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*Original Article*

# Antioxidant Activities, Total Polyphenol Profile and Anticancer Activity, of Leaf, Bulb and Root Extracts of *Tulbaghia violacea* from Bloemfontein

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## ABSTRACT

In this study, the effects of the home remedy herb *Tulbaghia violacea* on antioxidants, total polyphenol activity, and cancer were investigated. Using methanol/dichloromethane and aqueous solvents, the extracts were produced. The antioxidant activity of the extracts was assessed by the 2,2-diphenyl-1-picrylhydrazyl assay, and their phenol content by the gallic acid method. The extracts were found to be inactive or weak against the HeLa (cervix), human cancer cell lines TK-10 (renal), and PC3 (prostate). It is suggested that these three human cell lines be tested against extracts of water and methanol/dichloromethane at higher concentrations. The plant's leaf extract would also be the best substance to test against the human cell lines TK-10, PC-3, and HeLa. The IC50 values for two to three cell lines show that T. *violacea* plant extracts (>100 g/ml) have no effect on cells. T. *violacea* extract has greater antioxidant activity than the control. A thorough phenolic analysis showed that water leaf extract had the highest quantity of phenolics whereas bulb methanol/dichloromethane extract had the lowest. Both the methanol/dichloromethane and the aqueous extracts have the same characteristics for antioxidant activity. In order to enhance food's nutritional content and quality while also supporting excellent health, it has been found that phenolic compounds alter the color, flavor, and other sensory characteristics of the meal. Additionally, they help plants defend themselves against harm from ROS, molecular damage, microbial invasion, insects, and herbivores.

**Key words:** Antioxidants, *Tulbaghia violaceae*, Medicinal plants, Anticancer activity, Polyphenol.

## INTRODUCTION

Globally, the significance of employing medicinal plants to safeguard human health is growing.[1] According to studies by Cragg *et al.* (2005)[2] and Adeyemi *et al.* (2013),[3] more than 60% of currently used anti-cancer medications are derived in some way from natural sources such plants, microorganisms, and marine species. An important source of potent anti-cancer drugs has been plants. Camptothecin (CPT) was discovered to possess DNA topoisomerase I inhibition effect thereby stopping the progression of cancer in the early 1960's.[4]

In a quest for steroid-like compounds, thousands of plants were studied, and first discovery of this natural alkaloid was in the wood stem of *Camptotheca acuminata*, a Chinese tree of ornaments.[5] According to early research (Hertel *et al.*, 1990), mouse leukemia cells displayed strong anticancer properties and could be examined using a standard *in vitro* evaluation approach. These astounding results increased the public's curiosity in this herbal treatment as a potential anticancer drug. Hycamtin (topotecan), camptosar (irinotecan), and CPT-11 are three first-generation CPT analogues that has anti-cancer for ovaries, colon and lung. Their marketing is handled by Glaxosmithkline and Pfizer.[6] The Apocynaceae plant *Catharanthus roseus* (L.), which produces the anti-tumor alkaloids vinblastine and vincristine, is native to Madagascar, have been used to treat kaposi's sarcoma, advanced testicular cancer, lymphoma, leukemia, and lymphoma.[7] Another example of an herbal medicine discovery is the isolation of paclitaxel from the bark of the Pacific yew, Taxus brevifolia (Taxaceae).

Paclitaxel is reportedly necessary for the treatment of advanced breast, lung, and ovarian cancer.[7] According to reports from South Africa, the plant Combretum caffrum has anticancer properties, and the chemical combretastatin is possibly the most effective cytotoxic phytomolecule discovered so far.[8,9] It is important to note the characteristics that the local plants display because Bloemfontein, the capital of the Free State province and known as the "city of roses," is located in the middle of South Africa. It may have interesting plant biological activity as a result of the high levels of pollution, the high temperatures, and the numerous vehicles and cars passing through on their way to other provinces.

The majority of Africans have turned to using medicinal plants and going to public health facilities because doctors and over-the-counter medicine and medical treatments are frequently too costly for their ordinary life due to high unemployment and increased public transportation costs.[10,11] Increased usage of medical plant products is supported by many academics and dietary groups.[12] This was obvious during COVID-19 as South Africans and the Bloemfontein community relied on medical plants for survival.[1,11,13] Currently, roughly 64% of the world's population uses medicinal plant for their health problems. The have been a growing interest in plants natural product antioxidant activity and total phenolic content over the years.[14]

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According to Valko *et al.* (2007),[15] free radicals are highly reactive, unstable molecules that are produced naturally during metabolic processes. The regulation of the cellular decrease oxidation state was found to be maintained by the antioxidant enzyme systems, chemical compounds such endogenic enzymes or genuinely occurring nutritional antioxidants, as well as certain hormones during metabolic process (Lobo *et al.*, 2010). The increased creation of free radicals overwhelms the antioxidant defense, putting the physiological system under oxidative stress which may damage cellular lipids, proteins, or DNA by impeding their normal activity.[16,17] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by a range of mechanisms in the human body, including the spontaneous activation of a battery of detoxifying enzymes and the presence of antioxidant chemicals.[18] Despite the fact that several synthetic antioxidants are used, including butylated, vitamins, hydroxy anisole, propyl gallate, butylated hydroxytoluene, and tert-butylhydroquinone, it is thought that these compounds are dangerous to both humans and test subjects. It is essential to develop new drugs and use stronger natural antioxidants as a result. Studies on antioxidants may help us better understand the mechanisms underlying the therapeutic effects of medicinal plants.[19] The *T. violacea* plant antioxidant potential and total phenolic content will be assessed in this study. The IC50 of the extract is calculated and compared to the reference value using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, which was used to demonstrate the extract's antioxidant capacity. Total phenolics and antioxidants in the plant must be evaluated in order to find and use new natural sources of pharmaceuticals.[20]

# MATERIALS AND METHODS

## Plant material

Scientists from botanical garden in Bloemfontein of South Africa, verified the purchased plant as *Tulbaghia violacea* species. After being cleaned to eliminate soil, the material was divided into roots, bulbs, and the leaves. The plant components were dried in an oven at 30 to 60oC for five days. The dried plant material was hammer into a coarse powder, which was then kept at room temperature until extraction.

## Preparation of the plant extracts

In a Waring blender, water and methanol/dichloromethane 1:1 was added to the dried and ground plant materials. The mixture was then re-blended, the remaining solvent added, and steeped for 24 hours. The particles in each solution were eluted after 24 hours using medium filter paper (Bright Sign Number 102) and a Millipore funnel joined to a Millipore vacuum pump. Concentration of organic extracts were done in a rotational vacuum and then dried at room temperature in a vacuum oven. Followed by the use of freeze-dryer to concentrate the aqueous extracts until they dry. Before usage, the 6 plant extracts were stored at a temperature of -20 °C.

## Sulforhodamine (SRB) B assay

The SRB test was used to assess how the examined plant extracts affected growth inhibition. The SRB is based on the competence of the protein dye SRB (Acid Red 52) to electrostatically connect to protein-basic amino acid residues of cells fixed with trichloroacetic acid in a pH-dependent manner. It attaches to the fixed cellular protein in mildly acidic conditions, but in moderately basic settings, it can be solubilized and taken out of the cells for measurement. The SRB test was carried out at the Council for Scientific and Industrial Research (CSIR) using the NCI Drug Evaluation Branch's (NCI) methodology.

As a result of a scientific partnership between the CSIR and the NCI, the NCI contributed the human (TK10) cell lines and PC-3. In a panel for the SRB experiment, PC-3 (prostate), cancer cell lines TK10 (renal), and HeLa (cervical) were used to test the effects of plant extracts on the plant growth. At 100% relative humidity. 5% CO2, 95% air, and 37°C, Roswell Park Memorial Institute typically preserves cell lines in monolayer cell cultures with 5% foetal bovine serum, 2 mM L-glutamine, and 50 g/ml gentamicin. Cells (3–19 passages) were seeded in 96-well microtiter plates with plating densities of 7–10,000 cells per well for the screening experiment, and the plates were incubated overnight. Following which the cells were exposed to experimental plant extracts, which were dispersed in dimethyl sulfoxide (DMSO) and adjusted to five concentrations in medium. The control group's cells did not receives any medication. The blank contained simply media and no cells. There was a mention of parthenolide. The plant extracts were then added to the plates, which were then given a 48-hour incubation period. Before each well was cleaned, dried, and stained with SRB, live cells were settled to the floor of each well using frozen 50% trichloroacetic acid. After removing unbound dye and extracting protein-bound dye with 10 mM Tris base, the optical density at 540 nm was measured using a multi-well spectrophotometer.

## Statistical assay

Utilizing the GraphPad Prism tool, the data were examined. The four sets of plant extracts' cell growth activities are represented as IC50 values (concentration needed to induce 50% radical-scavenging activity) in Table 2. Non-linear regression was used to determine the 50% inhibition of cell growth (IC50).

## Assessment of antioxidant activity using the DPPH assay

Five (5) mL of the sample was combined with 120 mL of freshly made DPPH (0.1 mM in ethanol) and 120 mL of Tris-HCL buffer (50 mM, pH 7.4) in a 96-well plate. The plate was then allowed to sit at room temperature for 20 minutes in the dark. The absorbance at 513 nm was measured using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA), and the percent radical scavenging activity was determined as follows:

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

Positive control, quercetin was used at final concentrations ranging from 6.25 to 25 M. A buffer was used in place of the blank or control's 5 L sample. Experimental extracts were examined at absolute concentrations of 250 and 500 g/mL.

## Determination of total phenolic using the Folin-Ciocalteu assay

Gallic acid was used as the standard curve, with values ranging from 6.25 g/mL to 100 g/mL. To account for the 1:10 dilution factor, all extracts and the reference control (gallic acid) were produced in concentrations that were 10 times higher than those that were planned. The stock concentration was 100mg/mL when the sample extracts were made, and they were centrifuged for 10min at 12 000 x g after that. Then, 96-well plates were filled with 20uL of supernatant in triplicate. Additionally, supernatants were produced from each extract at dilutions of 1:1 and 1:10, respectively. Following the addition of 100 uL of FC reagent to each well, the plate underwent an additional five minutes of room temperature incubation. After gradually pouring 80uL of 7.5% Na2CO3 into each well, the plate was left to sit at room temperature in the dark for two hours. Then, using a BioTek® PowerWave XS spectrophotometer (Winooski, USA), the absorbance at 750 nm was computed.

# RESULTS AND DISCUSSION

Tables 3 through 6 and Graphs 1–7 display the anticancer activity of T. *violacea* crude extracts against cancer cell lines and healthy human fetal lung fibroblast cell lines. The detected activities were analyzed and categorized using the criteria as shown on table 2. The effects of

**Table 1:** ***Tulbaghia violacea*** **extraction material.**

| # Sample No. | Plant part | Solvent used for extraction | Yield elution (g) |
|---|---|---|---|
| 5 | Roots | Meth/dichlor (1:1) | 6.3 |
| 6 | Roots | Water | 3.5 |
| 3 | Bulbs | Meth/dichlor (1:1) | 4.9 |
| 4 | Bulbs | Water | 6.9 |
| 1 | Leaves | Meth/dichlor (1:1) | 6.3 |
| 2 | Leaves | Water | 7.2 |

*Meth/dichlor=Methanol/dichloromethane

**Table 2:** **CSIR standard criteria for anticancer activity.**

| $IC_{50}$ (µM) | Status | $IC_{50}$(µg/ml) | Status |
|---|---|---|---|
| > 100 | # Inactive | > 100 | #Inactive |
| < 100 | #Weak | < 100 | #Weak |
| > 50 | | >15 | |
| < 50 | #Moderate | < 15 | #Moderate |
| >10 | | > 6.25 | |
| < 10 | #Potent | < 6.25 | #Potent |

**Table 3:** **Summary of anticancer activity of the extracts from** ***T. violacea*****.**

| No. | Sample | Solvent | IC50 for TK-10 (µg/ml) | IC50 for PC-3 (µg/ml) | IC50 for HeLa (µg/ml) |
|---|---|---|---|---|---|
| | Parthenolide | Standard | 2.658 | 3.710 | 7.483 |
| 5 | Roots | Meth/dichlor (1:1) | >100 | >100 | >100 |
| 6 | Roots | Water | >100 | >100 | >100 |
| 3 | Bulbs | Meth/dichlor (1:1) | >100 | >100 | >100 |
| 4 | Bulbs | Water | >100 | >100 | >100 |
| 1 | Leaves | Meth/dichlor (1:1) | >100 | >100 | >100 |
| 2 | Leaves | Water | 62.37 | 49.14 | 64.8 |

*Meth/dichlor=Methanol/dichloromethane

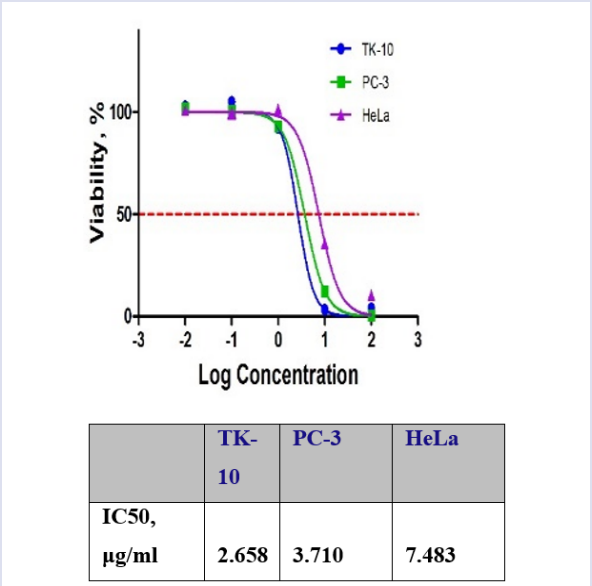
*T. violacea* leaf, root, and bulb extracts on the cancer cell lines TK10, PC3, and HeLa were therefore investigated. The extract was considered inactive when the findings of the IC50 value for two or three cell lines were larger than 100ug/ml. When tested against two or more cell lines, the extract's IC50 value was discovered to be greater than 15 g/ml but less than 100 g/ml.

## Determination of IC50 inhibition concentration

The parthenolide standard was tested on the TK-10, PC-3, and HeLa cell lines. With an IC50 value less than 6.25 g/ml, the standard drug (Parthenolide) (graph 1) demonstrated significant anticancer effect in all three cell types. The control results were in line with olive leaf extract, which, when used in combination with parthenolide, significantly reduced the viability of cervical cancer cells (Vizza *et al.*, 2019). In order to compare the growth-inhibitory properties of the plant extracts to Parthenolide's known activity, the SRB assay was used on a panel of three cancer cell lines: TK10 (renal), PC-3 (prostate), and HeLa (cervix).

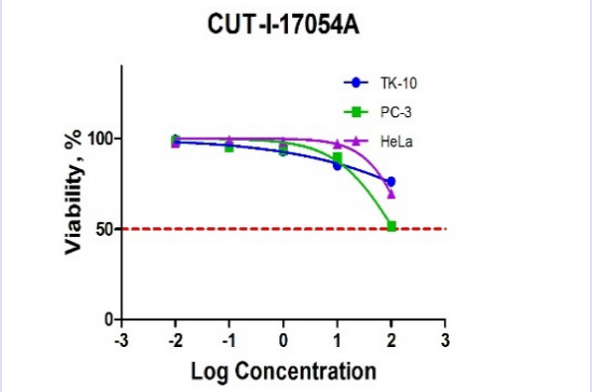
Three cell lines—TK-10, PC-3, and HeLa—were examined with the leaf extract in methanol/dichloromethane. Despite the extracts' generally modest potency, testing on the TK-10 and PC-3 cancer cell line demonstrated a specific anti-cancer activity. Methanol/dichloromethane (1:1) leaf extract was ineffective for all cell lines, as shown in Graph 2. 50% of the viable cells needed to be blocked by more than 100 ug/ml. This merely served to highlight Table 3's finding that the extract's performance was inferior to the control. The results of the investigation utilizing the dichloromethane/methanol leaf extract were different from the trial results using the methanol leaf extract. HeLa and the three distinct cell lines, HT-29 (colon cancer), MCF-7 (breast cancer), and WHCO3 (oesophageal cancer), shown growth suppression despite the use of a different solvent.[21]

When tested against the human cell lines TK-10 as opposed to HeLa and PC-3, the aqueous leaf extract shown more activity. The PC-3 behaved differently from the remaining two cell lines to the inhibitory effects of the water leaf extract as shown on graph 3. The study's findings, which demonstrated that T. *violacea* water leaf extract significantly and more efficiently repressed the human oral cell line at various concentrations,[22] did not match the results of the water leaf extract. The study's published findings, which prove that the water leaf extract of T. *violacea* significantly inhibited the human oral cell line at various concentrations, did not match the outcomes achieved from the water leaf extract.[22]



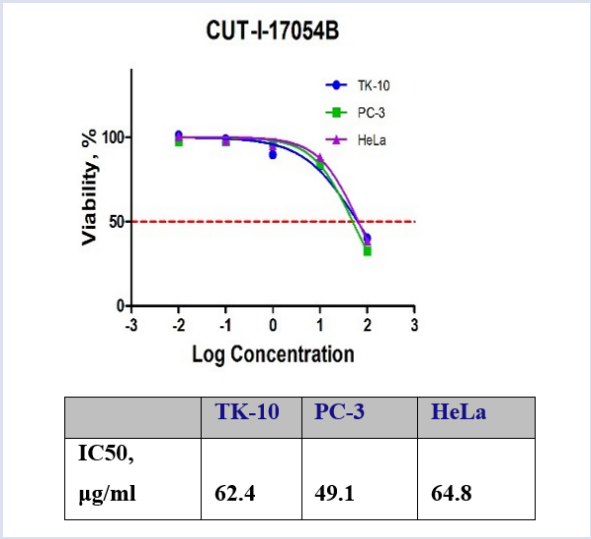

| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | 2.658 | 3.710 | 7.483 |

**Graph 1:** Parthenolide standard



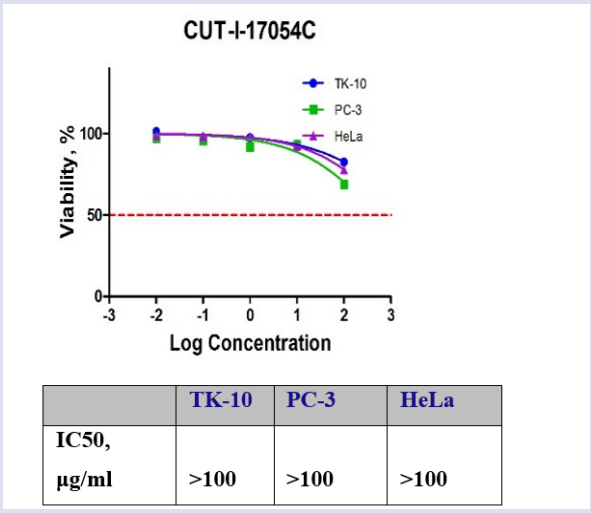

| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | >100 | >100 | >100 |

**Graph 2:** Anticancer activity of meth/dichlor leaf extract on TK10, PC3 and HELA
Z' factor: 0.98



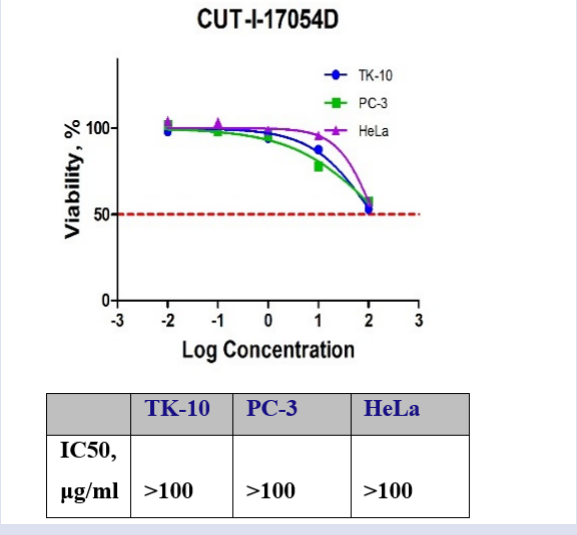

| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | 62.4 | 49.1 | 64.8 |

**Graph 3:** Anticancer activity of water leaf extract on TK10, PC3 and HELA



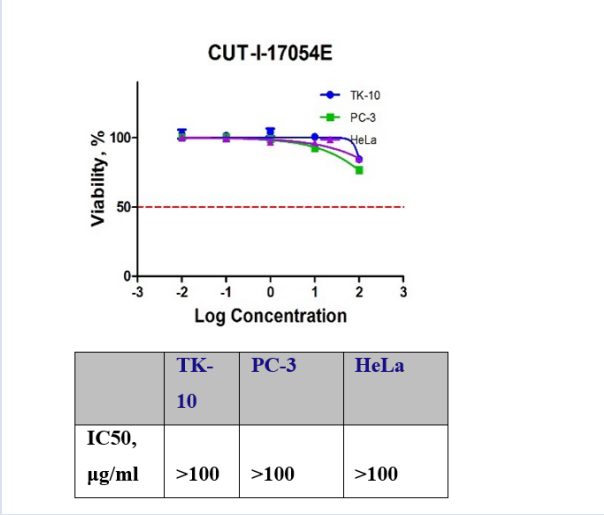

| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | >100 | >100 | >100 |

**Graph 4:** Anticancer activity of meth/dichlor (1:1) bulb extract of TK10, PC3 and HELA



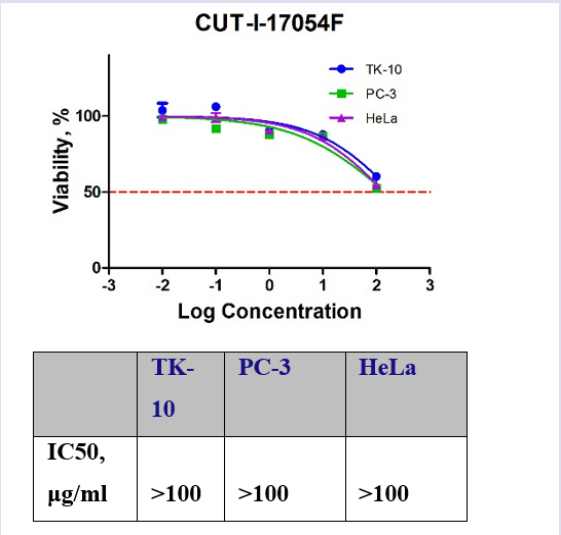

| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | >100 | >100 | >100 |

**Graph 5:** Anticancer activity of water bulb extract from *Tulbaghia violacea* on TK10, PC3 and HELA




| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | >100 | >100 | >100 |

**Graph 6:** Anticancer activity of meth/dichlor (1:1) root extract from *Tulbaghia violacea* on TK10, PC3 and HELA




| | TK-10 | PC-3 | HeLa |
|---|---|---|---|
| IC50, µg/ml | >100 | >100 | >100 |

**Graph 7:** Anticancer activity of water root extract on TK10, PC3 and HELA

The PC-3, TK-10, and HeLa cell lines were used to test the methanol/dichloromethane bulb extract. All of the bulb extracts were inert, however PC-3 was more potent than TK-10 and PC-3 against cancer. To give better activity, extract concentration should be increased as shown on graph 4.

Human cell lines TK10, PC-3, and HeLa were used to examine the activity of T. *violacea* water bulb extract. The water bulb extract performed better than the TK-10 and HeLa human cell lines when tested against PC-3 (graph 5). Extracts from water bulbs had little effect on TK-10 cells.

In PC-3, TK-10, and HeLa cell lines, the efficacy of the root extract in methanol/dichloromethane was investigated. Extracts from the root section, on the other hand, only showed minimal effect, and TK-10 and PC-3 responded more favorably than PC-3. The root extracts were innocuous when tested against the TK-10 cell line. As indicated on graph 6, it is advised to raise the extract concentration for better outcomes.

T. *violacea* aqueous root extract was tested on the TK10, PC-3, and HeLa human cell lines, and it was found to be active in each case. In

experiments against PC-3, root extracts fared better than the human (TK-10) cell lines and HeLa. Water root plant extract show weak activity against the TK-10 cell line.

On the PC-3, TK-10, and HeLa cell lines, T. *violacea* leaf, bulb, and root extracts were evaluated in aqueous and methanol/dichloromethane solutions. In a solution of methanol and dichloromethane, leaf extracts of T. *violacea* had no effect on the TK-10, PC-3, or HeLa cancer cell lines. The aqueous leaf extracts prevented the growth of the TK-10, PC-3, and HeLa cancer cell lines, which were utilized as test subjects. The methanol/dichloromethane bulb extracts performed badly when tested on the TK-10, PC-3, and HeLa cancer cell lines, according to the research. In tests, the aqueous bulb extracts did not kill the cancer cell lines TK-10, PC-3, or HeLa. When tested against the TK-10, PC-3, and HeLa cancer cell lines, the methanol/dichloromethane root extracts were unable to kill the cancer cells. The aqueous root extracts did not cause the cancer cell lines TK-10, PC-3, or HeLa to develop more slowly. The highest reduction of the HeLa cancer cell lines was seen with the aqueous leaf extract, followed by TK-10 and PC-3. Leaf extracts were the most active, as opposed to root and bulb extracts. The leaf is the best plant part to test against TK-10, PC-3, and HeLa cancer cells, according to their anticancer activity data. The three cell lines were tested using progressively higher quantities of plant extracts, with water remaining the most effective solvent. Traditional healers employ water, which is in line with the water extracts of T. *violacea's* greater inhibitory efficacy, as seen in table 3.

## Assessment of antioxidant activity using the DPPH assay

Different part of plants each extract listed in table 4 were examined for the free radicals (DPPH) scavenging activity and total phenols. The results of this assay are displayed in table 5, clearly showing the percentage activity of each extract.

At room temperature, the free radical DPPH is a stable compound. In ethanol, it produces a violet solution. A free radical that comes into contact with an antioxidant loses its ability to behave as a free radical and changes color to a light yellow as it takes an electron or hydrogen from the antioxidant to create a stable molecule. Extracts that produced a yellow tint were considered to have antioxidant properties in the current experiment. The antioxidant qualities of T. *violacea* leaf, bulb, and root extracts were assessed using percentage scavenging activities (table 5).

In contrast to the leaf water extracts, which had the maximum scavenging activity, the T. *violacea* bulb methanol/dichloromethane extracts showed the lowest. T. *violacea* crude extract was as effective as quercetin, with a maximum inhibition of 88.96% at 25ug/ml, versus 86.43% for quercetin at 25mM. In contrast to the usual, the T. *violacea* extract demonstrated significant antioxidant activity. These findings are consistent with those of Takaidza *et al.* (2018),[22] who discovered that T. *violacea* had the strongest DPPH [0.01 (43%) to 0.5 mg/mL (57%)] and ABTS [0.01 (46%) to 0.5 mg/mL (70%)] scavenging activities when compared to other Tulbaghia species.

## Total phenolic determination

Table 6 shows the absorbance of the reference material (gallic acid) in T. *violacea* at a maximum wavelength of 750 nm, and Figure 1 shows the reference calibration curve for determining the total phenolic content.

The total phenol concentration, as determined by the Folin-Ciocalteu reagent (standard curve equation: y = 0.0217, R2 = 0.9816; Figure 1), is represented in table 5 as gallic acid equivalents. Total phenolic concentrations for the leaf, bulb, and root extracts were discovered to be 21.8, 10.94, and 10.94 g/mL, respectively. Total phenolic concentrations for the leaf, stem, and root extracts in the methanol/dichloromethane extracts were reported to be 20.28, 7.93, and 13.36 g/mL, respectively.

**Table 4: List of plant extracts used in the DPPH and total phenolic assay against Vero cell.**

| Sample No. | Part of plant | Extraction solvent |
|---|---|---|
| 3 | Root | Meth/dichlor |
| 1 | Bulb | Meth/dichlor |
| 5 | Leaf | Meth/dichlor |
| 9 | Roots | Water |
| 12 | Bulb | Water |
| 10 | Leaf | Water |

*Meth/dichlor=Methanol/dichloromethane

**Table 5: Antioxidant activity of extracts expressed as percentage of DPPH scavenged.**

| Plant extract | Concentration | % DPPH scavenged* | Std Dev |
|---|---|---|---|
| #1 | 250µg/mL | 56.66 | 10.20 |
| | 500µg/mL | 75.3 | 4.8 |
| #3 | 250µg/mL | 82.6 | 1.8 |
| | 500µg/mL | 86.0 | 4.30 |
| #5 | 250µg/mL | 71.6 | 8.6 |
| | 500µg/mL | 74.0 | 20.6 |
| #9 | 250µg/mL | 58.4 | 4.5 |
| | 500µg/mL | 66.2 | 5.1 |
| #10 | 250µg/mL | 88.9 | 0.3 |
| | 500µg/mL | 88.5 | 1.1 |
| #12 | 250µg/mL | 81.5 | 5.8 |
| | 500µg/mL | 76.15 | 9.57 |
| Quercetin (positive control) | 6.25 µM | 48.7 | 9.7 |
| | 12.5 µM | 84.45 | 1.1 |
| | 25 µM | 86.4 | 0.7 |

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

**Table 6: Total phenolics determination using the FC method.**

| Plant extract | Ave. Abs 750 nm | phenolic conc (µg/mL) | Std dev of conc (µg/mL) |
|---|---|---|---|
| 1 | 0.17 | 7.93 | 0.12 |
| 9 | 0.24 | 10.94 | 1.46 |
| 12 | 0.24 | 10.94 | 0.83 |
| 3 | 0.29 | 13.26 | 1.09 |
| 5 | 0.44 | 20.28 | 0.98 |
| 10 | 0.47 | 21.75 | 0.88 |

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

From 7.93 to 10.94 g/ml, the total phenolic content varied between accessions. According to Table 6, water leaf extract had a higher content of phenolics, while methanol/dichloromethane bulb extract had the lowest. The total phenolic content of the water extracts in the leaf, stem, and roots was 3.59, 2.38, and 1.91 mg/g, respectively, according to Madike *et al.* (2017).[23] When 70% ethanol was one of the extracting solvents, the leaf, stem, and root extracts had total phenolic contents of 0.98, 0.34, and 0.15 mg/g, respectively.

Some T. *violacea* sections contain pharmacologically active substances as tannins, terpenoids, flavonoids, saponins, proteins, steroids, cardiac glycosides, phenols, and coumarins, according to Madike *et al.* (2017).[23] The plant's leaves held more active chemicals than the stems and roots in extracts made with water and 70% ethanol. The T. *violacea* extracts

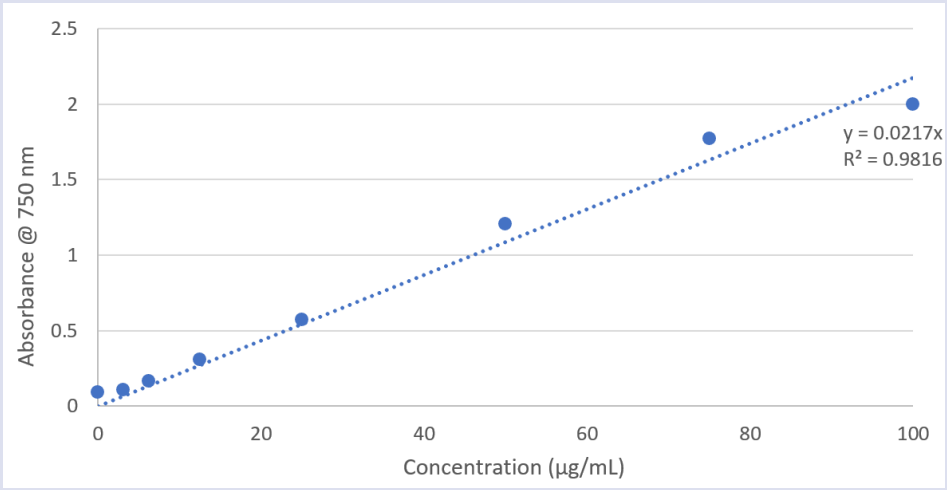



**Figure 1:** Normal curve for total phenolics determination using the FC assay and gallic acid as the standard control

utilized in this work are therefore likely to contain pharmacological elements, with water leaf extract having the highest levels of phenolics and antioxidants, as demonstrated in Tables 5 and 6 respectively.

## CONCLUSION

T. *violacea* extracts were often ineffective or weak against the human cell lines TK-10 (renal), PC3, and HeLa, as shown in Tables 2 through 6 and graphs 1 to 7. To compare these three human cell lines to aqueous and methanol/dichloromethane extracts, a higher concentration is indicated. An aqueous solvent was used to successfully extract the T. *violacea* plant. The leaf extract would also be the best plant component to test on the human cell lines TK-10, PC-3, and HeLa. The IC50 values for two to three cell lines show that T. *violacea* plant extracts are inert (>100 g/ml). Acokanthera and Gomphocarus, producers of cardiac glycosides, are widely used to create incredibly lethal arrow poisons in Africa. Bufadienolides, which are toxic to livestock and the cause of the severe poisoning illness known as nenta or krimpiekte (0.4% hit rate), are found in the Crassulaceae plants Kalanchoe paniculate, Kalanchoe thyrstflora, and Coryledon cuneate.[24] The aqueous leaf extract supports the traditional healers' use of the T. *violacea* plant to cure cancer and other diseases. Additional studies with different cell lines are required to identify the active components in T. *violacea* plant extracts. It has been observed that T. *violacea* extracts in both water and methanol/dichloromethane have antioxidant activity. Both the aqueous and methanol/dichloromethane extracts may also be used to assess total phenolic content. It is commonly recognized that phenolic compounds modify the color, flavor, and aroma of food while also enhancing nutritional content and providing positive health effects. They take involvement in plant defense mechanisms as well as defending plants against ROS, bacteria, insects, and herbivore damage.[25] The antibacterial effect of T. *violacea* will be further investigated on a variety of organisms that are particularly efficient opportunistic pathogens and consequently involved in a number of disorders.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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