UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e73445, 2024 • https://doi.org/10.5902/2179460X73445
Submitted: 02/01/2023 • Approved: 29/02/2024 • Published: 23/08/2024

Chemistry

Genotoxic and antiproliferative effects of *Alpinia zerumbet* (Zingiberaceae) essential oil in *Allium cepa* biotest

Efeito genotóxico e antiproliferativo do óleo essencial de *Alpinia zerumbet* (Zingiberaceae), em bioteste de *Allium cepa*

Márcia Aldeany Almeida de Sousa[I], **Fernando Souza Lima Silva**[I], **José Fábio França Orlanda**[I]

[I]Maranhão Tocantina Region State University, Imperatriz, MA, Brazil

ABSTRACT

The species Alpinia zerumbet, popularly known as colony, is quite abundant in northeastern Brazil and is widely used for medicinal purposes due to its hypotensive and cardiovascular effects, without, however, due scientific evidence. Therefore, the objective of this study was to evaluate the genotoxic and antiproliferative effects of A. zerumbet essential oil in test systems with Allium cepa. The chemical composition of the essential oil was determined by gas chromatography coupled with mass spectrometry (GC-MS). The antiproliferative and genotoxic effects were tested in seven treatments with three replications and five concentrations of essential oil (0.01, 0.05, 0,1, 0.5, and 1.0%) using onion bulbs. Two roots were analyzed from each bulb, with a count of 500 cells per slide/root, totaling 3,000 cells per treatment. The results showed that the main chemical constituents of the essential oil were 1.8 cineole (60.50%) and terpinen-4-ol (23.80%). In the assays with A. cepa, both the mitotic index (MI) and the cellular alteration percentages differed significantly in relation to the control, through the appearance of chromosomal and nuclear aberrations at the concentrations tested, revealing possible harmful effects on human health.

Keywords: *Alpinia zerumbet*; *Allium cepa*; Test system

RESUMO

A espécie *Alpinia zerumbet* popularmente conhecida como colônia é bastante abundante no nordeste brasileiro, largamente utilizada para fins medicinais, devido seu efeito hipotensor e cardiovascular, sem as devidas comprovações científicas. Com isso, o objetivo deste estudo foi avaliar o efeito genotóxico e antiproliferativo do óleo essencial de *A. zerumbet*, em sistemas testes com *Allium cepa*. A composição química do óleo essencial foi determinada por cromatografia gasosa acoplada à espectrometria de massas (CG-MS). O efeito antiproliferativo e genotóxico foi testado em sete tratamentos com três repetições, e cinco concentrações do óleo essencial (0.01, 0.05, 0,1, 0.5 and 1.0%) utilizando bulbos de cebola. De cada bulbo foram analisadas duas raízes, com contagem de 500 células por lâmina/raiz, totalizando 3.000 células por tratamento. Os resultados mostraram que os principais constituintes químicos do óleo essencial foram 1,8 cineol (60,50%)

e terpinen-4-ol (23,80%). Nos ensaios com *A. cepa*, tanto índice mitótico (IM) quanto as porcentagens de alterações celulares diferiram significativamente em relação ao controle, por meio do aparecimento de aberrações cromossômicas e nucleares nas concentrações testadas, revelando possíveis efeitos nocivos à saúde humana.

Palavras-chave: *Alpinia zerumbet*; *Allium cepa*; Sistema teste

1 INTRODUCTION

The botanical species *Alpinia zerumbet* (Pers.) Burtt & Smith, native to tropical regions of South and Southeast Asia, popularly known as colony, helicondia, jardineira, alpinia and shell ginger, belongs to the Zingiberaceae family. It is an aromatic herb with 2 to 3 meters in height, light green leaves, grouped in clumps, slightly aromatic flowers, arranged in the form of large yellow-pink clusters and is widely used for therapeutic purposes (Kawai et al., 2021; Cruz et al., 2020).

In folk medicine, it is used for respiratory disorders, expectoration, headache, fever, relaxation, rheumatism and as diuretic and vermifuge (Azevedo, Lins, 2020). *In vivo* and *in vitro* studies have demonstrated the therapeutic properties with antimicrobial, antinociceptive, antioxidant, antihypertensive, antispasmodic, anti-inflammatory, hypotensive, diuretic, sedative and cardiovascular activity (Nag et al., 2019; Xiao et al., 2018; Kerdudo et al., 2017).

The pharmacological use of *A. zerumbet* is attributed to its active principles, among which the essential oil (EO) commonly extracted from leaves, rhizomes, stems and flowers stands out. EO are mixtures of lipophilic and volatile substances, produced by aromatic plants as a result of their secondary metabolism, with wide application in the food, beverage, personal care products, cosmetics, perfumes, cleaning products and pharmaceutical compositions industries as a source of new bioactives molecules (Almeida, Almeida, Gherardi, 2020; Nascimento, Prade, 2019).

Chemical compounds present in EO have diverse biological activities, including: analgesic, antiviral, antispasmodic, anti-inflammatory, antioxidant, antimicrobial, healing, insecticidal, larvicide, vermifuge and, among others (Jahanafrooz et al., 2024;

Selles et al., 2024; Preti et al., 2018; Orlanda, Nascimento, 2015). However, it is necessary to carry out studies to prove its effectiveness and safety, regarding the toxic effects of substances harmful to human health (Viana et al., 2023; Viana et al., 2022).

The evaluation of cytotoxic and genotoxic effects is essential to understand the possible mechanisms of action, damage or mutations to nuclear DNA, in order to develop new safe and effective drugs (Kim et al., 2019; Ozaslan, Oguzkan, 2018; Turkez, Arslan, Ozdemir, 2017).

Bioassay with the *Allium cepa* plant test system as a bioindicator has shown good results to evaluate the cytotoxic, genotoxic and mutagenic effects of several medicinal plants. The in vivo assay employs the growth of *A. cepa* roots in direct contact with the substance of interest, allowing the prediction of possible damage to the cell's DNA (Fernandes et al., 2022; Parvan et al., 2020; Frota et al., 2019; Pastori et al., 2015).

The test is sensitive to detect numerous substances that cause chromosomal alterations, due to the kinetics of proliferation, rapid root growth, large number of dividing cells, excellent sensitivity, low cost, ease of handling and good correlation with mammalian cells (Cuchiara, Borges, Dobrows, 2012).

Thus, the present study aims to evaluate the genotoxic and antiproliferative effect of the essential oil *Alpinia zerumbet* in test systems with *Allium cepa*.

2 METHODOLOGY

2.1 Plant material

Leaves of *Alpinia zerumbet* were collected during the flowering of the plant, in the municipality of Senador La Roque, Maranhão, Brazil (5°26'34"S 47°17'27"O), in June 2021. A voucher specimen is kept at the UEMASUL Herbarium, accession number RB 433485.

2.2 Essential oil extraction

The essential oil was extracted from fresh plant material, subjected to hydrodistillation in a clevenger apparatus for 4 h, according to the methodology described by Orlanda and Nascimento (2015). The extraction yield was calculated in the mass/mass ratio by measuring the density, observing the volume obtained in the extraction system itself, and expressed in percentage (% v/p).

2.3 Chemical characterization of essential oil

Essential oil analysis was performed by gas chromatography coupled to mass spectrometry (GC/MS) in a Shimadzu QP-5000 GC system, equipped with a Shimadzu QP-5000 GC selective mass detector. Analysis parameters: 0.3 µL sample; split inlet 1:10; carrier gas: He (1 mL min^{-1}); HP5-MS fused silica capillary column (5% diphenyl and 95% dimethyl polysiloxane, 30 m x 0.25 mm, film thickness: 0.25 µm); analysis program: 40°C (Ti) for 5 min, 40-260°C, 4°C min^{-1} and 240°C isotherm for 7.5 min; injector and detector temperature: 280ºC; interface temperature: 250°C; ionization energy: 70 eV; database: NIST, 1998. The essential oil components were identified based on the retention index (RI), determined by using calibration curve of a homologous series of n-alkanes (C_8-C_{32}) injected into them. The chromatographic conditions of the samples and in the fragmentation models of the mass spectra were both compared with data from the literature. Essential oil components were identified by comparison of the mass spectra obtained with the equipment database using the NIST library.

2.4 Genotoxic and antiproliferative effect

Allium cepa (onion) bulbs were placed for rooting in glass pots (ø = 5 cm, height = 10 cm) filled with distilled water. The pots with the bulbs were kept in dark conditions at room temperature of 25 ± 1°C. When the adventitious roots reached a length of 2 to 3 cm were treated with *A. zerumbet* essential oil at concentrations of 0.01, 0.05, 0,1, 0.5 and 1.0% for 24 h. In the tests, the solvent Triton X-100 was used as a negative control

and 15% glyphosate was used as a positive control.

The treated roots were fixed in modified Carnoy's solution (ethanol:acetic acid – 3:1, v/v) for 24 h. Fixed roots were hydrolyzed with 1 mol L^{-1} HCl for 5 minutes to soften the tissues. Root tissue was stained with 2% acetic orcein for 30 minutes after washing with distilled water (Pastori et al., 2015).

Squash technique was used to prepare blades according to Guerra and Souza (2002), Armbruster et al. (1991), with modifications. For each treatment, three onion bulbs served as replicas.

Cytotoxic potential of the essential oil was studied by determining the mitotic index – MI (Equation 1) and percentage of occurrence of cellular changes (Equation 2). The analysis was performed using the Carl Zeiss Software Zen 2012 microscope connected to the ICC1 Camera (40-100X objective), by observing 500 cells per meristem root and F1 region of the bulb, totaling 3.000 cells analyzed in each treatment.

$$Mitotic\ index\ (\%) = \frac{\text{Number of dividing cells}}{\text{Total cells}}\ x\ 100 \quad (1)$$

$$Altered\ Cells\ (\%) = \frac{\text{Number of cells with cellular alterations}}{\text{Total dividing cells}}\ x\ 100 \quad (2)$$

2.5 Statistical analysis

A completely randomized design adopted. Data were submitted to analysis of variance (ANOVA) followed by the Tukey test ($p \leq 0.05$). Data were analyzed using the SisVar 5.6 software (Ferreira, 2011).

3 RESULTS AND DISCUSSION

3.1 Chemical composition

The essential oil from *A. zerumbet* leaves showed a yield of 0.22%, 0.92 ± 0.01 g mL^{-1} density at 25°C, a mild odor and a light yellow appearance. A similar result was obtained by Canuto et al. (2015) who showed a yield of 0.23%.

In total, nine chemical constituents were identified by GC-MS, terpenic in nature, containing mono and sesquiterpenes (Table 1). The major compounds found were 1,8 cineole (60.50%) and terpinen-4-ol (23.80%).

Table 1 – Chemical composition of *A. zerumbet* essential oil

N°	Component	RI (minutes)[a]	Percentage[b]	Molecular Formule
1	β-Pinene	17.33	5.12	$C_{10}H_{16}$
2	α Phellandrene	18.03	3.27	$C_{10}H_{16}$
3	1,3 Clycohexadiene, 1-methyl-4 – (1-methylethyl)	18.41	1.35	$C_{10}H_{16}$
4	O – Cymene	18.67	1.67	$C_{10}H_{14}$
5	d – Limonene	18.86	0.90	$C_{10}H_{16}$
6	Eucalyptol (1,8 cineole)	19.01	60.50	$C_{10}H_{18}O$
7	β-Linalool	21.26	1.10	$C_{10}H_{18}O$
8	Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl) – , (1α,2β,5α)-	22.37	2.29	$C_{10}H_{18}O$
9	Terpinen-4-ol	23.82	23.80	$C_{10}H_{18}O$

Source: Authors (2021)

Note: [a]Retention indices (RI); [b]% GC peak

The main chemical components of *A. zerumbet* essential oil most cited in the literature are two oxygenated monoterpenes, terpinen-4-ol and 1,8-cineole (Souza et al., 2018; Jezler et al., 2013; Santos et al., 2012; Rezende et al., 2011; Barcelos et al., 2010). Both compounds were found to be present in this study.

The volatile substances present in *A. zerumbet* essential oil may have an important influence on its biological activity. Terpinen-4-ol showed cardiac arrhythmogenic

activity and may have an influence on hypotensive effects (Gondim et al., 2017). In addition, terpinen-4-ol and 1,8-cineole have antimicrobial activity (Matasyoh et al., 2007; Janssen & Scheffer, 1985).

3.2 Antiproliferative effect

The evaluation of the antiproliferation effect of EO allows evaluating the action of chemical compounds on cell growth (Mohanty et al., 2023; Silva et al., 2019). Effect of *A. zerumbet* essential oil on the mitotic index (MI) differed significantly between treatments (Table 2).

Table 2 – Effect of *A. zerumbet* essential oil on the mitotic index of *A. cepa*

Treatments		CN	CP	Concentrations (%)				
				0.01	0.05	0.1	0.5	1.0
Cells	Interphase	2742	2977	2918	2944	2944	2961	2834
	Prophase	150	20	65	50	48	30	85
	Metaphase	8	0	7	3	0	0	2
	Anaphase	37	2	4	1	0	0	1
	Telophase	63	1	6	2	8	9	78
Total cells		3000	3000	3000	3000	3000	3000	3000
Mitotic index (%)		8.6a	0.77c	2.73bc	1.87c	1.87c	1.3c	5.53ab

Source: Authors (2021)

Note: CN = Negative control; CP = Positive control

In general, the IM of *A. cepa* cells showed significantly lower values compared to the negative control (CN = 8.6%). These results demonstrated that the essential oil reduced the cell division rate of *A. cepa* cells. Several studies have shown that essential oils reduce the mitotic index in *A. cepa* (ISSA et al., 2020; SHARMA et al., 2019; UBESSI et al., 2019). Issa et al. (2020) found that increasing concentrations of essential oil from *Vitex negundo* leaves (0.01 – 0.10 mg mL^{-1}) decreased IM in *A. cepa*. Sharma et al. (2019) observed that the volatile oil of *Hyptis suaveolens* (0.05 – 2.5 mg mL^{-1}) reduced the cell

division rate of *A. cepa* cells by up to 63.0% for the highest concentration tested.

The 1.0% essential oil concentration the highest MI value (5.53%) and did not present significant differences from the negative control, followed by the lowest concentration tested 0.01%. Intermediate essential oil concentrations (0.05, 0.1 and 0.1%) showed lower MI and did not differ from the positive control (CP = 0.77%). These results disagree with most of the studies cited with essential oil, where normally the highest concentrations are those with the lowest MI and generally the rate of cell division decreases with increasing concentration (Issa et al., 2020; Sharma et al., 2019). However, (it is possible to find) there are to find studies in the literature with similar results (Mohammed et al., 2015; Pastori et al., 2015; Chukwujekwu, Van Staden, 2014; Dias et al., 2014). Dias et al. (2014) observed that with increasing concentrations of *Mikania cordifolia* extract, the IM of *A. cepa* also increased. Mohammed et al. (2015) observed that a compound isolated from *Coleus forslohlii*, forskolin, had a higher MI in *A. cepa* at the highest concentrations tested. Pastori et al. (2015) found that the infusion of *Polygonum punctatum* had the highest MI for the highest concentration tested.

The reduction in the mitotic index indicates antiproliferative or cytotoxic activity of the essential oil (Issa et al., 2020; Sharma et al., 2019; Prajitha, Thoppil, 2016; Pastori et al., 2015; Chukwujekwu, Van Staden, 2014). According to Hoshina (2002), a decrease in the mitotic index (MI) is indicative of cytotoxicity of the substance, whereas an increase indicates that there was an induction of cell division, which can lead to the appearance of tumors in living beings. The antiproliferative effect of the essential oil demonstrated by the reduction in the mitotic index in *A. cepa* cells may occur due to changes in the cell cycle, such as blocking DNA synthesis or reducing the rate of cytokinesis (Issa et al., 2020; Liman et al., 2020; Prajitha, Thoppil, 2016; Mohammed et al., 2015).

All the tested concentrations of *A. zerumbet* EO showed lower MI in relation to the negative control, which may indicate possible cytotoxicity of the essential oil. Some studies carried out with *A. zerumbet* confirm that its essential oil has antiproliferative

activities. Castro et al. (2016) observed that *A. zerumbe*t essential oil at a concentration of 100 mg mL^{-1} completely inhibited the bacterial growth of *Staphylococcus aureus*. Victório et al. (2009) also verified antibacterial activity against *A. zerumbet*.

However, some studies carried out with EO of *A. zerumbet* obtained discordant results. Cavalcanti et al. (2012) found that concentrations of 500 µg mL^{-1} showed antiproliferative effects and DNA damage, while concentrations of 50 – 300 µg mL^{-1} did not induce genotoxicity in human leukocytes. Roman Junior *et al.* (2017) observed that compounds isolated from the extract of *A. zerumbet* leaves have antiproliferative activity against some lines of breast cancer and leukemia cells.

For Itoyama et al. (1997) the high concentration of some chemical compounds may have an inhibitory or stimulating effect on the cell cycle, as has happened with caffeine in *Drosophila prosaltans*. In addition to concentration, the chemical composition of the essential oil also has a strong influence on the biological activity of essential oils (Sousa et al., 2020; Orlanda, Nascimento, 2015; Bakkali et al., 2008).

3.3 Genotoxic effect

Micronucleus test in *A. cepa* has been considered an efficient system to evaluate the genotoxic effects promoted by changes in genetic material, due to exposure to various substances, based on the number of cells carrying chromosomal and nuclear aberrations (Schreiner et al, 2024; Freitas, Uchôa, Magalhães, 2020; Pathiratne, Hemachandra, 2015).

The effect of *A. zerumbet* essential oil on the change rate showed a significant difference between treatments (Table 3). The positive control (CP = 8.7%) showed the highest cell alteration date. The essential oil caused an increase in the cellular change rate in the root cells of *A. cepa*, when compared to its negative control (CN) which did not show any change in cell division, despite having the highest MI. These data corroborate several studies that confirm that essential oils have genotoxic activity (Issa

et al., 2020; Sharma et al., 2019).

Table 3 – Effect of *A. zerumbet* essential oil on the cellular alterations of *A. cepa*

Treatments	CN	CP	Concentrations (%)				
			0.01	0.05	0.1	0.5	1.0
Number of dividing cells	258	23	82	56	56	39	166
Disorganized cell division	-	2	2	2	-	-	10
Microkernel	-	-	-	1	2	-	-
Anaphase bridge with chromosome break	-	-	1	-	-	-	-
Total changes	0	2	3	3	2	0	10
Cellular change (%)	0e	8.7a	3.66d	5.36c	3.57d	0e	6b

Source: Authors (2021)

Note: CN = Negative control; CP = Positive control

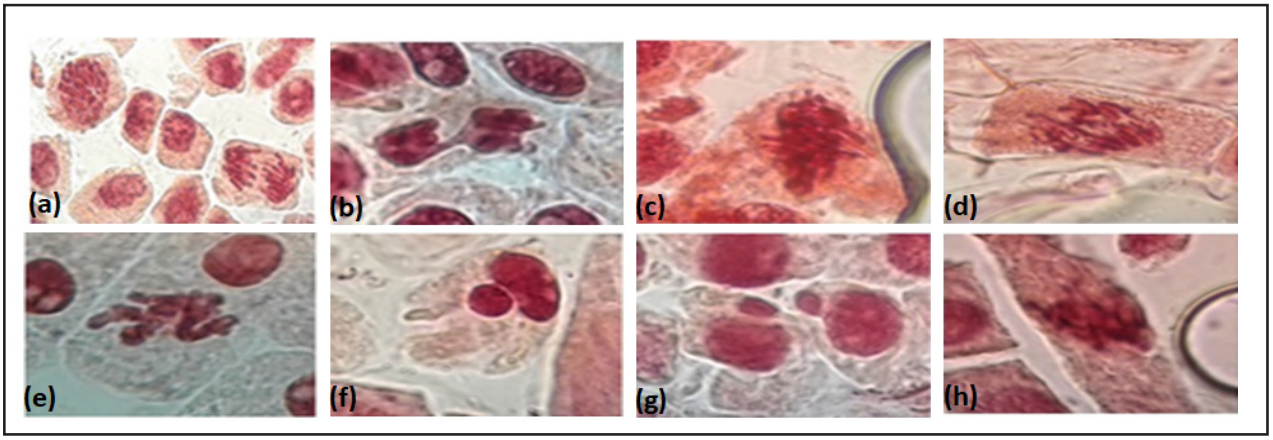
The changes that occurred in the positive control were expected, as several studies have already shown that glyphosate is capable of inhibiting cell division and inducing chromosomal changes in *A. cepa* meristematic cells (Mercado, Caleño, 2019; Pastori et al., 2015; Souza et al., 2010), such as breaks, chromosome bridges, and micronuclei (Mercado, Caleño, 2019)

Several studies have been carried out on the genotoxic effects on *A. cepa*, but most are with plant extracts and infusions. Pastori et al. (2015) found that the infusion of *Polygonum punctatum* with a lower concentration had a higher percentage of cellular changes. A similar result was found in Table 3, where the 0.05% concentration had a higher cellular change rate than those of 0.1 and 0.5%.

The main cellular alterations found in dividing cells were disorganized cell division (CP, 0.01, 0.05 and 1.0%), micronucleus (0.05 and 0.1%) and anaphase bridge

with chromosomal breakage (0.05%) according to Table 3 and Figure 1.

Figure 1 – Cellular changes in *A. cepa* bulbs treated with *A. zerumbet* essential oil. (a) cells in normal prophase and anaphase (CN); (b) disorganized division (CP); (c) disorganized cell division (0.01%); (d) anaphase bridge with chromosomal breakage (0.01%), (e) disorganized cell division (0.05%); (f) micronuclei (0.05%); (g) micronuclei (0.1%); (h) disorganized cell division (1.0%)



These changes are commonly found in *A. cepa* cells subjected to treatments with essential oils (Issa et al., 2020; Sharma et al., 2019) or extracts (Akwu et al., 2019; Prajitha, Thoppil, 2016).

However, some studies with the same species have reported that the volatile oil does not have genotoxic and mutagenic capacity (Cavalcanti et al., 2012). Cavalcanti et al. (2012) found that the *A. zerumbet* essential oil only has genotoxic effects at concentrations above 500 μg mL^{-1}, and that concentrations of 50 – 500 μg mL^{-1} have an antioxidant protective effect caused by H_2O_2.

Anaphase bridges are likely formed during chromatid organization the spindle, caused by rupture, fusion, uneven exchange of chromatid translocation (Liman et al., 2020; Chukwujekwu, Van Staden, 2014).

The genotoxic effect of *A. zerumbet* essential oil, as well as the antiproliferative, may be due to changes in the cell cycle, such as inhibition of DNA synthesis or mitotic

spindle, in addition to the generation of reactive oxygen species caused by treatment with the essential oil (Issa et al., 2020).

The indices of cellular alterations found indicated a possible genotoxic activity of the essential oil in a test assay with *A. cepa*, at least at the concentrations tested. Thus, the cellular alterations found in this study may be due to both the concentration and the chemical constitution of the essential oil.

4 CONCLUSIONS

This study demonstrated that *A. zerumbet* essential oil has antiproliferative activities in *A. cepa*, which makes it suitable for future studies in tumor cells, for example. However, due to its genotoxic activity, it can also affect non-target human cells. More studies are needed on the genotoxic and antiproliferative potential of *A. zerumbet* in other tests, as well as evaluation of the quantitative chemical composition of the essential oil and its individual constituents.

ACKNOWLEDGEMENTS

The authors thank the Foundation for Support to Scientific and Technological Research and Development of Maranhão – FAPEMA.

REFERENCES

Akwu, N. A, Naidoo, Y, & Singh, M. (2019). Cytogenotoxic and biological evaluation of the aqueous extracts of *Grewia lasiocarpa*: an *Allium cepa* assay. *S. Afr. J. Bot.*, 125, 371-380.

Almeida, J. C. de, Almeida, P. P. de, & Gherardi, S. R. M. (2020). Potencial antimicrobiano de óleos essenciais: uma revisão de literatura de 2005 a 2018. *NRE*, 17(2), 8623-8633.

Armbruster, B. L, Molin, W. T, & Bugg, M. W. (1991). Effects of the herbicide dithiopyr on cell division in wheat root tips. *Pestic. Biochem. Physiol.*, 39, 110-120, 1991.

Azevedo, M. V. M. P. da, & Lins, S. R. O. (2020). Aplicações terapêuticas da *Alpinia zerumbet* (colônia) baseado na medicina tradicional: uma revisão narrativa (2010-2020). *BJD*, 6(11), 84222-84242.

Bakkali, F, Averbeck, R, & Idaomar, M. (2008). Biological effects of essential oils – a review. *Food Chem. Toxicol.*, 46, 446-475.

Barcelos, F. F, Oliveira, M. L, Giovaninni, N. P. B, Lins, T. P, Filomeno, C. A, Schneider, S. Z, Pinto, V. D, Endringer, D. C, & Andrade, T. U. (2010). Estudo químico e da atividade biológica cardiovascular do óleo essencial de folhas de *Alpinia zerumbet* (Pers.) B.L.Burtt & R.M.Sm. em ratos. *Rev Bras Plantas Med.*, 12, 48-56.

Canuto, K. M, Pereira, R. de C. A, Rodrigues, T. H. S, Brito, E. S. de, Lima, Y. C. de, & Pimentel, F. A. (2015). Influência do horário de colheita das folhas na composição química do óleo essencial de colônia (*Alpinia zerumbet*). *Bol. Pesqui. Desenvolv.*, 102, 1-16.

Castro, K. N. de C, Lima, D. F, Vasconcelos, L. C, Santos, R. C, Pereira, A. M. L, Fogaça, F. H. dos S, Canuto, K. M, Brito, E. S. de, & Calvet, R. M. (2016). Composição química e eficácia do óleo essencial e do extrato etanólico de *Alpinia zerumbet* sobre *Staphylococcus aureus*. *Arq. Inst. Biol.*, 83, 1-7.

Cavalcanti, B. C, Ferreira, J. R. O, Cabral. I. O, Magalhães, H. I. F. (2012). Genetic toxicology evaluation of essential oil of *Alpinia zerumbet* and its chemoprotective effects against H_2O_2-induced DNA damage in cultured human leukocytes. *Food Chem. Toxicol.*, 50, 4051-4061.

Chukwujekwu, J. C, & Staden, J. V. (2014). Cytotoxic and genotoxic effects of water extract of *Distephanus angulifolius* on *Allium cepa* Linn. *S Afr J Bot*, 92, 147-150.

Cruz, J. D. da, Mpalantinos, M. A, Ramos, A. de S, Ferreira, J. L. P, Oliveira, A. A. de, Netto Júnior, N. L, Silva, J. R. de A, & Amaral, A. C. F. (2020). Chemical standardization, antioxidant activity and phenolic contents of cultivated *Alpinia zerumbet* preparations. *Ind Crops Prod*, 151, 1-9.

Cuchiara, C. C, Borges, C. S, & Bobrowski, V. L. (2012). Sistema teste de *Allium cepa* como bioindicador da citogenotoxicidade de cursos d'água. *Cienc. Tecnol. Agropecuaria*, 6(1), 33-38.

Dias, M. G, Canto-Dorow, T. S, Coelho, A. P. D, & Tedesco, S. B. (2014). Efeito genotóxico e antiproliferativo de *Mikania cordifolia* (L. F.) Willd. (Asteraceae) sobre o ciclo celular de *Allium cepa* L. *Rev Bras Plantas Med*, 16, 202-208.

Fernandes, V. M. P, Rocha, A. R. F. da S, Silva, V. E. S. da, Vale Júnior, E. P. do, Moreira, V. de A, Martins, F. A, & Lago, E. C. (2022). Potencial citotóxico, genotóxico e mutagênico de frações do extrato de Cróton L. (Euphorbiaceae). *Res., Soc. Dev.*, 11(4), 1-11.

Ferreira, D. F. (2011). Sivar: a computer statistical system. *Ciênc. Agrotec.*, 35(6), 1039-1042.

Freitas, F. A. P. S, Uchôa, I. S, & Magalhães, M.D.A.V. (2020). Importance of micro-nucleus testing as a preventive instrument in general and environmental. *Braz. J. Dev.*, 6(9), 68530-68542.

Frota, R. G, Amorim, Á. da S, Carneiro, J. K. R, & Oliveira, M. A. S. (2019). Citotoxicidade, genotoxicidade e mutagenicidade da infusão de *Plectranthus barbatus* – Lamiaceae (malva-santa) avaliada pelo sistema teste *Allium cepa*. *Rev. Ciênc. Méd. Biol.*, 18(1), 67-72.

Gondim, A. N. S, Lara, A, Santos-Miranda, A, Roman-Campos, D, Launton-Santos, S, Menezes-Filho, J. E. R, Vasconcelos, C. M. L. de, Conde-Garcia, E. A, Guatimosim, S, & Cruz, J. S. (2017). (-)-Terpinen-4-ol changes intracellular Ca^{2+} handling and induces pacing disturbance in rat hearts. *Eur. J. Pharmacol.*, 807, 56-63.

Guerra, M, Souza, M. J. de. (2002). *Como observar cromossomos*: um guia de técnicas em citgenética vegetal, animal e humana. Ribeirão Preto: Fundação de Pesquisas Científicas de Ribeirão Preto.

Hoshina, M. M. (2002). Avaliação da possível contaminação das águas do Ribeirão Claro – município de Rio Claro, pertencente à Bacia do rio Corumbataí, por meio de testes de mutagenicidade em *Allium cepa*. 2002. 90 p. [Trabalho de conclusão de curso, Instituto de Biociências da Universidade Estadual Paulista "Júlio Mesquita Filho"].

Itoyama, M. M, Bicudo, H. E. M. C, & Cordeiro, J. A. (1997). Effects of caffeine on mitotic index in *Drosophila prosaltans* (Diptera). *Brazil J Genet.*, 20(4), 655-657.

Issa, M, Chandel, S, Singh, H. P, Batish, D. R, Kohli, R. K, Yadav, S. S, & Kumari, A. (2020). Appraisal of phytotoxic, cytotoxic and genotoxic potential of essential oil of a medicinal plant *Vitex negundo*. *Ind Crops Prod*, 145, 1-8.

Janssen, A. M, & Scheffer, J. J. (1985). Acetoxychavicol acetate, an antifungal component of *Alpinia galanga*. *Planta Med.*, 51(6), 507-511.

Jahanafrooz, Z, Mousavi, M. M, Akbarzadeh, S, Hemmatzadeh, M, Maggi, F, & Morshedloo, M. R. (2024). Anti-breast cancer activity of the essential oil from grapefruit mint (*Mentha suaveolens* × piperita). FITOTERAPIA, 174(2), 1-10.

Jezler, C.N, Batista, R. S, Alves, P. B, Silva, D. da C, Costa, L. C. do B. (2013). Histochemistry, content and chemical composition of essential oil in different organs of *Alpinia zerumbet*. *Cienc. Rural*, 43(10), 1811-1816.

Kim, N. S, Shin, S, Shin, G, & Bang, O. (2019). Genotoxicity evaluation of a *Phragmitis rhizoma* extract using a standard battery of *in vitro* and *in vivo* assays. *J. Ethnopharmacol.*, 241, 1-28.

Kawai, H, Kuraya, E, Touyama, A, Higa, O, Hokamoto, K, Tokeshi, K, Yasuda, A, Naragaki, T, & Itoh, S. (2021). Improved yield and antioxidant activity ofessential oil from *Alpinia zerumbet* (Zingiberaceae) leaves by underwater shockwave pretreatment. *Food Bioprod. Process.*, 25, 134-140.

Kerdudo, A, Ellong, E. N, Burger, P, Gonnot, V, Boyer, L, Chandre, F, Adenet, S, Rochefort, K, Michel, T, & Fernandez, X. (2017). Chemical composition, antimicrobial and insecticidal activities of flowers essential oils of *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. from Martinique Island. *Chem. Biodiversity*, 14(4), 1-26.

Liman, R, Kursunlu, A. N, Ciğerci, I. H, Ozmen, M, & Acikbas, Y. (2020). Assessment of the cytotoxic and genotoxic potential of pillar[5]arene derivatives by *Allium cepa* roots and *Drosophila melanogaster* haemocytes. *Ecotoxicol Environ Saf*, 192, 1-7.

Matasyoh, J. C, Kiplimo, J. J, Karubiu, N. M, & Hailstorks, T. P. (2007). Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphoratus*. *Food Chem.*, 101(3), 1183-1187.

Mercado, S. A. S, & Caleño, J. D. Q. (2019). Cytotoxic evaluation of glyphosate, using *Allium cepa* L. as bioindicator. *Sci. Total Environ.*, 700, 1-34.

Mohammed, K. P, Aarey, A, Tamkeen, S, Jahan, P. (2015). Forskolin: genotoxicity assessment in *Allium cepa*. *Mutat Res Genet Toxicol Environ Mutagen*, 777 (1), 29-32.

Mohanty, S, Ray, A, Sahoo, C, Sahoo, A, Jena, S, Panda, P. C, & Nayak, S. (2023). Volatile profiling coupled with multivariate analysis, antiproliferative and anti-inflammatory activities of rhizome essential oil of four Hedychium species from India. *J. Ethnopharmacol.*, 317(12), 1-7.

Nascimento, A, & Prade, A. C. K. (2020). *Aromaterapia*: o poder das plantas e dos óleos essenciais. Recife: Fiocruz.

Nag, A, Chakrabarti, M, Banerjee, R, & Mukherjee, A. (2019). Evaluation of cytotoxicity and antioxidant properties of some Zingiberaceae plants. *Int. J. Green Pharm.*, 12(4), 870-875.

Orlanda, J. F. F, & Nascimento, A. R. (2015). Chemical composition and antibacterial activity of *Ruta graveolens* L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. *S Afr J Bot*, 99, 103-106.

Ozaslan, M, & Oguzkan, S. B. (2018). Use of plant extracts in alternative medicine. *Pak J Biol Sci*, 21(1), 1-7.

Parvan, L. G, Leite, T. G, Freitas, T. B, Pedrosa, P. A. A, Calixto, J. S, & Agostinho, L. de A. (2020). Bioensaio com *Allium cepa* revela genotoxicidade de herbicida com flumioxazina. *Rev Pan-Amaz Saude*, 1, 1-10.

Pastori, T, Kuhn, A. W, Tedesco, M, Hoffmann, C. E, Neves, L. A. S, Canto-Dorow, T. S, & Tedesco, S. B. (2015). Ação genotóxica e antiproliferativa de *Polygonum punctatum* Elliott (Polygonaceae) sobre o ciclo celular de *Allium cepa* L. *Rev Bras Plantas Med*., 17(2), 186-194.

Prajitha, V, & Thoppil, J. E. (2016). Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay. *S Afr J Bot*, 102, 18-25.

Pathiratne, A, Hemachandra, C. K, & Silva, N. (2015). Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. *Environ. Monit. Assess.*, 187(12), 1-12.

Preti, D, Romagnoli, R, Rondanin, R, Cacciari, B, Hamel, E, Balzarini, J, Liekens, S, Schols, D, Estévez-Sarmiento, F, Quintana, J, & Estévez, F. (2018). Design, synthesis, *in vitro* antiproliferative activity and apoptosis-inducingstudies of 1 – (30,40,50-trimethoxyphenyl)-3-(20-alkoxycarbonylindolyl)-2-propen-1-onederivatives obtained by a molecular hybridisation approach. *J Enzyme Inhib Med Chem*, 33(1), 1225-1238.

Rezende, M. E de, JasmimII, J. M, CaprinilII, G. P, Sousa, E. P. de, Schripsema, J, & ThiébautII, J. T. L. (2011). Teor e composição química do óleo essencial de alpínia em razão da adubação e da disponibilidade de água no solo. *Rev. Ceres*, 58(2), 208-215.

Roman Junior, W. A, Gomes, D. B, Zanchet, B, Schönell, A. P, Diel, K. A. P, Banzato, T. P, Ruiz, A. L. T. G, Carvalho, J. E, Neppel, A, Barison, A, & Santos, C. A. M. (2017). Antiproliferative effects of pinostrobin and 5,6-dehydrokavain isolated from leaves of *Alpinia zerumbet*. *Rev. Bras. Farmacogn.*, 27, 592-598.

Santos, M. S, Jezler, C. N, Oliveira, A. R. M. F, Oliveira, R. A, Mielke, M. S, & Costa, L. C. B. (2012). Harvest time and plant age on the content and chemical composition of the essential oil of *Alpinia zerumbet*. *Hortic. Bras.*, 30, 385-390.

Selles, S. M. A, Belhamiti, B. T, Kouidri, M, Amrane, A. A, Kadari, Y, Kaddour, Z, & Kabrit, S. (2024). Chemical compounds, antioxidant and scolicidal potencies of *Thymus fontanesii* essential oil. *Exp. Parasitol.*, 257, 1-15.

Schreiner, G. E, Eckert, G. L, Schuster, M. F, Baroni, S, Pelegrin, C. M. G. de, & Dartora, N. (2024). Cytotoxic and genotoxic effects of aqueous extracts of *Aloysia gratissima* (Gillies & Hook.) Tronc. using *Allium cepa* L. assay. *Pharmacol Res*, 2(1), 1-16.

Sharma, A, Singh, H. P, Batish, D. R, & Kohli, R. K. (2019). Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of *Hyptis suaveolens*. *Ecotoxicol Environ Saf*, 171, 863-870.

Silva, E. A. J, Estevam, E. B. B, Silva, T. S, Nicolella, H. D, Furtado, R. A, Alves, C. C. F, Souchie, E. L, Martins, C. H. G, Tavares, D. C, Barbosa, L. C. A, & Miranda, M. L. D. (2019). Antibacterial and antiproliferative activities of the fresh leaf essential oil of *Psidium guajava* L. (Myrtaceae). *Braz. J. Biol.*, 79(4), 697-702.

Sousa, M. M. A de, Mesquita, M. L. R, Orlanda, J. F. F, & Catunda Júnior, F. E. A. (2020). Chemical composition and phytotoxic activity of *Lippia origanoides* essential oil on weeds. *Aust. J. Crop Sci.*, 14(3), 3015-3024.

Souza, L. F, Laughinghouse, H. D, Pastori, P, Tedesco, M. T, Kuhn, A. W, Canto-Dorow, T. S, Tedesco, S. B. (2010). Genotoxic potential of aqueous extracts of *Artemisia verlotiorum* on the cell cycle of *Allium cepa*. *Int. J. Environ. Sci.*, 67, 871-877.

Souza, T. de A, Lopes, M. B. P, Ramos, A. de S, Ferreira, J. L. P, Silva, J. R. de A, Queiroz, M. M. C, Araújo, K. G. de L, & Amaral, A. C. F. (2018). Alpinia essential oils and their major components against *Rhodnius nasutus*, a vector of chagas disease. *Sci. World J.*, 1, 1-6.

Turkez, H, Arslan, M. E, & Ozdemir, O. (2017). Genotoxicity testing: progress and prospects for the next decade. *Expert Opin Drug Metab Toxicol,* 13(10), 1089-1098.

Ubessi, C, Tedesco, B. S, Silva, C. de B. da, Baldoni, M, Krysczun, D. K, Heinzmann, B. M, Rosa, I. A, & Mori, N. C. (2019). Antiproliferative potential and phenolic compounds of infusions and essential oil of chamomile cultivated with homeopathy. *J. Ethnofharmacol.*, 239, 1-32.

Viana, A. R, Noro, B. G, Santos, D, Wolf, K, Neves, Y. S. das, Moresco, R. N, Ourique, A. F, Flores, E. M. M, Rhoden, C. R. B, Krause, L. M. F, & Vizzotto, B. S. (2023). Detection of new phytochemical compounds from *Vassobia breviflora* (Sendtn.) Hunz: antioxidant, cytotoxic, and antibacterial activity of the hexane extract. *J. Toxicol. Environ. Health.*, 86(2-3), 51-68.

Viana, A. R, Bottari, N. B, Santos, D, Serafin, M. B, Rossato, B. G, Moresco, R. N, Wolf, K, Ourique, A, Hörner, R, Flores, E. M. de M, Schetinger, M. R. C, Vizzotto, B. S, & Krause, L. M. F. (2022). Insights of ethyl acetate fraction from *Vassobia breviflora* in multidrug-resistant bacteria and cancer cells: from biological to therapeutic. *J. Toxicol. Environ. Health.*, 85(23), 972-987.

Victório, C. P, Alviano, D. S, Alviano, C. S, Lage. C. L. S. (2009). Chemical composition of the fractions of leaf oil of *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. and antimicrobial activity. *Rev. Bras. Farmacogn.*, 19, 697-701.

Xiao, R, Wu, L, Hong, X, Tao, L, Luo, P, Shen, X. (2018). Screening of analgesic and anti inflammatory active component in fructus *Alpiniae zerumbet* based on spectrum-effect relationship and GC-MS. *Biomed. Chromatogr.*, 32(3), 1-26.

AUTHORSHIP CONTRIBUTIONS

1 – Márcia Aldeany Almeida de Sousa

Currently a substitute professor and member of the research group at the Environmental Chemistry Laboratory at the State University of the Tocantina Region of Maranhão - UEMAUSUL
https://orcid.org/0000-0002-7731-9920 • marciaaldeanybio@hotmail.com
Contribution: conceptualization, methodology, validation, data curation, formal analysis, investigation, writing – first formal writing, writing – review and editing, data visualization (infographics, flowcharts, tables, graphs)

2 – Fernando Souza Lima Silva

Currently a Master's student in Applied Chemistry at the State University of Santa Catarina - UDESC
https://orcid.org/0009-0001-3970-7674 • fernandosilva.20190002933@uemasul.edu.br
Contribution: conceptualization, methodology, validation, data curation, formal analysis, investigation, writing – first formal writing, writing – review and editing, data visualization (infographics, flowcharts, tables, graphs)

3 – José Fábio França Orlanda

Currently Associate Professor I at the State University of the Tocantina Region of Maranhão (UEMASUL) and Coordinator of the Environmental Biotechnology Laboratory (LABITEC)
https://orcid.org/0000-0002-6402-6192 • fabio.franca@uemasul.edu.br
Contribution: conceptualization, methodology, validation, data curation, formal analysis, investigation, writing – first formal writing, writing – review and editing, data visualization (infographics, flowcharts, tables, graphs), supervision, project administration, obtaining financing.

How to quote this article

Sousa, M. A. A. de ., Silva, F. S. L., & Orlanda, J. F. F. (2024). Genotoxic and antiproliferative effect of *Alpinia zerumbet* (Zingiberaceae) essential oil in *Allium cepa* biotest. *Ciência e Natura*, Santa Maria, 46, e73445. https://doi.org/10.5902/2179460X73445