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Biology-Genetics

Cytotoxity and Genotoxity of Aqueous and Hydroalcoholic Extracts From *Gallesia Integrifolia* (Spreng) Harms

Citotoxidade e genoxicidade de extratos aquosos e hidroalcoólicos de *Gallesia Integrifolia* (Spreng) Harms

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ABSTRACT

Gallesia integrifolia (Spreng) Harms is a medicinal plant commonly known in Brazil as *pau-d'alho*. This large tree species belongs to the family Phytolaccaceae, which is widely distributed in several Brazilian states. Studies carried out with extracts deriving from different parts of this plant have evidenced its acaricidal, larvicidal, antifungal, and bactericidal properties, among others. Thus, given its potential to be used as a therapeutic alternative, the aims of the current study are to trace the phytochemical profile and assess the cytogenotoxic and mutagenic effects of aqueous and hydroalcoholic extracts deriving from dry *G. integrifolia* leaves based on the *Allium cepa* system, germination assays conducted with *Lactuca sativa* L, and Random Amplified Polymorphic DNA (RAPD) as analysis tools. Results have indicated that aqueous and hydroalcoholic extracts from *G. integrifolia* leaves presented cytotoxic, genotoxic, and mutagenic effects at concentrations of 15 mg mL^{-1} and 30 mg mL^{-1}. The herein observed effects may be associated with phytochemical agents found in the tested extracts, as well as emphasize the importance of raising awareness about the indiscriminate use of medicinal plants. Thus, future research should be conducted to help isolate and better understand the structure of components capable of inhibiting cell division.

Keywords: Medicinal plant; Cytogenotoxicity; Mutagenicity; *Allium cepa*; RAPD

RESUMO

Gallesia integrifolia (Spreng) Harms, planta medicinal popularmente conhecida por pau-d'alho, é uma espécie arbórea de grande porte da família Phytolaccaceae, que tem ocorrência em diversos estados do Brasil. Estudos realizados com extratos obtidos de diferentes partes da planta têm demonstrado suas propriedades acaricida, larvicida, antifúngica, bactericida, entre outras. Sendo assim, diante

do seu potencial como alternativa terapêutica, este trabalho se propôs traçar o perfil fitoquímico e avaliar o efeito citogenotóxico e mutagênico de extratos aquosos e hidroalcóolicos de folhas secas de *G. integrifolia*, usando como ferramentas de análise o sistema *Allium cepa*, o ensaio de germinação com *Lactuca sativa* L e o Random Amplified Polymorphic DNA (RAPD). Os resultados demonstraram que os extratos aquoso e hidroalcóolico de *G. integrifolia* apresentam efeitos citotóxicos, genotóxicos e mutagênicos nas concentrações de 15 mg mL^{-1} e 30 mg mL^{-1}. Os efeitos observados podem estar relacionados aos agentes fitoquímicos presentes nos extratos e ressaltam também a importância da conscientização sobre o uso indiscriminado de plantas medicinais, o que permite ainda sugerir para pesquisas futuras, o isolamento e a elucidação estrutural de componentes com ações inibitórias sobre a divisão celular.

1 INTRODUCTION

Gallesia integrifolia (Spreng) Harms is commonly known in Brazil as *pau-d'alho,* given its characteristic garlic smell. This large tree species belongs to family Phytolaccaceae, which occurs naturally in several Brazilian states, from Ceará to Paraná (Carvalho, 1994; Barros et al. 2005). People use its leaves and bark in folk medicine to treat several diseases, such as influenza, pneumonia, gonorrhea, rheumatism and ulcers (Lorenzi, 2002; Raimundo et al. 2018). Studies conducted with extracts deriving from different parts of this plant have evidenced its acaricidal, larvicidal, antifungal and bactericidal potential, among others (Arunachalam et al. 2016; Bortolucci et al. 2020; Souza et al. 2022).

According to Arunachalam et al. (2016), the hydroalcoholic extract from *G. integrifolia* inner bark has shown broad antibacterial effect, and it favors its traditional use to treat bacterial infections. The aforementioned authors detected compounds like saponins, alkaloids, phenolic compounds and flavonoids, which may account, either alone or in combination, for the antibacterial activity of this extract. The essential oil extracted from its stem presents gastroprotective and healing properties, likely due to its antioxidant, mucogenic and anti-inflammatory effects (Arunachalam et al. 2017).

There are reports in the literature about *G. integrifolia's* antifungal action, as well as about its effectiveness against *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus ochraceus* and *Trichoderma viride*, which was 25 times higher than that of ketoconazole (Raimundo et al. 2018).

It is important conducting further studies focused on assessing pau-d'alho's cytogenetic and mutagenic potential, given *G. integrifolia's* potential to be used as therapeutic alternative in antifungal, antibacterial and gastroprotective treatments, among other diseases.

Thus, the aim of the present study was to assess the cytogenetic and mutagenic potential of both aqueous and hydroalcoholic extracts deriving from *G. integrifolia* leaves, based on using the *Allium cepa L.*, germination assay conducted with *Lactuca sativa* as assessment tools and Random Amplified Polymorphic DNA (RAPD*). Allium cepa* and *Lactuca sativa* are bioindicators widely used in toxicogenetic studies since they are low-cost, sensitive, reproducible tests validated by international agencies, such as Food and Drug Administration, International Program on Chemical Safety (IPCS, WHO) and United Nations Environmental Program (UNEP) (FDA, 1987; Bagatini et al. 2007; Omotola et al. 2021). RAPD, in its turn, refers to genomic DNA amplification based on arbitrary-sequence primers. It does not require prior knowledge about the investigated species' genetics. This low-cost technique is fast and requires minimal DNA amounts (Lacerda et al. 2002; Szulc et al. 2012; Pandey and Kumar, 2021).

2 MATERIAL AND METHODS

2.1 Sample collection and identification

G. integrifolia leaves at different development stages were collected at the campus of Rio de Janeiro Federal Rural University (UFRRJ), in Seropédica Country, Rio de Janeiro State, Brazil. The botanical material was recognized by herbarium technician Thiago Azevedo Amorim, who works at the Botany Department of UFRRJ; a voucher specimen was deposited in the university´s herbarium, under RBR n. 56195, with additional registration in SISGEN AE710AE.

2.2 Preparing *G. integrifolia* leaf extracts

G. integrifolia leaves were taken to the Plant Genotoxic Activity Laboratory (LAGEP) of UFRRJ, right after the collection procedure was over. They were spread on a table and left to dry in an area protected from sunlight, at room temperature (28 °C), for 72h (air drying). Then, the herein naturally dried leaves were placed in amber flask until extract preparation time.

2.2.1. Aqueous extract

Leaves were ground and placed in glass vials filled with warm distilled water (90 °C). Vials were capped right away and kept under this condition for at least 10 minutes to obtain the extracts. Crude extracts were cooled down to room temperature and filtered through cotton cloth to remove residues (Prista et al. 2011; Silva, M.C. et al. 2020). Fresh extracts, at concentrations of 15 mg mL^{-1}, 30 mg mL^{-1} (*Allium cepa*, RAPD and Germination assay) and 50 mg mL^{-1} (phytochemical analysis) were prepared on a daily basis, right before they were used in the experiments (Silva, M. C. et al. 2020).

2.2.2. Hydroalcoholic extract

Hydroalcoholic extract was obtained through maceration, based on using 60 g of the pulverized material and 700 mL of 70% ethanol, under the following conditions: at room temperature (28 ºC), for 72 hours, protected from light, with occasional stirring and without extracting solution renewal. After this time-period was over, the extract was filtered and evaporated in water bath, at 60 ºC; this process resulted in extract dry weight of 7.65 g (Stange et al. 2009, with modifications). Part of the dried extract was weighed and (re)dissolved in distilled water to get the concentration used in the experiment. Extracts - at concentrations of 15 mg mL^{-1}, 30 mg mL^{-1} (Allium cepa, RAPD and Germination assay) and 50 mg mL^{-1} (phytochemical analysis) - were prepared on a daily basis, right before they were used in the experiments.

2.3 Phytochemical screening of the investigated extracts

Phytochemical analysis was carried out with 50 mg of the produced extracts to identify and quantify the chemical constituents in the aqueous and hydroalcoholic extracts; it was done based on techniques described by Trease and Evans (1989), Matos (2009), Cai et al. (2011), Saklani et al. (2012), Kumar (2014) and Morsy (2014). The aqueous extract was dried by evaporation in water bath, at 50 °C. Screening was carried out to detect reducing sugars, saponins, tannins, cardiac glycosides, polysaccharides, anthraquinones, flavonoids, alkaloids, steroids and triterpenoids, and coumarins. Reducing sugars were detected based on using Benedict's reagent (Saklani et al. 2012). Saponins' presence was observed through froth formation in test tube subjected to vigorous stirring, based on using diluted samples (Cai et al. 2011; Saklani et al. 2012; Morsy, 2014). Tannins were identified based on using gelatin solution test, ferric chloride reagent and lead acetic test (Matos, 2009; Saklani et al. 2012; Morsy, 2014). Cardiac glycosides were detected based on using Kedde's reagent (Morsy, 2014). Polysaccharides were identified based on using lugol (Cai et al. 2011). Anthraquinones were detected through Bornträger test (Morsy, 2014). Flavonoids were detected through Shinoda test (Cai et al. 2011; Saklani et al. 2012; Morsy, 2014). Alkaloids were determined based on using Dragendorff, Mayer, Sonnenschein and Bouchardat's reagents (Trease and Evans, 1989; Saklani et al. 2012; Morsy, 2014; Kumar, 2014). Steroids and triterpenoids were detected through Liebermann-Burchard test (Cai et al. 2011; Saklani et al. 2012; Morsy, 2014). Finally, coumarins were detected based on using green fluorescence under UV light, at wavelengths of 254 and 365 nm (Matos, 2009; Morsy, 2014). Assays performed for each extract type were carried out in triplicate.

2.4 Germination assay conducted with *Lactuca sativa* L.

Petri dishes (150 x 20 mm) lined with three previously autoclaved sheets of germination paper were used for germination trials. *Lactuca sativa* L. seeds were disinfected in 1% hypochlorite solution for 3 minutes. Subsequently, they were washed in distilled water

and distributed on germination paper, which was soaked in 7 mL of distilled water (negative control), aqueous extract (15 mg mL^{-1} and 30 mg mL^{-1}) or hydroalcoholic extract (15 mg mL^{-1} and 30 mg mL^{-1}). Negative control was used for each concentration in both extracts. In total, 30 seeds were used on each plate by taking into consideration that seed germination rates can reach approximately 85%. It was done to ensure that 25 roots could be analyzed at the end of the experiment. The aforementioned plates were placed in B.O.D. (Biochemical Oxygen Demand) germination chamber, under controlled temperature and light (25 ± 2°C and 12h light/dark photoperiod) conditions; the root part was measured with the aid of pachymeter, after 7-day incubation period. Assays were performed in quadruplicate, for each concentration and extract type.

The herein applied statistical treatment was based on Student's t-test. $P < 0.05$ was considered statistically significant. Bioestat 5.0 software (Ayres et al. 2007) was used for statistical analysis purposes.

2.5 *Allium cepa* assay as bioindicator

Organically grown onions (approximately 2.0 cm, in diameter) were obtained in a local produce market to perform these tests. The outer layer of each bulb was removed with the aid of a paring knife, without damaging the root buds.

Bulbs were initially placed in a container filled with distilled water, for 48 hours, to allow the roots to grow; the water in the container was replaced on a daily basis. Then, bulbs were separated into control and treatment groups; each group comprised five onions. The negative control groups (one for each extract) remained in distilled water, whereas treatment groups were immersed in *G. integrifolia* leaf extract solutions (aqueous and hydroalcoholic) at concentrations of 15 mg mL^{-1} and 30 mg mL^{-1}, for each extract. The positive control group was immersed in ethyl methanesulfonate solution (EMS, 25 mM), which is a highly efficient mutagenic agent that acts directly in DNA molecules due to its alkylation activity. Solutions used in all groups (175 mL for each treatment) were replaced on a daily basis and temperature was maintained at 25 °C.

Root tips (from 2 cm to 2.5 cm, in length) were removed from the bulbs after 48-hour exposure to the respective solutions, fixed in ethanol: glacial acetic acid solution at 3:1 ratio (V/V), and stored at 4 °C; then, they were used to prepare slides, based on the method described by De Castro and Sousa et al. (2017), with modifications - this procedure was performed for each treatment. Five slides were prepared for each bulb, based on using five different root tips (one slide for each tip). Root tips were washed in distilled water, twice, for 5 minutes; hydrolyzed in HCl 5N, for 30 minutes; washed again, twice, in distilled water, for 5 minutes and then, placed on slides with the aid of tweezers. Subapical meristems were fragmented with the aid of a scalpel, stained with 2% acetic orcein and covered with coverslip.

All slides were coded and assessed under common optical microscope, at 100X magnification. Parameters used to determine the genotoxic and cytotoxic potential of the investigated extracts comprised chromosomal and cell changes, as well as changes in mitotic index. In total, 1,000 cells per bulb, or 5,000 cells per group, were analyzed. Results were expressed in absolute terms, except for the mitotic index (ID), which was expressed in percentage based on using the following formula: ID = dividing cells/total number of analyzed cells X 100. The most frequent anomalies are shown in the micrographs.

The herein applied statistical treatment was based on χ^2 test with Yates correction. $P < 0.05$ was considered statistically significant. Bioestat 5.0 software (Ayres et al. 2007) was used for statistical analysis purposes.

2.6 DNA extraction and RAPD

Approximately 100 mg of *A. cepa* roots subjected to different treatments were used for DNA extraction purposes. All DNA extraction procedures were performed in compliance with the manufacturer's recommendations (PureLinkTM Plant Total DNA Purification Kit, Invitrogen). DNA samples' quantification was carried out in Nanovue spectrophotometer (GE Healthcare).

RAPD was performed based on Koç and Pandir (2018), with modifications. In total, 10ng of DNA was used in each reaction, with final volume of 15 µL. Reactions

were standardized with 1.95 µL of arbitrary primer (10µM, Opc4 5'-CCGCATCTAC-3'), according to Tedeschi et al. (2014), as well as with 7.5 µL of GoTaq® G2 Green Master Mix Promega (5 U/µL). PCR conditions comprised one cycle at 94°C for 2.5 min, which was followed by 45 cycles (at 94°C, for 45 s; at 35°C, for 45 s; and at 72°C, for 45 s), as well as by a final extension step at 72°C, for 5 min. PCR products were separated based on electrophoresis in 2% agarose gel. The 1x Tris-Acetic Acid-EDTA (TAE) buffer was used for 3 hours and 20 minutes at 75 V, then, it was stained with 1x GelRed. Assay was performed in triplicate, for each extract type.

3 RESULTS

3.1 Phytochemical screening of the investigated extracts

Phytochemical analysis applied to both the aqueous and hydroalcoholic extracts detected coumarins, alkaloids, steroids and triterpenoids in them. The aqueous extract also presented reducing sugars and tannins (Table 1).

Table 1 – Phytochemical analysis applied to aqueous and hydroalcoholic extract from *Gallesia integrifolia* leaves at 50 mg mL^{-1}

Secondary compounds	Hydroalcoholic extract	Aqueous extract
Reducing sugars	-	+
Saponins	-	-
Cardiac glycosides	-	-
Coumarins	+	+
Polysaccharides	-	-
Alkaloids	+	+
Flavonoids	-	-
Tannins	-	+
Anthraquinones	-	-
Steroids and triterpenoids	+	+

(-) Absence or inconclusive, (+) Presence

3.2 Germination assay conducted with *Lactuca sativa* L.

L. sativa roots subjected to treatment with both extracts presented significantly shortened length after 7-day germination, in comparison to the negative control (Table 2). The hydroalcoholic extract presented an even more significant effect at both concentrations, since seeds in this case did not germinate and/or did not develop into seedlings.

Table 2 – Mean root size, in centimeters, after 7-day germination of *Lactuca sativa* plants subjected to different aqueous and hydroalcoholic extract concentrations from *Gallesia integrifolia*

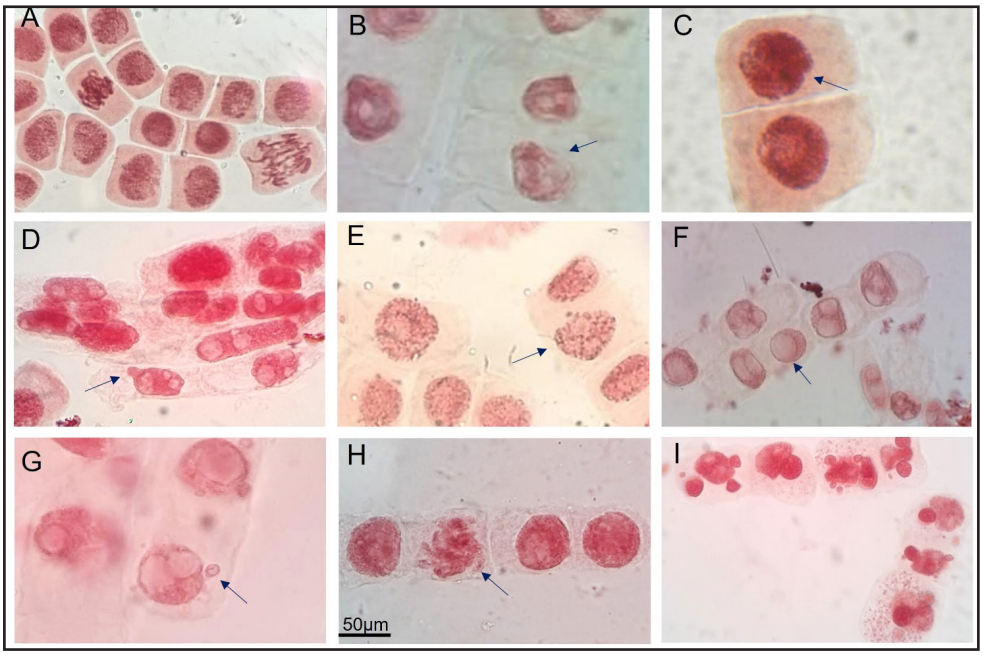
Aqueous extract			
NC	15 mgmL^{-1}	NC	30 mg mL^{-1}
1.142$^{(a)}$	0.621$^{(b)}$	1.486$^{(a)}$	0.491$^{(b)}$
Hydroalcoholic extract			
NC	15 mg mL^{-1}	NC	30 mg mL^{-1}
0.92$^{(a)}$	0$^{(b)}$	1.17$^{(a)}$	0$^{(b)}$

(a) and (b) – values followed by different letters in the same column significantly differ from each other (P<0.05) in T-Student test; NC – negative control; experiments were performed in quadruplicate

3.3 *Allium cepa* test system

Based on results recorded for the *A. cepa* assay, both the aqueous and hydroalcoholic extracts deriving from *G. integrifolia* leaves presented genotoxic and cytotoxic effects at the tested concentrations (15 mg mL^{-1} and 30 mg mL^{-1}). A significant number of nucleolar (large nucleoli) and nuclear changes, nuclear buds, cells undergoing cell death process, as well as mitotic index reduction, were observed for both extracts (Fig. 1, Tables 3 and 4). The aqueous extract also showed notched nuclei, whereas the hydroalcoholic extract showed micronucleated cells and cells with chromosome adhesion; these changes also indicate genotoxicity (Fig. 1, Tables 3 and 4).

Figure 1 – Changes in meristematic cells of *Allium cepa* subjected to the different ents



(A) negative control; (B) cells with nuclear changes (arrow) – positive control; (C-E) – aqueous extract: (C) notched nucleus (arrow); (D) cell with nuclear bud (arrow); (E) cell in karyorrhexis; (F-I) - hydroalcoholic extract: (F) cells with nucleolar changes (arrow) – large nucleoli; (G) micronucleated cell; (H) chromosome adherence (arrow); (I) cells in apoptosis.

Source: Authors' private collection

Table 3 – Cell changes and mitotic index observed for *Allium cepa* roots subjected to different treatments aqueous extract concentrations from *Gallesia integrifolia*

Tx	CONC	NoC	NB	CA	NaC	NO	CK	MI(%)
NC	dH_2O	$2555^{(b)}$	$87^{(b)}$	$0^{(b)}$	$70^{(b)}$	$8^{(b)}$	$10^{(b)}$	$4.1^{(a)}$
EMS	25 mM	$3811^{(a)}$	$97^{(b)}$	$24^{(a)}$	$398^{(c)}$	$21^{(c)}$	$7^{(b)}$	$2.26^{(b)}$
GI AQ	15 mg mL^{-1}	$2740^{(c)}$	$104^{(b)}$	$0^{(b)}$	$474^{(a)}$	$80^{(a)}$	$16^{(b),}$	$2.16^{(b)}$
GI AQ	30 mg mL^{-1}	$2536^{(b)}$	$149^{(a)}$	$2^{(b)}$	$190^{(d)}$	$49^{(c)}$	$145^{(a)}$	$0.9^{(c)}$

(a) (b) and (c) – values followed by different letters in the same column significantly differ from each other ($P<0.05$) in the χ^2 test; Tx – treatment; NC – negative control; EMS – ethyl methanesulfonate (positive control); GIAQ – *G. integrifolia* aqueous extract; CONC – concentration; dH_2O – distilled water; NoC – nucleolar changes (large nucleoli); NB - nuclear buds; CA - chromosome adherence; NaC - nuclear changes; NO – notched; CK - cell in karyorrhexis; and MI - mitotic index; 5000 cells were analyzed in each treatment

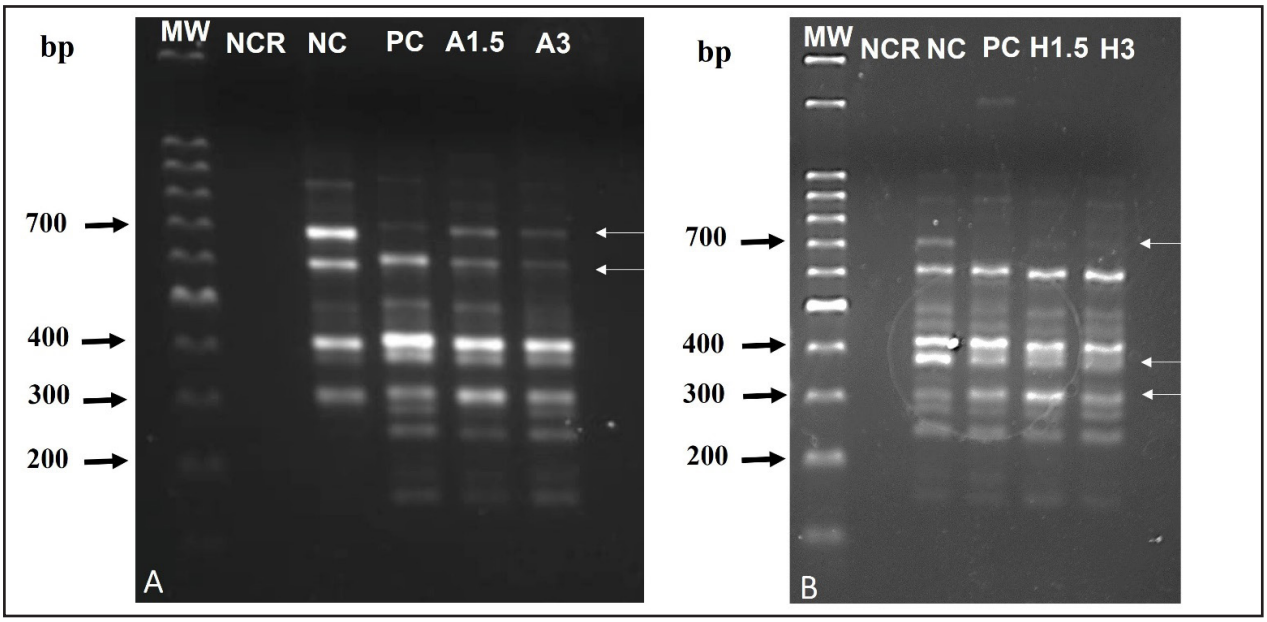
Table 4 – Cell changes and mitotic index observed for *Allium cepa* roots subjected to different treatments hydroalcoholic extract concentrations from *Gallesia integrifolia*

Tx	CONC	NoC	NB	CA	NaC	MC	AP	MI(%)
NC	dH_2O	1261$^{(d)}$	181$^{(a)}$	0$^{(c)}$	81$^{(d)}$	2$^{(b)}$	2$^{(d)}$	4,26$^{(a)}$
EMS	25 mM	3452$^{(a)}$	304$^{(b)}$	39$^{(a)}$	560$^{(c)}$	0$^{(b)}$	119$^{(c)}$	2,26$^{(b)}$
GIHA	15 mg mL^{-1}	2132$^{(c)}$	227$^{(c)}$	7$^{(b)}$	1209$^{(b)}$	11$^{(a)}$	571$^{(b)}$	0,26$^{(d)}$
GIHA	30 mg mL^{-1}	2237$^{(b)}$	125$^{(d)}$	8$^{(b)}$	1412$^{(a)}$	19$^{(a)}$	840$^{(a)}$	0,68$^{(c)}$

(a) (b) (c) and (d) – values followed by different letters in the same column significantly differ from each other ($P<0.05$) in the χ^2 test; Tx – treatment; NC – negative control; EMS – ethyl methanesulfonate (positive control); GIHA- hydroalcoholic extract of *G. integrifolia*; CONC – concentration; dH2O – distilled water; NoC- nucleolar changes (large nucleoli); NB- nuclear buds; CA- chromosome adherence; NaC – nuclear changes; MC – micronucleated cells; AP- apoptotic cells; e MI- mitotic index; 5000 cells were analyzed in each treatment

3.4 RAPD

Figure 2 – DNA fragments in agarose gel amplified by RAPD with primer Opc4



(A) gel comprising DNA fragments obtained from samples exposed to *G. integrifolia* aqueous extract. (B) gel comprising DNA fragments obtained from samples exposed to *G. integrifolia* hydroalcoholic extract; bp – base pairs; MW – molecular weight; NCR – negative control of the reaction, without DNA; NC – negative control roots; PC – positive control roots; A1.5 – roots treated with aqueous extract at 1.5%; A3 – roots treated with aqueous extract at 3%; H1.5 – roots treated with hydroalcoholic extract at 1.5%; H3 – roots treated with hydroalcoholic extract at 3%

Source: Authors' private collection (November 2022)

RAPD results have evidenced changes in DNA amplification profile among samples subjected to different treatments. The extracts, at different concentrations, as well as the positive control, triggered the emergence of bands and changed the intensity of other bands, in comparison to the profile associated with the genomic DNA extracted from roots belonging to the negative control group, as shown in Figure 2.

4 DISCUSSION

Results in the current study have evidenced that both the aqueous and the hydroalcoholic extracts deriving from *G. integrifolia* leaves presented cytotoxic, genotoxic and mutagenic effects at all tested concentrations.

Microscopic analyses applied to *A. cepa* meristematic cells have evidenced nucleolar and nuclear changes, nuclear buds and a significantly larger number of cells undergoing cell death in samples treated with both extract types than in the negative control group. Nucleolar (large nucleoli) and nuclear changes, as well as the presence of nuclear buds, are indicative of genotoxicity. The herein observed nuclear changes were featured by shapeless nuclei and nuclear vacuoles. Nuclear changes, together with nucleolar changes, are part of criteria adopted in cancer diagnosis processes (Filippin et al. 2000; Montanaro et al. 2008; Koh et al. 2011; Carotenuto et al. 2019; Radhakrishnan et al. 2023). According to Fenech (2001), nuclear buds result from the process to rule out excess of amplified DNA, which is likely a common micronucleus formation pathway. Although micronuclei can spontaneously emerge, their induction - as observed in the treatment conducted with hydroalcoholic extract - is often used to detect genotoxic damage resulting from exposure to a given mutagen (Heddle et al. 1983; da Silva et al. 2021; Tagorti and Kaya, 2022; Kwasniewska and Bara, 2022).

The hydroalcoholic extract also presented a larger number of cells showing chromosome adhesion. Chromosomal adhesion is a common sign of toxic effects on chromosomes. It can happen due to increased chromosomal condensation -

which ends up forcing the contraction of the total set of chromosomes and leads to adhesions - or to partial nucleoproteins' dissolution (Haq et al. 2016; Wijeyaratne and Wickramasinghe, 2020). In addition to the aforementioned changes, samples treated with aqueous extract presented significant number of cells with notched nucleus. This abnormality, which is also found in animal cells, indicates genotoxicity (Hemachandra and Pathiratne, 2016).

Components with genotoxic/mutagenic potential can also be detected through RAPD using. This technique has been used as tool to assess toxic effects of different chemicals on both plants and animals (Aksakal et al. 2013; Swaileh et al. 2013; Koç and Pandir, 2018). Koç and Pandir (2018) used RAPD to assess the effect of treatments conducted at increasing doses of food additives, such as sunset yellow (SY) and brilliant blue (BB), on *Allium cepa* meristematic cells. According to the aforementioned authors, the appearance/disappearance of, as well as the decrease/increase in, the observed band intensity between the negative control and the test groups has indicated the genotoxicity of the analyzed substances. These changes in band profile can be generated by point mutations or by modifications in DNA structure, such as disruptions, insertions, deletions and transpositions (Shahwar et al. 2022). Thus, results in the current study have confirmed the genotoxic/mutagenic potential of *G. integrifolia* aqueous and hydroalcoholic extracts.

The phytochemical profile analysis has evidenced the presence of coumarins, alkaloids, steroids and triterpenoids in both extracts, as well as of tannins in the aqueous extract. Similar profiles were observed by other authors who also analyzed extracts deriving from *G. integrifolia* leaves (Akisue et al, 1986; Ordoñez et al, 2006), although the analyzed extracts presented variations in the presence and absence of components, such as flavonoids. These variations can take place due to differences in the adopted solvent and extraction methodology. Bortolucci et al. (2020) used gas chromatography associated with mass spectrometry and observed diterpenes and triterpenes in leaf extracts. Most recently, Silva et al. (2023) detected saponin

(Esculentoside D) and flavonoid (Rutin) based on using UHPLC. One cannot rule out that extracts analyzed in the current study may hold these components, although at levels that could not be detected through the adopted techniques; moreover, they may have contributed to the herein observed effects.

Among all herein detected components, coumarins can be either beneficial or harmful to one's health, depending on their type, dose and matrix (Albarici et al 2010). Studies available in the literature associated coumarins with genotoxicity, mutagenesis and carcinogenesis, in different models (Borges et al. 2005; Montagner, 2007; Yordi et al. 2017; Hsieh et al. 2019; Ding et al. 2023). Alkaloids account for a wide range of secondary plant metabolites. Several alkaloids are known for their biological properties, such as significant toxicity or strong pharmacological ability (Rujjanawate et al. 2003). Fu (2004) and He et al. (2021) have chemically described several pyrrolizidine alkaloids and presented the likely mechanisms leading to their cytotoxic, mutagenic and tumorigenic activity. Pyrrolizidine alkaloids are common phytotoxins widely distributed in the plant kingdom (Fu, 2004).

Despite the lack of studies fully describing the chemical and pharmacological composition of alkaloids found in *G. integrifolia* extracts, the herein observed genotoxic and cytotoxic effects may also be linked to some components of this metabolite class. Steroids and triterpenoids form a secondary metabolite class that may be associated with several pharmacological effects (Rodrigues et al. 2010; Silva, F. C. O. et al. 2020), besides protecting some plant, animal and microorganism species from predators, pathogens and competitors (Gershenzon and Dudareva 2007).

Bhattacharya and Haldar (2012) used the *A. cepa* test system to assess the antimitotic and genotoxic effect of cucurbitacin-enriched extract, which is a triterpene deriving from *Trichosanthes dioica* (Roxb.) root. In addition to reduced mitotic index, which indicates cytotoxic activity, the aforementioned authors observed significant inhibition both in the length and number of roots. Triterpenes isolated from *Panax notoginseng* (Burk) have also shown moderate-to-strong cytotoxicity against

cancer cell lines (Silva, F. C. O. et al. 2020). It is important emphasizing that triterpenoid saponins are constituents widely distributed in the plant kingdom. Within this group, one finds esculentosides, which form a family comprising several pharmacological activities, such as the antibacterial, antiviral, antifungal, anticancer, antiparasitic, molluscicidal and insecticidal ones (Mahato and Garai 1998; Bailly and Vergoten, 2020). Silva et al. (2023) detected Esculentoside D in extract deriving from *G. integrifolia* leaves. Although no studies focused on investigating the pharmacological activity of this component were found in the literature, it is likely to have contributed to the cytotoxic effects presented by the investigated extract.

Cytotoxic effects herein observed for both extract types were identified based on the root size reduction recorded during the germination assay conducted with *L. sativa*, as well as on the mitotic index reduction observed in the test conducted with *A. cepa*, besides the significant presence of cells undergoing cell death processes, such as those undergoing karyorrhexis and apoptosis. According to Capobiango et al. (2009), cytotoxic compounds act in cells, in different ways; they can even induce cell death. These results resemble those observed by other authors (Arunachalam et al. 2017; De-Campos-Bortolucci et al. 2021 and Bortolucci et al. 2022; Silva et al. 2023).

It is worth emphasizing that the *G. integrifolia* hydroalcoholic extract, at all analyzed concentrations, had a more significant cytotoxic effect on, as well as caused a more significant mitotic index reduction in, *A. cepa,* besides fully inhibiting *L. sativa* germination. If one compares the phytochemical profile of the two herein analyzed extract types, it is possible seeing tannins in the aqueous extract, but not in the hydroalcoholic extract. Tannins comprise a heterogeneous group of phenolic compounds capable of forming insoluble complexes together with proteins, among other macromolecules (Monteiro et al. 2005; Maugeri et al. 2022). This feature, among others, is known to have positive effect on human health, such as curing intestinal infections by neutralizing bacterial activity in this environment; however, tannins' ability to bind to proteins, among other macromolecules, can also have irreversible toxic

effects (Monteiro et al. 2005; Pizzi 2019). Chung and collaborators (1998) have suggested that tannins appear to have dual effect. They are likely involved in cancer formation, hepatotoxicity or in anti-nutritional effects. On the other hand, they benefit human health due to their chemopreventive effect against carcinogenesis or microbial activities. Tannins also precipitate alkaloids and can be used as antidote in cases such as intoxication or poisoning by alkaloid plants (Lacerda et al. 2014). Silva et al. (2023) have analyzed crude extracts deriving from *G. integrifolia* leaves, flowers and fruits. Their results have evidenced the antioxidant potential of all investigated extract. They also associated this potential with phenolic compounds detected through UHPLC, such as the flavonoid "rutin" found in *G. integrifolia* leaves. Based on results in the current study, tannins found in the aqueous extract, likely in association with other phenolic compounds, can explain the less significant effect on *L. sativa* twinning, as well as the less significant mitotic index reduction observed for *Allium cepa*, in comparison to the hydroalcoholic extract. It is worth emphasizing that mitotic index reduction induced by aqueous extract has recorded the highest rate at aqueous extract concentration of 30 mg mL^{-1}, a fact that indicated dose-dependent effect. However, cells treated with hydroalcoholic extract were the ones mostly presenting mitotic index reduction, which recorded its highest reduction rate at hydroalcoholic extract concentration of 15 mg mL^{-1}. If one analyzes these two extract concentrations, this difference is often observed in studies conducted with crude plant extracts, since different extract concentrations present varying component interactions. Thus, there is no direct correlation between extract concentration increase and proportional increase in extract's effects. Similar results were reported by other researchers (Celik, 2012; Udo et al. 2015; Silva, M. C. et al. 2020).

5 CONCLUSIONS

The current findings have evidenced the cytogenetic effects of aqueous and hydroalcoholic extracts deriving from *G. integrifolia* leaves, as well as emphasized the

importance of raising awareness about the indiscriminate use of medicinal plants. Phytochemical analysis applied to the investigated extracts enabled finding coumarins, alkaloids, steroids and triterpenoids, as well as tannins in the aqueous extract. Since *G. integrifolia* remains poorly explored from the chemical and pharmacological perspectives, future studies focused on isolating and explaining the structure of components with inhibitory effects on cell division should be conducted to help better understanding this species' antitumor, antifungal, antibacterial and anti-inflammatory properties.

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