

Phytochemical Profile and Bioactivity of the Methanolic Leaf and Root Extracts of South African *Bulbine frutescens*

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ABSTRACT

Bulbine frutescens is an indigenous succulent medicinal plant in South Africa. The plant is commonly used traditionally for the treatment of skin related ailments. *B. frutescens* has been reported to possess antioxidant, anti-viral and antimicrobial properties. To date, most studies have been conducted on the leaves, while limited reports have been documented on the roots, as well as comparative analyses between both plant parts. The aim of this study was to determine the phytochemical content, antioxidant and antibacterial activities in the leaves and roots of *B. frutescens*. Methanolic extracts of each plant part were assessed on the phytochemical analysis, antioxidant (DPPH, hydrogen peroxide, metal chelating) and antibacterial activity against *S. aureus* and *E. coli*. The results from the phytochemical screening showed that the leaves contained more phytochemical groups than the roots. The roots displayed higher total phenolics (991.7 ± 54.1 mg/g GAE), total flavonoid (285.8 ± 60.6 mg/g QE) and total tannin contents (1698.75 ± 28.15 mg/g GAE) than the leaves. The roots also showed a stronger antioxidant activity in DPPH (3.43 ± 0.16 mg/ml) and metal chelating (0.54 ± 0.01 mg/ml) assays, compared to the leaves, which only showed a higher scavenging power against hydrogen peroxide radicals (1.48 ± 0.02 mg/ml) than the roots. Both plant parts showed intermediate zones of inhibition (10-19 mm) against both bacterial strains, with the exception of the leaves which had a 20.67 ± 0.67 mm inhibition against *S. aureus*. These results validate the uses of both leaves and roots of *B. frutescens* in traditional medicine and further suggests for the application of the roots in pharmaceutical and cosmetic industries.

Keywords: *Bulbine frutescens*, phytochemical, antioxidant, antibacterial, bioactivity.

INTRODUCTION

Bulbine frutescens is described as a subshrub with subterranean roots, and onion-like resemblance of green leaves which either grow erect or slightly erect inflorescences (Baijnath, 1987; Mocktar, 2000; Teffo *et al.*, 2021). The species is commonly dispersed in the Eastern Cape Province but can also be found in the western regions of South Africa (Baijnath, 1987; Mocktar, 2000; Teffo *et al.*, 2021). *B. frutescens* leaves are often used to treat acne, burns, cold sores, insect bites and ringworms and are also known to be a good anti-coagulant (Coopoosamy and Naidoo, 2012; Teffo *et al.*, 2021). The roots of the species are traditionally used to treat blood disorders,

convulsions, diabetes, nausea, rheumatism and venereal diseases (Watt *et al.*, 1962; Van Wyk *et al.*, 1997, 2009; Felhaber, 1997; Van Wyk *et al.*, 2000; Rood, 2008, Van Wyk, 2011). *B. frutescens* is one of the few *Bulbine* species that is used traditionally by indigenous people for the management of diabetes related illnesses in South Africa (Erasto *et al.*, 2005). *B. frutescens* leaves have been studied for a range of pharmacological activities, and has shown antioxidant, anti-viral, antimicrobial, anti-inflammatory and anti-cancer properties (Ghuman *et al.*, 2019; Shikalepo *et al.*, 2018; Mocktar, 2000; Rachuonyo *et al.*, 2016; Opinde *et al.*, 2016; Kushwaha *et al.*, 2019). However, very little is known on the phytochemical and biological

activities in the roots of the species, apart from the antimalarial, anti-trypanosomal and cytotoxic assessments of several isolated xanthone, knipholone and phenylanthraquinone compounds (Muyanatta *et al.*, 2005; Bringmann *et al.*, 2008; Abdissa *et al.*, 2014). In addition, a comparative analysis of the phytochemical and biological activities of both plant parts have yet to be reported. Thus the aim of this study was to determine the qualitative and quantitative phytochemical content, antioxidant and antibacterial activities in both leaves and roots of *B. frutescens* using standard procedures.

MATERIALS AND METHODS

Plant material collection and preparation

B. frutescens potted plants (n=4) were collected from the Wild Flower Nursery in the North West Province, South Africa, then maintained in the rooftop greenhouse where the plants were watered (200 ml each pot) every second day, at the University of the Witwatersrand, Johannesburg, South Africa until they fully matured. Once the plants had fully acclimatised, the leaves and roots were harvested and rinsed thoroughly with distilled water prior to laboratory analysis. The plant material was then dried using a hot air-drier (40 °C) and crushed into fine powder with an electric grinder.

Sample extraction

The extraction of phytochemical compounds was adopted from the method described by Siddhuraju *et al.* (2003) and Pakade *et al.* (2013). To produce extracts, three grams of the powdered leaf and root samples were mixed with 25 millilitres (ml) of 80% methanol. The extracts were then sonicated at 50 °C in a water bath for a period of 25 minutes. A volume of 20 ml of 80% acetone was added in the water bath. The extracts were then filtered using Whatman® qualitative filter paper, and the supernatant of all the extracts was collected. This method was repeated for re-extraction and fusion of the supernatants. Upon the completion of the extraction process, the extracts were stored in the refrigerator awaiting for phytochemical, antioxidant and antibacterial analyses.

Preliminary phytochemical screening

Test for Tannins (FeCl₃ test)

To test for the presence of tannins, 2 ml of the plant extract was mixed with 2 ml of distilled water and another 2 ml of 5% ferric chloride. A

green-blue colour indicated the presence of tannins (Roghini and Vijayalakshmi, 2018).

FeCl₃ Test for Phenolics

One millilitre of crude plant extract was placed into a test tube and mixed with a few drops of 10% ferric chloride. The appearance of a green blue or violet colour was an indication of phenolic compounds (Prabhavathi *et al.*, 2016).

Test for Flavonoids (HCl test)

A volume of 0.5 ml of crude plant extract was mixed with five drops of concentrated hydrochloric acid (HCl). The development of a red colour indicated the presence of flavonoids (Tepal, 2016).

Test for Steroids

Two millilitres of the crude plant extract was placed in a test tube and mixed with 2 ml of chloroform. A further 2 ml of concentrated H₂SO₄ was added to the test tube. A reddish-brown ring at the junction indicates the presence of steroids (Yadav *et al.*, 2011).

Test for Terpenoids

One millilitre of the crude plant extract was mixed with 0.5 ml of chloroform. Then, a few drops of concentrated H₂SO₄ acid was added to the mixture. The formation of a reddish-brown precipitate indicated presence of terpenoids (Prabhavathi *et al.*, 2016).

Salkowski's Test for Glycosides

For this method, 0.5 ml of crude plant extract was placed in a test tube and mixed with 2 ml of H₂SO₄. The presence of a reddish-brown colour forming indicated the presence of the steroidal aglycone part of the glycoside (Gul *et al.*, 2017).

Froth test for Saponins

A volume of 0.5 ml of Crude plant extract is placed in a test tube with five ml of distilled water. The test tube is shaken vigorously. Three drops of olive oil were placed inside the test tube and shaken vigorously. The presence of a stable foam was an indication of saponins (Gul *et al.*, 2017).

Test for Coumarins

One millilitre of crude plant extract was placed in a test tube and mixed with 1 ml of 10% sodium hydroxide. The formation of a yellow colour indicated the presence of coumarins (Roghini and Vijayalakshmi, 2018).

Test for Phlobatannins

One millilitre of crude plant extract was mixed with a few drops of 2% HCl. The appearance of red precipitate indicated the presence of phlobatannins (Roghini and Vijayalakshmi, 2018).

Test for Volatile Oils

One millimetre of crude plant extract was placed in a test tube and mixed with 0.2 ml of 1%

w/v sodium hydroxide. The presence of a precipitate indicated presence of volatile oils (Ayoade *et al.*, 2019).

Quantitative phytochemical analysis

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent assay was used for the approximation of TPC using the methanol extracts of *B. frutescens* leaves and roots. A solution of 750 μ l:10 Folin-Ciocalteu reagent and 2.5 mL of 7.5% aqueous sodium carbonate was added to methanol extracts of *B. frutescens* (200 μ L) which reacted in an Erlenmeyer flask closed at the opening with a rubber stopper (Pakade *et al.*, 2013). The mixture was diluted with 7 ml of distilled water, followed by placing the mixture in a dark compartment for two hours at ambient temperature. A spectrophotometer was used to determine the absorbance of the phenolic content in the extracts at a wavelength of 765 nm (Iqbal *et al.*, 2006). A linear equation inherited from the calibration curve formulated by using Gallic acid working stock solution: $y=0.0495 - 0.0259$, $r^2=0.9994$, was used to calculate the TPC from the methanol extracts.

Determination of Total Flavonoid Content (TFC)

The existing methodology developed by Siddhuraju *et al* (2003) that was slightly modified by Pakade *et al* (2013) was adopted for the approximation of TFC. A measurement of 0.3 ml of extracts was diluted with 4 ml of deionised water using a 10 mL volumetric flask. A volume of 0.3 ml of sodium nitrate (NaNO_3) was added and thoroughly mixed, followed by 3 ml of aluminium chloride (AlCl_3) solution after 5 minutes. Six minutes later, 2 ml of 1 M sodium hydroxide solution was placed inside the volumetric flask. Following the addition of the chemicals in sequential order, the reaction was made up to 10 ml, with deionised water, and stirred for 15 minutes. A UV-visible spectrophotometer was used to measure the TFC of the extract mixture at a wavelength of 510 nm (Iqbal *et al.*, 2006). A linear equation inherited from the calibration curve formulated using quercetin standards: $y= 0.2388 - 0.0019$, $r^2 = 0.9997$, was used to calculate the TFC from different methanolic extractions.

Determination of Total Tannin Content (TTC)

The method modified by Lahare *et al* (2021) was used to determine the concentration of total tannins in the methanolic extracts of *B. frutescens*.

Aliquots of 0.1 ml of the extracts were dissolved in 7.5 ml of distilled water, followed by the addition of 0.5 ml of Folin-Ciocalteu reagent. A volume of 1 ml of 35% sodium carbonate was added into the mixtures, and then diluted to 10 ml using distilled water. The mixtures were shaken thoroughly, and incubated at room temperature for 30 minutes. The absorbance of the mixtures and gallic acid (standard) were recorded at a wavelength of 725 nm using a spectrophotometer. A linear equation obtained from the gallic acid curve was used to determine the concentration of total tannins: $y= 0.046x - 0.0264$, $r^2 = 0.9833$.

Determination of Total Proanthocyanidin Content (TPAC)

The total proanthocyanidin content was determined using the method by Oyedemi *et al* (2010). A volume of 0.5 ml of the methanolic extracts of *B. frutescens* were mixed with 3 ml of 4% vanillin-methanol solution, followed by 1.5 ml of HCl. The mixtures were vortexed and incubated for 15 minutes at room temperature prior to measuring the absorbance at a wavelength of 500 nm. The linear equation from the catechin calibration curve was used to determine the concentration of total proanthocyanidins: $y= 0.9554x + 0.0003$, $r^2 = 0.9927$.

In-Vitro Antioxidant activity

DPPH scavenging activity assay

A 2, 2 diphenylpicrylhydrazyl (DPPH) free radical assay developed by Brand-Williams *et al* (1995) was followed with some modifications. In this method, the methanolic extracts of *B. frutescens* were used to determine the scavenging potential of DPPH. Fifty milligrams (mg) of DPPH was added to 100 ml of 80% methanol to prepare the stock solution. The stock solution was then diluted with 80% methanol where the dilution mixture had a ratio of 1:5 (stock solution: 80% methanol). A reaction mixture was made containing 10, 20, 30, 40 and 50 μ L of the extracts and 700 μ L of the work solution which was filled with 80% methanol to make up a volume of 1 ml. The mixture was then stored in the dark for 45 minutes, followed by the measurement of the absorbance at a wavelength of 517 nm using a spectrophotometer. A blank solution was made using 80% methanol and the control solution was prepared by mixing the work solution and 80% methanol. The inhibition (%) was determined using the following equation:

$$\text{Scavenging (\%)} = \frac{\text{Ab sample} - \text{Ab blank}}{\text{Ab control}} \times 100$$

Hydrogen peroxide scavenging assay

The potential of *B. frutescens* to scavenge hydrogen peroxide was determined using the method by Ruch *et al* (1989). The 40mM solution of hydrogen peroxide was prepared by adding phosphate buffer (pH 7.4) and 30% aqueous hydrogen peroxide solution. The methanolic leaf, and root extracts of *B. frutescens* (10, 20, 30, 40 and 50 μ L) were added to the hydrogen peroxide solution (600 μ L, 40mM) and then the absorbance of each solution was recorded following a 10 minute incubation at a wavelength of 230 nm using a spectrophotometer. The blank solution used contained the phosphate buffer without hydrogen peroxide and the positive control contained the 40mM hydrogen peroxide solution. The scavenging activity of the extracts were calculated using the following equation:

$$\% \text{ scavenged H}_2\text{O}_2 = \frac{(A_c - A_s)}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the extract mixed with 40mM hydrogen peroxide.

Metal chelating assay

The method by Dinis *et al* (1994) was modified and used to assess the chelate scavenging power of the methanolic leaf and roots of *B. frutescens*. Varying concentrations (10, 20, 30, 40, 50 μ L) of the extracts were added to a solution of 2 mM ferric (II) chloride (50 μ L). A reaction was initiated by the addition of 200 μ L of ferrozine (5 mM). The mixture was vigorously shaken and then left to stand at room temperature for 10 minutes. The absorbance of the solutions was measured spectrophotometrically at 562 nm, with the blank solution being 80% methanol and the positive control being the mixed ferric chloride and ferrozine solutions without the extracts. The percentage chelating effect of the extracts were calculated using the following equation:

$$\% \text{ chelating effect} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample of the extracts.

Qualitative antibacterial analysis**Test organisms**

Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922) were obtained from Thermo-Fisher Laboratory specialties (Pty) Ltd, Johannesburg, South Africa. The microorganisms were sub-

cultured in two media, namely Muller-Hinton (*E. coli*) and Baird-Parker (*S. aureus*) agar respectively (agar was procured from Thermo-Fisher Laboratory specialties (Pty) Ltd).

Screening of antibacterial activity

The agar well diffusion method formulated by Okeke *et al* (2001) and adapted by Jain *et al* (2015) with slight modifications by Adeleye and Risenga (2022) was used in this study. Bacterial plates containing *S. aureus* and *E. coli* were incubated at 37°C for 24h. Following the growth of the bacteria on the agar plates, six millimetre diameter wells were bored on the plates using a sterile borer. A 100 μ L volume of the crude extracts were impregnated into the wells. In order to achieve efficient diffusion of the extracts, the plates were allowed to stand for one hour in the fridge before transferring them into the incubator for another 24h at 37°C. A zone of inhibition, which indicates the potency of the extract against the bacterial strains, was measured in millimetres (mm). Dimethyl sulfoxide (DMSO) was used as a negative control.

Statistical analysis

The collected data from this experiment was analysed by one-way analysis of variance (ANOVA) and Tukey post-hoc test using R Studio. All numerical values for the quantitative phytochemical analysis, antioxidant and antibacterial activities were expressed in mean \pm standard error (SE). All of the abovementioned parameters were repeated three times ($n = 3$) for accuracy purposes.

RESULTS AND DISCUSSION**Phytochemical screening**

The results obtained from the phytochemical screening of the leaves and roots of *B. frutescens* show the presence of all the tested phytochemical groups overall (Table I). The leaves displayed most of the compounds identified, with stronger presence of phenolics and coumarins followed by a moderate presence of tannins, glycosides and volatile oils. Terpenoids, steroids and phlobatannins were not detected in the leaves. The root extract had slightly less phytochemicals identified than the leaves, with tannins, steroids, terpenoids, saponins, coumarins and phlobatannins being present. It is interesting to note that the presence of coumarins in the roots was as high as in the leaves. The presence of phytochemical compounds in the leaves and roots of *B. frutescens* attribute to the plant's medicinal properties (Asres *et al.*, 2005; Casano *et al.*, 2010;

Table I. Phytochemical screening of the leaves and roots of *B. frutescens*.

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

(+): low presence; (++): moderate presence; (+++): high presence; (-): absence

Table II. Quantitative phytochemical analysis of the leaves and roots of *B. frutescens*.

Plant part	TPC (mg/g GAE)	TFC (mg/g QE)	TTC (mg/g GAE)	TPAC (mg/g CE)
Leaves	831.4 ^a ± 53.4	215.0 ^a ± 12.9	1027.5 ^b ± 15.61	645.83 ^a ± 2.68
Roots	991.7 ^a ± 54.1	285.8 ^a ± 60.6	1696.75 ^a ± 28.15	296.94 ^b ± 4.73

Mean values of the same superscript letter in each column (a) show no significant difference (Tukey's post-hoc test; $p > 0.05$), whereas mean values with different superscript letters in each column (a-b) show a significant difference (Tukey post-hoc test; $p < 0.05$).

Pasetto *et al.*, 2014; Rashed *et al.*, 2012; Vlietinick *et al.*, 1998; Shikalepo *et al.*, 2018). So far, only three compounds have been qualitatively identified in *B. frutescens* leaves namely, tannins, phenolics and flavonoids (Shikalepo *et al.*, 2018). The current study has revealed that *B. frutescens* contains more phytochemical compounds such as steroids, terpenoids, glycosides, saponins, coumarins, phlobatannins and volatile oils. Although most of these compounds have been identified in the leaves, the roots also have a substantial presence of the compounds. To date, the phytochemical screening of the roots have not been reported, hence this study shows and validates the uses of *B. frutescens* roots for the treatment of various diseases and illnesses. This further illustrates the usefulness of this plant species, especially to the pharmaceutical and cosmetic industries since each of the identified compounds possess specific medicinal properties. Out of all the compounds identified in both plant parts of *B. frutescens*, coumarins were shown to have a higher presence in both leaves and roots. Coumarins are recognized for having multiple pharmacological activities, some of which include anti-inflammatory, anticoagulant, antimicrobial, antiviral, anticancer, antihypertensive, and anti-hyperglycaemic

properties (Al-Warhi *et al.*, 2020; Sharifi-Rad *et al.*, 2021). This further shows that *B. frutescens* could have greater applications to industry.

Quantitative phytochemical analysis

Total phenolic content (TFC)

The highest TPC was found in the root extract (991.7 ± 54.1 mg/g GAE) whereas the leaves presented a TPC of 831.4 ± 53.4 mg GAE/g (Table II). No significant difference was noted between the TPC of the leaves and roots ($p > 0.05$). Phenolic compounds are mostly known for their antioxidant properties, however, they also possess other health-promoting properties, ranging from anti-inflammatory to tumour-inhibiting activities, to name a few (Heijnen *et al.*, 2001; Chun *et al.*, 2003; Harbone and Williams, 2000; Chen, 2004; Ghasemzadeh *et al.*, 2010). The roots of *B. frutescens* having a higher TPC in this study opens more possibilities for further research on the plant part in assessing its medicinal properties, with the aim to hopefully introduce them into industrial product development.

Total flavonoid content (TFC)

The results from the TFC showed a similar pattern to TPC, with the roots having a higher content (285.8 ± 60.6 mg/g QE) than the leaves

(215 ± 12.9 mg/g QE) (Table II). Similar to the TPC, no significant difference was reported for TFC between both plant parts ($p > 0.05$). Flavonoids being a subgroup of phenolic compounds, have been reported to have antimicrobial, anti-inflammatory and antioxidant activities, to which the latter is most sought after in flavonoid research (Sulaiman and Balachandran, 2012).

Total tannin content (TTC)

The roots of *B. frutescens* showed to have a significantly higher TTC (1698.75 ± 28.15 mg/g GAE) in comparison to the leaves (1027.5 ± 15.61 mg/g GAE) ($p < 0.05$) (Table II). Tannins are very important compounds, as their medicinal applications range from antidiabetic, antioxidant and antidiarrhoeic activities (Pizzi, 2021; Teffo *et al.*, 2022). The higher concentration of tannins in *B. frutescens* roots validates the plant part in its uses in traditional medicine, and its application to industry could potentially lead to the progression of medical and pharmaceutical sciences.

Total proanthocyanidin content (TPAC)

The TPAC in the leaves were higher than in the roots (645.83 ± 2.68 mg/g CE and 296.94 ± 4.73 mg/g CE respectively) (Table II). A significant difference was reported when comparing the two plant parts ($p < 0.05$). This is the first study to report on the content of proanthocyanidins in both plant parts of *B. frutescens*. Proanthocyanidins have a multitude of medicinal properties, which proposes for further research of these compounds in medicinal plants. Ultraviolet protection, antibacterial, antiviral, anti-allergic and anti-coagulant activities are some of the beneficial properties that proanthocyanidins possess (Shi *et al.*, 2003; Rauf *et al.*, 2019; Fine, 2000; Teffo *et al.*, 2022). Although further quantitative phytochemical analyses is recommended for *B. frutescens*, especially with regards to the leaves, there have been several advancements in the isolation of bioactive compounds from *B. frutescens* roots, such as the sulphated phenylanthraquinones sodium ent-knipholone 6'-O-sulphate, sodium 4'-O-demethylknipholone-4'- β -D-glucopyranoside 6'-O-sulphate, sodium 4'-O-demethylknipholone 6'-O-sulphate and sodium isoknipholone 6'-O-sulphate (Muyanatta *et al.*, 2005), Joziknipholone A and B (Bringmann *et al.*, 2008) and xanthone (8-hydroxy-6-methylxanthone-1-carboxylic acid) and phenylanthraquinone (6',8-O-dimethylknipholone) (Abdissa *et al.*, 2014). These compounds have already been assessed on their antimalarial, anti-trypanosomal and cytotoxic

activities. This presents the opportunity to conduct more advanced quantitative phytochemical research with the aim to assess these useful compounds on a variety of pharmacological assessments, including antioxidant and antibacterial activities, to have a better insight on the potential of isolated compounds and how they contribute to the species overall medicinal properties.

In-vitro antioxidant activity

The leaf and root extracts of *B. frutescens* were assessed and compared on their antioxidant activity against the DPPH, hydrogen peroxide and metal chelating radicals. The root extract of *B. frutescens* showed a slightly higher scavenging power against DPPH radical than the leaf extract (IC₅₀ values of root and leaf extract were 3.43 ± 0.16 and 4.55 ± 0.21 mg/ml respectively) (Figure 1-A). The results from the hydrogen peroxide assay showed that the leaves had the lowest IC₅₀ value of 1.48 ± 0.22 mg/ml, when compared against the roots (2.05 ± 0.21 mg/ml), indicating a greater scavenging activity (Figure 1-B). No significant difference was reported between both plant parts against hydrogen peroxide ($p > 0.05$). The root extract exhibited the greatest scavenging activity in the metal chelating assay, with a recorded IC₅₀ value of 0.54 ± 0.01 mg/ml, whereas the leaf extract had a slightly higher value of 1.35 ± 0.09 mg/ml (Figure 1-C). Of the three antioxidant assays performed, only the DPPH and metal chelating assays showed significant differences between the leaves and roots of *B. frutescens* ($p < 0.05$). Several authors have reported on the antioxidant potential of *B. frutescens* leaves (Shikalepo *et al.*, 2018; Ghuman *et al.*, 2019). Although limited studies have shown that the roots have a good antioxidant activity, the results from the current study validates its uses in traditional medicine as well as its possible applications in various industries, such as cosmetics, food and beverage, and pharmaceuticals. While studies such as the one by Ghuman *et al.* (2019) show that leaves have a higher antioxidant activity against nitric oxide radicals compared to the roots (26.32 and 42.51 μ g/ml respectively), the current study showed that the roots possessed a stronger scavenging power against DPPH and metal chelating radicals than the leaves. Oxidative radicals are known to cause several illnesses and diseases such as cardiovascular, neurodegenerative diseases such as Alzheimer's disease, aging and autoimmune diseases (Pham-Huy *et al.*, 2008).

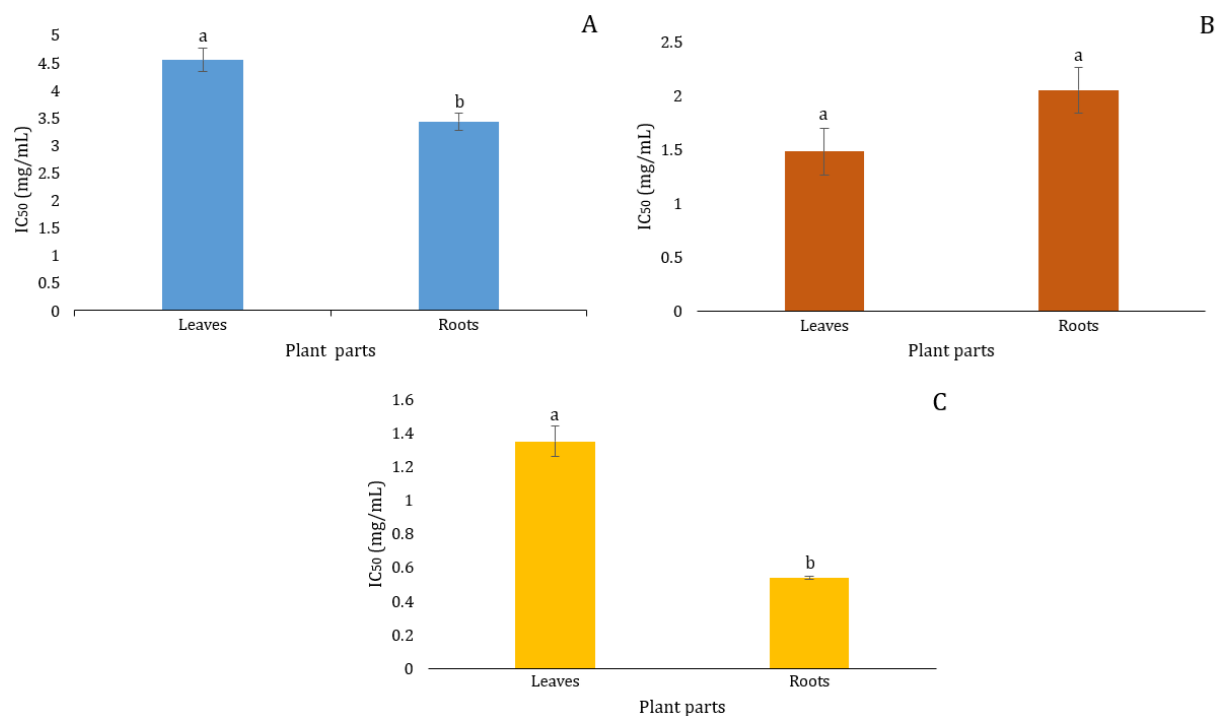


Figure 1. *In-vitro* antioxidant activity in the leaves and roots of *B. frutescens*: DPPH (A), hydrogen peroxide (B), and metal chelating (C). Mean values of the same letter (a) show no significant difference (Tukey’s test; $p > 0.05$), whereas mean values of different letters (a-b) show a significant difference (Tukey’s test; $p < 0.05$).

Table III. Antibacterial activity of the leaves and roots of *B. frutescens* against Gram-positive *S. aureus* and Gram-negative *E. coli*.

Plant part	<i>S. aureus</i>	<i>E. coli</i>
Leaves	20.67 ^a ± 0.67	13.33 ^b ± 0.33
Roots	14.33 ^b ± 0.33	15.67 ^a ± 0.67

Mean values of different superscript letters in each column (a-b) show a significant difference (Tukey’s test; $p < 0.05$).

Antibacterial activity

The zones of inhibition observed from the leaf and root methanolic extracts of *B. frutescens* against Gram-positive *S. aureus* and Gram-negative *E. coli* (Table III). The leaves had the greatest zone of inhibition against *S. aureus*, at 20.67 ± 0.67 mm, demonstrating that the bacterial strain is susceptible to the extract. The roots showed an intermediate zone of inhibition of 14.33 ± 0.33 mm. For *E. coli*, the roots displayed a greater inhibition against the strain at 15.67 ± 0.67 mm when compared to the leaves (13.33 ± 0.33 mm). A significant difference was observed for both bacterial strains ($p < 0.05$). The results from this study presented greater zones of inhibition compared to the study by Mocktar (2000). All results from this study presented greater inhibition

with respect to *S. aureus* and *E. coli*, with leaves having a 20.67 mm zone of inhibition and the roots (14.33 mm) against *S. aureus* compared to 8.5 mm (leaves- cold water extract), 7.5 mm (leaves- hot water extract) and 11.5 mm (roots- organic extract) from the study by Mocktar (2000). Contrasting results were observed for the current study against *E. coli*, with leaves showing a 13.33 mm inhibition, while the roots had 15.67 mm, compared to Mocktar (2000), where the leaves had a zone of inhibition of 8.5 mm (cold water extract) and 8.0 mm (hot water extract), whereas the roots exhibited 9.5 mm (aqueous extract). The variation between these results could obviously stem from the preparation of the sample and extract. The preparation of the plant samples prior to the extraction process is one of the factors that change

the bioactivity of plants (Yuan *et al.*, 2015). Additionally, solvents possess various polarities, which alter the phytochemical yield and medicinal properties (Truong *et al.*, 2019; Teffo *et al.*, 2022). The intermediate zones of inhibition values obtained from the current study against all bacterial strains, with the exception of *B. frutescens* leaves against *S. aureus*, indicates that the sensitivity of the bacterial strains to the plant could result in uncertain therapeutic effects (Rodloff *et al.*, 2008). This means that the leaves and roots of *B. frutescens* can still be used in traditional medicine to prevent diseases and illness associated with *S. aureus* (e.g. skin infections and food poisoning) and *E. coli* (e.g. diarrhoea, acute urinary tract sepsis) (Tong *et al.*, 2015; Kaper *et al.*, 2004). The leaves, however, showed a susceptible inhibition against *S. aureus*, indicating that the plant part has a higher chance of therapeutic success against the strain (Rodloff *et al.*, 2008). The results further elucidates that the leaves can be greatly recommended for its application in the pharmaceutical and cosmetic industries for the production of antibiotics and other antibacterial products.

CONCLUSION

The purpose of this study was to demonstrate comparative analysis of the phytochemical, antioxidant and antibacterial activities of the leaves and roots of *B. frutescens*, as there are currently very limited reports on this particular research area, since most of the documented studies have focused on the leaves of the species. The phytochemical screening results showed that the leaves had an overall higher presence of the tested phytochemical groups than the roots. Interestingly, coumarins exhibited the highest quantity in both plant parts. The quantitative analyses displayed higher total phenolic, total flavonoid and total tannin content values in the roots than in the leaves. The antioxidant activity of *B. frutescens* followed a similar pattern as the TPC, TFC and TTC analyses, whereby the roots had a stronger scavenging power against DPPH and iron radicals, compared to the leaves. The plant species as a whole however, showed an overall good antioxidant activity. Both plant parts displayed intermediate zones of inhibition against Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria, with the exception of the leaf extract, which showed a susceptible inhibition against *S. aureus*. This means that *B.*

frutescens has the potential to be used in the manufacturing of bacteria-resistant drugs. The overall results from this study validate the uses of *B. frutescens* in traditional medicine and opens avenues for its applications in pharmaceutical and cosmetic industries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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