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Original Article

Cytotoxic effect of *Vernonanthura polyanthes* leaves aqueous extracts

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(With 3 figures)

Abstract

Vernonanthura polyanthes, popularly known as assa-peixe, is a medicinal plant that has been widely used by Brazilian Cerrado population for treatment of diseases without a detailed evaluation of their effectiveness, toxicity, and proper dosage. Thus, more studies investigating the safety of *V. polyanthes* aqueous extract before the use are needed. The purpose of this study was to evaluate the toxicity, cytotoxicity and genotoxicity of *V. polyanthes* leaves aqueous extract using the *Artemia salina* and *Allium cepa* assays. For the *A. salina* assay, three groups of 10 larvae were exposed to *V. polyanthes* leaves aqueous extract at the concentrations of 5, 10, 20, 40, and 80 mg/ml. For the *A. cepa* assay, 5 onion bulbs were exposed to *V. polyanthes* leaves aqueous extract at 10, 20, and 40 mg/ml, and then submitted to macroscopic and microscopic analysis. As result it was identified a toxicity and cytotoxicity of *V. polyanthes* dependent on the extract concentration. The *A. salina* assay suggests that the concentration of 24 mg/ml of the *V. polyanthes* extract is able to kill 50% of naupllis; while the *A. cepa* assay suggests that *V. polyanthes* leaves aqueous extract is toxic at concentrations higher than 20 mg/ml; however the cytotoxic effect in *A. cepa* root cells was observed at 40 mg/ml of the extract. It is important to say that the *V. polyanthes* leaves aqueous extract concentration commonly used in popular medicine is 20 mg/ml. Thus, the popular concentration used is very close to toxicity limit in *A. salina* model (24 mg/ml) and is the concentration which showed toxic effect in *A. cepa* root cells (20 mg/ml). No genotoxic activity of *V. polyantes* leaves aqueous extract was observed in the conditions used in this study. Because of the antiproliferative action and no genotoxic activity, *V. polyanthes* leaves aqueous extract may present compounds with potential use for human medicine. However more detailed studies need to be performed to confirm this potential.

Keywords: *Allium cepa*, *Artemia salina,* assa-peixe, Cerrado, cytotoxicity.

Efeito citotóxico do extrato aquoso de folhas de *Vernonanthura polyanthes.*

Resumo

Vernonanthura polyanthes, popularmente conhecida como assa-peixe, é uma planta medicinal amplamente utilizada pela população brasileira do Cerrado para o tratamento doenças, sem uma avaliação detalhada de sua eficácia, toxicidade e dosagem adequada. Dessa forma, são necessários estudos para investigar a segurança do uso do extrato aquoso de *V. polyanthes*. O objetivo deste estudo foi avaliar a toxicidade, citotoxicidade e genotoxicidade do extrato aquoso de folhas de *V. polyanthes* utilizando os ensaios de *Artemia salina* e *Allium cepa*. Para o ensaio de *A. salina*, três grupos de 10 larvas foram expostos ao extrato aquoso de folhas de *V. polyanthes* nas concentrações de 5, 10, 20, 40 e 80 mg/ml. Para o ensaio de *A. cepa*, 5 bulbos de cebola foram expostas ao extrato aquoso de folhas de *V. polyanthes* nas concentrações de 10, 20 e 40 mg/ml, e então submetidos a análise macroscópica e microscópica. O ensaio de *A. salina* sugere que a concentração de 24 mg/ml do extrato de *V. polyanthes* é capaz de matar 50% dos náuplios; enquanto o ensaio de *A. cepa* sugere que o extrato aquoso das folhas de *V. polyanthes* é tóxico em concentrações superiores a 20 mg/ml. O efeito citotóxico nas células da raiz de *A. cepa* foi observado apenas na concentração de 40 mg/ml. É importante dizer que a concentração de extrato aquoso de folhas de *V. polyanthes* comumente usada na medicina popular é de 20 mg/ml. Assim, a concentração popular utilizada está muito próxima do limite de toxicidade no modelo de *A. salina* (24 mg/ml) e é a mesma concentração que apresentou efeito tóxico nas células da raiz de *A. cepa* (20 mg/ml). Não foi observada atividade genotóxica do extrato aquoso de folhas de *V. polyantes* nas condições utilizadas neste trabalho. Por causa da ação antiproliferativa e ausência de atividade genotóxica, o extrato aquoso de folhas de *V. polyanthes* pode ser uma boa fonte natural de compostos antitumorais e pode apresentar potencial para uso na medicina. No entanto, estudos mais detalhados precisam ser realizados para confirmar esse potencial.

Palavras-chave: *Allium cepa, Artemia salina,* assa-peixe, Cerrado, citotoxicidade.

1. Introduction

Medicinal plants have played an important role in the prevention, treatment and also cure of different diseases. The plants used in medicine, food supplements, cosmetics, and other health related products has increased along the time (Jones et al., 2004). Approximately 50% of all drugs currently in clinical trials are derived from plants (Shakya, 2016) and there is speculation that more than two billion people worldwide resort to medicinal plants to treat diseases (Lambert et al., 1997).

The bioactive phytocompounds present in medicinal plants include an array of compounds, such as tannins, lignans, coumarins, quinones, stilbenes, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins, and proanthocyanins. However, it is important to say that those same compounds also can cause adverse issues for human health, if bad administered. For example, rosemary (Cardoso et al., 2014), and guaco (Dalla Nora et al., 2010), commonly considered medicinal plants, reveals toxic effects dependent on the dose.

The present study is designed to appraise the toxicity and cytogenotoxicity potential of a folk medicinal plant, *Vernonanthura polyanthes* (Spreng.) A.J. Vega & Dematt. known commonly as assa-peixe. The genus *Vernonia* went through a taxonomical reclassification and the South America species were segregated in 22 new genera, including *Vernonathura* (Robinson, 1999). The taxonomic classification of *Vernonia* and *Vernonathura* genus are yet complex and unclear (Martucci et al., 2104). But the compounds found in species of booth genus have been usually used in popular medicine. *Vernonanthura polyanthes* presents oval leaves, rough and hairy spear-shaped and occurs primarily in Brazilian Cerrado biome (Vega and Dematteis, 2010). The white or pink inflorescences are arranged at the apices of the branches in small capitula (Alves and Neves, 2003).

Vernonanthura polyanthes extracts are used in ethnomedicine to treat persistent coughs, pneumonia, bronchitis, gastric disorders, kidney stones, malaria, fever, wounds and fractures (Lorenzi and Matos, 2008). In addition, the plant is indicated by common sense as diuretic and anti-rheumatic (Jorgetto et al., 2011; Oliveira et al., 2011). Focusing on the pharmacological research, this species demonstrated to be a potential vasodilatation agent, able to manage blood pressure (Romanezi da Silveira et al., 2003). Moreover, this species demonstrated antibacterial (Romanezi da Silveira et al., 2003), antifungal (Silva et al., 2012), leishmanicidal (Moreira et al., 2017), antinociceptive and anti-inflammatory activities (Temponi et al., 2012; Rodrigues et al., 2016). In addition, *V. polyanthes* extracts promotes gastroprotection without significant effects on gastric acid secretion (Barbastefano et al., 2007).

In relation to the toxicity, previous study identified that the *V. polyanthes* aqueous extract showed no toxic, genotoxic, and antigenotoxic activity in the experimental conditions tested using the wing somatic mutation and recombination test SMART/wing (Guerra-Santos et al., 2016). On the other hand, other study demonstrated that the *V. polyanthes* leaves ethanolic extract was toxic to mice with LD_{50} of 2.78 g/kg (Temponi et al., 2012). That contradictory information needs to be best analyzed to verify the *V. polyanthes* toxicological potential. Actually, several different methodologies should be used to ensure accurate information about the toxicity of medicinal plants.

Here we selected two different methodologies to evaluate the *V. polyanthes* toxicological potential: *Allium cepa* and *Artemia salina* assays. Those methods are very useful for toxicity and cytogenotoxicity screening and they are simple, inexpensive and minimum laboratory facilities are required for its performance (Ribeiro et al., 2016; Neves et al., 2014; Leme and Marin-Morales, 2009). Moreover, the results obtained using those *in vivo* models show a high degree of conformity with the results obtained from mammalian assays (Freires et al., 2017; Fedel-Miyasato et al., 2014; Leme and Marin-Morales, 2009).

2. Material and Methods

2.1. Plant collection and preparation of aqueous extract

Samples of *Vernonanthura polyanthes* (Spreng.) A.J. Vega & Dematt leaves were collected from specimes located at Campus Henrique Santillo, Universidade Estadual de Goiás, Anápolis, Goiás, Brasil (16° 23' 0,16"S 48° 56' 37,8"W, 1073 m) in November 2015 and plants no present flowers at the moment of collect. The collected leaves were identified and a voucher specimen was deposited in UEG Herbarium under identification number 10512. After the identification, the leaves were dried under air circulation and powdered using a knife mill. The aqueous extract solution was prepared by infusion with boiling water. After a rest of 20 min, the solution was filtered using the Millipore membrane filter (0.45 µm pore size) and briefly used. The *V. polyanthes* leaves aqueous extract concentration commonly used in popular medicine is 20 mg/ml (ANVISA, 2011, Guerra-Santos et al., 2016). Then, it was tested five different concentrations in *A. salina* assay (5, 10, 20, 40, and 80 mg/ml) and three different concentrations in *A. cepa* assay (10, 20, and 40 mg/ml).

2.2. Phytochemical screening and secondary metabolites quantification

The *V. polyanthes* leaves aqueous extract was evaluated to detect the presence of the secondary metabolites. The screening was performed for alkaloids (Bouchardat, Draggendorff and Mayer reaction), anthraquinones (Borntraeger reaction), coumarins (NaOH reactions), flavonoids (cianidin and sulfuric acid reactions, A-1 and A-II), terpenes, phenolic compounds (precipitation reaction with ferric chloride), and tannins (iron salts reaction, protein precipitation, B-1 and B-II), using methodologies previously described (Costa, 2001; Matos, 1988; Matos and Matos, 1989). Also, the total phenols, flavonoids and tannins were quantified using methodologies previously described by Waterman and Mole (1994) and Rolim et al. (2005).

2.3. Toxicity evaluation using Artemia salina

Eggs (25 mg) from *A. salina* were acquired from local pet shops and hatched at 25 - 30°C in saline water (pH 8.0). After 24 h, the newly hatched larvae were collected and used in the lethality assay according to procedures described by Meyer et al. (1982). Groups of 10 larvae were exposed to *V. polyanthes* leaves aqueous extract (5, 10, 20, 40, and 80 mg/ml) diluted in natural seawater and, after 24 h, the survival rates (%) were recorded. The negative control (NC) was the saline water used and the positive control (PC) was a potassium dichromate ($K_2Cr_2O_7$) solution (5 mg/ml). Three independent experiments were performed in experimental triplicate. The averages for the number of dead individuals of *A. salina* were compared by ANOVA followed by Tukey's test, using the statistical program SISVAR (Ferreira, 2011). In addition, Statistica software was used to calculate the minimum lethal concentration (LC_{50}).

2.4. Mitochondrial activity evaluation using Allium cepa

The mitochondrial respiratory chain activity using *A. cepa* was adapted from the method reported by Prajitha and Thoppil (2017). Briefly, *A. cepa* bulbs were growth in water for 72 h. The germinated bulbs were exposed to *V. polyanthes* aqueous extract at 10, 20, and 40 mg/ml for 3 h. Sterile mineral water and sodium azide (1 mg/mL) were used as negative (NC) and positive (PC) controls, respectively. To evaluate the mitochondrial activity, the roots with the same size were stained with 2,3,5-triphenyl tetrazolium chloride (TTC, 0.5%) for 15 min at 35 °C in the dark. After the staining, the roots images were captured in stereoscope and the quantitative analyses were performed measuring complex triphenyl formazan released after washing with 95% ethanol for 30 min in dark. The detection of the dye was made at 490 nm in spectrophotometer, and the experiment was performed in triplicate. The treated and control groups were compared using one way analysis of variance (ANOVA) of ranks, followed by Tukey's test. P values of less than 0.05 ($P<0.05$) were considered statistically significant.

2.5. Cytotoxic and genotoxic evaluation using Allium cepa assay

Allium cepa bulbs were germinated in water for 48 h, and after exposed to three different concentrations of *V. polyanthes* leaves aqueous extract (10, 20, and 40 mg/mL). The controls were: sterile mineral water (NC) and sodium azide (PC, 1mg/ml). After treatment, the roots were collected for the microscopic analyses. Then, the treated roots were collected and placed in Carnoy's fixative solution (3:1 ethanol:glacial acetic acid v/v) at -2 °C. The roots were subsequently hydrolyzed in HCl 5N for 1 min and washed with sterile mineral water. After this, the roots were placed on a microscope slide and one drop of acetic acid 45% was added. Then, the roots were macerated with rusty needles. A coverslip were placed on the material and a pressure was made to help separate the cells. After, the cover slips were removed after freezing in liquid nitrogen and stained with Giemsa (5%) for 5–10 min. The microscope slides were observed under an optical microscope. The following parameters were observed: mitotic index (MI); frequency of chromosomal aberrations (CA); and nuclear abnormalities (NA). To obtain those information 1000 cells were evaluated from each bulb, totalizing 5 bulbs per treatment (5,000 cells). The data were compared using t test and $p < 0.05$ was considered statistically significant.

3. Results

The phytochemical screening of *V. polyanthes* leaves aqueous extract revealed the presence of phenolic compounds, flavonoids, terpenoids, tannins and coumarins. Alkaloids and saponins were not identified in this samples. The total phenolic content, flavonoids and tannins were determined, indicating 27.73 ± 0.11%, 2.35 ±0.00% and 4.81 ± 0.02% in *V. polyanthes* leaves aqueous extract, respectively. After first screening for secondary metabolites, the *V. polyanthes* leaves aqueous extract was submitted to *Artemia salina* and *Allium cepa* assay to assess its toxicological potential.

The lethality of *V. polyanthes* leaves extract on brine shrimps nauplii was directly proportional to the concentrations of the extract used in this work (Figure 1). All *A. salina* nauplii were alive after 24 h of experiment in the negative control, and all nauplii exposed to the positive control died. The LC_{50} obtained for *V. polyanthes* leaves aqueous extracts was 24 mg/ml. This means that the medicinal popular dose (20 mg/ml) is very close to toxicity limit identified for *Artemia salina* at the present study (LC_{50} = 24 mg/ml). However, the toxicity of *V. polyanthes* leaves aqueous extract was smaller than the toxicity of positive control (LC_{50} = 0.19 mg/ml) which

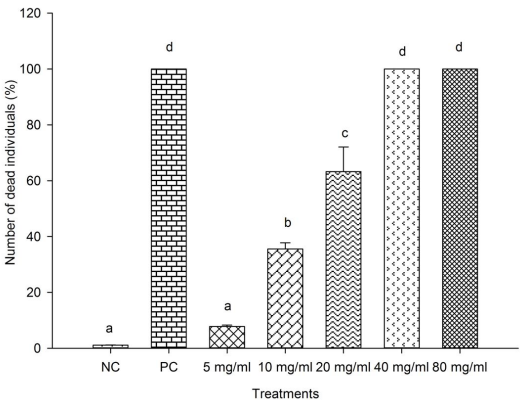

Figure 1. Effects of *Vernonanthura polyanthes* leaves aqueous extract on the mortality of *Artemia salina* assay. Nauplii were treated with 5, 10, 20, 40, and 80 mg/ml of *V. polyanthes* for 24 h. PC: positive control (potassium dichromate, 5mg/ml); NC: negative control (saline water). Averages were compared using one-way ANOVA followed by Tukey´s test. Averages followed by the same letters do not differ statistically among themselves.

is a drug commonly used in this assay able to cause nauplii death at low concentration (potassium dichromate, 5 mg/ml). In addition, statistical analysis showed no significant differences in survival rate between negative control and 5 mg/ml of *V. polyanthes,* showing that in this concentration this extract is not toxic to *A. salina* nauplii. On the other hand, no statistical differences were observed between positive control and 40, and 80 mg/ml of *V. polyanthes,* which means that in this concentration the *V. polyanthes* extract is highly toxic.

The effect of *V. polyanthes* aqueous extract on the viability of *A. cepa* cells was also investigated using a mitochondrial respiratory chain activity assay. For this, the conversion of TTC to insoluble red colored triphenyl formazan by the meristematic cells was measured in the root. We observed a concentration-dependent decrease in mitochondrial activity in the onion roots treated with *V. polyanthes* aqueous extract (Figure 2A). The results obtained are in agreement with the *A. salina* assay and showed a significant decrease in viability of *A. cepa* cells at 20 and 40 mg/ml. In this assay, the *V. polyanthes* aqueous extract was not toxic at 10 mg/ml.

With the objective of investigating the possible mechanism involved in toxicity of the *V. polyanthes* aqueous extract we performed a microscopic analysis of the *A. cepa* meristematic cells. The results showed no statistical difference in MI between 10 and 20 mg/ml and the negative control (Table 1), which suggested that on those concentrations the *V. polyanthes* extracts is not cytotoxic. However, it was observed a decrease in the MI rate in cells subjected to the concentration of 40 mg/ml (Table 1), which suggest that the highest concentration of the *V. polyanthes* aqueous extract used in this work was able to inhibit the cell division and so is cytotoxic.

The genotoxicity was evaluated by the frequency of chromosomal aberrations and nuclear abnormalities. *V. polyanthes* leaves aqueous extracts at all concentrations showed very few chromosomal aberrations and nuclear abnormality frequency, not differing statistically from the negative control (Table 1). Three main types of chromosome aberrations were recorded: stickiness, bridges and lagging chromosomes (Figure 3). This result indicates that *V. polyanthes* leaves aqueous extracts is no genotoxic at all the concentrations tested in this work.

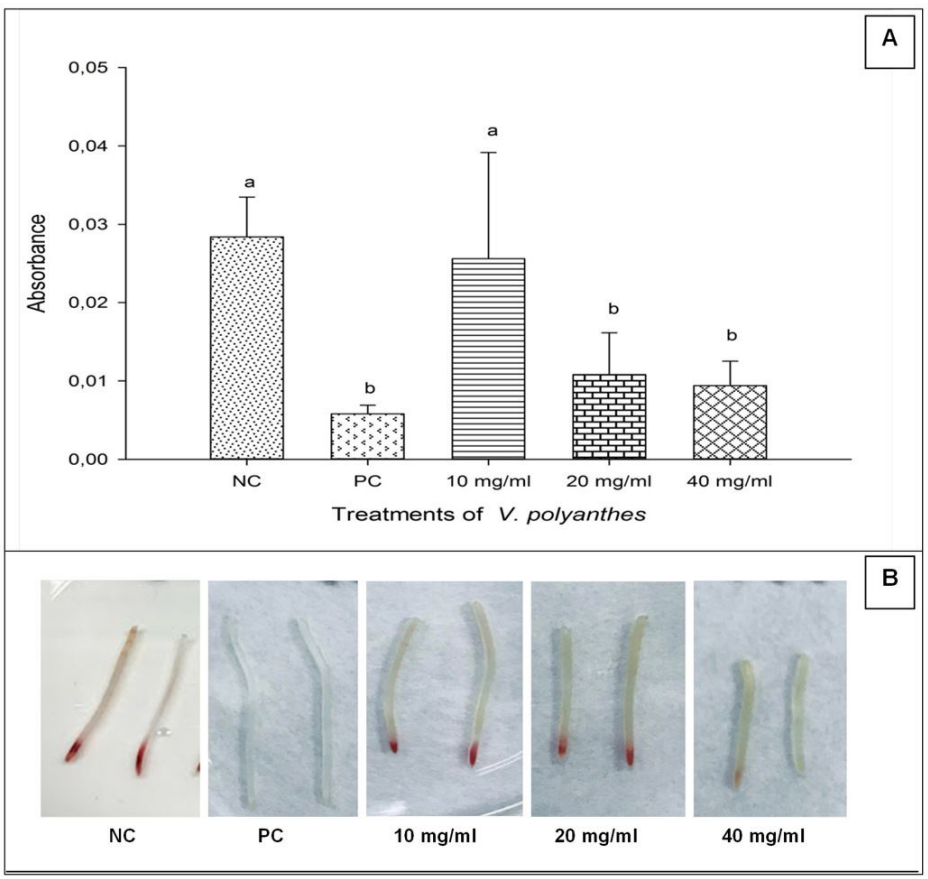

Figure 2. Effects of the *Vernonanthura polyanthes* leaves aqueous extract on *Allium cepa* viability using the triphenyltetrazolium chloride (TTC) staining. Bulbs were treated with 10, 20, and 40 mg/ml of *V. polyanthes* leaves aqueous extract for 3 h. (A) Quantitative analyses were performed measuring complex triphenyl formazan released by the root tips. Averages were compared using one-way ANOVA followed by Tukey´s test. Averages followed by the same letters do not differ statistically among themselves; (B) Representative pictures of *Allium cepa* root tips after TTC staining.

Table 1. Cytogenetic analysis of *Allium cepa* meristematic cells exposed to different concentrations of *Vernonanthura polyanthes* leaves aqueous extract.

Treatments	Number of cells	Interphase Cells	Division cells	Cytotoxicity	Genotoxicity	
				Mitotic index (MI)	% Chromosomal aberrations (CA)	% Nuclear abnormalities (NC)
NC (water)	5000	3865	1135	22.7 ± 6.4[a]	0.06 ± 0.01[a]	0.02 ± 0.008[a]
PC (sodium azide)	5000	4730	270	5.4 ± 4.4 [b]	0.30 ± 0.03[b]	0.28 ± 0.05[b]
10 mg/mL	5000	4170	830	16.6 ± 3.4[a]	0,14 ± 0.03[a]	0.02 ± 0.01[a]
20 mg/mL	5000	4190	810	16.2 ± 4.1[a]	0,12 ± 0.02 [a]	0.02 ± 0.006[a]
40 mg/mL	5000	4880	120	2.4 ± 3.1[b]	0.16 ± 0.006[a]	0.06 ± 0.01[a]

Same letters represent no significant difference using one-way ANOVA followed by Tukey´s test ($p < 0.05$). NC: negative control; PC: positive control; and 10, 20, and 40 mg/ml of V. polyanthes leaves aqueous extract.

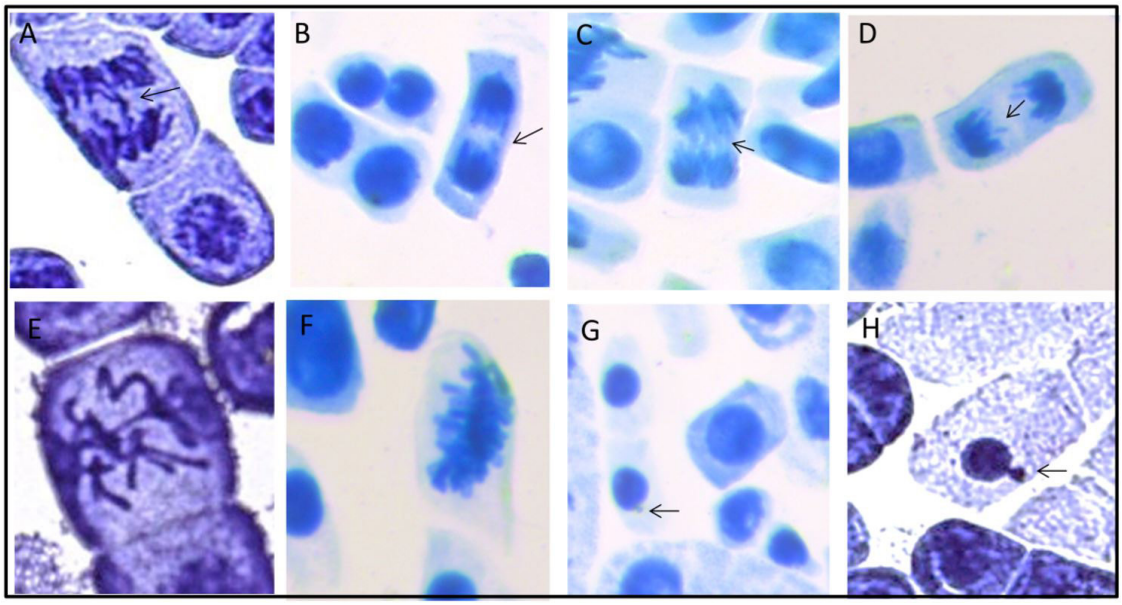

Figure 3. The most common chromosomal and nuclear aberrations observed in the *Allium cepa* meristematic cells exposed to *Vernonanthura polyanthes* leaves aqueous extract. (A), (B), and (C): Chromosome bridge; (D) Lagging chromosome; (E) Abnormal mitosis; (F) Chromosome stickness; and (G) and (H) nuclear bud.

4. Discussion

In this study, the *in vivo A. salina* and *A. cepa* assays were used to assess the toxic, cytotoxic, and genotoxic potential of the *V. polyanthes* leaves aqueous extract. This species has been empirically used by Brazilian Cerrado population for treatment of several diseases without a detailed evaluation of their effectiveness, toxicity, and proper dosage. Then, more studies investigating the safety of *V. polyanthes* aqueous extract are needed to safeguard the wellbeing of its users. Trying to assess the effects of *V. polyanthes* in the form it is actually administered we prepared plant extract in the same way as the therapeutic teas used by the general population In addition, we used different concentrations from that of the popular use to evaluate the effect of concentration in tea toxicity.

The *A. salina* assay suggests that concentrations over 24 mg/ml are toxic for shrimp and kill 50% of nauplii; while the viability of *A. cepa* assay suggests that concentrations higher than 20 mg/ml cause significant cell death. Since the concentration commonly used in popular medicine is 20 mg/ml, it is necessary a precaution in the administration of this infusion and more studies using human cells need to be performed to detect the proper dosage for humans.

A previous study using *Drosophila* as a model to evaluate the toxic potential of *V. polyanthes* leaves aqueous extract showed no toxic, genotoxic, and antigenotoxic activity in the experimental conditions tested using the wing somatic mutation and recombination test SMART/wing (Guerra-Santos et al., 2016). Different from that, at the present work we identified a toxic and cytotoxic activity of *V. polyanthes*. This disagreement can happen, once there is no single test to detect the full spectrum of toxicity of the substance (Dearfield et al., 2002).

The cytotoxic and antiproliferative activity of the *V. polyanthes* leaves aqueous extract observed in the

present work can be due to the phytochemicals present in this species. We identified the presence of phenolic compounds, flavonoids, terpenoids, tannins and coumarins. The total phenolic content was 27.73 ± 0.11%. Phenolic compounds consist of minimum of one aromatic ring and can be classified into several groups such as phenolic acids, flavonoids, coumarins, tannins, lignins and stilbenes (Shahidi and Yeo, 2016). The phenolic compounds have attracted attention of scientific community due to their effective antioxidant capacity (Shahidi and Yeo, 2016). We identified that *V. polyanthes* leaves aqueous extracts has 2.35% of flavonoids and 4.81% of tannins. In addition to those phytochemical screenings, previous characterization also have been shown that different *V. polyanthes* leaves extracts present compounds such as: flavones chrysoeriol-7-O-glycuronyl, acacetin-flavones 7-O-glycuronyl, sesquiterpenes, triterpenes, lactones piptocarphin A and piptocarphin B, glaucolide A, chlorogenic acids, coumarins, glycosides, steroids, saponin glycosides, and alkaloids (Gallon et al., 2018; Martucci et al., 2014; Igual at al., 2013; Souza et al., 2008). Despite this variety of metabolites, some studies pointed that flavonoids and terpenoids are the main compounds present in *V. polyanthes* extracts (Barbastefano et al., 2007; Bohlmann et al., 1981).

Up to now, it was identified more than 8000 flavonoids, which have been presented different biological activities (Chen et al., 2016). The wide functional and pharmacological activities of flavonoids are mainly due to their structural variations and chemical modifications, such as acylation, methylation, glycosylation and hydroxylation (Malla et al., 2012). Those wide conformational variations allow the flavonoids to exhibit different activities, such as: antioxidant, antitumor, antiallergic, anti-inflammatory, antibacterial, antiarteriosclerotic and antiestrogenic (Bhouri et al., 2011; Zhu et al., 2015). Then, flavonoids can interfere in biological cell activity depending on their nature and concentration.

Regard to terpenes, in plants, in high concentration they can be toxic, and are important to defense against herbivores and pathogens (Paduch et al., 2007). For human helthcare, the terpenoids exhibit important pharmacological activities, such as antimicrobial, antiviral, anti-fungal, anti-inflammatory, antihyperglycemic and antiparasitic activities, as well as are effective in chemoprevention and chemotherapy (Paduch et al., 2007). There are some terpenoids such as paclitaxel and its derivate which are commonly used as anticancer drugs (Perveen, 2018). However, complementary studies are necessary to determine the correlation of the flavonoids and terpenoids with the antiproliferative cell activity.

In addition, our results showed that *V. polyanthes* aqueous extract present no genotoxic effect on *A. cepa* root. As discussed before, the presence of flavonoids and terpenoids could account for the lack of mutagenic effects caused by infusions of these extract (Teixeira et al., 2003; Perveen, 2018). Similarly to what was observed in this work, *Costus spiralis* (Sousa et al., 2017), *Sambucus australis* (Tedesco et al., 2017), *Luchea divaricata* (Frescura et al., 2012) and *Solidago microglossa* (Bagatini et al., 2009) medicinal extracts also presented cytotoxic but no genotoxic or mutagenic effect using *A. cepa* test.

The scientific literature has been shown that many of the agents used in cancer therapy are derived from natural compounds extract from plants, such as: vinca alkaloid family isolated from *Catharanthus roseus* (Noble 1990; Stanton et al., 2011), and the taxanes paclitaxel originally identified from plants of the genus *Taxus* (Baird et al., 2010). *Vernonia* genus which is a sister genus of *Vernonanthura* also has different species with antitumor properties, such as *V. zeylanica* (Abeysinghe et al., 2019), *V. extensa* (Thongnest et al., 2019), *V. cinerea* (Pouyfung et al., 2019), *V. amygdalina* (Yedjou et al., 2018), *V. anthelmintica* (Ito et al., 2016), *V. condensate* (Thomas et al., 2016), *V. divaricata* (Lowe et al., 2014) and *V. scorpioides* (Pagno et al., 2006). In the similar way to those species, the identification of cytotoxic and antiproliferative properties of *V. polyanthes* leaves aqueous extract may suggest that this species also could be able to inhibit the tumor cells proliferation, however this capacity must be yet deeply investigated with other assays using cancer cells.

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