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ANTIOXIDANT AND PHENOLIC CONSTITUENTS OF *HYPOXIS COLCHICIFOLIA*

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Abstract

Hypoxis colchicifolia is extensively used in traditional medicine in Southern Africa. Providing a rationale and indicating the toxicity of the plant is essential for its future use as a natural therapeutic agent. In this study *H. colchicifolia* corm and leaf extracts were qualitatively assessed for phytochemical constituents and total phenolic content. The antioxidant potential of the extracts was evaluated using the 1,1-Diphenyl-2-picryl-hydrazyl assay, 2,2′-azinobis-(3-ethylbenzothiazolline)-6-sulfonic acid assay, phosphomolybdenum assay, cupric ion reducing capacity assay and the ferric reducing antioxidant power assay. All extracts indicated the presence of key phytochemical constituents and no toxicity. The extracts showed good antioxidant potential against various free radicals. Most extracts exhibited a lower IC_{50} than that of the positive control (rutin) against free radicals, thus indicating the good antioxidant potential of the extracts. The extracts of leaves and corms cannot be used interchangeably due to differences in the phytochemical composition. Acetone and methanol extracts show the most favorable results demonstrating the solvents' effectiveness in the phytochemical extraction of *H. colchicifolia*. The study also sheds light on the use of *H. colchicifolia* leaves as a natural alternative, which has not been previously explored. The *H. colchicifolia* extracts exhibit promising antioxidant potential, validating their use as a tonic for good health.

Keywords: *Hypoxis colchicifolia*, antioxidant, phytochemical, African potato

Introduction

Antioxidants are nature's way of defending cells against attack by reactive oxygen species (ROS). When the free radicals in the body outnumber the antioxidant defenses, this state is referred to as oxidative stress (Shahidi and Ambigaipalan, 2015). Oxidative stress causes serious cell damage, leading to a variety of human

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diseases like Alzheimer's, Parkinson's, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorder (Surveswaran *et al*., 2007, Pandey and Rizvi, 2009). The body naturally circulates a variety of nutrients for their antioxidant properties and manufactures antioxidant enzymes in order to control these destructive chain reactions (Hässig *et al*., 1999).

Vitamin C, vitamin E, carotenes and lipoic acid are well known and well researched antioxidants. Most of these antioxidants are found in the foods and beverages that we consume and improve our health as well as wellbeing.

Secondary plant metabolites are usually classified by their biosynthetic pathways. Phenolics, terpenoids and alkaloids are the three large molecule families considered, with glycosides, saponins, as well as tannins being part of them, according to their specific structure (Bourgaud *et al*., 2001). Biological studies of secondary metabolites have revealed a broad spectrum of physiological and pharmacological properties. The antioxidant potential of a plant extract is due to the various free radical scavenging molecules present (Choi *et al*., 2002), with each phytochemical constituent in the extract having certain biological activities due to their chemical structure (Tiwari *et al*., 2011).

Hypoxis colchicifolia is commonly referred to as broad leaved *Hypoxis*, 'inkomfe', 'igudu', 'ingcobo' and 'ilabatheka', in Zulu, both in coastal and inland regions, such as the Eastern Cape, KwaZulu-Natal, as well as the Free State. *Hypoxis colchicifolia* is a robust plant, relatively taller than other *Hypoxis* species, about 250-500 mm in height and growing singly, almost glabrous, unlike other *Hypoxis* species (Singh, 2007, Singh, 2006). It is one of the four most sought after plant species in traditional medicine (Ncube *et al*., 2013). *Hypoxis colchicifolia* corms have a few known constituents such as Haemanthine, hypoxoside and its aglycone rooperol (Chavan *et al*., 2013; Amusan *et al*., 2005). The phytochemical identification has not been carried out on the leaves and flowers, as most studies have focused on the corms, due to their extensive use in traditional medicine. The leaves are not used traditionally, though; they may contain important phytochemical constituents that are beneficial to human health. Therefore, the aim of this study was to compare the phytochemical composition, toxicity, and antioxidant potential of *H. colchicifolia* corm and leaf extracts. *In vitro* antioxidant assays can only classify antioxidants for their particular reaction mechanism; therefore, the use of multiple antioxidant assays is necessary in antioxidant screening.

Materials and methods

Collection of plant material

Hypoxis colchicifolia was collected and identified using taxonomic keys by Professor H. Baijnath from the School of Life Science, University of KwaZulu-Natal (UKZN). The sampling site was located in Mooiriver, KwaZulu-Natal, South Africa. A voucher specimen of the authenticated plant material was deposited at

the Ward Herbarium at UKZN (Westville campus) (Voucher number- Baijnathsn-01).

Preparation of plant material

Fresh corm

Corms of *Hypoxis colchicifolia* were washed, dried, peeled and grated. The grated corms were ground to a pulp using a mortar and pestle. The fresh corm (150 g) pulp was mixed with different solvents (acetone, methanol, distilled water) in the 1:4 w/v ratio. This was stirred for 48 h on a rotary shaker then samples were filtered using Whatman No. 1 filter paper. The solvents were then evaporated using a Bauchi Rotary evaporator, the remaining extract air being dried further.

Dried samples

The dried corms were peeled, grated and allowed to dry completely. The dried corms were coarsely ground in an industrial grinder (Retsch Gmbh, West Germany), then stored in labelled Schott bottles in a cool dark place until further use. The leaves were thoroughly washed and dried completely. The dried leaves were then ground. The milled sample was stored in an airtight bottle until further use. The milled corms (20 g) and leaves (20 g) were extracted in a 1:20 ratio using acetone, methanol and distilled water. The samples were allowed to shake at 200 rpm for 48 h on a rotary shaker, and subsequently filtered. The solvents from the respective filtrates were evaporated using a rotary evaporator to concentrate the extract.

Phytochemical screening

The phytochemical screening was conducted using standard qualitative methods by Tiwari *et al*. (2011) with minor modifications.

Test for alkaloids: The extracts were treated with Dragendroff's reagent, and the formation of a red precipitate indicated the presence of alkaloids.

Test for saponins: Five mililiters of the extract was shaken vigorously and observed for a stable persistent froth, three drops of olive oil were added and shaken vigorously, after which the formation of an emulsion was observed.

Test for tannins: 10 mL of aqueous solution of extracts were boiled and filtered. A few drops of 0.1% ferric chloride were added and observed for a blue-black or brownish green colouration.

Ferric chloride test: the extracts were treated with 3-4 drops of ferric chloride solution. The formation of a blue-black color indicated the presence of phenols.

Test for terpenoids: The extracts were suspended in chloroform and concentrated sulphuric acid was carefully added to form a layer. A reddish-brown color at the interface indicated the presence of terpenoids.

Test for cardiac glycosides: An aqueous solution of extracts was added to 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated sulphuric acid. The formation of a brown ring indicated the presence of glycosides.

Test for reducing sugars: The extracts were boiled with Benedict's reagent, an orange- red precipitate indicated the presence of reducing sugars.

Brine shrimp lethality assay

The safety of the extracts was tested using the Brine Shrimp lethality Assay method by Meyer *et al*. (1982), with minor modifications. Artificial seawater was obtained by adding 23 g NaCl, 11 g $MgCl_3 6H_2O$, 4 g Na_2SO_4, 1.3 g $CaCl_2.2H_2O$ and 0.7 g KCl to distilled water, which was brought to 1 L with the pH adjusted to 9 using 0.1 M Na_2CO_3 solution. The extracts were re-suspended in 2% DMSO, in artificial seawater. The samples were prepared in 1 mg/mL and 10 mg/mL concentrations. Brine shrimp were hatched in artificial seawater over a period of 48 h by adding 100 mg brine shrimp eggs to 100 mL artificial seawater. The incubation took place at room temperature.

The assay was conducted by adding 10 brine shrimp nauplli into 4.9 mL artificial seawater and 100 µL sample in a 6-well plate. Potassium chromate (in the same concentration as the sample) was used as a positive control, and the artificial seawater instead of the sample was used as the negative control.

Total phenol content

The total phenolic content was determined by the method by Ainsworth and Gillespie (2007) with minor modifications. Folin Ciocalteu reagent (10%, 2.5 mL) was added to 500 µL of sample (1 mg/mL), and subsequently 2 mL of 2% Na_2C0_3 was also added. The reaction was then incubated at 45˚C for 15 min. Thereafter, the absorbance was found to be 765 nm. A gallic acid standard curve using 100-1000 µg/mL concentrations of gallic acid was used with the results expressed as milligram of gallic acid equivalents (mg GAE per g).

Antioxidant activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The DPPH assay was performed according to the method by Oboh (2006) with minor modifications. DPPH (0.1 mM, 1mL) was prepared in methanol and then added to 1 mL of the samples (200, 400, 600, 800 and 1000 µg/mL). The reaction mix was kept in the dark for 30 min, and finally the absorbance was found to be 517 nm. Rutin (1mg/mL) was used as a positive control, and methanol was used as a negative control.

$$Inhibition\,(\%) = \frac{Absorbance(control) - Absorbance\,(sample)}{Absorbance\,(control)} \times 100$$

2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay

The ABTS' free radical scavenging ability was determined according to the method by Re *et al*. (1999) with minor modifications. Briefly, the ABTS solution was prepared by mixing equal parts of 7 mM ABTS and 2.45 mM Potassium persulphate for 16 h. The absorbance of this solution was measured at 734nm. The solution was then diluted to a concentration that yielded an absorbance of 0.7 (±0.02) at 735 nm. Diluted ABTS (3mL) solution was added to 1 mL of the sample (200, 400, 600, 800, 1000 µg/mL). The samples were read at 734 nm after 5

minutes of incubation. Rutin (1 mg/mL) was used as a positive control and methanol was used as a negative control.

$$Percentage\,scavenging = \frac{Absorbance\,(control) - Absorbance(sample)}{Absorbance\,(control)} \times 100$$

Phosphomolybdenum (PM) assay

The PM assay was conducted using the method by Sudha et al. (2011) with minor modifications. A molybdate solution was prepared by adding 1 mL each of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate to 20 mL of distilled water. The final volume was 50 mL. Molybdate solution (1 mL) was added to 1 mL of sample (200, 400, 600, 800, 1000 µg/mL). This was then incubated at 95˚C for 90 min.The samples were cooled and showed values of 695 nm. Rutin (1 mg/mL) was used as a positive control, and methanol was used as a negative control.

$$Inhibition(\%) = [Absorbance\,(sample) - Absorbance\,(control)] \times 100$$

Cupric ion reducing antioxidant capacity (Cuprac) assay

The Cuprac assay was performed according to the method devised by Phatak and Hendre (2014). Briefly, 1 mg of 10 mM cupric chloride, 7.5 mM neocuprine (made in methanol), 1mM ammonium acetate buffer (pH 7) and 2 mL of distilled water were added to 100 µL of sample (200, 400, 600, 800, 1000 µg/mL). The samples were incubated at room temperature for 30 min and the absorbance was 450 nm. Rutin (1mg/mL) was used as a positive control, and methanol was used as a negative control.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was conducted according to the method by Benzie and Strain (1996) with minor modifications. A 300 mM acetate buffer was prepared by adding 3.1 g sodium acetate trihydrate to 16 mL glacial acetic acid. The pH was adjusted to 3.6 and the final volume was brought to 1 L with distilled water. Freshly prepared 10 mM TPTZ (2, 4, 6-tripyridyl-s- triazine) in 40 mM HCL and 20 mM $FeCl_3.6H_2O$ in distilled water were made. The TPTZ reagent was prepared by adding 10 parts of acetate buffer to 1 part each of TPTZ and $FeCl_3$ solution to make up the FRAP reagent. The FRAP reagent (3 mL) was added to 100 µL of the samples (200, 400, 600, 800, 1000 µg/mL). The samples were read at 593 nm, incubated at 37˚C and read again after 4 minutes. Ascorbic acid was used as the standard and the FRAP value for Ascorbic acid was 2.

$$FRAP = \frac{\Delta Absorbance_{593}\,(sample)}{\Delta Absorbance_{593}\,(standard)} \times Frap\,value\,of\,the\,standard$$

Statistical analysis

The results were analyzed by ANOVA (Graph Pad Prism software, San Diego, CA, USA). All analysis was done in triplicate; the mean ± standard deviation was

calculated. IC_{50} was also calculated using Graph Pad Prism. The lower the IC_{50} concentration, the more potent the extract as a therapeutic substance**.**

Results and discussion

Phytochemical screening

All *H. colchicifolia* extracts tested positive for saponins, tannins, flavonoids, terpenoids, phenols and reducing sugars (Table 1). All acetone and methanol extracts were positive for cardiac glycosides and anthraquinones. Only corm extracts were positive for alkaloids, while all leaf extracts were negative for alkaloids.

Table 1. Phytochemical constituents in *H. colchicifolia* extracts using different solvent systems.

Test	FCA	FCM	FCAQ	DLA	DLM	DLAQ	DCA	DCM	DCAQ
Alkaloids	+	+	+	-	-	-	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	-	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+	+	+	+
Anthraquinones	+	+	-	+	+	-	+	+	-
Phenols	+	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+	+

Fresh corm acetone extract (FCA), fresh corm methanol extract (FCM), fresh corm aqueous extract (FCAQ), dried leaf acetone extract (DLA), dried leaf methanol extract (DLM), dried leaf aqueous extract (DLAQ), dried corm acetone extract (DCA), dried corm methanol extract (DCM), dried corm aqueous extract (DCAQ). The values represent the mean of replicate reading (n=3).

In a study by Zimudzi (2014), crude methanolic extracts of *Hypoxis hemerocallidea*, *Hypoxis obtusa*, *Hypoxis rigidula* and *Hypoxis galpinri* tested positive for reducing sugars, tannins, cardiac glycosides, saponins and terpenoids; and negative for flavonoids, alkaloids and anthraquinones. The *Hypoxis colchicifolia* corm and leaf extracts in our study tested positive for all the phytochemical constituents tested, the only exception being the absence of alkaloids in the leaves. The phytochemical constituents identified in the study have some association with biological activities attributed to plant chemistry. Tannins possess antinociceptive, antioxidant and anti-inflammatory properties; terpenoids have anti-inflammatory and anti-microbial properties. Saponins possess anticancer, anti-diabetic, anti-inflammatory, anti-microbial and antioxidant properties; cardiac glycosides are considered to be important in treating heart conditions (Tiwari *et al*., 2011).

The assessment of the aerial and subterranean parts of *Hypoxis* by Katerere and Eloff (2008) show a clear difference between the biological activity and the chemistry of these parts. *Hypoxis hemerocallidea* corms and leaves tested for chemical composition on TLC found them to be distinctly different, with leaf samples being more complex than corms. There were no apparent differences in the chemical composition of the ethanol and acetone extracts of either corms or leaves in the study by (Katerere, 2013, Katerere and Eloff, 2008).

Brine shrimp lethality assay

All *H. colchicifolia* extracts tested at different concentrations showed no mortality of the brine shrimp, indicating the non-toxic effect that extracts possess (Table 2). Potassium chromate used as the positive control was lethal from the lowest concentration tested.

Table 1. Brine shrimp lethality assay, Percentage death by *H. colchicifolia* extracts by different solvent systems.

Concentration (µg/mL)	FCA	FCM	FCAQ	DLA	DLM	DLAQ	DCA	DCM	DCAQ	Positive control
0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	100
1000	0	0	0	0	0	0	0	0	0	100
10000	0	0	0	0	0	0	0	0	0	100
100000	0	0	0	0	0	0	0	0	0	100

Fresh corm acetone extract (FCA), fresh corm methanol extract (FCM), fresh corm aqueous extract (FCAQ), dried leaf acetone extract (DLA), dried leaf methanol extract (DLM), dried leaf aqueous extract (DLAQ), dried corm acetone extract (DCA), dried corm methanol extract (DCM), dried corm aqueous extract (DCAQ). The values represent the mean of replicate reading (n=3).

Jooste (2012) showed that the corms of *H. hemerocallidea* showed cytotoxicity on brine shrimp lethality at 10, 100 and 1000 µg/mL (Jooste, 2012). However, Ramulondi *et al*. (2018) found that an aqueous extract of *H. hemerocallidea* corms had a low brine shrimp lethality assay mortality percentage of 4 and 5% after 24 and 48 h respectively. The organic solvent extract had a higher mortality, of 29 and 54% mortality after 24 and 48 h respectively. A study by Zimudzi (2014) revealed that the extracts of *Hypoxis* corms are nontoxic to brine shrimp nauplli. In this study, all the extracts tested were nontoxic, their highest concentration being 100 000 µg/mL.

Total phenol content

The methanol extract of dried corms of *H. colchicifolia* has the highest phenolic content out of all the extracts evaluated (204.80±1.73 mg/g); the aqueous extract of dried leaves has the lowest phenolic content (103.67±1.15 mg/g). The statistical analysis of the results showed no significant difference between FCA and FCM,

FCAQ and DCM, DLA and DLM; nor between DCA and DCAQ (Table 3). The remaining extracts showed significant differences p <0.0001.

Table 2. Total phenolic content of *H. colchicifolia* extracts by different solvent systems.

Sample	Solvent type	GAE (gallic acid equivalent) mg/g
Fresh Corms	Acetone	186.53±5.08^{c}
	Methanol	186.87±0.81^{c}
	Aqueous	198.53±1.89^{a}
Dried Leaves	Acetone	112.80±0.33^{g}
	Methanol	115.13±3.45^{g}
	Aqueous	103.67±1.15^{i}
Dried Corms	Acetone	157.47±2.05^{e}
	Methanol	204.80±1.73^{a}
	Aqueous	160.60±1.23^{e}

The data represent the mean±standard deviation (n=3). Superscript letters indicate significant difference (p<0.0001).

The total phenolic content of the three fresh corm extracts was similar and showed no significant difference. The total phenolic content of the leaf extracts was lower (±50%) than that of the fresh and dried corm extracts. The aqueous extract of dried leaves had the lowest phenolic content. In a study by Laher *et al*. (2013), the total phenolic content of fresh *H. hemerocallidea* corms and leaves had a significantly higher total phenolic content than that of dried corms and leaves. Phenolic compounds are considered responsible for antioxidant activity and effective free radical scavenging; hence, the quantity of total phenols present gives an indication of the samplesț antioxidant ability. Fresh corms had a total phenolic content of 173.59 mgGAE/g. Fresh *H. hemerocallidea* leaves had a higher flavonoid content than that of dried leaves. An assessment of 7 *Hypoxis* species for the total phenolic content by (Nsibande *et al*., 2018) found the total phenolic content ranging from 134.79 to 396 µg/g in the corm extracts of the *Hypoxis* species evaluated. In the study *H. hemerocallidea* had a total phenolic content of 204.56 µg/g , thus keeping in line with the findings of this study.

Antioxidant activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The acetone extract of the dried corms of *H. colchicifolia* shows the highest DPPH free radical inhibition potential, and the fresh corm aqueous extract shows the poorest inhibition potential (Figure 1). The IC_{50} for DPPH in descending order are as follows: fresh corm aqueous (23.16 µg/mL), fresh corm methanol (21.25 µg/mL), dried corm aqueous (20.16 µg/mL), dried corm methanol (18.91 µg/mL), dried leaf aqueous (18.76 µg/mL), fresh corm acetone (18.72 µg/mL), dried leaf methanol (18.44 µg/mL), dried leaf acetone (17.89 µg/mL) and dried corm acetone (17.56 µg/mL). The IC_{50} of the positive control rutin was 21.79 µg/mL. There was no significant difference in the results of DLA and DLM, DLA and DLAQ; and DLM and DLAQ. So, it can be concluded that there was no significant difference

between all leaf extracts. The fresh and dried corm extracts had significant differences ($p<0.0001$) between each other, as well as between the leaf extracts and the positive control.

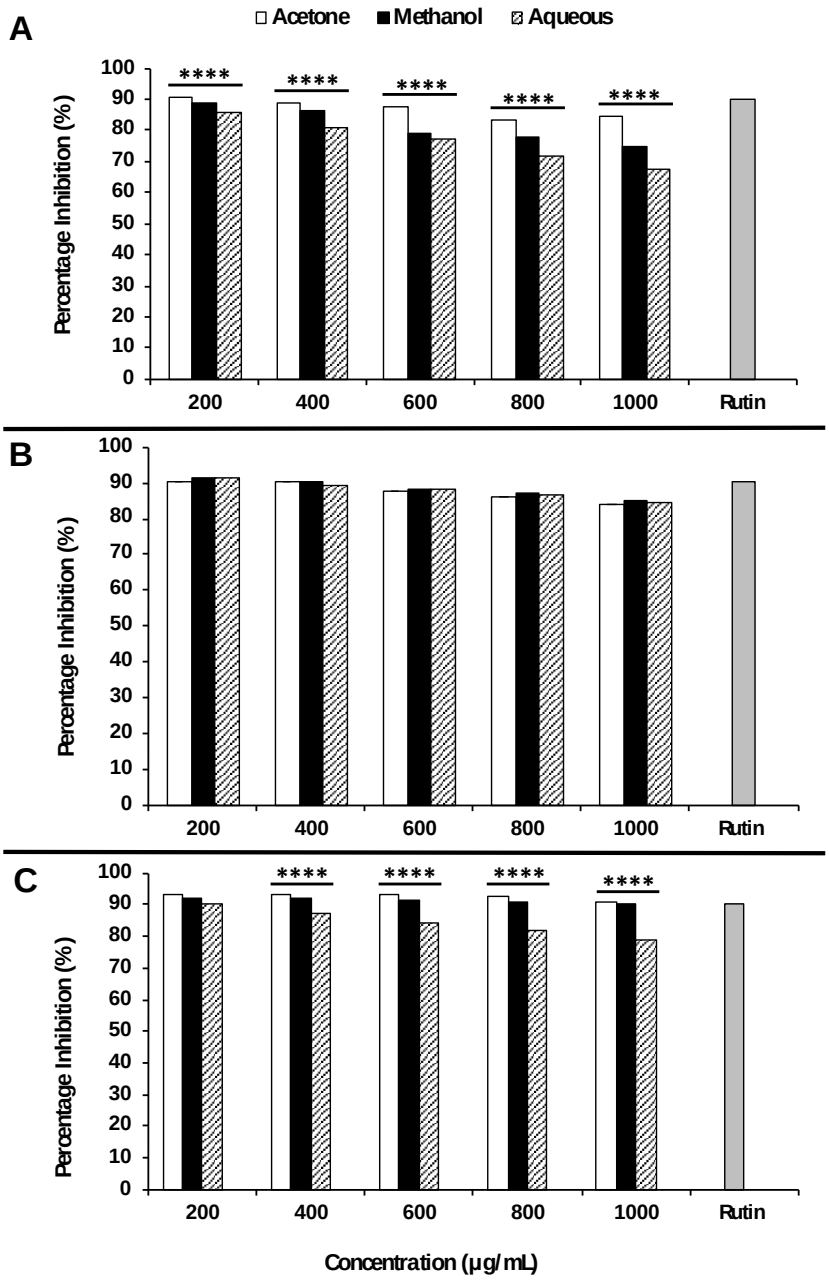

Figure 1. DPPH Inhibition by *H. colchicifolia* extracts [A - Solvent extracts of fresh corms; B - solvent extracts of dried leaves; C - solvent extracts of dried corms]. The values represent the mean ± standard deviation of replicate reading (n=3) (**** indicates significant differences $p<0.0001$).

However, the dried leaf extracts possessed a lower total phenolic content, but had as good a DPPH scavenging potential as the other extracts tested. The acetone extracts of the fresh corms, dried leaves, as well as dried corms had better potential than aqueous and methanol extracts. The acetone extract of dried corms had the lowest IC_{50} for DPPH scavenging. There was no significant difference between each of the nine extracts and the extracts as compared to rutin. A study by Laporta *et al*., 2007 showed that extracts of *H. hemerocallidea* have greater antioxidant activity than extracts of green tea and olive leaf (Laporta *et al.*, 2007).

An antioxidant inhibition screening against DPPH and ABTS free radicals by (Madikizela and McGaw, 2019) found that aqueous extracts of *H. colchicifolia* corms were most effective against free radicals, with *H. colchicifolia* showing a weak DPPH scavenging potential. Acetone, ethanol, hot and cold-water extracts were evaluated, with aqueous extracts having the lowest IC_{50} values for antioxidant inhibition. Hot water extract of *H. colchicifolia* had an IC_{50} of 12.18 µg/mL against ABTS free radicals and the cold-water extract had an IC_{50} of 19.75 µg/mL against DPPH free radicals.

2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay

The methanol extract of dried leaves exhibited the greatest inhibitory effect (Figure 2). The IC_{50} for ABTS inhibition in descending order are as follows: fresh corm methanol (153 µg/mL), fresh corm acetone (120.1 µg/mL), dried corm aqueous (118.6 µg/mL), dried corm methanol (106.4 µg/mL), dried corm acetone (106.4 µg/mL), fresh corm aqueous (104.5 µg/mL), dried leaf acetone (89.98µg/mL), dried leaf aqueous (85.57 µg/mL) and dried leaf methanol (74.46 µg/mL). The IC_{50} for the positive control rutin was 30.69 µg/mL. Leaf extracts were most effective against ABTS cations, with the methanol extract having the lowest IC_{50}, followed by aqueous and acetone extract. Fresh corm extracts were the least effective in scavenging ABTS radicals. There was a significant difference between all the extracts tested, including the positive control. The assessment of *Hypoxis argentea* for antioxidant potential using DPPH and ABTS methods found that the ethanol and aqueous extracts of corm had high antioxidant potential, as well as an ABTS inhibition of more than 90% at a 500 µg/mL concentration. The extracts showed moderate DPPH scavenging potential (Akinrinde *et al*., 2018). These findings were similar to that of this study, as corm extracts exhibited high antioxidant potential.

Phosphomolybdenum (PM) assay

The acetone extract of dried corms of *H. colchicifolia* was shown to have the highest TEAC against phosphomolybdenum free radicals, while the aqueous extract of fresh corms exhibited the lowest potential (Table 4). All acetone extracts had a higher potential than that of rutin (150.53±1.76 µg/mL). The acetone extract of dried corms inhibited PM free radicals better than other extracts, and had the highest trolox equivalent. There was a significant difference (p<0.0001) between all the extracts tested, including the positive control.

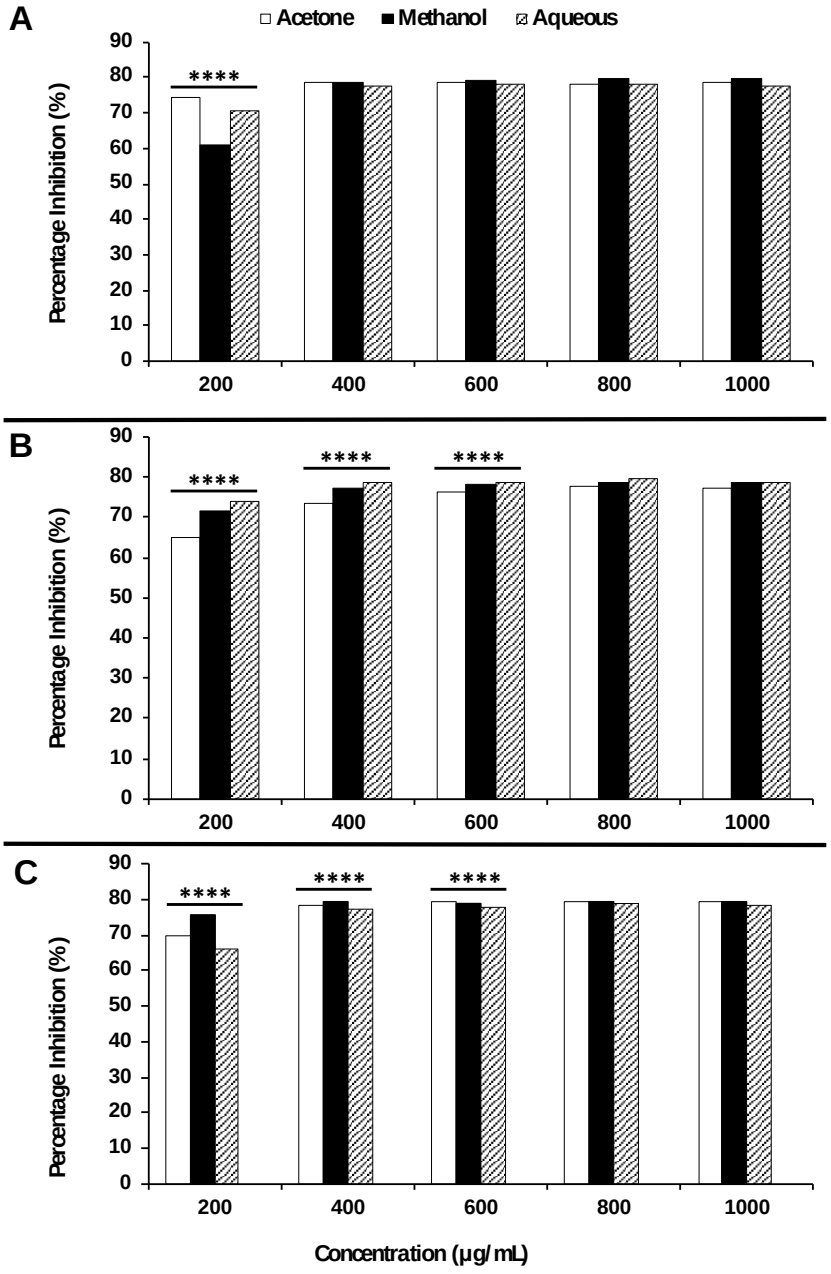

Figure 2. ABTS Inhibition by *H. colchicifolia* extracts [A - Solvent extracts of fresh corms; B - solvent extracts of dried leaves; C - solvent extracts of dried corms]. The values represent the mean ± standard deviation of replicate reading (n=3) (**** indicates significant differences $p<0.0001$).

Table 4. Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against phosphomolybdenum free radicals.

Sample	Concentration (µg/mL)	Acetone	Methanol	Aqueous
Fresh corm extracts	**0**	0	0	0
	200	54.30±1.32^{ab}	80.11±1.32^{bc}	68.82±0.88^{ac}
	400	109.14±3.07^{ab}	95.16±0.44^{b}	96.24±3.07^{ac}
	600	132.26±5.27^{ab}	113.98±0.88^{b}	110.22±3.07^{ac}
	800	152.69±1.76^{ab}	124.73±0.88^{bc}	103.76±3.07^{ac}
	1000	178.49±7.90^{ab}	130.11±3.51^{bc}	106.45±4.39^{ac}
Dried leaf extracts	**0**	0	0	0
	200	74.73±3.07^{a}	72.94±2.92^{a}	65.05±1.32^{bc}
	400	117.74±7.46^{ab}	76.88±0.44^{a}	82.80±4.39^{bc}
	600	151.61±2.63^{ab}	104.30±2.63^{ac}	67.20±2.19^{bc}
	800	162.90±4.83^{ab}	106.45±2.63^{a}	105.91±1.32^{bc}
	1000	162.37±0.88^{ab}	147.85±2.19^{ac}	116.67±4.83^{bc}
Dried corm extracts	**0**	0	0	0
	200	107.53±3.51^{ac}	63.44±0.88^{b}	58.60±0.44^{ac}
	400	127.96±0.00^{ac}	70.43±1.32^{b}	66.13±0.44^{ac}
	600	143.01±1.76^{ac}	81.18±1.32^{b}	84.41±1.32^{ac}
	800	160.22±0.88^{ac}	88.71±0.44^{b}	91.94±1.32^{ac}
	1000	180.65±2.63^{ac}	131.72±0.44^{bc}	120.43±6.15^{ac}

The values represent the mean±standard deviation of replicate reading (n=3). Superscript letters indicate significant difference (p<0.0001).

Cupric ion reducing antioxidant capacity (Cuprac) assay

All extracts had a poor TEAC as compared to that of rutin (978.79±3.89 µg/mL); however, the methanol extract of fresh corms of *H. colchicifolia* had the highest TEAC against cupric ion free radicals, while the methanol extract of dried leaves had the lowest TEAC potential (Table 5). There was no significant difference between FCA and FCM, DLM and DCM; nor between DCM and DCAQ.

The remaining extracts tested had a significant difference (p<0.0001) when compared to each other and the positive control. In a study by Güçlü *et al*. (2006) on methanol extracts of dry and fresh apricots, it was shown that TEAC against cupric ion free radicals ranged between 2.67 and 52.47 µmol/g. The methanol extracts of 21 Macedonian medicinal plants tested against cupric ion free radicals by Tusevski *et al*. (2014) had results ranging from 52.89 to 1068.58 µmol/g, TEAC with *Origanum vulgare* having the highest equivalent. A study by Zengin et al. (2015) on solvent extracts of three medicinal plants (*Hedysarum varium*, *Onbrychis hypargyea* and *Vicia truncatula)* showed EC_{50} values ranging between

0.69 and 3.01(mg/mL), with *Hedysarum varium* having the best cupric potential in the study.

Table 5. Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against cupric ion free radicals.

Sample	Concentration (µg/mL)	Acetone	Methanol	Aqueous
Fresh corm extracts	**0**	0	0	0
	200	107.78 ± 9.79^{b}	105.03 ± 8.92^{ac}	42.65 ± 2.83^{bc}
	400	184.84 ± 10.72^{b}	185.29 ± 5.77^{ac}	99.07 ± 2.59^{bc}
	600	254.55 ± 5.54^{b}	261.89 ± 2.97^{ac}	118.79 ± 4.05^{bc}
	800	325.65 ± 1.72^{b}	303.17 ± 9.98^{ac}	136.68 ± 1.95^{bc}
	1000	359.13 ± 3.61^{b}	362.80 ± 3.24^{ac}	225.66 ± 9.08^{bc}
Dried leaf extracts	**0**	0	0	0
	200	27.97 ± 5.15^{ab}	36.23 ± 4.05^{a}	7.44 ± 1.13^{ab}
	400	70.63 ± 2.25^{a}	58.24 ± 7.37^{ac}	57.79 ± 7.48^{ab}
	600	124.29 ± 3.89^{a}	90.81 ± 3.61^{a}	81.18 ± 5.54^{ab}
	800	187.59 ± 2.97^{a}	110.53 ± 7.86^{ac}	118.33 ± 8.13^{ab}
	1000	$228.87\pm16.85^{a}_{b}$	$167.87\pm14.49^{a}_{c}$	188.05 ± 3.24^{ab}
Dried corm extracts	**0**	0	0	0
	200	49.53 ± 7.89^{a}	38.98 ± 2.25^{b}	8.25 ± 2.83^{a}
	400	104.11 ± 12.01^{a}	66.96 ± 7.97^{b}	60.08 ± 1.72^{a}
	600	$183.00\pm14.23^{a}_{b}$	95.40 ± 6.83^{b}	98.15 ± 20.81^{ac}
	800	$227.95\pm10.07^{a}_{b}$	135.76 ± 1.72^{b}	133.01 ± 2.39^{ac}
	1000	288.49 ± 3.43^{ab}	176.12 ± 13.96^{b}	$248.13\pm12.15^{a}_{c}$

The values represent the mean±standard deviation of replicate reading (n=3). Superscript letters indicate significant difference (p<0.0001).

Ferric reducing antioxidant power (FRAP) assay

The methanol extract of *H. colchicifolia* dried corms had the highest ferric ion free radical scavenging ability, while the acetone extract of dried leaves had the lowest scavenging ability (Table 6).

There was no significant difference between the following extracts: FCA and DLA, FCA and DLM, DLA and DLM, DLM and DCA, DLM and DCAQ; and DCA and DCAQ. The rest of the extracts tested had a significant difference (p<0.0001) between each other, and between each of them and the positive control.

Table 6. Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against ferric ion free radicals.

Sample	Concentration (µg/mL)	Acetone	Methanol	Aqueous
Fresh corm extracts	**0**	0	0	0
	200	0.46±0.02^{a}	0.68±0.05^{a}	0.31±0.10^{a}
	400	0.52±0.01^{a}	0.88±0.35^{a}	0.66±0.07^{ab}
	600	1.05±0.17^{a}	1.22±0.09^{a}	0.83±0.10^{a}
	800	1.30±0.16^{ac}	1.73±0.11^{a}	2.28±0.16^{ab}
	1000	1.36±0.17^{ab}	2.26±0.19^{a}	2.49±0.17^{ab}
Dried leaf extracts	**0**	0	0	0
	200	0.52±0.01^{a}	0.44±0.03^{a}	0.41±0.01^{a}
	400	0.64±0.02^{a}	0.80±0.07^{a}	0.63±0.01^{a}
	600	0.83±0.07^{a}	1.12±0.11^{ab}	1.39±0.15^{a}
	800	1.07±0.15^{a}	1.39±0.09^{ab}	1.75±0.10^{a}
	1000	1.24±0.11^{a}	1.44±0.13^{ab}	2.00±0.04^{ac}
Dried corm extracts	**0**	0	0	0
	200	0.68±0.10^{a}	0.73±0.11^{a}	0.60±0.01^{a}
	400	1.05±0.05^{a}	1.19±0.13^{a}	0.93±0.06^{a}
	600	1.32±0.35^{a}	1.65±0.06^{a}	1.06±0.05^{ac}
	800	1.43±0.31^{a}	2.05±0.11^{ab}	1.29±0.28^{ac}
	1000	1.54±0.13^{a}	2.78±0.11^{ab}	1.38±0.19^{ac}

The values represent the mean±standard deviation of replicate reading (n=3). Superscript letters indicate significant difference (p<0.0001).

In this study, the methanol extract of dried corms manifested the highest ferric ion scavenging potential, and the acetone extract of fresh corms also had a greater scavenging potential than that of rutin (2.31±0.10 µmol/mL). In this study, acetone extracts generally had poor potential, however there were no significant differences as compared to some of the other solvent extracts. Aqueous extracts of *H. hemerocallidea* and active compound hypoxoside were evaluated for their antioxidant potential against DPPH and FRAP free radicals by (Nair *et al*., 2007). *Hypoxis hemerocallidea* extracts showed dose dependent free radical scavenging, with hypoxoside not having any antioxidant potential when tested alone (Nair *et al*., 2013). No single assay can capture the different modes of action of the antioxidant.

Antioxidants work by various mechanisms; by free radical scavenging activity, donating hydrogen to radicals metal chelating activity, reducing power, quenching singlet oxygen, and inhibition of β-carotene (Badarinath *et al*., 2010).

Conclusions

The composition of *Hypoxis colchicifolia* corm and leaf extracts was shown to be phytochemically different, the solvent used for extraction being the determining factor. Only the acetone and methanol extracts were shown to have a full spectrum of phytochemical constituents. The plant is shown to be nontoxic and safe to use, with extracts exhibiting promising antioxidant potential; the acetone and methanol corm extracts yielded the most favourable results. This could be the reason why only the corms are used in traditional medicine. The leaf extracts have moderate phenolic content and may have promising biological activity, even though their antioxidant ability is limited. Further studies are recommended to assess the quantitative phytochemical composition and the full potential of *H. colchicifolia* as an antioxidant.

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