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Research Article

Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of *Tulbaghia violacea*

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ABSTRACT

Tulbaghia violacea has been used extensively in South African traditional medicine for treatment of a number of ailments. Few studies have examined the bioactive compounds present in the plant. This study assessed the phytochemicals present in the leaves, stems and roots of *T. violacea.* The phytochemicals were extracted separately with distilled water and 70% ethanol by maceration. A wide variety of pharmacologically active compounds such as tannins, terpenoids, flavonoids, saponins, proteins, steroids, cardiac glycosides, phenols and coumarins were present in some of the *T. violacea* plant parts. However, phlobatannins, leucoanthocyanins, alkaloids, carbohydrates and anthocyanins were absent in the plant. This study showed that most of the phytocompounds were present in the stem and roots of *T. violacea* compared to the stem and roots. This is significant for conserving the species since the leaves of the plant can be harvested for medicinal use while the rest of the plant is left intact for regeneration of the plant. This study also showed that the two solvents extracted different amounts and types of phytochemicals from the different parts of the plant suggesting that a single solvent may not be able to extract all the known bioactive compounds from a plant.

Keywords: *Tulbaghia violacea*, phytochemicals, maceration, bioactive compounds.

INTRODUCTION

Tulbaghia violacea, has been used extensively in South African traditional medicine for Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) patients and to treat various ailments such as fever, colds, asthma, cryptococcal meningitis, tuberculosis, oesophagus cancer and high blood pressure[1-5]. The common English name for *T. violacea* is "wild' or 'society' garlic. This name apparently originated from the belief that, despite its garlic-like flavour, the consumption of *T. violacea* is not accompanied by the development of bad breath, as is the case with the consumption of commercial garlic (*A. sativum*)[6]. When crushed on the skin the leaves of *T. violacea* can cure sinus headaches, repel fleas, ticks and mosquitoes[7].

According to the World Health Organization (WHO, 1978), about 80% of the world's rural population currently relies on medicinal plants as their complementary or alternative source of health care[8,9]. Medicinal plants contain bioactive non-nutrient and biologically active compounds known as phytochemicals which contain a broad spectrum of chemical structures and protective/disease preventative properties. Thus, conducting preliminary phytochemical screening of plants is an important aspect in determining the chemical constituents in plant materials. Preliminary phytochemical screening of plants is also necessary for the discovery and development of novel therapeutic agents with improved efficacy[10,11]. The present study reports on the phytochemical screening as well as the total phenol and flavonoid contents from water and 70% ethanol extracts of the leaves, stems, and roots of *T. violacea*.

MATERIALS AND METHODS

Plant material

Tulbaghia violacea plants were collected from The Vaal University of Technology greenhouse. The plant species was identified with the help of AP Goosen Herbarium as *Tulbaghia violacea* (ST0008). The plants were firstly washed gently under running tap water to remove dust and soil and then rinsed with distilled water. The leaves, stems and roots were separated and cut into small pieces. The different samples were frozen, freeze dried and pulverised into a fine powder with a laboratory electric blender. The powdered plant materials were stored in air tight bottles. Five grams of the plant samples (leaves, stems, and roots) were extracted separately with distilled water and 70% ethanol by maceration (24 hrs for each solvent) with constant shaking. The homogenates were then filtered through Whatman® filter paper (0.45 µm pore size) and the extracts (0.05 g/ml) were all stored at 4ºC.

Qualitative Phytochemical Analysis

All the extracts (0.05 g/ml) were subjected to preliminary phytochemical screening following standard methods[12-15] for detection of the following constituents.

Steroids

Five milliliters of chloroform and 5 ml of H_2O_4 were added to 500 µl of the prepared plant extracts. The presence of steroids was indicated by a colour change from violet to blue or green or a ring of blue/green or if the upper layer

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turns red and the sulphuric layer was yellow with a green fluorescence.

Saponins

About 3 ml of plant extracts were added to 3 ml of distilled water and shaken vigorously. The formation of a stable persistent froth was taken as a positive test for saponins.

Alkaloids

Approximately 3 ml of extracts were added to 3 ml of 1% HCl and heated for 20 min. The mixtures were then cooled and used to perform the following tests:

Mayer's test

To the filtrate in test tube I, 1 ml of Mayer's reagent was added drop by drop. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Wagner's test

To the filtrate in tube III, 1 ml of Wagner's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Protein

Xanthoproteic test

A few drops of nitric acid were added to 2 ml of plant extracts and a colour change to yellow was observed.

Anthocyanin

Approximately 2 ml of the prepared plant extracts were added to 2 ml of 2N HCl and ammonia. The appearance of a pink red coloration that turned blue violet indicated the presence of anthocyanin.

Coumarin

About 3 ml of 10% NaOH were added to 2 ml of plant extracts. The formation of a yellow colour was an indication for the presence of coumarins.

Carbohydrates

Fehling test

Two milliliters of each plant extract were hydrolyzed with dilute HCl, neutralized with alkali, and then heated with Fehling's solution A and B. The formation of a red precipitate was an indication for the presence of a reducing sugar.

Flavonoid

Alkaline reagent test

Three milliliters of plant extract was treated with 1 ml of 10% NaOH solution. The formation of an intense yellow colour was an indication of the presence of flavonoids.

Leucoanthocyanin

Approximately 5 ml of isoamyl alcohol were added to 5 ml of plant extracts. The appearance of a red upper layer indicated the presence of leucoanthocyanin.

Cardiac Glycosides

Keller-Killani Test

Two milliliters of plant extract were treated with 2 ml glacial acetic acid containing a drop of $FeCl_3$. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

Phlobatannins

Two milliliters of 1% HCl were added to 3 ml of plant extracts and boiled. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Terpenoids

Approximately 2 ml of chloroform and 3 ml of H_2O_4 were added to 5 ml of plant extracts. A reddish-brown coloration was taken as positive test for terpenoids.

Test for phenols and tannins

Ferric chloride test

Two milliliters of 5% solution of $FeCl_3$ were added to 1 ml crude extracts. A black or blue-green colour indicated the presence of tannins and phenols.

Quantitative Phytochemical Analysis

Determination of total phenolic content (TPC)

The concentration of TPC in all the plant extracts was measured using a UV spectrophotometer, based on oxidation/reduction reaction[16] using Folin-Ciocalteu reagent[17]. To 500 µl of diluted extracts (10 mg in 10 ml solvent), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 ml of Na_2CO_3 (7.5%) were added. The samples were incubated for 5 min at 50ºC and then cooled. Distilled water (500 µl) was used as a negative control for the experiment. The absorbance of the standard gallic acid solution (0.5 mg/ml) was measured using 500 µl of 50, 100, 150, 200, 250 and 300 µg/ml methanolic gallic acid solutions. All determinations were performed in triplicate and a standard curve was established. The total phenol value was obtained from the regression equation: $y = 0.0106x + 0.1246$ and expressed as mg/g gallic acid equivalent using the formula, $C = cV/M$; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (µg/ml) established from the calibration curve, V = volume of extract (0.5 ml) and m = the weight of pure plant methanolic extract (0.05 g).

Determination of total flavonoid content

Total flavonoid content was measured according to the Aluminium Chloride colorimetric method [18] with some modification. A 1.5 ml aliquot of 20 g/l $AlCl_3$ ethanol solution was added to 500 µl of the plant extracts (5 g in 100 solvent) and distilled (500 µl) water was used as the negative control. The extracts were evaluated at a final concentration of 0.05 g/ml. The absorbance of the standard quercetin solutions was recorded after 60 min at 420 nm using 20, 40, 60, 80 and 100 µg/ml methanolic quercetin solutions and a calibration curve was established. The total flavonoid content expressed as quercetin equivalent (QE) was calculated based on the calibration curve using the following equation: $y = 0.0175x - 0.0061$, where x is the absorbance and y is the concentration (mg QE) of the methanolic quercetin solutions.

RESULTS

Qualitative Phytochemical Analysis and percentage yields

The yields obtained from the leaves, stems and roots with water ranged from 33.94 to 41.67% w/w, while that with ethanol ranged from 15.20 to 26.08% w/w (Table 1). The results of the phytochemical screening tests (strong, weak and negative) obtained from the water and 70% ethanol

extracts of the leaves, stems, and roots of *T. violacea* are presented in Table 1.

Quantitative Phytochemical Analysis

The total phenolic and flavonoid content of the three parts of the plant are shown in Table 2. The amount of phytochemicals varied not only among the leaves, stems and roots but also depended on whether water or ethanol was used as the extractant. The highest phenolic content appeared in the leaves in the water and ethanol extracts followed by the stems and roots. The leaves had the highest total flavonoid content compared to the stems and roots. However, the ethanol extracts had a higher amount of flavonoid in the leaves compared to the water extracts. A similar phenomenon was observed for the water extracts.

Total Flavonoid Content

The quercetin absorbance readings are shown in Table 3 and the standard calibration curve is shown in Figure 1. The flavonoid content of the water extracts in terms of quercetin equivalent (the standard curve equation: $y = 0.0175x - 0.0061$, $R^2 = 0.9892$; Figure 1) were found to be 0.47, 0.027 and 0.025 mg/g for the leaf, stem and root extracts, respectively. In the 70% ethanol extracts, the flavonoid content was found to be 0.66, 0.053 and 0.038 mg/g for the leaf, stem, and root extracts, respectively.

Total Phenolic Content

The absorbance of the standard compound (gallic acid) at λmax =760 nm in *T. violacea* is presented in Table 4 and the standard calibration curve for quantification of Total Phenolic content is shown in Figure 2. Table 2 shows the content of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 0.0106x + 0.1246$, $R^2 = 0.9949$; Figure 2). The total phenolic content for the water extracts was found to be 3.59, 2.38 and 1.91 mg/g in the leaf, stem and root extracts, respectively. In the 70% ethanol extracts, the total phenolic content was found to be 0.98, 0.34 and 0.15 mg/g in the leaf, stem and root extracts, respectively.

DISCUSSION

The analysis and characterization of bioactive compounds from plants is important to ascertain their medicinal value[19]. This study showed that pharmacologically active compounds such as tannins, terpenoids, flavonoids, saponins, proteins, steroids, cardiac glycosides, phenols and coumarins were present in some organs of *T. violacea* (Table 1). However, phlobatannins, leucoanthocyanins, alkaloids, carbohydrates and anthocyanins were absent in all plant parts (Table 1). An interesting aspect of this study is that the leaves of the plant contained more active compounds than those present in the stems and roots when both water and 70% ethanol were used as the extractants. This has importance in conserving the species. Many medicinal plants are being overexploited and are in danger of becoming extinct[20]. Since most of the bioactive compounds are present in the leaves of *T. violacea*, it is therefore possible to harvest the leaves while leaving the other parts, especially the underground rhizome of the plant, intact to regenerate itself.

Various chemicals have been used to extract bioactive compounds from plants. In this study, the water and 70% ethanol showed differential extraction of some of the compounds not only within the same organ but also in the different organs of the plant. For example, the ethanol extracted less saponins in the leaves while it extracted more flavonoids in the stems when compared to water. The differential extractions may be due to degrading enzymes that may be active or denatured in either of the two extractants. For example, the enzyme polyphenol oxidase degrades polyphenols in water extracts, whereas in ethanol they are inactive[21]. The important lesson from this study is that a single solvent may not necessarily extract all the useful bioactive compounds from a plant. Several solvents may have to be used to obtain the best yields of specific compounds. This study also showed that the roots yielded the least number of compounds overall.

With the exception of the 70% ethanol extracts, saponins were present in all parts of the plant with higher quantities observed in the water extracts of the leaves and roots (Table 1). Several studies have outlined the biological importance of saponins that include anti-inflammatory, anti-diabetic, anti-HIV and anti-atherosclerotic properties[22,23].

Flavonoids were found in all plant extracts, with the highest quantities observed in the water extracts of the leaves and 70% ethanol extracts of the leaves and stems (Table 1). Flavonoids have been reported to possess a wide variety of biological activities among which are antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic and antioxidant, antiviral, anticarcinogenic, anticancer as well as anti-diarrheal properties[24-28]. This corresponds to the diverse use of the leaves of *T. violacea* for the treatment of oral fungal infections, gastrointestinal ailments, fever and colds[6,29-31].

Proteins were present in all plant parts, with higher quantities observed in the ethanol extract of the leaves. This means that the nutritional value of these species as a protein supplement cannot be ignored. Studies have reported that the protein hydrolytes from various sources possess antioxidant activity[32,33]. Coumarins were also present in all plant parts. Overall, the *T. violacea* plant has high quantities of coumarins with smaller quantities observed only in the 70% ethanol extract of the roots. There are several biological activities that have been reported for coumarins, among which are anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, Cytochrome P450 inhibiting, antihyperglycemic[34,] antioxidant, estrogenic, dermal photosensitizing, antihelmentic, hypnotic, analgesic, hypothermic, antiulcer[35] anticlotting, hypotensive and antitumor activities[36].

Cardiac glycosides were present in high quantities in all the parts of *T. violacea* except in the water extract of the leaves. Glycosides are natural cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia[37-39]. The presence of cardiac glycosides in *T.*

Table 1: Results of preliminary phytochemical screening of water and 70% ethanol extracts from the leaves, stems, and roots of *Tulbaghia violacea.*

No	Compounds	Leaves		Stems		Roots	
		Water	Ethanol	Water	Ethanol	Water	Ethanol
1	Saponins	++	+	+	+	++	−
2	Flavonoids	++	++	+	++	+	+
3	Proteins	+	++	+	+	+	+
4	Coumarins	++	++	++	++	++	+
5	Cardiac glycoside	+	++	++	++	++	++
6	Terpenoids	++	++	++	+	++	+
7	Phlobatannins	−	−	−	−	−	−
8	Steroids	−	−	−	++	−	+
9	Phenols	++	++	++	+	+	−
10	Tannins	++	++	++	+	+	−
11	Carbohydrates	−	−	−	−	−	−
12	Alkaloids						
	• Mayer's reagent	−	−	−	−	−	−
	• Dragendorff's reagent	−	−	−	−	−	−
	• Wagner's reagent	−	−	−	−	−	−
13	Leucoanthocyanins	−	−	−	−	−	−
14	Anthocyanins	−	−	−	−	−	−
	Percentage yields (% w/w)	33.94	26.08	41.67	22.33	39.44	15.20

++ = Strong positive test, + = Weak positive test, − = Negative tests

Table 2: Phenolic and flavonoid contents of the water and 70% ethanol extracts from the leaves, stems, and roots of *T. violacea.*

Plant parts	Phenolic Content (mg of GAE/g of dry extract) ± SD		Flavonoid content (mg of QE/g of dry extract) ± SD	
	Water	Ethanol	Water	Ethanol
Leaves	3.59 ± 0.1*a*	0.98 ± 0.06*a*	0.47 ± 0.01*a*	0.66 ± 0.01*a*
Stems	2.38 ± 0.05*a*	0.34 ± 0.02*a*	0.027 ± 0.02*ab*	0.053 ± 0.01*ab*
Roots	1.91 ± 0.1*a*	0.15 ± 0.02*a*	0.025 ± 0.01*ac*	0.038 ± 0.01*ac*

Data represents the mean ± SD mg of Gallic acid equivalent per gram of dry weight (mg GAE/g) and Quercetin equivalent per gram of dry weight (mg QE/g) of the extracts, $n = 3$. Small letter *a* indicates statistically significant groups according to the *t*-test: Two-Sample Assuming Equal Variance ($p < 0.05$). Non-significant groups are represented by a*b* and *ac* ($p > 0.05$).

violacea may support the usefulness of this plant for the treatment of cardiac diseases[40].

Terpenoids were present in high quantities in most parts of the plant except for the lower quantities observed in the ethanol extracts of the stems and roots. They are known to possess a wide range of biological activities including antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, anti-inflammatory, immunomodulatory properties[41,42,] antimalarial, inhibition of cholesterol synthesis, antibacterial[43] and insecticidal properties[44]. They are also important in the prevention and therapy of several diseases, including cancer[45].

Steroids were only observed in the ethanol extract of the stems with a slightly lower amount in the ethanol extract of the roots. They are responsible for reducing cholesterol levels, for regulating the immune response[46] and some steroids also have immune-enhancing benefits[47,48] Tannins were absent in the 70% ethanol extract of the roots but were present in all the other parts of the plant. The presence of tannins in plants can be affected by the developmental stage of the plant and also by environmental factors[49,50]. Tannins possess a wide variety of biological activities among which are antimicrobial[51], anti-viral[52] antibacterial[53] and antiparasitic effects[54]. Studies have also investigated and reported on the ability of tannins to inhibit HIV replication selectivity and their use as a diuretic[55].

Similar to tannins, phenols were absent in the 70% ethanol extract of the roots but were present in the other parts of the plant (Table 1). It is not clear why the ethanol extract of the roots did not contain phenol (Table 1) since ethanol extracts of the leaves and stems did contain phenol. Several studies have reported that environmental factors, such as soil composition, temperature, rainfall and the incidence of ultraviolet radiation can influence the concentration of phenolic compounds in plants[56,57]. There are various biological activities have been reported for phenols in plants, among which are antitumor, antiviral, antimicrobial[58] and hypotensive effects[59] as well as antioxidant properties[60].

The only phytochemical studies on *T. violacea* were conducted by Soyingbe[61] and Ncube *et al.*[62]. Soyingbe[61] examined the essential oils from the roots of *T. violacea*

Table 3: Absorbance of standard compound (Quercetin) at λ_{max} = 415 nm

Sample no.	Concentration of Quercetin (µg/ml)	Absorbance at 415 nm
1	0	0.000
2	20	0.374
3	40	0.657
4	60	0.963
5	80	1.508
6	100	1.706

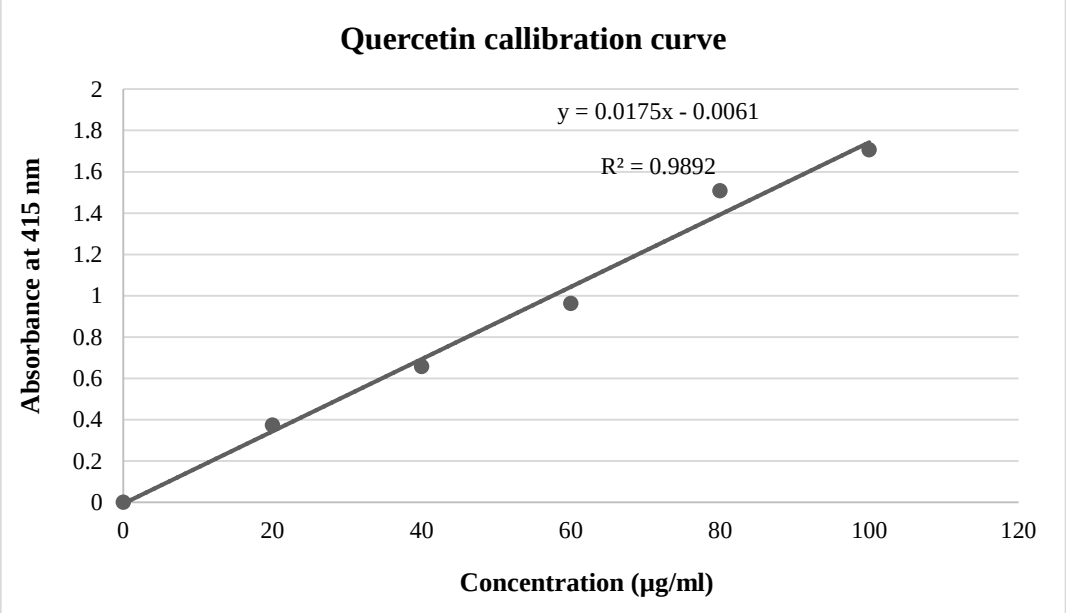

Figure 1: Standard calibration curve for quantification of Flavonoid content.

and reported the presence of tannins, alkaloids, and flavonoids while anthraquinones, cardiac glycosides and saponins were absent. Ncube *et al.*[62] examined the seeds and the whole plant (leaves and roots) of *T. violacea* and recorded the presence of phenols, flavonoids, gallotannins, condensed tannins and saponins. The results of Soyingbe [61] and Ncube *et al.*[62] are quite similar to those reported in this research except for the absence of alkaloids in all the plant parts of *T. violacea*. One of the reasons may be that Soyingbe[61] used essential oils of *T. violacea* instead of plant extracts for the phytochemical analysis. Environmental extremities such of light, temperature, and drought[63] may influence the synthesis/ content of certain compounds[64]. The class, content and quantity of the compounds may be different depending on the ecological factors present in the area where the plant is cultivated[65]. Whether environmental conditions and genetic variation are factors to be considered for the variation in this research study is a matter of conjecture and needs further studies.

Table 2 revealed that the total phenolics ranged from 1.91 to 3.59 mg/g gallic acid equivalent for the water extracts and from 0.98 to 0.15 mg/g gallic equivalent for the ethanol extracts. The concentration of total flavonoids ranged from 0.47 to 0.025 mg/g quercetin equivalent for the water extracts and from 0.66 to 0.038 mg/g quercetin equivalent for the ethanol extracts. This data reveals that the water and 70% ethanol extracts were less effective in extracting flavonoids and phenols from the stems and roots than from the leaves of the plant. However, the data obtained in this study was different from those reported by Olorunnisola *et al.*[66] who used 100% methanol extracts from fresh and dried *T. violacea* roots and from that of Narendhirakannan *et al.*[67] who used ethanol extracts from three varieties of *Allium sativum* L. The amounts of phenolic compounds from this study was lesser than what was obtained in the whole plant (leaves and bulbs), the fresh and dried root samples as well as in the three varieties of *A. sativum.* These differences may be attributed to the microclimate, processing method as well as the type of solvent employed[68,69] and genetic variation[70]. The high levels of phenolic compounds in the leaves support the medicinal importance of the plant in management and treatment of oxidative stress induced disorder[66]. In a study conducted by Ncube, Ngunge, Finnie and Van Staden [62], 50% methanol extracts from the whole plant (bulb and leaves) of *T. violacea* were used to determine total phenolic compounds. However, this study used an aqueous-methanol solution and different units making it difficult to conduct a comparison of the data obtained with what was reported in this study.

CONCLUSION

The phytocompounds present in *T. violacea* suggests that the plant is a potential source of chemotherapeutic compounds. The quantitative/semi quantitative analysis of these phytocompounds will be an interesting area for further study[71]. In this study, the leaves of *T. violacea* were

Table 4: Absorbance of standard compound (Gallic acid) at λ_{max} = 760 nm.

Sample no.	Concentration of Gallic acid (µg/ml)	Absorbance at 760 nm
1	0	0.000
2	50	0.765
3	100	1.196
4	150	1.755
5	200	2.285
6	250	2.834
7	300	3.224

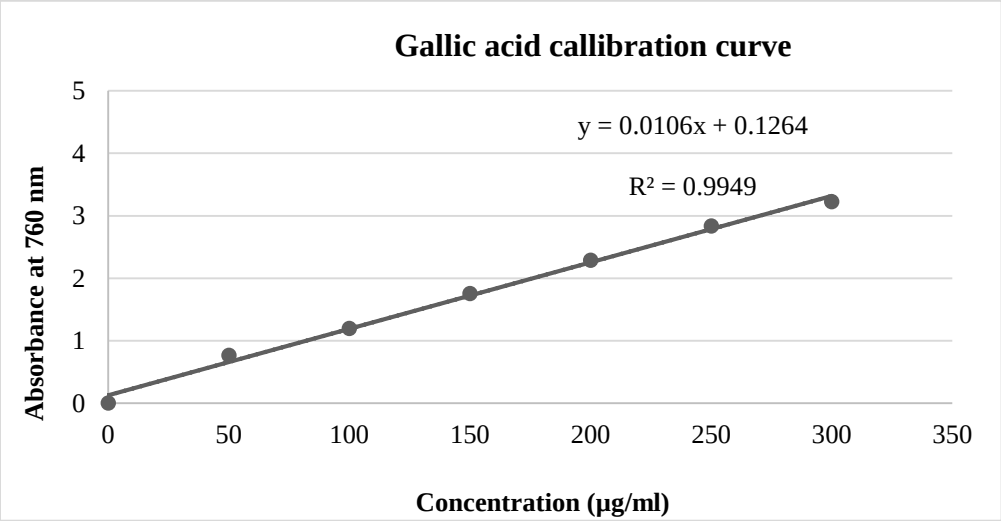

Figure 2: Standard calibration curve for quantification of Total Phenolic content.

found to contain the most phytocompounds validating their traditional use in the treatment of various ailments such as fever, colds, asthma, tuberculosis, oesophagus cancer, high blood pressure, stomach problems such as gastroenteritis, abdominal pains. Further research is required to exploit the biomedical applications of *T. violacea*. The two solvents used in this study were able to extract different bioactive compounds. For example, the water extract of the leaves showed the highest yield of total phenolic content (3.53 ± 0.1 mg of GAE/g of extract) while the highest yields of total flavonoid content (0.66 ± 0.01 mg of QE/g of extract) was obtained with 70% ethanol. This constitutes vital information for those wishing to extract compounds from the plant. The anti-HIV activity of saponins is an interesting area for further research as their presence was observed in all the plant parts of *T. violacea.* Studies should also be conducted to isolate, identify, characterize, and elucidate the structure of these bioactive compounds. There are different factors that will affect the quantity and composition of the phytocompounds present in an extract. Among these are the types of extraction, time of extraction, temperature, nature of the solvent, solvent concentration and lastly polarity of the solvent[31]. To broaden this research, different extraction methods should be considered for further verification of the results obtained in this study; these may include; infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, etc.[72]. The use of different solvents may also be considered as solvents are selective for the extraction of specific compounds. Extraction solvents such as; acetone, chloroform, ether, dichloromethanol, butanol or methanol may be utilized. However, methanol is cytotoxic, making it unsuitable for extraction in certain kind of studies[69].

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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