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RESEARCH ARTICLE OPEN ACCESS

Bioactive Compound Characterization and Phytopharmacological Potentials of *Tulbaghia violacea* Fruits and Seeds

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Received: 3 December 2024 | **Revised:** 29 January 2025 | **Accepted:** 11 February 2025

Keywords: antibacterial | antioxidant | ESKAPE pathogens medicinal plants | *Tulbaghia violacea*

ABSTRACT

Tulbaghia violacea is an established medicinal plant that is indigenous to southern Africa. All its plant parts have been profiled for their phytochemical constituents and medicinal potentials except for the seeds and fruits. Thus, this study assessed the seeds (air-dried) and fruits (freeze-dried), extracted with six solvents, for their bioactive compounds, antioxidant capacities, and antibacterial activities. All the 10 tested phytochemicals were detected across the six solvents, with more phytochemicals detected in the fruits. The fruit aqueous extract gave the highest yield (37.4%), while the hexanoic fruit extract had the lowest extraction yield (3.27%). The fruit had higher phenolic content across the solvents except in methanol. Conversely, except in hexanoic extracts, the seed had higher total proanthocyanidin contents across the solvents. In addition, the fruit had a higher total antioxidant capacity than the seeds, similar to the observation in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. *T. violacea* fruit and seeds showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*, but this activity was dose-dependent. However, neither the fruit nor the seed extract had any antibacterial effect on *Klebsiella pneumoniae*. This study showed that *T. violacea* fruits and seeds may be additional resources with medicinal benefits for human use.

1 | Introduction

Tulbaghia genus, in the Amaryllidaceae [1], is indigenous to southern African countries such as Lesotho, Malawi, Botswana, Swaziland, Zimbabwe, and South Africa. It is endemic to the Eastern Cape of South Africa but well distributed in other provinces such as Gauteng, Limpopo, and Western Cape [2–4]. The genus comprises about 30 species, of which *T. violacea* is one of the most studied species for medicinal purposes [3–5].

Tulbaghia violacea Harv. derived its binomial name from (i) Ryk Tulbagh—governor of the Cape of Good Hope (1699–1771) and (ii) the violet flowers of the species, respectively. It is commonly called "wild or society garlic." Other names include "isihaqa/sikwa," "moelela/sefothafotha," "ivimba/mpunzi," and "knoflook/wilde knoffel" in IsiZulu, IsiSotho, IsiKhoxa, and Afrikaans, respectively [4]. It is a perennial bulb that is characterized by long and narrow leaves that give a garlic-like smell. Its purple flowers are held on thin and long (≈ 30 cm) stalks. Its fruits are small (≈ 1–2 cm long) triangular capsules that are dry at maturity. The capsules split open at maturity, each containing 10–20 black, oval and flat seeds that are 2–3 mm long [6] (Figure 1).

T. violacea is a well-known medicinal plant in southern Africa and it is popular among the traditional folks of South Africa. For example, the Xhosa people use *T. violacea* as an immune booster, to relieve toothache and as a blood pressure regulator. It is used

FIGURE 1 | Diagram showing *T. violacea* (A) blooming plants, (B) fruits, and (C) seeds.

to treat arthritis, intestinal worms and headaches by the Sotho people. Zulus use it to treat indigestion, stomachache, cough, asthma, cold and as a topical treatment for wound infections. Culturally, the Zulus uses it as a protective charm, believing it wards off evil. It is also used as a snake-repellant [2]. In addition, *T. violacea* is used for culinary purposes, in which the leaves and flowers are used in salads, soups, stews, and garnishes [5]. It is also used as an ornamental plant because of its bright purple flowers [7]. The species, easily identified by its conspicuous and radiant violet flowers, can be seen in gardens, potted plants at homes, and public places like malls, hospitals, schools, and garages, among many others.

Various parts of *T. violacea*, that is, the leaves, stems, roots, bulbs, flowers, and flower stalks, have been investigated for phytochemical constituents and medicinal potential [6, 8, 9]. Some of these phytochemicals include phenolic compounds, flavonoids, glycosides, volatile oils, quinones, and many more. Phenolic compounds are useful in the cosmetic, food, and pharmaceutical industries. The compounds have antiaging, anti-inflammatory, and antioxidant benefits, among others [10]. Flavonoids, which are derivatives of phenolic compounds, have over 9000 derivatives. They are beneficial for humans due to their anticancer, antimicrobial, cardioprotective, antioxidant, antidiabetic, and antiviral effects [11]. Glycosides have similar benefits to flavonoids but in addition, they have antiseptic and analgesic properties [12]. Volatile oils, which are obtainable in some plant families and species, have therapeutic applications, including the treatments of joint, gastrointestinal, psychiatric, respiratory, and muscle problems [13]. Quinones are not only found in plants but also in algal, fungal, and bacterial organisms. These phytochemicals have antimalarial properties in addition to some properties of phenolic and flavonoid compounds [14].

However, there is no evidence of phytochemical screening for fruits and seeds. From personal observations, this may be due to *T. violacea* being mainly cultivated for ornamental purposes because of its flowers. Allowing the growth of *T. violacea* to the fruiting stage implies that the flowers will wither, which is a loss of its ornamental purpose. Thus, to prevent such loss, flowers are continually trimmed off at the earliest sign of aging. This process consequently prevents fruiting and seed production.

This research aimed to screen *T. violacea* fruits and seeds for phytochemical constituents, potential antioxidant, and biological activities in vitro. As the population of the world is increasing, so is the demand for, and use of, resources. Particularly, the demand for medicinal plant resources is on the rise because of their cost-effectiveness, little or no side effects, health benefits, as well as an increase in awareness and growing interest of the pharmaceutical companies [15]. However, these perceived benefits often drive overexploitation. Unfortunately, fruits and seeds are not often screened for phytochemical constituents and medicinal potentials in comparison with other plant parts such as leaves and roots. Hence, such a study on fruits and seeds, as conducted in this research, can help to expand and maximize the available resources from a single species, thus minimizing overexploitation.

2 | Results and Discussion

2.1 | Qualitative Analysis

Some authors have carried out qualitative phytochemical screening on the various parts of *T. violacea*. According to [16], flavonoids, terpenoids, saponins, and quinones were detected in the methanolic extracts of roots, rhizomes, stems, and leaves of *T. violacea*. In addition, glycosides, tannins, and steroids were moderately present in the acetone extract of the leaves [3], coumarin in aqueous and ethanol extracts of leaves [8], volatile constituents and sterols in the hexane extracts of flowers and callus of flowers [17], phlobatannins, cardiac glycosides and phytosterols in the aqueous and methanolic extracts of flowers and flower stalks [6]. In the current investigation, most of the phytochemicals are also present in the fruits and seeds of *T. violacea*, with the fruits showing more phytochemicals than the seeds (Table 1), which was similar to the observation of Ojo et al. [18]. Water (aqueous) extracted more phytochemicals than the other five solvents (Table 1). Notably, all the previous authors [3, 6, 8, 16, 17] cited earlier did not detect alkaloids in their analyses. In contrast, alkaloids were detected in trace amounts in the acetonic extract of the fruits and seeds, including the methanolic extract of the seeds, but more abundantly in the aqueous extract of the seeds (Table 1). Phytochemicals that are poisonous and lethal to animals and humans when consumed have been associated with those in the alkaloid group [19–21]. Hence, the detection of alkaloids, particularly in the *T. violacea* seeds may be an indication of caution regarding the consumption of the seeds.

TABLE 1 | Qualitative screening of phytochemicals in *Tulbaghia violacea* fruits and seeds.

PC	Met	Aq	Eth	EA	Ace	Hex	Met	Aq	Eth	EA	Ace	Hex
	***T. violacea* fruits**						***T. violacea* seeds**					
Saponins	—	—	+	++	—	—	—	++	—	+	—	+
Terpenoids	—	++	—	—	—	—	—	+	—	—	—	+
Glycosides	+++	+++	+++	+	+++	—	—	+++	—	+	—	—
Steroids		++		+	+++		—	+	—	+	+++	++
Volatile oils	+	+	+	+	++	++	—	—	—	—	—	—
Coumarins	++	++	++	++	++	+	—	+	—	—	—	—
Phlobatannins	+	—	+	+	+	+	—	—	—	—	—	—
Alkaloids	—	—	—	—	+	—	+	+++	—	—	+	—
Phenolics	++	+++	++	+	+	—	+	++	+	+	+	+
Tannin												
Bromine H_2O test	—	+	—	—	—	—	—	+	—	—	+	—
$FeCl_3$ test	—	+++	—	—	—	—	—	++	—	—	—	—
Quinones	+	+++	+	+	++	—	++	+++	++	++	+++	—
Cardiac glycosides	+++	+++	+++	++	+++	—	—	++	+++	+	++	—
Flavonoid												
NaOH test	—	+++	—	—	—	—	—	+	—	—	—	—
C. H_2SO_4 test	—	+++	—	+	+++	—	+	+++	++	++	+++	—
C. HCl test	—	—	—	—	—	—	—	—			—	—
Yield (%)	23.43	37.4	7.03	3.93	5.77	3.27	10.4	29.63	7.73	6.77	5.37	8.73

Abbreviations: Ace, acetone; Aq, aqueous; EA, ethyl acetate; Eth, ethanol; Hex, hexane; Met, methanol; PC, phytochemicals.

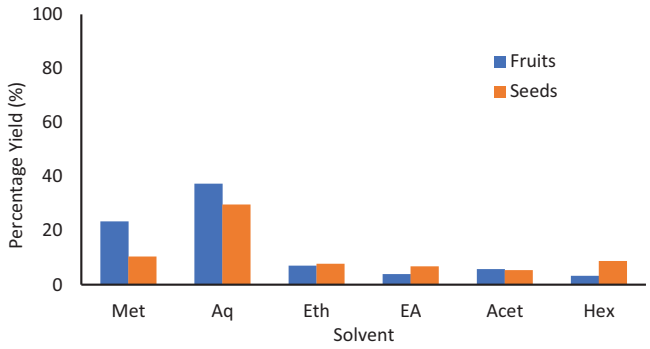

FIGURE 2 | Percentage yield of extracts from the seeds and fruits of *Tulbaghia violacea*.

The polar, aqueous solvent extracted the highest quantity of phytochemicals (37.4% and 29.63% in fruits and seeds, respectively), while the nonpolar solar ethyl acetate and hexane fruit extracts were the least (3.93% and 3.27%, respectively) (Figure 2). Polar solvents often extract more phytochemicals than nonpolar solvents. However, other factors can influence extraction yield, such as temperature, pH, particle size, and method of extraction [22].

2.2 | Quantitative Analysis

The total phenolic content (TPC) in *T. violacea* ranged from 4.00 to 35.82 mg gallic acid equivalent per mL (mg GAE/mL). Methanol extracted more TPC, while hexane extracted the least. TPC was higher in the fruits than in the seeds in almost all the solvents, except methanolic extracts, which had higher TPC in the seeds. The differences between the TPC of the fruits and seeds were significant for each solvent, except for aqueous and hexane extract (Table 2). The total flavonoid content (TFC) ranged from 2.54 to 5.44 mg quercetin equivalent per mL (mg QE/mL). Water (aqueous solvent) extracted the highest TFC. The difference in TFC between seeds and fruits of *T. violacea* was insignificant for each solvent (Table 2). In most cases, the values of flavonoid content were smaller than phenolic content. This is because flavonoids are a subset of the phenolic group of phytochemicals [23, 24].

In *T. violacea* fruits and seeds, the highest total tannin content (TTC) was in aqueous extract, while the least was recorded for hexane and methanolic extracts of fruits and seeds, respectively (Table 2). On the other hand, the lowest total proanthocyanidin content (TPAC) was recorded in aqueous extract for both fruits and seeds. This is because proanthocyanidins are a group of tannins that are not hydrolyzable [25]. Ethyl acetate and acetone solvents extracted the highest TPAC in fruits and seeds, respectively (Table 2).

The presence of bioactive phytochemicals (Tables 1 and 2) confers medicinal properties on *T. violacea*. These include antioxidant, anti-inflammatory, antibacterial, anti-HIV, antithrombotic, antidiabetic, anticancer, and anticoagulant properties [2, 3, 6, 8, 16, 26–28]. The concentration of total phenolics, flavonoids, tannins, and proanthocyanidins in the fruits and seeds of *T.*

TABLE 2 | Quantification of total phenolic (TPC), total flavonoid (TFC), total tannin (TTC), and total proanthocyanidin (TPAC) contents of *Tulbaghia violacea* fruits and seeds.

Solvent	TPC (mg GAE/mL)	TFC (mg QE/mL)	TTC (mg GAE/mL)	TPAC (mg CE/mL)
Met-Fr.	28.24 ± 1.43^{a}	3.70 ± 0.67^{aegh}	3.20 ± 0.26^{ad}	0.12 ± 0.01^{ab}
Met-Sd	35.82 ± 1.31^{b}	3.55 ± 0.21^{abegh}	1.70 ± 0.06^{b}	0.13 ± 0.00^{a}
Aq-Fr	5.59 ± 0.18^{c}	5.31 ± 0.06^{c}	5.16 ± 0.02^{c}	0.08 ± 0.01^{b}
Aq-Sd	4.91 ± 0.36^{c}	5.44 ± 0.35^{c}	5.44 ± 0.22^{c}	0.11 ± 0.00^{ab}
Eth-Fr	22.11 ± 1.49^{d}	3.4 ± 0.28^{adegh}	2.72 ± 0.20^{defg}	0.18 ± 0.01^{c}
Eth-Sd	19.45 ± 0.39^{e}	4.00 ± 0.16^{agh}	2.99 ± 0.27^{ae}	0.47 ± 0.01^{d}
EA-Fr	10.02 ± 0.32^{f}	3.22 ± 0.31^{afh}	3.22 ± 0.12^{a}	0.38 ± 0.02^{e}
EA-Sd	3.44 ± 0.4^{c}	2.84 ± 0.03^{bdfi}	2.29 ± 0.02^{fhi}	0.49 ± 0.01^{d}
Acet-Fr	13.90 ± 0.25^{g}	2.54 ± 0.15^{f}	2.91 ± 0.11^{ag}	0.27 ± 0.02^{f}
Acet-Sd	4.41 ± 0.06^{c}	2.87 ± 0.03^{efi}	2.56 ± 0.08^{deh}	0.74 ± 0.01^{g}
Hex-Fr	4.11 ± 0.15^{c}	$4.2.4 \pm 0.28^{g}$	2.37 ± 0.23^{di}	0.13 ± 0.02^{a}
Hex-Sd	4.00 ± 0.10^{c}	3.63 ± 0.18^{ghi}	1.88 ± 0.01^{bf}	0.12 ± 0.00^{ab}

Note: Different superscripts within each column implied significant differences.
Abbreviations: CE, catechin equivalent; Fr, fruit; GAE, gallic acid equivalent; QE, quercetin equivalent; Sd, seed.

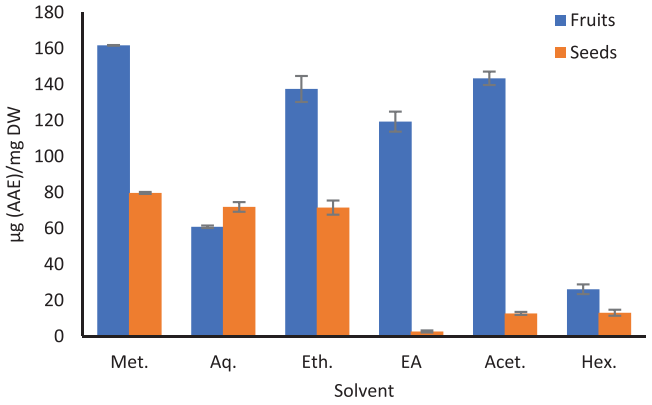

FIGURE 3 | Total antioxidant capacity of 1 mg/mL *Tulbaghia violacea* fruit and seed.

violacea (Table 2) were comparable with those found in roots, leaves, stems, rhizomes, bulbs, flowers, and flower stalks of *T. violacea* [3, 6, 8, 16, 26, 28].

TABLE 3 | IC_{50} of *Tulbaghia violacea* fruits and seeds on the scavenging of DPPH and reduction of Fe^{3+}.

Solvents	IC_{50} + SD (mg/mL)	
	DPPH	Metal chelating
Met-Fr	7.57 ± 0.10	2.06 ± 0.17
Aq-Fr	14.24 ± 0.07	61.98 ± 1.86
Eth-Fr	74.39 ± 105.65	18.98 ± 0.38
EA-Fr	5.69 ± 0.25	32.93 ± 8.89
Acet-Fr	6.43 ± 0.15	12.83 ± 0.10
Hex-Fr	16.47 ± 6.39	4.67 ± 0.02
Met-Sd	16.41 ± 0.29	5.47 ± 0.03
Aq-Sd	47.72 ± 27.13	1.07 ± 0.08
Eth-Sd	56.59 ± 19.46	10.09 ± 0.15
EA-Sd	93.65 ± 64.94	20.02 ± 23.31
Acet-Sd	39.41 ± 19.09	2.34 ± 0.05
Hex-Sd	11.45 ± 2.67	42.75 ± 20.81

2.3 | In Vitro Antioxidant Assays

The fruits of *T. violacea* showed a higher total antioxidant capacity (TAC) than the seeds. The highest capacity (161.73 µg ascorbic acid equivalent [AAE] per mg dry weight [DW]) was recorded for the methanolic extract of the fruit, while the least (2.71 µg AAE/mg DW) was in the ethyl acetate extract of the seeds (Figure 3).

Specifically, the antioxidant potential of 1 mg/mL *T. violacea* extracts was tested against oxidants such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Fe^{3+} molecules (Figures 4 and 5; Table 3). Oxidants, also known as reactive oxygen species (ROS), are unstable molecules that are produced naturally in the body. ROS are beneficial to the body at low concentrations [29]. However, at higher concentrations, they cause oxidative stress, which has damaging, degenerative effects on the human body [30]. Naturally, the body produces enzymatic antioxidants to reduce the harmful effects of ROS [31]. However, insufficient antioxidant activities result in the negative effects of ROS in the body [32].

The antioxidant activity of *T. violacea* against DPPH showed that the fruits (Figure 4A) scavenged more DPPH than the seeds (Figure 4B) in all the solvents used. In addition, methanol, ethyl acetate, and acetone fruit extracts, at 0.1 mg/mL, scavenged higher DPPH molecules (35.17%, 42.46%, and 42.70%,

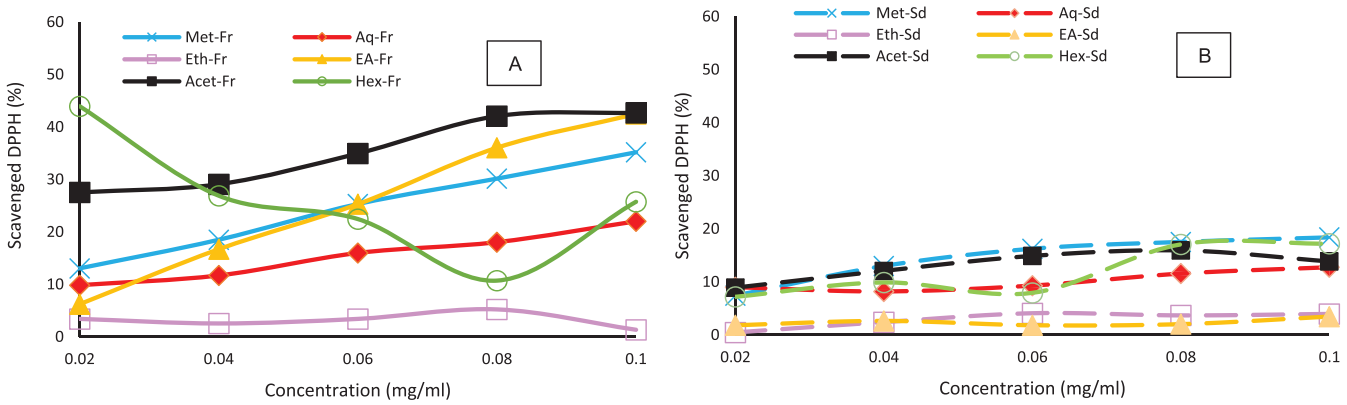

FIGURE 4 | Scavenging of DPPH by phytochemicals from *Tulbaghia violacea* (A) fruits and (B) seeds.

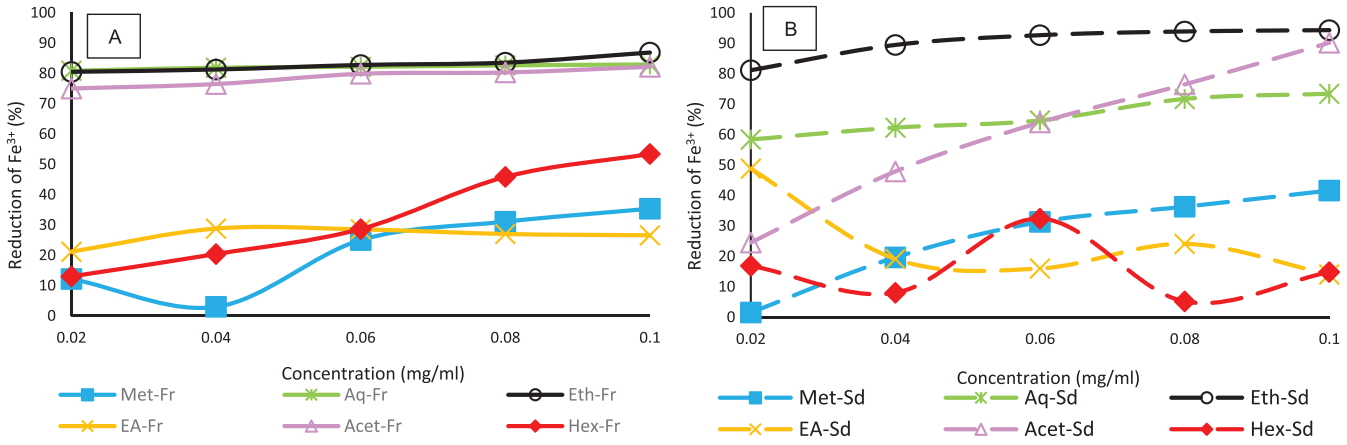

FIGURE 5 | Reduction of Fe^{3+} to Fe^{2+} by phytochemicals extracted from *Tulbaghia violacea* fruits (A) and seeds (B), using six different solvents.

respectively) than other solvents (Figure 4A). Similarly, the same concentration (100 μg/mL) of acetone and aqueous leaf extracts of *T. violacea* had about 45%–50% inhibition against DPPH [3, 33]. According to Olorunnisola et al. [34], the methanolic extracts of the fresh and dried *T. violacea* rhizomes had between 60%–70% scavenging of DPPH at 50 μg/mL.

The reducing effect of *T. violacea* extracts on Fe^{3+} was highest in ethanol extracts of both fruits and seeds (82.74% and 92.65% respectively), and in the aqueous and acetone fruit extracts (82.92% and 79.82%, respectively) (Figure 5). Unlike in DPPH, the fruit and seed extracts seemed to have the same reducing effect on Fe^{3+} (Figure 5). Acetone extract, especially from the fruit of *T. violacea*, maintained its relatively high antioxidant activities (Figures 3–5). The reducing ability of *T. violacea* could be an indication of its potential to eliminate excessive heavy metal accumulation both in humans and in the environment [35].

The half maximal inhibitory concentration, that is, the IC_{50}, is the concentration of extracts required to have a 50% counter-effect, such as scavenging or inhibitory, on damaging oxidative molecules like DDPH. IC_{50} values have an inversely proportional relationship with the potency of plant extracts. Thus, in *T. violacea*, ethanol, acetone, and methanol fruit extracts had a higher scavenging effect against DPPH (IC_{50} were less than 10 mg/mL) while ethyl acetate seed extract had the least (93.65 mg/mL). On the other hand, methanol and hexane fruit extracts and aqueous, acetone, and methanol seed extracts showed higher potential in reducing Fe^{3+} (IC_{50} = 2.06, 4.67, 1.07, 2.34, and 5.47 mg/mL, respectively) while aqueous fruit had the least (61.98 mg/mL) (Table 3).

The combination of percentage inhibition (DPPH) or reduction (Fe^{3+}) and IC_{50} indicates the antioxidant potential of *T. violacea* fruits and seeds. Plant extracts are nonenzymatic antioxidant materials, which modulate the activities of unstable and deleterious oxidative molecules. The modulation is by donating electrons to the oxidative molecules and by disrupting their chain reactions, leading to their reduction and stability, thus inhibiting their harmful effects on the body [31].

2.4 | Antibacterial Assay

The seeds of *T. violacea* presented higher antibacterial activity against *Escherichia coli* in comparison with the fruits (Table 4). The antibacterial effects on *Staphylococcus aureus* were similar for both fruit and seed extracts (Table 4). The antibacterial activity was dose-dependent, with the 100 μL extracts having higher effects than the lower doses in most cases. For example, all the lower doses of the fruit extracts had no effect on *E. coli* except the highest tested dose of 100 μL. Similarly, the same highest tested

TABLE 4 | The antibacterial effect of *Tulbaghia violacea* crude extracts on *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, measured by the zone of inhibition (mm).

	Met-Fr	Aq-Fr	Eth-Fr	EA-Fr	Ac-Fr	Hex-Fr	Met-Sd	Aq-Sd	Eth-Sd	EA-Sd	Ac-Sd	Hex-Sd
E. coli												
5 μL	0	0	0	0	0	0	0	2.5 ± 3.53	0	9.0 ± 12.73	0.5 ± 3.53	9.0 ± 12.73
10 μL	0	0	0	0	0	0	13.0 ± 1.41	8.0 ± 0.0	0	8.0 ± 11.31	13.0 ± 0	15.5 ± 3.54
20 μL	0	0	0	0	0	0	17.5 ± 0.71	9.0 ± 1.41	13.0 ± 0	20.5 ± 12.02	17.0 ± 1.41	11.0 ± 15.56
100 μL	14.67 ± 1.15	0	13.67 ± 1.53	12.67 ± 0.58	9.67 ± 0.58	15.33 ± 3.21	17.67 ± 1.53	15.0 ± 1.0	16.33 ± 1.53	18.67 ± 1.15	12.0 ± 1.0	13.0 ± 1.73
S. aureus												
5 μL	0	0	0	0	0	0	0	9.5 ± 0.71	0	0	0	0
10 μL	0	9.5 ± 0.71	0	0	0	4.5 ± 6.36	0	0	0	0	0	0
20 μL	0	11.5 ± 2.12	0	11.5 ± 0.71	0	0	0	12.0 ± 0	0	0	0	0
100 μL	10.67 ± 0.58	10.0 ± 1.0	9.67 ± 0.58	9.0 ± 1.0	9.33 ± 0.58	0	13.33 ± 0.58	0	16.33 ± 0.58	16.67 ± 2.31	9.33 ± 0.58	0
E. faecalis												
5 μL	0	0	0	0	0	0	0	0	0	0	0	0
10 μL	0	0	0	0	0	0	0	0	0	0	0	0
20 μL	0	0	0	0	0	0	0	0	0	0	0	0
100 μL	21.33 ± 2.08	21.67 ± 3.21	19.67 ± 1.15	22.33 ± 2.08	18.67 ± 1.15	19.0 ± 2.0	22.67 ± 4.16	19.67 ± 1.53	22.67 ± 3.06	26.33 ± 1.53	0	0
K. pneumonia												
5 μL	0	0	0	0	0	0	0	0	0	0	0	0
10 μL	0	0	0	0	0	0	0	0	0	0	0	0
20 μL	0	0	0	0	0	0	0	0	0	0	0	0
100 μL	0	0	0	0	0	0	0	0	0	0	0	0

dose of fruit and seed extracts was effective against *Enterococcus faecalis*. However, none of the tested doses, as well as extract solvents, had any antibacterial effect on *Klebsiella pneumoniae* (Table 4). Considering only the 100 μL dose, ethyl acetate seed extract had the highest zones of inhibition (ZOIs) against *E. coli* (18.67 ± 1.15 mm), *S. aureus* (16.67 ± 2.31 mm), and *E. faecalis* (26.33 ± 1.53 mm). Pure solvents without the extract, which were used as negative controls, showed no ZOIs and hence did not exhibit antibacterial potential (data not included).

The tested microorganisms are clinically significant microorganisms that affect human health, require treatment, and thus influence drug use and discovery. *E. coli* are Gram-negative bacteria that are naturally found in the human's intestine. They are usually harmless and sometimes are beneficial to humans. The pathogenic strains, however, are associated with food poisoning, urinary tract infections (UTIs), diarrhea, neonatal meningitis, and skin infections [36–38]. *S. aureus* are Gram-positive bacteria that start off as natural commensal colonizers of humans to harmful pathogens [39]. They cause mild (such as skin and wound infections) to severe and sometimes, lethal diseases such as sepsis, pneumonia, lung infections, and heart failure [39–41]. Gram-positive *E. faecalis* are anaerobic commensal bacteria that inhabit the oral cavity and the gastrointestinal tract (GIT) [42]. They are often opportunistic pathogens and are known as the leading cause of nosocomial infections [43]. They are pathogens of the dental root canal systems and are responsible for unsuccessful endodontic treatments [42, 44]. *K. pneumoniae*, a habitual commensal colonizer of the mammalian gut, is a Gram-negative pathogen that is responsible for a wide range of infections and diseases [45, 46]. Some of these include nosocomial infections such as surgical wound infections, community-acquired infections such as UTIs, pneumonia, and invasive infections such as liver abscess [46].

Three of the tested bacteria—*E. faecalis*, *S. aureus*, and *K. pneumoniae*—are part of the ESKAPE pathogens. These are critically multidrug-resistant bacterial species that require combination therapies for effective treatments [47, 48]. Antibacterial resistance is also increasing in *E. coli*, drawing the attention of the World Health Organization (WHO) [36]. The ineffectiveness of *T. violacea* fruit and seed extracts against *K. pneumoniae* (Table 4) is not surprising considering its notoriety for multitherapy resistance. According to WHO, there is a rising global concern about the hypervirulent strain of *K. pneumoniae* [49]. The bacterium develops resistance through various mechanisms such as membrane protein (porin) mutation, biofilm formation, as well as the acquisitions of plasmid, efflux pump, and multiple resistant genes [50, 51]. The dosage-dependent antibacterial effects on the other two ESKAPE pathogens (*S. aureus* and *E. faecalis*) (Table 4) could offer a glimmer of hope for combating the threats of these microorganisms to human well-being. *E. coli* presented with the highest susceptibility to *T. violacea* seed extracts (Table 4). This implies that the antibacterial resistance of *E. coli* is lesser when compared with the tested members of the ESKAPE pathogens. Various groups of phytochemicals have antibacterial properties [52].

The mechanisms of the antibacterial activities include inhibitory effects on various processes such as cell wall, nucleic acid and bacterial protein synthesis, as well as the disruption of membranes, enzymes and metabolic processes [52, 53]. Different phytochemical groups such as flavonoids, alkaloids, coumarins, and polyphenols, among others, have specific mechanisms of action against pathogens [52, 54–56].

Hence, plant materials with a substantial number of phytochemical groups, such as *T. violacea* fruits and seeds, would have multi-antibacterial mechanisms of action, making them suitable for multi-therapy options against resistant bacteria. The qualitative and quantitative analyses, as well as TAC, suggested that the fruits had higher phytochemicals than the seeds. However, the antibacterial effects did not always follow the observed pattern. For example, the seeds presented more antibacterial effects against *E. coli* than the fruits. This implies there are other factors, such as different specific phytochemical isolates in the tested materials, which may influence the antibacterial potential. *T. violacea* has been shown to possess antibacterial properties through the assessment of its leaves, bulbs, hypocotyl, essential oil, flowers, and stalks [6, 9, 28, 57–59]. The compound, marasmicin, is associated with the antibacterial properties of *T. violacea* [9, 60]. Some factors, such as the freshness of plant materials, drying temperature, and the texture of ground plant materials, could affect the formation of marasmicin, and subsequently, the antibacterial potential of *T. violacea* [60]. Hence, the results from various authors may not be comparable due to the variability of factors.

3 | Conclusion

This study showed that the fruits and seeds of *T. violacea* could be added medicinal resources for human use. They contained similar phytochemical constituents like those found in the leaves, stems, roots, flowers, stalks, and rhizomes. They also presented with high antioxidant and antibacterial potential. However, these plant parts seem not to be readily available because *T. violacea* is usually cultivated for ornamental purposes. Also, the detection of alkaloids in the seeds, which were not detected in the fruits nor other documented plant parts of *T. violacea*, suggested a need for caution in consuming the seeds. This work has its limitations because it was purely laboratory-based. Hence, its findings need to be further investigated in living systems before validation. Also, since samples were collected from a single location, the results, therefore, would not represent what would be obtained in broader sampling sites. Finally, quantified phytochemicals were not exhaustive due to cost.

4 | Experimental

All the reagents and chemicals used in this study were of analytical grades and were bought from Sigma-Aldrich, USA. All absorbance readings were taken with a Thermo-Fisher Scientific Genesys (GEN10S UV-VIS) spectrophotometer. Phosphomolybdate reagent was made by combining equal volumes of 28 mM sodium phosphate, 0.6 M H_2SO_4, and 4 mM ammonium molybdate. A stock solution of 2,2-diphenyl-1-picryhydrazyl (DPPH) was made by dissolving 50 mg of DPPH in 100 mL of 80% methanol. The DPPH work solution was prepared by mixing one part of the stock solution with four parts of 80% methanol. Each experiment was carried out in triplicates except for the qualitative analysis.

Plant material collection, preparation, extraction, and yield: Whole plants of *T.* violacea (Figure 1A) were identified and validated by Dr. Ida Risenga. Specimen samples with voucher number IR/2023/01 were deposited at the herbarium of the University of the Witwatersrand. The fruits of *T. violacea* were harvested from the Cresta shopping mall, Johannesburg, South Africa, in January 2024. Seeds were extracted from mature, brown, and dry fruits, while the green fruits were left intact (Figure 1B). The seeds (Figure 1C) were air-dried in the oven (Binder oven) for 3 days at 33 ± 2°C, while the fruits were freeze-dried (Zirbus technology freeze-dryer) at −83°C for 3 days. The dried materials were subsequently ground to fine powder.

Three polar solvents: water, methanol and ethanol, and three medium- to nonpolar solvents: acetone, ethyl acetate and hexane were used for the crude extraction of phytochemicals from the powdered material of *T. violacea*. To achieve this, 3 g of seed powder and 30 mL of each solvent were combined, agitated in an orbital shaker at 150 rpm for 72 h, and subsequently centrifuged at 3500 rpm for 5 min. The supernatants served as the crude extracts, which were kept in the refrigerator until needed while the residues were discarded. The same procedure was repeated for the fruit powder. To determine the yield of phytochemicals (dry extracts) from the crude extracts, the solvents were evaporated from the crude extracts and the yield was subsequently calculated as follows:

$$\%\,\text{yield} = (\text{dry extract (without solvent)} / \text{weight of ground sample}) \times 100$$

Qualitative phytochemical screening: Various color tests for alkaloids, tannins, saponins, flavonoids, glycosides, steroids, volatile oils, coumarins, phlobatannins, quinones, terpenoids, cardiac glycosides, and phenolics were carried out using standard procedures according to various authors [6, 61–66].

4.1 | Quantitative Phytochemical Screening

TFC: In a test tube, 4 mL of distilled water (dH_2O) and crude extract (0.3 mL) were combined. Then, 5% $NaNO_3$ (0.3 mL) was also added, shaken together and left for 5 min. Thereafter, 10% $AlCl_3$ (3 mL) was added to the mixture in the test tube and left to rest for 6 min, after which 1 M NaOH solution (2 mL) was added. The mixture was finally made up to 10 mL by the addition of 0.4 mL dH_2O and then incubated in the dark at room temperature for 1 h. The absorbance of the solution was measured at 510 nm wavelength, using 80% methanol as the blank. TFC was calculated from the quercetin standard calibration curve equation:

$$y = 0.2388x - 0.0019\,(r^2 = 0.9997)$$

TPC: The Folin–Ciocalteu (FC) reagent assay was used to determine TPC. Each crude extract (0.2 mL) and 7.5% Na_2CO_3 (2 mL) were combined. Then, diluted FC reagent (0.75 mL) and distilled water (7 mL) were added. The FC reagent was diluted to 10% strength before its use. The solution was incubated for 2 h in the dark and at room temperature. The absorbance of the solution was taken at 765 nm wavelength using 80% methanol as the blank. The TPC was calculated from the gallic acid standard calibration curve equation:

$$y = 0.0495x - 0.0259\,(r^2 = 0.9994)$$

TTC: The method of Lahare [67], adapted and adopted by Teffo [64], was used to determine the TTC from the crude extract of *T. violacea* fruits and seeds. The crude extract (0.1 mL) was added to dH_2O (7.5 mL). Thereafter, undiluted FC reagent (0.5 mL) and 35% Na_2CO_3 (1 mL) were added. The volume was then made up to 10 mL by the addition of dH_2O (0.9 mL). The solution was incubated in the dark and at room temperature for 30 min. The absorbance of the solution was measured at a wavelength of 725 nm. A gallic acid standard calibration curve was used to calculate the TTC:

$$y = 0.046x - 0.0264\,(r^2 = 0.9833)$$

TPAC: TPAC was determined using the Oyedemi's method [68]. Each crude extract (0.5 mL) was added to 4% vanillin–methanol solution (3 mL), this was followed by the addition of HCl (1.5 mL). The mixture was mixed vigorously using a vortex and incubated for 15 min in the dark and at room temperature. The absorbance of the mixture was measured at a wavelength of 500 nm. A catechin calibration curve equation was used to calculate the TPAC as follows:

$$y = 0.9554x + 0.0003\,(r^2 = 0.9927)$$

4.2 | In Vitro Antioxidant Assays

TAC by phosphomolybdate method: In a capped test tube, 0.1 mL each of *T. violacea* fruit and seed extracts, at a concentration of 1 mg/mL, was added to 1 mL of phosphomolybdate reagent. The test tubes were then placed in a preheated water bath at 95°C for 90 min. The tubes were thereafter removed and were left to cool completely. Then, the absorbance readings of the solutions were taken at 695 nm. Eighty percent methanol was used as blank, while a linear equation generated from the ascorbic acid calibration curve was used to calculate the TAC:

$$y = 0.0036x + 0.1161\,(r^2 = 0.9985)$$

DPPH scavenging activity assay: Seven hundred microliters of the DPPH work solution was added to 50 µL each of various concentrations (0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) of each *T. violacea* fruit and seed extracts. The final volume was made up to 1 mL by the addition of 250 µL of respective solvents. The reaction was allowed to take place at room temperature and in the dark for 45 min. The absorbance of the mixture was then measured at a wavelength of 517 nm. The control reaction was the combination of 700 µL DPPH work solution and 300 µL of solvent (without the extract), while 80% methanol was used for the blank. The percentage scavenging of DPPH was calculated as:

$$\%\,\text{activity} = ([A_0 - A_1] / A_0) \times 100$$

where A_0 and A_1 represented the absorbance of the control and the extracted sample, respectively. A linear graph equation, generated from the plot of percentage activity against extract concentrations, was used to calculate IC_{50}.

Iron (III) ion reduction assay: The method of Pavithra and Vadivukkarasi [69], as reported by Rakesh et al. [70] was modified for the assay. Using culture bottles, 50 µL of various concentrations (0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL) of *T. violacea* fruit and seed extracts were each made up to 1 mL with the various solvents used for extraction. Thereafter, 50 µL of 2 mM $FeCl_3$ was added to each mixture, then vortexed for thorough mixing, after which 200 µL of 5 mM ferrozine was added and shaken thoroughly again with a vortex. The bottles were then incubated for 10 min in the dark and at room temperature. The absorbance of each sample was measured at a wavelength of 562 nm. The control was the combination of 1 mL of each solvent, 50 µL of 2 mM $FeCl_3$ and 200 µL of 5 mM ferrozine, while 80% methanol was used as the blank. The percentage reduction of Fe^{3+} was calculated as:

$$\%\,\text{reduction} = [1 - (A_0/A_1)] \times 100$$

where A_0 and A_1 represented the absorbance of the control and the extracted sample, respectively. A linear graph equation, generated from the plot of percentage activity against extract concentrations, was used to calculate IC_{50}.

4.3 | Antibacterial Assays

Two Gram-negative bacterial microorganisms: *E. coli* (ATCC 11775) and *K. pneumoniae* (ATCC 13883), and two Gram-positive organisms: *S. aureus* (ATCC 12600) and *E. faecalis* (ATCC 19433) were used for this antibacterial assay.

Kirby-Bauer disk diffusion test: Each microorganism was inoculated into a nutrient broth for 24 h to promote growth. Using 0.9% physiological saline (NaCl) solution, the microorganism broth was diluted to approximately 0.5 McFarland standard of turbidity to allow for uniform cell density across all tested microorganisms. Thereafter, 100 µL of the diluted bacterial broth was pipetted unto solidified Mueller–Hinton (MH) agar in a 90-mm Petri dish. Each Petri dish had been earlier divided into four quadrants before the inoculation of bacterial broth. The inoculant was spread across the agar with the aid of a "hockey stick" cell spreader and then allowed to dry. After drying, 0, 5, 10, and 20 µL of plant extracts were pipetted onto absorbing paper discs, and the discs were placed in each quadrant accordingly. The quadrant with the zero amount of plant extracts contained only the solvent used for the extract and represented the negative control. The Petri dishes were incubated at 37°C for 24 h. Thereafter, the ZOIs, which indicated the antibacterial potency of *T. violacea* fruit and seed extracts, were measured in mm with a ruler [71].

Agar well diffusion: One hundred 100 microliters of each bacterial broth was smeared on the surface of MH agar. A sterile blue pipette tip was then used to bore four 6-mm diameter wells in each petri dish. The four wells were filled with 100 µL each of *T. violacea* fruit and seed extract. The cultured plates were sealed with Parafilm and placed in the refrigerator for 1 h, to allow for the diffusion of various extracts into the media. The cultures were subsequently incubated for 24 h at 37°C, after which clear ZOIs were measured [72].

Acknowledgments

The authors wish to thank the University of the Witwatersrand (WITS) Research Funds and the School of Animal, Plant and Environmental Sciences, (WITS) for making this research a possibility.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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