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Research Article

In Vitro Total Phenol, Antioxidant and Antibacterial Activities of *Tulbaghia violacea* Crude Extracts

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ABSTRACT

This study was designed to assess the antibacterial activity of *Tulbaghia violacea*, to evaluate its total phenol activity, antioxidant activity, and antibacterial activity. the antioxidant and antibacterial activities were assessed using the 2,2-diphenyl-1-picrylhydrazyl assays and *p*-Iodonitrotetrazolium chloride assay respectively. The total phenolic evaluation found that the aqueous and the bulb methanol/dichloromethane extract contained the lowest amount of phenolics, and leaf extract had the largest amount. In contrast to the norm, *T. violacea* extract showed a significant level of antioxidant activity. With several plant extracts, the antibacterial activity produced intriguing results, suggesting that they may be unique to Gram-positive bacteria. The aqueous extracts of the leaf and bulb both exhibited antibacterial action against the Gram-negative bacterium Klebsiella pneumoniae, but not on a Gram-positive organism (Staphylococcus aureus). At lower concentrations, the extract from water's roots exhibited a comparable result. The elevated concentrations of the root methanol/dichloromethane extract decreased the proportion cell viability of *S. aureus* more than *K. pneumoniae*. Dichloromethane bulb and methanol extracts appeared to be efficient on *S. aureus*. These findings suggest that *T. violacea* may be an essential extract for developing as an antibacterial agent.

Keywords: *Medicinal plant, Total phenol, antioxidant, antibacterial activity*

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INTRODUCTION

Therapeutic herbs contain phytochemicals, which are bioactive non-nutrient and physiologically active molecules with a broad range of chemical compositions, including disease and protection capabilities (Madike *et al*., 2017). Herbs naturally create significant bioactive substances called phytochemicals. They act as defences against pathogenic assault and outside stress (Pandey and Kumar 2013). The body can be protected from the harmful effects of free radicals by powerful natural antioxidants called phytochemicals (Soni and Sosa 2013). An important phytochemical antioxidant that plants make is phenol (Naczk and Shahidi 2004). They are more efficient because they have features that suppress lipid peroxidation, can bind metal ions, and are anti-free radical (Tlili *et al*., 2013). The peroxidation of phospholipid bilayers brought on by ROS can be stopped by phenolic substances like quercetin and caterchin, according to studies (Perkins *et al*.,2012). Due to a lack of innovative antibiotics for Gram-negative pathogens therapy and the challenges associated with the emergence of multi-drug resistance, novel antibacterial strategies must be developed for the treatment of these illnesses (Masoko 2017).

The creation of secondary metabolites as a defence mechanism against environmental stressors may partially account for the high concentration of antibiotics seen in herbal products (Dzoyem *et al*.,2016). This characteristic makes herbal products a significant and fascinating focus in medication discovery and development, together with their extreme biodiversity and tremendous structural variation (Levy and Marshall 2004). It is expected that 26% of deaths each year are caused by infectious infections. Projections show that 26% of all annual deaths worldwide are caused by infectious diseases (Murry and Lopez 1997). In Africa, bacterial illnesses have a big impact because there aren't enough therapies and a lot of the ones that are now available don't work anymore because of the rise of drug resistance (Hendrick and Yang 2012). Antibiotic-resistant bacteria are a severe and spreading public health hazard that exists globally (Van Vuuren Muhlarhi 2017). Undoubtedly, microorganisms that are resistant to several drugs, notably Gram-positive infections, are making it harder to treat infectious diseases (Magiorakos *et al*., 2011).

By the year 2050, antimicrobial-resistant bacterial illnesses are anticipated to be the major cause of death

worldwide (Das *et al*.,2010). *Pseudomonas aeruginosa, S. aureus, Acinetobacter baumannii, and K. pneumoniae* are some notable bacterial strains that have been documented to be easily transmissible and resistant (Mulaudzi *et al*., 2011). Researchers have recently become attracted by the adaptability of therapeutic herbs. To do this, secondary metabolites produced by herbs had to be isolated, identified, and used as active ingredients in therapeutic compositions (Essawi and Srour 2000). Studies have indicated that methicillin resistance was the cause of more than 50% of all S. aureus infections acquired in hospitals (Olutayo *et al*.,2013). Herbal remedies are frequently utilized around the world to treat bacterial, fungal, and viral illnesses. Herbal remedies are frequently utilized around the world to treat bacterial, fungal, and viral illnesses. These botanical extracts form the basis for the therapeutic substances used to treat cervicitis vaginitis, herpes simplex virus I, and urinary tract infections (Takaidza *et al*.,2018). The screening and assessment of the inhibitory effects on test microorganisms rely heavily on antibacterial evaluations.

The two organisms *K. pneumoniae* and *S. aureus* characterized as Gram-negative and Gram-positive bacteria were in this study. These assays, it is crucial to use a well- as a positive control, a well-known antibacterial substance guarantee the experiment was carried out properly and to allow direct comparison of extracts.

MATERIALS AND METHODS

Plant material: The validity of the *T. violacea* plant material was confirmed by specialists at the National Botanical Garden in Bloemfontein, South Africa. The plant material was divided into roots, bulbs, and leaves after being properly cleaned to get rid of any impurities. The plant components were dried for five days in an oven with a temperature range of 30 to 60 degrees Celsius. Before extraction, the plant was dried-up then parts were crushed into a coarse powder in a hammer mill and kept at room temperature.

Extraction of plant material: Following the crushing of the plant different parts, the powdered materials were subjected to the extraction solvents in the Waring blender in a ratio of 1. The remaining solvent was added after blending, and the mixtures were then allowed to soak for 24 hours. After 24 hours, the particles from each solution were collected using a medium Millipore funnel, Bright Sign No. 102 filter paper, using a Millipore vacuum pump. The plant organic extracts were concentrated using rotating vacuum at 50–60ºC, and then followed by a further drying at room temperature. The aqueous extract was concentrated till dry using a freeze-dryer. All the eluted plant extracts were then kept at -20°C cold room until for future use (Table 1).

Using the Folin-Ciocalteu assay, total phenolic content is determined: A standard curve made of gallic acid with concentrations ranging from 6.25 g/mL to 100 g/mL was employed. All extracts were made at ten times the required concentrations, together with gallic acid as the standard control, to account for the 1:10 dilution factor. The stock concentration was 100 mg/mL when the sample extracts were made, and they were centrifuged at 12 000 X g for 10 minutes after that. The 96-well plates' wells were then filled with 20 L of supernatant in triplicate. The supernatants of each extract were likewise produced in dilutions of 1:1 and 1:10. Each well of the plate received 100 L of the FC reagent, which was then let to stand at room temperature for 5 minutes. A 100uL dose of the FC reagent was applied to each well of the plate, and it was let to sit at room temperature for 5 minutes. Before the plate completed a 2-hour incubation period at room temperature and in the dark, 80 L of 7.5% Na2CO3 was gently added to each well. A BioTek® PowerWave XS spectrophotometer was then used to measure the absorbance at 750 nm (Winooski, USA).

Table 1:
A list of plant extracts used

Sample No.	Solvent used (extraction)	Part of plant
#12	#Water	#Bulb
#10	#Water	#Leaf
#9	#Water	#Roots
#5	#Methanol/ dichloromethane	# Leaf
#3	#Methanol/ dichloromethane	# Root
#1	#Methanol/dichloromethane	#Bulb

DPPH test for measuring antioxidant activity: A 96-well plate was filled with the following ingredients: 5 mL of sample, 120 mL of newly made DPPH (0.1 mM in ethanol), and 120 mL of Tris-HCL buffer (50 mM, pH 7.4). After that, the mixture was left to rest for another 20 minutes in the dark and at room temperature. A BioTek® PowerWave XS spectrophotometer from Winooski, Vermont, USA, was used to measure the absorbance at 513 nm and calculate the percentage of radical scavenging activity:

% DPPH scavenged is equal to 100 x (blank-sample/blank).

The 5 µL sample for the blank/control was changed to buffer. 250 and 500 µg/mL final concentrations of plant extracts were evaluated. As a positive control, quercetin was employed at final concentrations ranging from 6.25 to 25 µM.

Sensitivity testing of extracts on microorganisms using *p-Iodonitrotetrazolium* chloride: The two organism *S. aureus* (ATCC 11632) *and K. pneumoniae* (ATCC 10031), were used to test the antibacterial activity of plant extracts. Bacterial organisms were raised on Mueller-Hinton (MH) agar plates at a temperature of 37°C. The 24 hours-streaked plate, MH broth (Merck, USA) was inoculated and allowed to grow at 37°C for 16 hours (log phase). Vancomycin hydrochloride and Gentamicin sulphate served as positive controls for *S. aureus* and *K. pneumoniae* respectively (Sigma, USA). A 0.2 m filter was used to sterilize antibiotic solution that had been dissolved in double-distilled water at a stock concentration of 2 mg/mL. To determine the minimum inhibitory concentration (MIC) value, working antibiotic concentrations were produced in MH broth.

Then, the appropriate 96-well sterile plate were filled with 50 mL of each extracts and 40 mL of MH broth. This was followed by a series of extract dilutions to determine the

concentration range, as depicted in Figures 1 and 2. The cultures were assessed and adjusted in accordance with the 0.5 McFarland standard solution to achieve 1.5 x 108 cells/mL. A 50mL aliquot of the relevant microorganisms was added to each test well. *p-iodonitrotetrazolium* chloride (INT) was produced at 0.2 mg/mL working concentration in double dH_2O, filter-sterilized, and then plates were sealed and incubated at 37 °C for 24 hours.

A color change (from yellow to purple/pink to show the elimination of the dye by living bacteria) was then noticed after an additional 30 to 60 minutes at 37 °C and 50 L of INT added to each well. No alteration in color indicated that bacterial growth was being slowed down. Utilizing a BioTek® PowerWave XS spectrophotometer, absorbance (abs) at 600 nm was measured (Winooski, USA).

The following formula was used to calculate percentage inhibition:

% inhibition =

1 - (test well abs/mean abs triplicate bacteria only well) x 100.

RESULTS

Total phenolic determination: Table 2 displays the absorbance of the reference substance (gallic acid) at maximum = 750 nm in T. violacea, and Figure 1 displays the standard calibration curve for determining the total phenolic content.

The total phenol concentration was determined using the Folin-reagent Ciocalteu's, and Table 2 presented the data in terms of gallic acid equivalents (standard curve equation: y = 0.0217, R2 = 0.9816; figure 1). Total phenolic concentrations for the aqueous extracts of the root, bulb, and leaf were reported to be 10.94, 10.94, and 21.75g/mL, respectively. In the methanol/dichloromethane extracts, the total phenolic content of the root, bulb, and leaf extracts was found to be 20.28, 7.93, and 13.36 g/mL, respectively.

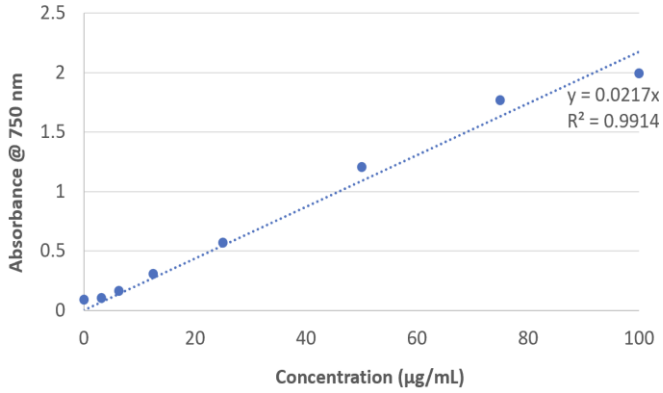

Figure 1:
The standard curve for the determination of total phenolics in the FC assay uses gallic acid as the standard control.

DPPH test for measuring antioxidant activity: Table 3's results for this experiment clearly indicate each extract's percentage DPPH scavenging activity.

The unstable free radical DPPH is stable at normal temperature and generates a violet solution in ethanol. A free radical loses its status when it interacts with an antioxidant because the latter gives it an electron or hydrogen, converting it into a stable molecule and changing its color to a pale yellow (Olutayo *et al*., 2013). In the current study, extracts that produced a yellow color were thought to have antioxidant properties. The antioxidant qualities of T. violacea's root, bulb, and leaf extracts were determined using scavenging activities (Table 3).

Table 2:
Using the FC technique, determine the total phenolics content

Sample number (Extract)	Abs @ 750 nm (Ave)	Phenolic concentration (µg/mL)	Std dev of concentration (µg/mL)
#12	0.24	10.94	0.83
#10	0.47	21.75	0.88
#9	0.24	10.94	1.46
#5	0.44	20.28	0.98
#3	0.29	13.26	1.09
#1	0.17	7.93	0.12

#12 – Water bulb extract. #10 – Water leaf extract; #9 – Water root extract; #5 – Methanol/dichloromethane leaf extract; #3 – Methanol/dichloromethane root extract; #1 – Methanol/dichloromethane bulb extract.

Table 3:
Extracts' antioxidant activity as a percentage of DPPH scavenged

Extract	Concentration (µg/mL)	% DPPH scavenged*	Std Dev
#12	250	81.53	5.78
	500	76.05	9.47
#10	250	88.9	0.30
	500	88.49	1.07
#9	250	58.44	4.45
	500	66.19	5.14
#5	250	71.59	8.64
	500	73.62	20.56
#3	250	82.62	1.82
	500	85.91	4.30
#1	250	56.56	10.20
	500	75.34	4.76
Quercetin (positive control)	6.25 µM	48.65	9.71
	12.5 µM	84.35	1.08
	25 µM	86.43	0.65

#12 – Water bulb extract; #10 – Water leaf extract; #9 – Water root extract; #5 – Methanol/dichloromethane leaf extract; #3 – Methanol/dichloromethane root extract; #1 – Methanol/dichloromethane bulb extract.

Determination of Antibacterial activity: A Gram-negative and Gram-positive bacterium were used to assess the extracts' antibacterial properties. Figure 2 below shows the antibacterial effectiveness of six T. violacea extracts against S. aureus at dosages ranging from 0.0156 mg/ml to 2 mg/ml. As a positive control, vancomycin, with a MIC of 2 g/ml, was used. Figure 1's dichloromethane extract 1 demonstrated that MIC action against S. aureus depended on extract concentrations. At concentrations between 0.0125 mg/mg and 0.5 mg/ml, both water root extract and methanol/dichloromethane root extract failed to prevent bacterial growth. However, they did at levels of between 1 mg/ml and 2 mg/ml

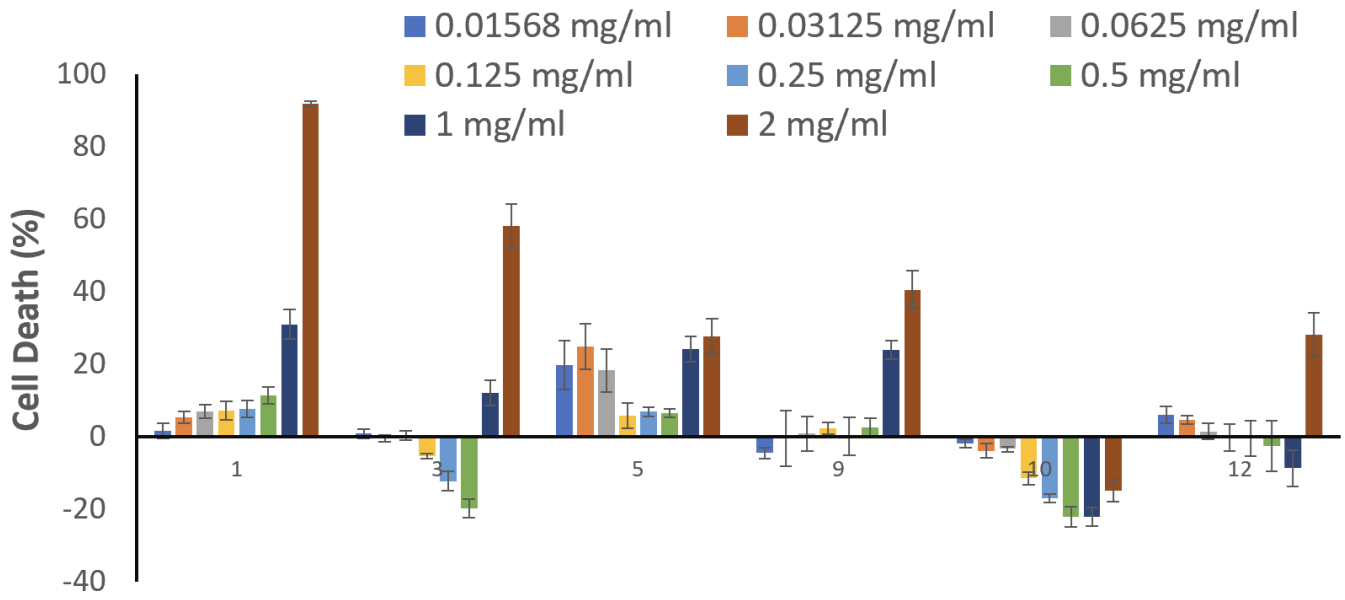

Figure 2:
Six extracts were tested for their ability to fight against S. aureus at concentrations between 0.0156 mg/ml and 2 mg/ml. At its MIC of 2 g/ml, vancomycin was utilized as a positive control. The error bars show the standard deviation of the quadruplicate values that were found in one experiment.

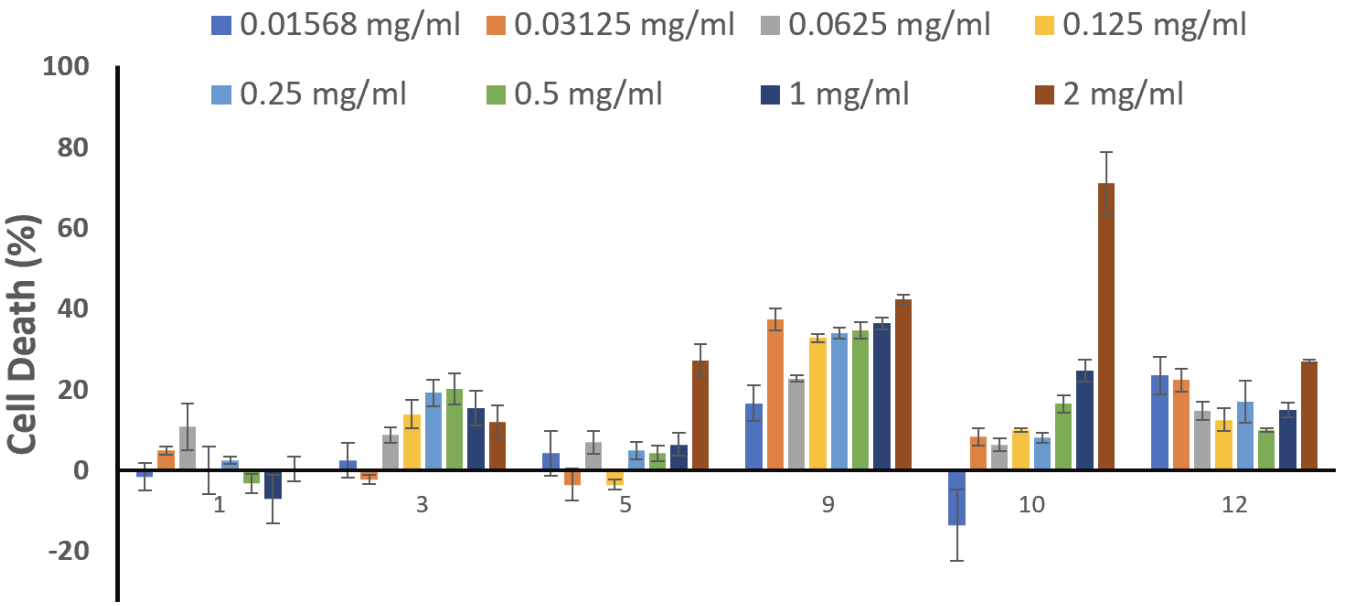

Figure 3:
Six extracts were tested for antibacterial activity against K. pneumoniae at concentrations between 0.0156 mg/ml and 2 mg/ml. At its MIC of 2 g/ml, gentamicin was utilized as a positive control. Error bars show the standard deviation of data that were determined in quadruplicate and in a single experiment.

DISCUSSION

The water leaf extract showed to possess the most phenolics, whereas methanol/dichloromethane bulb extract contained the least. The phenolic content for the aqueous leaf, bulb, and roots extracts largely agrees with this investigation, claim Madike *et al* (2017). In research by Madike *et al*., certain T. violacea specimens contained flavonoids, tannins, saponins, terpenoids, proteins, steroids, phenols, cardiac glycosides, and coumarins (2017). All parts of the plant were devoid of phlobatannins, leucoanthocyanins, alkaloids, sugars, and anthocyanins. The plant's leaves contained more chemically

active compounds than the stems and roots, which were extracted with water and 70% ethanol. The highest levels of scavenging activity were seen in T. violacea leaf water extracts, whereas the lowest values were seen in bulb methanol/dichloromethane extracts. It seems that T. violacea's raw extract works equally as well as quercetin. When compared to the standard, the T. violacea extract had stronger antioxidant activity. According to a study by Takaidza *et al*. (2018), T. violacea was the Tulbaghia species with the greatest potential to scavenge DPPH, and these findings support that finding. The T. violacea extracts utilized in this study therefore probably contain pharmacological components, and water leaf extract probably has higher phenolic and antioxidant activity.

Except for water leaf extract, which displayed higher concentration-dependent activity, other extracts exhibited unpredictable variable activities. This shows that water leaf extract had an inhibitory effect on *K. pneumoniae* but no effect on *S. aureus*. According to a study, T. violacea dichloromethane bulb extracts were found to have the strongest antibacterial activity on K. pneumoniae and S. aureus all winter long (Ncube *et al*. 2011). This is related to aqueous leaf extract and dichloromethane methanol/dichloromethane bulb extract. The type of solvent employed in the extraction method has a significant impact on the effectiveness of the outcomes of plant-derived botanical substances sources. Water is the principal solvent employed by traditional healers, however the researcher discovered that in this and previous studies, extracts from organic solvents had greater antibacterial activity than extracts from aqueous solutions. These results can be explained by the polarity of the chemicals extracted by each solvent, as well as their innate bioactivity and capacity to disperse in the various test media (Das *et al*., 2010.

In conclusion, the research demonstrated that the antioxidant activity capabilities of T. violacea were present in both the water and methanol/dichloromethane extracts. The water and methanol/dichloromethane extracts both possess potential for total phenolic analysis. It is well known that phenolic chemicals improve nutritional content and quality through modifying colour, flavour, and other sensory attributes as well as by promoting good health. Additional research will be conducted on microorganisms that are very effective opportunistic pathogens and are consequently connected to several diseases to ascertain the antibacterial impact of T. violacea.

At the quantities utilized in this investigation, the T. violacea extracts were effective against *K. pneumoniae* and *S. aureus*. Interesting results came from the antibacterial activity, with several plant extracts exhibiting Gram-specificity. T. violacea' s water leaf and water bulb extracts both demonstrated efficacy against Gram-negative *K. pneumoniae* but not against *S. aureus* (Gram-positive). At lower quantities, aqueous root extract produced a comparable outcome. *S. aureus* saw a more marked rise in the percentage cell mortality when exposed to high concentrations of methanol/dichloromethane extract than did *K. pneumoniae*. Against *S. aureus*, methanol/dichloromethane extract also appeared to have effect.

The study on T. violacea plant extract indicate the therapeutic potential of several botanicals used in herbal treatment. These results offer a sound foundation for selecting potential plant species for further pharmacological and phytochemical study. The results of the study support the use of the extract under consideration since some plant extracts contain chemicals with antibacterial properties that could be used as antimicrobial agents in cutting-edge pharmaceuticals to treat viral illnesses brought on by infections. The strongest extracts are useful for pharmacological research and therapeutic antibacterial isolation. When used against Gram-negative K. pneumoniae but not S. aureus, water extracts from the water leaf and bulb of T. violacea were both effective.

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