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*Research Article*

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

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## 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-diphenylpicryhydrazyl 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.

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## INTRODUCTION

Medicinal plants have been widely used since prehistoric times[1]. Their usage is common in most African homes as they are easily accessible and less expensive than Western medicine[2]. Ethnomedicine studies how ethnic groups have survived and continue using traditional medicine[3]. 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 Africa[8]. It is a fast-growing perennial plant native to the Eastern Cape, Kwazulu-Natal, and Limpopo provinces of South Africa[9]. It thrives under the full sun and resists environmental stresses such as droughts[7]. This species is characterized by its small tubular violet or lilac flowers[10]. These flowers are usually in clusters of

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10-15, resting on a green stalk that can grow as long as 30 cm[11] (**Figure 1**). Its flowers are in full bloom during the hottest times, around January to April[7]. Its green leaves are long and leathery, producing a strong garlic-onion-like scent when bruised[9]. *Tulbaghia violacea* has triangular-shaped capsules that split open when ripe, releasing black seeds for propagation[12]. 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 night[11].

Most parts of *T. violacea* (i.e., leaves, bulbs, roots) are documented to have medicinal importance[6]. This includes the treatment of esophageal cancer, sinus headaches, stomach aches, asthma, fever, colds, high blood pressure, and tuberculosis[13]. *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 AIDS[14]. 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 pathogens[15]. Distinct parts of *T. violacea* have biologically active compounds such as flavonoids, saponins, terpenoids, tannins, phenolics, and cardiac glycosides[16]. These phytochemicals allow it to have antioxidant, antibacterial, antifungal, anticancer, and anthelmintic properties[14].

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*.



**a** **b**

**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 ($H_2O_d$) 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 $H_2O_d$. 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 $H_2O_d$ 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 $H_2SO_4$. A red-brown precipitate indicated terpenoids.

*Glycosides*: In a test tube, 2 mL of $H_2SO_4$ 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 $H_2SO_4$ 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% $FeCl_3$. 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 ($H_2SO_4$ test)*: About 1 mL of the plant extract was added to 1 mL of concentrated $H_2SO_4$. 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 $H_2SO_4$, and a single drop (~50 µL) of 5% $FeCl_3$ 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 $H_2SO_4$.

*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 $H_2O_d$ and 2.5 mL of concentrated $H_2SO_4$ 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 (Na2CO3). To this mixture, 0.75 mL of Folin-Ciocalteu's (FC) phenol reagent was added then the entire mixture was diluted with H2Od 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**).

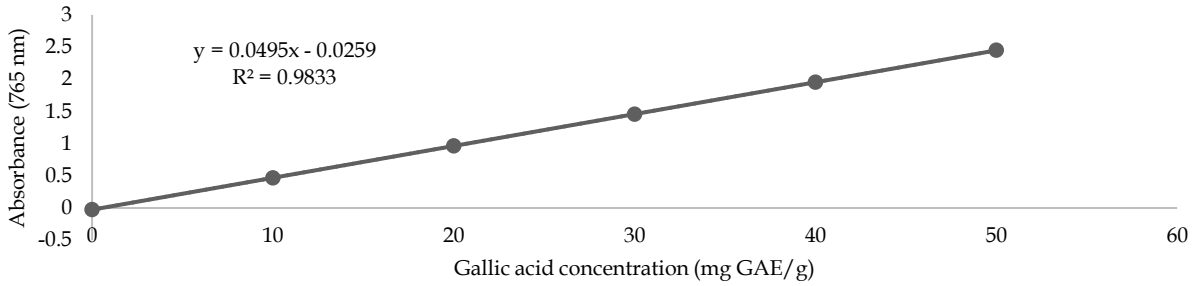



**Figure 2.** Standard curve for total phenolic content.

*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 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**).

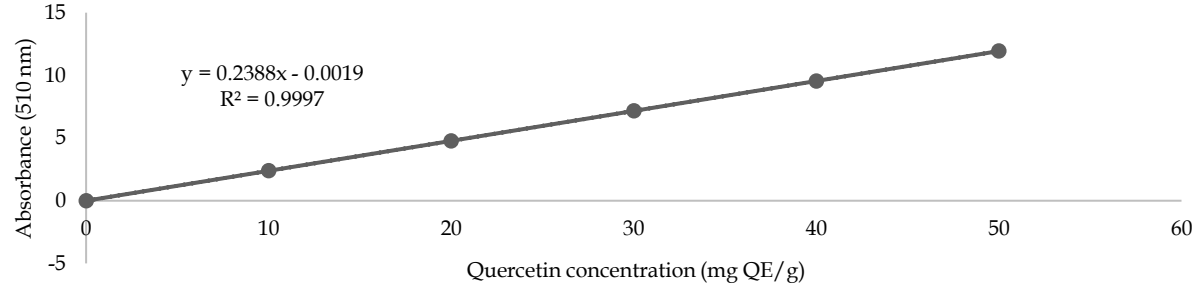



**Figure 3.** Standard curve for total flavonoid content.

*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**).

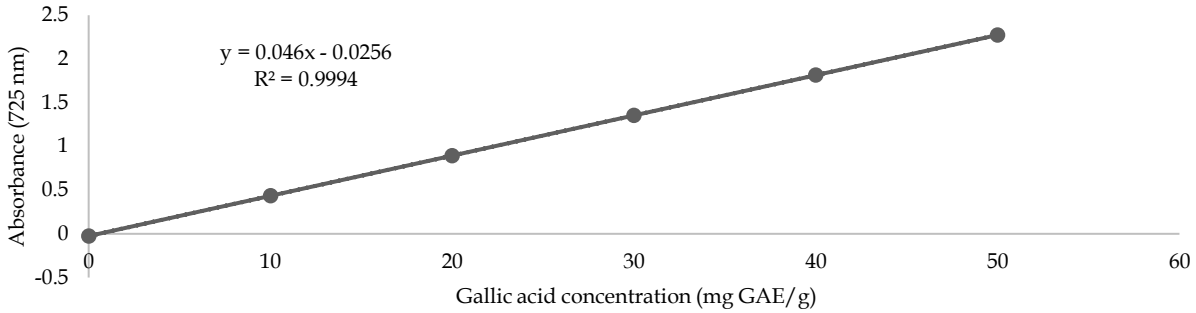



**Figure 4.** Standard curve for total tannin content.

*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**).

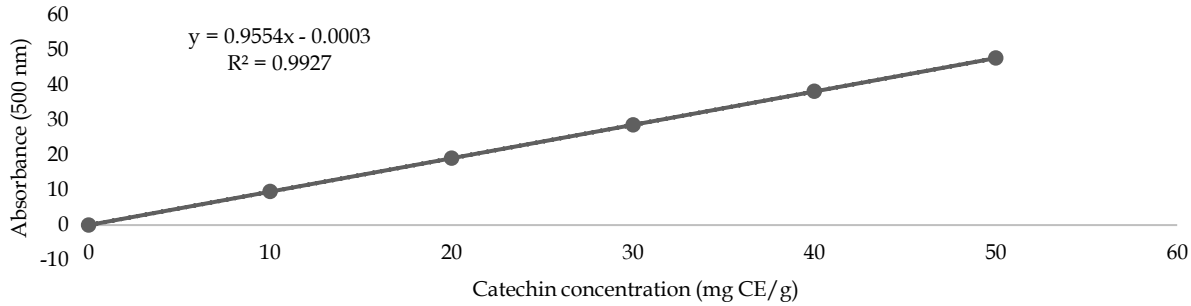



**Figure 5.** Standard curve for total proanthocyanidin content.

*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 Âcc was the absorbance of the control, and Âss was the absorbance of the test compound (plant extract).

$$\%\ DPPH = \frac{Âcc - Âss}{Âcc} x100\% \qquad [1]$$

*Hydrogen peroxide assay*: A 30% $H_2O_2$ solution was prepared by mixing 30 mL of concentrated $H_2O_2$ with 70 mL of $H_2O_d$. Then, a 40 mM $H_2O_2$ solution was prepared by mixing 4.53 mL of the 30% $H_2O_2$ solution with 995.47 mL of phosphate buffer (pH 7.4). About 600 µL of 40 mM $H_2O_2$ 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 $H_2O_2$ 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 $H_2O_2$. **Equation 2** was used to calculate the percentage of $H_2O_2$ reducing the power of the extract, in which Âcc was the absorbance of the control, and Âss was the absorbance of the test compound (plant extract).

$$\% H_2O_2 = \frac{Âcc - Âss}{Âcc} x100\% \quad [2]$$

*Metal chelating assay*: A 2 mM iron chloride solution was prepared by dissolving 0.03244 g of $FeCl_3$ in 100 mL of $H_2O_d$. 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 $FeCl_3$. As a reaction initiator, 200 µL of 5 Mm ferrozine solution (prepared by adding 0.246 g of ferrozine to 100 mL of $H_2O_d$) was added to the mixture of the plant extracts and 2 mM $FeCl_3$. The mixed solution was shaken vigorously and left to set for 10 minutes in the dark at room temperature. The mixed $FeCl_3$ 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 Âcc was the absorbance of the control, and Âss was the absorbance of the test compound (plant extract).

$$\% Chelating = \frac{Âcc - Âss}{Âcc} x100\% \quad [3]$$

*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 ($p \leq 0.05$). 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 methods[21]. 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 HIV[13]. Previous research has also shown that tannins can treat kidney-related ailments[20]. 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 deceases. 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 repellent[24], 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 tract[27]. 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 properties[30]. More phytochemicals were detected in the flowers as compared with stalks. The detected phytocompounds 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 drugs[31]. 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 properties[21]. Gallic acid possesses antioxidant and anti-inflammatory properties, thus increasing the health benefits with limited side effects compared to modern-day medicine[32]. 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 uses[22]. Quercetin is a type of flavonoid that has antioxidant properties thus increasing the health benefits with fewer side effects[33]. 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 eyesight[23]. 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 effects[34]. 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 metabolism[35]. Oxidative stress can result from over-accumulating free radicals in the body, which can be fatal to cells and thus cause illnesses[36]. 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 properties[37]. 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 activities[38].

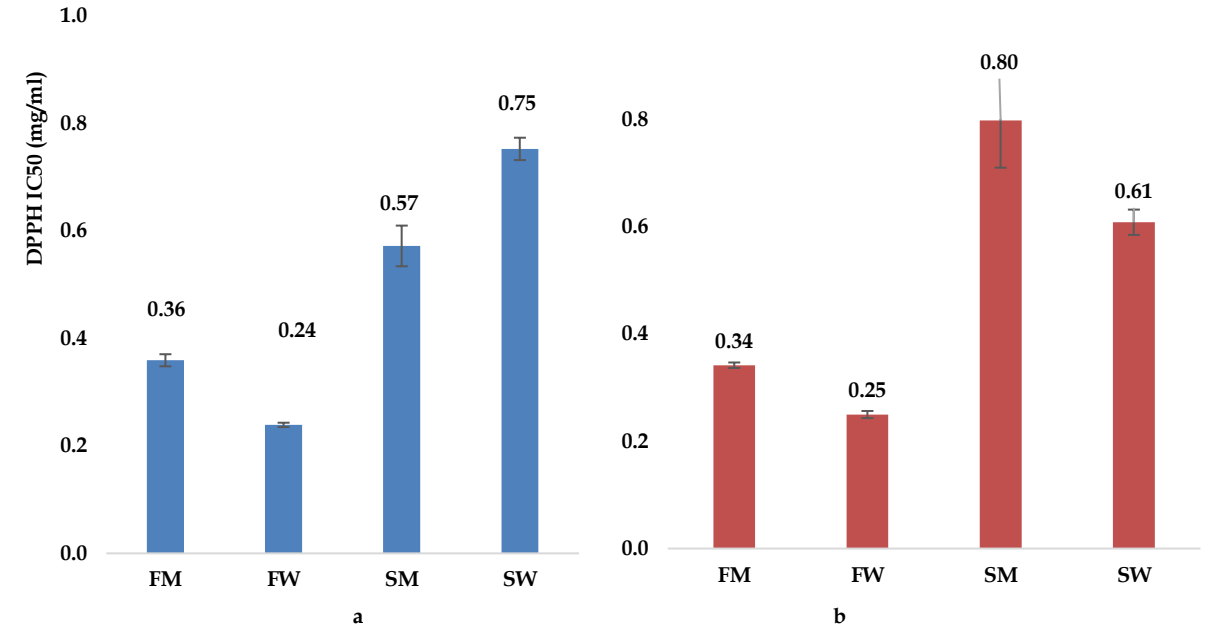



**Figure 6.** DPPH $IC_{50}$ values of (**a**) air-dried and (**b**) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

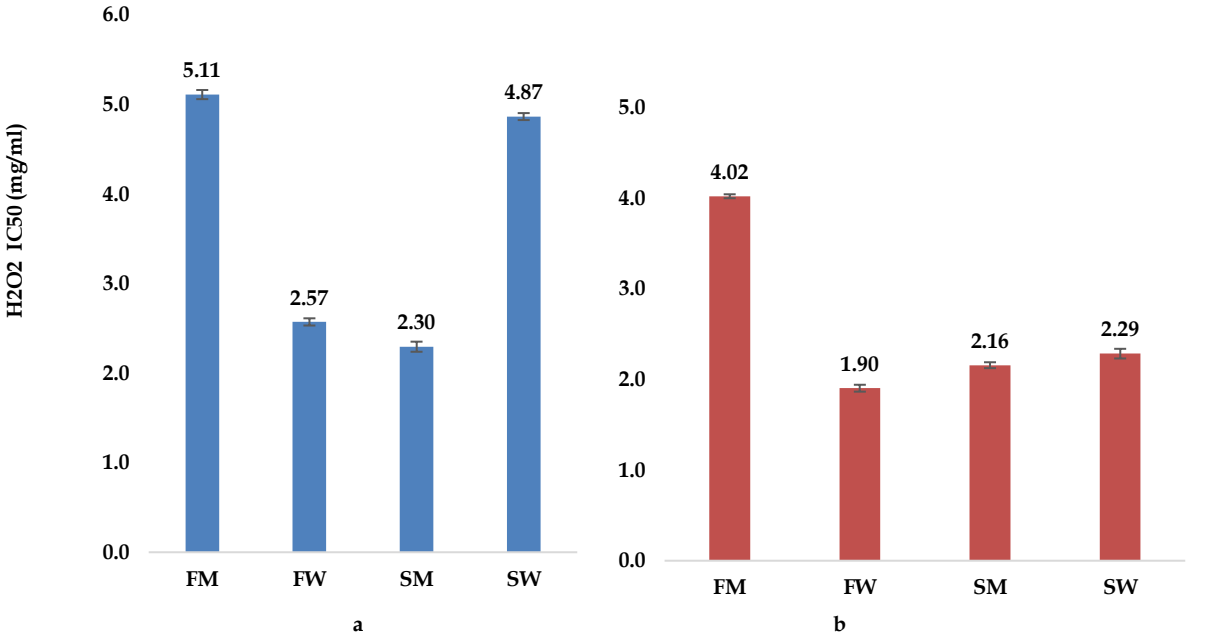



**Figure 7.** $H_2O_2$ $IC_{50}$ values of (**a**) air-dried and (**b**) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

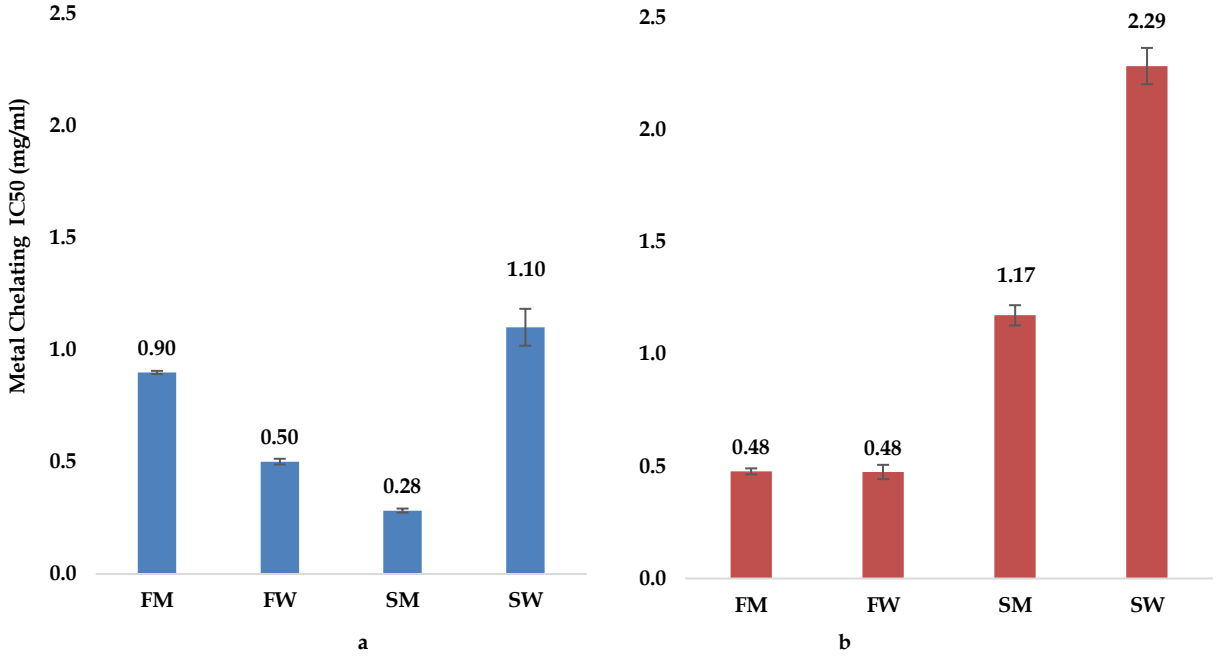



**Figure 8.** Metal chelating $IC_{50}$ values of (**a**) air-dried and (**b**) freeze-dried flowers and flower stalks of *T. violacea* ($p \leq 0.05$). FM: flower-methanol; FW: flower-water; SM: stalk-methanol; SW: stalk-water.

For both drying methods, the methanolic and aqueous extracts of the flowers and stalks of *T. violacea* had $IC_{50}$ values below 1 mg/mL, thus indicating an excellent scavenging activity against DPPH radicals[38]. The very low $IC_{50}$ 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 $IC_{50}$ values than the stalk ($p < 0.001$). This was consistent in both drying methods and solvents that were used. The higher $IC_{50}$ 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 $IC_{50}$ values below 10 mg/mL (upper limit of $IC_{50}$), thus indicating a strong scavenging activity against $H_2O_2$. The results of this study show that the stalks had significantly lower $IC_{50}$ values than the flowers ($p < 0.001$). For the aqueous extracts, the results in this study showed that the flowers had significantly lower $IC_{50}$ values as compared with the stalks ($p < 0.001$). The scavenging activity of $H_2O_2$ 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 $H_2O_2$[40]. Apart from the freeze-dried aqueous extracts, for both drying methods, the $H_2O_2$. Scavenging activity exhibited strong positive correlations with the quantified phytochemicals (**Tables IV** and **V**). There were perfect strong positive correlations ($r = 1$) between the $H_2O_2$ 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 $IC_{50}$ values below 10 mg/mL (upper limit of $IC_{50}$), thus indicating a strong scavenging activity against iron oxidants. Except for the air-dried methanolic extracts, the flowers had significantly lower $IC_{50}$ 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* ($p \leq 0.05$).

| Air-dried samples | | | | |
|---|---|---|---|---|
| **Antioxidant assay** | **TPC** | **TFC** | **TTC** | **TPAC** |
| | | **Flower-Methanol** | | |
| DPPH | 0.737 | 0.808 | 0.808 | 1* |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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* ($p \leq 0.05$).

| Freeze-dried samples | | | | |
|---|---|---|---|---|
| **Antioxidant assay** | **TPC** | **TFC** | **TTC** | **TPAC** |
| | | **Flower-Methanol** | | |
| DPPH | 0.845 | 0.95 | 0.881 | 0.737 |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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 |
| $H_2O_2$ | 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 infections[41]. *Staphylococcus aureus* is linked with skin conditions such as skin and soft tissue infections (SSTI), a common infection[21]. 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** | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *E. coli* | *S. aureus* |
| 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, $H_2O_2$, 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.

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## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020;17(10):3376. DOI: 10.3390/ijerph17103376; PMCID: PMC7277765; PMID: 32408690

2. Cock IE, Van Vuuren SF. The traditional use of southern African medicinal plants for the treatment of bacterial respiratory diseases: A review of the ethnobotany and scientific evaluations. J Ethnopharmacol. 2020;263:113204. DOI: 10.1016/j.jep.2020.113204; PMCID: PMC7383173; PMID: 32730881

3. Mahapatra AD, Bhowmik P, Banerjee A, Das A, Ojha D, Chattopadhyay D. Chapter 3 - Ethnomedicinal Wisdom: An Approach for Antiviral Drug Development. In: Khan MSA, Ahmad I, Chattophadhyay, editors. New Look to Phytomedicine: Advancements in Herbal Products as Novel Drug Leads. Cambridge, Massachusetts: Academic Press; 2019. pp. 35–61. DOI: 10.1016/B978-0-12-814619-4.00003-3; PMCID: PMC7149776

4. Magtalas MC, Balbin PT, Cruz EC, Clemente RF, Buan AKG, Garcia JP, et al. Ethnomedicinal plants used for the prevention and treatment of anemia in the Philippines: a systematic review. Trop Med Health. 2023;51(1):27. DOI: 10.1186/s41182-023-00515-x; PMCID: PMC10176884; PMID: 37170350

5. Fibrich BD, Lall N. Chapter 10 - Maximizing Medicinal Plants: Steps to Realizing Their Full Potential. In: Lall N, editor. Medicinal Plants for Holistic Health and Well-Being. Cambridge, Massachusetts: Academic Press; 2018. pp. 297–300. DOI: 10.1016/B978-0-12-812475-8.00010-X

6. Madike LN, Takaidza S, Ssemakalu C, Pillay M. Genotoxicity of aqueous extracts of Tulbaghia violacea as determined through an Allium cepa assay. S Afr J Sci. 2019;115(1/2):4391. DOI: 10.17159/sajs.2019/4391

7. Danquah CA, Minkah PAB, Agana TA, Moyo P, Ofori M, Doe P, et al. The Phytochemistry and Pharmacology of Tulbaghia, Allium, Crinum and Cyrtanthus: 'Talented' Taxa from the Amaryllidaceae. Molecules. 2022;27(14):4475. DOI: 10.3390/molecules27144475; PMCID: PMC9316996; PMID: 35889346

8. Styger G, Aboyade OM, Gibson D, Hughes G. Tulbaghia--A Southern African Phytomedicine. J Altern Complement Med. 2016;22(4):255-61. DOI: 10.1089/acm.2014.0406; PMID: 27008423

9. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of Tulbaghia violacea Harv. (Wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med. 2015;15:408. DOI: 10.1186/s12906-015-0932-9; PMCID: PMC4647322; PMID: 26577219

10. Frontini A, De Bellis L, Luvisi A, Blando F, Allah SM, Dimita R, et al. The green leaf vlatile (Z)-3-Hexenyl acetate is differently emitted by varieties of Tulbaghia violacea plants routinely and after wounding. Plants. 2022;11(23):3305. DOI: 10.3390/plants11233305; PMCID: PMC9739665; PMID: 36501344

11. Phelan S, Douglas G, Hunter A. Micropropagation and growth regulation of Tulbaghia violacea Silver Lace. Acta Hortic. 2007;764:113-8. DOI: 10.17660/ActaHortic.2007.764.13

12. Kulkarni MG, Sparg SG, van Staden J. Influence of temperature and watering frequencies on seed germination and seedling growth of Ornithogalum longibracteatum and Tulbaghia violacea. Sci Hortic. 2005;107(1):103-9. DOI: 10.1016/j.scienta.2005.08.001

13. Madike LN, Takaidza S, Pillay M. Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of Tulbaghia violacea. Int J Pharmacogn Phyto Res. 2017;9(10):1300-8. DOI: 10.25258/phyto.v9i10.10453

14. Krstin S, Sobeh M, Braun MS, Wink M. Tulbaghia violacea and Allium ursinum Extracts Exhibit Anti-Parasitic and Antimicrobial Activities. Molecules. 2018;23(2):313. DOI: 10.3390/molecules23020313; PMCID: PMC6017524; PMID: 29393867

15. Turabi KS, Sannakki JA, Aich J, Garse S, Iyer D, Devarajan S. Chapter 10 - Computational screening of phytochemicals for anti-bacterial drug discovery. In: Egbuna C, Rudrapal M, Tijjani H, editors. Phytochemistry, Computational Tools and Databases in Drug Discovery. Amsterdam: Elsevier; 2023. pp. 213–43. DOI: 10.1016/b978-0-323-90593-0.00011-3

16. Takaidza S, Mtunzi F, Pillay M. Analysis of the phytochemical contents and antioxidant activities of crude extracts from Tulbaghia species. J Tradit Chin Med. 2018;38(2):272-9. DOI: 10.1016/j.jtcm.2018.04.005

17. 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

18. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020;8(2):603–8. DOI: 10.22271/chemi.2020.v8.i2i.8834

19. Khan H, Pervaiz A, Intagliata S, Das N, Venkata KCN, Atanasov AG, et al. The analgesic potential of glycosides derived from medicinal plants. Daru. 2020;28(1):387–401. DOI: 10.1007/s40199-019-00319-7; PMCID: PMC7214601; PMID: 32060737

20. Rajkumar G, Panambara PAHR, Sanmugarajah V. Comparative Analysis of Qualitative and Quantitative Phytochemical Evaluation of Selected Leaves of Medicinal Plants in Jaffna, Sri Lanka. Borneo J Pharm. 2022;5(2):93–103. DOI: 10.33084/bjop.v5i2.3091

21. Basson DC, Teffo TK, Risenga IM. A Phytochemical Screening, Antioxidant and Antibacterial Activity Analysis in the Leaves, Stems and Roots of Portulacaria afra. J HerbMed Pharmacol. 2022;12(1):109–17. DOI: 10.34172/jhp.2023.10

22. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020;25(22):5243. doi: 10.3390/molecules25225243; PMCID: PMC7697716; PMID: 33187049

23. Teffo TK, Dukhan S, Ramalepe P, Risenga IM. 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

24. Al-Snafi AE. Oils and Fats Contents of Medicinal Plants, as Natural Ingredients for Many Therapeutic Purposes- a Review. IOSR J Pharm. 2020;10(7):1-41.

25. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in Pharmacological Activities of Terpenoids. Nat Prod Commun. 2020;15(3):1934578X2090355. DOI: 10.1177/1934578x20903555

26. Fan M, Yuan S, Li L, Zheng J, Zhao D, Wang C, et al. Application of Terpenoid Compounds in Food and Pharmaceutical Products. Fermentation. 2023;9(2):119. DOI: 10.3390/fermentation9020119

27. Haruna S, Aliyu BS, Bala A. Plant Gum Exudates (Karau) and Mucilages, Their Biological Sources, Properties, Uses and Potential Applications: A Review. Bayero J Pure Appl Sci. 2016;9(2):159-65. DOI: 10.4314/bajopas.v9i2.30

28. de Jongh S, Vissers MN, Rol P, Bakker HD, Kastelein JJP, Stroes ESG. Plant Sterols Lower LDL Cholesterol without Improving Endothelial Function in Prepubertal Children with Familial Hypercholesterolaemia. J Inherit Metab Dis. 2003;26(4):343-52. DOI: 10.1023/a:1025155002348; PMID: 12971422

29. Cabral CE, Klein MRST. Phytosterols in the Treatment of Hypercholesterolemia and Prevention of Cardiovascular Diseases. Arq Bras Cardiol. 2017;109(5):475-82. DOI: 10.5935/abc.20170158; PMCID: PMC5729784; PMID: 29267628

30. Ríos JL. Effects of Triterpenes on the Immune System. J Ethnopharmacol. 2010;128(1):1-14. DOI: 10.1016/j.jep.2009.12.045; PMID: 20079412

31. Farooqui AA. Chapter 9 - Use of phytochemicals for the treatment of insulin resistance–linked visceral and neurological disorders. In: Farooqui AA, editor. Insulin Resistance as a Risk Factor in Visceral and Neurological Disorders. Cambridge, Massachusetts: Academic Press; 2020. pp. 385–438. DOI: 10.1016/b978-0-12-819603-8.00009-2

32. Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, et al. Pharmacological Effects of Gallic Acid in Health and diseases: a Mechanistic Review. Iran J Basic Med Sci. 2019;22(3):225-37. DOI: 10.22038/ijbms.2019.32806.7897; PMCID: PMC6528712; PMID: 31156781

33. David AVA, Arulmoli R, Parasuraman S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. Pharmacogn Rev. 2016;10(20):84-9. DOI: 10.4103/0973-7847.194044; PMCID: PMC5214562; PMID: 28082789

34. Fan FY, Sang LX, Jiang M. Catechins and Their Therapeutic Benefits to Inflammatory Bowel Disease. Molecules. 2017;22(3):484. DOI: 10.3390/molecules22030484; PMCID: PMC6155401; PMID: 28335502

35. Aruoma OI. Free radicals, Oxidative stress, and Antioxidants in Human Health and Disease. J Am Oil Chem Soc. 1998;75(2):199-212. DOI: 10.1007/s11746-998-0032-9; PMCID: PMC7101596; PMID: 32287334

36. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Faizal A, Modu B, et al. Oxidative stress, Free Radicals and antioxidants: Potential Crosstalk in the Pathophysiology of Human Diseases. Front Chem. 2023;11:1158198. DOI: 10.3389/fchem.2023.1158198; PMCID: PMC10206224; PMID: 37234200

37. Olszowy-Tomczyk M. How to Express the Antioxidant Properties of Substances properly? Chem Papers. 2021;75(12):6157–67. DOI: 10.1007/s11696-021-01799-1

38. Aykul S, Martinez-Hackert E. Determination of half-maximal Inhibitory Concentration Using biosensor-based Protein Interaction Analysis. Anal Biochem. 2016;508:97–103. DOI: 10.1016/j.ab.2016.06.025; PMCID: PMC4955526; PMID: 27365221

39. Takaidza S, Mtunzi F, Pillay M. Analysis of the Phytochemical Contents and Antioxidant Activities of Crude Extracts from Tulbaghia Species. J Tradit Chin Med. 2018;38(2):272-9. DOI: 10.1016/j.jtcm.2018.04.005; PMID: 32186066

40. Okoro IO. Effects of Extraction Solvents on the Antioxidant and Phytochemical Activities of Manihot Esculenta Leaves. Iran J Toxicol. 2020;14(1):51–8. DOI: 10.32598/ijt.14.1.51

41. Gambushe SM, Zishiri OT, El Zowalaty ME. Review of Escherichia coli O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective. Infect Drug Resist. 2022;15:4645-73. DOI: 10.2147/idr.s365269; PMCID: PMC9420067; PMID: 36039321