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Anti-bacterial and anti-oxidant activity of *Hypoxis hemerocallidea* (Hypoxidaceae): Can leaves be substituted for corms as a conservation strategy?

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Abstract

Hypoxis hemerocallidea Fisch. & C.A. Mey (Hypoxidaceae) is an important plant species in traditional medicine in southern Africa. The use of the corms of hypoxis, commonly known as "African potato" is so popular that the species is threatened by over-harvesting. This study was aimed at comparing the chemical composition and biological (anti-bacterial and anti-oxidant) activities of fresh and dried aerial, and dried underground parts of hypoxis with the view of promoting the use of the former, as a conservation strategy. Leaves (fresh and dried) and dried corms were milled and extracted with acetone and then ethanol and analysed by TLC with two different solvent systems. The extracts were also then tested against four species of nosocomial bacteria in a micro-dilution assay and also for DPPH anti-oxidant activity. There were clear differences in the chemistry and biological activity of leaves versus corms of hypoxis. Ethanol extracts of fresh leaves showed overall best activity in the anti-bacterial assay with MIC < 0.63 mg/ml. Components in the acetone extracts of both leaves and corms showed good anti-oxidant activity. Substituting leaves for corms in the medicinal use of hypoxis is not recommended because of the lack of phytochemical and bioactive similarities.

Keywords: African potato; Anti-bacterial; Anti-oxidant; *Hypoxis hemerocallidea*; Hypoxis; MIC; Conservation

1. Introduction

Hypoxis hemerocallidea Fisch. & C.A. Mey (Hypoxidaceae) is previously known as *H. rooperi* and more commonly known as hypoxis, African potato, Yellow star flower (English), Lilabatseka, Zifozonke (Swazi) and Inkomfe (Zulu). It is widely distributed in the savanna regions of South Africa, Swaziland and Zimbabwe and is increasingly also grown as a pot plant. *H. hemerocallidea* is a stemless, geophytic, perennial herb with large corms (tubers) which are dark brown to black on the outside and bright yellow inside (Van Wyk et al., 1997).

Infusions of the mature corms are used in African traditional medicine as emetics, to treat dizziness, burns, wounds, wasting disease, anxiety, depression and insanity, diabetes mellitus, cancer, polyarthritis, hypertension and asthma (Grierson and Afolayan, 2003; Ojewole, 2006; Ojewole et al., 2006). Botanical products of this species are formulated and marketed for the amelioration of prostate disorders (benign prostate hypertrophy (BPH) in particular) and urinary infections as well as immune modulation (Bouic et al., 1996; Schulz et al., 2001). It is thought that the phytosterols in hypoxis with their recognized 5α-reductase and aromatase inhibition activity are responsible for amelioration of BPH. These sterols have also been shown to possess immune modulating properties which may have some use in TB treatment (Bouic et al., 1996; Donald et al., 1997). Anti-inflammatory and anti-diabetic properties have been demonstrated with aqueous extracts in mice and rats (Ojewole, 2006; Steenkamp et al., 2006). There are also unsubstantiated reports that hypoxis extracts stabilize CD4 lymphocytes in HIV/AIDS (Ojewole, 2002). It is for its purported anti-HIV/AIDS activity

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that the African potato has become symbolic of the ascendance of African herbal medicine over allopathic medicine.

Reported constituents of hypoxis include the norlignan glycoside, hypoxoside which is hydrolysed in the gastrointestinal tract (GIT) to rooperol which possesses anti-mutagenic and anti-inflammatory activity (Albrecht et al., 1995). The phytosterols, for which hypoxis is a rich source, are known to be responsible for its use in the management of testicular tumours and benign prostatic hyperplasia (BPH) (Rhodes et al., 1993; Retief, 2001). Unidentified lectin-like compounds have been shown to inhibit staphylococcal growth (Gaidamashvili and Van Staden, 2002).

Because of over-harvesting in the wild hypoxis is a threatened species. This study was aimed at investigating the chemical and biological differences between the corms (which are currently the more widely used part) and the aerial parts. There have been no chemical or pharmacognosy studies on the latter. In the event that there is no difference between these plant parts, the promotion of the use of aerial parts can be initiated as a conservation measure which may ensure sustainable harvesting. Anti-bacterial and anti-oxidant tests were also done as part of this study as there is little data on the activity of hypoxis extracts against nosocomial infections and oxidative stress.

2. Materials and methods

2.1. Extraction and chemical profiling

H. hemerocallidea originally collected from Mpumalanga province, north-eastern South Africa and grown as pot plants in the Phytomedicine laboratory at Onderstepoort, University of Pretoria were used for this study. The plants were uprooted and the leaves separated from the corms. The leaves were split into two and extracted either freshly harvested (9.6 g) (coded F) or after oven-drying at about 50 °C for several days (4.1 g) (designated D). The corms (22.6 g) were sliced and then extracted after drying in the oven at about 50 °C. In each case the plant material was extracted three times with a total of 400 ml of acetone followed by 400 ml of ethanol.

Aliquots of 100 µg were loaded on TLC, developed in either chloroform:ethylacetate:formic acid (CEF) (20:16:4) or ethylacetate:methanol:water (EMW) (40:5.4:4) (Katerere and Eloff, 2004) and then sprayed with vanillin spray (0.1 g vanillin dissolved in 28 ml methanol and 1 ml sulphuric acid).

2.2. Anti-microbial assays

The minimum inhibitory concentration (MIC) was determined by serial dilution micro-plate assay with *p*-iodonitrotetrazolium (INT) (Sigma) as growth indicator according to the method by Eloff (1998). In brief, the test solutions (100 µl) at an initial concentration of 10 mg/ml were serially diluted with water to 50% in a 96-well plate. The actively growing bacterial cultures (100 µl) were added to each well making a further 50% dilution. The plate was covered and incubated overnight at 37 °C after which 40 µl of a 0.2 mg/ml INT solution was added to each well and the colour change observed after 2 h of incubation at 37 °C. The MIC was read as the lowest well in which there is no colour change. *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 25922), *Escherichia coli* (ATCC 27853) and *Enterococcus faecalis* (ATCC21212) were used as test organisms as they are important nosocomial pathogens. Ampicillin (Rolab, South Africa) and neomycin (Aspen, South Africa) were used as positive controls for Gram-positive and Gram-negative bacteria respectively.

The number of anti-bacterial compounds present was determined by the direct bioautography method of chromatograms using *S. aureus* (Begue and Kline, 1972).The extracts were loaded onto a TLC plate and developed using CEF. The plate was thoroughly dried overnight and then the chromatograms were sprayed with a dense culture of *S. aureus*, incubated overnight at 37 °C and then sprayed with 0.2 mg/ml *p*-iodonitrotetrazolium (INT) (Sigma). Clear zones indicated compounds which inhibited bacterial growth.

2.3. Free radical scavenging assay

The DPPH assay as previously used by Braca et al. (2002) was employed with the colour change of 2, 2-diphenyl-picrylhydrazyl radical (DPPH) (Sigma) which is purple in colour being reduced to diphenylpicryl hydrazine which is yellow in colour. The extracts were loaded onto a TLC plate which was then developed in EMW. After it was dried, the plate was sprayed with 0.2% DPPH in methanol and the colour change observed over a 30 min period.

3. Results

3.1. Chemical profile

The chemical composition of the corms and the leaves on TLC were distinctly different; leaf samples were more complex than the corm extracts (Table 1). There were no apparent differences in the chemical composition of the acetone and ethanol extracts of the corms and leaves. Phytosterols, which were found to show up as pink bands on TLC after spraying with vanillin (Retief, 2001) were absent in the aerial parts but present in the corms.

3.2. Anti-microbial assays

Both the corms and the leaf (whether fresh or dry) extracts had activity against the four selected cultures (Table 2). The

Table 1
Chemical complexity of extracts of *H. hemerocallidea* separated by EMW or CEF

Sample	EMW	CEF
Dried corm — A	9	2 (1 UV)
Dried corm — E	9	3 (4 UV)
Fresh leaf — A	5 (1 UV)	6 (2 UV)
Fresh leaf — E	5 (1 UV)	6 (2 UV)
Dried leaf — A	8 (3 UV)	8 (3 UV)
Dried leaf — E	11	2

The complexity was judged by counting the number of distinct spots visualized on TLC under UV and after spraying with vanillin. A = acetone, E = ethanol extracts.

Table 2
Minimum inhibitory concentrations (MIC) of acetone (A) and ethanol (E) extracts of bulb and aerial parts of *H. hemerocallidea*

	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
Dried corm — A	0.31	0.63	2.5	>5
Dried corm — E	0.63	1.25	2.5	>5
Fresh leaf — A)	2.5	2.5	2.5	2.5
Fresh leaf — E	0.63	0.31	0.31	0.63
Dried leaf — A	1.25	2.5	0.31	0.63
Dried leaf — E	2.5	0.63	2.5	>5
Ampicillin*	1.56	6.25	–	–
Neomycin*	–	–	0.20	0.78

All concentrations are in mg/ml except for positive controls∗ which are in μg/ml.

acetone extract of the dried corms showed activity at 0.31 mg/ml against *S. aureus* and was by far the most active of all the extracts against this micro-organism. The ethanol fraction of fresh leaves was also highly active against *E. faecalis* and *E. coli* at 0.31 mg/ml. *E. coli* and *P. aeruginosa* were both highly resistant to all the extracts but were sensitive to ethanol extracts of fresh leaves and to acetone fractions of dried leaf.

The ethanol extract of the fresh leaves was the only one to show activity in the bioautogram with a single clear band (R_f 0.45) against *S. aureus*. This correlates with the micro-plate result which showed the same extract to have an MIC of 0.63 mg/ml.

3.3. Free radical scavenging assay

In the DPPH anti-oxidant assay, the acetone extracts of the fresh leaf eluted in EMW had three bands of high activity. Both corm extracts also had high activity confined to the origin on TLC implying that activity is due to highly polar constituents. Rooperol, a dicatechol aglycone of hypoxoside showed higher anti-oxidant activity compared to the glycoside in the Trolox equivalent anti-oxidant capacity (TEAC) assays and the oxygen radical absorbance capacity (ORAC) assay (Laporta et al., 2007). Both rooperol and hypoxoside also showed good radical scavenging activity in the thiobarbituric acid-reactive substances (TBARS) assay. Extracts of *H. hemerocallidea* have demonstrated superior anti-oxidant activity to extracts of both olive leaf and green tea (Laporta et al., 2007).

4. Discussion

The ethanolic extracts of fresh leaves of hypoxis were consistently the most active against all four bacteria tested with MIC ≤ 0.63 mg/ml. For crude extracts, activity below 1 mg/ml is generally considered good and worth pursuing in further fractionation studies. The acetone extract of fresh leaves on the other hand was inactive. Lectin-like proteins from the corms of hypoxis have shown *in vitro* activity against *S. aureus* (Gaidamashvili and Van Staden, 2002). The corm extracts in this study showed moderate activity against *S. aureus* and this may confirm the earlier results. This result lends credence to the use of hypoxis in the treatment of wounds and burns (Grierson and Afolayan, 2003).

Activity against both *E. faecalis* and *E. coli* may be supportive of the use of hypoxis in urinary tract infections (UTI). *E. coli* is frequently isolated in monobacterial urinary tract infections (Keegan et al., 2003; Steenkamp et al., 2006) and is estimated to cause 80% of all UTIs (Persaud et al., 2006). Steenkamp et al. (2006) have previously demonstrated activity against *E. coli* by both ethanolic and aqueous extracts of hypoxis corms.

There is no literature information on the phytochemical constituents of hypoxis leaves because the interest of researchers has hitherto focussed on corm extracts. The corms are known to possess sterols, stanols and sterolins as well as the norlignan glycoside, hypoxoside (Bouic et al., 1996; Nair et al., 2007). The activity of the leaves seen here should spur focus to shift to studies on their phytochemistry and biological activity which appear to be distinct from the underground parts.

The clear chemical and bioactive differences between aerial and underground parts of hypoxis mean that it is not possible to substitute one part with the other. This contrasts with the work on *Prunus africanum* where young leaves have been reported to be an alternative source of sterols and sterolins (Stewart, 2003). This report which compared leaves and bark of *P. africanum*, a popular herb for BPH phytomedicines in Europe has led to more sustainable use of this threatened species. In the case of hypoxis this will not be possible because of the differences reported here. The only other viable alternative would be the domestication and propagation of the species.

5. Conclusion

The leaves of *H. hemerocallidea* showed a different chemical profile from that of the corms. Proper phytochemical isolation studies followed by HPLC profiling are required to understand these differences and are worth pursuing. The use of leaves is more rational in the treatment of infections than the current use of corms. The demonstration of some activity by ethanol and acetone leaf extracts against the Gram-negative bacteria merits further study.

References

Albrecht, C.F., Theron, E.J., Kruger, P.B., 1995. Morphological characterisation of the cell-growth inhibitory activity of rooperol and pharmacokinetic aspects of hypoxoside as an oral prodrug for cancer therapy. South Afr. Med. J. 85, 853–860.

Begue, W.J., Kline, R.M., 1972. The use of tetrazolium salts in bioautographic procedures. J. Chromatogr. 64, 182.

Bouic, P.J., Etsebeth, S., Liebenberg, R.W., Albrecht, C.F., Pegel, K., Van Jaarsveld, P.P., 1996. β-Sitosterol and β-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. Int. J. Immunopharmacol. 18, 693–700.

Braca, A., Sortino, C., Poloiti, M., Morelli, I., Mendez, J., 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol. 79, 379–381.

Donald, P.R., Lamprecht, J.H., Freestone, M., Albrecht, C.F., Bouic, P.J., Kotze, D., Van Jaarsveld, P.P., 1997. A randomized placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. Int. J. Tuberc. Lung Dis. 1, 518–522.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extract for bacteria. Planta Med. 64, 711–714.

Gaidamashvili, M., Van Staden, J., 2002. Interaction of lectin-like proteins of South African medicinal plants with *Staphylococcus aureus* and *Bacillus subtilis*. J. Ethnopharmacol. 80, 131–132.

Grierson, D.S., Afolayan, A.J., 2003. An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. J. Ethnopharmacol. 67, 327–332.

Katerere, D.R., Eloff, J.N., 2004. Variation in the chemical composition, antibacterial and anti-oxidant activity of fresh and dried Acacia leaf extracts. S. Afr. J. Bot. 70, 303–305.

Keegan, S.J., Graham, C., Neal, D.E., Blum-Oehler, G., N'Dow, J., Pearson, J.P., Gally, D.L., 2003. Characterization of *Escherichia coli* strains causing urinary tract infections in patients with transposed intestinal segments. J. Urol. 169, 2382–2387.

Laporta, O., Pérez-Fons, L., Mallvia, R., Caturla, N., Micol, V., 2007. Isolation, characterisation and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). Food Chem. 101, 1425–1437.

Nair, V.D.P., Foster, B.C., Arnason, J.T., Mills, E.J., Kanfer, I., 2007. *In vitro* evaluation of human cytochrome P450 and *P*-glycoprotein-mediated metabolism of some phytochemicals in extracts and formulations of African potato. Phytomedicine 14, 498–507.

Ojewole, J.A., 2002. Anti-inflammatory properties of *Hypoxis hemerocallidea* corm (African potato) extracts in rats. Methods Find. Exp. Clin. Pharmacol. 24, 685–687.

Ojewole, J.A., 2006. Antinociceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae) corm ['African potato'] aqueous extract in mice and rats. J. Ethnopharmacol. 103, 126–134.

Ojewole, J.A., Kamadyaapa, D.R., Musabayane, C.T., 2006. Some *in vitro* and in vivo cardiovascular effects of *Hypoxis hemerocallidea* Fisch & CA Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models. Cardiovasc. J. South Afr. 17, 166–171.

Persaud, K.C., Pisanelli, A.M., Evans, P., Travers, P.J., 2006. Monitoring urinary tract infections and bacterial vaginosis. Sens. Actuators, B, Chem. 116, 116–120.

Retief, A.C., 2001. Analysis of sterols and sterolins in *Hypoxis hemerocallidea* and related herbal medicine. MSc Thesis. University of Pretoria, Department of Pharmacology.

Rhodes, L., Primka, R.L., Berman, C., Vergult, G., Gabriel, M., Pierre-Malice, M., Gibelin, B., 1993. Comparison of finasteride (Proscar), a 5 alpha reductase inhibitor, and various commercial plant extracts in *in vitro* and *in vivo* 5 alpha reductase inhibition. Prostate 22, 43–51.

Schulz, V., Hänsel, R., Tyler, V.E., 2001. Rational Phytotherapy — A Physician's Guide to Herbal Medicine, 4th ed. Springer Verlag, Berlin.

Steenkamp, V., Gouws, M.C., Gulumian, M., Elgorashi, E.E., Van Staden, J., 2006. Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostatic hyperplasia and prostatitis. J. Ethnopharmacol. 103, 71–75.

Stewart, K.M., 2003. The African cherry (*Prunus africana*): can lessons be learned from an over-exploited medicinal tree? J. Ethnopharmacol. 89, 3–13.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

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