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In vitro antiproliferative activity of Amaryllidaceae species against the K562 human leukaemia cell line

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A R T I C L E   I N F O

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A B S T R A C T

Acute lymphocytic leukaemia is the most common leukemic cancer reported in children. Chemotherapy is the preferred treatment even though it continues to pose negative side effects of toxicity. Medicinal plants are reported to provide alternative treatment with lower toxicity levels. The three *Amaryllidaceae* species; *Crinum bulbispermum, Boophone disticha, and Amaryllis belladonna Linnaeus* have been reported for their anti-leukemic properties. These claims, however, lack supporting scientific data. The study aimed to investigate the anti-proliferative activity of the three Amaryllidaceae species against the human K562 leukaemia cells, as well as their phytochemical composition. The plants' roots, bulbs and leaves were extracted with water, and sequentially with selected organic solvents. Cell antiproliferation was investigated using the SRB assay. Thin Layer Chromatography was performed to compare chemical profiles of different plant parts, and of plant samples collected from different geographic areas. Most plant parts tested positive for terpenoids and flavonoids. Only the bulbs contained phytosterols and alkaloids. Plant samples of *C. bulbispermum* obtained from two geographic areas had similar chemical profiles. Water bulb extract of *C. bulbispermum* and *B. disticha* showed over 70% cell growth inhibition at concentration of 10 μg/ml, while their methanol extracts showed over 50% cell growth inhibition at 100 μg/ml and 10 μg/ml. Methanol root extract of *A. belladonna L* exhibited 100% cell growth inhibition at the concentration of 50 μg/ml and over 80% at 25 μg/ml concentration. In general, the polar extracts exhibited highest activity. The cell antiproliferation results obtained in this study support the use of the selected *Amaryllidaceae* species to treat leukemia as currently practiced in traditional medicine. The consistency of the constituents of the species, despite of their collection points, could enable standardization of traditional medicines.

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1. Introduction

Acute lymphocytic leukaemia (ALL) is caused by an accumulation of lymphoblasts in the bone marrow and is most malignant in the childhood stages of life (Brentjens et al., 2013). ALL is the most common pediatric leukemic cancer in the United States accounting for an estimation of about 6590 diagnosis and 1430 deaths in the year 2016 (Ahmed, Yagoub, AlSaid, Mohammed, and Osman, 2016). Chemotherapy as the primary treatment regimen used to treat ALL has fundamental drawbacks; it is common cause of morbidity and mortality in cancer patients due to its elevated toxicity levels (Cordier and Steenkamp, 2018). The intolerable side effects associated with chemotherapy call for efforts to discover safer and efficacious alternative treatments for leukaemia.

The *Amaryllidaceae* family is by far one of the most recognizable bulbous family known globally for its exploitation in traditional medicine and unique bioactive alkaloid constituents (Nair, van Staden, Bonnet, and Wilhelm, 2017). The medicinal potential of this family was realized through the commercialization of galanthamine as an Alzheimer's drug with potent and selective inhibitory activity against acetylcholinesterase enzyme (Nair and van Staden, 2013). Promising chemotherapeutic candidates of the family reside with the class of alkaloids such as pancratistatin, which showed cell line specific antiproliferative properties and potential for clinical development (Atanasov et al., 2015 and Nair and van Staden, 2013).

The three selected species from Amaryllidaceae family: *C. bulbispermum, B. disticha* and *A. belladonna L* have been used traditionally for treatment of numerous cancers including leukemia (Griffin et al., 2007 and Shawky, Takla, Hammoda, and Darwish, 2018). Previous studies reported that several biologically active alkaloids have been isolated from all three species (Neergaard et al., 2009). Over thirty-one alkaloids

Abbreviations: ALL, Acute lymphocytic leukaemia; DCM, Dichloromethane; MeOH, Methanol; g, Grams; v/v, Volume to volume; DMEM, Dulbecco's Modified Eagle Medium; FBS, Fetal bovine serum; %, Percentage; μL, Microliters; h, Hours; Min, Minutes; ml, Milliliters; μg/ml, Microgram per milliliter; °C, Degrees Celsius; wt/vol, Weight per volume; SRB, Sulforhodamine B; TLC, Thin Layer Chromatography; cm, Centimeters; R_f, Retention factor; CT, Cape Town; PTA, Pretoria

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which have primary and secondary functions of anti-inflammation, antiproliferation and anti-neoplastic effects, have been identified in *A. belladonna L* for example (Tallini et al., 2017). Several common alkaloids such as buphanamine and distichamine have been isolated from *B. disticha* with potential acetylcholinesterase inhibitory activity (Adewusi, Fouche, and Steenkamp, 2012). The plant *C. bulbispermum* also produce a series of alkaloids which exert a wide range of interesting possible physiological and anti-tumor effects (Cortes et al., 2015).

The study investigates the anti-proliferative activity of extracts of *C. bulbispermum, B. disticha* and *A. belladonna L* against leukaemia cell line K562, as potential sources of anticancer therapeutic agents, and to characterize their phytochemical composition to determine the classes of compounds present: which could be responsible for the anti-proliferative activity. Phytochemical analysis was also performed to compare the constituents in the different parts of the plants, and to compare *Crinum bulbispermum* plant extracts obtained from two geographic areas to assess consistency in the composition of the species.

2. Materials and methods

2.1. Plant materials

The study was conducted at the Central University of Technology, and the University of the Free State, Bloemfontein, South Africa. Two *Crinum bulbispermum* plants were collected from different geographic locations in South Africa; Kirstenbosch National Botanical Gardens, Cape Town and Grow Wild Indigenous Plants Nursery, Pretoria. *Boophone disticha* and *Amaryllis belladonna L* were collected from Kirstenbosch National Botanical Gardens, Cape Town between March and June 2017. All three plants were identified/authenticated by Mrs Magdil Pienaar, a Botanist at the University of the Free State. Upon arrival at the laboratory, plant materials were washed with tap water to remove debri. Plants were then dried at room temperature and ground to fine powder. Bulbs of all three plants were divided into two samples; one sample was dried while the other was used as wet materials.

2.2. Extraction

To extract, maceration method was used as adapted from Azwanida (2015). The dried plant material was finely grounded and weighed (10 g), then extracted with water (200 ml), sequentially with organic solvents (200 ml) in their increasing order of polarity, starting with hexane, dichloromethane, methanol: dichloromethane (MeOH: DCM or 1:1 (v/v)) and methanol. The plant-solvent mixtures were left on the orbital shaker for 48 h and filtered. The filtrates from the aqueous extract were concentrated via freeze drying while the filtrate from the organic extracts were concentrated by rotatory evaporation. The percentage yields derived were calculated.

2.3. Cell culture

The cell culture environment was maintained at 37 °C in humidified atmosphere containing 5% CO_2. The growth medium DMEM 10% FBS and 0.6% streptomycin was used to culture the cell line. Cells were grown until 80% confluence was reached (Freshney, 2005).

2.4. SRB assay

The SRB assay method was used in this study due to its rapidness and increased sensitivity for measuring drug-induced cytotoxicity in culture. The cells (1×10^4 cells/mL) in growth medium (200 μL) were seeded in individua1 wells of 96-well microplates and incubated for 24 h to allow attachment. After 24 h, the media was aspirated and the cells were treated with various concentrations of the plant extracts (100, 10 and 1 μg/ml) in triplicates. After 48 h of incubation, the cells were fixed with cold (4 °C) 50% trichloroacetic acid and placed at 4 °C for 2 h. Plates were then rinsed with running tap water, air dried, and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Plates were rinsed five times with 1% acetic acid to remove unbound dye, then air dried. Bound dye was solubilized with 200 μl of 10 mM unbuffered Tris base (pH 10.5) for 5 min. Absorbance was measured at 510 nm using a CLS microplate reader. The SRB assay provided a rapid and sensitive method for measuring the drug-induced cytotoxicity in suspension cultures in 96-well microtiter plates (Spectra-Max-190). Methodology was adopted from Skehan et al., 1990.

2.5. Thin layer chromatography

Extracts were profiled using Thin Layer Chromatography (TLC). Extracts of *A. belladonna L, C. bulbispermum* and *B. disticha* were subjected on TLC silica gel aluminium plates (8 cm × 10 cm), eluted using two solvent systems; chloroform: ethyl acetate: methanol (4:4:2) (v/v/v), and chloroform: methanol (95:5) (v/v) in another tank. The plates were taken out when mobile phase reached 1 cm from the top of the plate, air-dried, and then viewed under UV light and Fluorescence: absorbance was read at 250 nm wavelength. The separation of components was measured by the retention factor (R_f) values (Kumar, Jyotirmayee, and Sarangi, 2013).

Retention factors were calculated using the following formula:

$$\text{Rf value} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the mobile phase}}$$

2.6. Phytochemical analysis

Phytochemical analysis was carried according to the Bhandary, Bhat, Sharmila, and Bekal, 2012 to analyse the presence of various secondary metabolites. Major classes of phytochemicals including alkaloids, pentose, glycosides, anthraquinones, tannins, flavonoids, phenolic compounds, terpenoids, saponins and phytosterols were tested in the three plants.

2.7. Statistical analysis

Data were represented as arithmetic mean ± standard deviation. The test was performed in triplicates.

3. Results

3.1. Phytochemical analysis

Phytochemical analysis results of *C. bulbispermum* in Table 1 displayed uniformity in the presence of flavonoids, terpenoids, phytosterols and alkaloids in *C. bulbispermum* collected from Pretoria and Cape Town. The bulbs of *A. belladonna* contained the most phytochemicals, except anthraquinones, which were absent in all three species.

Table 1
Phytochemical analysis results of the two plants showed that root, bulb and leaf plant material of C. bulbispermum and A. belladonna L were examined for the below 9 phytochemicals. (+) and (–) indicate presence and absence of phytochemical, respectively.

Test	*Crinum bulbispermum*				*Amaryllis belladonna L*		
	Pretoria		Cape Town		Bulbs	Roots	Leaves
	Roots	Bulbs	Roots	Bulbs			
Flavonoids	+	+	+	+	+	–	–
Pentose	–	–	–	–	–	–	+
Glycosides	–	–	–	–	+	+	–
Tannins	–	–	–	–	+	+	–
Terpenoids	+	+	+	+	+	–	–
Phytosterols	–	+	–	+	+	–	–
Saponins	–	–	–	–	+	+	+
Anthraquinones	–	–	–	–	–	–	–
Alkaloids	–	+	–	+	+	+	+

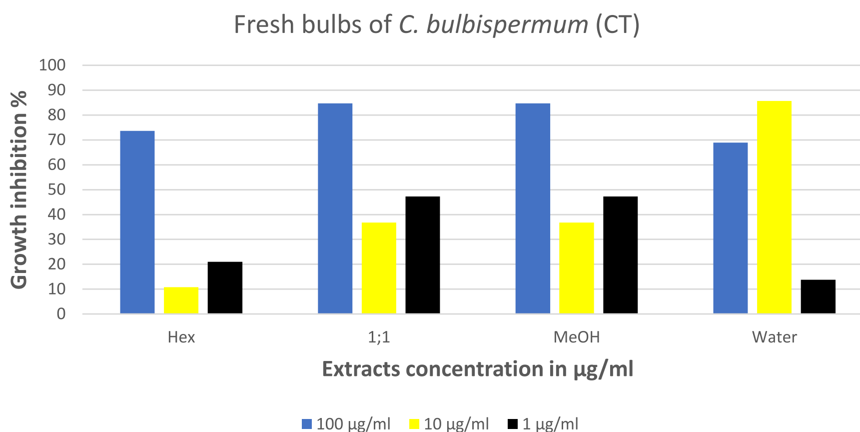

Fig. 1. Fresh bulb extracts of *C. bulbispermum* (hexane, DCM: MeOH, MeOH and H_2O) cell growth expressed in growth inhibition percentage (%).

3.2. Anti-Proliferation activity

High levels of cell growth inhibition percentage in the figures below indicate increased cytotoxic activity. Fig. 1 shows the cell growth inhibition percentages following 48 h exposure to fresh bulb extracts (hexane, DCM: MeOH, MeOH and water) of *C. bulbispermum* collected from Cape Town (CT). The DCM: MeOH, and MeOH extracts exhibited the highest inhibition percentages of above 80% at 100 μg/ml extract concentration, while the MeOH extract also exhibited over 45% growth inhibition at 1 μg/ml extract concentration. Interestingly, the water extract showed 85% growth inhibition at extract concentration of 10 μg/ml.

Fig. 2 below illustrates activity of four extracts of *C. bulbispermum* collected from Pretoria (Pta) region. The highest cell growth inhibitions were recorded at 100 μg/ml concentration of the hexane extract. None of the 10 and 1 μg/ml extract concentrations recorded growth inhibitions higher than 50%. See Fig 2 below.

Results in Fig. 3 shows cell growth inhibition percentage following exposure to dry bulb extracts of *C. bulbispermum* (CT). The DCM: MeOH (1:1) extract exhibited over 80% growth inhibition at 100 μg/ml concentration. Both DCM: MeOH and MeOH extracts exhibited over 50% growth inhibition at a lower extract concentration of 10 μg/ml. Interestingly, at the lowest extract dose concentration of 1 μg/ml tested, the water extract displayed 35% growth inhibition.

Fig. 4 below shows high growth inhibition properties of the DCM: MeOH extracts of the *C. bulbispermum* dry bulbs. Over 60% inhibitory activity was recorded across all three extract concentrations (100, 10 and 1 μg/ml) of this extract.

Fig. 5 shows growth inhibition activity of extracts of *B. disticha.* There was increased activity with increasing polarity, as displayed by MeOH and water extracts at 10 and 1 μg/ml concentrations. The water extract displayed the highest cytotoxicity, surpassing 50% growth inhibition at all concentrations.

Growth inhibition activities of the root extracts of *B. disticha* are shown in Fig. 6. All extracts exhibited above 50% growth inhibition at 100 μg/ml concentrations. Much like the fresh bulbs water extract in Fig. 5, the root water extract in Fig. 6 displayed over 50% growth inhibition at 10 μg/ml.

Fig. 7 records the growth inhibition activities of dry bulbs of the *A. belladonna L* plant across six concentrations (100, 50, 25, 12.5, 6.25 and 3.125 μg/ml). The highest growth inhibitions (above 50%) were recorded in the semipolar to polar extracts; DCM and methanol extracts at 100 μg/ml concentration.

The average inhibition percentage across all four extracts decreased with decreasing concentrations. The hexane extract showed proliferation instead of inhibition in four of the six extract concentrations measured.

Fig. 8 below displays growth inhibitory activity of *A. belladonna L* wet bulb extracts. The DCM: MeOH and MeOH extracts showed more than 50% growth inhibition at concentrations of 25 μg/ml and above. The lower extract dose concentrations showed low activity.

Fig. 9 illustrates cell growth inhibition of the roots part of the A. belladonna L plant. The results showed high growth inhibition by the methanol extract from 6.25 μg/ml to 100 μg/ml.

Fig. 10 depicts the results of growth inhibitory activity of water extracts of all three extracts parts of *A. belladonna L* and standard

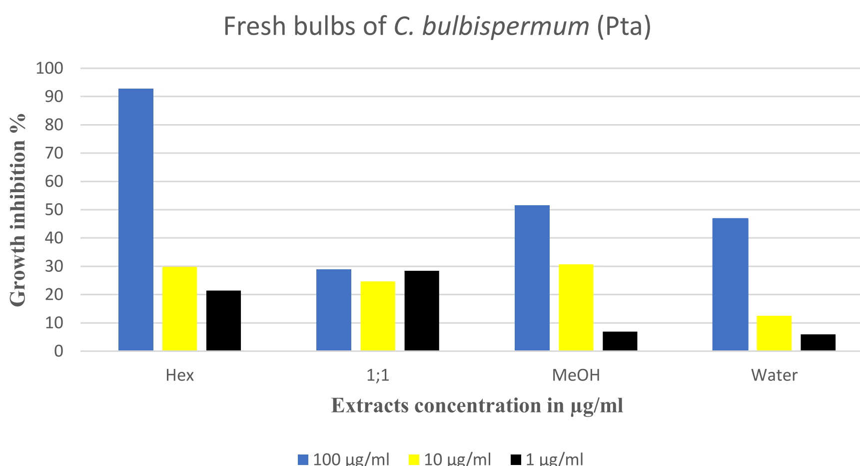

Fig. 2. Cell growth inhibition activity of fresh bulbs extracts of *C. bulbispermum* (Pta) in percentage (%) at varying concentrations.

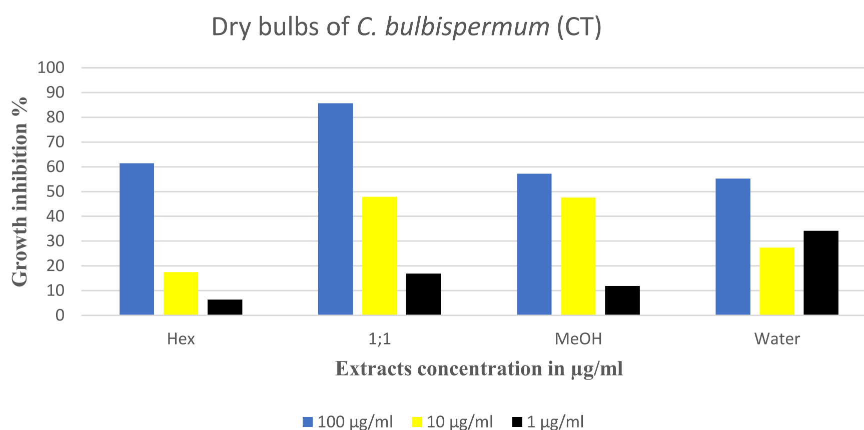

Fig. 3. Cell growth inhibition activity of dry bulbs extracts of *C. bulbispermum* (CT) in percentage (%) at varying concentrations.

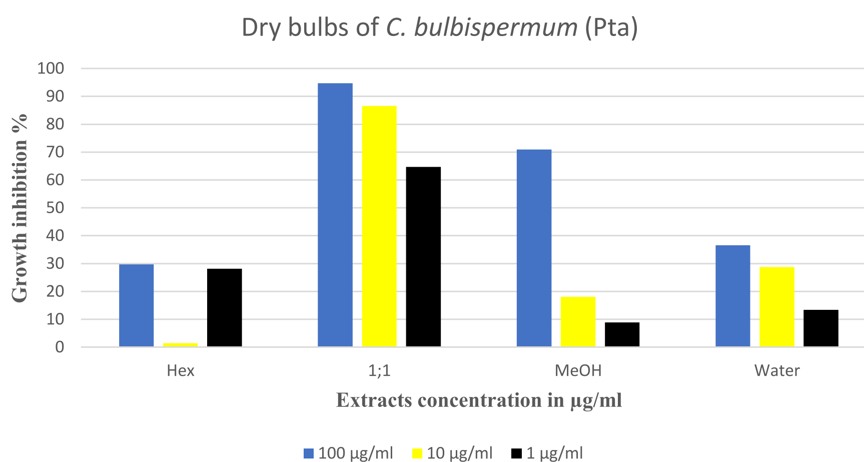

Fig. 4. Cell growth inhibition activity of dry bulbs extracts of *C. bulbispermum* (Pta) in percentage (%) at a range of concentrations.

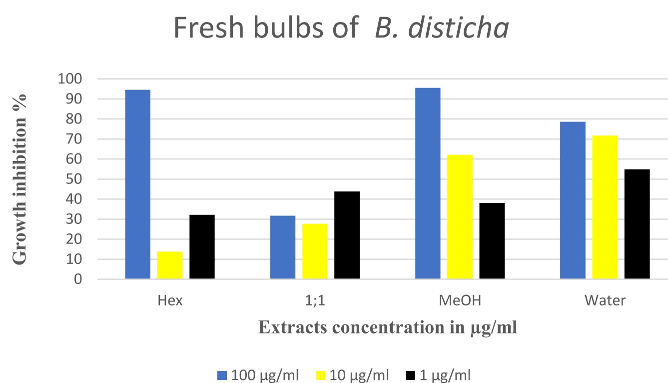

Fig. 5. Cell growth inhibition activity of fresh bulbs extracts of *B. disticha* in percentage (%) at varying concentrations.

drug, doxorubicin. The figure shows that all the water extracts from the different plant-parts of *A. belladonna L* have antiproliferative properties. Dry bulbs and roots are more cytotoxic than the wet bulbs as seen across all extract dose concentrations.

3.3. Thin layer chromatography (R_f values)

Standardization of herbal health products is often a concern as the level of plant constituents is influenced by environmental factors in different geographic areas. TLC was performed, and the R_f values were used to compare chemical profiles of samples; the roots and bulbs of *C. bulbispermum* collected in two geographical areas; Pretoria and Cape Town, for presence and levels of chemical constituents.

Results (Table 2) show that in *C. bulbispermum* plants collected from both regions, there was general homogeneity in the number bands observed as confirmed by similar Rf values in the same extracts. Bulb extracts in both regions exhibited more bands than the root extracts.

Results presented in Table 3 show higher numbers of constituents in bulbs and roots extracts especially when comparing DCM and MeOH extracts. The DCM bulbs extract showed the fewest band separations (see number of R_f values) in the table. The comparison in

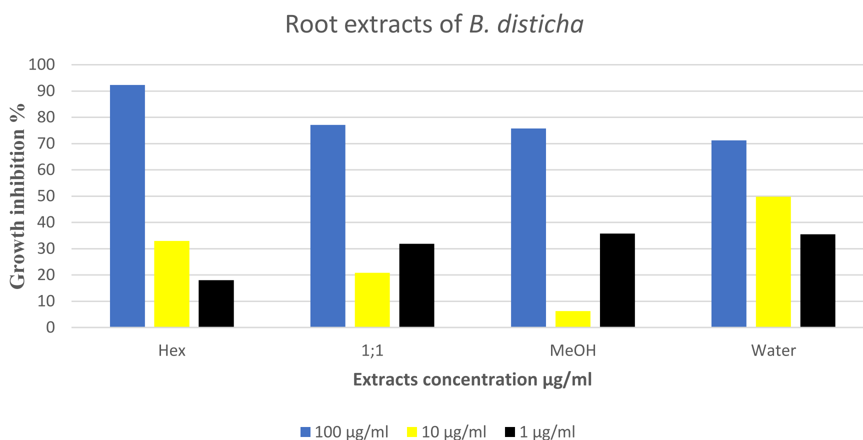

Fig. 6. Cell growth inhibition activity of root extracts of *B. disticha* in percentage (%) at varying concentrations.

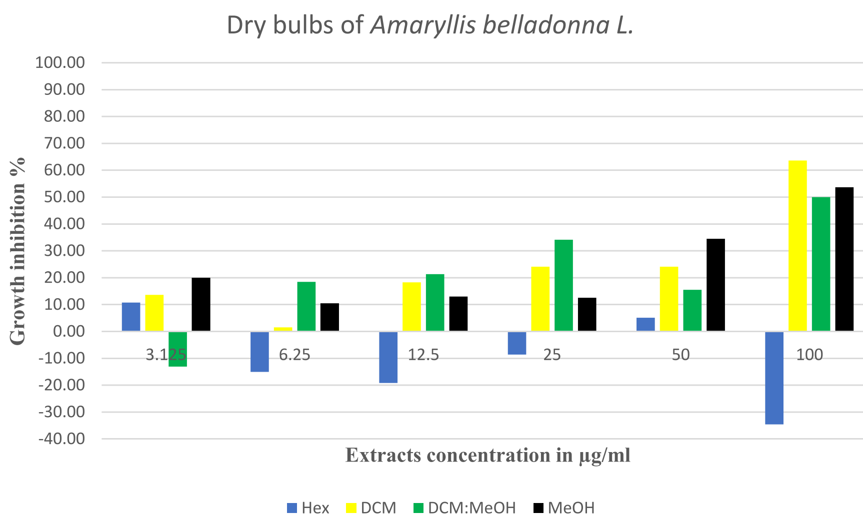

Fig. 7. Cell growth inhibition activity of dry bulbs extracts of *A. belladonna L* in percentage (%) at varying concentrations.

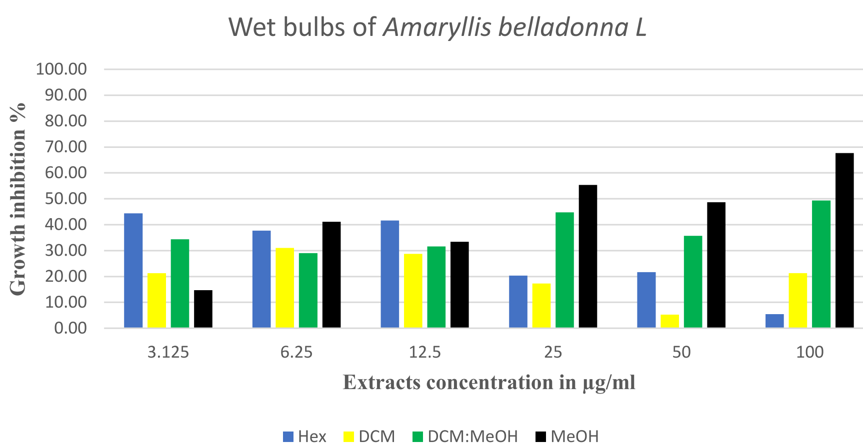

Fig. 8. Cell growth inhibition activity of wet bulbs extracts of *A. belladonna L* in percentage (%) at varying concentrations.

Table 3 shows the similarity in the number of bands observed and R_f values e.g. MeOH bulb extract (Pretoria) vs MeOH bulb extract (Cape Town).

Bands at the same position in the two extracts suggest existence of possibly similar phytochemical compounds. The similarity in the intensity suggest equivalent levels of the phytochemicals (see Figs. 11,12). The band uniformity observed in the TLC plates demonstrate similarity of chemical composition of in the plants.

Similarity in chemical composition is demonstrated more clearly in subfigures 5 A, B and D. The position of the bands, the colours and the intensities of the bands are similar thus indicating the relatedness of the phytochemicals present.

4. Discussion

The phytochemical analysis conducted on the three Amaryllidaceae species revealed the presence of secondary metabolites such as

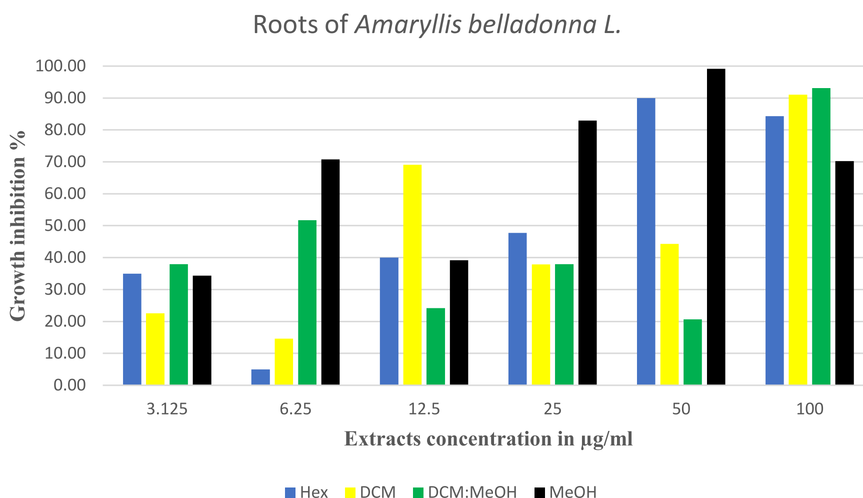

Fig. 9. Cell growth inhibition activity of roots extracts of *A. belladonna L* in percentage (%) at varying concentrations.

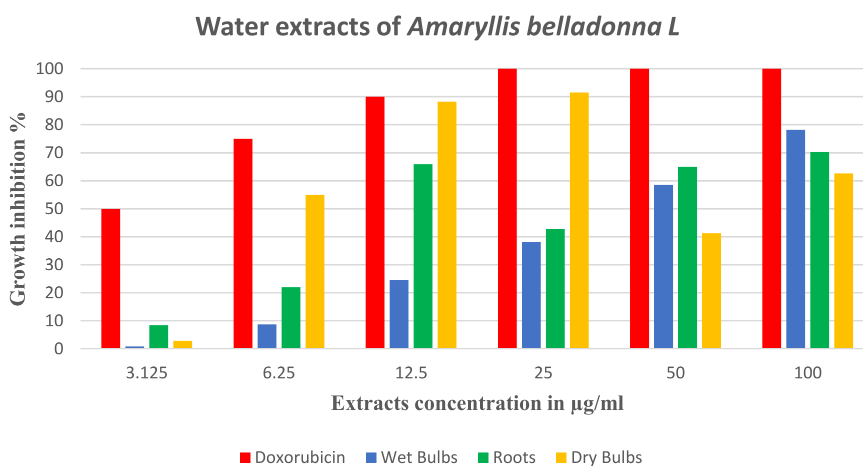

Fig. 10. Cell growth inhibition activity of water extracts of *A. belladonna L* in percentage (%) at varying concentrations.

Table 2
Retention values (cm) for DCM, DCM: MeOH, MeOH and water extracts of C. bulbispermum roots and bulbs.

Extract	*Crinum bulbispermum*			
	Pretoria		Cape Town	
	Roots	Bulbs	Roots	Bulbs
DCM	0.21, 0.5	0.22, 0.46, 0.5, 0,72, 0.86	0.21, 0.6, 0.70	0.22, 0,45, 0.5, 0.71, 0.88
DCM: MeOH	0.07, 0.14, 0.22, 0.51, 0.71, 0.79	0.14, 0.21, 0.22, 0.46, 0.71, 0.80, 0.86, 0.92	0.07, 0.14, 0.22, 0.51, 0.71, 0.79	0.14, 0.21, 0.29, 0.45, 0.71, 0.86, 0.97
MeOH	0.07, 0.14, 0.17,0.21, 0.71, 0.79	0.07, 0.14, 0.17, 0.35, 0.4, 0.46, 0.79, 0.86	0.07, 0.14, 0.17, 0.23, 0.71, 0.79	0.07, 0.14, 0.17,0.21, 0.35, 0.4, 0.46, 0.79, 0.86
Water	0.14, 0.19, 0.42	0.16, 0.2, 0.29, 0.36, 0.45	0.07, 0.14, 0.36, 0.4, 0.46, 0.97	0.07, 0.14, 0.21, 0.36, 0.4, 0.46, 0.57, 0.97

Table 3
Retention values (cm) for DCM, DCM: MeOH and MeOH extracts of B. disticha from plants collected in Pretoria and Cape Town, respectively.

Extract	*Boophone disticha*			
	Pretoria		Cape Town	
	Roots	Bulbs	Roots	Bulbs
DCM	0.29, 0.36, 0.5, 0.64	0.36, 0.5, 0.57	0.26, 0.34, 0.70	0.22, 0.35, 0.5, 0.71, 0.86
DCM: MeOH	0.07, 0.1, 0.3, 0.36, 0.43, 0.5, 0.57, 0.64, 0.79	0.07, 0.21, 0.29, 0.36, 0.57, 0.64, 0.79, 0.86	0.07, 0.12, 0.28, 0.50, 0.71, 0.79	0.14, 0.21, 0.25, 0.5, 0.71, 0.86, 0.97
MeOH	0.07, 0.1, 0.3, 0.43, 0.5, 0.57, 0.64, 0.79	0.07, 0.21, 0.29, 0.57, 0.64, 0.79, 0.86	0.07, 0.14, 0.17,0.21, 0.71, 0.79	0.07, 0.25, 0.43, 0.55, 0.66, 0.80, 0.86

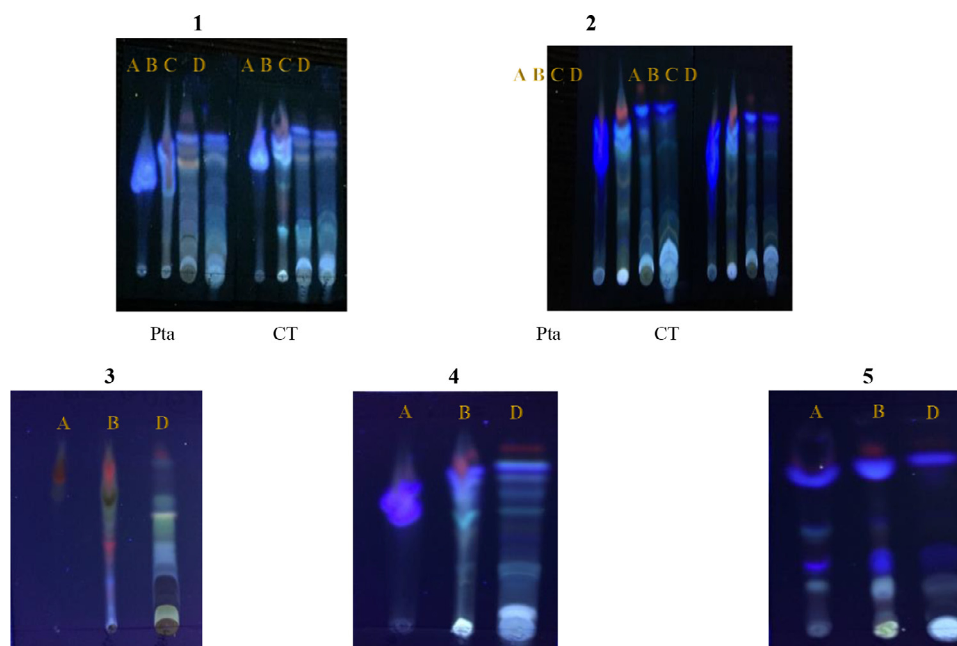

Fig. 11. The subfigures (**1**–**7**) above exhibit TLC plates elucidation of **A**- Hexane, **B**- DCM, **C**- DCM: MeOH and **D**- MeOH extracts of *C. Bulbispermum* and *B. disticha*. Pta=Pretoria and CT= Cape Town. (**1**) *C. Bulbispermum* Dry roots (Pta and CT) (**2**) *C. bulbispermum* dry bulbs (Pta and CT) **(3)** *B. disticha* dry bulbs (Pta) **(4)** *B. disticha* dry roots (Pta) **(5)** *B. disticha* dry roots (CT).

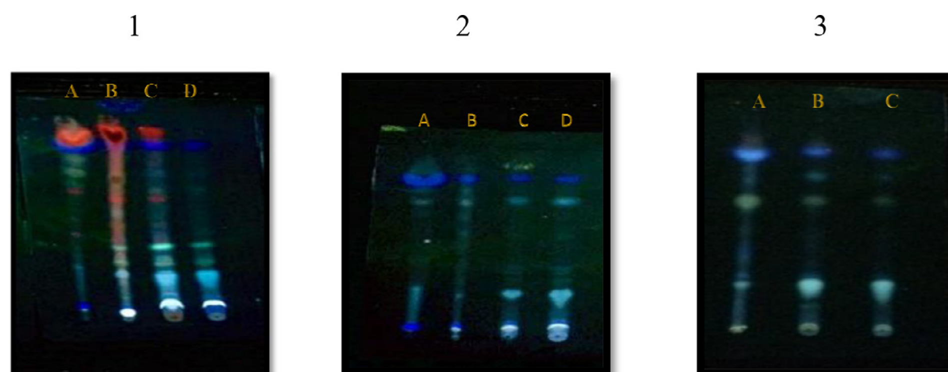

Fig. 12. The subfigures (**1**–**4**) above show TLC plates elucidation of **A**- Hexane, **B**- DCM, **C**- DCM: MeOH and **D**- MeOH solvents of the *A. belladonna L* plant extract. (**1**) *A. belladonna L* leaves (**2**) *Amaryllis belladonna L* dry bulbs (**3**) *A. belladonna L roots* extracts.

flavonoids, alkaloids and Terpenoids, which have been associated with cell antiproliferation (Atanasov et al., 2015 and Nair and van Staden, 2013). Our results show that *A. belladonna L* dry bulbs and roots were rich in these chemical constituents, which could explain the high antiproliferative activity observed. The phytochemical results concurred with the report by Maroyi, 2016 on the alkaloid and non-alkaloid composition of *C. bulbispermum* and *A. belladonna L* plants. Biomarkers were not probed in this study. However, the literature suggested the presence of alkaloids in this family of plants provide a rationale for the observed cytotoxicity. Interestingly, the roots, in which alkaloids were not detected still showed cytotoxicity, which suggests that there are other phytochemicals contributing to the activity observed. This is not surprising, as synergistic activity of constituents is common in plants.

Generally, cell antiproliferative activity increased with increasing extract concentration. Only ≥ 50% cell proliferative activity at concentration of ≤10 μg/ml for Figs. 1–7, and of ≤12.5 μg/ml for Figs. 8-10 were considered, as the aim was to evaluate growth inhibition% at the lowest concentrations. More attention was placed on polar extracts as the secondary objective is to discover water soluble chemo agents to improve drug distribution. Hexane extracts fatty-acids mainly pose challenges of not dissolving in less harmful solvents (Ramos-López, González-Chávez, Cárdenas-Orteg, and Zavala-Sánchez, 2012; Shinde, Devaiah, and Kilaru, 2017). It was interesting therefore to observe increasing antiproliferative activity with increasing polarity of extracts of different parts; e.g. water bulbs and roots extracts of *B. disticha*. Notably, *B. disticha* showed more proliferation in this study than previously reported by Botha, Kahler, Du Plooy, Du Plooy, and Mathibe, 2005. The *A. belladonna L* plant phytochemical composition results were generally consistent across the different parts of the plant, with methanolic extracts showing higher inhibitory activity in wet bulbs and roots. Water extracts of all plant parts showed considerably high growth inhibition activity.

There were similar phytochemical compositions of plants parts of *C. bulbispermum* collected from different geographical locations. There was also similar antiproliferative activity of the extracts (Figs. 1–4), especially the dry bulbs DCM: MeOH extracts. This consistency is critical for development and standardization of plant-based medicines.

The *C. bulbispermum* bulbs indicated the presence of more constituents, evident by high number of bands observed on TLC plates (Table 2). The R_f values obtained from *C. bulbispermum and B. disticha*

from Pretoria and Cape Town were similar. The sample-runs look similar for all five extracts (see Tables 2 and 3). Although the phytochemical TLC profiles of all the five extracts looked similar in terms of the presence of similar number and pattern of bands, the intensities of the bands were different which could explain why the extracts expressed different cell growth inhibitory activities. Similarly the presence of pharmacologically important phytochemicals such as flavonoids and alkaloids insinuate that similarity in antiproliferative activity could be expected. The bulb samples in both plants had additional bands, and the increased number of bands correlated to the increased cytotoxicity in the bulb extracts. Furthermore, the uniformity of constituents suggest that the TLC profiles can be used to authenticate this species regardless of location of collection.

Although plant-based studies encourage the use of leaves as they are easily accessible and for conservation purposes, this study focused on roots and bulbs as these are the parts used in Traditional medicines for these species (Kariuki and Njoroge, 2011). Extensive use and research of these plants are resulting in negative environmental impacts, which call for conservation strategies and mechanisms for sustainable use. Propagation protocols are also necessary as viable means to meet the demand (Raimondo et al., 2009). The significance of this study's comparative evaluation of the phytochemical and cytotoxic activities of these plants are important for future attempts to suggest plant part substitution as means of conserving these highly collected plant species (Williams, 2007).

Amaryllidaceae constituents have been examined against several leukemia cell lines in previous studies to investigate antiproliferative effects (Breiterová et al., 2020). What sets this article apart is the use of Amaryllidaceae species against K562 acute lymphocytic leukaemia cell line instead of the previously reported MOLT-4 and Jurkat cell lines. In agreement to our results are reports by Breiterová et al., 2020 which reported good growth inhibition (50%) results of Amaryllidaceae based alkaloids against Jurkat cell line. Broadening the scope of topic to reveal the importance and use of South African-based Amaryllidaceae species lays the groundwork for standardization, comparative analysis and conservation of such plants in the pharmacological space.

5. Conclusions

Maroyi (2016) reported a need for systematic data linking of the ethnomedicinal uses of *A. belladonna L, C. bulbispermum* and *B. disticha* to the advancement of leukaemia treatment. This can only be done by further studies to improve our knowledge of mechanisms of action these plants employ, the levels of toxicity, composition of bioactive constituents, the clinical relevance of the observed *In vitro* activity.

The preliminary results of the *In vitro* antiproliferative activity of the three species of *Amaryllidaceae* family against human leukaemia K562 cell line obtained in this study supported their use towards treatment of ALL either as adjuvants or at increased concentrations. *In vivo* experiments will have to be performed to obtain appropriate effective dosages. The contributions of this study showed evidence of the consistency in constituents of *C. bulbispermum* and *B. disticha* plants collected from different geographical locations.

Declaration of competing interest

The authors declare no conflict of interest.

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