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African Potato (*Hypoxis* Spp): Diversity and Comparison of the Phytochemical Profiles and Cytotoxicity Evaluation of four Zimbabwean Species.

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ABSTRACT

The *Hypoxis* corm has a catalogue of medicinal uses and also serves as a source of food. Most scientific research on *Hypoxis* has centred on *Hypoxis hemerocallidea* although many other species are exploited medicinally. *Hypoxis* corms look similar and this has resulted in different species being prescribed and sold as the same species raising important public health issues of the efficacy and quality of the medicines sold. The aim of this study was to investigate and compare the phytochemical profiles and cytotoxicity of *Hypoxis hemerocallidea*, *Hypoxis rigidula, Hypoxis galpinii* and *Hypoxis obtusa*. Corm methanolic extracts of the four species were qualitatively screened for different phytochemicals following standard methods. Tests indicated presence of terpenoids, saponins, cardiac glycosides, tannins and reducing sugars and negative for alkaloids, flavonoids and anthraquinones in the four *Hypoxis* species. Cytotoxic effects of the plants were assessed through Brine shrimp lethality (BSL) bioassay. LC_{50} values ranged from 6.3 -409 mg/ml showing that the plants are non toxic.

INTRODUCTION

Hypoxis (commonly known as the African potato) is a pantropically distributed genus belonging to the plant family Hypoxidaceae (Nordal and Zimudzi, 2001). The genus is of wide distribution throughout Africa and southern Africa is considered to be its main centre of diversity and endemism (Singh, 2009). The genus is characterised by the presence of yellow hairy flowers with six stamens, trilocular ovaries without a beak and free perianth segments (Nordal *et al.*, 1985). Zimbabwe is endowed with a wealth of indigenous medicinal plant species and *Hypoxis* is among the commonly prescribed medicines by traditional healers. Traditional medicine plays a critical role in the primary health care delivery system in Zimbabwe especially now with the spiralling costs of prescription drugs and the poor or nonexistent health care infrastructure in some rural areas. It has been estimated that about 85% of the general population in the country use African traditional medicines (Mdluli, 2002). African potato rootstock extracts, powders, infusions and decoctions have been used for years by African traditional healers for the treatment, management and/or control of a variety of human ailments including cancers, nervous disorders, immune-related illnesses, heart weaknesses, urinary tract infections (Singh, 1999), HIV/AIDS, intestinal parasites, common cold, nausea, vomiting, infertility, depression, wounds, anxiety, and many more (Drewes *et al.*, 2008, Boukes and Venter, 2011). Recently, some studies have validated the therapeutic potency of the 'African potato' with some indicating antioxidant, anti-inflammatory, antimicrobial, antinociceptive, anticonvulsant and antidiabetic properties of *Hypoxis* (Drewes *et al.*, 2008). Most of the medical benefits of *Hypoxis* have been attributed to *H.hemerocallidea*, a species that has been thoroughly scientifically evaluated. Species delimitation in *Hypoxis* is problematic and despite several attempts, the systematics of the genus remains largely unresolved (Nordal *et al.* 1985, Nordal & Zimudzi, 2001, Wiland-Szymanska, 2001). This is mainly due to the lack of distinct morphological boundaries separating species, reticulate evolution and the occurrence of apomixis (Zimudzi, 1994). The lack of clear species identity in the genus has created problems for traditional healers and medicine vendors who harvest different species and sell them all as the same plant commonly referred to as the African potato.

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This potentially creates problems of substitution and adulteration of the medicinal plants consequently compromising the quality and safety of the medicines concerned. Although the accurate description of the African potato has been published (Zimudzi and Kativu, 2002; Singh, 2009), the indiscriminate harvesting of *Hypoxis* species continues to be a problem. Studies elsewhere in the region (Oluwule *et al*., 2007, Sathekge, 2010) have shown that a number of *Hypoxis* species share similar phytochemicals. This study compares the phytochemical profiles of four commonly harvested *Hypoxis* species in Zimbabwe, namely; *H.obtusa, H.hemerocallidea, H. goetzei* and *H. rigidula* and evaluates their cytotoxicity so as to assess whether the substitution that occurs has any effects on public health.

MATERIALS AND METHODS

Collection of plant material

Hypoxis corms were collected from transplants growing at greenhouses at the University of Zimbabwe's department of Biological Sciences and at the National botanic gardens. The plants were identified by botanists in the department and voucher specimens were deposited at the University of Zimbabwe teaching herbarium. The corms were chopped into small pieces and oven-dried at temperatures around 50^0C for one week.

Extraction

Dried samples were milled into a fine powder by pounding manually with a clean pestle and mortar. Some 10g of ground material was extracted with 200 ml analytical grade methanol solvent at room temperature for 48 hours using the cold maceration method. The extracted material was filtered using Whatman filter paper (No: 1) and the Buchner vacuum filter, and stored at 4^0C. The residue was further soaked in an equal volume of the same solvent for 24 hours. After filtration, the two extracts were pooled and concentrated using a rotary evaporator at 40^0C under reduced pressure.

Phytochemical screening

Standard qualitative methods as described by Sowofora (1993) and Tiwari *et. al* (2011) were adopted for phytochemical screening. The crude extract was tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehlings test, anthraquinones with Borntragor's test, terpenoids with Salkowski test, flavonoids with ammonia and suphuric acid, saponins with foam test, tannins with Ferric Chloride test, alkaloids with Mayer's and Draggendorff's tests and cardiac glycosides with Keller- Killian's test.

Brine shrimp lethality test

The cytotoxicity of the crude extract was assessed on brine shrimp nauplii (*Artemia salina*) according to brine shrimp lethality bioassay (Meyer *et al*., 1982). Artificial sea water was prepared by dissolving 12 g sodium chloride in 1 L of distilled water and adjusting the pH to 8.5 using 40 % sodium hydroxide. Some 2 g of brine shrimp eggs were hatched in 1L of sterile sea water in a flask. The cysts were kept under bright light, and were continuously agitated and aerated using an aquarium pump. The nauplii hatched within 48 h at room temperature. The crude extract was dissolved in 1% aqueous dimethyl sulfoxide (DMSO) in artificial sea water to obtain the following concentrations of extract: 1000, 500, 100, 50, and 10 μg/ml. Some 0.5 ml of each solution was transferred, to clean sterile vials containing 4.5 ml of aerated seawater. Shrimp nauplii were drawn through a glass capillary and placed in each vial. The experiments were performed in triplicate for each extract concentration. Potassium dichromate (5mg/ml) and 1% DMSO in seawater were used as positive and negative controls, respectively. After 24h, the vials were examined against a lighted background using a hand held magnifying glass, and the number of nauplii that survived in each counted.

Data analysis

Results were analyzed using Microsoft Excel (2007). The cytotoxicity data were analysed using linear regression analysis. The regression equations were used to calculate the lethal concentration

RESULTS AND DISCUSSION

Descriptions of the plants

Figure 1 below shows photographs of the four species of *Hypoxis* analysed in this study. The four species are morphologically distinct and are distributed throughout southern Africa with some records extending into east Africa. *H.rigidula* Baker are generally tall slender plants bearing a rigid, cylindrical false stem. The leaves are strongly ribbed with newly formed leaves erect and densely hairy whereas mature leaves are less hairy, straplike and recurved above their middle. The plants are often many flowered with black seeds. The species frequently inhabits open grasslands on well-drained areas, rocky slopes and on the edges of shrubland, wetlands and stream banks. *H.hemerocallidea* Fisch., Mey. & Ave-Lall. species are robust plants with broad, shiny, slightly hairy leaves arranged one above the other in three ranks. Inflorescences and flowers are clothed in long whitish hair. The plants occupy a variety of habitats including open grassland, sand dunes, thickets and forest margins. *H. galpinii* Baker are also robust plants with long, lanceolate leaves that clasp at the base to form a short false stem, widening above into a funnel shape. Leaf lamina often with four prominent veins and long, sparse whitish hairs. Leaves turning purplish-red on drying. Inflorescences densely covered with white hairs. *H. galpinii* grows mainly on sandy soils at high altitudes, in montane. Individuals belonging to *H.obtusa* Burch are morphologically variable and could warrant the consideration of several taxa. Generally, however, the leaves are stiff and prominently ribbed with whitish hairs on margins and lower surface of the midrib. The species is also generally many flowered. The plants grow on clayey, sandy and lateritic soils, mostly in undisturbed open woodland and grassland.

Fig 1. Photographs of the four species of Hypoxis studied. A-*H.rigidula*, B-*H.hemerocallidea*, C-*H.galpinii*, D-*H.obtusa*.

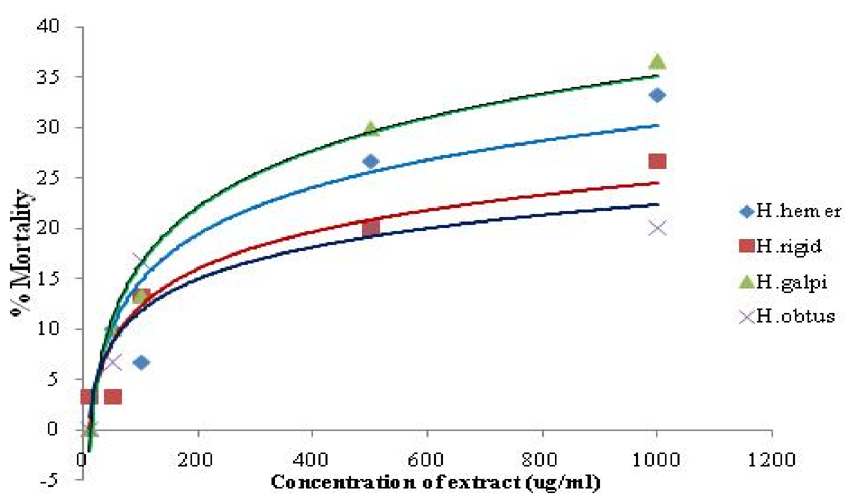

Fig 2. Mortality of brine shrimp nauplii in different concentrations of corm extract from the four *Hypoxis* species. H.hemer-*H.hemerocallidea*, H.rigid.-*H.rigidula*, H.galpi-*H.galpinii*, H.obtus.-*H.obtusa*.

Phytochemical screening

The screening of plant secondary metabolites is essential in understanding their pharmacological properties as secondary metabolites are responsible for the medicinal properties of plants. In this study all extracts tested positive for terpenoids, saponins, cardiac glycosides, tannins and reducing sugars and negative for alkaloids, flavonoids and anthraquinones (Table 1).

Table. 1: Results of phytochemical screening tests on *Hypoxis spp* methanol crude.

Phytochemical test	Observations			
	H.hemerocallidea	*H.obtusa*	*H.rigidula*	*H.galpinii*
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Saponins	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinones	-	-	-	-
Cardiac glycosides	+	+	+	+
Tannins	+	+	+	+
Reducing sugars	+	+	+	+

Table. 2: Cytotoxicity of *Hypoxis spp* extracts determined by the brine shrimp lethality test.

Species	LC_{50} (µg/ml)	Regression equation	R^2
H.hemerocallidea	19245.26	y=6.6973ln(x)-16.069	0.8685
H.rigidula	121784.1	y=5.2994ln(x)-12.056	0.8937
H.galpinii	6251.153	y=8.097ln(x)-20.772	0.9798
H.obtusa	408995.4	y=4.5887ln(x)-9.2927	0.8891

The four *Hypoxis* species contain similar phytoconstituents but this does not necessarily mean that the plants have similar pharmacological properties. This is because the present study was a qualitative assessment of the phytochemicals; quantitative assessments may show differences in the phytochemical contents of the species. Most importantly, however, is that the phytochemicals identified here are large and diverse groups and further analysis of particular compounds in these groups needs to be done to make accurate comparisons on the biological activity of the phytochemicals.

Hypoxoside, a glucoside compound isolated from a number of African species of *Hypoxis* including *H.hemerocallidea*, *H.rigidula* and *H. angustifolia* (Marini-Bettolo *et al*., 1982, Nair and Kanfer, 2006), has for a long time been suspected to be the active ingredient in *Hypoxis*. However, other gylcosides like acuminoside and nyasoside isolated from *H.acuminata* and *H.nyasica* respectively have been isolated and could also have biological activity. Phytosterols have also been implicated in some of the therapeutic and pharmacological properties of *Hypoxis* like immunomodulation, antiinflammation, anti-pyretic activities (Mkhize *et a*l., 2013) and these have been shown to vary among *Hypoxis* species. It is highly unlikely that the multitude of medicinal properties of *Hypoxis* extracts could be attributed to only these compounds. It is possible that these compounds act in synergy with other compound. Certainly, more laboratory and clinical studies are required to clarify this.

All the phytochemicals identified in this study, however have been associated with some of the biological activities attributed to these plants. Tannins are reported to have antinociceptive, anti-inflammatory and antioxidant properties (Owira and Ojewole, 2009), saponins have antioxidant, anti-inflammatory, anticancer, antidiabetic and antimicrobial properties (Ali *et a*l., 2011), whilst terpenoids have antimicrobial, analgesic and antiinflammation properties (Sermakkani and Thangapandian, 2010). Cardiac glycosides on the other hand have been reported to be important in treating heart ailments (Schneider and Wolfling, 2004).

Brine shrimp lethality

The brine shrimp cytotoxicity assay is widely considered a convenient method for preliminary assessment of toxicity and has been used for the detection of fungal toxins, food additives, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials (Rajeh *et al.*, 2012). Figure 2 shows that the mean percent mortality of the brine shrimp nauplii increases with increase in extract concentration for all the species of *Hypoxis* studied. This shows that the extracts are biologically active and toxic to some extent. The brine shrimp mortality was 100% in the Potassium dichromate standard and there was no mortality in the negative control vials.

Table 2 presents the calculated LC_{50}, regression equations and R^2 value. All the R^2 values are closer to 1, showing that the regression lines presented on Figure 2 best fit the original data points. The LC_{50} values vary from 6.3 mg/ml in *H.galpinii* to 409 mg/ml in *H.obtusa.*

Standard brine shrimp lethality bioassay stipulates that plant extracts with LC_{50} values less than 1mg/ml are considered bioactive in toxicity evaluations (Meyer et al., 1982). Based on this benchmark, all the *Hypoxis* extracts are non-toxic since all their LC_{50} values were greater than 1 mg/ml. Drewes *et al.*, (1984) and Smit *et al.*, (1995) have also reported low toxicity values of *Hypoxis*. Toxicity of plant crude extracts is usually attributed to the presence of alkaloids and saponins (Musa 2012; Olaleye 2007. In this study alkaloids were not found, so the little toxicity observed could be attributed to the saponins present.

CONCLUSIONS

The *Hypoxis* species *H.hemerocallidea, H.rigidula, H.galpinii and H.obtusa* show similar phytochemical profiles and are non-toxic. The expectation from these results is that the observed problems of adulteration of the species in the traditional medicine markets should have no impact on public health. However, as highlighted above the methodology employed here only identifies major groups of phytochemicals which are known to be diverse in their chemical constituents so it is not possible to make firm conclusions on the safety of these substitutions to public health on the basis of this study. More detailed laboratory work to decipher the detailed chemical constituents is required.

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