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Original Article

Anticancer, cytotoxicity, and genotoxicity assays of *Tulbaghia violacea* extracts

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ABSTRACT

It has been shown that herbal medicine is a rich source of treatments with several chemical structures and bioactive elements that are effective against a variety of illnesses. Using the Sulforhodomine B(SBR) assay, *T. violacea* was examined for anticancer activity in this study to ascertain the growth-inhibiting properties of the water and methanol/dichloromethane plant extracts. Thermo Fischer Scientific product, the NucRed Live 647 Probe was used in the genotoxicity experiment to measure the growth of micronuclei. The SRB test was performed to evaluate the cytotoxic activity on the WI38 cell line. When examined with water and methanol/dichloromethane extracts, PC3 (prostate), HeLa (cervix), and the human cell lines TK-10 (renal) showed no activity. No genotoxicity was evident in the extracts made from water or methanol/dichloromethane. When tested against, neither the water extract nor the methanol/dichloromethane extract of the WI-38 cell line. Aqueous proved to be an effective solvent for *T. violacea* plant extraction. The plant's leaf extracts were recommended as the best extract to test on the PC-3, HeLa human cell lines, and TK-10. This study, therefore, agrees with the traditional methods of extraction and the preferred plant part used by traditional healers.

Keywords: *Tulbaghia violacea*, Extracts, Aqueous, Methanol/dichloromethane

Introduction

Many South Africans rely on natural medicines for their essential medical requirements [1, 2]. Inappropriate collection of plants, processing while fresh or dried, and storage techniques, as well as unintended contaminants in the products, have harmed consumers' perceptions of African herbal remedy products competing in international markets [3]. Numerous factors, including reasonably excellent ease of access to medicinal plants, affordability, and substantial indigenous education and expertise among the locals, can be used to explain why such a large percentage of people need herbal remedies [4, 5]. Secondary metabolites found in herbal remedies are not benign molecules [6]. Herbal remedies have established chemical resistance to prevent ailment or destroy threatening species [7, 8].

The host's normal cells are badly harmed by most of the cancer chemotherapies [9]. The most significant challenge in treating cancer is eliminating malignant cells while sparing healthy cell damage [10, 11]. These affordable cancer treatments are inaccessible to those who reside in underdeveloped and resource-constrained communities due to their high cost [12]. The entire range of critical human organs might be disturbed by the toxic components included in herbal treatments. Some individuals risk damaging vitally significant functional bodily systems, such as the central nervous system (CNS), by interfering with the body's nerve functions [13, 14]. Metabolic toxins and Cytotoxins which have an impact on organs including the kidneys, liver, heart, and lungs, come after neurotoxins, which have an impact on the brain and central nervous system [15]. The solubility of the toxin in body fluids is another aspect that may affect the severity of toxins, the rate of poisoning, and the phase of the target [16]. Cell death, genotoxic uncertainty, and the development of cancer can result from the development of micronuclei [17]. As part of the search

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for new and unique biological activities, the anticancer, cytotoxicity, and genotoxicity capabilities of crude extracts of *T. violacea* 's leaves, bulbs, and roots will be examined in this work

Materials and Methods

Plant collection and preparation

The herbal material from *T. violacea* has been validated by a South African expert at Bloemfontein National Botanical Garden. The acquired raw material was separated into roots, bulbs, and leaves after being cleaned to get rid of the soil. The herbal components were dried in an oven set between 30 to 60°C for a week. The dried herbal components were crushed to a granular powder in a hammer mill and put in storage in the 20-25°C room before extraction.

Extraction

Waring blender was used to combine the dried and ground herbal ingredients with some of the extraction solvents (methanol/dichloromethane (1:1) and water). After blending, the remaining solvent was combined, and the solutions were left to soak for 24 hours. Over the next 24 hours, the particles in each solution were removed using a Millipore funnel with medium filter paper (Bright sign # 102) coupled to a Millipore vacuum pump. The rotating vacuum was used to concentrate plant extracts at 50–60°C and followed by further drying in vacuo at room temperature. A freeze-dryer was used to concentrate the water *T violacea* extracts until they were dry. The six herbal extracts **(Table 1)** were kept at a temperature of -20°C pending usage.

Table 1. *Tulbaghia violacea* extraction using solvents such as water and methanol/dichloromethane

Sample No.	Solvent for extraction	Part	Yield (g)
#6	Water	Roots	3.4951
#5	Methanol/dichloromethane (1:1)	Roots	6.2868
#4	Water	Bulbs	6.8882
#3	Methanol/dichloromethane (1:1)	Bulbs	4.8616
#2	Water	Leaves	7.1516
#1	Methanol/dichloromethane (1:1)	Leaves	6.2716

Assay: Sulforhodamine B

The effectiveness of *T. violacea* herbal extracts as growth inhibitors was investigated using the SRB assay. The protein-dye SRB (Acid Red 52)'s ability to bind to protein-basic amino acid residues of trichloroacetic acid-fixed cells pH-dependently electrostatically is the basis of the SRB. In slightly basic environments, it can be taken out of cells and solubilized for measurement; however, in gently acidic environments, it would then bind to the stable cellular protein. The National Cancer Institute's (NCI) Drug Evaluation Branch's methodology was used to conduct the sulforhodamine assay at the Council for Scientific and Industrial Research (CSIR).

The TK10 (renal), HeLa (cervix) cancer cell lines, and PC3 (prostate) were acquired from the NCI as part of a joint research collaboration project between the NCI and the CSIR. The ability of the herbal extracts to stop cell growth was evaluated using the SRB test in a panel of three cancer cell lines, including the TK10, HeLa cancer cell lines, and PC3. Cell lines were maintained daily using Roswell Park Memorial Institute (RPMI) at 37°C, anaerobic condition (5% CO_2), aerobically at 95% air, and 100% relative humidity, which comprises 50 g/ml gentamicin, 2 mM L-glutamine, and5% fetal bovine serum. The screening test was performed as follows, the cells (3–19 passages) were plated in 96-well microtiter plates with densities between 7–10,000 cells/well, followed by incubation of the plates for 24 hours. The examined herbal extracts were dissolved in Dimethyl sulfoxide (DMSO) and diluted in a medium to attain five concentrations the next day, and then administered to the cells. The control cells were those that had no treatment applied to them. The blank contained no cells and was entirely devoid of media. The parthenolide used as a reference was used. The plates were cleaned, dried, and dyed with SRB after the herbal extracts had been applied and incubated for 48 hours. The bottom of each well was then coated with cold, 50% trichloroacetic acid, which was used to adhere to viable cells. Unbound dye was removed, and protein-bound dye was extracted with a 10 mM Tris base to determine optical density at a wavelength using a multi-well spectrophotometer at 540nm.

Genotoxicity assay

Thermo Fischer Scientific's NucRed Living 647 Probe is a far-red nuclear dye that penetrates both dead and live cells. It was applied to gauge the micronuclei growth of cells. Vero cells which are derived from the kidney cells of an African green monkey were sown at a density of 4,000 cells per well and given the night to attach. Each extract was applied to the cells at different concentrations namely 200, 100, 50, 25, and 12.5 µg/ml followed by incubation *at* 37°C for 48 hours. NucRed was used to dye the medium and treated cells using a working solution in 1 ml of PBS (+Ca + Mg). A hundred microlitres (100 ul) of the NucRed working solution was added to each well in the aspirate medium as well as in the treatments, then later all were incubated at 37°C for 15 to 30 minutes.

Data statistical analysis

Analysis of the data was carried out using the GraphPad Prism program. The four categories of the herbal extracts' cell growth activities are reported as IC_{50} (50% radical-scavenging impact) values **(Table 2)**. Non-linear regression was used to calculate the 50% cell growth inhibitor (IC_{50}).

Table 2. Benchmark for anticancer activities according to CSIR

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

Results and Discussion

Anticancer activity

Table 3 provide the results of tests obtained by determining the anticancer activity of extracts against healthy human fetal lung fibroblast cell lines cancer and cell lines. As a benchmark, Parthenolide was used. The activities investigated were interpreted and categorized using the anticancer activity criteria adopted from the CSIR **(Table 2)**. The TK10, HeLa cancer cell lines, and PC3 were evaluated against both methanol/dichloromethane and water root, bulb, and leaf extracts. The extract was declared inactive when the IC_{50} value of 2 or 3 cell lines was found to be more than 100 g/ml. An extract's results of IC_{50} value when tested against 2 or more cell lines were found to be higher than 15 g/ml but lower than 100 g/ml. This extract was deemed poor **(Table 2)**.

The determination of IC_{50} concentration

Table 3. Anticancer activity of the *T. violacea* extracts

No.	Sample	Solvent	IC_{50} TK-10 (µg/ml)	IC_{50} PC-3 (µg/ml)	IC_{50} HeLa (µg/ml)
1	Leaves	Methanol/ dichloromethane (1:1)	>100	>100	>100
2	Leaves	Water	62.37	49.14	64.8
3	Bulbs	Methanol/ dichloromethane (1:1)	>100	>100	>100
4	Bulbs	Water	>100	>100	>100
5	Roots	Methanol/ dichloromethane (1:1)	>100	>100	>100
6	Roots	Water	>100	>100	>100
	Parthenolide	**Standard**	**2.658**	**3.710**	**7.483**

As indicated in **Table 3**, the following cell lines PC-3, TK-10, and HeLa were examined using equal ratios of the methanol/dichloromethane and water root, bulb, and leaf extracts. The common drug, parthenolide, **(Table 3)**, demonstrated potent anticancer properties. When tested on the PC-3, TK-10, and HeLa cancer cell lines, the *T. violacea* leaf extract in methanol/dichloromethane was ineffective. While tested against the TK10, HeLa cancer cell lines, and PC3, the water leaf extract demonstrated inhibitory efficacy. When tested against the PC-3, TK-10, and HeLa cancer cell lines, the methanol/dichloromethane bulb extracts failed. When tested against the cancer cell lines PC-3, TK-10, and HeLa, the water bulb extracts likewise performed poorly. When tested against the TK-10, PC-3, and HeLa cancer cell lines, the methanol/dichloromethane root extracts failed. When tested against the TK10, HeLa cancer cell lines, and PC-3, the water root extracts also displayed minimal effects. The HeLa, TK-10, and PC-3 cancer cell lines were more effectively inhibited by the water leaf extract. When compared to bulb and root extracts, the leaf extract showed the most activity. These results on anticancer activity presented that the leaf extract is the optimum composition of the herbal to employ for PC-3, TK-10, and HeLa cancer cell lines further testing. The three cell lines should be tested using a higher concentration of the herbal extracts, and the optimum solvent to utilize is still water. The water extracts from the plant had a greater inhibitory effect, which related to the use of water by traditional healers.

Cytotoxicity

According to the CSIR standards, the investigation of the various extracts was carried out **(Table 4)**. Weakly cytotoxic is defined as any extract having the IC_{50} value of more than 30µg/ml and then lower than 100*µg/ml* once evaluated against a cell line **(Table 4)**. An extract is regarded as highly cytotoxic if its IC_{50} value is bigger than 5µg/m*l* but less than 30 µg/ml **(Table 4)**. All extracts with the IC_{50} below 5µg/ml were deemed to be extremely dangerous.

Table 4. Standard cytotoxicity standards based on IC_{50}

IC50 (µg/ml)	Status
> 100	#Low hazard
< 100	#Weak hazard
> 30	
< 30	#Moderate hazard
>5	
<5	#High hazard

#(IC_{50}) :50% inhibition concentration

Table 5. Cytotoxicity assay from *T.violace* extracts against WI-38 cell line

No.	The solvent used for extraction	Plant part extracted	IC50 (µg/ml)
1	Methanol/dichloromethane	Leaf	>100
2	Water	Leaf	>100
3	Methanol/dichloromethane	Bulb	>100
4	Water	Bulb	>100
5	Methanol/dichloromethane	Root	>100
6	Water	Root	>100
7	**Standard**	**Emetine**	**2.66**

As demonstrated in **Table 5**, *T. violacea* root, bulb, and leaf extracts in methanol/dichloromethane and water at identical ratios were tested on the WI-38 cell line, a typical human fetal lung fibroblast. To test 50 percent inhibitory dosages against WI-38 cell lines at various concentrations, Emetine **(Table 5)** was utilized as a control. None of the *T. violacea* extracts were dangerous when examined against the WI-38 cell line. These findings diverge from those of Madike *et al.*, 2020, who demonstrated using a different cell line that the fraction of viable

cells dropped as the amount of the extract increased being ethanolic in their study [18].

Genotoxicity

Figure 1 shows how the growth of micronuclei was evaluated using the ImageXpress microscope and NucRed nuclear dye. These results overwhelmingly demonstrate that all extracts in **Table 6** failed to stimulate the formation of micronuclei, with the possible exception of methanol/dichloromethane, which may be genotoxic.

Table 6. A list of the plant extracts tested for genotoxicity on Vero cells.

Sample No.	Extraction solvent	Part
#12	Water	Bulb
#10	Water	Leaf
#9	Water	Root
#5	Methanol/dichloromethane	Leaf
#3	Methanol/dichloromethane	Root
#1	Methanol/dichloromethane	Bulb

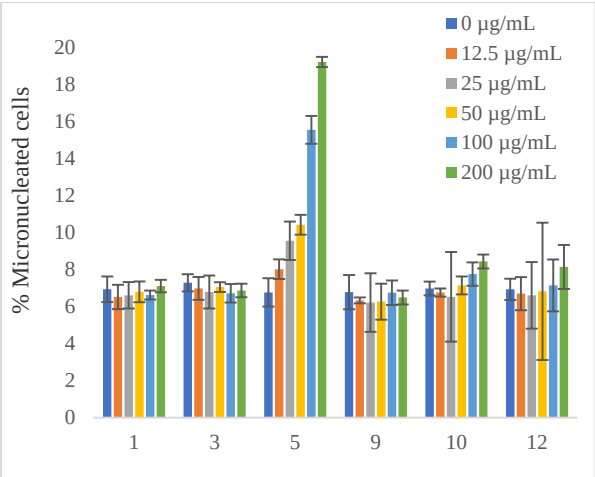

Figure 1. Micronuclei formed after Vero cells were exposed to extract at various doses (as demonstrated). Standard deviation from quadruplicate results was displayed in error bars.

Figure 1 showed the micronucleated cells at 7%, which was even evident at 0 ug/ml, which clearly shows false positive results displayed as induced micronucleus. The overall elimination of the 7% could have been attributed to the circumstances surrounding incubation conditions and another possible trial and error in the lab. There was a 1% rise in the negative control on the water extracts of both the leaf and bulb, which could have been overlooked when error bars are considered. The DNA damage caused by all extracts, except for dichloromethane and methanol, on leaves is less significant. By contrasting water leaf extract, which exhibited no genotoxicity, with the chemical on the leaf, it can be assumed that methanol/dichloromethane contributed to the genotoxicity. The findings of this study corroborated those of a study by Madike *et al.*, 2019, that showed that stem extracts resulted in an elevated percentage of micronucleated cells than root and leaf extracts [19]. The findings of this study corroborated those of a study by Madike *et al.,* 2019 that showed that stem extracts produced a greater percentage of micronucleated cells than leaf and root extracts [14].

Conclusion

According to **Table 3**, most extracts were ineffective against TK-10 in the human cell lines HeLa and PC3 cancer cell lines. It is recommended to test all the human cell lines against water and methanol/dichloromethane at a higher concentration. Water was found to be the best solvent for extracting *T. violacea* plant material. Additionally, the plant's leaf extract showed the best results when tested against the HeLa, PC-3, and TK-10 human cell lines. The allegations made by customary healers on the use of *T. violacea* herbal for the healing of cancer and other illnesses are endorsed by the water extract on the leaf.

To find the *T. violacea* herbal extracts' active ingredients, additional research employing other cell lines is crucial. When evaluated on the WI-38 cell line, the *T. violacea* both the methanol/dichloromethane and the water extracts were not harmful. Additionally, because they did not cause the development of micronuclei, all most all extracts of *T. violacea* could not be deemed genotoxic. This result suggests that more investigation is possible to ascertain the phenols and total antioxidants contained in *T. violacea* in the treatment of the diseases. The results of the tests for cytotoxicity and genotoxicity backed up the continued belief of safety in using herbs for human consumption. This study demonstrated that *T. violacea* is safe to use topically to treat diseases.

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Conflict of interest: None

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Ethics statement: None

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