

# Phytochemical Composition and Antioxidant Activities of *Dianthus Thunbergii* Hooper and *Hypoxis Argentea* Harv Ex Baker: Plants Used for the Management of Diabetes Mellitus in Eastern Cape, South Africa

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## ABSTRACT

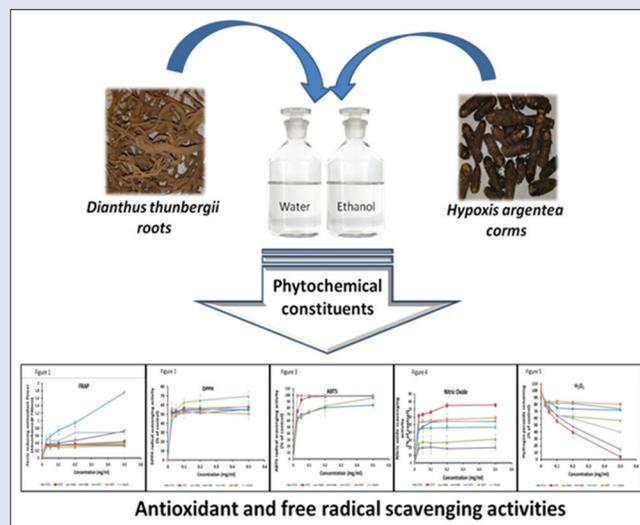
**Background:** Inhabitants of the Eastern Cape Province of South Africa use the roots of *Dianthus thunbergii* and corms of *Hypoxis argentea* to treat diabetes mellitus and other ailments. **Objective:** The objective of this study was to analyze the phytochemical composition and antioxidant activities of the aqueous and ethanol extracts of the roots and corms of two plants. **Materials and Methods:** Total phenolics, flavonoids, flavonols, proanthocyanidins, tannins, and alkaloids were determined by standard methods. The scavenging activities of the extracts against 1,1 diphenyl-2-picrylhydrazyl (DPPH), 2'-azino-bis (3-ethylbenthiazoline-6-sulfonic acid (ABTS), nitric oxide (NO), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and their ferric-reducing antioxidant potentials (FRAPs) were measured. **Results:** The ethanol extract of *H. argentea* had the highest content of phenolics (66.71 ± 2.71 mg gallic acid equivalent/g) and tannins (1.18 ± 0.07 mg TAE/g), while the ethanol extract of *D. thunbergii* gave higher contents of flavonoids and proanthocyanidins (62.21 ± 1.75 mg Qe/g and 432.62 ± 2.43 mg Ca/g, respectively). Flavonols were the most predominant in the aqueous extract of *H. argentea* (25.51 ± 1.92 mg Qe/g). We observed a concentration-dependent response in the ABTS- and H<sub>2</sub>O<sub>2</sub>-scavenging activities and FRAP values of the extracts and standards (Vitamin C, butylated hydroxytoluene, and rutin). The ethanol extracts of both plants generally demonstrated better antioxidant activities against H<sub>2</sub>O<sub>2</sub>, NO, and ABTS while also possessing better reducing power than the aqueous extracts. The aqueous extract of *D. thunbergii*, however, showed the best DPPH scavenging activity. **Conclusion:** The higher content of phytochemicals and antioxidant capacity obtained for the ethanol extracts of *D. thunbergii* and *H. argentea* may prove to be valuable information in selecting suitable extraction solvents for the medicinal applications of both plants.

**Key words:** Antioxidants, diabetes, *Dianthus thunbergii*, *Hypoxis argentea*, phytochemicals, radical scavenging

## SUMMARY

- Ethanol extracts of *Hypoxis argentea* had the highest levels of phenolics and tannins
- Ethanol extracts of *Dianthus thunbergii* had the highest levels of flavonoids and proanthocyanidins
- Ethanol extracts of both plants possess better antioxidant activity against hydrogen peroxide, nitric oxide, and ABTS as well as higher reducing power than the aqueous extracts

- Aqueous extract of *Dianthus thunbergii* had the highest free radical scavenging activity as measured with DPPH.



**Abbreviations used:** ABTS: 2'-azino-bis (3-ethylbenthiazoline-6-sulfonic acid); BHT: Butylated hydroxytoluene; DPPH: 1,1 diphenyl-2-picrylhydrazyl; DTA: *Dianthus thunbergii* aqueous extract (16.6%); DTE: *Dianthus thunbergii* ethanol extract (2.4%); Fe<sup>3+</sup>-TPTZ: Ferric tripyridyltriazine; FRAP: Ferric-reducing antioxidant potentials; GAE: Gallic acid equivalent; HAA: *Hypoxis argentea* aqueous extract (3.2%); HAE: *Hypoxis argentea* ethanol extract (1.8%); Qe: Quercetin equivalence; ROS: Reactive oxygen species; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid.

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## INTRODUCTION

Plants have developed secondary biochemical pathways to synthesize a variety of chemicals that enable them to cope with challenges such as herbivores, pathogen attacks, and local nutrient fluctuations in their immediate environment.<sup>[1]</sup> These phytochemicals, including phenols, flavonoids, tannins, saponins, alkaloids, phenolic glycosides, terpenoids, and other metabolites,<sup>[2,3]</sup> are sources of compounds

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that possess medicinal properties against several diseases that affect humans. The pathogenesis of most diseases involves oxidative and inflammatory components, usually arising from excessive production of reactive oxygen species (ROS).<sup>[4]</sup> ROS include activated oxygen metabolites called free radicals, such as superoxide anion radicals ( $O_2^-$ ) and hydroxyl radicals (OH), as well as nonradicals including hydrogen peroxide ( $H_2O_2$ ) and singlet oxygen ( $^1O_2$ ). Nonradicals have the potential to participate in reactions that eventually give rise to free radicals. Free radicals and nonradicals alike are capable of reacting *in vivo* with tissue macromolecules, forming products such as guanine adducts with DNA, or lysyl adducts with proteins.<sup>[5]</sup>

The body of aerobic organisms is equipped with antioxidant defense mechanisms (enzymic and nonenzymic) that protect against tissue damage by ROS.<sup>[6]</sup> These are, however, not always sufficient to counteract the severity of oxidative challenge that characterizes many disease or toxic states. The imbalance that results between production of oxidants (ROS) and antioxidants, in favor of the oxidants, is described as oxidative stress and is a major underlying factor in the pathogenesis of many chronic diseases such as diabetes, cancer, and atherosclerosis.<sup>[3]</sup>

In recent times, considerable research efforts have been directed at finding naturally occurring compounds, especially of botanical origin, which offer protection against diseases through their antioxidative and/or anti-inflammatory properties. Dietary supplementation with phytochemicals represents an effective protective strategy to complement the activities of natural antioxidant defense systems, as well as a viable alternative to synthetic drugs which generally have limitations of associated adverse effects.<sup>[7,8]</sup>

The South African population is one of those plagued with a rising incidence of chronic noncommunicable diseases such as diabetes mellitus.<sup>[9]</sup> A considerable proportion of the population resorts to the use of herbs for the treatment of various diseases such as diabetes. Ethnobotanical surveys have revealed a large number of these plants reported to be useful in the treatment of diabetes mellitus.<sup>[10,11]</sup> *Dianthus thunbergii*, of the family *Caryophyllaceae*, belongs to a genus of over 300 species, which are traditionally used for medicinal purposes as infusions for the treatment of chest complaints, severe colic, soothing of wounds, urolithiasis, boils and carbuncles, eczema, and itching.<sup>[12,13]</sup> Extracts from the fresh crushed roots are reportedly used against diabetes and other conditions and are taken as 2 teaspoonfuls orally three times daily.<sup>[11]</sup> *Hypoxis argentea* is one of the several species of the genus *Hypoxis* which are popular in African herbal medicine for the treatment of septic sores, headaches, dizziness, testicular tumors, tuberculosis, asthma, and diabetes.<sup>[14-16]</sup> The corm, the underground part, is usually sought for medicinal use. For diabetes treatment, it is reportedly boiled in water and the infusion is administered orally until a patient is healed. Despite traditional claims for the effectiveness of *D. thunbergii* and *H. argentea* against diabetes mellitus, they have not been investigated scientifically for their phytochemical composition and biological activities. As part of ongoing comprehensive analysis of the medicinal potentials of these plants, the present study aimed to quantify the content of major phytochemicals and also to evaluate the antioxidant activities of the aqueous and ethanol extracts of the roots and corms of *D. thunbergii* and *H. argentea*. These properties were compared between the two plants and correlations were made between the phytochemical contents and antioxidant activities of the plants.

## MATERIALS AND METHODS

### Chemicals

Folin–Ciocalteu reagent, 1,1 diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), vanillin,

ferric chloride, butylated hydroxytoluene (BHT), rutin, Vitamin C potassium ferricyanide, trichloroacetic acid (TCA), thiobarbituric acid, glacial acetic acid, sodium nitroprusside, tannic acid, gallic acid, quercetin, sodium carbonate, aluminum chloride, and potassium acetate were purchased from Merck (Pty) Ltd., Gauteng, South Africa. All the other chemicals and solvents were of analytical grade.

### Collection of plant materials and preparation of extracts

The roots of *D. thunbergii* and corms of *H. argentea* were collected in May, 2015, at Alice, Eastern Cape, South Africa. They were authenticated at the Giffen Herbarium, University of Fort Hare, South Africa. Both plants were identified with voucher specimen numbers CRY-2502 for *D. thunbergii* and HYP-1230 for *H. argentea*. The roots and corms were initially washed free of soil attachments and then oven-dried to constant weight at 30°C. The dried plant materials were then milled into fine powder using an electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc., China). A composition of 200 g of each of the powdered plant materials was extracted separately in distilled water and 99.99% ethanol using an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 h. The suspensions in ethanol were thereafter filtered using Whatman No. 1 filter papers in a Buchner funnel. The aqueous suspensions were initially filtered through a thick layer of sterile cotton wool before their filtration with the filter paper. The ethanol extracts were concentrated to dryness using a rotary evaporator (Heidolph Laborota 4000, Heidolph Instruments, GmbH and Co, Germany) while the aqueous extracts were initially frozen at -40°C and then dried using a freeze dryer. The different extracts were reconstituted in their respective solvents for use in the assays conducted in this study.

### Phytochemical screening

#### Total phenolic content

The content of phenols in the different extracts was determined spectrophotometrically by the Folin–Ciocalteu reagent according to the method of Ozkok *et al.*<sup>[17]</sup> A calibration curve was prepared with gallic acid as standard (0.025–0.125 mg/ml in 70% methanol v/v). To 0.5 ml of each of the gallic acid concentrations or extracts (mg/ml), 2.5 ml Folin–Ciocalteu reagent (previously prepared as 10% v/v dilution in distilled water) was added. Thereafter, 2 ml anhydrous sodium carbonate (7.5%) was added, producing a blue-colored solution. The mixtures were vortexed thoroughly and placed in a water bath for 30 min at 45°C. The absorbance was then read at 765 nm. The equation of the calibration curve obtained ( $Y = 14.885x$ ;  $R^2 = 0.9961$ ) was used to establish the gallic acid equivalence (mg/ml). The total phenolic content was calculated using the formula:  $T = C \times V/m$ , where  $T$  is the total phenolic content,  $V$  is the volume of the extract (ml) used in the assay,  $C$  is the gallic acid equivalent (GAE) (mg/ml), and  $m$  is the weight of the pure plant extract used in the assay. Values were expressed as GAE per gram of dry plant extract (mg GAE/g). All assays were performed in triplicate.

#### Total flavonoid content

Flavonoid contents in the extracts were determined using the aluminum chloride method as described by Ozkok *et al.*<sup>[17]</sup> A calibration curve was prepared with quercetin (0.025–0.125 mg/ml in 80% methanol v/v). Briefly, 0.5 ml of the extracts (prepared at a concentration of 1 mg/ml) or the standard at the different concentrations was mixed with 3 ml of 95% ethanol, 0.2 ml of aluminum chloride (prepared as a 10% aqueous dilution), and 0.2 ml of 1 M potassium acetate, and the whole mixture was made up to 10 ml with distilled water. The resulting solutions, prepared in triplicate, were yellow and were thoroughly vortexed and allowed to stand

for 30 min at room temperature, after which the absorbance was read at 420 nm. The equation of the calibration curve obtained ( $Y = 11.922x$ ;  $R^2 = 0.9955$ ) was used to establish quercetin equivalence (mg/ml) and the total flavonoid content was calculated using the formula:  $T = C \times V/m$ , where  $T$  is the total flavonoid content,  $V$  is the volume of the extract (ml) used in the assay,  $C$  is the quercetin equivalent (mg/ml), and  $m$  is the weight (g) of the pure plant extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (mg Qe/g).

#### Total flavonol content

Total flavonols were determined using the method of Wintola and Afolayan,<sup>[18]</sup> with slight modifications. A calibration curve was prepared with quercetin (0.025–0.125 mg/ml in 80% methanol v/v). Plant extracts were prepared at a final concentration of 1 mg/ml. 2 ml of the extract or the standard was mixed with 3 ml of 95% ethanol, 0.2 ml of aluminum chloride (10% w/v), and 0.2 ml of sodium acetate (50 g/L). The resulting mixture was made up to 10 ml with distilled water and vortexed thoroughly. All assays were done in triplicate and were allowed to stand for 2.5 h at room temperature, after which the absorbance was read at 440 nm. The equation of the calibration curve obtained ( $Y = 13.128x$ ;  $R^2 = 0.9990$ , where  $Y$  is the absorbance and  $x$  is the concentration) was used to establish quercetin equivalence (mg/ml). The total flavonol content was calculated using the formula:  $T = C \times V/m$ , where  $T$  is the total flavonol content,  $V$  is the volume of the extract (ml) used in the assay,  $C$  is the quercetin equivalent (mg/ml), and  $m$  is the weight (g) of the pure plant extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (mg Qe/g).

#### Total tannins

The method described by Wintola and Afolayan<sup>[18]</sup> was used to quantify the tannin content in the plant extracts. A standard curve was prepared using tannic acid (0.002–0.010 mg/ml in distilled water) as standard. Briefly, 0.2 g of each extract in triplicate was dissolved in 20 ml of 50% methanol. This was placed in a water bath at 80°C for 1 h, after which the mixtures were filtered into 100 ml volumetric flasks. To the standard concentrations of tannic acid as well as the filtrates from the extracts, 20 ml of distilled water, 2.5 ml of Folin–Ciocalteu reagent, and 10 ml of 17% sodium carbonate were added in that order. All mixtures in triplicate were then made up to 100 ml with distilled water and were allowed to stand for 20 min. A bluish green color was developed at the end of the reaction and the absorbance of the mixtures was read at 760 nm. Tannic acid equivalence (mg/ml) was established from the equation of the standard curve ( $Y = 154.45x$ ;  $R^2 = 0.9585$ ), where  $y$  is the absorbance and  $x$  is the tannic acid equivalent in mg/ml. Total tannin content ( $T$ ) in milligram tannic acid equivalent per gram of dry extract was calculated using the formula:  $T = C \times V/m$ , where  $V$  is the volume of the extract (ml) used in the assay,  $C$  is the tannic acid equivalent (mg/ml), and  $m$  is the weight (g) of the pure plant extract used in the assay.

#### Total proanthocyanidins

Total proanthocyanidins were measured using the method described by Oyedemi *et al.*<sup>[19]</sup> The extracts were prepared at a final concentration of 1 mg/ml. To 0.5 ml of this in triplicate, 3 ml of 4% vanillin-methanol solution and 1.5 ml of hydrochloric acid were added. These were thoroughly mixed and allowed to stand for 15 min at room temperature. The absorbance was read at 500 nm. Total proanthocyanidin content as catechin equivalent was evaluated from the equation  $Y = 0.5825x$ ;  $R^2 = 0.9277$ , where  $y$  is the absorbance and  $x$  is the catechin equivalent (mg/ml). The amount of proanthocyanidin in the extracts in mg/g was calculated with the following formula:  $T = C \times V/m$ , where  $V$  is the volume of the extract (ml) used in the assay,  $C$  is the catechin equivalent (mg/ml), and  $m$  is the weight (g) of the pure plant extract used in the assay.

## Alkaloids

Alkaloid content was determined using the method of Harbone<sup>[20]</sup> with slight modifications. Briefly, 0.5 g of the dried extract was dissolved in 20 ml of 20% acetic acid in ethanol v/v. The mixture was allowed to stand for 4 h after which it was filtered. The filtrate was then placed in a water bath for about 30 min at boiling temperature. Thereafter, concentrated ammonium hydroxide was added dropwise which produced some effervescence and precipitation. The collected precipitate was washed with dilute ammonium hydroxide and then filtered with already weighed filter papers. The residue left in the filter papers was then dried in an oven and the resulting dried papers with residue were also weighed. The alkaloid content was determined using the formula: Alkaloid (%) = final weight of the residue/initial weight of the extract used  $\times$  100. All the determinations were done in triplicate.

## Antioxidant activity assays

The antioxidant capacities of the different extracts were measured using DPPH radical scavenging activity, ferric-reducing power, ABTS radical scavenging activity,  $H_2O_2$ , and nitric oxide (NO) scavenging activities. These measurements were made against standard antioxidants including Vitamin C, BHT, and Rutin.

### 1,1 diphenyl-2-picrylhydrazyl radical scavenging activity

The method described by Liyana-Pathiranan and Shahidi<sup>[21]</sup> was used in this assay. 1 ml of the extracts or the standards at different concentrations (0.025–0.50 mg/ml), prepared in triplicates, was mixed with 1 ml of DPPH (0.135 mM) prepared in methanol. The mixtures were vortexed thoroughly and left in the dark for 30 min at room temperature. The absorbance was then measured spectrophotometrically at 517 nm. The percentage of DPPH scavenging activity of the extract or standard was calculated with the following formula: % DPPH radical scavenging activity =  $[(AC-AS)/AC] \times 100$ , where  $AC$  is the absorbance of the control and  $AS$  is the absorbance of the test samples (extract or standard).

### Ferric-reducing antioxidant power

The method described by Aiyegoro and Okoh<sup>[22]</sup> was used for the determination of the ferric-reducing activities of the plant extracts. The assay is based on the reduction of ferric-tripyridyltriazine ( $Fe^{3+}$ -TPTZ) complex by the action of electron-donating antioxidants at low pH to the ferrous form. Each extract or standard was initially prepared in distilled water in increasing concentrations from 0.025 to 0.5 mg/ml. 1 ml of each of the extract or the standard at the different concentrations was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide. The mixture was incubated for 20 min at 50°C. This was followed by the addition of 2.5 ml of TCA (10% w/v) and centrifugation at 3000 rpm for 10 min. Thereafter, 2.5 ml of the supernatant was withdrawn and mixed with 2.5 ml of distilled water and 0.5 ml  $FeCl_3$  (0.1% w/v). The absorbance was read at 700 nm with distilled water as blank. An increase in absorbance with increasing concentration of extract or standard corresponds to the formation of the bluish-green color of the reduced form of TPTZ. The average absorbance of the reactions performed in triplicate was obtained and plotted against the different concentrations of each extract and standard.

### 2'-azino-bis (3-ethylbenthiazoline-6-sulfonic acid) radical scavenging activity

This assay was performed according to the method of Adedapo *et al.*<sup>[23]</sup> It is based on the reaction between ABTS and potassium persulfate, to produce the ABTS radical cation ( $ABTS^+$ ), a bluish-green chromogen,

which is converted to a colorless solution in the presence of an antioxidant reductant.<sup>[24]</sup> A working solution was prepared by mixing equal amounts of 7 mM ABTS and 2.4 mM potassium persulfate. These were allowed to react for about 12 h in the dark at room temperature. 1 ml of the resulting solution was mixed with 60 ml methanol and the absorbance was adjusted to  $0.706 \pm 0.001$  units at 734 nm by addition of drops of the original ABTS/potassium persulfate solution. Thereafter, 1 ml of each extract and the standards prepared at different concentrations (0.025–0.50 mg/ml) in methanol was mixed with 1 ml of the ABTS/methanol solution. The absorbance of the resulting solutions was read at 734 nm after about 7 min. ABTS radical scavenging activity was indicated by different degrees of decolorization of the dark-green color of the ABTS solution. All assays were done in triplicate. The percentage of ABTS radical scavenging activity was calculated from the following equation: % ABTS scavenging activity =  $[(A_c - A_s)/A_c] \times 100$ , where  $A_c$  is the absorbance of the control (ABTS + methanol) and  $A_s$  is the absorbance of the test samples (extract or standard).

### Hydrogen peroxide scavenging activity

H<sub>2</sub>O<sub>2</sub> scavenging activity was determined according to the method described by Wintola and Afolayan.<sup>[18]</sup> H<sub>2</sub>O<sub>2</sub> in the presence of an antioxidant is converted to water and oxygen, thus decreasing the concentration of H<sub>2</sub>O<sub>2</sub> as the concentration of antioxidant increases. 1 ml of the extract or the standard prepared at different concentrations (0.025–0.5 mg/ml) in distilled water was mixed with 0.6 ml of 40 mM H<sub>2</sub>O<sub>2</sub> (prepared in 0.1 mM phosphate buffer, pH 7.4). This was left to react for 10 min after which the absorbance was read at 230 nm using a UV-VIS 3000 PC spectrophotometer, against a blank containing phosphate buffer without H<sub>2</sub>O<sub>2</sub>. The inhibition of H<sub>2</sub>O<sub>2</sub> was calculated as the percentage of H<sub>2</sub>O<sub>2</sub> remaining in the solutions<sup>[25]</sup> using the following formula: % H<sub>2</sub>O<sub>2</sub> remaining =  $[(A_c - A_s)/A_c] \times 100$ , where  $A_c$  is the absorbance of the control (phosphate buffer + H<sub>2</sub>O<sub>2</sub>) and  $A_s$  is the absorbance of the test samples (extract or standard).

### Nitric oxide scavenging activity

The method described by Wintola and Afolayan<sup>[18]</sup> was used for the assay of the NO radical scavenging activity of the extracts. The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions. The nitrite ions are detected in solution by the Griess reagent which contains sulfanilamide and naphthylethylene diaminedihydrochloride. Compounds that scavenge NO compete with oxygen, leading to a reduced production of nitrite ions (Ebrahimzadeh *et al.*,<sup>[26]</sup>). For the assay, (0.5 ml of the extracts or standard was mixed with 2 ml of 10 mM sodium nitroprusside [prepared in 0.5 mM phosphate-buffered saline, pH 7.4]). The mixture was incubated for 2.5 h at 25°C. 0.5 ml of the mixture was mixed with 0.5 ml of Griess reagent (prepared by mixing 1 ml sulfanilic acid [0.33% in 20% glacial acetic acid] with 1 ml of naphthalene diaminedihydrochloride [0.1% w/v]). The mixture was incubated for 30 min at room temperature and the absorbance was measured at 540 nm. The percentage of NO scavenging ability of the plant extracts and standard compounds was calculated using the following formula: % NO scavenged =  $[(A_c - A_s)/A_c] \times 100$ , where  $A_c$  is the absorbance of the control reaction and  $A_s$  is the absorbance of the test samples (extract or standard).

### Statistical analysis

All values have been expressed as mean  $\pm$  standard deviation of three replicates. Microsoft Excel Professional for Windows (Version 2013) was used for statistical analyses and determination of IC<sub>50</sub> for the antioxidant assays. Significant differences ( $P < 0.05$ ) were examined by ANOVA using GraphPad Prism Software (GraphPad Software, Inc; www.graphpad.com).

## RESULTS

The yields of the extracts obtained are *D. thunbergii* aqueous extract (DTA; 16.6%), *D. thunbergii* ethanol extract (DTE; 2.4%), *H. argentea* aqueous extract (HAA; 3.2%), and *H. argentea* ethanol extract (HAE; 1.8%).

### Phytochemical constituents

The phytochemical composition of the different extracts of *D. thunbergii* roots and *H. argentea* corms is presented in Table 1. The results indicate significant differences ( $P < 0.05$ ) in the contents of the different phytochemicals between the two plants and the extraction solvents used in this study, water and ethanol.

The ethanol extracts of each plant had significantly higher content ( $P < 0.05$ ) of phenols than the aqueous extracts. HAE had the highest total phenolic content while DTA had the least. The results, however, indicate a significantly higher content ( $P < 0.05$ ) of total phenols in *H. argentea* than in *D. thunbergii*. Total flavonoid contents in the extracts were in the following order DTE > HAE > HAA > DTA, implying that the aqueous extracts of each plant had higher contents of flavonoids than the ethanol extracts. The contents of flavonols, on the other hand, were significantly higher ( $P < 0.05$ ) in the aqueous extracts than the ethanol extracts. HAA had the highest content of total flavonols while HAE had the least amount. High concentrations of proanthocyanidins were detected in the two plants, the majority of which were concentrated in the ethanol extracts in the following order: DTE > HAE > HAA > DTA.

Tannin content was generally low in all the extracts compared to the other phytochemicals evaluated. As with most phytochemicals, the ethanol extracts contained higher contents of tannins than the aqueous extracts, the amount in *H. argentea* being slightly higher than in *D. thunbergii*. The percentage of alkaloids detected in the plant extracts also showed that the ethanol extracts had significantly higher alkaloid contents ( $P < 0.05$ ) than the aqueous extracts. There were no significant differences in the alkaloid contents of both plants when extracted with the same solvent. This indicates a trend which suggests that ethanol may extract greater amounts of phytochemicals, despite the relatively lower yields of ethanol extracts produced from the original plant material, compared to the aqueous extracts.

### Antioxidant activities

The antioxidant activities of the plant extracts compared favorably with those of the standard antioxidants (Vitamin C, BHT, and Rutin) employed in the antioxidant assays.

### Ferric-reducing power

As shown in Figure 1, the extracts and standards exhibited a concentration-dependent increase in their ferric-reducing abilities, indicated by an increase in absorbance with increasing concentration of extract or standard. Vitamin C exhibited the most potent ferric-reducing ability of all other compounds and extracts tested. The overall order of the ferric-reducing abilities at the highest concentration (0.50 mg/ml) was as follows: Vitamin C > HAE > Rutin > DTE > HAA > DTA > BHT. This result suggests that the ethanol extracts of both *D. thunbergii* and *H. argentea* possessed higher ferric-reducing capacities than the aqueous extracts. This observation appears to bear significant correlation with the total phenolic and flavonoid contents of the extracts.

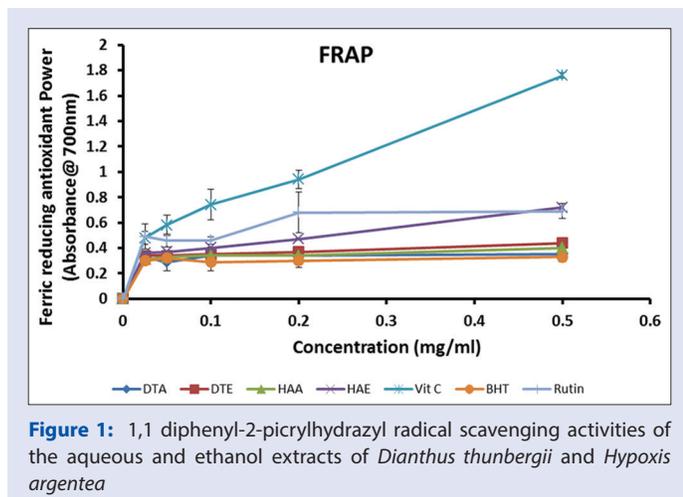
### 1,1 diphenyl-2-picrylhydrazyl scavenging activity

The DPPH radical scavenging activities of the extracts and standards are presented in Figure 2. At the maximum concentration tested (0.50 mg/ml), HAA exhibited the highest DPPH scavenging

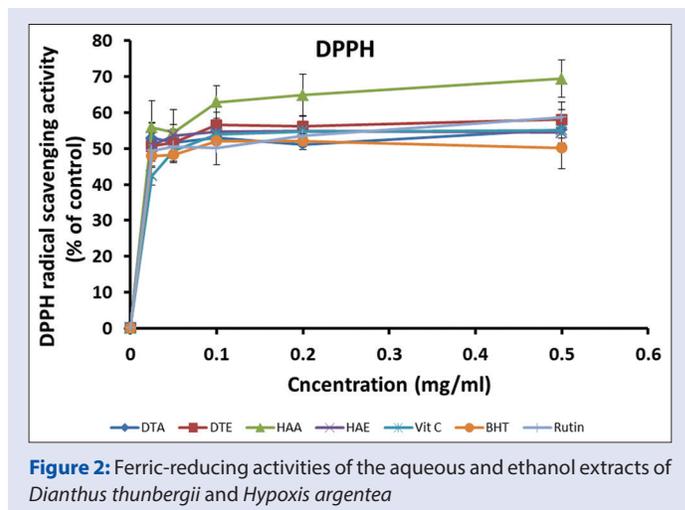
**Table 1:** Phytochemical composition of aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*

Phytochemical constituents	DTA	DTE	HAA	HAE
Total phenols (mg GAE/g)	9.71±0.98 <sup>a,c</sup>	28.01±0.37 <sup>a,d</sup>	18.01±0.44 <sup>b,c</sup>	66.71±2.71 <sup>b,d</sup>
Total flavonoids (mg Qe/g)	16.56±1.31 <sup>a,c</sup>	62.21±1.75 <sup>a,d</sup>	27.70±2.20 <sup>c</sup>	28.39±1.17 <sup>d</sup>
Total flavonols (mg Qe/g)	14.71±1.85 <sup>c</sup>	13.06±0.21 <sup>d</sup>	25.51±1.92 <sup>b,c</sup>	10.09±0.92 <sup>b,d</sup>
Total tannins (mg TAE/g)	0.72±0.04 <sup>a,c</sup>	1.07±0.07 <sup>a</sup>	0.89±0.01 <sup>b,c</sup>	1.18±0.07 <sup>b</sup>
Total proanthocyanidins (mg Ca/g)	14.31±3.57 <sup>a,c</sup>	432.62±2.43 <sup>a,d</sup>	38.63±3.64 <sup>b,c</sup>	311.87±3.57 <sup>b,d</sup>
Alkaloids (%)	14.33±1.74 <sup>a,c</sup>	25.42±2.25 <sup>a</sup>	15.70±4.44 <sup>b,c</sup>	24.15±2.17 <sup>b</sup>

Values are expressed as mean±SD of three replicates. Superscripts represent significant differences between <sup>a</sup>DTA and DTE; <sup>b</sup>HAA and HAE; <sup>c</sup>DTA and HAA; and <sup>d</sup>DTE and HAE at  $P < 0.05$ . mg GAE/g: Milligram gallic acid equivalent per gram of extract; mg Qe/g: Milligram quercetin equivalent per gram of extract; mg TAE/g: Milligram tannic acid equivalent per gram of extract; mg Ca/g: Milligram catechin equivalent per gram of extract; DTA: *Dianthus thunbergii* aqueous extract; DTE: *Dianthus thunbergii* ethanol extract; HAA: *Hypoxis argentea* aqueous extract; HAE: *Hypoxis argentea* ethanol extract; SD: Standard deviation



**Figure 1:** 1,1 diphenyl-2-picrylhydrazyl radical scavenging activities of the aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*



**Figure 2:** Ferric-reducing activities of the aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*

activity ( $69.42 \pm 5.26\%$ ), followed by Rutin ( $58.69 \pm 4.25\%$ ), DTE ( $58.06 \pm 2.83\%$ ), DTA ( $55.18 \pm 2.23\%$ ), Vitamin C ( $55.06 \pm 2.25\%$ ), HAE ( $54.19 \pm 2.83\%$ ), and BHT ( $50.19 \pm 5.77\%$ ), in decreasing order. It appears that this effect correlated with the contents of flavonols in the extracts.  $IC_{50}$  values [Table 2], however, indicate that DTA and HAE required the lowest concentration (0.005 mg/ml) to produce 50% inhibition, followed by Vitamin C (0.092 mg/ml).

### 2'-azino-bis (3-ethylbenthiazoline-6-sulfonic acid) radical scavenging activity

The results of this assay [Figure 3] show that all the extracts and standards possessed considerable ABTS radical scavenging activities, even at the low concentrations, and this activity compared favorably with the standards. At the highest concentration tested (0.50 mg/ml), all the standards produced up to 99% inhibition, while values for DTA, DTE, HAA, and HAE were ( $84.14 \pm 4.56\%$ ), ( $98.16 \pm 0.14\%$ ), ( $95.52 \pm 2.02\%$ ), and ( $99.15 \pm 0.14\%$ ), respectively. Among the extracts, HAE had the lowest  $IC_{50}$  value (0.0125 mg/ml), similar to the standards, followed by DTE (0.175 mg/ml), DTA (0.020 mg/ml), and HAA (0.020 mg/ml) [Table 2].

### Nitric oxide inhibition

The percentage NO inhibitory activities of the extracts and standards [Figure 4] were in the following order: DTE > BHT > DTA > Rutin > Vitamin C > HAA > HAE, at the highest concentration (0.50 mg/ml). This seems to suggest that extracts of *D. thunbergii* exhibited a more potent NO inhibitory activity than those of *H. argentea* at higher concentrations. DTE also possessed the lowest  $IC_{50}$  [Table 2], indicating its strong NO scavenging activity. In general, increasing the concentration of the extracts or standards did not seem to significantly affect their NO inhibitory potentials.

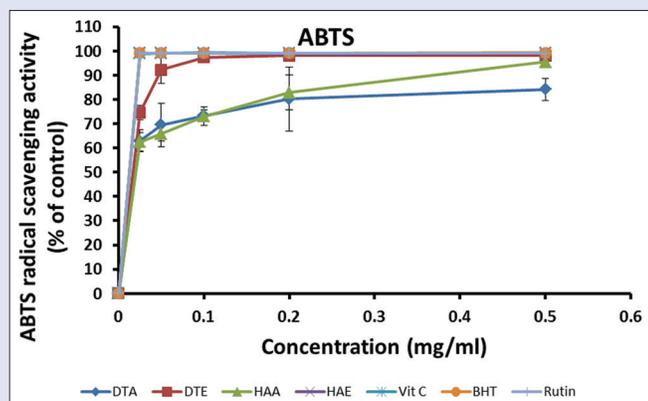
### Hydrogen peroxide scavenging activity

As shown in Figure 5, the extracts and standards exhibited a concentration-dependent inhibition of  $H_2O_2$ , as the contents of  $H_2O_2$  remaining in the reaction mixtures reduced with increasing concentration of extracts or standards. At the highest concentration (0.50 mg/ml), the order of inhibition was as follows: DTE > HAE > Rutin > HAA > DTA > Vitamin C > BHT, suggesting that the ethanol extracts possessed higher  $H_2O_2$  scavenging abilities than that of the aqueous extracts. The result also indicates that the plant extracts had better antioxidant activity than most of the standards with respect to removal of  $H_2O_2$ .

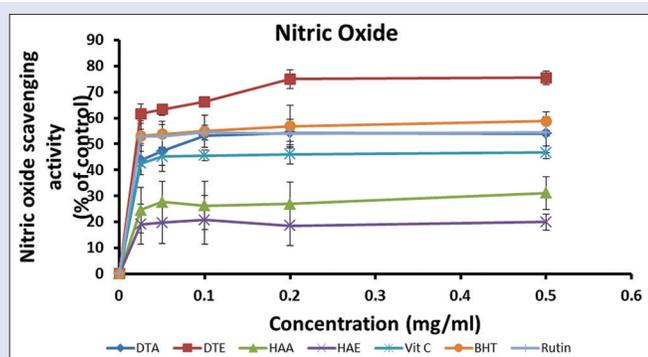
## DISCUSSION

The pathogenesis of many chronic ailments such as diabetes mellitus usually involves oxidative stress mechanisms.<sup>[27]</sup> In oxidative stress, the production of ROS such as hydroxyl radicals, superoxide radicals, and  $H_2O_2$  exceeds the capacity of exogenous antioxidants to detoxify them, leading to cellular or tissue damage due to lipid peroxidation and other mechanisms.<sup>[5]</sup> The use of herbal preparations as alternative or complementary therapeutic strategies against several diseases is a common practice in many African communities. Various groups of phytochemicals in plant extracts are believed to possess potent antioxidant and anti-inflammatory activities, which makes them useful in disease prevention and treatment.<sup>[28]</sup> For example, some flavonoids, tannins, alkaloids, saponins, and polycyclic glycosides have been reported to have hypoglycemic and antidiabetic activities.<sup>[29]</sup>

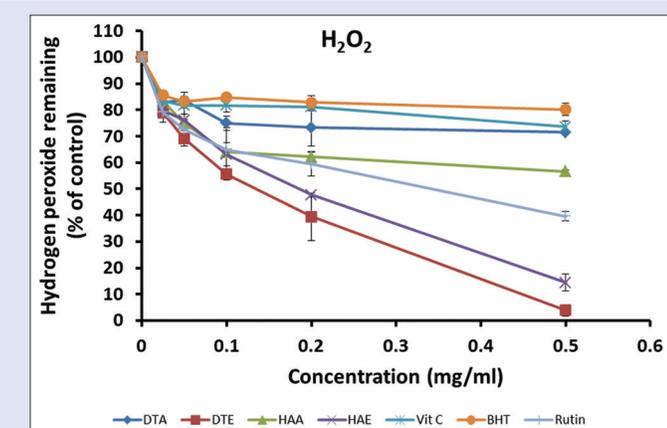
In this study, we evaluated the phytochemical composition and antioxidant activities of extracts from the roots of *D. thunbergii* and corms of *H. argentea*, two commonly used plants in the Eastern Cape



**Figure 3:** 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activities of the aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*



**Figure 4:** Nitric oxide inhibitory activities of the aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*



**Figure 5:** Hydrogen peroxide scavenging activities of the aqueous and ethanol extracts of *Dianthus thunbergii* and *Hypoxis argentea*

**Table 2:** IC<sub>50</sub> values for antioxidant activities of the extracts

Extract/standard	IC <sub>50</sub> (mg/ml)			
	DPPH	H <sub>2</sub> O <sub>2</sub>	Nitric oxide	ABTS
DTA	0.0050	0.9400	0.0700	0.0200
DTE	0.0150	0.1300	0.0200	0.0175
HAA	0.0100	0.5500	0.9800	0.0200
HAE	0.0050	0.1900	***	0.0125
Vitamin C	0.0600	1.1500	0.4250	0.0125
BHT	0.0750	1.7300	0.0250	0.0125
Rutin	0.0350	0.3400	0.0250	0.0125

\*\*\*Values too high and not relevant. IC<sub>50</sub>: Concentration of extract or standard producing 50% antioxidant activity; DTA: *Dianthus thunbergii* aqueous extract; DTE: *Dianthus thunbergii* ethanol extract; HAA: *Hypoxis argentea* aqueous extract; HAE: *Hypoxis argentea* ethanol extract; BHT: Butylated hydroxytoluene; DPPH: 1,1 diphenyl-2-picrylhydrazyl; ABTS: 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide

tannins, and alkaloids, compared to the aqueous extracts. The differences in the extracting abilities may be related to the differences in polarity of the components extracted and that of the solvents.<sup>[30]</sup> Total flavonol contents of the extracts, however, were higher in the aqueous extracts than in the ethanol extracts. This latter observation is similar to findings in another research.<sup>[31]</sup> The aqueous extract of *H. argentea* contained the highest amount of flavonols followed by the aqueous extract of *D. thunbergii*.

Flavonols, a class of flavonoids, are a group of poly-hydroxylated phenolics which are highly water soluble as a result of their hydroxyl groups. They are, therefore, more soluble in aqueous solvents than in organic solvents.<sup>[32]</sup> Comparison of total flavonol content with DPPH radical scavenging activities of the extracts at 0.5 mg/ml revealed a positive correlation. The DPPH radical is relatively stable. Its unpaired electron can become paired upon reaction with hydrogen-donating antioxidants.<sup>[33]</sup> Flavonoids have the ability to easily donate the hydrogen atoms in their hydroxyl groups<sup>[34]</sup> and therefore, it appears that this group of phytochemicals must have played a major role in the DPPH radical scavenging activities of the extracts in this study.

The order of increasing total phenolic content in the extracts was DTA (9.71 mg/g) < HAA (18.10 mg/g) < DTE (28.01 mg/g) < HAE (66.71 mg/g). In line with this result, extractions with ethanol or ethanol in water (50% v/v) have been reported to yield higher total phenolic contents than extraction with water alone.<sup>[35]</sup> At 0.5 mg/ml, we observed a highly positive correlation between the total phenolic contents of the extracts and their ferric-reducing abilities. The ability of compounds to convert ferric ion into ferrous form is a good indicator of antioxidant potential.<sup>[36]</sup> This ferric-reducing ability has been attributed to the presence of phenols in plant extracts, as well as the number and position of the hydroxyl groups in these compounds.<sup>[37]</sup> This finding is consistent with reports from several other researchers.<sup>[36,38-40]</sup>

The concentration of proanthocyanidins was higher in the ethanol extracts of both plants compared to that of the aqueous extracts, being highest in the ethanol extract of *D. thunbergii*. The tannin contents of the extracts were considerably low compared to the other groups of phytochemicals. The ethanol and aqueous extracts of each plant had similar contents of alkaloids. Although almost all plant species possess these different phytochemicals, their quantitative distribution varies, and this is believed to be responsible for the different medicinal potentials they exhibit.<sup>[41]</sup> Tannins have been reported to also possess the ability to reduce Fe<sup>3+</sup> to the Fe<sup>2+</sup> form and can inhibit the 5-lipoxygenase in arachidonic acid pathways, which highlights their importance in inflammatory processes. Interestingly, we found a similar trend of positive correlation between tannin content and the ferric-reducing abilities of the extracts,

province of South Africa for the management of diabetes mellitus. We aimed to compare the parameters between the two plants and the solvents (water and ethanol) used for the preparation of these plants for medicinal use. We also attempted to find any correlations between the antioxidant activities and the content of the major phytochemicals in these extracts. With respect to the extracting ability of the solvents, we observed that the ethanol extracts of the two plants contained significantly higher contents of phenols, flavonoids, proanthocyanidins,

as obtained for total phenolic contents. This, therefore, suggests that the antioxidant activity of the extracts is a combination of the roles played by the different groups of phytochemicals, rather than any one group.

The NO radical is produced *in vivo* by phagocytes and endothelial cells and it is very important in inflammatory processes. However, its overproduction can result in tissue damage seen in many inflammatory conditions including diabetes and arthritis.<sup>[42]</sup> This radical can combine with the superoxide radical to form the highly reactive peroxynitrite anion (ONOO<sup>-</sup>).<sup>[43]</sup> In our study, the ethanol extract of *D. thunbergii* exhibited the most potent NO inhibitory activity, which was even higher than those of Rutin, Vitamin C, and BHT. The NO scavenging activity was lowest in the extracts of *H. argentea*. The significantly higher contents of flavonoids and proanthocyanidins in the ethanol extracts of *D. thunbergii* might have been responsible for this observation. This finding suggests that *D. thunbergii* than *H. argentea* may be more beneficial in counteracting inflammatory signaling processes involving NO.

H<sub>2</sub>O<sub>2</sub> is a nonradical which is not very reactive, but becomes toxic when it forms the highly reactive hydroxyl radical in the presence of transition metals such as Fe<sup>2+</sup>.<sup>[44]</sup> We observed a concentration-dependent pattern of H<sub>2</sub>O<sub>2</sub> scavenging activity in the extracts and standards used in this study. This activity was highest in the ethanol extract of *D. thunbergii* which gave the least amount of H<sub>2</sub>O<sub>2</sub> remaining in the reaction mixtures at the highest concentration tested and the lowest IC<sub>50</sub>, while the ethanol extract of *H. argentea* also demonstrated high activity. It is noteworthy that the activity of these extracts in scavenging H<sub>2</sub>O<sub>2</sub> was higher than those exhibited by the standards, again highlighting the significant antioxidant capacities of the plants. Further confirmation of the potent antioxidant activities of these extracts was indicated by their pronounced ABTS radical scavenging activities, even at the lowest concentrations. This effect was comparable to those of the standard antioxidants, Vitamin C, BHT, and Rutin.

## CONCLUSION

Our study shows that extracts from *D. thunbergii* and *H. argentea* are easily accessible sources of phytochemicals possessing significant antioxidant activities, attributes which may prove useful for their effectiveness in the treatment of diseases such as diabetes mellitus as claimed by traditional healers in the Eastern Cape. With respect to the solvents used for extraction, the ethanol extracts had higher contents of phytochemicals than the aqueous extracts, an observation which correlated positively with the higher antioxidant activities exhibited by the ethanol extracts. The two solvents used in this study have been chosen to represent those commonly used by the local traditional healers in the preparation of these plants for medicinal use.

Further studies are, however, required to determine the best extraction solvents for these plants. Overall, both plants exhibited superior antioxidant activities than the other in different respects, depending on the nature of radical being scavenged. This may be related to the variations in their respective contents of the different phytochemicals. This latter observation may explain, in part, the traditional approach of mixing different plants in concoctions, rather than the use of individual plants for the treatment of different diseases. Further studies are currently ongoing to investigate the acclaimed roles of the plants used in this study in the treatment of diabetes mellitus.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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