

Research Article

Comparative Phytochemical Profiling and Biological Activities in the Flowers and Stalks of *Tulbaghia violacea*

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Keywords:

Antibacterial
Antioxidant
Phytochemical screening
Tulbaghia violacea

Abstract

Tulbaghia violacea is indigenous to Southern Africa and has been used extensively in traditional medicine in this region. Extensive research has been documented on the bioactive compounds found in the leaves and roots but not in the flowers and stalks. Thus, this study assessed the phytochemical profile and biological activities in the flowers and stalks of *T. violacea*. Methanolic and aqueous extracts of the air and freeze-dried *T. violacea* were screened for phytochemicals, and then antioxidant and antibacterial assays were performed. Phytochemicals such as phenols, tannins, flavonoids, coumarins, and terpenoids are present in either of the tested plant parts. The flowers contain most of the phytochemicals being tested and a higher total phenolic, tannin, and proanthocyanidin content than the stalks. The flowers exhibit the strongest scavenging activity against 2,2-diphenylpicrylhydrazyl radicals and metal oxidants. The hydrogen peroxide scavenging activities show that the aqueous flower extracts have a higher radical scavenging activity than stalks. In contrast, the methanolic stalk extracts have a higher antioxidant activity than the flowers. Antibacterial activity is only exhibited in the flowers, showing resistant and intermediate inhibition zones of *Escherichia coli* and *Staphylococcus aureus* growth, respectively. This study validates the use of *T. violacea* in traditional medicine, and these results are significant for conserving the species as specific plant parts can be harvested to treat specific ailments. This study suggests the potential application of *T. violacea*, particularly the flowers and stalks, in the pharmaceutical and cosmetic sectors.

Received: November 3rd, 2023

1st Revised: February 4th, 2024

Accepted: February 13th, 2024

Published: February 29th, 2024



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INTRODUCTION

Medicinal plants have been widely used since prehistoric times¹. Their usage is common in most African homes as they are easily accessible and less expensive than Western medicine². Ethnomedicine studies how ethnic groups have survived and continue using traditional medicine³. Ethnomedicine and ethnobotany go hand in hand as it is how different ethnic groups view and approach health-related issues, especially with preventing and curing diseases by using plants that contain bioactive compounds^{4,5}. As per the World Health Organization (WHO), approximately 80% of the global population depends on ethnomedicine practices as their primary source of health care. Plants that are utilized in South African traditional medicines include *Tulbaghia violacea*⁶.

Commonly known as the 'wild' or 'society' garlic, *T. violacea* is a monocotyledonous plant of the Amaryllidaceae family^{6,7}. It is one of the species native to Southern Africa⁸. It is a fast-growing perennial plant native to the Eastern Cape, Kwazulu-Natal, and Limpopo provinces of South Africa⁹. It thrives under the full sun and resists environmental stresses such as droughts⁷. This species is characterized by its small tubular violet or lilac flowers¹⁰. These flowers are usually in clusters of

10-15, resting on a green stalk that can grow as long as 30 cm¹¹ (Figure 1). Its flowers are in full bloom during the hottest times, around January to April⁷. Its green leaves are long and leathery, producing a strong garlic-onion-like scent when bruised⁹. *Tulbaghia violacea* has triangular-shaped capsules that split open when ripe, releasing black seeds for propagation¹². Its brightly colored, sweetly scented, and nectar-rich flowers allow the plant to be pollinated by bees and butterflies during the day and moths at night¹¹.

Most parts of *T. violacea* (i.e., leaves, bulbs, roots) are documented to have medicinal importance⁶. This includes the treatment of esophageal cancer, sinus headaches, stomach aches, asthma, fever, colds, high blood pressure, and tuberculosis¹³. *Tulbaghia violacea* is also one of the medicinal plants that have shown antimicrobial activities against pathogens that result in infection in individuals with HIV and AIDS¹⁴. This plant can have all these functions as it contains phytochemicals and secondary metabolites produced naturally by plants to resist stressors such as herbivory and pathogens¹⁵. Distinct parts of *T. violacea* have biologically active compounds such as flavonoids, saponins, terpenoids, tannins, phenolics, and cardiac glycosides¹⁶. These phytochemicals allow it to have antioxidant, antibacterial, antifungal, anticancer, and anthelmintic properties¹⁴.

Extensive research has been done on this plant's bulbs, leaves, and roots with substantial scientific documentation. However, although both the leaves and flowers are edible and have been used traditionally in ethnomedicine, there is no scientific documentation on the phytochemical profile and biological activities in flowers and stalks of *T. violacea*². Therefore, the present study was to comparatively assess the phytochemical profile and biological activities in the flowers and stalks of *T. violacea*.



Figure 1. (a) *Tulbaghia violacea* in natural habitat and (b) their flowers and stalks.

MATERIALS AND METHODS

Materials

The stalks and attached flower heads of *T. violacea* were collected at the University of the Witwatersrand (26.1929 °S, 28.0305 °E), Johannesburg, South Africa, in March 2023, when the flowers were in full bloom. Fresh aerial parts were authenticated by Dr. Ida Risenga at the same university. The voucher specimen (IR/2023/01) and the plant species were deposited at the university's medicinal plant laboratory.

Methods

Preparation of plant material

Collected flowers and stalks were washed with distilled water (H₂O_d) before being separated cautiously. These were dried using the hot air drier (40°C) and freeze-drying (-83°C). Dried plant materials were ground into fine powder and kept in separate containers at room temperature.

Preparation of plant extracts

The extraction of the separate ground powder was prepared using two solvents: 80% methanol and H₂O_d. About 3 g of each plant powder was extracted using 25 mL of each solvent inside 100 mL Schott bottles. The mixture was agitated on an orbital shaker at 150 rpm for 48 hours and centrifuged for 5 minutes at 3500 rpm. Samples were then filtered through Whatman® No.1 filter paper.

Qualitative analysis of phytochemicals

Recommended laboratory procedures^{17,18} were followed to carry out preliminary phytochemical screening of methanolic and aqueous extracts of *T. violacea*.

Saponins (froth test): About 0.5 mL of the plant extract was added to 5 mL of H₂O_d and then shaken vigorously for 15 minutes. A foam layer confirmed the presence of saponins.

Terpenoids (chloroform test): In a test tube, 0.5 mL of chloroform was mixed with 1 mL of the plant extract and three drops (~150 µL) of concentrated H₂SO₄. A red-brown precipitate indicated terpenoids.

Glycosides: In a test tube, 2 mL of H₂SO₄ was added to 0.5 mL of the plant extract. A red-brown color confirmed the presence of glycosides.

Steroids: To 1 mL of the plant extract, 10 drops of chloroform and five drops of H₂SO₄ were added. A blue-brownish ring confirmed the presence of steroids.

Volatile oils: About 1 mL of the plant extract was mixed with 0.2 mL of 10% NaOH. The formation of a precipitate indicated that volatile oils were present.

Coumarins (NaOH test): About 1 mL of 10% NaOH was mixed with 1 mL of the plant extract; the formation of a yellow top layer was indicative of the presence of coumarins.

Phlobatannins (HCl test): Five drops (~250 µL) of 2% HCl was added to 1 mL of the plant extract. A red precipitation indicated the presence of phlobatannins.

Alkaloids (Mayer's test): A drop (~50 µl) of Mayer's reagent was added to 1 mL of the plant extracts. A creamy precipitate confirmed alkaloids as present.

Phenolics (Ferric chloride test): In a test tube, 1 mL of the plant extract was mixed with three drops (~150 µL) of 10% FeCl₃. A dark blue-green or violet color confirmed the presence of phenolics.

Tannins (Bromine water test): In a test tube, 10 mL of bromine water was added to 1 mL of the plant extract. A decolorization of the mixture indicated the presence of tannins.

Quinones (H₂SO₄ test): About 1 mL of the plant extract was added to 1 mL of concentrated H₂SO₄. The presence of quinones was indicated by the formation of a red color.

Cardiac glycosides (Keller-Killani test): About 2 mL of glacial acetic acid, a mL of concentrated H₂SO₄, and a single drop (~50 µL) of 5% FeCl₃ were added to 0.5 mL of the plant extract. A brown ring confirmed the presence of cardiac glycosides.

Flavonoids (Alkaline reagent test): In a test tube, 2 mL of 2% NaOH was added to 1 mL of the plant extract. A color change from yellow to colorless after adding a few drops of diluted HCl was indicative of the presence of flavonoids.

Carbohydrates: The presence of carbohydrates was confirmed by a formation of purple color when two drops of Molisch's reagent were added to 2 mL of the plant extract and 1 mL of concentrated H₂SO₄.

Fixed oils and fats (Stain/spot test): The plant extract was filtered through a filter paper. An oil stain confirmed that fixed oils and fats were present.

Gums and mucilage (Alcohol test): About 1 mL of H₂O_d and 2.5 mL of concentrated H₂SO₄ were added to 1 mL of the plant extracts. A white precipitate showed the presence of gums and mucilage.

Resins: Three drops of glacial acetic acid and 1 mL of concentrated H_2SO_4 were added to 1 mL of the plant extract. An orange/yellow color confirmed the presence of resins.

Triterpenoids and phytosterol: About 1 mL of chloroform and three drops of concentrated H_2SO_4 were added to 1 mL of the plant extract. The solution was shaken vigorously and left to set for a few seconds. A yellow or red color confirmed the presence of triterpenoids or phytosterols, respectively.

Anthocyanins: About 1 mL of 2 N HCl was added to 1 mL of the plant extract. A color change from reddish pink to violet after adding a few drops of ammonia indicated the presence of flavonoids.

Cholesterol: About 1 mL of chloroform, five drops of glacial acetic acid, and two drops of H_2SO_4 were added to 1 mL of the plant extract. A red color was indicative of the presence of cholesterol.

Quantitative analysis of phytochemicals

Total phenolic content: About 0.3 mL of the prepared plant extracts were added to a solution of 7.5% sodium carbonate (Na_2CO_3). To this mixture, 0.75 mL of Folin-Ciocalteu's (FC) phenol reagent was added then the entire mixture was diluted with H_2O_d to a final volume of 7 mL. The mixture was then left to incubate for 2 hours in the dark. Using a Genesys 10s UV-Vis spectrophotometer, the absorbance of the sample was taken at 765 nm. The total phenolic content (TPC), expressed in milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g), was calculated using the following linear regression obtained from the gallic acid standard curve graph (Figure 2).

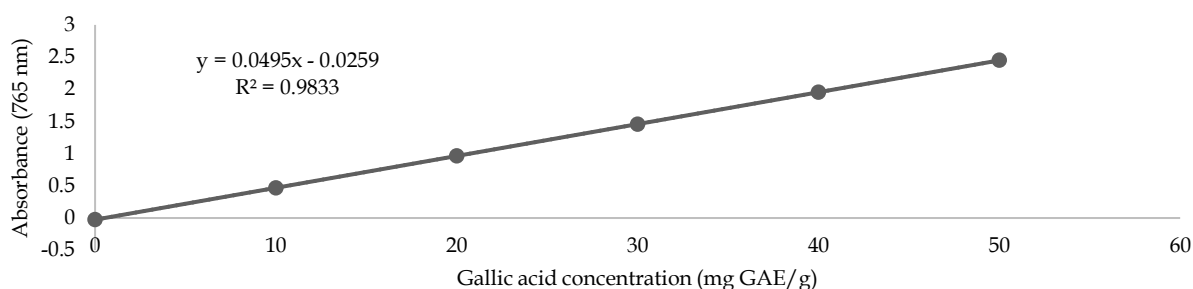


Figure 2. Standard curve for total phenolic content.

Total flavonoid content: The aluminum chloride (AlCl_3) colorimetric assay was followed. A 5% (w/v) sodium nitrate (NaNO_3) solution was prepared by adding 100 mL of H_2O_d to 5 g of NaNO_3 . In a test tube, 0.3 mL of the prepared plant extracts were combined with the prepared 5% NaNO_3 , which was then left to set for 5 minutes. About 3 mL of 10% (w/v) AlCl_3 solution (prepared by dissolving 10 g of AlCl_3 in 100 mL of H_2O_d) was added to the test tube that contained the extract and NaNO_3 . This test tube was then left to rest for 6 minutes. After that, 2 mL of 7.5% sodium hydroxide (NaOH) was added to the test tube. To the entire mixture, 0.75 mL of diluted FC reagent and H_2O_d were added to reach a final volume of 10 mL and were then left to incubate for 1 hour in the dark at room temperature. As described earlier, the absorbance readings were measured at 510 nm against the blank, which was 80% methanol. Total flavonoid content (TFC), expressed in milligrams of quercetin equivalents per gram of dry weight (mg QE/g), was calculated using the following linear regression obtained from the quercetin standard curve graph (Figure 3).

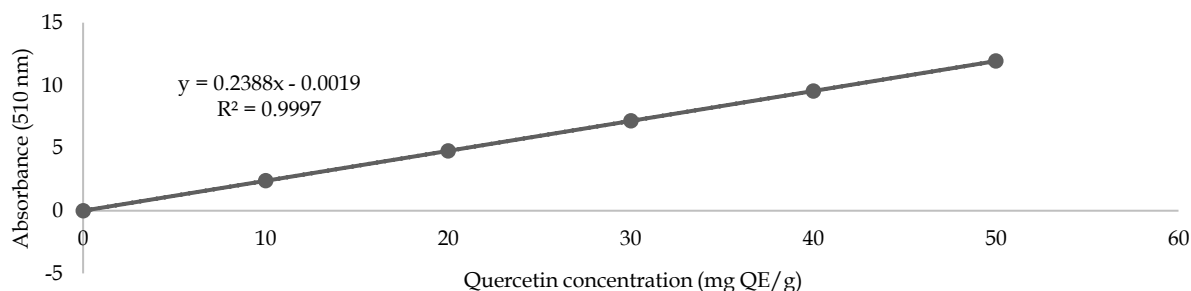


Figure 3. Standard curve for total flavonoid content.

Total tannin content: About 0.1 mL of the prepared plant extracts were diluted with 7.5 mL of H_2O_d before adding 0.5 mL of Folin-Ciocalteu's phenol reagent. About 0.1 mL of 35% (w/v) sodium carbonate (Na_2CO_3) solution (prepared by adding 10 mL of H_2O_d to 3.5 g of Na_2CO_3) was added to the mixture of extract and FC phenol reagent. The entire mixture was made up of 10 mL with H_2O_d . The absorbance of the mixture was measured at 725 nm, as described earlier, against the blank, which was 80% methanol. Total tannin content (TTC), which was expressed in mg GAE/g, was calculated using the following linear regression obtained from the gallic acid standard curve (Figure 4).

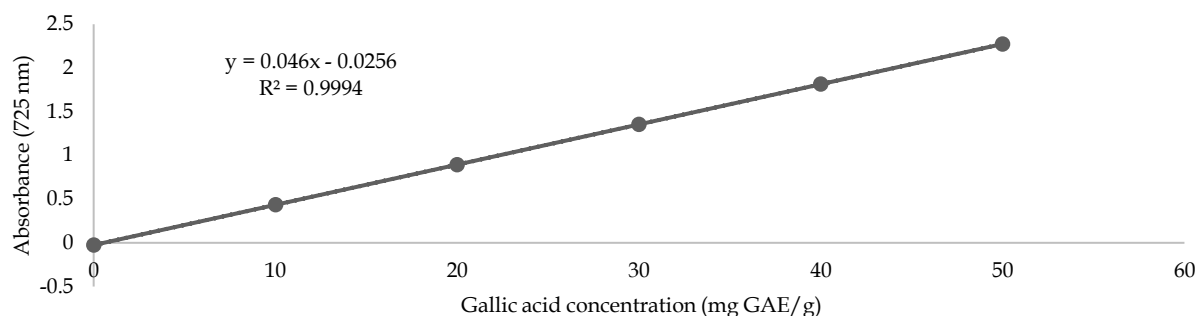


Figure 4. Standard curve for total tannin content.

Total proanthocyanidin content: About 3 mL of 4% vanillin-methanol (w/v) (prepared by adding 100 mL of water to 4 g of vanillin-methanol) was mixed with 0.5 mL of the prepared plant extracts, then 1.5 mL of HCl was added. This mixture was vortexed and then left to incubate for 15 minutes in the dark at room temperature. The absorbance of the mixture was measured at 500 nm as described earlier against the blank which was 80% methanol. Total proanthocyanidin content (TPAC), which was expressed in milligrams of catechin equivalents per gram of dry weight (mg CE/g) was calculated using the following linear regression obtained from the catechin standard curve (Figure 5).

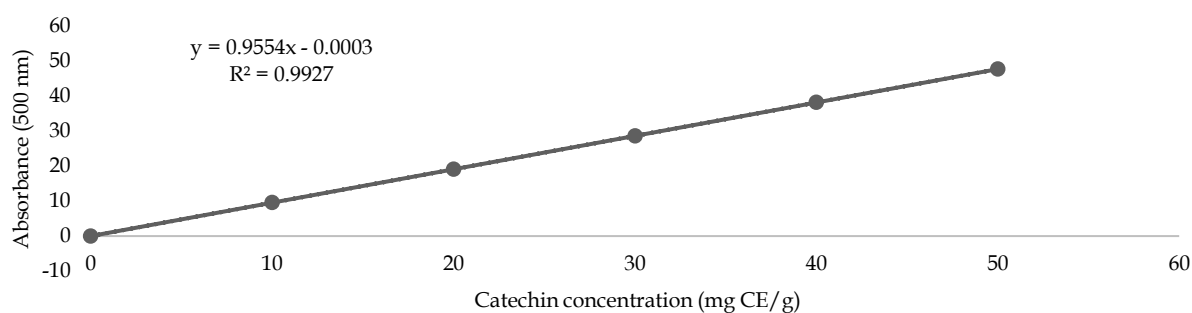


Figure 5. Standard curve for total proanthocyanidin content.

Antioxidant assays

2,2-diphenylpicrylhydrazyl (DPPH) scavenging assay: To determine the DPPH scavenging activities of the plant extracts, the DPPH solution was prepared by mixing 50 mg of DPPH and 100 mL of 80% methanol, which was then shaken vigorously (stock solution). This solution was diluted 1 : 5 times with 80% methanol (work solution). About 70 μL of the work solution was added to the different volumes (10, 20, 30, 40, and 50 μL) of the plant's extracts. The work solution without the plant extracts was used as a control. The extract and DPPH solution mixture was left to incubate for 45 minutes in the dark at room temperature. The absorbance of the mixture was measured at 517 nm, as described earlier. Equation 1 was used to calculate the DPPH scavenging percentage of the extract, in which $\hat{A}_{cc}$ was the absorbance of the control, and $\hat{A}_{ss}$ was the absorbance of the test compound (plant extract).

$$\% \text{ DPPH} = \frac{\hat{A}_{cc} - \hat{A}_{ss}}{\hat{A}_{cc}} \times 100\% \quad [1]$$

Hydrogen peroxide assay: A 30% H₂O₂ solution was prepared by mixing 30 mL of concentrated H₂O₂ with 70 mL of H₂O_d. Then, a 40 mM H₂O₂ solution was prepared by mixing 4.53 mL of the 30% H₂O₂ solution with 995.47 mL of phosphate buffer (pH 7.4). About 600 µL of 40 mM H₂O₂ solution was added to the different volumes (10, 20, 30, 40, and 50 µL) of the plant extracts, and these were left to set for 10 minutes. About 40 mM H₂O₂ served as a control. The absorbance of the mixture was measured at 230 nm as described earlier, against the blank which was the phosphate buffer without the H₂O₂. Equation 2 was used to calculate the percentage of H₂O₂ reducing the power of the extract, in which $\hat{A}_{cc}$ was the absorbance of the control, and $\hat{A}_{ss}$ was the absorbance of the test compound (plant extract).

$$\% H_2O_2 = \frac{\hat{A}_{cc} - \hat{A}_{ss}}{\hat{A}_{cc}} \times 100\% \quad [2]$$

Metal chelating assay: A 2 mM iron chloride solution was prepared by dissolving 0.03244 g of FeCl₃ in 100 mL of H₂O_d. For determining the iron-reducing power of *T. violacea*, different volumes (10, 20, 30, 40, and 50 µL) of the plant extracts were mixed with 0.05 ml of the 2 mM FeCl₃. As a reaction initiator, 200 µL of 5 Mm ferrozine solution (prepared by adding 0.246 g of ferrozine to 100 mL of H₂O_d) was added to the mixture of the plant extracts and 2 mM FeCl₃. The mixed solution was shaken vigorously and left to set for 10 minutes in the dark at room temperature. The mixed FeCl₃ and ferrozine solution without the extracts served as a control. The absorbance of the mixture was measured at 562 nm, as described earlier. Equation 3 was used to calculate the percentage metal chelating effect of the extract, in which $\hat{A}_{cc}$ was the absorbance of the control, and $\hat{A}_{ss}$ was the absorbance of the test compound (plant extract).

$$\% Chelating = \frac{\hat{A}_{cc} - \hat{A}_{ss}}{\hat{A}_{cc}} \times 100\% \quad [3]$$

Preliminary antibacterial assays

An agar well diffusion method was followed to determine the antimicrobial activity of flowers and stalks of *T. violacea*. This was assessed from gram-negative (*Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*). The Mueller-Hinton (MH) and Baird-Parker (BP) agar were used to culture the *E. coli* and *S. aureus*, respectively. The bacteria strains were inoculated on cooled petri dishes with the MH and BP agar, respectively, before incubating for 24 hours at 37°C, which is the normal human body temperature. Subsequently, holes were punched into the agar plates using sterilized 6 mm diameter pipette tips. About 100 µL of the plant extracts were then added to the punched holes, and the Petri dishes were left to set for 10 minutes before being incubated for 48 hours at 37°C in a binder oven. The 80% methanol was used as a negative control, while the antibiotic rifampicin (100 µg/mL) was used as a positive control. After 48 hours, zones of inhibition (ZOI) on the plates were measured in mm to determine the antibacterial activity of the plant extracts.

Data analysis

The results are expressed in mean ± SD with n = 3. All experiments were done in triplicates. The quantitative analysis and antioxidant activity results were analyzed using paired t-tests (p ≤ 0.05). Pearson correlations were conducted to determine the relationship between phytochemical constituents and antioxidant activity. All statistical analyses were conducted on R studio version 4.12.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

A qualitative analysis was used to evaluate the presence or absence of phytochemicals in the flowers and stalks of *T. violacea*. Phytochemical screening results at varying intensities (Strong presence, moderate presence, weak presence, and absent) are displayed in Table I. Both the methanolic and aqueous extracts of both plant parts showed the absence of saponins, volatile oils, alkaloids, carbohydrates, and resins and this detection was consistent in both drying methods. The absence of alkaloids and carbohydrates coincides with a study performed by Madike *et al.*¹³ for other plant parts of *T. violacea*.

Table I. Phytochemical screening analysis of freeze- and air-dried methanolic and aqueous extracts of flowers and flower stalks of *T. violacea*.

	Plant Part	Flower		Stalk		Flower		Stalk	
		Drying method							
		Air Dried				Freeze-dried			
Solvent		M	W	M	W	M	W	M	W
Phytochemicals	Saponin								
	Terpenoid								
	Glycosides								
	Steroids								
	Volatile oils								
	Coumarins								
	Phlobatannins								
	Alkaloids								
	Phenolics								
	Tannins								
	Quinones								
	Cardiac glycosides								
	Flavonoids								
	Carbohydrates								
	Fixed oils and fats								
	Gums and Mucilage								
	Resins								
	Triterpenoids								
	Phytosterols								
	Anthocyanin								
	Cholesterol								
	+++	Strong presence							
	++	Moderate presence							
	+	Weak presence							
	-	Absence							
M : methanol; W : water									

Despite the solvents or the drying methods, glycosides, phenolics, tannins, flavonoids, and fixed oils and fats were extracted from both plant parts. Glycosides, which were very strongly detected in the flowers, are known for their antinociceptive and anti-inflammatory properties and have the potential for treating diabetes mellitus^{19,20}. Phenolic compounds, known to have anti-inflammatory, antimicrobial, and antioxidant properties, showed a strong presence in both plant parts and for both drying methods²¹. Tannins, which showed a more substantial presence in the flowers, are known for their antiparasitic, antiviral, and antimicrobial properties and can be used to stop the replication of HIV¹³. Previous research has also shown that tannins can treat kidney-related ailments²⁰. Flavonoids, the largest group of phenolic compounds, were strongly detected in the methanolic flower extracts across the two drying methods. Flavonoids have been shown to exhibit antioxidant, analgesic, antidiarrhea, and antimicrobial properties and have been used in cancer and Alzheimer's disease treatments^{22,23}. Therefore, this data suggests that the stalks of *T. violacea* can potentially treat the above-mentioned diseases. Fixed oils and oils were more strongly detected in the stalks than in flowers. They possess antifungal and antibacterial properties and can be used as an insect repellent²⁴, suggesting that stalks could have antifungal, antibacterial, and insect-repellent properties.

Terpenoids, gums, mucilage, phytosterol, anthocyanidins, and cholesterol were only detected in flowers. Terpenoids have anticancer, anti-inflammatory, and antioxidant properties^{25,26}. Gums and mucilage, only present in the aqueous extracts, can treat irritated mucous membranes in the throat and digestive tract²⁷. Phytosterols and cholesterol, which fluctuated in their strength of presence, can be used to lower cholesterol levels^{28,29}. Anthocyanidins, only present in the methanolic extracts, have antioxidant, anticancer, anti-obesity, and anti-inflammatory properties. Triterpenoids were the only compounds that were detected in the stalks and not flowers. These compounds have antiviral, anti-inflammatory, and antitumor properties³⁰. More phytochemicals were detected in the flowers as compared with stalks. The detected phytochemicals are natural chemicals that can be used in pharmacological fields or the production of bioactive compounds, and these are preferred and have fewer side effects than synthetic drugs³¹. Therefore, the presence of these phytochemicals supports the use of *T. violacea* flowers and stalks in ethnomedicine.

Quantitative analysis of phytochemicals

The quantitative phytochemical analysis of air and freeze-dried flowers and stalks are displayed in [Tables II](#) and [III](#), respectively. Phenolic compounds have numerous pharmacological effects, including their antioxidant, anti-inflammatory, and antidiabetic properties²¹. Gallic acid possesses antioxidant and anti-inflammatory properties, thus increasing the health benefits with limited side effects compared to modern-day medicine³². The results in this study show that for both air and freeze-dried methanolic and aqueous extracts, the flowers had a significantly higher total phenolic content as compared with the stalks ($p < 0.001$).

Table II. Quantitative phytochemical analysis of methanolic and aqueous extracts of air-dried flowers and stalks of *T. violacea* ($p \leq 0.05$).

Air-dried samples			
Phytochemical Constituents	Flowers		
	Methanol	Water	
Phenol (mgGAE/g)	40.6±0.013	41.41±0.015	
Flavonoid (mgQE/g)	8.9±0.068	7.58±0.19	
Tannin (mgGAE/g)	40.07±0.017	34.03±0.012	
Proanthocyanidin (mgCE/g)	0.7±0.013	0.36±0.0091	
Phytochemical Constituents	Stalks		
	Methanol	Water	
Phenol (mgGAE/g)	27.26±0.017	28.52±0.016	
Flavonoid (mgQE/g)	5.79±0.067	9.29±0.14	
Tannin (mgGAE/g)	22.64±0.02	28.07±0.013	
Proanthocyanidin (mgCE/g)	0.46±0.0057	0.2±0.0015	

Table III. Quantitative phytochemical analysis of methanolic and aqueous extracts of freeze-dried flowers and stalks of *T. violacea* ($p \leq 0.05$).

Freeze-dried samples			
Phytochemical Constituents	Flowers		
	Methanol	Water	
Phenol (mgGAE/g)	34.62±0.02	37.29±0.0027	
Flavonoid (mgQE/g)	2.54±0.022	2.42±0.085	
Tannin (mgGAE/g)	44.27±0.011	33.37±0.065	
Proanthocyanidin (mgCE/g)	0.66±0.002	0.46±0.015	
Phytochemical Constituents	Stalks		
	Methanol	Water	
Phenol (mgGAE/g)	26.27±0.037	24.82±0.0058	
Flavonoid (mgQE/g)	2.5±0.067	2.52±0.19	
Tannin (mgGAE/g)	28.89±0.18	13.87±0.46	
Proanthocyanidin (mgCE/g)	0.24±0.0062	0.36±0.28	

Flavonoids, a phenolic compound, possess antimicrobial, analgesic, and antioxidant properties, among other pharmacological uses²². Quercetin is a type of flavonoid that has antioxidant properties thus increasing the health benefits with fewer side effects³³. The results in this study show that for the methanolic extracts of the air-dried extracts, the flowers had a significantly higher total flavonoid content than the stalks ($p < 0.001$). For both air and freeze-dried aqueous extracts, the stalks had a significantly higher total flavonoid content as compared with the flowers ($p < 0.05$). The low TFC for freeze-dried extracts coincides with a study performed by Madike *et al.*¹³ for other plant parts of *T. violacea*. Tannins possess antiparasitic, antiviral, and antimicrobial properties, among other functions. This study's results show that for air and freeze-dried methanolic and aqueous extracts, the flowers had a significantly higher total tannin content than the stalks ($p < 0.001$). Proanthocyanidins have been documented to possess anti-allergic, antioxidant, and antimicrobial properties. They also have pharmacological uses such as the improvement of eyesight²³. Catechin has been reported to possess antioxidant properties and can be used in the prevention of congestive heart failures thus increasing the health benefits with fewer side effects³⁴. The presence of all these phytochemicals at varying concentrations can be used in pharmaceutical industries to promote human health. The results in this study show that for both air and freeze-dried methanolic and aqueous extracts, the flowers had a significantly higher total proanthocyanidin content as compared with the stalks ($p < 0.001$).

Analysis of antioxidant activity

The DPPH radicals, H_2O_2 , and iron oxidant scavenging activity of air and freeze-dried flowers and stalks are displayed in Figures 6 to 8, respectively. Free radicals are unstable and reactive molecules produced during metabolism³⁵. Oxidative stress can result from over-accumulating free radicals in the body, which can be fatal to cells and thus cause illnesses³⁶. Medicinal plants contain phytochemicals that have antioxidant activities. The plant extracts' ability to scavenge for and neutralize free radicals in the body gives us a general idea of their antioxidant properties³⁷. The samples' ability to scavenge the DPPH, H_2O_2 , and metal radicals is expressed by IC_{50} values. An IC_{50} value, the "half-maximal inhibitory concentration", indicates how much of an extract is needed to inhibit a detrimental biological activity by 50%. Low IC_{50} values indicate high antioxidant activities³⁸.

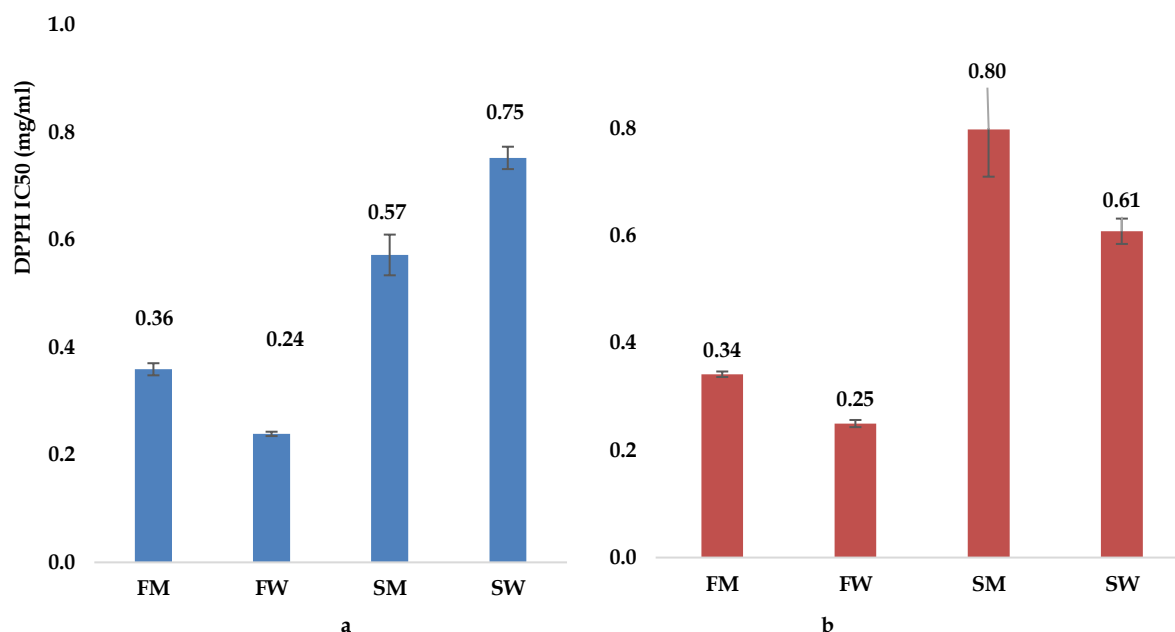


Figure 6. DPPH IC_{50} values of (a) air-dried and (b) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

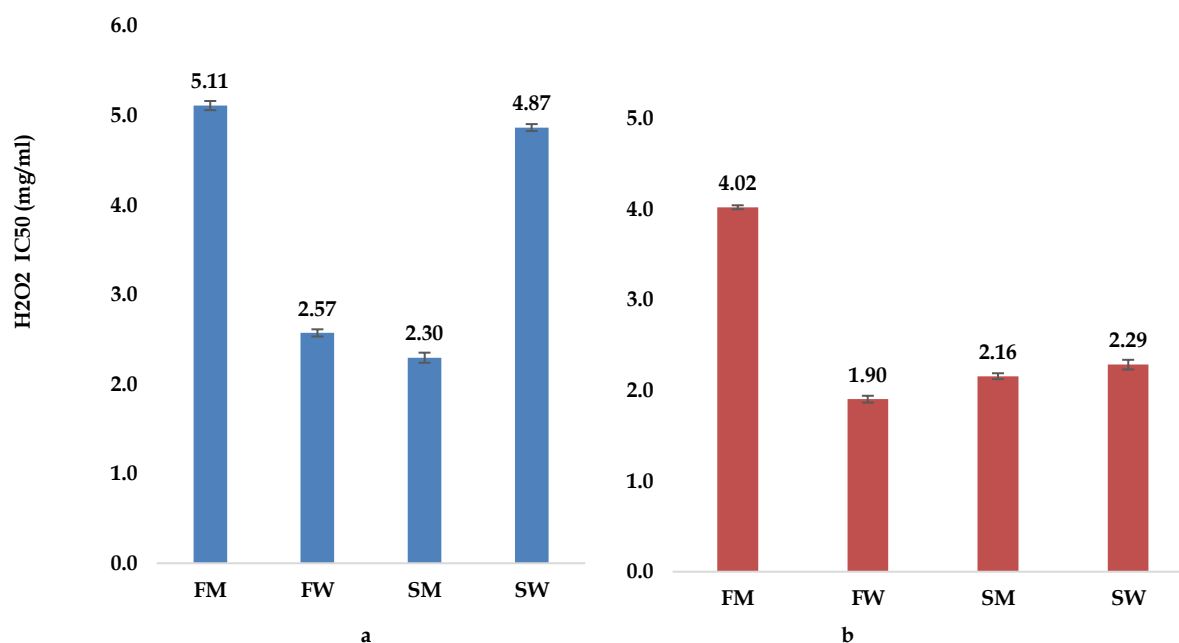


Figure 7. H_2O_2 IC_{50} values of (a) air-dried and (b) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

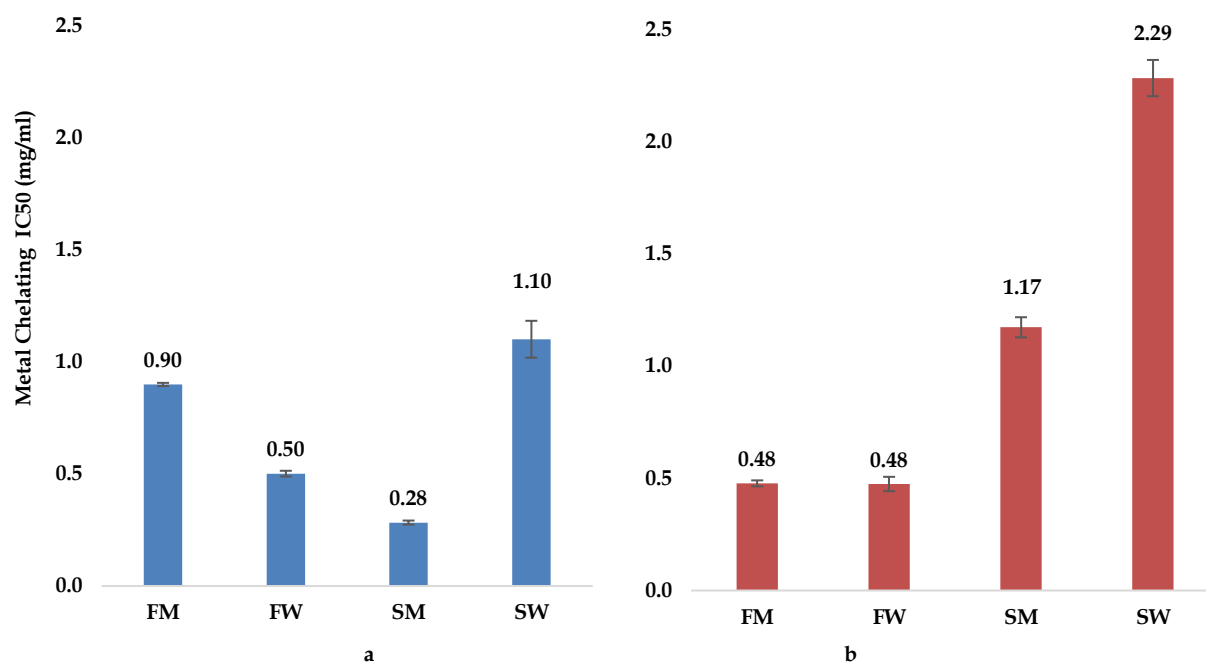


Figure 8. Metal chelating IC₅₀ values of (a) air-dried and (b) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

For both drying methods, the methanolic and aqueous extracts of the flowers and stalks of *T. violacea* had IC₅₀ values below 1 mg/mL, thus indicating an excellent scavenging activity against DPPH radicals³⁸. The very low IC₅₀ values coincide with a study performed by Takaidza *et al.*³⁹, where *T. violacea* as a whole plant was used. The results in this study show that the flowers had significantly lower IC₅₀ values than the stalk ($p < 0.001$). This was consistent in both drying methods and solvents that were used. The higher IC₅₀ values in the stalks can be attributed to the lower phytochemical presence (Table I). The DPPH scavenging activity exhibited strong positive correlations with most of the quantified phytochemicals, and perfect strong positive correlations ($r = 1$) are exhibited between the DPPH scavenging activity and TPAC for air-dried methanolic extracts of the flowers and the TFC for freeze-dried aqueous extracts of the stalks (Tables IV and V). This shows that the tested plant parts' ability to scavenge for and neutralize DPPH radicals can be attributed to the presence of phytochemicals as they possess antioxidant activities.

For both drying methods, the methanolic and aqueous extracts of the flowers and stalks had IC₅₀ values below 10 mg/mL (upper limit of IC₅₀), thus indicating a strong scavenging activity against H₂O₂. The results of this study show that the stalks had significantly lower IC₅₀ values than the flowers ($p < 0.001$). For the aqueous extracts, the results in this study showed that the flowers had significantly lower IC₅₀ values as compared with the stalks ($p < 0.001$). The scavenging activity of H₂O₂ is dependable on the solvent used for extraction. Thus, since water and methanol have different polarities, this then affects the tested plant parts' scavenging power against H₂O₂⁴⁰. Apart from the freeze-dried aqueous extracts, for both drying methods, the H₂O₂ Scavenging activity exhibited strong positive correlations with the quantified phytochemicals (Tables IV and V). There were perfect strong positive correlations ($r = 1$) between the H₂O₂ scavenging activity and the TFC, TTC, and TPAC for the air-dried aqueous flower extracts; the TTC for the air-dried methanolic stalk extracts; and the TPAC of the air-dried aqueous stalk extracts (Tables IV and V). This shows that the tested plant parts' ability to scavenge for and neutralize H₂O₂ can be attributed to the presence of phytochemicals.

For both drying methods, the methanolic and aqueous extracts of the flowers and stalks had IC₅₀ values below 10 mg/mL (upper limit of IC₅₀), thus indicating a strong scavenging activity against iron oxidants. Except for the air-dried methanolic extracts, the flowers had significantly lower IC₅₀ values than the stalks ($p < 0.01$). Apart from the freeze-dried methanolic extracts, the iron oxide scavenging activity exhibited very strong positive correlations with most of the quantified phytochemicals for both drying methods. This shows that the tested plant parts' ability to chelate iron oxidants can be attributed to the presence of phytochemicals.

Table IV. Pearson correlation coefficients (r) between TPC, TFC, TTC, TPAC, and antioxidant activities of the methanolic and aqueous extracts of air-dried flowers and stalks of *T. violacea* ($p \leq 0.05$).

Antioxidant assay	Air-dried samples			
	TPC	TFC	TTC	TPAC
Flower-Methanol				
DPPH	0.737	0.808	0.808	1*
H ₂ O ₂	0.95	0.979	0.979	0.911
MC	0.907	0.948	0.949	0.953
Flower-Water				
DPPH	0.737	0.994	0.994	0.993
H ₂ O ₂	0.808	1*	1*	1*
MC	0.938	0.962	0.962	0.962
Stalk-Methanol				
DPPH	0.563	0.847	0.673	0.616
H ₂ O ₂	0.99	0.963	1*	0.997
MC	0.977	0.98	0.997	0.989
Stalk-Water				
DPPH	0.778	0.945	0.993	0.693
H ₂ O ₂	0.992	0.891	0.771	1*
MC	0.994	0.972	0.898	0.973

*: Perfect strong correlation

Table V. Pearson correlation coefficients (r) between TPC, TFC, TTC, TPAC, and antioxidant activities of the methanolic and aqueous extracts of freeze-dried flowers and stalks of *T. violacea* ($p \leq 0.05$).

Antioxidant assay	Freeze-dried samples			
	TPC	TFC	TTC	TPAC
Flower-Methanol				
DPPH	0.845	0.95	0.881	0.737
H ₂ O ₂	0.941	0.995	0.963	0.866
MC	0.442	0.648	0.507	0.277
Flower-Water				
DPPH	0.908	0.945	0.778	0.804
H ₂ O ₂	0.648	0.721	0.442	0.481
MC	0.908	0.945	0.778	0.804
Stalk-Methanol				
DPPH	0.59	0.786	0.721	0.661
H ₂ O ₂	0.751	0.901	0.854	0.808
MC	0.997	0.941	0.97	0.986
Stalk-Water				
DPPH	0.951	1*	0.932	0.994
H ₂ O ₂	0.99	0.985	0.98	0.998
MC	0.916	0.995	0.981	0.978

*: Perfect strong correlation

Preliminary antibacterial assays

Escherichia coli is a common bacteria strain and is linked with urinary infections⁴¹. *Staphylococcus aureus* is linked with skin conditions such as skin and soft tissue infections (SSTI), a common infection²¹. Flowers were the only tested plant part that showed the inhibition of *E. coli* and *S. aureus* (Table VI). Zones of inhibitions for *E. coli* were only exhibited in the flower methanolic extracts, which fall under the resistant category. Zones of inhibitions for *S. aureus* were only exhibited in the aqueous extracts for flowers, and these fall under the intermediate category. This shows that the aqueous extracts have the potential to be used to cure skin conditions such as SSTI.

Table VI. Zone of inhibition (mm) of the methanolic and aqueous extracts of freeze-dried flowers and stalks of *T. violacea* against *E. coli* and *S. aureus*.

Drying method	Air-dried		Freeze-dried	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
Flower-methanol	10±0.1	-	11±0.1	-
Flower-water	-	12±0.2	-	13±0.2
Stalk-methanol	-	-	-	-
Stalk-water	-	-	-	-
Antibiotic	12.67±0.58	12.67±0.58	12.67±0.58	12.67±0.58

CONCLUSION

Consuming *T. violacea* would be beneficial as it contains phytochemicals that allow the plant to have therapeutic properties. Flowers had more phytochemicals present, higher antioxidant activity (DPPH, H₂O₂, and metal chelating) than stalks, and were the only plant part with antibacterial activity. The results of this study can be highly beneficial to communities that rely on medicinal plants as their source of health care as they can use each plant part for specific ailments. This can also be beneficial to pharmaceutical industries for the promotion of human health.

ACKNOWLEDGMENT

The authors acknowledge the University of the Witwatersrand, School of Animal Plant and Environmental Sciences, Medicinal Plants Laboratory, for providing facilities (NRF Reference number: TTK190401426371). A special appreciation to HCSA Kenkijin Bursary Trust for funding with reference number IT000358/2016(G).

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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