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Phytochemical analysis and biological activities of various parts of *Bulbine natalensis* (Baker): A comparative study

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ABSTRACT

Introduction: *Bulbine natalensis* is a succulent plant native to South Africa, used to treat various skin conditions. The purpose of this investigation was to analyse the phytochemical content, as well as antioxidant and antibacterial activities of various parts of *B. natalensis*.
Methods: Phytochemical screening tests were performed to investigate the presence of ten compounds from *B. natalensis* methanol extract. Total phenolic, flavonoid, tannin, and proanthocyanidin content assays were followed to determine their concentrations in *B. natalensis*. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), and iron chelating assays were used to assess the antioxidant activity. To determine the antibacterial properties of *B. natalensis* against *Staphylococcus aureus* and *Escherichia coli*, the agar-well diffusion method was adopted.
Results: The phytochemical screening revealed more compounds present in the leaves. The underground stems yielded higher concentrations of total phenolics (1909.2 ± 4.8 mg gallic acid equivalent [GAE]/g), total flavonoids (259.7 ± 27.2 mg quercetin equivalent [QE]/g), and total proanthocyanidins (858.3 ± 1.7 mg catechin equivalent [CE]/g) in comparison to the leaves and roots. The roots showed stronger DPPH (0.36 ± 0.02 mg/mL) and H_2O_2 (0.24 ± 0.04 mg/mL) scavenging activities. Only the underground stems and roots showed inhibition against *S. aureus* with the values of 15.33 ± 0.67 mm and 15.67 ± 0.33 mm, respectively, whereas the leaves displayed the highest inhibition against *E. coli* (18.33 ± 0.88 mm).
Conclusion: The methanolic extracts of *B. natalensis* leaves, underground stems, and roots possess significant phytochemical content, in conjunction with antioxidant and antibacterial activities.

Implication for health policy/practice/research/medical education:
The results validate the uses of the underground plant organs of *B. natalensis*, and suggest the application of all plant parts in traditional medicine and healthcare industries.
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Introduction

Bulbine natalensis (Baker) is a perennial succulent native to South Africa, characterised by its close resemblance to *Aloe,* with its broad, fleshy yellow-green leaves (1,2). The plant species is commonly referred to as the broad-leaved "Bulbine" (English), "ibhucu" (Zulu), "ingcelwane" (Xhosa), and "rooiwortel" (Afrikaans) (3). The term "rooiwortel", translated to "red root" was named due to the orange colour of the underground stem when cut open (3,4). *B. natalensis* is regarded as a medicinal plant because of its wide range of uses in traditional medicine. The leaves are mostly used for the treatment of wounds, rashes, ringworms, and other skin ailments due to the presence of gel fluid (2). The underground stem is commonly used as a fertility booster for men (2,5). The roots of *B. natalensis* are used to treat vomiting, diarrhoea, convulsions, venereal diseases, blood disorders, and diabetes-related illnesses (1,2,5,6). *B. natalensis* has been proven to have various biological activities, including antioxidant, antimicrobial, wound-healing, fertility, anti-platelet aggregation, and

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anti-inflammatory properties (2,5,7-10). There have been numerous studies covering the phytochemical analysis, as well as the antioxidant and antibacterial activities of *B. natalensis* leaves (1,7,11). However, limited reports were documented on the species' subterranean plant organs. Additionally, a comparative analysis of each plant part is yet to be reported. Considering that the whole plant is used in traditional medicine, it is imperative to assess the species' individual parts on their phytochemical and biological activities, which could be beneficial to indigenous communities, and pharmaceutical and cosmetic industries. The purpose of this investigation was to analyse the qualitative and quantitative phytochemical content, as well as the antioxidant and antibacterial activities of *B. natalensis*.

Materials and Methods

Sample preparation

Bulbine natalensis was procured from the Witkoppen Wildflower Nursery in Randburg, South Africa during the summer period (February). The plant was left to acclimatise in the Oppenheimer Life Sciences greenhouse for one month to mature. Following this process, the plant part was harvested and rinsed with deionised water (MeOH). The plant parts were hot air-dried at 40 ˚C for a period of four days, and then ground to powder with an electric grinder.

Preparation of methanolic extracts

The crude methanolic extracts were prepared by a previously described method (12,13). Three grams (3 g) of the respective powdered samples were placed in autoclave bottles in which 25 mL of 80% methanol (MeOH) was added. The extracts were placed in a water bath (Multifunctional ultrasonic cleaner water bath (TUC-100)) mixed with 20% acetone and sonicated at 50 ˚C for 25 minutes. Filter paper (Whatman® no. 1) was used to filter out the supernatants from the solid material, and the supernatants were refrigerated at 16 ˚C before phytochemical and bioactivity analyses were conducted.

Qualitative phytochemical analysis

Ferric chloride ($FeCl_3$) test for tannins

The method by Roghini and Vijayalakshmi (14) was adopted with slight modifications for this test. Two millilitres (2 mL) of deionised water and 2 mL of 5% $FeCl_3$ were added to a glass vial and mixed with 2 mL of the methanolic extract. The presence of a green or blue colour was an indication of tannins.

$FeCl_3$ test for phenolics

The method expressed by Prabhavathi et al (15) was followed with slight adjustments to determine the presence of phenolics. One millilitre of the MeOH extract was mixed with five drops of 10% $FeCl_3$. The presence of a green, blue, or violet colour was an indicator of phenolic compounds.

Hydrochloric acid (HCl) flavonoids test

Five hundred microliters of MeOH extract were placed in a glass vial, followed by five drops of concentrated HCl. A red colour change indicated the presence of flavonoids (16).

Test for steroids

In a glass vial, 2 mL of the methanolic extract was mixed with 2 mL of chloroform ($CHCl_3$) and 2 mL of sulphuric acid (H_2SO_4). The formation of a red-brown ring at the juncture of the mixtures indicated the presence of steroids (17).

Test for terpenoids

One millilitre of the methanolic extract was mixed with 0.5 mL of $CHCl_3$ and five drops of H_2SO_4. The presence of terpenoids was indicated by a red-brown precipitate (15).

Salkowski's test for glycosides

A volume of 0.5 mL of the methanolic extract was mixed with 2 mL of H_2SO_4. A red-brown colour indicated the presence of the steroidal aglycone part of the glycoside (18).

Froth test for saponins

In a glass vial, 0.5 mL of methanolic extract was mixed with 5 mL of deionised H_2O. After vigorously shaking the vial, three drops of olive oil were added, and the vial was shaken again. The presence of saponins was indicated by a stable foam (18).

Sodium hydroxide (NaOH) test for coumarins

A volume of 1 mL of the methanolic extract was mixed with 1 mL of 10% NaOH. A yellow colour change in the mixture indicated the presence of coumarins (14).

Test for phlobatannins

One millilitre of methanolic extract was placed in a glass vial, followed by five drops of 2% HCl. A red precipitate indicated the presence of phlobatannins (14).

Test for volatile oils

In a glass vial, 1 mL of the methanolic extract was mixed with 0.2 mL of 1% NaOH. The appearance of a precipitate indicated the presence of volatile oils (19).

Quantitative phytochemical analysis

For the quantification of the select phytochemical groups, an ultraviolet (UV) spectrophotometry approach was adopted with the methods from several authors being followed. The absorbance values obtained from the spectrophotometer were used to determine the

concentration of these compounds in various plant parts of *B. natalensis*.

Total phenolic content (TPC) analysis

A volume of 750 µL solution of Folin-Ciocalteu (1:10) was mixed with 2.5 mL of 7.5% saturated sodium carbonate (Na_2CO_3) and 0.3 mL of methanolic *B. natalensis* extract. The mixture reacted in a volumetric flask closed with a rubber stopper (13) and then diluted with 7 mL of deionised H_2O. The mixture was placed in a dark cabinet for two hours at room temperature. The absorbance reading of the mixtures post-incubation was measured at a wavelength of 765 nm. The following equation from the gallic acid calibration curve was used to calculate the TPC from each MeOH extract:

$y=0.0495 – 0.0259$, $r^2= 0.9994$.

Total flavonoid content (TFC) analysis

The quantification of total flavonoids was performed using the method described by Siddhuraju et al (12) with adjustments by Pakade et al (13). A volume of 300 µL of the respective methanolic extract was mixed with 4 mL of deionised H_2O in a 10 mL volumetric flask. Sodium nitrate ($NaNO_3$ – 300 µL) was then added to the flask, mixed, and then left to stand for five minutes before adding 3 mL of aluminium chloride ($AlCl_3$). Six minutes after adding the $AlCl_3$, 2 mL of 1 M NaOH solution was added to the flask, and the contents were made up to 10 mL with deionised H_2O. The mixture was shaken for 15 minutes before measuring the absorbance at a wavelength of 510 nm (20) using a UV spectrophotometer. A quercetin standard calibration curve was used to calculate TFC for each extract: $y= 0.2388 – 0.0019$, $r^2 = 0.9997$.

Total tannin content (TTC) analysis

In order to quantify the TTC in *B. natalensis* MeOH extract, a modified method by Lahare et al (21) was followed. One hundred microliters (100 µL) of each extract were placed in a glass vial and then 7.5 mLof deionised H_2O and 500 µL of Folin-Ciocalteu reagent were added. One millilitre (1 mL) of 35% Na_2CO_3 was added in the vial before topping up to 10 ml with deionised H_2O. The contents in each vial were thoroughly shaken and incubated at room temperature for 30 minutes. A gallic acid calibration curve was used to determine the TTC with the following linear equation:

$y= 0.046x – 0.0264$, $r^2 = 0.9833$.

Total proanthocyanidin content (TPAC)

The methodology by Oyedemi et al (22) was adopted for the determination of total proanthocyanidins in *B. natalensis*. The methanolic extract (0.5 mL) was mixed with 3 mLof 4% vanillin-methanol solution and 1.5 ml of HCl. A vortex was used to mix the contents before a 15-minute incubation period at room temperature. A wavelength of 500 nm was used to obtain the absorbance of the extracts, and the following linear equation from a standard catechin calibration curve was used to calculate the TPAC: $y= 0.9554x + 0.0003$, $r^2 = 0.9927$.

In vitro antioxidant activity analysis

An inhibition concentration or IC_{50} value is the minimum quantity of a substance needed to impede half of a biological process (23). The results for the antioxidant activity of *B. natalensis* were presented as such, following the conduction of three assays. A UV-spectrophotometer was used to record the absorbance values required to determine the IC_{50} concentration.

2, 2 diphenylpicryhydrazyl scavenging activity assay

Sight modifications were made after following the method of Brand-Williams et al (24) for the DPPH assay. A stock solution consisting of 50 mg of DPPH and 100 mL of 80% MeOH was further diluted with 80% MeOH (1:5; stock solution: 80% MeOH) to make a work solution. A range of volumes of *B. natalensis* extracts (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) were mixed with 0.7 mL of the work solution and then topped up to 1 mL with 80% MeOH. The mixtures were incubated in a dark cabinet for 45 minutes, and the absorbance of each mixture was measured at a wavelength of 517 nm. The % inhibition for each extract was calculated using the following equation:

$$Scavenging\ (\%) = [(Absample – Abblank)/Abcontrol] \times 100$$

where Absample was the absorbance of the extract mixture, Abblank was 80% MeOH and Abcontrol was the work solution mixed with 80% MeOH.

Hydrogen peroxide (H_2O_2) assay

The investigation of the H_2O_2 scavenging potential of *B. natalensis* was conducted following the method by Ruch et al (25) with some modifications. A phosphate buffer (pH 7.4) and 30% saturated H_2O_2 solution were used to prepare a 40 mM solution of H_2O_2. The MeOH extracts with volumes ranging between 10 to 50 µL were mixed with 0.6 ml of 40 mM H_2O_2 solution. The mixtures were incubated for 10 minutes, and the absorbance was measured at a wavelength of 230 nm. A blank solution was made with phosphate buffer without the 40 mM H_2O_2 solution. The following equation was used to determine the inhibition % of the extracts:

$$\%\ Scavenged\ H_2O_2 = [(A_c – A_s)/Ac] \times 100$$

where A_c represents the absorbance of the control (phosphate buffer mixed with 40 mM H_2O_2 solution, and A_s is the absorbance of the extracts.

Iron chelating assay

A modified method of the chelating power of *B. natalensis* extracts was conducted following the developed methodology by Dinis et al (26). Varying concentrations (10-50 µL) of the MeOH extracts were initially mixed with a 50 µL solution of 2 mM of ferric (II) chloride. A volume of 0.2 mL of 5 mM of ferrozine was added to initiate the reaction, which was thoroughly shaken and left to incubate at room temperature for 10 minutes. The absorbance was measured at 562 nm, and a blank solution of 80% MeOH was prepared. To determine the % inhibition of chelated iron, the following equation was used:

$$\% \ Chelation = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the control absorbance (ferric (II) chloride mixed with ferrozine) and A_1 is the extract absorbance.

Qualitative antibacterial activity assay

Test for pathogens

Two strains of bacterial pathogens, gram-positive *Staphylococcus aureus* (ATCC 25923) and gram-negative *Escherichia coli* (ATCC 25922) were cultured in two agar media, namely Baird-Parker (*S. aureus*) and Muller-Hinton (*E. coli*), respectively. Both test microorganisms and agar were purchased from Thermo-Fisher Laboratory Specialties (Pty) Ltd.

Investigation of antibacterial activity

The agar-well diffusion method developed by Jain et al (27) with slight modifications by Adeleye and Risenga (28) was adopted for this assay. The cultured agar plates with each respective pathogen were incubated for 24 hours at 37 ˚C. Once the successful growth of the pathogen was observed, 6 mm diameter wells were bored in the agar using a sterile borer. The respective methanolic extract and dimethyl sulfoxide (DMSO-negative control) (100 uL each) were added to the wells and the plates were refrigerated for one hour to allow for the diffusion of the extracts. Following this process, the plates were once again incubated in the same conditions as previously mentioned for another 24 hours. The zone of inhibition, which served as an indicator of antibacterial activity, was measured in millimetres (mm) using Vernier callipers.

Statistical analysis

R Studio® statistical software was used to perform one-way analysis of variance (ANOVA) and Tukey HSD post hoc tests to determine the overall significance across all plant parts (indicated by the $P < 0.05$) and where the difference lies amongst the plant parts (indicated by superscript letters). The quantitative phytochemical, *in vitro* antioxidant, and antibacterial activity analyses were replicated three times, and all values were represented in mean ± standard error (SE).

Results

Qualitative phytochemical analysis

Table 1 summarises the qualitative phytochemical analysis of the MeOH leaf extract of *B. natalensis*. The leaves showed a higher presence of tannins, phenolics, and coumarins than the underground stems and roots, followed by an average presence of glycosides. Flavonoids, steroids, and volatile oils were also present in the leaves. Only glycosides, saponins, terpenoids, coumarins, and phlobatannins were detected in the underground stem extract, and surprisingly the roots only showed the presence of tannins, terpenoids, steroids, saponins, and glycosides.

Total phenolic content

The results from the *B. natalensis* extracts showed that the underground stems contained the highest TPC of 1909.2 ± 4.8 mg GAE/g, whereas the roots followed with 1002.8 ± 24.7 mg GAE/g and then the leaves with 867 ± 64.5 mg GAE/g (Table 2). Although the ANOVA results indicated a significant difference among all plant parts ($P < 0.05$), Tukey's test revealed that only the underground stems had a significant difference in comparison to the leaves and roots.

Total flavonoid content

The underground stem extract of *B. natalensis* recorded a TFC of 259.7 ± 27.2 mg QE/g, followed by the roots with 238.3 ± 43.3 mg QE/g and then the leaves with 134.4 ± 21.4 mg QE/g. The ANOVA showed no significant difference in the TFC ($P > 0.05$) (Table 2).

Total tannin content

The leaves contained the greatest TTC (3003.3 ± 38.5 mg GAE/g; Table 2), with the underground stems trailing behind (2890 ± 53.1 mg GAE/g) and then the roots (1645 ± 34.5 mg GAE/g). Overall, a significant difference was reported across the plant parts ($P < 0.05$); however, only the roots showed a significant difference when compared

Table 1. Qualitative phytochemical analysis of MeOH leaf extract of *B. natalensis*

Phytochemical group	Leaves
Tannins	+++
Phenolics	+++
Flavonoids	+
Steroids	+
Terpenoids	-
Glycosides	++
Saponins	-
Coumarins	+++
Phlobatannins	-
Volatile oils	+

Present (+); average presence (++); high presence (+++); undetected (-).

Table 2. Quantitative phytochemical content of the MeOH extract of *Bulbine natalensis*

Plant part	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)	TPAC (mg CE/g)
Leaves	867^{a} ± 64.5	134.4^{a} ± 21.4	3003.3^{a} ± 38.5	362.3^{a} ± 9.5
Stems	1909.2^{b} ± 4.8	259.7^{a} ± 27.2	2890^{a} ± 53.1	858.3^{b} ± 1.7
Roots	1002.8^{a} ± 24.7	238.3^{a} ± 43.3	1645^{b} ± 34.5	373.3^{a} ± 1.7

GAE, gallic acid equivalent; QE, quercetin equivalent; CE, catechin equivalent; TPC, total phenolic content; TFC, total flavonoid content; TTC, total tannin content; TPAC, Total proanthocyanidin content.
The mean values of different superscript letters (a-b) in each column show a significant difference (Tukey's post hoc test, $P < 0.05$), whereas values with similar superscript letters (a) show no significant difference (Tukey's post hoc test, $P > 0.05$).

with the leaves and underground stems, based on Tukey's post hoc test.

Total proanthocyanidin content

The results from the total proanthocyanidin content complemented the trend in the concentration from the different plant parts as the TPC and TFC. The underground stems had a higher concentration of 858.3 ± 1.7 mg CE/g, followed by the roots (373.3 ± 1.7 mg CE/g) and the leaves (362.3 ± 9.5 mg CE/g; Table 2), respectively. Similar to the TPC, the underground stem was the only plant part to have a significant difference in comparison to the leaves and roots ($P < 0.05$).

In vitro antioxidant activity

As previously mentioned, the lower the IC_{50} value the stronger the radical quenching power of the extracts. The DPPH, H_2O_2, and iron chelating properties of *B. natalensis* MeOH extracts were investigated. The root extract of *B. natalensis* showed a lower IC_{50} value (0.36 ± 0.02 mg/mL) against the DPPH radical than the leaves (3.88 ± 0.16 mg/mL) and the underground stems (35.86 ± 0.29 mg/mL) with the latter exhibiting the weakest antioxidant activity (Table 3). *B. natalensis* scavenged the hydrogen peroxide radical much better than DPPH, which was evident in the IC_{50} values falling under 1 mg/mL indicating excellent antioxidant activity. The *B. natalensis* root extract had the lowest radical quenching activity with an IC_{50} value of 0.24 ± 0.04 mg/mL, while the leaves showed an IC_{50} concentration of 0.29 ± 0.05 mg/mL and a concentration of 0.61 ± 0.05 mg/mL for the underground stems. Once again, *B. natalensis* displayed an excellent scavenging potential in the iron chelating assay with the leaves, roots, and underground stems all having IC_{50} values less than 1 mg/mL (0.19 ± 0.001 mg/mL, 0.33 ± 0.01 mg/mL, and 0.89 ± 0.02 mg/mL, respectively). All three assays showed an overall significant difference in *B. natalensis* ($P < 0.05$).

Antibacterial activity

The antibacterial activities of various *B. natalensis* plant parts were tested against gram-positive *S. aureus* and gram-negative *E. coli*. The zones of inhibition were measured following a 24-hour incubation after the extracts were impregnated in the wells and the results were recorded in Table 4. *B. natalensis* extracts showed intermediate zones of inhibition with the exception of the leaves against *S. aureus*, which showed no activity. The leaves had the greatest inhibitory activity against *E. coli* at 18.33 ± 0.88 mm. All plant parts had a significant difference for each bacterial strain when compared to each other ($P < 0.05$).

Discussion

The presence of tannins, phenolics, and coumarins in *B. natalensis* leaves was revealed to be in excess compared to the species' subterranean counterparts. Tannins were proclaimed to possess a number of beneficial properties,

Table 3. Antioxidant activity (IC_{50}) of *Bulbine natalensis* MeOH extracts

Plant part	DPPH (mg/mL)	H_2O_2 (mg/mL)	Iron chelating (mg/mL)
Leaves	3.88^{b} ± 0.16	0.29^{a} ± 0.05	0.19^{c} ± 0.001
Stems	35.86^{a} ± 0.29	0.61^{b} ± 0.05	0.89^{a} ± 0.02
Roots	0.36^{c} ± 0.02	0.24^{a} ± 0.04	0.33^{b} ± 0.01

DPPH, 2,2-diphenyl-1-picrylhydrazyl; H_2O_2 Hydrogen peroxide.
Mean values in each column followed by different superscript letters (a-c) are significantly different (Tukey's post hoc test, $P<0.05$).

Table 4. Antibacterial activities of *Bulbine natalensis* MeOH extracts against *Staphylococcus aureus* and *Escherichia coli*

Plant part	*S. aureus*	*E. coli*
Leaves	No activity	18.33^{a} ± 0.88
Stems	15.33^{a} ± 0.67	15.33^{ab} ± 0.88
Roots	15.67^{b} ± 0.33	13.33^{b} ± 0.88

Mean values in each column followed by different superscript letters (a-b) are significantly different (Tukey's post-hoc test).

some of which include cancer therapy, antioxidant, antimicrobial, wound-healing, and antidiabetic properties (29). Tannins are also utilised in food service industries as food additives in wine, beer, fruit juice, and so on (29,30). Phenolics are one of the most well-known antioxidants and are attributed to plant anti-oxidative activities, which enable them to successfully act as reducing agents and hydrogen donors (31-33). Phenolics are good alternatives in the treatment of inflammatory diseases (34). Coumarins are known to possess anticancer, anti-inflammatory, antibacterial, and neuroprotective properties (35). Coumarins have also been reported to control the mitigation and attack of breast cancer cells (35). Similar to the findings from the phytochemical screening of *B. natalensis* underground stems of the present study, Yakubu and Afolayan (5) reported the absence of phenolics, flavonoids, and steroids in the aqueous extracts. Surprisingly, phlobatannins were not detected in the latter study; however, the phytochemical group was identified in this study. Another report on the phytochemical screening of the leaves by Lazarus (1) showed that terpenoids, phlobatannins, and steroids were not detected in the water, chloroform, hexane, and ethyl acetate extracts. However, in contrast to their findings, this study reported the presence of steroids in the methanolic leaf extracts. The reasons for the differences in the detection of compounds could be based on a number of factors. The use of various solvents and the method of extraction determine the presence or absence of phytochemicals, as a result of their varying solubility in different solvents (36,37). In addition, the method of preparing the plant material (drying method and drying time) prior to extraction can also influence the synthesis or degradation of compounds, as well as their bioactivity (38). Further research should be conducted on the phytochemical screening of *B. natalensis*, using various methods of plant preparation and extraction, as they could possibly produce different results.

The root and underground stems of *B. natalensis* showed a higher quantity of total phenolics, total flavonoids, and total proanthocyanidin content compared to the leaves. Flavonoids are greatly used in the healthcare industry, due to having potential anti-inflammatory, antioxidant, and anti-cancer properties (39). The present study is the first of its kind to report the presence of proanthocyanidins in all plant parts of this species. Proanthocyanidins have been reported to possess antioxidant, anti-diabetic, anti-platelet aggregating, and anticancer properties (40). Overall, the quantities of the phytochemical compounds in *B. natalensis* further validate the species' uses in traditional medicine and could open avenues for their potential applications in the pharmaceutical and cosmetic industries.

The development of cancer, diabetes, neurodegenerative, and cardiovascular diseases is the result of the accumulation of free radicals to which antioxidants prevent oxidative stress and the development of such illnesses and diseases (41-43). Lazarus (1) reported that the IC_{50} values of *B. natalensis* leaves were all greater than 5 mg/mL for hexane, ethyl acetate, and water extracts. Another study by Ghuman et al (10) showed that *B. natalensis* roots had a stronger antioxidant activity against nitric oxide (28.64 µg/mL) compared to the leaves (54.79 µg/mL). In the current study, the roots and leaves exhibited the lowest IC_{50} against DPPH compared to the leaves and underground stems. Similar results were recorded for the hydrogen peroxide assay, where the roots had the greatest scavenging activity, followed by the leaves and the underground stems. All plant parts exhibited excellent iron radical scavenging activity. These results validate the plant's use in traditional medicine. The results from this study further elucidate that the *B. natalensis* roots have overall excellent antioxidant activity and imply that the plant part might be beneficial to the pharmaceutical industry. The underground stems and leaves also showed good antioxidant activities indicating that the plant species can be useful in antioxidant product development.

The intermediate zones of inhibition imply that the plant parts, although having antibacterial activity, have an uncertain effect against the bacterial strains (44). The current results showed higher antibacterial activities in the underground plant parts against *S. aureus* and all three plant parts against *E. coli* with zones of inhibition between 13 and 19 mm as compared to the results reported by Mocktar (7). In contrast to the current results, a study by Mocktar (7) showed that the organic solvent extracts of *B. natalensis* did not inhibit *E. coli* bacterial growth. However, an intermediate inhibition was reported for the underground stems (10.5 mm) and roots (11 mm) against *S. aureus* from the organic extracts. Although *B. natalensis* displays sufficient antibacterial activity to inhibit and relieve skin infections in the traditional context, the species' application for the advancement of antibiotic medication would still need to be further analysed.

Conclusion

In the current study, the highest presence of tannins, phenolics, and coumarins was observed in the leaves, indicating that the leaves could have the greatest potential for application to industry, although the underground stems and roots had some potentials considering that the identified compounds in both plant parts can have applications to industry. The TPC, TFC, and TPAC results revealed that the underground stems had the highest concentration of phenolics, flavonoids, and proanthocyanidins, followed by the roots and the leaves. This could be the result of plant morphology functions, meaning that the underground organs store most of the nutrients and compounds. The roots showed excellent antioxidant activity against all three radicals, indicating their potential uses in antioxidant manufacturing in

pharmaceutical industries. Overall, *B. natalensis* does have good antioxidant activity validating its traditional uses. The intermediate zones of inhibition recorded from the antibacterial assay infer that *B. natalensis* has the potential to inhibit bacterial growth to some degree, and can also be introduced to pharmaceutical and cosmeceutical industries for the production of antibiotic products. Future recommendations would include the expansion of the phytochemical analysis of the species (both qualitative and quantitative) by adopting various plant preparation and extraction methods, as multiple factors can influence the phytochemical profile of medicinal plants. Quantitative antibacterial methods such as minimum inhibitory concentration should also be considered for further research on the antimicrobial potential of the plant species.

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Authors' contributions

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Writing-original draft: Thabiso Katlego Teffo.
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Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, and redundancy) have been completely observed by the authors.

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References

1. Lazarus GG. In Vitro Anti-Platelet Aggregation Activity of the Extracts of *Bulbine natalensis* [dissertation]. University of Zululand; 2012. p. 1-134.
2. Teffo TK, Dukhan S, Ramalepe P, Risenga I. Possible implications of climate change on the medicinal properties of *Bulbine* species with a particular focus on *Bulbine abyssinica*, *Bulbine frutescens* and *Bulbine natalensis* in South Africa. J Pharmacogn Phytochem. 2021;10(5):49-56. doi: 10.22271/phyto.2021.v10.i5a.14208.
3. Mosa RA, Lazarus GG, Gwala PE, Oyedeji AO, Opoku AR. In vitro anti-platelet aggregation, antioxidant and cytotoxic activity of extracts of some Zulu medicinal plants. J Nat Prod. 2011;4:136-46.
4. van Wyk BE, van Oudtshoorn B, Gericke N. Medicinal Plants of South Africa. Pretoria, South Africa: Briza Publications; 1997. p. 300.
5. Yakubu MT, Afolayan AJ. Effect of aqueous extract of *Bulbine natalensis* (Baker) stem on the sexual behaviour of male rats. Int J Androl. 2009;32(6):629-36. doi: 10.1111/j.1365-2605.2008.00910.x.
6. Erasto P, Adebola PO, Grierson DS, Afolayan AJ. An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape province, South Africa. Afr J Biotechnol. 2005;4(12):1458-60.
7. Mocktar C. Antimicrobial and Chemical Analyses of Selected *Bulbine* Species [dissertation]. University of KwaZulu-Natal; 2000. p. 1-118.
8. Pather N, Viljoen AM, Kramer B. A biochemical comparison of the in vivo effects of *Bulbine frutescens* and *Bulbine natalensis* on cutaneous wound healing. J Ethnopharmacol. 2011;133(2):364-70. doi: 10.1016/j.jep.2010.10.007.
9. Kandari LS. Effect of vermicompost leachate on biomass and antibacterial properties of five bulbous medicinal plants: a case study from KwaZulu Natal, South Africa. Iran J Sci Technol Trans A Sci. 2018;42(3):1049-56. doi: 10.1007/s40995-016-0106-8.
10. Ghuman S, Ncube B, Finnie JF, McGaw LJ, Mfotie Njoya E, Coopoosamy RM, et al. Antioxidant, anti-inflammatory and wound healing properties of medicinal plant extracts used to treat wounds and dermatological disorders. S Afr J Bot. 2019;126:232-40. doi: 10.1016/j.sajb.2019.07.013.
11. Bodede O, Prinsloo G. Ethnobotany, phytochemistry and pharmacological significance of the genus *Bulbine* (Asphodelaceae). J Ethnopharmacol. 2020;260:112986. doi: 10.1016/j.jep.2020.112986.
12. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem. 2003;51(8):2144-55. doi: 10.1021/jf020444+.
13. Pakade V, Cukrowska E, Chimuka L. Comparison of antioxidant activity of *Moringa oleifera* and selected

vegetables in South Africa. S Afr J Sci. 2013;109(3):1-5. doi: doi:10.10520/EJC133141.
14. Roghini R, Vijayalakshmi K. Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. Int J Pharm Sci Res. 2018;9(11):4859-64. doi: 10.13040/ijpsr.0975-8232.9(11).4859-64.
15. Prabhavathi RM, Prasad MP, Jayaramu M. Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. Adv Appl Sci Res. 2016;7(4):11-7.
16. Tepal P. Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of *Musa paradisiaca*. Malays J Anal Sci. 2016;20(5):1181-90. doi: 10.17576/mjas-2016-2005-25.
17. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3(12):10-4.
18. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. ScientificWorldJournal. 2017;2017:5873648. doi: 10.1155/2017/5873648.
19. Ayoade ET, Akinyemi AO, Oyelere FS. Phytochemical profile of different morphological organs of *Moringa oleifera* plant. J Phytopharmacol. 2019;8(6):295-8.
20. Iqbal S, Bhanger MI. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J Food Compost Anal. 2006;19(6-7):544-51. doi: 10.1016/j.jfca.2005.05.001.
21. Lahare RP, Yadav HS, Bisen YK, Dashahre AK. Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus* from Durg district, Chhattisgarh, India. Sch Bull. 2021;7(1):1-6.
22. Oyedemi SO, Bradley G, Afolayan AJ. In-vitro and-vivo antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol. 2010;4(2):70-8.
23. Martinez-Morales F, Alonso-Castro AJ, Zapata-Morales JR, Carranza-Álvarez C, Aragon-Martinez OH. Use of standardized units for a correct interpretation of IC50 values obtained from the inhibition of the DPPH radical by natural antioxidants. Chem Zvesti. 2020;74(10):3325-34. doi: 10.1007/s11696-020-01161-x.
24. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi: 10.1016/s0023-6438(95)80008-5.
25. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-8. doi: 10.1093/carcin/10.6.1003.
26. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994;315(1):161-9. doi: 10.1006/abbi.1994.1485.
27. Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N. Comparative evaluation of antibacterial efficacy of six indian plant extracts against *Streptococcus mutans*. J Clin Diagn Res. 2015;9(2):ZC50-3. doi: 10.7860/jcdr/2015/11526.5599.
28. Adeleye OC, Risenga IM. Screening of phytochemical profile and biological activities in the leaves, stems and roots of South African *Portulacaria afra* using four extraction solvents. Biomed Pharmacol J. 2022;15(3):1561-72. doi: 10.13005/bpj/2494.
29. Pizzi A. Tannins medical/pharmacological and related applications: a critical review. Sustain Chem Pharm. 2021;22:100481. doi: 10.1016/j.scp.2021.100481.
30. Ricci A, Parpinello GP, Palma AS, Teslić N, Brilli C, Pizzi A, et al. Analytical profiling of food-grade extracts from grape (*Vitis vinifera* sp.) seeds and skins, green tea (*Camellia sinensis*) leaves and Limousin oak (*Quercus robur*) heartwood using MALDI-TOF-MS, ICP-MS and spectrophotometric methods. J Food Compost Anal. 2017;59:95-104. doi: 10.1016/j.jfca.2017.01.014.
31. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-56. doi: 10.1016/0891-5849(95)02227-9.
32. Galleano M, Verstraeten SV, Oteiza PI, Fraga CG. Antioxidant actions of flavonoids: thermodynamic and kinetic analysis. Arch Biochem Biophys. 2010;501(1):23-30. doi: 10.1016/j.abb.2010.04.005.
33. Gharekhani M, Ghorbani M, Rasoulnejad N. Microwave-assisted extraction of phenolic and flavonoid compounds from *Eucalyptus camaldulensis* Dehn leaves as compared with ultrasound-assisted extraction. Lat Am Appl Res. 2012;42(3):305-10.
34. Bhuyan DJ, Basu A. Phenolic compounds potential health benefits and toxicity. In: Utilisation of Bioactive Compounds from Agricultural and Food Production Waste. Boca Raton, FL: CRC Press; 2017. p. 27-59.
35. Ambriz-Pérez DL, Leyva-López N, Gutierrez-Grijalva EP, Heredia JB. Phenolic compounds: natural alternative in inflammation treatment. A review. Cogent Food Agric. 2016;2(1):1131412. doi: 10.1080/23311932.2015.1131412.
36. Sharifi-Rad J, Cruz-Martins N, López-Jornet P, Lopez EP, Harun N, Yeskaliyeva B, et al. Natural coumarins: exploring the pharmacological complexity and underlying molecular mechanisms. Oxid Med Cell Longev. 2021;2021:6492346. doi: 10.1155/2021/6492346.
37. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. J Food Qual. 2019;2019:8178294. doi: 10.1155/2019/8178294.
38. Teffo TK, Dukhan S, Ramalepe P, Risenga I. Comparative phytochemical analysis, antioxidant and antibacterial activities in the leaves, underground stems and roots of *Bulbine abyssinica*. Biomed Pharmacol J. 2022;15(3):1323-35. doi: 10.13005/bpj/2469.
39. Papoutsis K, Pristijono P, Golding JB, Stathopoulos CE, Bowyer MC, Scarlett CJ, et al. Effect of vacuum-drying, hot air-drying and freeze-drying on polyphenols and antioxidant capacity of lemon (*Citrus limon*) pomace aqueous extracts. Int J Food Sci Technol. 2017;52(4):880-7. doi: 10.1111/ijfs.13351.
40. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41.
41. Beecher GR. Proanthocyanidins: biological activities associated with human health. Pharm Biol. 2004;42(Suppl 1):2-20. doi: 10.3109/13880200490893474.

42. Anderson FA Jr, Spencer FA. Risk factors for venous thromboembolism. Circulation. 2003;107(23 Suppl 1):I9-16. doi: 10.1161/01.cir.0000078469.07362.e6.
43. Houston MC. The role of nutrition, nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. Altern Ther Health Med. 2013;19 Suppl 1:32-49.
44. Feghhi-Najafabadi S, Safaeian L, Zolfaghari B. In vitro antioxidant effects of different extracts obtained from the leaves and seeds of *Allium ampeloprasum* subsp. *persicum*. J Herbmed Pharmacol. 2019;8(3):256-60. doi: 10.15171/jhp.2019.37.
45. Rodloff A, Bauer T, Ewig S, Kujath P, Müller E. Susceptible, intermediate, and resistant - the intensity of antibiotic action. Dtsch Arztebl Int. 2008;105(39):657-62. doi: 10.3238/arztebl.2008.0657.