Research, Society and Development, v. 9, n. 9, e353997322, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i9.7322

Potencial citogenotóxico da casca de *Terminalia actinophylla* (Mart.) em *Allium cepa* L.

Cytogenotoxic potential of *Terminalia actinophylla* (Mart.) bark in *Allium cepa* L.

Potencial citogenotóxico de la corteza de *Terminalia actinophylla* (Mart.) en *Allium cepa* L.

Received: 06/08/2020 | Reviewed: 14/08/2020 | Accept: 17/08/2020 | Published: 21/08/2020

Francisca Mairana Silva de Sousa
ORCID: https://orcid.org/0000-0001-6550-0098
State University of Piauí, Brazil
E-mail: mairanassousa@hotmail.com
José Rafael da Silva Araujo
ORCID: https://orcid.org/0000-0002-3049-6866
Federal University of Pernambuco, Brazil
E-mail: jrafaelquadros@hotmail.com
Luciana Maria Fortes Magalhães Castelo Branco Couto
ORCID: https://orcid.org/0000-0003-0350-1591
State University of Piauí, Brazil
E-mail: lucianafortesmagalhaes@hotmail.com
Regina Maria Silva Sousa
ORCID: https://orcid.org/0000-0002-6300-0361
State University of Piauí, Brazil
E-mail: reginasousa484@gmail.com
Maria das Dores Alves Oliveira
ORCID: https://orcid.org/0000-0002-2847-6567
State University of Piauí, Brazil
E-mail: maralves013@gmail.com
Joaquim Soares da Costa Júnior
ORCID: https://orcid.org/0000-0002-9849-201X
Federal Institute of Piauí, Brazil
E-mail: jquimjr@gmail.com

Francielle Aline Martins
ORCID: https://orcid.org/0000-0002-0113-8023
State University of Piauí, Brazil
E-mail: franufv@yahoo.com.br
Pedro Marcos de Almeida
ORCID: https://orcid.org/0000-0001-5431-6818
State University of Piauí, Brazil
pedromarcosalmeida@yahoo.com.br

Resumo

Terminalia actinophylla (Mart), comumente conhecida como "chapada", é usada na medicina popular para o tratamento de diarreia, diabetes e anti-inflamatória. No entanto, estudos sobre sua toxicidade ainda são incipientes. O presente trabalho foi realizado com o objetivo de avaliar o perfil fitoquímico e o efeito citogenotóxico do extrato etanólico da casca de *T. actinophylla* utilizando a semente de *Allium cepa* L. Sementes de *A. cepa* foram submetidas a diferentes concentrações de extrato (1,25, 2,5, 5 e 10 mg/mL), controle negativo (CN - água destilada) e trifluralina (CP - controle positivo), durante 24 horas de exposição. O perfil fitoquímico do extrato foi obtido para identificar os principais metabólitos secundários. A citotoxicidade (índice mitótico) e a genotoxicidade (alterações cromossômicas) foram analisadas por meio da coleta de 5.000 células meristemáticas. A análise estatística foi realizada pelo teste de Kruskal-Wallis ($p<0,05$). Os fitoquímicos presentes no extrato foram taninos, saponinas e açúcares redutores. Apenas a concentração mais baixa (1,25 mg/mL) de *T. actinophylla* foi citotóxica em comparação com CN. Houve um aumento significativo na média total de alterações cromossômicas para concentrações mais baixas (1,25 e 2,5 mg/mL) em comparação com o CN. Provavelmente, os fitoquímicos presentes no extrato interferiram no ciclo celular e causam danos ao DNA de *A. cepa* em concentrações mais baixas. No entanto, mais estudos devem ser realizados em mamíferos, uma vez que o extrato com fins medicinais pode ter efeitos nocivos no organismo.

Palavras-chave: Alterações cromossômicas; Ciclo celular; Dano ao DNA; Extrato etanólico; Índice mitótico.

Abstract

Terminalia actinophylla (Mart), commonly known as "chapada", is used in folk medicine for the treatment of diarrhea, diabetes and anti-inflammatory. However, studies on its toxicity are still incipient. The present work was carried out with the objective of evaluating the phytochemical profile and the cytogenotoxic effect of the ethanolic extract of the *T. actinophylla* bark using the *Allium cepa* L. seed. Seeds of *A. cepa* were subjected to different extract concentrations (1.25, 2.5, 5 and 10 mg/mL), a negative control (NC - distilled water) as well as trifluralin (PC - positive control), during 24 h of exposure. The phytochemical profile of the extract was obtained to identify the main secondary metabolites. The cytotoxicity (mitotic index) and the genotoxicity (chromosomal alterations) were analyzed by means of the collection of 5,000 meristematic cells. The statistical analysis was carried out using the Kruskal-Wallis test ($p<0.05$). The phytochemicals present in the extract were tannins, saponins and reducing sugars. Only the lowest concentration (1.25 mg/ml) of *T. actinophylla* was cytotoxic in comparison with NC. There was a significant increase in the total average of chromosomal changes to lower concentrations (1.25 and 2.5 mg/ml) compared to NC. Probably, phytochemicals in the extract interfere with the cell cycle and cause DNA damage in *A. cepa* in lower concentrations. However, if bad studies must be carried out on mammals, since the extract with medicinal purpose can have harmful effects on the organism.

Keywords: Cell cycle; Chromosomal alterations; DNA damage; Ethanolic extract; Mitotic index.

Resumen

Terminalia actinophylla (Mart), comúnmente conocida como "chapada", se usa en la medicina popular para el tratamiento de la diarrea, la diabetes y antiinflamatoria. Sin embargo, los estudios sobre su toxicidad aún son incipientes. El presente trabajo tuvo como objetivo evaluar el perfil fitoquímico y el efecto citogenotóxico del extracto etanólico de la corteza de *T. actinophylla* usando la prueba *Allium cepa* L. Las semillas de *A. cepa* fueron sometidas a diferentes concentraciones de extracto (1.25, 2.5, 5 y 10 mg/mL), un control negativo (CN - agua destilada) así como trifluralin (CP - control positivo), durante 24 h de exposición. El perfil fitoquímico del extracto se obtuvo para identificar los principales metabolitos secundarios. La citotoxicidad (índice mitótico) y la genotoxicidad (alteraciones cromosómicas) se analizaron mediante el recuento de 5.000 células meristemáticas. El análisis estadístico se realizó con la prueba de Kruskal-Wallis ($p<0.05$). Los fitoquímicos presentes en

el extracto fueron taninos, saponinas y azúcares reductores. Solo la concentración más baja (1,25 mg/ml) de *T. actinophylla* fue citotóxica en comparación con CN. Se observó un aumento significativo en la media total de las alteraciones cromosómicas a las concentraciones más bajas (1,25 y 2,5 mg/ml) en comparación con CN. Probablemente, los fitoquímicos en el extracto interfieren con el ciclo celular y causan daño al DNA en *A. cepa* en las concentraciones más bajas. Sin embargo, se deben realizar más estudios en mamíferos, una vez que el extracto con fines medicinales puede tener efectos nocivos en el organismo.

Palabras clave: Alteraciones cromosómicas; Ciclo celular; Daño en el DNA; Extracto etanólico; Índice mitótico.

1. Introduction

The use of plant extracts and phytochemicals for medicinal purposes as prevention, treatment and cure of diseases is one of the oldest practices of traditional folk medicine (Kich et al., 2017). These compounds are still used in the development of drugs in the fight against cancer, infectious, immunological, cardiovascular, neurological and inflammatory diseases (Almeida et al., 2017; Santos et al., 2020). According to data from the World Health Organization (WHO), approximately 80% of the population in developing countries use herbal medicines for basic health care (Sarimahmut et al., 2016).

On the other hand, possible adverse effects caused by using medicinal plants are related to cell cycle disorder or genetic material instability known as cytogenotoxic effects (Almeida et al., 2016; Mendonça et al., 2016). Therefore, evaluations of the safety of these compounds is important.

Terminalia is the second largest genus of the family Combretaceae. It comprises approximately 200 species found in tropical and subtropical regions, distributed in the South and Central Americas (Madrigal et al., 2010), Southeast Asia (Manosroi et al., 2010), Egypt, India, Pakistan and the African continent (Pfundstein et al., 2010). The different parts of *Terminalia* (leaf, bark, fruit and seeds) present medicinal properties as antimalarial (Abiodun, 2011), hypoglycemic (Ramachandran et al., 2012), antidepressant (Das & Kumar, 2013), hepatoprotective (Nishanth et al., 2014), cardioprotective (Shukla et al., 2015), trypanocidal (Rayan et al., 2015), anti-inflammatory (Abiodun et al., 2016), and antiviral agents (Gupta et al., 2016).

Terminalia actinophylla (Mart.), commonly known in Brazil as ‘chapada’, is a endemic tree, being found in Cerrado and Caatinga biomes (Marquete & Loiola, 2015). In

Brazilian Cerrado, the dried and powdered bark has been used in popular medicine as antidiarrheal and wound-healing agent (Pádua et al., 2013), while the leaves have shown promise for photoprotection and acetylcholinesteratic activity (Silva et al., 2015; Farias et al., 2016). Phytochemical studies in this species revealing high content of phenols and flavonoids in the leaves (Silva et al., 2015; Farias et al., 2016) and presence of tannins, flavonoids and saponins in the bark (Pádua et al., 2013).

Although *T. actinophylla* has therapeutic advantages, different secondary metabolites found in this species may be potentially toxic, mutagenic, carcinogenic and/or teratogenic. In this sense, toxicogenetic tests are necessary to provide complementary information on the safety and use of this species based on the identification of concentrations and dosages that may induce adverse effects.

Among the methods available to evaluate cytogenotoxicity and mutagenicity, the bioassay using *Allium cepa* L. (onion) stands out for its low cost, reliability and good correlation with other test systems, such as Swiss mice (Fedel-Miyasato et al., 2014), cell culture (Malini et al., 2010) and fish erythrocytes (Hemachandra & Pathiratne, 2016), besides eliminating the need for use and sacrifice of animals (Leme & Marin-Morales, 2009). Moreover, the test-system with *A. cepa* is validated by the World Health Organization, the United Nations Environment Programme and the US Environmental Protection Agency (Leme & Marin-Morales, 2009). According to Roberto et al. (2016), this test-system also allows the simultaneous evaluation of cytotoxic, genotoxic and mutagenic effects of a certain compound, environmental samples or natural products, eliminating the need of different assays.

Considering the medicinal importance of *T. actinophylla* and the need of performing studies to assess toxicity and damage to the genome, the present study aimed to carry out a phytochemical prospection and evaluate the cytotoxic and genotoxic potential of the ethanolic extract from *T. actinophylla* bark in meristematic cells of *A. cepa.*

2. Material and Methods

2.1 Biological Material

Leaves, bark and flowers of *T. actinophylla* were collected at the municipality of Picos (PI, Brazil) in January of 2016. The exsiccate (no. 21642) was identified by Prof. Francisco Soares Santos-Filho and is deposited at the Herbarium Graziela Barros at the Federal

University of Piauí. The seeds of *A. cepa* cv. Vale Ouro IPA-11 used in the bioassay were provided by the Agronomic Institute of Pernambuco (Recife – PE, Brazil).

2.2 Preparation of the Ethanolic Extract and Phytochemical Prospection of *T. actinophylla*

Bark of *T. actinophylla* was dried in oven (45ºC) at the Genetics Laboratory of UESPI (Teresina – PI, Brazil) for five days. The dried material was ground in a blender until obtaining a powder. Next, 870 g of the bark powder were extracted in 96% ethanol, at a proportion of 1:3 of plant material to solvent (m/v), at room temperature for 32 h. The extract was filtrated and concentrated in a rotary evaporator (40°C), then kept in oven (72 h) to produce a completely dry mass (82.3 g), thus yielding the ethanolic extract from *T. actinophylla* bark (EEBTa). Subsequently, 500 mg of the extract were diluted in 50 mL of distilled water, yielding the highest concentration (10 mg/mL). The further concentrations (5, 2.5 and 1.25 mg/mL) were then obtained by serial dilution of the highest concentration in distilled water, to be used in the *A. cepa* assay.

Tests phytochemical were performed at the Chemistry Laboratory of the Federal Institute of Piauí (IFPI) in Teresina (PI, Brazil) to assess the qualitative presence of the main primary and secondary metabolites (alkaloids, anthraquinones, saponins, phenols, tannins, reducing sugars, polysaccharides, proteins and amino acids, and catechins) present in EEBTa, realized in accord with Pereira et al. (2020).

For alkaloids, the extract was dissolved in HCl, filtered and exposed to Dragendorff, Mayer and Bouchardat reagents. The positive result is white or yellow color (Mayer reagent), red color (Dragendorff), and brown or black (Bouchardat).

For anthraquinones, the extract was boiled with HCl for a few minutes, filtered, and allowed it to cool. Then $CHCl_3$ and 10% ammonia solution was added. The material was shaken for bright pink color observation, which indicates the anthraquinones presence. While saponins, the extract was submitted to agitation with distilled water in a bottle for 2 min for a layer of foam identification, that indicates the presence of saponins.

For phenols and tannins, iron solution was exposed to extract and the mixture was compared to a control test with distilled water. The presence of blue to red precipitate indicates phenols, whereas a dark blue precipitate indicates water soluble tannins.

For reducing sugars, the extract was diluted in distilled water, then, Fehling A reagent + Fehling B reagent were added and the mixture was boiled about 5 min. The identification of a brick-red precipitate indicates the presence of reducing sugars. Already polysaccharides, diluted extract (with distilled water) was exposed to Lugol's solution. Development of blue color indicates the presence of polysaccharides.

Then proteins and amino acids, the extract was diluted in aqueous ninhydrin solution, then boiled for about 2 min. The development of a violet color indicates presence of proteins and amino acids.

For flavonoids, the extract was diluted in methanol and exposed to HCl and magnesium. The appearance of a brown color indicates the presence of flavonoids. Finally, catechins detection, the extract was diluted in methanol, then vanillin solution and HCl were added. The presence of catechins is indicated by a red color.

2.3 *A. cepa* Assay

One hundred seeds of *A. cepa* were germinated on Petri dishes containing filter papers moistened with distilled water, at room temperature at the Laboratory of Genetics of UESPI. After germination, the seeds were transferred to the two controls (negative and positive) and to the four concentrations (1.25, 2.5, 5 and 10 mg/mL) of the EEBTa for a period of 24 h with a Petri dish for each concentration and control. Distilled water was used as a negative control (NC) and the herbicide trifluralin (0.84 ppm), a substance with aneugenic and clastogenic action was used with positive control (PC) (Fernandes et al., 2009). The root tips were fixed in ethanol: acetic acid solution (3:1) and stored at -20ºC until the preparation of the slides.

For slide preparation, the root tips were washed three times in distilled water, for 5 min each time, and hydrolyzed at 60 °C for 10 min in HCl 1 N. After hydrolysis, the root tips were again washed in distilled water and transferred to amber glass bottles containing Schiffs reagent, in which they remained for 2 h in the dark. After this time, the root tips were washed until complete removal of the reagent, transferred onto slides, squashed with one drop of 2% acetic carmine, and mounted with Entellan® (Fernandes et al., 2009).

Cytotoxicity (mitotic index - MI) and genotoxicity (chromosome alterations - CA) were evaluated by scoring 5,000 meristematic cells (500 cells/slide; 10 slides per treatment) under a light microscope (Olympus CX 21) at 400X magnification. The last one includes alterations resulting from aneugenic effects (*e.g.* C-metaphases, metaphase with chromosome adherences, loss chromosomes, multipolar anaphases, binucleate cells, polyploid metaphases)

or clastogenic effects (*e.g.* chromosome fragments in metaphase or anaphase and chromosome bridges). Micronuclei can be result from either aneugenic or clastogenic effects. Alterations resulting from aneugenic (interference with the fibers of the mitotic spindle) and clastogenic effects (breaks in the genetic material) denoted genotoxicity (Fernandes et al., 2009).

2.4 Statistical Analysis

The phytochemical prospection was a qualitative analysis. The datas of cytotoxicity and genotoxicity were analyzed in a completely randomized experimental design, under a quantitative analysis. The data were evaluated by non-parametric test of Kruskal-Wallis, followed by a posteriori test of Student-Newman-Keuls ($P < 0.05$) using the BioEstat 5.3 program (Ayres et al., 2007). All experimental was realized with Pereira et al., (2018) considerations.

3. Results

The results of the phytochemical profile of EEBTa demonstrated the presence of saponins, tannins and reducing sugars (Table 1).

Table 1. Qualitative phytochemical detection in *T. actinophylla* ethanolic bark extract.

Metabolites class	**Findings**
Alkaloids	-
Anthraquinones	-
Saponins	+
Phenols	-
Tannins	+
Reducing sugars	+
Polysaccharides	-
Proteins and amino acids	-
Catechins	-

+, presence; -, absence. Source: the authors

In the cytotoxicity test, only the lowest concentration (1.25 mg/mL) of EEBTa was cytotoxic, leading to significant decrease of the mitotic index (MI) when compared to NC

(Table 2). Regarding to chromosome alterations (CA), significant increase in the total mean of CA was observed at the lowest concentrations (1.25 and 2.5 mg/mL) compared to NC (Table 2).

When chromosome alterations were analyzed separately, metaphases with chromosomes adherences and C-metaphases (1.25, 2.5 and 5 mg/mL) as well as polyploid cells (1.25 and 5 mg/mL) were significant, whereas binucleated cells were not significant at any of the evaluated EEBTa concentrations (Table 3).

Table 2. Mean of mitotic index and total chromosomal alterations in meristematic cells of *Allium cepa* radicles after 24 h exposure to the ethanolic extract from *T. actinophylla* bark.

Concentration (mg/mL)	**Mitotic Index (Mean ± SD)**	**Chromosomal Alteration (Mean ± SD)**
NC	187.04 ± 25.00	6.23 ± 3.05
1.25	110.09 ± 32.18*	33.34 ± 5.10**
2.5	178.74 ± 30.58	14.34 ± 3.46*
5	211.45 ± 17.39	11.76 ± 2.24
10	175.26 ± 46.56	7.71 ± 2.64
Trifluralin	270.70 ± 44.54*	41.36 ± 7.43**

NC: Negative control (Distilled water). **Trifluralin:** positive control (0.84 ppm). **SD:** standard deviation. *Significant by Kruskal-Wallis test with *a posteriori* Student-Newman-Keuls test (*****p < 0.05; **p < 0.01). The results refer to analysis of 5,000 cells per treatment. Source: the authors.

Alterations in the chromosome segregation during anaphase and/or telophase were recorded, such as loss, bridges and multipolarity (Table 3). Among the observed alterations, chromosome loss (1.25 and 2.5 mg/mL) and chromosome bridges at all tested concentrations were significant. Furthermore, nuclear buds (1.25, 2.5 and 5 mg/mL) and micronuclei (MN; 1.25, 2.5, 5 and 10 mg/mL) presented significant alterations at the indicated concentrations, whereas chromosome fragments were significant at 1.25, 2.5 and 10 mg/mL. In addition, the Lobulated nucleus were not significant at any of the evaluated EEBTa concentrations (Table 3).

Table 3. Mean of the types of chromosomal alterations in meristematic cells of *Allium cepa* after 24 h exposure to the ethanolic extract from *T. actinophylla* bark.

Chromosomal Alteration	Negative control	Ethanolic Extract (mg/mL)				Positive Control
	Distilled Water	1.25	2.5	5	10	Trifluralin (0.84 ppm)
Chromosome adherence	1.59 ± 0.78	7.50 ± 1.79*	2.30 ± 1.14*	1.14 ± 1.31*	0.0 ± 0.0	5.75 ± 2.19*
C-metaphase	0.19 ± 0.19	3.15 ± 1.44*	1.15 ± 0.11*	0.57 ± 1.02*	0.0 ± 0.0	11.49 ± 4.32*
Polyploid cell	0.0 ± 0.0	0.18 ± 0.19*	0.0 ± 0.0	0.19 ± 0.29*	0.0 ± 0.0	4.11 ± 3.24*
Chromosome loss	0.19 ± 0.29	1.67 ± 0.74*	0.35 ± 0.36*	0.38 ± 0.11	0.0 ± 0.0	1.31 ± 1.08*
Binucleated cell	0.38 ± 0.18	1.85 ± 1.18	2.12 ± 1.77	3.88 ± 2.88	1.56 ± 0.83	1.31 ± 1.04
Multipolar anaphase	0.0 ± 0.0	0.09 ± 0.19	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Nuclear bud	0.85 ± 0.21	2.97 ± 1.26*	2.83 ± 1.56*	1.42 ± 0.36*	0.82 ± 0.40	4.27 ± 2.98*
Chromosome bridge	0.66 ± 0.18	3.70 ± 1.43*	1.24 ± 0.86*	0.95 ± 0.49*	0.46 ± 0.38*	2.14 ± 1.13*
Micronuclei	1.79 ± 1.09	10.47 ± 2.22*	3.89 ± 1.03*	2.46 ± 1.19*	5.04 ± 2.21*	10.43 ± 2.84*
Chromosome fragment	0.47 ± 0.79	1.39 ± 0.33*	0.35 ± 0.22*	0.28 ± 0.26	0.0 ± 0.0*	0.41 ± 0.58
Lobulated nucleus	0.0 ± 0.0	0.0 ± 0.0	0.18 ± 0.17	0.48 ± 0.42	0.0 ± 0.0	1.15 ± 1.82

*Significant by Kruskal-Wallis test with *a posteriori* Student-Newman-Keuls test (* $p < 0.05$). The results refer to analysis of 5,000 cells per treatment. Source: the authors.

4. Discussion

In recent years, there has been growing concern regarding the empirical use of plants for medicinal purposes due to their exhibiting toxic, carcinogenic and/or mutagenic properties (Sponchiado et al., 2016). Considering that about 80% of the world's population depends on medicinal plants for basic healthcare (Sarimahmut et al., 2016), toxicogenetic studies must be performed to assess the safety of these compounds. Owing to the different therapeutic applications of *T. actinophylla* bark and the lack of respective scientific information, the *A. cepa* bioassay was used in the present study to evaluate the effects of EEBTa on its capacity of causing damage.

Considering that many secondary metabolites of medicinal plants can be toxic to the human organism (Agra et al., 2007), a prospection of the possible responsible for the toxicogenetic activities observed in this work was carried out. The *T. actinophylla* bark extract showed saponins, tannins and reducing sugars. These metabolites are recognized for having protective effects on DNA, which are exceptionally toxic to the human body (Konoshima et al., 1996; Nepka et al., 1999).

Considering the MI, at the highest concentrations of EEBTa (2.5, 5 and 10 mg/mL), did not confirm a cytotoxic effect on the meristematic cells of *A. cepa*. It is possible that the tannins, saponins and reducing sugars of EEBTa did not interfere with the DNA synthesis

process or blockage at the G2/M phase of the cell cycle, allowing the cells to enter the division process (Bianchi et al., 2016). Nevertheless, the lowest concentration (1.25 mg/mL) was cytotoxic, as it presented significant reduction of MI in comparison to NC. At this concentration, the saponins and tannins of EEBTa possibly acted in various ways: by inhibiting the progression of the cell cycle, as observed for different saponins and tannins found to act as antitumor (cytotoxic) agents, promoting the accumulation of cells in phase S; hindering the progression of G2/M; inhibiting the activity of cyclin-dependent kinases (CDKs); and inducing apoptosis by activation of caspases (Sakagami et al., 2012; Yildırım & Kutlu, 2015).

The results of the present study suggest that the activity of saponins and tannins was concentration-dependent, being cytotoxic only at the lowest concentration in meristematic cells of *A. cepa*, as also observed in tumor cell cultures with concentrations lower than in this study (Tiwary et al., 2015; Yildırım & Kutlu, 2015). Likewise, Atoyebi et al. (2015) and Almeida et al. (2016) also reported the cytotoxic activity of the mentioned metabolites isolated from medicinal plant extracts on meristematic cells of *A. cepa*.

The reducing sugars found in EEBTa are metabolites that, besides constituting energy sources, also act together with the hormone auxin in the signaling pathway that activates cyclins and CDKs specific to the progression of the cell cycle in plants (phases G1, S and G2/M) (Wang & Ruan, 2013). As tannins and saponins, the reducing sugars of EEBTa at the lowest concentration (1.25 mg/mL) may have interfered with the above-mentioned signaling pathway, which may have delayed the cell cycle and consequently reduced the MI in the present study.

With regard to genotoxicity (total mean of chromosome alterations), Cavallo et al. (2010); Bianchi et al. (2015) and Bianchi et al. (2016) have demonstrated that higher concentrations of a given compound may induce less damage to the DNA than lower concentrations, as observed here. For these authors, this observation is due to the increased activity of antioxidant enzymes in response to the high potential of damage induced by a higher concentration. This way, secondary metabolites of EEBTa may have caused oxidative stress in the cells, leading to a significant increase in the activity of antioxidant enzymes in meristematic cells of *A. cepa* at the highest concentrations (5 and 10 mg/mL), resulting in absence of genotoxic effect. In turn, at the lowest concentrations (1.25 and 2.5 mg/mL), the lower stimulation of the antioxidant enzymes allowed genotoxic action of the EEBTa metabolites.

A similar result regarding absence of genotoxicity was found by Pádua et al. (2013) in somatic cells of *Drosophila melanogaster* for the ethanolic extract from bark of this same species at the concentrations of 2.4, 4.8, 9.5 and 19 mg/mL. However, in the present study, the lowest concentrations (1.25 and 2.5 mg/mL) were genotoxic in *A. cepa*. This difference may be associated to the metabolization of the ethanolic extract from *T. actinophylla* carried out by the cytochrome P450, present only in *D. melanogaster*.

When the chromosome alterations (CA) were analyzed isolatedly, the majority of the significant CA were found to occur at the lowest concentrations. The previously mentioned phytochemicals probably caused aneugenic effects (arising from alterations in the spindle fibers) and/or clastogenic action (resulting from chromosome breakages) in *A. cepa* cells; this was also observed, with the same phytochemicals, by Almeida et al. (2016) for the ethanolic extract of *Jatropha gossypiifolia* L. leaves. Furthermore, studies have demonstrated interference of tannins (Khan et al., 2000; Sawadogo et al., 2012), saponins (Li et al., 2010; Bäcker et al., 2016) and reducing sugars (Dutta et al., 2005; Lee & Chan, 2015) with the DNA, leading to its damage. The action of saponins as inhibitors of the complex of DNA topoisomerase I (Bäcker et al., 2016) or topoisomerase II (Li et al., 2010) can be highlighted, promoting breakages and interfering with the polymerization of the spindle fibers in meristematic cells of *A. cepa* (Żabka et al., 2014).

Hence, it is possible that the three mentioned compounds (tannins, saponins and reducing sugars) exercise clastogenic and/or aneugenic activity. The metabolites of EEBTa, in an isolated and/or synergistic manner, probably interfered with the process of spindle fiber assembly, resulting in alterations such as C-metaphases, and caused errors in cytokinesis (polyploid cells). Owing to their greater chromosome content, polyploid cells tend to show a higher contraction of both chromosomes as well as chromatids, resulting in chromosome adherence (Żabka et al., 2014; Bianchi et al., 2015; Bianchi et al., 2016), as observed in this study. Significant occurrence of the three latter CA was observed at the lowest concentrations (1.25, 2.5 and 5 mg/mL), except for polyploid cells at 2.5 mg/mL, which were not significant. Moreover, polyploid cells tend to expel the excess of genetic material in the shape of nuclear buds (Leme & Marin-Morales, 2009), as also verified here.

The occurrence of chromosome loss reinforces the aneugenic action of EEBTa on the spindle fibers at the lowest concentrations (1.25 and 2.5 mg/mL). In turn, chromosome bridges, which arise from the breakage-fusion-bridge cycle (Bianchi et al., 2015; Bianchi et al., 2016), were significant in all tested concentrations, indicating, besides aneugenic activity, also a clastogenic effect of EEBTa. We point out that the reduction in the mean number of

chromosome bridges with increasing concentration evinced greater action of EEBTa at the lowest concentrations. Chromosome fragments may arise from chromosome bridges (Fernandes et al., 2009) and, in the present study, the fragments were significantly more frequent than in NC only at the lowest concentration (1.25 mg/mL), reinforcing the clastogenic action of EEBTa.

Micronuclei (MN) may arise from chromosome loss, breakage and/or nuclear buds (Leme & Marin-Morales, 2009). In this study, the EEBTa induced the formation of MN at all concentrations, and in higher frequency at 1.25 mg/mL. At this (lowest) concentration, the observed MN may have originated from chromosome loss, buds and/or breakages, emphasizing an aneugenic and clastogenic action of the secondary metabolites of EEBTa. In turn, at the highest concentrations MN arose from chromosome loss and/or nuclear buds (2.5 mg/mL) or nuclear buds (5 mg/mL), pointing to a possible aneugenic origin at these levels. At the highest concentration (10 mg/mL), the significant presence of MN may be due to the occurrence of chromosome bridges, evincing a possible clastogenic origin. Moreover, at the highest concentrations, particularly at 10 mg/mL, most of the CA were not significant, probably indicating high activity of antioxidant enzymes in *A. cepa* cells, which reduced the effect of EEBTa metabolites on the fibers of the mitotic spindle. However, they did not hinder the action of these metabolites on the formation of chromosome bridges and MN.

5. Conclusion

The secondary metabolites of EEBTa possibly altered the cell cycle, reducing MI at the lowest concentration (1.25 mg/mL), and acted in an aneugenic and/or clastogenic manner, especially at the lowest concentrations of EEBTa. This way, considering that the extract of *T. actinophylla* is applied empirically in popular medicine, and that the test system using *A. cepa* presents good correlation with the tests performed in mammals (Fedel-Miyasato et al., 2014), we remark that the use of this plant should be made with caution.

Thus, since the compounds of *T. actinophylla* induce DNA damage in *A. cepa* cells, it is recommended that this plant be used with caution for medicinal purposes. However, complementary toxicological tests should be conducted using *in vitro* cell culture and/or *in vivo* tests to detect probable mammalian DNA damage. In addition, the identification of major compounds is recommended to understand the possible mechanisms of action observed in the present work.

Conflict of Interest

The authors declare no conflicts of interest.

References

Abiodun, O., Gbotosho, G., Ajaiyeoba, E., Happi, T., Falade, M., Wittlin, S., & Oduola, A. (2011). *In vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. *Pharmaceutical biology*, 49(1), 9-14.

Abiodun, O. O., Rodríguez-Nogales, A., Algieri, F., Gomez-Caravaca, A. M., Segura-Carretero, A., Utrilla, M. P., & Galvez, J. (2016). Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of *Terminalia catappa* L. (Combretaceae): *in vitro* and *in vivo* evidences. *Journal of ethnopharmacology*, *192*, 309-319.

Agra, M. D. F., Freitas, P. F. D., & Barbosa-Filho, J. M. (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Revista Brasileira de Farmacognosia*, *17*(1), 114-140.

Almeida, P. M., Araújo, S. S., Santos, I. R. M. R., Marin-Morales, M. A., Benko-Iseppon, A. M., Santos, A. V., & Brasileiro-Vidal, A. C. (2016). Genotoxic potential of leaf extracts of *Jatropha gossypiifolia* L. *Genetics and Molecular Research*, *15*(1), 1-8.

Almeida, A. C. A., de-Faria, F. M., Dunder, R. J., Manzo, L. P. B., Souza-Brito, A. R. M., & Luiz-Ferreira, A. (2017). Recent trends in pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease. *Evidence-Based Complementary and Alternative Medicine*, *2017*, ID 8528210.

Atoyebi, S. M., Oyeyemi, I. T., Dauda, B. A., & Bakare, A. A. (2015). Genotoxicity and anti-genotoxicity of aqueous extracts of herbal recipes containing *Luffa cylindrica* (L), *Nymphaea lotus* (L) and *Spondias mombin* (L) using the *Allium cepa* (L) assay. *African Journal of Pharmacy and Pharmacology*, *9*(15), 492-499.

Ayres, M., Ayres, J. R. M., Ayres, D. L., & Santos, A. D. (2007). *BioEstat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas*. Belém, PA, Sociedade Civil Mamirauá

Bäcker, C., Drwal, M. N., Preissner, R., & Lindequist, U. (2016). Inhibition of DNA–Topoisomerase I by Acylated Triterpene Saponins from *Pittosporum angustifolium* Lodd. *Natural products and bioprospecting*, *6*(2), 141-147.

Bianchi, J., Fernandes, T. C. C., & Marin-Morales, M. A. (2016). Induction of mitotic and chromosomal abnormalities on *Allium cepa* cells by pesticides imidacloprid and sulfentrazone and the mixture of them. *Chemosphere*, *144*, 475-483.

Bianchi, J., Mantovani, M. S., & Marin-Morales, M. A. (2015). Analysis of the genotoxic potential of low concentrations of Malathion on the *Allium cepa* cells and rat hepatoma tissue culture. *Journal of Environmental Sciences*, *36*, 102-111.

Cavallo, D., Ursini, C. L., Fresegna, A. M., Ciervo, A., Maiello, R., Rondinone, B., & Iavicoli, S. (2010). Direct-oxidative DNA damage and apoptosis induction in different human respiratory cells exposed to low concentrations of sodium chromate. *Journal of Applied Toxicology: An International Journal*, *30*(3), 218-225.

Das, A., & Kumar, S. M. (2013). Anxiolytic, antidepressant and *in vivo* antioxidant activity of the ethanolic extract of stem bark of *Terminalia tomentosa* Roxb. *Indo American Journal of Pharmaceutical Research*, *3*(10), 8037-8045.

Dutta, U., Cohenford, M. A., & Dain, J. A. (2005). Nonenzymatic glycation of DNA nucleosides with reducing sugars. *Analytical biochemistry*, *345*(2), 171-180.

Farias, R. R. S. (2016). *Estudo Fitogeográfico e Químico-Farmacológico de Três Espécies da Família Combretaceae (Combretum duarteanum Cambess., C. mellifluum Eichler. e Terminalia actinophylla Mart.)*: uma análise comparativa nos Cerrados Setentrional e Meridional do Estado do Piauí. (Tese de doutorado). Universidade Federal do Piauí, PI, Brasil.

Fedel-Miyasato, L. E. S., Formagio, A. S. N., Auharek, S. A., Kassuya, C. A. L., Navarro, S. D., Cunha-Laura, A. L., & Oliveira, R. J. (2014). Antigenotoxic and antimutagenic effects of *Schinus terebinthifolius* Raddi in *Allium cepa* and Swiss mice: a comparative study. *Genetics and Molecular Research*, *13*, 3411-3425.

Fernandes, T. C. C., Mazzeo, D. E. C., & Marin-Morales, M. A. (2009). Origin of nuclear and chromosomal alterations derived from the action of an aneugenic agent—Trifluralin herbicide. *Ecotoxicology and environmental safety*, *72*(6), 1680-1686.

Gupta, A. M. I. T., & Chaphalkar, S. R. (2016). Inhibition of antigen specific T cell population using *Calotropis gigantea* and *Terminalia arjuna*. *Journal of Biology and Nature*, *5*(1), 14-19.

Hemachandra, C. K., & Pathiratne, A. (2016). Combination of physico-chemical analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet assay/nuclear abnormalities tests for cyto-genotoxicity assessments of treated effluents discharged from textile industries. *Ecotoxicology and environmental safety*, *131*, 54-64.

Khan, N. S., Ahmad, A., & Hadi, S. M. (2000). Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. *Chemico-Biological Interactions*, *125*(3), 177-189.

Kich, D. M., Bitencourt, S., Caye, B., Faleiro, D., Alves, C., Silva, J., & Santos, R. C. V. (2017). Lymphocyte genotoxicity and protective effect of *Calyptranthes tricona* (Myrtaceae) against H_2O 2-induced cell death in MCF-7 cells. *Molecular and cellular biochemistry*, *424*(1-2), 35-43.

Konoshima, T. (1996). Anti-tumor-promoting activities of triterpenoid glycosides; cancer chemoprevention by saponins. In R. George, & K. Y. Waller (Eds), *Saponins used in traditional and modern medicine* 87-100. Boston, Springer.

Lee, S. C., & Chan, J. C. (2015). Evidence for DNA damage as a biological link between diabetes and cancer. *Chinese medical journal*, *128*(11), 1543.

Leme, D. M., & Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research*, *682*(1), 71-81.

Li, M., Miao, Z. H., Chen, Z., Chen, Q., Gui, M., Lin, L. P., & Ding, J. (2010). Echinoside A, a new marine-derived anticancer saponin, targets topoisomerase2α by unique interference with its DNA binding and catalytic cycle. *Annals of oncology*, *21*(3), 597-607.

Nepka, C. H., Asprodini, E., & Kouretas, D. (1999). Tannins, xenobiotic metabolism and cancer chemoprevention in experimental animals. *European journal of drug metabolism and pharmacokinetics*, *24*(2), 183-189.

Madrigal, Q. J. (2010) Combretaceae. In: Hammel, B. E., et al. (Eds.). *Manual de Plantas de Costa Rica. Vol. V.* Saint Louis: Monographs in Systematic Botany from the Missouri Botanical Garden, 119, 55–64.

Malini, M., Marin-Morales, M. A., Mantovani, M. S., Jamal, C. M., Nati, N., Passos, T. D. S., & Matsumoto, S. T. (2010). Determination of the antimutagenicity of an aqueous extract of *Rhizophora mangle* L. (Rhizophoraceae), using in vivo and in vitro test systems. *Genetics and molecular biology*, *33*(1), 176-181.

Manosroi, A., Jantrawut, P., Akazawa, H., Akihisa, T., & Manosroi, J. (2010). Biological activities of phenolic compounds isolated from galls of *Terminalia chebula* Retz. (Combretaceae). *Natural product research*, *24*(20), 1915-1926.

Marquete, N., & Loiola, M. I. B. (2015) Combretaceae. In: *Lista de Espécies da Flora do Brasil*, Jardim Botânico do Rio de Janeiro. Recuperado de http://floradobrasil.jbrj.gov .br/jabot/floradobrasil/FB16895

Mendonça, E. D., da Silva, J., Dos Santos, M. S., Carvalho, P., Papke, D. K. M., Ortmann, C. F., & Ferraz, A. D. B. F. (2016). Genotoxic, mutagenic and antigenotoxic effects of *Cecropia pachystachya* Trécul aqueous extract using *in vivo* and *in vitro* assays. *Journal of ethnopharmacology*, *193*, 214-220.

Nishanth, R. P., Prasad, T., Jyotsna, R. G., Reddy, P. K., & Reddanna, P. (2014). Hepatoprotective effects of *Terminalia chebula* fruit extract against 2-AAF–induced hepatic damage in Albino mice: role of MDR1 and COX-2. *Journal of herbs, spices & medicinal plants*, *20*(4), 402-420.

Pádua, P. F. M. R., Dihl, R. R., Lehmann, M., de Abreu, B. R. R., Richter, M. F., & de Andrade, H. H. R. (2013). Genotoxic, antigenotoxic and phytochemical assessment of *Terminalia actinophylla* ethanolic extract. *Food and chemical toxicology*, *62*, 521-527.

Pereira, M. L., Monteiro, C. N., Siqueira, C. F. N., Ribeiro, M. S., Lopes, A. P., Sousa, R. M. S., ... & Almeida, P. M. (2020). Evaluation of effects of *Poincianella bracteosa* (Tul.) LP Queiroz leaves in *Allium cepa* and *Mus musculus*. *Biotechnic & Histochemistry*, 1-10.

Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. (2018). *Metodologia da pesquisa científica*. Santa Maria, RS, UFSM, NTE.

Pfundstein, B., El Desouky, S. K., Hull, W. E., Haubner, R., Erben, G., & Owen, R. W. (2010). Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida*): characterization, quantitation and determination of antioxidant capacities. *Phytochemistry*, *71*(10), 1132-1148.

Ramachandran, S., Rajasekaran, A., & Manisenthilkumar, K. T. (2012). Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. *Asian Pacific journal of tropical biomedicine*, *2*(4), 262.

Rayan, P., Matthews, B., McDonnell, P. A., & Cock, I. E. (2015). *Terminalia ferdinandiana* extracts as inhibitors of *Giardia duodenalis* proliferation: a new treatment for giardiasis. *Parasitology research*, *114*(7), 2611-2620.

Roberto, M. M., Jamal, C. M., Malaspina, O., & Marin-Morales, M. A. (2016). Antigenotoxicity and antimutagenicity of ethanolic extracts of Brazilian green propolis and its main botanical source determined by the *Allium cepa* test system. *Genetics and molecular biology*, *39*(2), 257-269.

Sakagami, H., Kushida, T., Makino, T., Hatano, T., Shirataki, Y., Matsuta, T., & Mimaki, Y. (2012). Functional analysis of natural polyphenols and saponins as alternative medicines. *A Compendium of Essays on Alternative Therapy*, 269.

Sarimahmut, M., Balikci, N., Celikler, S., Ari, F., Ulukaya, E., Guleryuz, G., & Ozel, M. Z. (2016). Evaluation of genotoxic and apoptotic potential of *Hypericum adenotrichum* Spach. in vitro. *Regulatory Toxicology and Pharmacology*, *74*, 137-146.

Sawadogo, W. R., Schumacher, M., Teiten, M. H., Dicato, M., & Diederich, M. (2012). Traditional West African pharmacopeia, plants and derived compounds for cancer therapy. *Biochemical pharmacology*, *84*(10), 1225-1240.

Santos, D. L., da Silva, G. D. N. F., Moraes, J. S., da Souza, K. O., da Silva Rodrigues, E. M., Fecury, A. A., & de Araújo, M. H. M. (2020). Traditional herbal medicine in a community in northeastern Pará: the use of *Eleutherine plicata* Herb. in the treatment of Amebiasis. *Research, Society and Development*, *9*(7), 620974539.

Shukla, S. K., Sharma, S. B., Singh, U. R., Ahmad, S., & Dwivedi, S. (2015). *Terminalia arjuna* (Roxb.) Wight & Arn. augments cardioprotection via antioxidant and antiapoptotic cascade in isoproterenol induced cardiotoxicity in rats. *Indian Journal of Experimental Biology*, *53*, 810-818.

Silva, J. N. (2015). *Atividade Antioxidante e Citotóxica de Extratos de Plantas do Semiárido Brasileiro com Potencial para Desenvolvimento de Fitoterápicos*. (Dissertação de mestrado). Centro de Ciências da Saúde, Universidade Federal do Piauí, PI, Brasil

Sponchiado, G., Adam, M. L., Silva, C. D., Soley, B. S., de Mello-Sampayo, C., Cabrini, D. A., & Otuki, M. F. (2016). Quantitative genotoxicity assays for analysis of medicinal plants: A systematic review. *Journal of ethnopharmacology*, *178*, 289-296.

Tiwary, B. K., Bihani, S., Kumar, A., Chakraborty, R., & Ghosh, R. (2015). The *in vitro* cytotoxic activity of ethno-pharmacological important plants of Darjeeling district of West Bengal against different human cancer cell lines. *BMC complementary and alternative medicine*, *15*(1), 22.

Wang, L., & Ruan, Y. L. (2013). Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in plant science*, *4*, 163.

Yıldırım, I., & Kutlu, T. (2015). Anticancer agents: saponin and tannin. *International Journal of Biological Chemistry*, *9*, 332-340.

Żabka, A., Polit, J. T., Bernasińska, J., & Maszewski, J. (2014). DNA topoisomerase II-dependent control of the cell cycle progression in root meristems of *Allium cepa*. *Cell biology international*, *38*(3), 355-367.

Percentage of contribution of each author in the manuscript

Francisca Mairana Silva de Sousa – 20%

José Rafael da Silva Araujo – 20%

Luciana Maria Fortes Magalhães Castelo Branco Couto – 10%

Regina Maria Silva Sousa – 10%

Maria das Dores Alves Oliveira – 10%

Joaquim Soares da Costa Júnior – 10%

Francielle Aline Martins – 10%

Pedro Marcos de Almeida – 10%