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Environmental stress effect on the phytochemistry and antioxidant activity of a South African bulbous geophyte, *Gethyllis multifolia* L. Bolus

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ABSTRACT

Gethyllis multifolia is a South African bulbous geophyte with medicinal properties and on which very limited research has been conducted. This research investigated the effect of drought and shade, which are experienced in the natural habitat, on the antioxidant properties, as well as the isolation of natural compounds from certain plant parts. The total polyphenol, flavonol/flavone and flavanone contents, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) and radical cation scavenging ability (ABTS) were measured in the leaves, bulbs and roots (dry weight) of *G. multifolia* under photo- and drought stress. A significantly higher total polyphenol content was observed in the roots under the photo- and drought stresses when compared to the control. When all the plant parts were compared, the highest total polyphenol content was observed in the drought-stressed roots of *G. multifolia*. An increased antioxidant capacity was observed in the root system of *G. multifolia* where the FRAP, ORAC and ABTS were found to be significantly higher during drought stress when compared to the control. Phytochemical investigation of the leaves, bulbs and roots of *G. multifolia* revealed the presence of tannins, flavonoids, phenolics, saponins, glycosides (phenolic and terpenoid) as well as essential oils, while the test for alkaloids was negative. Further in -depth studies on the roots of *G. multifolia* led to the isolation of three known flavonoids, of which one was also isolated as its endogenously acetylated derivative. Their structures were elucidated by chemical and spectroscopic methods as 2,3-dihydro-7-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (**1**), (1-[2.4-dihydroxyphenyl]-3-phenylpropan-1-one) (**2**), 2,3-dihydro-5,7-dihydroxy-2-phenyl-4*H*-1-benzopyran-4-one or pinocembrin (**3**) and 5,7-diacetoxy-2,3-dihydro-2-phenyl-4*H*-1-benzopyran-4-one (**4**). This investigation indicated how environmental conditions can be manipulated to enhance the antioxidant properties of certain plant parts for future cultivation of this species and the isolation of the four natural compounds elucidated its medicinal potential and created a platform for future *in vivo* research.

1. Introduction

1.1. Medicinal uses and chemical composition

The genus *Gethyllis* belongs to the plant family Amaryllidaceae and is better known as "Kukumakranka" by the Khoi-San people. The genus comprises 37 currently accepted species in Southern Africa (Du Plessis and Duncan, 1989), among which many are considered to be endangered. Presently, very little is known about the chemical composition and bioactivities of this genus (Van Wyk et al., 1997). The word "Kukumakranka" is described by farmers as meaning "goed vir my krank maag" in Afrikaans, one of South Africa's eleven languages, which translates to "cure for my upset stomach" in English (Van der Walt, 2003). Watt and Breyer-Brandwijk (1962) reported that "Kukumakranka brandy", which is made from the fruit of *Gethyllis afra* and *Gethyllis ciliaris*, is believed to contain oils and esters of low molecular weight, and is an old Cape remedy that was used for colic and indigestion. According to Rood (1994) the early Cape colonialists used an alcoholic infusion of the fruit of *Gethyllis linearis* and *Gethyllis spiralis* as a remedy for digestive disturbances. In more recent times, a diluted infusion of the flower has been used for teething problems, and the skin of the fruit as a local application on boils, bruises and insect bites. Further reports by Rood (1994) indicated that the fruit was boiled by the Khoi-San and used as an aphrodisiac, while Van der Walt (2003) mentioned that *G. ciliaris* was used as a tonic for fatigue. Further pharmaceutical studies by Elgorashi and Van Staden (2004) revealed some anti-inflammatory and antibacterial activities in certain *Gethyllis* species and reported that the findings were in agreement with their uses as a traditional medicine. Previously, the following compounds: dihydroxydimethylbenzopyran-4-one, isoeugenitol, its 5-*O*-glycoside and 9Z-octadec-9-enamide, had been isolated from the roots and

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bulbs of *G. ciliaris* (Elgorashi et al., 2007). During head-space analysis of the volatiles from the fruits of *G. afra* and *G. ciliaris*, the following major compounds were characterized for *G. afra*: α-pinene, n-butyl n-butyrate, isoamyl acetate, β-pinene and 2-methylbutyl butyrate and for *G. ciliaris*: pentacosane; ethyl octanoate; ethyl isovalerate; ethyl hexanoate and ethyl benzoate. It was further reported that these compounds are responsible for the sweet/banana/piney odors of the fruit of these two species (Kamatou et al., 2008).

1.2. Botanical aspects

The interpretation of the species *Gethyllis multifolia* L. Bolus was last used in its 'Red Data Assessment' of 1996 and was classified as 'Vulnerable' (Hilton-Taylor, 1996). In the latest 'Red List of Southern African Plants' of 2009, *G. multifolia* has provisionally been subsumed under *Gethyllis campanulata*, but *G. multifolia* has not formerly been placed into synonomy with *G. campanulata* (SANBI, 2009). According to Du Plessis and Delpierre (1973) *G. multifolia* is a deciduous, winter-growing, summer-blooming and bulbous geophyte (Fig. 1A and B), 120 mm in height and indigenous to South Africa. The flowers measure 60–80 mm in diameter (Fig. 1C), colored white to cream with 12 anthers (six pairs) and the flowering period is from November to January (summer) (Goldblatt and Manning, 2000). The highly fragrant, tasty and edible fruit berries (Fig. 1D) are produced from mid-March to mid-April (autumn) at the onset of the new growing season (Van Reenen, 1975).

1.3. Biological properties

An antioxidant capacity-and -content study of plant parts of *G. multifolia* revealed higher polyphenol content and antioxidant activity in its root system when compared to the leaves and bulbs (Daniels et al., 2011). This study further revealed the highest total polyphenols and antioxidant activity in the fruits and flowers, which is comparable to blueberries, strawberries and raisins. According to Babajide et al. (2010), the brine shrimp lethality assay, which indicates toxicology levels of bioactive compounds, revealed that methanolic extracts of *G. multifolia* whole plants indicated a high potential for antimicrobial and antiviral activities. Plants possess different antioxidant properties, depending on their antioxidant molecule content, which is strongly affected by the plant's growing conditions (Lin et al., 2006). Environmental stress factors such as shade, abnormal salt levels, high temperature and drought, may result in the generation of reactive oxygen species (ROS) in plants which in turn may cause oxidative stress when in excess. In plant cells, oxidative stress reactions are associated with the production of toxic free radicals (Price et al., 1989). Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to scavenge ROS and protect their cells against oxygen toxicity (Fridovich, 1975). According to Di Carlo et al. (2001) the relationship between plant stress acclimation and human health comprises a broad array of metabolites some of which possess "desirable" pharmacological properties. Many examples can be found in nature as in the case of hyperforin, which is the active ingredient in St. John's wort (*Hypericum perforatum*) and is known for alleviating mild depression. When St. John's wort plants are subjected to heat stress it substantially increases hyperforin concentration in the shoots (Zobayed et al., 2005).

1.4. Justification for the research

According to Hilton-Taylor (1996) *G. multifolia* is threatened in its natural habitat, which stresses the need for future cultivation of this species by pharmaceutical companies, traditional healers and farmers. Should certain environmental stresses increase the antioxidant content or activity of this species, it can be incorporated in future cultivation practices to induce increased antioxidant levels in essential plant parts during production. To date no published data are available on how important biological properties, such as antioxidant activity of this *Gethyllis* species, are affected by environmental stress factors. Thus, the aim of this study was to investigate the changes in the antioxidative capacity and levels in the leaves, bulbs and roots of *G. multifolia* during controlled photo- and drought environmental stresses over one growth season. Furthermore, phytochemical screening was undertaken to isolate and characterize some natural compounds from the dried leaves,

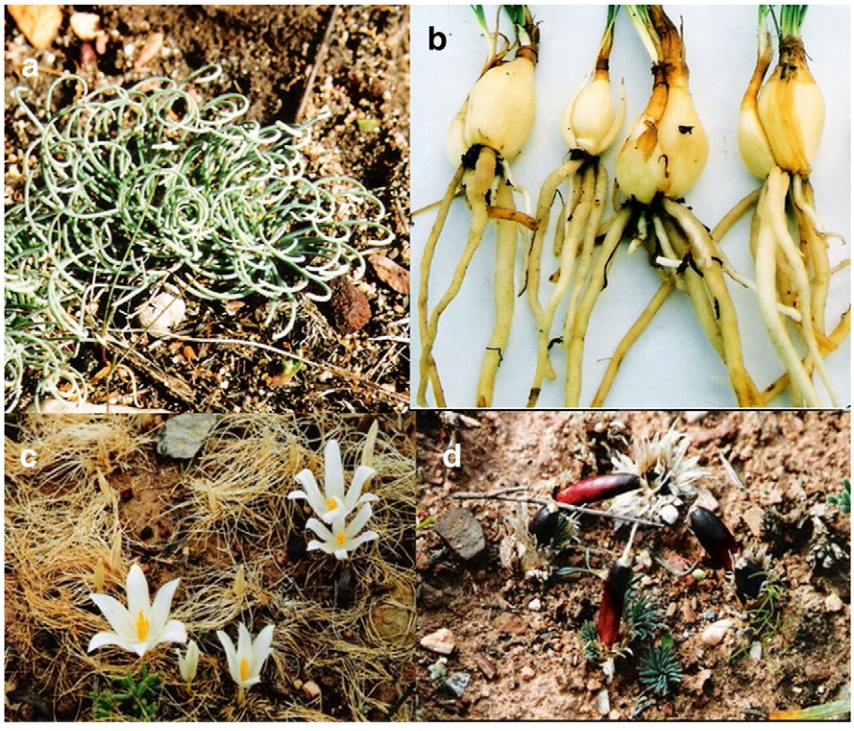

Fig. 1. The foliage emerging during March and April (a), roots and bulb (b), flowers emerging during early December (c) and the club-shaped berry (d) of *Gethyllis multifolia* protruding from the soil during March and April.