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In Vitro Investigation of the Antioxidant and Cytotoxic Potential of *Tabernaemontana ventricosa* Hochst. ex A. DC. Leaf, Stem, and Latex Extracts

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Abstract: *Tabernaemontana ventricosa* (Apocynaceae) a latex-bearing plant is used in traditional medicine for its therapeutic benefits in reducing fever and hypertension and wound healing. Due to limited information on the plant's pharmacological activities, this study aimed to investigate the antioxidant potential of the leaf, stem, and latex extracts of *T. ventricosa*, using the Folin-Ciocalteu (total phenolics), aluminum chloride colorimetric (total flavonoids), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays. The cytotoxic activity was evaluated in the human HEK293 (embryonic kidney), HeLa (cervical carcinoma), and MCF-7 (breast adenocarcinoma) cell lines using the MTT assay. The latex extracts possessed the highest total phenolic content (115.36 ± 2.89 mg GAE/g), followed by the stem hexane extracts (21.33 ± 0.42 mg GAE/g), the chloroform leaf (7.89 ± 0.87 mg GAE/g), and the chloroform stem (4.69 ± 0.21 mg GAE/g) extracts. The flavonoid content was substantially high ranging from 946.92 ± 6.29 mg QE/g in the stem hexane, 768.96 ± 5.43 mg QE/g in the latex, 693.24 ± 4.12 mg QE/g in the stem chloroform, and 662.20 ± 1.00 mg QE/g in the leaf hexane extracts. The DPPH assays showed the highest percentage of inhibition at 240 µg/mL, for the stem hexane (70.10%), stem methanol (65.24%), and stem chloroform (60.26%) extracts, with their respective IC_{50} values of 19.26 µg/mL (stem hexane), 6.19 µg/mL (stem methanol), and 22.56 µg/mL (stem chloroform). The FRAP assays displayed minimal inhibition ranging from 4.73% to 14.40%, except for the latex extracts which displayed moderate inhibition at 15 µg/mL (21.82%) and substantial inhibition at 240 µg/mL (98.48%). The HeLa and MCF-7 cell lines were the most sensitive to the extracts, with the hexane, chloroform, and methanol leaf and stem, and latex extracts significantly affecting the percentage cell survival. Overall, the various parts of *T. ventricosa* exhibited strong antioxidant activity correlating to its cytotoxicity. Further studies should focus on the isolation of specific antioxidant compounds that could be investigated for their anticancer potential.

Keywords: antioxidant; cytotoxicity; DPPH; flavonoids; FRAP; phenols

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1. Introduction

Cancer is a composite terminal disease whereby irregular cells rapidly divide and often attack several tissues or migrate into various other locations [1]. There are three significant and common causes of cancer, which include improper diet [2] genetic character [3], and ecological factors [4] such as ultraviolet (UV)-light, infectious agents, and pollution [4]. The

hasty incline in the critical contributors to this disease has directly increased cancer rates over the past decade [5–7]. According to the global cancer statistics (2018), approximately 18.1 million people are likely to be diagnosed with cancer, and an additional 10 million cancer-related deaths were anticipated in 2020 [5,8–10]. Global cancer statistics have revealed that lung cancer is the foremost cause of cancer mortality (18.4%), closely followed by breast cancer (11.6%), stomach cancer (8.2%), liver cancer (8.2%), prostate cancer (7.1%), and colorectal cancer (6.1%) [10]. According to Made et al. [11], nearly 38,000 (8%) of the total deaths in South Africa in 2014 were associated with cancer. Regardless of the widespread usage of chemotherapy, radiation therapy, and immunotherapy, these treatments introduce significant consequences ranging from multidrug resistance (MDR), exhaustive side effects, and the generation of reactive oxygen species (ROS), which ultimately induce detrimental effects to DNA (deoxyribonucleic acid) and cellular signaling pathways [12–14].

Recently, the discovery of novel compounds from medicinal plant extracts has been considered for the treatment of cancer [7,15,16]. These compounds are often preferred over conventional techniques since natural compounds display minimal side effects and act as a modulator of MDR [17–19]. Approximately 60% of anticancer agents are often generated from plant-based compounds [20–23]. The most common plant-derived anticancer products include vinca alkaloids, combretastatin, taxanes, camptothecin, and epipodophyllotoxin [24–27]. Additionally, phenolics, flavonoids, and volatile essential oils are often utilized in cancer research due to their rich source of antioxidants [28–30]. These compounds are exploited to combat ROS, resulting in the reduction of oxidative stress, improvement of immune function, and an increase in longevity [31–33]. According to Kam et al. [34], several species belonging to the genus *Tabernaemontana* contain indole alkaloids, which may suppress the growth and development of tumor cells.

Tabernaemontana ventricosa Hochst. ex A. DC. (Forest toad tree) is a latex-bearing plant belonging to the Apocynaceae and a genus consisting of medium-sized flowering trees within *Tabernaemontana* [35]. This species emits a pungent sweet scent from tubular salver-shaped whitish-yellow flowers [36]. Approximately 100 species of the *Tabernaemontana* genus are primarily distributed in tropical and subtropical regions of the world, including Africa, Asia, and America [17,36]. The *Tabernaemontana* species *T. ventricosa* and *T. elegans* are reported to occur in South Africa [1,36,37]. Previous phytochemical analysis on *T. ventricosa* indicated the presence of major alkaloidal components and large quantities of triterpenes [38]. Parts of *T. ventricosa* are reportedly used in traditional medicine systems in KwaZulu-Natal to palliate fever, reduce blood pressure, treat wounds, and heal sore eyes [36,39]. Insufficient research is available on *T. ventricosa*, although a few studies have investigated the biological activity of certain chemical compounds. Van Beek et al. [40] reported on the alkaloidal compounds conopharyngine and akuammincine, which showed healing and opioid activity, respectively.

Considering the scarcity of information available on the pharmacological activities of *T. ventricosa* and the crucial discoveries of curative agents from innovative plant-based products, the current investigation aimed to determine the antioxidant activities of the hexane, chloroform, methanol leaf and stem, and latex extracts of *T. ventricosa* using various protocols such as the Folin-Ciocalteu (total phenolics), aluminum chloride colorimetric (total flavonoids), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays. The cytotoxic activity of all extracts was also evaluated in the human cell lines; HEK293 (human embryonic kidney cells), HeLa (cervical carcinoma), and MCF-7 (breast adenocarcinoma) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

2. Materials and Methods

Leaves, stems, and latex exudate from fully grown *T. ventricosa* plants (Figure 1) of wild origin were collected from the University of KwaZulu-Natal (Westville campus), South Africa (29°49′03.3″ S 30°56′32.7″ E). A combination of emergent, young, and mature leaves and stems were collected for analysis. The plant material and exudate were taxonomically identified, and a voucher specimen (18222) was deposited at the Ward herbarium, School of

Life Sciences, University of KwaZulu-Natal. The plant material was inspected for any signs of microbial and fungal contamination. Thereafter the leaves and stems were separately air-dried for three months at 23 °C and ground at high speed into a fine powder using a grinder (Mellerware, Model: 29105, Durban, South Africa). The powdered material was kept in an airtight consol^{TM} glass jar, out of direct sunlight, at 23 °C, until further use. The latex exudate was aseptically collected by careful incisions in the soft stems of the plant with a sharp blade. The latex was diluted in distilled water (1:1; *v*/*v*), and the sample was centrifuged at 5000 rpm/ref for 10 min using an Eppendorf centrifuge (Model: 5415R, Marshall Scientific, Hampton, NH, USA). The pellet was removed, and the supernatant was stored at −8 °C until further use.

Figure 1. *Tabernaemontana ventricosa* captured at the University of KwaZulu-Natal (UKZN)-Westville Campus, Durban, South Africa. Co-ordinates: 29°49′03.3″ S 30°56′32.7″ E. (**A**) Dichotomously branched tree, (**B**) punctured leaf showing white latex exudate, (**C**) white salver-shaped flowers, and (**D**) obliquely ellipsoid follicle fruit joined at the base.

2.1. Solvent Extraction

Powdered leaf (10 g) and stem (10 g) samples were placed separately into round bottom flasks and dissolved in 100 mL of hexane. The flasks containing the solvent mixture were attached to a Soxhlet apparatus, heated at 60 °C for four sessions (3 h each), and filtered after each session using Whatman No. 1 filter paper. The extraction process was repeated using chloroform and methanol solvents, separately. The resulting leaf and stem extracts for each solvent were stored individually in sterilized consol^{TM} glass jars, in a dark room.

2.2. Evaporation and Concentration

Following extractions, the leaf and stem extracts, and latex supernatants were entirely air-dried in a well-ventilated dark room (~23 °C) for approximately 30 days. The dried

extracts were stored in airtight glass bottles at 4 °C. The percentage yield of the extracts was calculated as in the equation below:

$$\text{Extract yield } (\%) = \frac{\text{Weight of dried extract (mg)}}{\text{Weight of powdered material (mg)}} \times 100$$

2.3. Quantification of Total Phenolics, Total Flavonoids, and Antioxidant Assays

2.3.1. Preparation of Stock Solutions

Various stock solutions such as leaf hexane (LH), leaf chloroform (LC), leaf methanol (LM), stem hexane (SH), stem chloroform (SC), and stem methanol (SM) were prepared by resuspending 1 mg of dried crude extracts (i.e., hexane, chloroform, and methanol) in 1 mL of methanol solvent respectively. Previously collected and prepared latex (LX) supernatants were adjusted to 1 mg/mL. All the stock solutions (1 mg/mL) were homogenized using a vortex (Model: VM-1000, Taipei, Taiwan), and various concentrations (15, 30, 60, 120, and 240 µg/mL) were prepared for total phenolics, total flavonoids, antioxidant and cytotoxic assays.

2.3.2. Total Phenolic Content: Folin-Ciocalteu Method

The Folin-Ciocalteu method was used to determine the total phenolic content of the respective extracts [41]. Approximately 150 µL of diluted Folin-Ciocalteu reagent (10%) and 0.7 M of sodium carbonate (Na_2CO_3) was added to 30 µL sample extracts. The mixtures were incubated at 23 °C, in the dark for 30 min. Thereafter, the absorbance (Abs) of the samples was measured at 765 nm using a Synergy HTX Multi-mode reader, BioTek Instruments Inc., Winooski, VT, USA. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight using the equation:

$$\text{Ctp} = \text{C} * \frac{\text{V}}{\text{M}}$$

where Ctp = total phenolic content (mg/g) in GAE (gallic acid) equivalent

C = concentration of gallic acid obtained from calibration curve in mg/mL
V = volume of extract in mL
M = mass of extract in gram

2.3.3. Total Flavonoid Content: The Aluminum Chloride Colorimetric Assay

The aluminum chloride colorimetric assay was used to determine the total flavonoid content of the respective extracts, the protocol of Arruda et al. [42], with modifications was followed. Diluted extracts (25 µL) were mixed with 100 µL ultrapure millipore water (Milli *Q* water) and 7.5 µL of 5% $NaNO_2$. After 5 min, approximately 7.5 µL mL of 10% $AlCl_3$ was added to the reaction and left undisturbed for a further 6 min. Thereafter, 50 µL of 1 M NaOH and 60 µL of ultrapure millipore water (Milli *Q* water) were added respectively. The solutions were mixed, and the absorbance of samples was measured at 510 nm using a Synergy HTX Multi-mode reader, BioTek Instruments Inc., Winooski, VT, USA. Ethanol was used as a blank and the total flavonoid content was expressed as mg quercetin equivalents (QE/per gram of dry weight) using the equation:

$$\text{Ctf} = \text{C} * \frac{\text{V}}{\text{M}}$$

where Ctf = total flavonoid content (mg/g) in QE (quercetin) equivalent

C = concentration of quercetin obtained from calibration curve in mg/mL
V = volume of extract in mL
M = mass of extract in gram.

2.3.4. The 2,2′-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity

The free radical scavenging activity of *T. ventricosa* extracts (latex excluded due to pro-oxidant activity) was examined using DPPH, with adjustments of the method described in Akwu et al. [43]. Ascorbic acid served as the positive control at different concentrations in methanol (15, 30, 60, 120, and 240 µg/mL). The DPPH solution (0.3 mM) was prepared in 99% methanol. Briefly, 50 µL of 0.3 mM DPPH was added to 100 µL of each extract. The solutions were mixed well and incubated in the dark at 23 °C for 30 min. The colorimetric observation from purple to yellow indicated the scavenging potential of the extracts. The absorbance was measured at 517 nm using a Synergy HTX Multi-mode reader (BioTek Instruments Inc., Winooski, VT, USA) against a blank (no sample/standard). The IC_{50} was determined using the inhibition curves. The scavenging activity of the extracts was established using the following equation:

$$\text{DPPH Scavenging activity \%} = \left[\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}\right] * 100$$

where Abs control is the absorbance of the DPPH and methanol.

Abs sample is the absorbance of DPPH radical in the presence of the sample or standard.

The percentage obtained of the scavenging extracts was compared with the positive control.

2.3.5. Ferric (Fe^{3+}) Reducing Antioxidant Power (FRAP) Assay

The FRAP activity of the extracts was determined using the method described in Akwu et al. [43]. Briefly, 25 µL of each extract (15, 30, 60, 120 and 240 µg/mL) was mixed with 25 µL of 0.2 M sodium phosphate buffer (pH 6.6). Thereafter, 50 µL of 1% potassium ferricyanide was added to the mixture, which was incubated at 50 °C for 30 min. The reaction was halted with the addition of 25 µL of 10% trichloroacetic acid, 25 µL of ultrapure millipore water (Milli *Q* water), and 5 µL of 0.1% iron (III) chloride ($FeCl_3$). This was then mixed and incubated at 23 °C for 10 min, and the absorbance was measured at 700 nm using a Synergy HTX Multi-mode reader (BioTek Instruments Inc., Winooski, VT, USA). The results were expressed as a percentage of the absorbance of the extracts of gallic acid (standard) using the formula:

$$\text{\% Inhibition} = \left[\frac{(\text{Abs of sample})}{\text{Abs of gallic acid}}\right] * 100$$

2.4. MTT Cytotoxicity Assay

Three human cell lines viz., embryonic kidney (HEK293), breast adenocarcinoma (MCF-7), and cervical carcinoma (HeLa) were procured from the ATCC, Manassas, VA, USA. For use in the cytotoxicity assays, all three cell lines were grown to confluency in 25 cm^2 tissue culture flasks using Eagle's minimum essential medium (EMEM), containing 10% (*v/v*) gamma-irradiated FBS and 1% antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin) in a HEPA Class 100 Steri-Cult CO_2 incubator (Thermo-electron Corporation, Waltham, MA, USA), at 37 °C in 5% CO_2. Upon confluency, the cells were trypsinized and seeded into clear 96-well plates and incubated at 37 °C overnight. The cells were then prepared by replenishing the growth medium with a fresh complete medium (EMEM + 10% FBS + 1% antibiotics). Thereafter, 100 µL of the seven extracts with varying concentrations ranging from 15 to 240 µg/mL were added to the cells and incubated at 37 °C for 36 h. The cells (100 µL) without treatment (extract) were used as the positive control (c.a 100% viability). All assays were conducted in triplicate.

The methods briefly described by Mosmann [44] and Daniels and Singh [45], were used to evaluate the cytotoxic potential activity of the extracts from the leaves, stems, and latex of *T. ventricosa* in the three cell lines. Following 48 h of incubation at 37 °C, the growth medium was aspirated and replaced with 100 µL of medium containing 10 µL MTT solution (5 mg/mL in PBS), and the cells containing the treatment and negative control

were incubated for a further 4 h at 37 °C in 5% CO_2. The medium-MTT mixture was then removed and replaced with 100 µL dimethylsulphoxide (DMSO). The absorbances were then measured at 570 nm using a Mindray MR-96A microplate reader (Vacutec, Hamburg, Germany). Graphs were used to determine the concentration at which there was an inhibition of 50% in cell growth (IC_{50}). The viability of the cell lines was directly related to the absorbance. Percentage cell survival was calculated using the equation below:

$$\% \text{ cell survival} = \frac{\text{Average optical density of control cells only}}{\text{Average optical density of treated cells}} \times 100$$

2.5. Statistical Analyses

The results were presented as means ± standard deviation, n = 3. Statistical analyses were performed using two-way ANOVA which was used to compare the various treatments using different concentrations. The ANOVA test was followed by Tukey's honest significant difference range *post hoc* tests. Values were considered significant when the *p*-value was less than 0.05 (* $p < 0.05$ or ** $p < 0.01$). R Statistical computing software of the R Core Team, 2020, version 3.6.3 was used for the analyses.

3. Results and Discussion

3.1. Quantification of Plant Extracts

The highest percentage yield was obtained from the methanol extracts of leaves (20.17%) and stems (18.64%) respectively (Table 1). Overall, the metabolite yield detected throughout the crude extracts was higher in the leaves comparable to the stems. Moreover, the latex extract displayed a substantial percentage yield of 15%. Akwu et al. [43] suggested that the leaves and latex may contain a larger variety and quantity of polar compounds when compared to the stems.

Table 1. Percentage yield of the extracts of the leaves, stems, and latex of *T. ventricosa*.

Crude Extracts	Leaves	Stems	Latex	Leaves	Stems	Latex
	Dried Extract Yield (mg)			Yield (%)		
Hexane	50.00	40.00	0.15	5.28	4.36	15.00
Chloroform	80.00	70.00		8.78	7.28	
Methanol	200.00	180.00		20.17	18.64	

3.2. Total Phenolic Content

Phenolic compounds are essential as they are responsible for the absorption and neutralization of free radicals, the quenching of singlet and triplet oxygen molecules, or the decomposition of peroxides [46,47]. The phenolic compounds present within plants often aid in several biological properties such as antimicrobial, antiviral, antiallergic, anti-inflammatory, and anticancer activity [46,47]. The present study assessed the total phenolic content using the Folin-Ciolcalteu method which ranged from weak to significant phenolic content (Table 2). The highest total phenolic content was observed for the latex extracts (115.36 ± 2.89 mg GAE/g), followed by the stem hexane extracts (21.33 ± 0.42 mg GAE/g), with the chloroform leaf (7.89 ± 0.87 mg GAE/g) and chloroform stem (4.69 ± 0.21 mg GAE/g) extracts exhibiting moderate phenolic content, whereas the lowest levels of phenolics were found in the leaf hexane and methanol extracts (Table 2). A similar report by Banik et al. [48] investigated the antioxidant potential of *Tabernaemontana recurva* whole plant extracts. The total phenolic assay revealed a moderate number of phenols (5.89 ± 0.29 mg GAE/g) [48]. In another study, Sari et al. [49] evaluated the total phenolic content of *Tabernaemontana catharinensis* extracts which presented a value of 23.34 mg GAE/g. However, in comparison to Boligon et al. [50], significant differences were observed for the same species, *T. catharinensis*, which ranged from 135.57 to 562.780 mg GAE/g. The total phenolic content of other *Tabernaemontana* species was

much higher than that observed in the present study, except for the latex extracts. Thus, it is highly likely that these differences could be due to the variation in plant material used [51].

Table 2. Total phenolic content of the leaf, stem, and latex extracts of *T. ventricosa*.

Crude Extracts	Total Phenols (mg GAE/g)		
	Leaves	Stems	Latex (Only)
Hexane	1.01 ± 0.82	21.33 ± 0.42	115.36 ± 2.89
Chloroform	7.89 ± 0.87	4.69 ± 0.21	
Methanol	1.75 ± 0.13	0.99 ± 0.16	

Data displayed as mean ± SD of triplicates.

3.3. Total Flavonoid Content

Flavonoids are classified as a group of phenolics and are strong antioxidants that are often used to prevent the occurrence of cardiovascular and liver diseases [46,47]. The total flavonoid content in the current study showed significant values ranging from 152.22 ± 0.76 to 946.92 ± 6.29 mg QE/g. The highest number of flavonoids were observed for the stem hexane (946.92 ± 6.29 mg QE/g), latex (768.96 ± 5.43 mg QE/g), stem chloroform (693.24 ± 4.12 mg QE/g), and leaf hexane (662.20 ± 1.00 mg QE/g) extracts respectively whereas the lowest amount was observed for the leaf and stem methanol extracts (Table 3). The total flavonoid content of *T. ventricosa* extracts showed contrasting results compared to studies by Sathishkumar and Baskar [52]. In their study, the total flavonoid content in the fresh leaves of *Tabernaemontana heyneana* was much lower with a value of 4.4 ± 0.17 mg QE/g [52]. However, it has been reported that the accumulation of flavonoids is often associated with drought-tolerant plants, such as the *Arabidopsis* species [53].

Table 3. Total flavonoid content of the leaf, stem, and latex extracts of *T. ventricosa*.

Crude Extracts	Total Flavonoids (mg QE/g)		
	Leaves	Stems	Latex (Only)
Hexane	662.20 ± 1.00	946.92 ± 6.29	768.96 ± 5.43
Chloroform	332.83 ± 0.96	693.24 ± 4.12	
Methanol	152.22 ± 0.76	262.19 ± 2.36	

Data displayed as mean ± SD of triplicates.

3.4. Antioxidant Activity

Several studies investigated the antioxidant potential of *Tabernaemontana* species using many techniques, thus a great variation in results has been observed in the literature [17,51,54–56]. Herein, the antioxidant potential of the hexane, chloroform, and methanol extracts from the leaf and stem, and latex of *T. ventricosa* was investigated at various concentrations (15, 30, 60, 120 to 240 µg/mL) using DPPH scavenging activity FRAP assays. These assays are commonly used to determine the inhibition, generation, or scavenging ability against ROS [17].

The results for the DPPH and FRAP assays were variable (Figures 2 and 3). However, the % inhibition for all extracts was dose-dependent across both assays. For the DPPH assays, the stem methanol (54.28%), stem hexane (50.83%), and stem chloroform (49.08%) displayed the highest % inhibition at the lowest concentration (15 µg/mL), whereas at the higher concentrations (240 µg/mL), stem hexane (70.10%), stem methanol (65.24%), and stem chloroform (60.26%) showed the highest % inhibition (Figure 2). In comparison to the control (ascorbic acid), the values of the stem extracts were within range, however, the leaf extracts displayed very weak inhibition (Figure 2). Overall, for the DPPH assays, the stem crude extracts revealed a better % inhibition compared to the leaf and latex extracts. The IC_{50} values of the extracts were significantly larger than the positive control ascorbic acid

(3.11 µg/mL) (Table 4). However, the stem extracts displayed better IC_{50} values compared to the leaf extracts, with the stem methanol (6.19 µg/mL), stem hexane (19.26 µg/mL), and stem chloroform (22.56 µg/mL) displaying moderate activity (Table 4).

Table 4. IC_{50} values of the antioxidant activities of the various extracts from leaves, stems, and latex of *T. ventricosa*.

Crude Extract	DPPH (µg/mL)	FRAP (µg/mL)
Leaf hexane	538.66	>1000
Leaf chloroform	>1000	>1000
Leaf methanol	>1000	>1000
Stem hexane	19.26	>1000
Stem chloroform	22.56	>1000
Stem methanol	6.19	>1000
Latex	ND	42.22
Ascorbic acid	3.11	NA
Gallic acid	NA	29.44

ND = not determined; NA = not applicable.

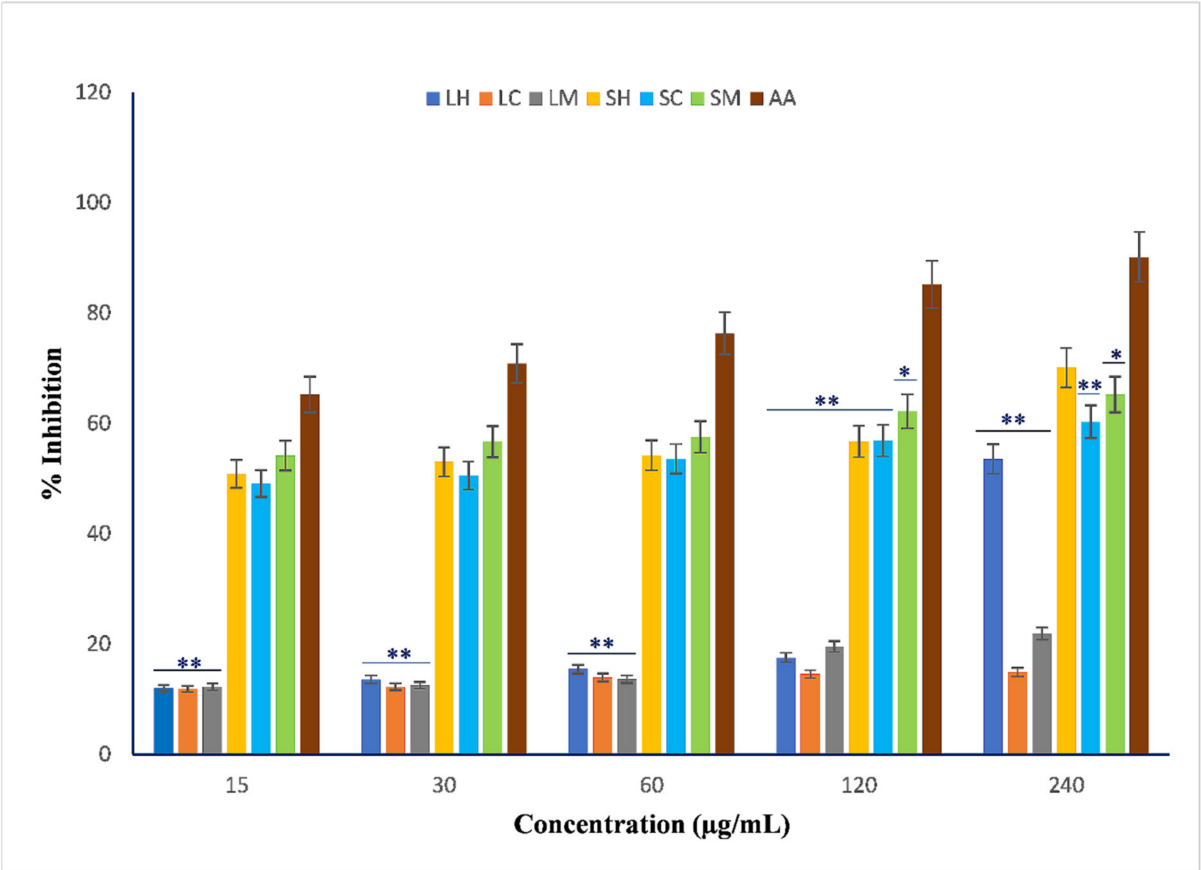

Figure 2. In vitro antioxidant activity (% inhibition_DPPH) using leaf hexane (LH), leaf chloroform (LC), leaf methanol (LM), stem hexane (SH), stem chloroform (SC), stem methanol (SM), and latex (LX) extracts of *T. ventricosa*. Standard = ascorbic acid (AA). Values were considered significant when the *p*-value was less than 0.05 (* $p < 0.05$ and ** $p < 0.01$ compared to each treatment and concentration).

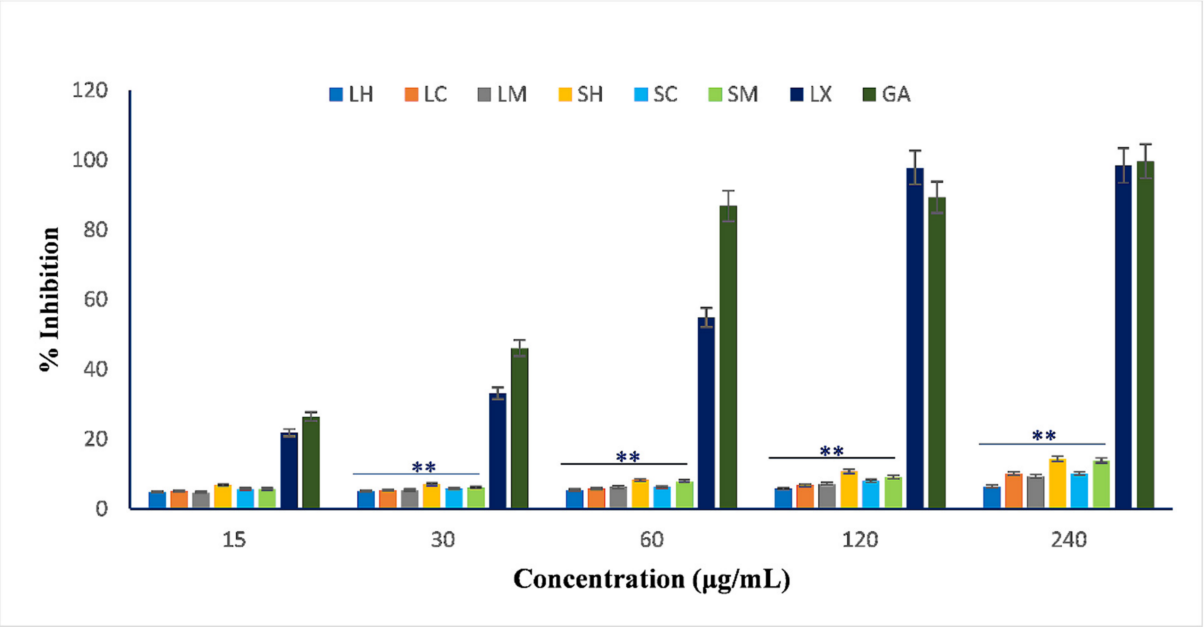

Figure 3. In vitro antioxidant activity (% inhibition_FRAP) using leaf hexane (LH), leaf chloroform (LC), leaf methanol (LM), stem hexane (SH), stem chloroform (SC), stem methanol (SM), and latex (LX) extracts of *T. ventricosa.* Standard = gallic acid (GA). Values were considered significant when the *p*-value was less than 0.05 (** $p < 0.01$ compared to each treatment and concentration).

Studies have shown that *T. catharinensis* is among one of the most intensively studied species within the genus [17]. The results of the current study are similar to those reported by Santos et al. [57]. In their study, the root ethanolic crude extracts and several isolated alkaloidal compounds from *T. catharinensis* were evaluated using the DPPH assay, which displayed significant inhibition ranging from 50% to 82% [57]. Boligon et al. [54] investigated the leaf crude extracts and fractions of *T. catharinensis* using several antioxidant techniques. The DPPH results revealed substantial IC_{50} values ranging from 4.64 ± 1.25 mg/mL to 27.78 ± 0.93 mg/mL, which are consistent with the values exhibited by the stem extracts herein [54]. Additional reports by Nicola et al. [55] indicated good antioxidant potential of *T. catharinensis* branches, leaves, and fractions. The most significant value was obtained by the alkaloid fraction which displayed an IC_{50} value of 37.18 ug/mL [55]. Raju and Rao [51] investigated the antioxidant potential using *Tabernaemontana divaricata* latex. In their study, the latex extracts showed moderate inhibition (28 ± 3.0%) [51]. However, in the present study very poor antioxidant values were observed for the latex extracts (DPPH assay not shown for latex).

While moderate to significant results were observed for the DPPH assays, the FRAP assay displayed minimal % inhibition across extracts and concentrations ranging from 4.73% to 14.40 %, except for the latex extracts which displayed moderate inhibition at 15 µg/mL (21.82%) and substantial inhibition at 240 µg/mL (98.48%), comparable with the control which displayed a percentage inhibition of 26.30% (15 µg/mL) and 99.64% (240 µg/mL) (Figure 3). The % inhibition of the latex extracts was very similar to that of the control (gallic acid) (Figure 3). Raju and Rao [51], likewise observed antioxidant activity in the latex extracts, thus indicating a significant quantity of antioxidant molecules. The results further revealed that the ferric ion reducing power of the extracts is consistent with the low quantity of phenolic compounds observed in Table 2, except for the latex extracts. A previous study revealed that *T. divaricata* ethanolic, hexane, and ethyl acetate stem extracts exhibited weak ferric reducing antioxidant capacities when compared to butylated hydroxytoluene (BHT) [58], which are very similar to the current FRAP results. However, Sari et al. [49] reported high levels of antioxidant activity in *T. catharinensis* using many techniques such as DPPH, FRAP, and many more. The IC_{50} values for the FRAP assay were

very weak (>1000 µg/mL), with all extracts besides the latex extracts (42.22 µg/mL) which showed moderate activity when compared to the control gallic acid (29.44 µg/mL) (Table 4). Comparatively, the DPPH and FRAP assays showed similar results, which indicated that the stem extracts exhibited a better antioxidant potential rather than the leaf extracts.

The differences in the antioxidant capacity in previous studies and the present investigation are highly likely due to the variation in geographical location and environmental conditions (soil and climate) of the plant sample/type of plant organ used [49–51]. Furthermore, according to Dutta and Ray [47], extracts often display a variation in their antioxidant potential, which is regularly associated with the usage of various base solvents and several extraction methods, that usually alters the composition of phytochemical compounds and therefore scavenges free radicals differently based on their polarity. Reports have shown that antioxidants are optimally extracted using polar solvents [50,54,55,59]. A study by Dutta and Ray [47] confirmed that leaf methanolic extracts using fractions of *Manilkara hexandra* displayed significant antioxidant potential. Furthermore, due to the complex mechanisms of the oxidation process, the comparison of the antioxidant potentials of the extracts, fractions, and isolated compounds of certain species and across species are very challenging since various methods are often utilized, and this often leads to a large variation in the antioxidant capacity of many species [17].

Overall, significant differences ($p < 0.05$ and $p < 0.01$) were observed for the extracts and concentrations for both the DPPH and FRAP assays. However, despite the variation among antioxidant techniques leading to the differences in antioxidant potential the results of the present study indicate that the leaf, stem, and latex extracts of *T. ventricosa* contain varying degrees of antioxidant potential and is therefore suggested as a natural source of antioxidants due to its probable oxidizing agents.

3.5. MTT Cytotoxicity Activity

Despite the advancements in contemporary medicine and sciences, we are frequently challenged by the complications of major side effects and many other factors which are often associated with ordinary cancer treatments such as surgery (removal of tumors), chemotherapy, radiotherapy, and immunotherapy [15]. Thus, researchers are determined on screening several medicinal plants to discover novel natural anticancer agents [7,15,16,56]. The family Apocynaceae and genus *Tabernaemontana* are well-known for their medicinal and pharmacological properties such as cytotoxic and anticancer activities [17]. A prominent example includes the compound vincristine (Oncovin®) found in *Tabernaemontana* which is an antitumor agent that is often used for oncology treatments and subsequently halts the process of metaphase which results in the prevention of rapidly dividing cancerous cells [28,60,61]. Due to the significant anticancer and cytotoxic potentials of *Tabernaemontana* species, the present study aimed to evaluate the cytotoxicity of the leaf, stem, and latex extracts of an unstudied species, *T. ventricosa*. For the MTT assays, various extracts with a range of concentrations (15, 30, 60, 120 to 240 µg/mL) were screened in three human cell lines, HEK293 (embryonic kidney), MCF-7 (breast adenocarcinoma), and HeLa (cervical carcinoma).

The cytotoxic potentials of all extracts of the leaves, stems, and latex of *T. ventricosa* on the various cell lines are displayed in Figure 4. All extracts showed moderate activity at the lowest concentrations, whereas at higher concentrations substantial cytotoxicity in the cells was observed. Thus, this is suggestive of a dose-dependent relationship for all extracts. The results of the current study revealed that at the lowest concentrations (15 µg/mL), HeLa cells treated with stem hexane extracts showed the most sensitivity (61.29%), while at the highest concentrations (240 µg/mL), HeLa cells treated with latex extracts showed substantial cell sensitivity (30.27%) (Figure 4). Overall, it appears that cell lines HeLa and MCF-7 were most sensitive to the extracts, with the hexane, chloroform, methanol leaf and stem, and latex extracts affecting the percentage cell survival considerably, and therefore contains effective cytotoxic activity (Figure 4). Furthermore, it was noted that significant differences were observed for the cytotoxic analyses of all extracts within each concentration and across all cell lines ($p < 0.01$ and/or $p < 0.05$).

Similarly, Thind et al. [62], investigated the cytotoxic activity of the leaves of *T. divaricata* using various solvents such as hexane, chloroform, methanol, and ethyl acetate. In their study, a range of concentrations were used (10 to 100 µg/mL) against several cell lines such as: HCT-15 (colon), HT-29 (colon), 502, 713 (colon), MCF-7 (breast), and PC-3 (prostate) [62]. Their results showed that hexane extracts did not display any cytotoxicity unlike the present study however, the methanol extracts displayed 71% and 76% of cell inhibition in 502, 713 and HT-29 colon cancer cell lines, respectively [62]. Moreover, the chloroform extracts also showed inhibition in HCT-15 (72%) and HT-29 (71%) cancer cell lines [62]. Likewise, in the present study, these extracts also showed moderate to significant activity in the cell lines. Furthermore, recently Rosales et al. [7], investigated the isolated compounds from *T. catherinensis.* The results showed that the isolated compound affinisine displayed selective cytotoxic activity in A375 cells (melanoma), with survival rates above 50%, which are in line with the results of the current study [7].

The extracts of *T. ventricosa* displayed a range of cytotoxic activity, showing low (IC_{50} > 100), moderate (IC_{50} > 50–100), and substantial (IC_{50} < 50) proliferative effects. Significant cytotoxic effects were displayed by the latex extracts (17.20 ± 1.63 µg/mL) in the MCF-7 cell line, whereas moderate activity was observed for the leaf hexane extracts (54.81 ± 0.71 µg/mL) in the HeLa cell line, and the stem hexane extracts (83.33 ± 1.49 µg/mL) for the HEK293 cell line (Table 5). However, weak IC_{50} values showing low proliferative effects were noted for the stem chloroform extracts in MCF-7 (1402.88 ± 3.44 µg/mL), latex extracts (231.10 ± 11.71 µg/mL) in HEK293, and stem methanol extracts (164.66 ± 1.49 µg/mL) against HeLa cell lines (Table 5).

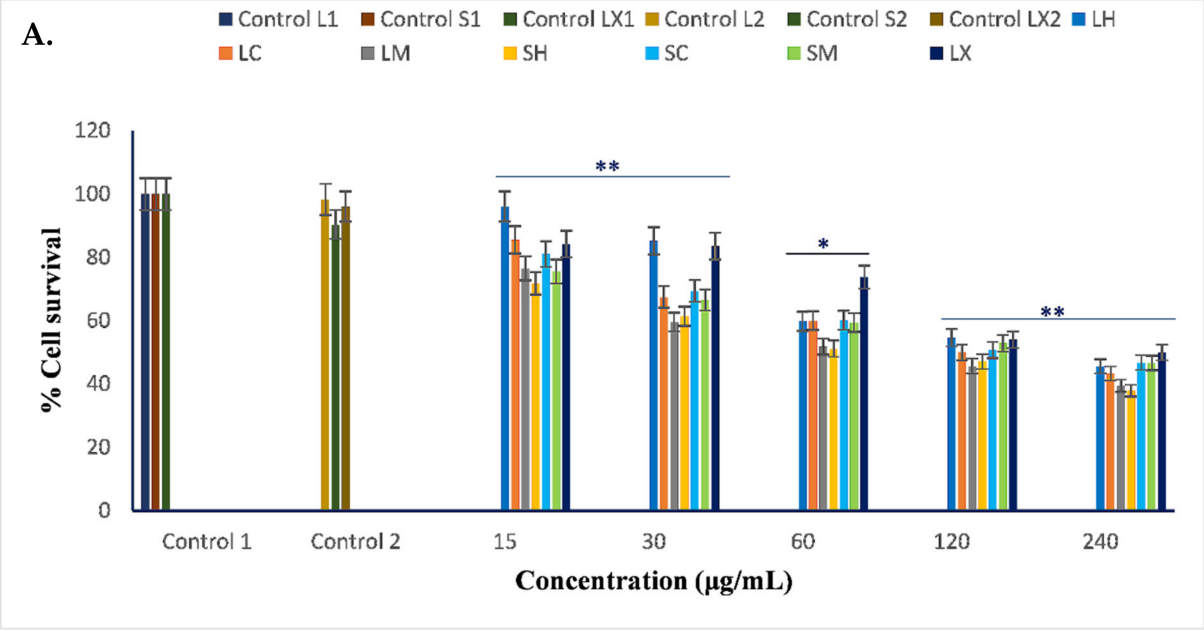

Figure 4. *Cont.*

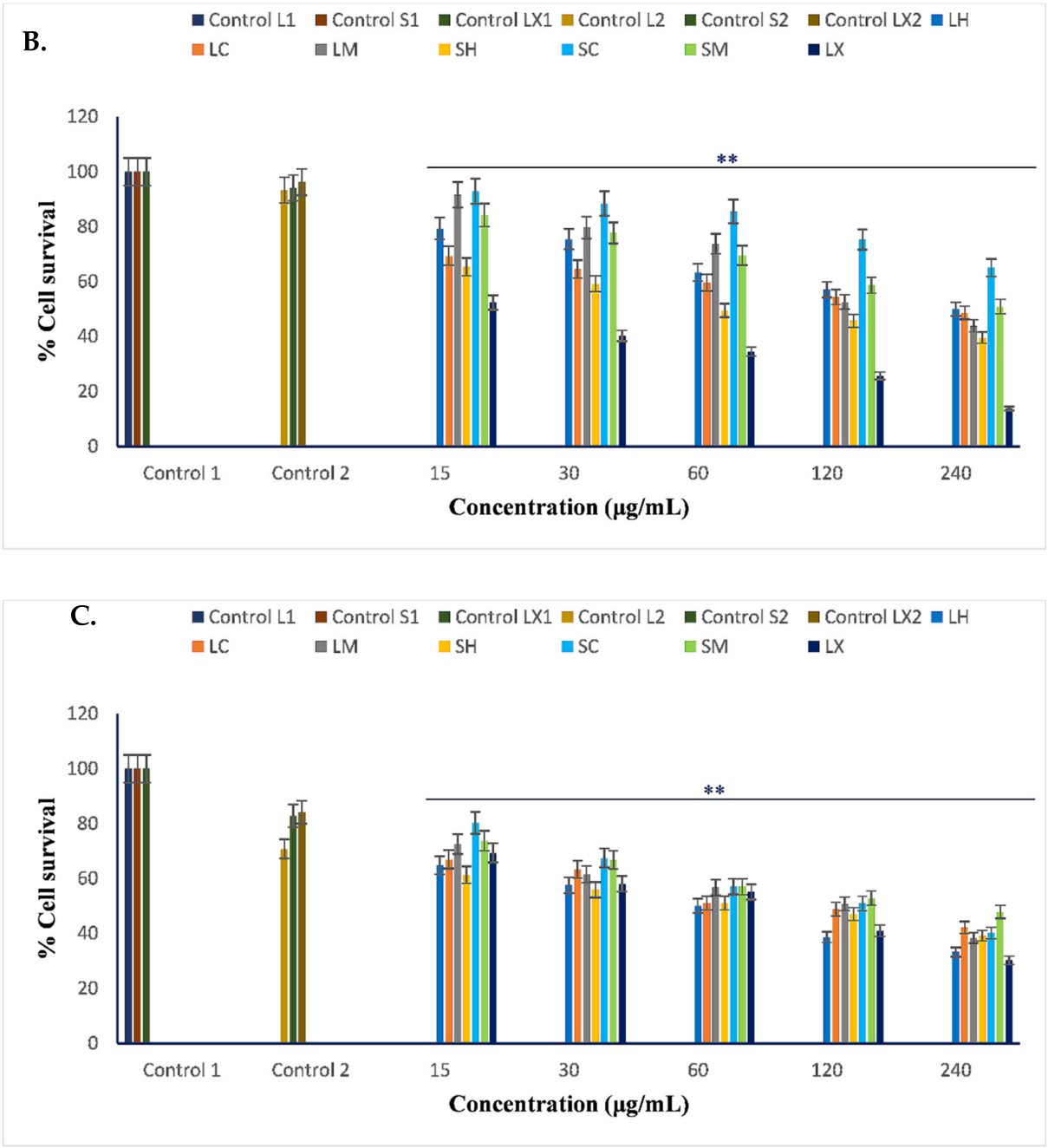

Figure 4. In vitro cytotoxicity (% cell survival) of leaf hexane (LH); leaf chloroform (LC); leaf methanol (LM); stem hexane (SH); stem chloroform (SC); stem methanol (SM), and latex (LX) extracts of *T. ventricosa*. Control 1 = cells only; Control 2 = cells + DMSO. (**A**) HEK293, (**B**) MCF-7, and (**C**) HeLa cell lines. Values were considered significant when the *p*-value was less than 0.05 (* $p < 0.05$ and ** $p < 0.01$ compared to each treatment and concentration).

Table 5. IC_{50} values of the cytotoxic activity of the crude leaf and stem, and latex extracts of *T. ventricosa*.

Crude Extracts	Cell Lines		
	HEK293	MCF-7	HeLa
	Concentration µg/mL		
Leaf hexane	156.90 ± 3.41	232.13 ± 1.97	54.81 ± 0.71
Leaf chloroform	129.39 ± 1.22	208.54 ± 2.73	97.60 ± 2.16
Leaf methanol	86.23 ± 1.76	168.24 ± 2.23	101.82 ± 1.12
Stem hexane	83.33 ± 1.49	73.20 ± 3.08	67.63 ± 0.76
Stem chloroform	150.75 ± 2.13	1402.88 ± 3.44	115.86 ± 1.40
Stem methanol	161.01 ± 7.46	260.70 ± 5.64	164.66 ± 1.49
Latex	231.1 ± 11.71	17.20 ± 1.63	63.50 ± 1.37

Data displayed as mean ± SE of triplicates.

According to Pallant et al. [63], previous research relating to the cytotoxic activity of *Tabernaemontana* species has been variable. These values do however correspond with previous reports [63]. Earlier studies of the *Tabernaemontana* species have displayed significant cytotoxicity [63]. A study by Kingston et al. [64] investigated the cytotoxicity of *Tabernaemontana arborea* extracts in P-388 cells (murine lymphocytic leukemia) which revealed a substantial IC_{50} value of 8 µg/mL. However, reports by Pallant et al. [63], which investigated the aqueous extracts of *T. elegans*, a sister species of *T. ventricosa* revealed weak toxicity of human lymphocytes with IC_{50} values ranging from 80 to 160 µg/mL, similar to the present study. However, most recently Andima et al. [65], investigated the cytotoxic activity of isolated compounds ibogamine, voacristine, and vobasine from *T. ventricosa* stembark, roots, twigs, and leaves extracts (mixture). The study confirmed low proliferative (>100) effects for isolated compounds, except for voacristine (23.00 µg/mL) which displayed significant cytotoxic activity in the HepG2 (human liver cancer cells) [65]. It is highly likely, that voacristine is responsible for the cytotoxic activity displayed by the extracts in the present study however, the variations may be due to the concentration of various alkaloids within the species, environmental factors, type of plant material used, method of extraction and solvent type [63,66]. However, despite these factors, previous studies have indicated that the *Tabernaemontana* species have shown promising cytotoxic activity, with IC_{50} values below 20 µg/mL, suggesting the strong potential of these species [63].

According to the literature, phenolic compounds possess several biological properties which include anticancer properties. Furthermore, flavonoids are well-known for their detoxifying effects which are associated with the inhibition of the transcription factors that are responsible for the activation of tumor promoters, while indole alkaloids such as ibogamine, voacristine, and vobasine are often used for both their antioxidant and cytotoxic activities [7,17,52,65,67,68]. The cytotoxic potential displayed predominantly by the leaf and stem hexane extracts, and latex extracts correlate with the antioxidant assays, which also revealed antioxidant activity for these extracts. Moreover, due to the moderate phenolic content, substantial flavonoid content, strong antioxidant potential (DPPH and FRAP), and several previously isolated indole alkaloids of *T. ventricosa*, the phytochemical compound types mentioned above are likely to be responsible for the moderate to significant cytotoxic and antioxidant activity revealed herein [17,63,65].

4. Conclusions

The present study indicated that the leaf, stem, and latex extracts of *T. ventricosa* contain significant cytotoxic and antioxidant potentials. Overall, the stem and latex extracts showed higher antioxidant and cytotoxic potential as compared to the leaf extracts. Furthermore, the total phenolic and flavonoid content, and % inhibition of antioxidants (DPPH and FRAP

assays) displayed by the extracts could be attributed to several phytochemical compounds. It can be suggested that previously isolated indole alkaloids from *T. ventricosa* such as ibogamine, vobasine, and voacristine contribute to the strong antioxidant potential of this species, which in turn promote the cytotoxic activity of *T. ventricosa* extracts. The current study revealed a substantial connection between the antioxidant activity of the leaf and stem hexane and latex extracts and their cytotoxic activity. However, further studies should focus on the elucidation of the compounds and their respective antioxidant and cytotoxic properties to determine the specific correlation involved in these processes. This study constitutes the first report on the in vitro antioxidant and cytotoxic potential of the leaf, stem, and latex extracts of *Tabernaemontana ventricosa*.

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