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Phytochemical analysis and anti-oxidant activities of *Albuca bracteata* Jacq. and *Albuca setosa* Jacq bulb extracts used for the management of diabetes in the Eastern Cape, South Africa

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ABSTRACT

Objective: The bulb of *Albuca bracteata* Jacq., and *Albuca setosa* commonly used in Eastern Cape Province of South Africa for the treatment of several types of disease conditions including diabetes, was investigated for their phytochemical contents and antioxidant activities.

Methods: The antioxidant activity was determined by evaluating the effect of various solvent extracts (acetone, methanol, ethanol and water) on 2,2 – diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, 2,2′ – azino-bis (3-ethylbenzthiazoline-6-sulfonic acid diammonium salt (ABTS), ferric reducing power, hydrogen peroxide and nitric oxide scavenging activity while the various extracts were also analysed for phytochemical their contents.

Results: The results obtained indicate that polyphenols, proanthocyanidins, saponins and alkaloids are present in higher concentrations. The composition of phenols (117 mg/g), flavonols (26.28 mg/g), proanthocyanidins (84.85 mg/g) and flavonoids (5.36 mg/g) were significantly higher in the acetone extract while saponin and alkaloids contents were higher in the aqueous extract. Antioxidant studies of the extracts for nitric oxide, hydrogen peroxide, ABTS and DPPH showed high antioxidant potential in a concentration-dependent manner that was not significantly different from Vitamin C, BHT and Rutin used as standard references. The methanol extract showed higher ferric reducing potential compared to the aqueous extract but was significantly different from Vitamin C, BHT and Rutin.

Conclusion: This implies that the bulb of *Albuca bracteata* may serve as a natural source of antioxidants, which together with the numerous phytochemicals present, could account for its folkloric use as a medicinal plant.

1. Introduction

Medicinal plants have been reported to have antioxidant activities, which have been attributed to the presence of flavonols, flavones, anthocyanins, catechins and isocatechins [1]. These antioxidants due to their ability to chelate catalytic metals, scavenge reactive oxygen species and stop spontaneous reactions by acting as final electron acceptors to prevent oxidative damage caused by free radicals [2]. Reactive oxygen species (ROS) are continuously generated in the body through enhanced glucose auti-oxidation, increased mitochondrial superoxide production and uncoupled endothelial nitric oxide synthase activity [3]. Excess production of ROS and/or inadequate production of antioxidant defence leads to oxidative damage of various biomolecules including lipids, proteins and DNA [4]. Oxidative damage is, therefore, a critical etiological factor involved in degenerative diseases such as diabetes, cancer, atherosclerosis, arthritis, neurodegenerative diseases and ageing [5]. Diabetes mellitus is a metabolic disease associated

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with persistent hyperglycaemia, due to the inability of the body to either synthesize insulin or insensitivity to the action of insulin or both [6]. Increasing evidence has shown that in poorly controlled or persistent hyperglycaemia, increased formation of advanced glycation end products (AGEs), ROS and lipid peroxidation products exacerbate intracellular oxidative stress. The complications of diabetes mellitus such as glaucoma, cataract formation, neuropathy and nephropathy can therefore, be ameliorated by antioxidants. Available synthetic antioxidants which are commonly used such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are not without side effects and have been reported to be unsafe [5], therefore, there is a need to substitute these synthetic antioxidants with naturally occurring ones obtained from plants. *Albuca bracteata* (*A. bracteata*) and *Albuca setosa* (*A. setosa*) are plants reported to have been used by traditional healers in the Eastern Cape Province of South Africa to treat diabetes mellitus [7,8]. These plants are widely distributed throughout the Eastern Cape. *A. setosa* has been reportedly used for ritual washing, protection against bad luck and to heal wounds in a similar manner to *Aloevera* [9,10] while *A. bracteata* has been reportedly used as an aphrodisiac and to manage cancer [11]. Before the commencement of this study, however, there is paucity of data in literature that validates the bioactive compounds of these plants, comparative study of both plants and their antioxidant properties responsible for their folkloric usage. In this study, *A. bracteata* and *A. setosa* were investigated for antioxidant activity and their phytochemical contents analysed.

2. Materials and methods

2.1. Chemicals

2, 2, Diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid and BHT, Folin-Ciocalteu reagent and hydrogen peroxide (H_2O_2) were obtained from Merck Limited, (South Africa), while methanol (HPLC grade) was obtained from Sigma–Aldrich (South Africa). All other chemicals and reagents used in this study were of analytical grade.

2.2. Collection of plant materials

A. bracteata was collected from a forest near Alice while *A. setosa* was collected from Grahamstown (Eastern Cape Province, South Africa). They were identified and authenticated in the Department of Botany, University of Fort Hare where voucher specimen (voucher number ORN14/425 and AS15/01) were deposited. The plant materials were thinly sliced and oven-dried at 40 °C for 72 h, then macerated in a Hamilton Beach Commercial Blender type GB27 model HBF 400-CE(Hamilton Beach, Canada).

2.3. Preparation of plant extracts

Eighty grams of the powdered samples were weighed for extraction in 1 L either of distilled water and methanol, then placed on a mechanical shaker for 48 h at room temperature. The solutions were then filtered through a Buchner funnel using Whatman No. 1 filter paper under vacuum. The aqueous filtrates were freeze dried while the methanolic extracts were dried in a rotatory evaporator. The dried powdered plant materials were stored at −4 °C and later reconstituted in the relevant solvents just before the various analyses.

2.4. Phytochemical screening

2.4.1. Determination of total phenol content

The total phenols in the extracts were determined with Folin Ciocalteau reagent using the modified method of Wolfe *et al.*, [12]. Aliquots of the extracts were mixed with 5 mL Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). To this mixture, 4 mL of sodium carbonate (75 g/L) was added, vortexed for 10 s and allowed to stand for 30 min at 40 °C to develop colour. The absorbance was measured at 765 nm (Hewlett Packard UV–Vis spectrophotometer). Total phenolics content of the extracts was expressed as mg tannic acid equivalents (TAE) per gram of sample in dry weight (mg/g) using the equation derived from the calibration curve:

$y = 0.1216x$, $R^2 = 0.9365$, where x is the concentration of the tannic acid equivalent and y is the absorbance. The experiment was conducted in triplicate and the results expressed as mean ± SD values. The total phenolic content was calculated as gallic acid equivalent (TAE) by the following equation:

$$T = C \times V/M$$

T is the total phenolic content in mg/g of the extracts as TAE, C is the concentration of gallic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract in g.

2.4.2. Determination of total flavonoid content

Total flavonoids content was measured using $AlCl_3$ as described by Ordonez *et al.* [13]. A 0.5 mL aliquot of the extracts were mixed with 0.5 mL of 2% aluminium chloride ($AlCl_3$) prepared in ethanol. This mixture was allowed to stand for 1 h at room temperature. The formation of a yellow color indicated the presence of flavonoids. The absorbance was then read at 420 nm. Total flavonoid content were calculated as quercetin equivalents (mg/g) using the equation from the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$. Where y is the absorbance and x is the concentration of quercetin equivalent.

2.4.3. Determination of total tannin contents

Tannin content was estimated using the method described by Swain [14]. A 500 mL beaker containing 0.2 g of the extract and, 20 mL of 50% methanol was covered and shaken on a mechanical shaker to ensure thorough mixing, then placed in a water bath at 78 °C for 1 h. The mixture was then filtered into a 100 mL volumetry flask, to which 20 mL of distilled water, 2.5 mL Folin Ciocalteu reagent and 10 mL of 17% $NaCO_3$ were added and mixed thoroughly. The mixture was made up to the mark with distilled water and allowed to stand for 20 min to develop a bluish green colour. The absorbance was measured at 760 nm. Tannin standards were used to prepare a standard curve and the total tannin content were calculated as tannic acid equivalents (mg/g) using the equation from the calibration curve: $y = 0.087x–0.06$, $R^2 = 0.9277$, where y was the absorbance and x is the concentration of the tannic acid equivalent.

2.4.4. Determination of proanthocyanidin content

The total proanthocyanidin was determined using the procedure reported by Sun *et al.* [15]. 0.5 mL of 0.1 mg/mL of the extract was mixed with 3.0 mL of 4% vanillin-methanol solution, 1.5 mL of hydrochloric acid and then vortexed. After allowing the mixture to stand for 15 min at room temperature, the absorbance was measured at 500 nm. Total proanthocyanidin content was calculated as catechin equivalents (mg/g) using the equation from the calibration curve: y = 0.5825x, R^2 = 0.9277, where x is the concentration of the catechin equivalent and y is the absorbance.

2.4.5. Determination of total flavonol content

Total flavonol was estimated using the method of Kumaran and Karunakaran [16]. The reacting mixture consisted of 2.0 mL of the extract or standard, 2.0 mL of 2% $AlCl_3$ prepared in ethanol and 3.0 mL (50 g/L) sodium acetate solution. This was allowed to stand for 2 h at 20 °C. The absorbance was measured at 440 nm. Total flavonol content was calculated as quercetin equivalents (mg/g) using the equation from the calibration curve: y = 0.0255x, R^2 = 0.9812, where x is the concentration of the quercetin equivalent (mg/mL) and y is the absorbance.

2.4.6. Determination of saponin content

Saponin content was determined according to the method described by Obadoni and Ochuko [17]. Briefly, 50 mL of 20% ethanol (in distilled water) was added to 5 g of the sample. The solution was heated for 4 h in a water bath with continuous stirring at 55 °C, this was then filtered and re-extracted in 50 mL of 20% ethanol (in distilled water). The extracts were combined and reduced to 10 mL at 90 °C. The concentrate was then transferred into a 250 mL separating funnel, 5 mL diethyl ether was added and vigorously shaken. The ether layer was discarded while the aqueous layer was repeatedly purified using 15 mL *n*-butanol and later washed with 10 mL of 5% aqueous NaCl. The solution was heated in a hot water bath to evaporation and was later oven-dried to a constant weight at 40 °C.

The saponin content of the sample was calculated using the equation:

Saponin content (mg/g) = (weight of residue/weight of sample)

2.4.7. Determination of alkaloid content

The alkaloid content of the plant extract was determined according to the method of Harborne [18]. Briefly, 200 mL of 20% acetic acid in ethanol was added to 5 g of the powdered sample and was allowed to stand for 4 h. This was then filtered and concentrated in a water bath until the volume was one – quarter of the original volume. Concentrated ammonium hydroxide was then added dropwise until precipitation was completed. The solution was then filtered and the precipitate collected and weighed.

The alkaloid content was calculated using the equation:

Alkaloid content (mg/g) = (weight of precipitate/weight of sample)

2.5. Antioxidant analysis

2.5.1. Determination of diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

To determine the scavenging activity of DPPH free radical of the extract, the method of Mbaebie *et al.* [14] was used. DPPH in methanol (0.135 mM) was prepared and 1.0 mL of the DPPH solution was then mixed with 1.0 mL of the extract prepared in methanol containing 0.02–0.1 mg of the plant extracts and standard reference Vit. C and BHT. The reaction mixture was then vortexed thoroughly, left in the dark for 30 min at room temperature and the absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability to scavenge DPPH radical by the plant extract was calculated from the equation:

$$\text{DPPH radical scavenging activity } (\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right) / \text{Abs}_{\text{control}}\right] \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical + methanol. $\text{Abs}_{\text{sample}}$ is absorbance of DPPH radical + sample (extract or standard).

2.5.2. Determination of ABTS scavenging activity

The ABTS scavenging activity of the plant extracts was determined according to the methods of Yang *et al.* [19]. The working solution was prepared by mixing 7 mM of ABTS and 2.4 mM of potassium persulfate in ratio 1:1 in distilled water. The mixture was allowed to react in the dark for 12 h at room temperature. After 12 h, 3 mL of the working solution was further diluted with 150 mL methanol to obtain an absorbance of 0.706 ± 0.002 units at 734 nm using a spectrophotometer. This was adjusted by mixing drop wise of ABTS previously prepared. 1 mL of the working solution was then added to the extracts of varying concentrations (0.2–1.0 mg/mL) and allowed to react in the dark. The absorbance was measured at 734 nm after 7 min. The $ABTS^+$ scavenging capacity was compared with BHT and ascorbic acid. The percentage inhibition was calculated as follows:

$$ABTS^+ \text{ scavenging activity } (\%) = \left[1 - \left(\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}\right)\right] \times 100$$

where $\text{Abs}_{\text{sample}}$ is absorbance of $ABTS^+$ + sample (extract or standard). $\text{Abs}_{\text{control}}$ is absorbance of $ABTS^+$ + methanol.

2.5.3. Ferric reducing potential assay

The reducing power of the extract was estimated as described by Hossain *et al.* [20]. The extract was prepared in different concentrations ranging from 0.02 to 0.1 mg/mL. To 1 mL of each concentration, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide ($K_3Fe(CN)_6$) (1% w/v) were added. The resulting mixture was incubated for 20 min at 50 °C, 2.5 mL of trichloroacetic acid (10% w/v) was added and centrifuged at 3000 rpm for 10 min (Labline Centrifuge model no. CF – 622). 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl3 (0.1% w/v). The absorbance was measured at 700 nm against appropriate blank solution using a Vis/3000 spectrophotometer. An increase in absorbance of

the reaction mixture is an indication of higher reducing power of the extract. BHT, ascorbic acid and rutin were used as positive controls.

2.5.4. Determination of nitric oxide scavenging activity

Nitric oxide scavenging activity of the extract was determined by the modified method of Chidambaram *et al.* [21]. 2 mL of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was added to 0.5 mL of the extract and standards of varied concentrations (0.2–1.0 mg/mL) and then incubated for 2.5 h at 25 °C of the incubated mixture, 1 mL was then taken, mixed with 1 mL of Griess reagent (equal volume of 0.33% sulphanilic acid prepared in 20% glacial acetic acid and 0.1% (w/v) naphthalenediamine dichloride) and incubated at room temperature for 30 min. The absorbance was measured at 540 nm and percentage nitric oxide inhibition by the extract was calculated using the equation

$$\text{NO scavenging activity } (\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of NO radicals; $\text{Abs}_{\text{sample}}$ is the absorbance of NO radical + sample (extract or standard).

2.5.5. Determination of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of the extract was investigated using the modified method of Sarma *et al.* [22]. 4 mM hydrogen peroxide stock solution was prepared in 0.1 M phosphate buffer (pH 7.4). 2 mL of each sample was added to 0.6 mL of the hydrogen peroxide solution, incubated for 15 min at room temperature, then absorbance was measured at 230 nm. Percentage inhibition of hydrogen peroxide was calculated as:

$$\text{Hydrogen peroxide scavenging activity } (\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

where the $\text{Abs}_{\text{control}}$ is the absorbance of H_2O_2 radicals; $\text{Abs}_{\text{sample}}$ is the absorbance of H_2O_2 radical + sample (extract or standard).

2.6. Anti-inflammatory activity

2.6.1. Protein denaturation method

The modified method as previously described by Alhakmani *et al.* [23] was used to determine the protein denaturation assay. An aliquot of 50 μL of the reaction mixture containing 1% aqueous solution of bovine serum albumin (pH 6.3) was reacted with 100 μL of the samples with concentrations ranging from 0.1 to 0.5 mg/mL. These were incubated at 37 °C for 30 min and then heated at 57 °C for 5 min. After cooling the samples, 250 μL of phosphate buffer solution (pH 6.4) was added. The turbidity was measured with spectrophotometer at 660 nm. Distilled water was used as the negative control. Different concentrations of diclofenac sodium ranging from 100, 200, 300, 400, and 500 μg/mL were used as reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage of inhibition } (\%) = 1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

2.7. Statistical analysis

All experiments were performed in triplicates and data were expressed as mean ± SD (standard deviation). Statistical analysis was carried out for ANOVA (analysis of variance) using Minitab student release 12 (V12.11). The probability of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

3. Results

3.1. Phytochemical contents

Plants exhibit their medicinal properties through the synthesis of various secondary metabolites that are used primarily as an adaptive or protective mechanisms from their immediate environments. The phytochemical contents of the aqueous extractions of *A. bracteata* and *A. setosa* revealed the presence of flavonols, flavonoids, phenolics, proanthocyanidins, saponins and tannins as shown in Table 1.

A. bracteata contained the highest saponin and flavonol contents with the saponin content highest overall of all the phytochemicals determined while *A. setosa* contained more phenols,flavonoids, proanthocyanidins, tanins and alkaloids contents than *A. bracteata*. Table 1 also shows the phytochemicals contents of the aqueous extracts of *A. bracteata* and *A. setosa* compared to their metanolic counterparts. It is therefore evident that these phytochemicals exhibit different solubilities in different solvents. For instance the aqueous extract of *A. bracteata* and *A. setosa* contained 167.323 ± 0.105 and 281.449 ± 1.687 mg/g TAE while the metanolic extracts contained 85.262 ± 0.069 and 72.457 ± 0.235 mg/g TAE

Table 1

Phytochemical analysis of different solvent extracts of the bulb of *Albuca bracteata* and *Albuca setosa.*

Aqueous			Methanol	
	Albuca bracteata	*Albuca setosa*	*Albuca bracteata*	*Albuca setosa*
Phenols[1]	167.323 ± 0.105	281.449 ± 1.687	85.262 ± 0.069	72.457 ± 0.235
Flavonols[2]	164.09 ± 0.029	124.31 ± 0.126	1.386 ± 0.015[b]	1.59 ± 0.064[b]
Flavonoids[2]	43.4 ± 0.026	128.39 ± 0.029	0.68 ± 0.015	1.853 ± 0.039
Proantho cyanidins[3]	118.83 ± 0.58	124.66 ± 0.00	18.06 ± 0.58	43.88 ± 0.67
Tannin[1]	7.4 ± 0.000	10.7 ± 0.00245	0.102 ± 0.0002	0.095 ± 0.0006
Saponin	415.5 ± 0.01	147.43 ± 0.06	115.48 ± 0.06	63.0 ± 0.16
Alkaloids	136.37 ± 0.01	211.365 ± 0.80	34.44 ± 0.03	44.439 ± 0.03

Data expressed as mean ± SD; $n = 3$, values with the same superscripts do not differ significantly ($P < 0.05$), the subscript indicates [1]Expressed as mg/g of the extracts as tannic acid equivalent, [2]Expressed as mg/g of the extracts as quercetin equivalent, [3]Expressed as mg/g of the extracts as catechin equivalent.

respectively. The aqueous extracts showed higher extractable phytochemical contents than the metanolic extracts.

3.2. Antioxidant assay

3.2.1. DPPH radical scavenging activity

The extracts of *A. bracteata* and *A. setosa* were investigated against DPPH activity and the percentage inhibitory activity observed for DPPH compared with BHT and Vitamin C is shown in Figure 1. The antioxidant potential of the aqueous bulb extracts of *A. bracteata* and *A. setosa* determined were used to calculate the IC_{50} (Table 2) which is the concentration required to attain 50% inhibitory effect. Rutin has the least IC_{50} followed by the aqueous extract of *A. setosa* indicating that *A. setosa* with IC_{50} of 0.330 mg/ml was the most active of all the plant extracts followed by the methanolic extract of *A. setosa*. The aqueous extract of *A. bracteata* with IC_{50} of 0.650 mg/mL was more active than the methanolic extract.

3.2.2. ABTS radical scavenging activity

The extracts of *A. bracteata* and *A. setosa* were effective scavengers of ABTS radicals as shown in Figure 2. The aqueous extracts of both plants showed better activity against ABTS radicals compared to the standards used as evident from their IC_{50}. The IC_{50} was in the order 0.0809, 0.1326, 0.170, 0.222, 0.3357 and 0.3817 mg/mL for *A. setosa* aqueous bulb extract, *A. brceata* aqueous bulb extract, BHT, rutin, *A. setosa* methanol extract and *A. bracteata* methanol extracts respectively.

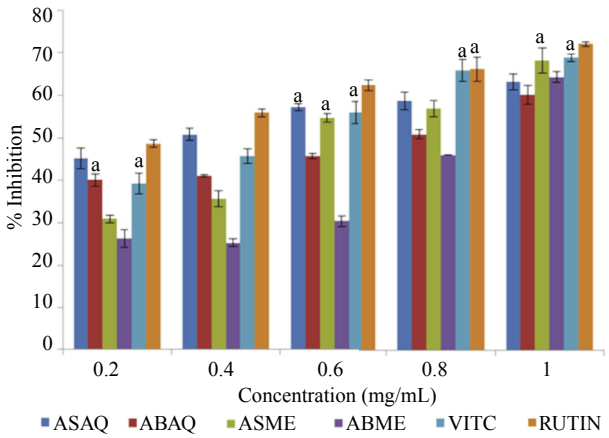

Figure 1. DPPH scavenging activity of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of triplicate samples. Bars with the same superscripts in the same concentration do not differ significantly ($P < 0.05$). ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*, Vit C = Vitamin C.

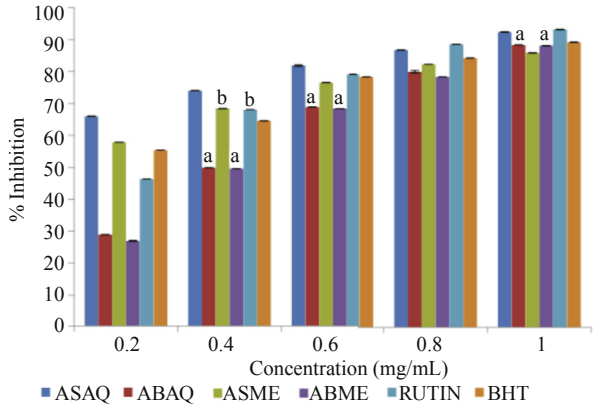

Figure 2. ABTS scavenging activity of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of triplicate samples. Bars with the same superscripts in the same concentration do not differ significantly ($P < 0.05$). ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*, BHT = butylated hydroxyltoluene.

3.2.3. Ferric reducing antioxidant potential assay

The ferric ion reducing potential of the extracts were significantly lower than both rutin and Vitamin C used as reference compounds as shown in Figure 3. The ability of the extracts to reduce Fe^{3+} to Fe^{2+} was observed as colour change from yellow to blue of the test solution depending on the concentration of the plant extracts. Comparing the extracts, *A. setosa* methanol extract showed higher reducing power followed by the metanolic extract of *A. bracteata*. However, *A. setosa* aqueous extract has the least IC_{50} among the extracts investigated (Table 2). All extracts of *A. setosa* had better better reducing power than the extracts of *A. bracteata*.

3.2.4. Nitric oxide inhibition activity

The aqueous extracts of both plants showed significantly lower nitic oxide inhibitory activity compared to their respective methanolic extracts Figure 4. However, the aqueous extract of *A. bracteata* together with the methanolic extract of *A. setosa* have IC_{50} comparable with BHT (Table 2).

Table 2

IC_{50} Scavenging activity of *Albuca bracteata* and *Albuca setosa*.

Sample		DPPH	ABTS	Nitric oxide	Hydrogen peroxide	Reducing power	Anti-inflammatory
Albuca bracteata	Aqueous	0.650	0.1326	0.564	1.2306	0.344	1.234
	Methanol	0.90	0.3817	0.655	0.4092	0.381	0.747
Albuca setosa	Aqueous	0.330	0.0809	0.614	1.3448	0.416	0.885
	Methanol	0.538	0.3357	0.570	0.7708	0.4923	0.555
Rutin		0.2383	0.222	–	–	0.548	–
BHT		–	0.170	0.543	0.3833	–	–
Vit. C		0.4004	–	0.723	0.2324	0.309	–
Diclofenac							0.314

IC_{50} is defined as the concentration (mg/mL) sufficient to obtain 50% of a maximum scavenging capacity.
–: values not determined.

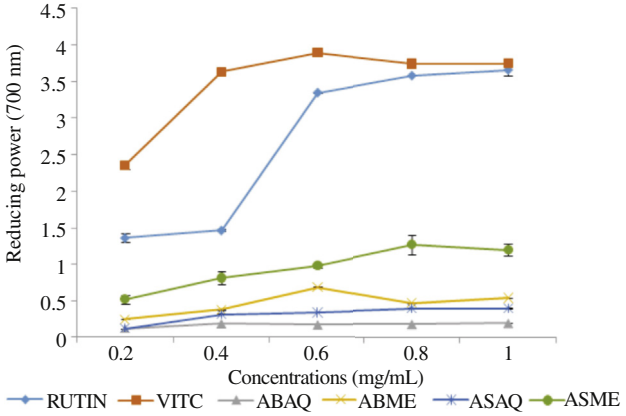

Figure 3. Reducing power of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of triplicate samples ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*, Vit C = Vitamin C.

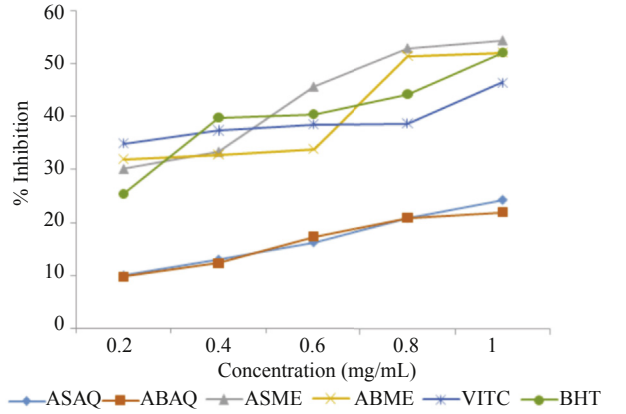

Figure 4. NO_2 scavenging activity of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of triplicate samples ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*, Vit C = Vitamin C.

3.2.5. Hydrogen peroxide inhibition activity

The percentage inhibition of hydrogen peroxide of the extracts is presented in Figure 5. All the extracts have lower percentage inhibtion except *A. bracteata* methanolic extract however, they all have higher IC_{50}than the standards (Table 2). The methanolic extracts of both *A. bracteata* and *A. setosa* showed lower IC_{50} than their respective aqueous extracts.

3.3. In vitro anti-imflammatory activity

All the plant extracts showed lower percentage inhibition compared to diclofenac (Figure 6). The metanolic extracts of both *A. bracteata* and *A. setosa* showed lower IC_{50} than their respective aqueous extracts (Table 2). Overall, the metanolic extract of *A. setosa* showed the best activity followed by the methanolic extract of *A. bracteata*. The aqueous extract of *A. setosa* showed better activity than the aqueous extract of *A. bracteata* (Table 2).

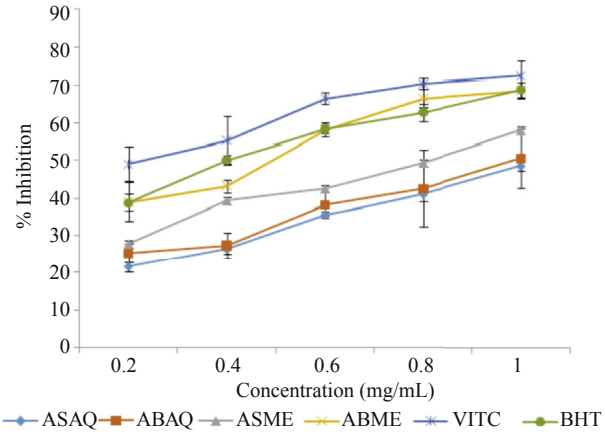

Figure 5. H_2O_2 scavenging activity of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of triplicate samples ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*, Vit C = Vitamin C.

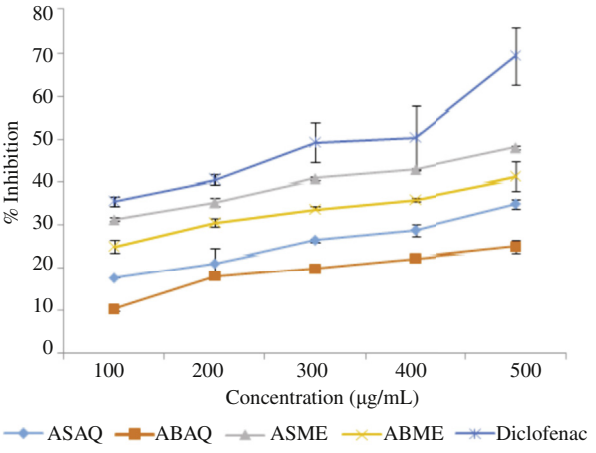

Figure 6. Protein denaturation activity of *Albuca bracteata* and *Albuca setosa*. Results are expressed as mean ± SD of $n = 3$. ASAQ: aqueous extract of *Albuca setosa*, ABAQ: aqueous extract *Albuca bracteata*, ASME: methanolic extract of *Albuca setosa*, ABME: methanolic extract *Albuca bracteata*.

4. Discussion

The use of medicinal plants in the management of ailments cut across different cultures globally [24]. Most of these plants have been reported to exhibit high antioxidant properties such as the reduction of DPPH radicals, ABTS, NO_2 and ferric ion reducing power [24–26]. Therefore, the ferric reducing power, DPPH, ABTS, NO_2, H_2O_2 scavenging activities of the extracts investigated were used as models for anti-oxidant capacity. In this study, the aqueous bulb extract of *A. setosa* showed higher activity against DPPH and ABTS radicals in all the extracts comparable to the standards, this could be due to the high phenolic and alkaloid contents present in the aqueous extracts of *A. setosa*. Polyphenols have been reported to possess DPPH and ABTS radical scavenging activities either by donating hydrogen to the nitrogen-centred free radicals of DPPH converting it to a stable diamagnetic molecule known as diphenyl-picryl hydrazine or by single electron transfer to ABTS radical [27–29]. The high ABTS radical scavenging activity of all the extracts compared to DPPH maybe due to the different mode af action and this supports earlier reports that ABTS is more reactive than DPPH [30]. But it was evident that these extracts can scavenge both

ABTS and DPPH similar to the report of Wintola and Afolayan [26], who suggested that the ability of a plant extract to scavenge DPPH radical could also reflect its ability to scavenge and inhibit ABTS radical formation. The reducing power result shows that these extracts possess antioxidant activity in a concentration-dependent manner indicating that these extracts have the ability to reduce oxidative damage in cells. Oxidative damage has been implicated in various diseases such as heart problems, cancers and complications of diabetes. The reducing power is a measure of the plant's ability to reduce Fe^{3+} to Fe^{2+} and is associated with the presence of a redundant molecule or complex that serves as the electron donor and/or free radical scavengers. This ferric ion reducing ability may be due to the presence of phenolics compounds which might act as electron donor. This result therefore, supports the reports that reducing power ability of a plant correlates with the phenolic content [31–33]. Nitric oxides are generated by the endothelial cells, macrophages and neurons where they serve as important chemical messengers. Nitric oxide is also implicated in the regulation of various physiological processes but excess concentrations can lead to disease conditions [34]. The activity of *A. bracteata* aqueous extract and *A. setosa* methanolic extract against nitric oxide were comparable with BHT but higher than vitamin C indicating promising ameliorative effect on oxidative stress arising from excess nitric oxide synthesis. However, the aqueous extracts activity against hydrogen peroxide is not promising at the concentrations investigated but the methanolic extracts of these plants against hydrogen peroxide was comparable to the standards. This could be as a result of the solubility of the active principles in methanol. The ability of hydrogen peroxide to penetrate the cellular membrane accounts for its toxicity and could be more toxic if it is converted into hydroxyl radicals in biological cells [35]. Hydroxyl radical is thus the more toxic than hydrogen peroxide and other reactive oxygen species and capable of damaging biological molecules including DNA, proteins, and polyunsaturated fatty acids [24,25,35]. In alternative medicine, liquid hydrogen peroxide has been used when mixed with water to treat skin and mouth infections [36] due to its ability to penetrate the cellular membrane. Proteins lose their tertiary and secondary structures when denatured. This is often associated with oxidative stress during degenerative diseases. Hence, protein denaturation has been reported to be the cause of inflammation [37,38]. Therefore, as part of the investigation on the anti-inflammation activity, the ability of the plant extracts to inhibit protein denaturation was studied. The anti-inflammatory activity of these extracts is similar to previous report by Gambhire *et al.*, [38] and could be attributed to the presence of the phtochemical constituents. Phenolics, alkaloids and saponins have been reported to posess anti-inflammatory activity in plant extracts [38–40]. Phytochemicals are secondary metabolites that occur naturally in plants and have been implicated in a variety of functions such as stimulation of protective enzymes, inhibition of nitrosation or formation of DNA adducts [41]. The phytochemical analysis of various solvent extractions of the bulb of *A. bracteata* and *A. setosa* revealed the presence of phenols, saponin, alkaloids, flavonols and flavonoids. The antioxidant capacity of these phytochemicals has been reported to be essential in ameliorating several diseases conditions such as diabetes, cancer and arteriosclerosis [42]. The differences observed in the phytochemical contents may be attributed to microclimate change, the nature of the soil [43] and processing methods and/or solvents of extraction. Saponins are synthesized by plants and are used for protection against pathogens thus serving as natural antibiotics [44]. This natural tendency for saponin in plants to ward off microbes may suggest that the high saponin content of *A. bracteata* aqueous extract makes it a good potential antifungal or antibacterial agent. Foam formation in aqueous solutions, lowering of cholesterol binding (thus interfere with cell growth and division), hemolytic activity, bitterness and ability to inhibit or kill cancer cells have also been associated with the presence of saponins [45]. Alkaloids in plants have been associated with analgesic effect and bactericidal activities [46]. Most plants reported to have medicinal values have been shown to contain traces of alkaloids [47], therefore, the higher alkaloid content in *A. setosa* suggests its medicinal value.

Phytochemicals which are naturally present in plants are used by plants to fight external (pests) and internal (free radicals) stresses. However, these agents have been found to possess antioxidant attributes in the management of human diseases. These phytochemicals present in the bulb extracts of *A. bracteata* and *A. setosa* may therefore, explain the benefit and medicinal value of these plants in the management and treatment of disorders induced by oxidative stress. These results could also justify the usage of *A. bracteata* and *A. setosa* in the treatment of diabetes as claimed by the traditional healers in Eastern Cape region.

Conflict of interest statement

The authors declare that there is no competing interest.

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