germination rates of seeds or failure of cuttings to produce primary root. There are some reports on the micropropagation of *H. procumbens* (Bairu *et al*., 2009); Jain *et al*., 2009;Levieille and Wilson,2002). These approaches need to be supplemented with *ex situ* cultivation and evaluation of the ability of the cultivated plant to produce tubers. *Ex situ* cultivation procedures should be preceded by proper evaluation of the plants for their ability to produce their bioactive constituents and the results should be compared to their wild counterparts. This study aims at assessing the antioxidant activity and phytochemical profiles of the *ex situ* cultivated plants in comparison to wild species.

Ethnobotanical data & species information

Harpagophytum procumbens (Burch.) DC.Ex. Meisn is one of the most important medicinal plants native to Southern Africa. Its storage tubers are harvested, dried and sold by an estimated10-15 thousand plant gatherers who rely on the plant as a primary source of income (Bairu *et al.,*2010).

Species name: *Harpagophytum procumbens* (Burch.) Dc.Ex.Meisn.

Family name: Pedaliaceae

Common names: Sengaparile, Lengakapitse (Tswana); Kalahari Devil's claw(English) Xwate (Bushman).

Part used and mode of preparation: Tubers are boiled in water.

Devil's claw has been used as part of traditional medicine for centuries. Clinical trials indicated that extracts of the tubers are active in the treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation and heart disease (Stewart and Cole, 2005). The literature indicates that the anti-inflammatory activity is attributed to the presence of glycoside forms of iridoids harpagide (Boje *et al.,* 2003) acetylated phenolic glycosides (Munkombwe, 2003) terpenoids (Clarkson *et al*., 2003 and procumbine (Levieille and Wilson, 2002). Some animal experiments have reported antioxidant activity of *H. procumbens* (Bhattacharya and Bhattacharya,1998; Langmead *et al*., 2002) although these results have not been confirmed by others (Jadot and Lecomte,1992; Betancor-Fernandez *et al*., 2003). In 2001, in Germany, Devil's claw became the third most frequently used medicinal plant with sales of roughly 30 million Euro and an overall industry growth of 113% between 1999 and 2000 (Strohbach and Cole, 2007). Despite these encouraging economic benefits, not a lot of research has been done to assess ways of propagating it on larger scale and evaluating the phytochemical and antioxidant profiles of wild and domesticated plants.

Description*: H. procumbens* is a perennial herb with succulent tap root. The annual and creeping stems can be 2 m long. They grow from a primary or mother tuber, whose tap root can be up to 2 m deep. Secondary tubers called "babies" develop on fleshy roots growing from the primary tuber. They can be up to 60 cm long and 8 cm wide. The secondary tubers contain stachyose, a photosynthetic storage product thought to be an adaptation to drought conditions. The leaves are simple and opposite, up to 6.5 cm long and 4 cm wide. They are deeply or shallowly lobed. The flowers are tubular, 5-6 cm long and are normally light purple or pink (sometimes white) but yellow inside the tube. Flowering occurs in summer during November to April and fruits in January with first deep green fruits getting dark brown when mature. The fruits are large (7cm) in diameter and have four rows of curved arms with recurved (hooky) spines. The seeds are dark brown or black (Motlhanka *et al ,* 2011).

MATERIALS AND METHODS

Plant collection

The tubers of *H. procumbens* (wild species) were excavated from Seolwane lands in Eastern Botswana (S22°39′ 12.0″;E027°42′13.9″).The plants were collected during October. The habitat is largely sandy loam soils. The tubers were collected by means of digging 1m x 1m x1m depth around the live plant. The whole plant was removed intact and the shoots were detached from the mother plant using a secateur. Care was taken not to detach the secondary tubers from the primary stem. The tubers were then covered with a moist perforated mussel cloth. These tubers were then divided into two groups (Group 1 & 2); Group 1 kept for *ex situ* cultivation whilst group 2 sample was kept for experimental analysis. The plant was identified by its vernacular name and later validated at the Gaborone National Art Gallery and Herbarium and Voucher specimen DMT2012-002 was deposited.

Cultivation

Tubers (Group 1) from which the shoots have been removed were transported to an experimental botanical plot in Sebele, Gaborone, 474km from their native habitat. The tubers were propagated by means of placing them in pre-dug wells (1mx1mx1mdepth). The tubers were placed vertical with the stem head just protruding above the ground surface. Then the wells were refilled with soil and slightly compacted. The plants were watered once a week for a month and left. After 4 months, the plants had re-established and they were allowed to grow for two years. After two years tubers and shoots from these *ex situ* cultivated plants were subjected to TLC phytochemi-

cal screening and antioxidant analysis. The results from the *ex situ* cultivated plants were compared with those from the wild.

Extract preparation

The shoots (with leaves) and tubers from the wild plants and *ex situ* cultivated plants were sun dried and ground into separate fine powders (shoots powder and tuber's powder) to produce four samples:[Powder of shoots from the wild, powder of tubers from wild, powder of shoots from ex situ, powder of tubers from *ex situ*]. Each sample powder was extracted using a soxhlet apparatus for 12 hours in either methanol or chloroform. The resulting 8 crude extracts were concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The residues in the form of powder or greasy material were preserved in sterile bottles in a cool dark room until use.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, ascorbic acid, were purchased from Sigma Chemical Co. (St Loius, MO,USA); Folin-Ciocalteus's reagent, sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany); Silica gel 60, UV_{254nm}.(Merck). All other reagents were of analytical grade.

TLC-phytochemical profiling

Chloroform and methanolic shoots and tuber extracts of *ex situ* and wild plants were analysed by thin layer chromatography (TLC) with mobile phase system consisting of $CHCl_3$:MeOH:H_2O (50:30:1).

Determination of total phenolics

Total phenol contents in the extracts were estimated using the modified Folin-Ciocalteu method (Waterhouse, 2009). An aliquot (20µl) of the extract was mixed with 100µl Folin-Ciocalteu reagent in a clean cuvette and mixed well. Then 300µl of (0.2g/ml) Sodium Carbonate was added. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using Hewlett Packard UV-Vis spectrophotometer. Samples of extracts were evaluated at a final concentration of 0.1mg/ml. The total phenolic content was determined from the calibration curve and presented as Gallic Acid Equivalence mg/L).

DPPH radical scavenging assay

Free radical scavenging activity of the methanol extracts was measured in terms of the hydrogen radical scavenging ability using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Blois, 2002). Briefly a 0.1mM solution of DPPH in methanol was prepared and 1.0ml of this solution was added to 0.5ml of samples in different concentrations. After 20 min the absorbance was measured at 525 nm. The percent scavenging power of the extracts was calculated using the following relation: DPPH radical scavenging activity (%)=[($Abs_{control}$-Abs_{sample})]/($Abs_{control}$)]x100, where $Abs_{control}$ is the absorbance of DPPH radical +methanol; Abs_{sample} is the absorbance of DPPH radical+ sample/standard.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) [n=4]. To determine statistical difference between means *(p at 0.2),* ANOVA was performed using SAS statistical package.

RESULTS

TLC-phytochemical profiling

The TLC plates were developed from a solvent system comprising [$CHCl_3$:CH_3OH:H_2O](50:30:1).

From this phytochemical profile (Figure 1), there was no difference between the phytochemical composition of wild pants and *ex situ* cultivated plants.

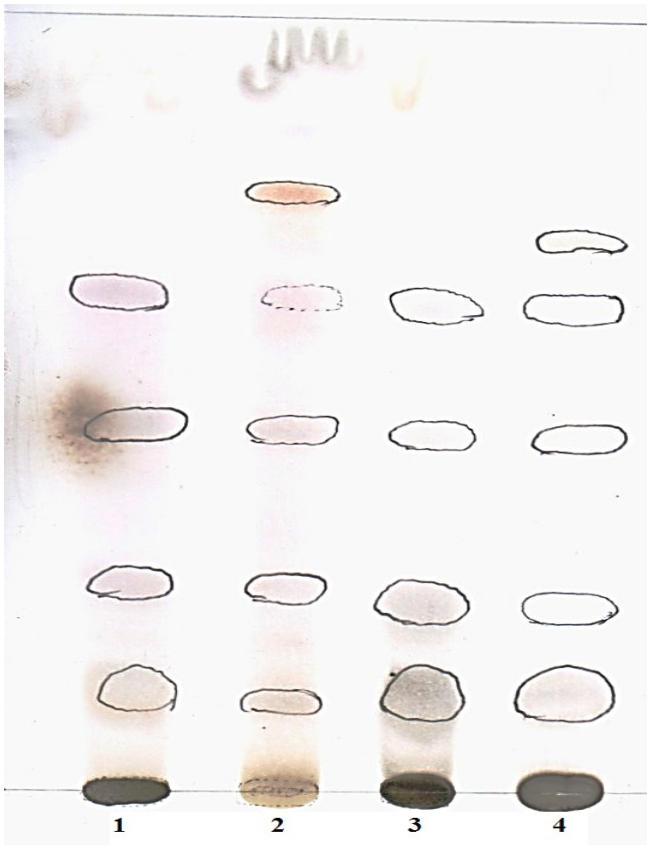

Figure 1. Phytochemical profiling of wild and *ex situ* cultivated *Harpagophytum procumbens.* **1**=Wild tubers; **2**=Wild Shoots; **3**=*Ex situ* Tubers; **4**=*Ex situ* Leaves (all tested samples were methanol extracts).

Determination of total phenolic content

There was no significant difference between the total phenolic contents of tubers from the wild and *ex situ* cultivated plants. There was also no significant difference between the total phenolic contents of leaves obtained from the wild *H.procumbens* and *ex situ* cultivated plants (Table 1). The results suggest that relocating these plants from the wild to the botanical garden did not result in phytochemical loss. The consistency in retention of phytochemicals is relevant for domestication.

Table 1. Comparative Total phenolic contents between *ex situ* and wild *H. procumbens*.

Sample Type	Mean ± standard deviation (mg GAE/L)
Ex situ Chloroform Leaf extract	1213.25[a]±9.53
Wild Chloroform Leaf extract	1184.25[a]±49.00
Ex situ Chloroform tuber extract	1479.75[b]±18.63
Wild Chloroform tuber extract	1528.50[b]±45.60
Ex situ Methanol Leaf extract	2686.00[c]±10.49
Wild Methanol Leaf extract	2562.25[c]±158.77
Ex situ Methanol tuber extract	3297.00[d]±54.56
Wild Methanol tuber extract	3366.75[d]±22.68

Means with the same letter are not significantly different. Values are mean ± SD (n=4) followed by different letters imply the significances between values (P_{value} at 0.2).

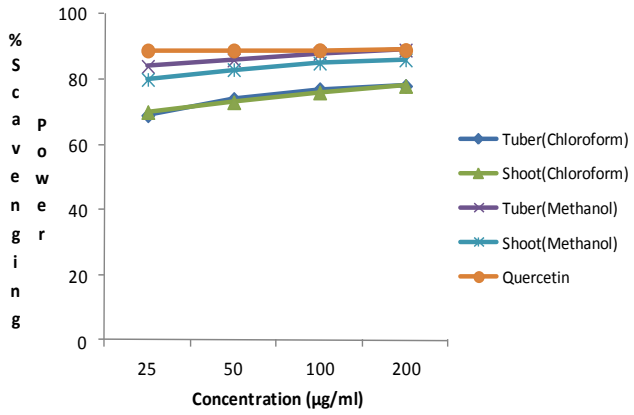

Figure 2. Free radical scavenging power of extracts of plants from the wild.

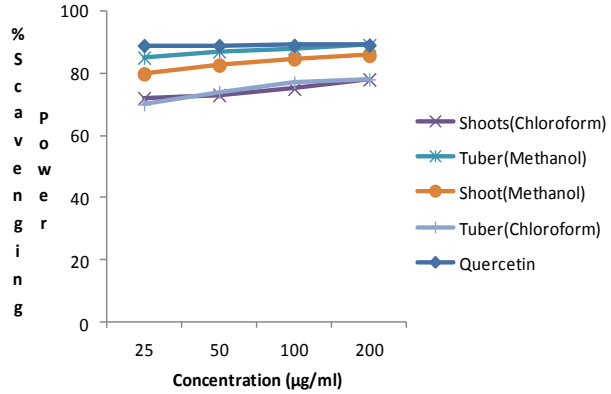

Figure 3. Free radical scavenging power of extracts from *ex situ* cultivated plants.

DPPH radical scavenging assay

There were no significant differences between the scavenging potencies of *ex situ* cultivated plants and wild plants (Figures 2 & 3). At all tested concentrations, scavenging potencies of chloroform extracts were lower than those of the methanol extracts. The observed antioxidant activity could be attributed to presence of phenolic compounds in the tested samples. Strong evidence exist that polyphenolic compounds from plants play crucial role as antioxidants (Kuruman and Joel, 2006: Kraus and Speteller, 1990). These compounds are polar and are easily extracted using polar solvents such as methanol.

DISCUSSION

Despite the extensive data demonstrating important therapeutic and encouraging economic benefits of *H. procumbens*, research on this plant is largely focused on its pharmacology and regulatory harvesting. Information on the biology, phytochemical properties and bioactivity profiles of the *ex situ* cultivated plants is scanty (Kathe *et al*., 2003). In the light of over exploitation of this plant, conservation strategies should be adopted. *In situ* preservation through development of improved forms of controlled use of naturally growing plants, and *in domo* conservation through development of cultivation practices as a means to conserve the species within the human domain are strategies for conservation (Wiersum, 2003).The IUCN red data list criteria in South Africa assessed the conservation status of *H. procumbens* as the least concern even though this plant is rated as near threatened by the department of Forestry and range resources (Botswana). From Figure 1, the chemical profiling of the plants using Thin layer Chromatography, there was consistency in the chemical composition of both wild and *ex situ* cultivated plants. Analysis of the total phenolic contents (Table 1) of wild and *ex situ* cultivated plants also showed consistency in the quantities of the phenolic compounds between wild and *ex situ* cultivated plants. At concentrations between 50 and 100µg/ml, the methanol extracts of both wild and *ex situ* cultivated plants showed very good free radical scavenging capacities (≥80%)comparable to control (Fig.2&3). At all tested concentrations, chloroform extracts showed lower free radical scavenging activities than the methanol extracts. This high content of total phenolics in methanol extracts correlates well with the observed higher DPPH scavenging potencies of methanol extracts.

CONCLUSIONS

Domestication of this plant can reduce the pressure on wild populations and should receive first priority. Field trials on domestication should be coupled with qualitative profiling to validate consistency in phytochemical composition. The total phenolic contents of wild plants

were comparable to the *ex situ* cultivated plants. The consistency in the presence of compounds in these plant materials is of interest from both the pharmacological and conservation point of view given the role played by these compounds in oxidative stress. These results indicate that *ex situ* cultivation can be both a conservation strategy and can provide an alternative and sustainable source of therapeutically active compounds.

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