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Cytogenotoxic potential of *Malva parviflora* L. cultivated under different light conditions

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ABSTRACT. For many centuries, the treatment with medicinal plants was the only means accessible to various ethnic groups, and nowadays plants are still widely used in the treatment of diseases, besides being the raw material for several drugs. However, many of these plants may present unknown genotoxic effects, making their consumption a health risk. Species such as *Malva parviflora* L. have been used by the general population for medicinal purposes: antifungal, anti-inflammatory, analgesic, and antioxidant. This study had the objective of analyzing the cytogenotoxic potential of aqueous extracts of this *Malva* species using the *Allium cepa* test, which serves as a bioindicator of the genotoxicity of plant extracts. Forty-four seedlings of *M. parviflora* were grown for 36 days between November and December 2020, in a greenhouse in two groups: half of the plants were protected with a shading screen, and the rest were not. The aqueous extracts were prepared from the aerial part and roots (fresh and dehydrated), at the concentration of 5 g L^{-1}. The bulbs of *A. cepa* were left for 24 hours in the treatments, plus the negative (distilled water) and positive (glyphosate 1.5%) treatments. The roots of the bulbs were collected, placed in the fixative for 24 hours, transferred to 70% alcohol, and stored in a refrigerator. The slides were prepared by the crushing technique, and cells in the division and with chromosome alteration were analyzed, mitotic and genotoxic index were calculated, and statistical analysis was done by the Chi-square and Scott-Knott tests. From the results obtained, it is possible to conclude that the aqueous extracts of *M. parviflora* at a concentration of 5 g L^{-1} present antiproliferative activity, but are not genotoxic. The cultivation mode is relevant, that is, with and without shading the fresh leaves present a difference in cell proliferation, but the same does not occur with the dried leaves.

Keywords: *Allium cepa* cells; medicinal plants; extracts; mitotic index; genotoxicity.

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Introduction

Medicinal plants are less or non-toxic drug resources that are easily accessible, economical, and safe (Fathi, Ghane, & Pishkar, 2022). For many centuries, the treatment with these plants was the only resource available to various ethnic groups, and currently, plants continue to be frequently used for the treatment of pathologies in many countries (Gasparetto, Martins, Hayashi, Otuky, & Pontarolo, 2011; Souza et al., 2020), including Brazil. Besides being part of the culture, their use can be influenced by the high cost of medicines, the difficulty of access to medical consultations (Oliveira, Bovini, Bortoluzzi, Boff, & Boff, 2019), and the growing trend of the return of the use of natural resources as an alternative to synthetic medicines (Liu, Wang, & Kang, 2022).

Malvaceae Juss. is a botanical family with a rich diversity of species used for various purposes, such as medicinal, textile, and ornamental (Oliveira et al., 2019). As one of the 10 richest angiosperm families in Brazil (The Brazil Flora Group, 2021), it is composed of about 280 genera (World Flora Online, 2022a) and 861 species (Flora e Funga do Brasil, 2022). It has a cosmopolitan distribution, with a high number of species in the tropics (Oliveira et al., 2019). The genus *Malva* comprises about 140 species (World Flora Online, 2022b), and can be considered as annual or biennial herbaceous (Liu et al., 2022). The species of the genus are widely used for their diuretic, laxative, antiseptic, anti-inflammatory, and healing effects (Sharifi-Rad et al., 2019).

Malva parviflora L. is known as mallow, little mallow, and other popular names. The species is native to Europe and Asia, but naturalized in several countries and is now almost cosmopolitan (World Flora Online, 2022c). In Brazil, it is cultivated in the Midwest (Mato Grosso do Sul State), Southeast (Minas Gerais, Rio de Janeiro, and São Paulo States), and South (Paraná, Rio Grande do Sul, and Santa Catarina States) regions. It is decumbent to erect herbaceous, 0.4 to 1.8 m tall, reniform and glabrous leaf and has white flowers with lilac

parts (Oliveira et al., 2019). The dried powder or infusion made from leaves and roots is used to clean wounds and bruises (Zayed, Eisa, & Hezma, 2017). Extracts of the plant also exhibit antifungal, anti-inflammatory, analgesic, and antioxidant functions (Zayed, Eisa, & Shabaka, 2012). Abkar, Hanif, Ali, and Ishtiaq (2014) report it is used for headaches, fever, intestinal infections, ulcers, bronchitis, sore throat, and cough. The main compounds found in the species that generate these functions are scopoletin and oleanolic acid (Medrano-Jimenez et al., 2019), in addition to Naringenin (majority compound), p-coumaric acid, Apigenin-7-glucoside, Luteolin, Cinnamic acid and Gentisic acid (Naser, Mahdi, & Alasadi, 2022). Normally, the compound present in greater quantity is responsible for the bioactivity of the aqueous extract. Naringenin has various pharmacological effects, such as anti-inflammatory, antimicrobial, and hepatoprotective (Salehi et al., 2019; Ke et al., 2017; Pinho-Ribeiro et al., 2016).

However, many of these plants may have unknown cytogenotoxic effects, making their use a health risk. One test that has been widely used to assess the toxicity of plant extracts is the *Allium cepa* test, which serves as a bioindicator of genotoxicity (Hister et al., 2017; Pereira, Rodrigues, Freitas, & Tedesco, 2019).

The test is performed to indicate the genotoxic and antiproliferative potential of plants and is an excellent plant bioindicator, validated by the World Health Organization (WHO) and with good sensitivity and great correlation with mammalian tests (Tedesco & Laughinghouse IV, 2012).

Plant species can synthesize a variety of metabolites that are classified according to their function, and secondary metabolites are used for their defense, besides other functions (Miranda, Santana, Machado, Coelho, & Carvalho, 2013). Thus, differences in shading can alter these metabolites and their quantity and may affect genotoxicity, as in Pereira, Hister, Ubessi, and Tedesco (2022), in which the aqueous extract of *Phyllanthus tenellus* Roxb. grown under shading conditions showed antiproliferative activity and genotoxic effect in the *Allium cepa* test. It is noteworthy that clastogenic agents can act on chromosomes, being considered genotoxic, and that cytotoxicity refers to the inhibition of cell division.

Research on the genotoxicity of *M. parviflora* is scarce and necessary. Thus, the objectives of this study were to verify if the shading and the plant part interfere with the cytogenotoxic potential of aqueous extracts of *M. parviflora*.

Material and methods

Growing in a greenhouse

The cultivation of *M. parviflor*a was done in the period between November and December 2020, from seedlings of the same batch, purchased in the local commerce of Santa Maria (Rio Grande do Sul State, Brazil). The plants were grown in 3 L plastic pots, with the substrate composed of peat, limestone, and composted pine bark, in the plastic greenhouse of the Biology Department of the *Universidade Federal de Santa Maria* (UFSM) and received water at least once a day. Two groups with 22 plants each were made, totaling 44. In group A, a 70% black Sombrite shading screen was used so that the seedlings would receive less incidence of sunlight, and those in group B received the natural sunlight that fell on the greenhouse. In total, the plants stayed for 36 days in the greenhouse, the first six of which were for acclimatization. The minimum average temperature of the period was 17.8°C, and the maximum was 29.6°C.

Collection and storage

After 36 days the plants were collected, and half of the material was stored in a freezer and the other part was dried in a controlled environment in the Laboratory of Plant Cytogenetics and Genotoxicity (Labcitogen) of UFSM, at a temperature of approximately 20°C, and then stored in a dry place. One exsiccata was deposited in the SMDB Herbarium (UFSM), with accession number 22143.

Genotoxicity test

The analysis of the cytogenotoxic potential of *M. parviflora* was performed at Labcitogen/UFSM, using the *Allium cepa* test system. The bulbs had their old roots scraped and were placed in distilled water for re-rooting for four days in April 2021. After this period, they were transferred to the aqueous extracts of *M. parviflora*, which were prepared from the aerial part (leaves and stems) and the roots, at the concentration of 5 g L^{-1}. The plant material was weighed, and the extracts were made by decoction for 10 minutes with distilled water. The negative control of the experiment was distilled water and for the positive control, glyphosate at a concentration of 1.5%, according to Pereira et al. (2022). For the test, repetitions with four bulbs of *A. cepa* were made for each treatment. There were 10 treatments: T1 = Negative (distilled water), T2 = Positive

(glyphosate 1.5%), T3 = Fresh aerial part with shading, T4 = Fresh aerial part without shading, T5 = Dry aerial part with shading, T6 = Dry aerial part without shading, T7 = Fresh roots with shading, T8 = Fresh roots without shading, T9 = Dry roots with shading, and T10 = Dry roots without shading. The rooted bulbs remained in contact with the treatments for 24 hours. Subsequently, the roots were collected and fixed in ethanol: acetic acid (3:1) for 24 hours at room temperature. The material was then passed to other flasks containing 70% ethanol and stored under refrigeration until used for analysis.

Slide preparation

For the analysis of *A. cepa* cells, two slides were made per bulb and 500 cells were counted per slide, totaling 4.000 cells per treatment. The slides were prepared using the methodology adapted from Guerra and Souza (2002) where the roots were hydrolyzed in 1N HCl for 5 minutes, then washed in distilled water and had their meristematic region removed, which was placed on a glass slide and stained with 2% acetic orcein. After that, the crushing technique was performed, which consisted of grinding the material with the help of a small glass rod. A glass slide was placed over this material and the excess dye was removed. With the aid of optical microscopy (Leica DM500) at 40X magnification the cells were analyzed, taking into consideration the cell cycle: interphase and cell division (prophase, metaphase, anaphase, and telophase), and chromosomal alterations found (breaks, micronuclei, anaphase, and telophase bridges).

Statistical analysis

The design used in the *A. cepa* test was entirely randomized, with four repetitions. Two slides were analyzed in each repetition. Data regarding cell division, and mitotic index (MI= number of cells in the division, divided by the total number of analyzed cells x 100), were compared by the Chi-square test ($p < 0.05$), using the statistical program BIOESTAT 5. O (Ayres, Ayres, Ayres, & Santos, 2007). Chromosomal irregularities, and genotoxic index (GI= number of cells with alteration, divided by the total number of analyzed cells x 100), were compared by the Scott-Knott test ($p < 0.05$), using the SISVAR 5.6 statistical program (Ferreira, 2014).

Results and discussion

Although a large number of medicinal plants are known and used in folk medicine, more detailed studies of the biological and pharmacological activity of many remain lacking (Ciappina et al., 2017; Santos et al., 2022). Most of them are not sufficiently studied for cytotoxicity and mutagenicity but can be monitored by the *Allium cepa* test system (Tedesco et al., 2015).

Observing Table 1, it is possible to notice that treatments T1 (Negative Control), T2 (Positive Control), T9 (Dry roots with shade), and T10 (Dry roots without shade) presented the highest values of mitotic index (cell proliferation) and did not differ from each other, either from T2 (Positive Control), T3 (Fresh aerial part with shade), and T7 (Fresh roots with shade) (x^2: T1XT2 = 0.093; T1XT3 = 0.004; T1XT7 = 0.546; T1XT9 = 0.004; T1XT10 = 0.429; T2XT3 = 0.134; T2XT7 = 0.188; T2XT9 = 0.060; T2XT10 = 0.922; T3XT7 = 0.639T3XT9 = 0.015; T3XT10 = 0.353; T7XT8 = 0.176; T7XT9 = 0.460; T9XT10 = 0.512; T7XT10 = 1.939).

T8 (Fresh roots without shade) did not differ from T2, T3, and T7, but differed from T1, presenting a mitotic index (MI) of 2.82%. However, it differed from T4 (Fresh aerial part without shade), T5 (Dry aerial part with shade), and T6 (Dry aerial part without shade), which presented the lowest values. Of these, there was a decrease in MI compared to water, and this decrease indicates antiproliferative activity.

The results obtained from the analyses of the aerial part and roots of *M. parviflora* grown in the conditions with and without shading in the period November and December 2020 under the *A. cepa* test system are recorded in Table 1. In it, the different treatments made with the aqueous extracts are shown, in addition to the negative (distilled water) and positive (glyphosate 1.5%) treatments, and the results of these treatments are presented: the mitotic index, number of cells in interphase and division.

Comparing the aqueous extracts made from the aerial part (T3 to T6) versus those prepared with roots (T7 to T10), T3 to T6 had overall lower MI. Except for T3, all other extracts of the mallow aerial part were statistically different from the negative control, decreasing cell division. Only T10 had higher MI than water, from T9 was similar, and from T7 and T8 was lower, but despite this, they are not statistically different. These results differed from Bispo et al. (2021), in which the aqueous extract of *Erythrina fusca* increased MI.

Table 1. Number of *Allium cepa* L. cells analyzed under different conditions of the aqueous extract of *Malva parviflora* L. at a concentration of 5 g L^{-1}, with cells in interphase, in division, and the mitotic index, in each treatment.

Treatment	Analyzed Cells	Interphase Cells	Cells in Division	Mitotic Index (%)
T1- NC	4000	3859	141	3.52 a*
T2- PC	4000	3864	136	3.4 ab
T3- FAPS	4000	3858	142	3.55 ab
T4- FAPWS	4000	3916	84	2.1 de
T5- DAPS	4000	3923	77	1.92 ef
T6- DAPWS	4000	3910	90	2.25 d
T7- FRS	4000	3869	129	3.22 ab
T8- FRWS	4000	3887	113	2.82 bc
T9- DRS	4000	3860	140	3.5 a
T10- DRWS	4000	3848	152	3.8 a

Source: Elaborated by the authors (2022). T1 = Negative Control (distilled water), T2 = Positive Control (Glyphosate 1.5%), T3 = Fresh aerial part with shade, T4 = Fresh aerial part without shade, T5 = Dry aerial part with shade, T6 = Dry aerial part without shade, T7 = Fresh roots with shade, T8 = Fresh roots without shade, T9 = Dry roots with shade, and T10 = Dry roots without shade. *Means followed by the same letter do not differ significantly at the 5% level by the Chi-square test.

On the aqueous extracts made with the dried aerial part (T5 and T6), both with and without shading, there was a reduction in MI compared to T1, so it is possible to say that they are antiproliferative, differing from Trapp, Hister, Laughinghouse IV, Boligon and Tedesco (2020), in which aqueous extract of dried *Plectranthus barbatus* did not affect the MI. Extracts of the dried roots, on the other hand, with and without shading (T9 and T10) were similar to T1, also differing from Trapp et al (2020).

The treatments without shading had lower MI (T4 = 2.1, T6 = 2.25, and T8 = 2.82%) compared to T1 (3.52%), except T10 (3.8%), in which the value was close to T1. This result agrees with Pereira et al. (2022), in which the genotoxicity of *Phyllanthus tenellus* under different luminosities was analyzed, and the plants without shading also caused a reduction in the MI of *A. cepa*. The cause of this lower MI is likely to be because the greenhouse cultivation was done between November and December 2020 (late spring), which had a higher incidence of direct light and consequently higher temperatures, which caused more stress in this group than in the shaded group. This stress causes the plants to produce more secondary metabolites, which influence the cell cycle of *A. cepa* (Pereira et al., 2022). On the other hand, the shaded group (T3, T5, T7, and T9), which had less stress because they received less sunlight, had similar values to T1 and cannot be considered antiproliferative.

In summary, aqueous extracts of mallow aerial part without shading, fresh or dry material (T4 and T6), dry aerial part extract with shading (T5), and fresh root extract without shading (T8) were the treatments that most reduced MI, being considered cytotoxic. On the other hand, aqueous extracts prepared from dried roots, regardless of shading (T9 and T10), can be considered safe for consumption, since they did not show cytotoxic activity. As well as extracts from fresh material from plants with shading, they are also not cytotoxic.

The treatments with extracts of mallow plants with shading (T3, T7, and T9), had similar MI among themselves and with distilled water, except in T5. The root MI of bulbs treated with extracts from unshaded plants (T4, T6, T8, and T10), on the other hand, varied. Li, Kong, Fu, Sussman, and Wu (2020) point out that medicinal plants are composed of a wide variety of chemical compounds related to external factors, such as environmental conditions and plant age. Controlling abiotic factors is important for maintaining the quality of cultivated medicinal plants, as they can vary according to external factors.

It is noticed that the shading caused differences in the aerial part of aqueous extracts (T3 to T6), but not concerning the roots (T7 to T10). In addition, the drying of the aerial part (T5 and T6) potentiated the effect of cytotoxicity of *M. parviflora*, because the compounds became more concentrated. According to Naser et al. (2022), the aerial part of the species has flavonoids, tannins, saponins, alkaloids, and phenols, as well as roots, which have a lower concentration.

Table 2 shows the data of the chromosomal alterations observed, in percentage, caused by the different treatments. It can be observed that irregular cells appeared in all treatments, including the negative control in water, as also occurred in Tedesco et al. (2015), Sharma, Sharma, and Pal Vig (2018), Silva et al. (2018), Hister, Trapp, Boligon, and Tedesco (2019), and Pinheiro et al. (2020), but emphasizing, these values represent less than 1% (0.35 to 0.92%). Only in glyphosate, which is known to be genotoxic, did a GI value above 1% appear.

According to Table 2, all treatments are statistically similar, and all the chromosomal alterations were less than 1% (except the positive treatment, T2). The treatments made with the aqueous extracts of the aerial part of *M. parviflora* (T3 to T6) had similar GI, greater than that of water, but without statistical difference. Thus,

from a statistical point of view, although some irregularities were found, it is not possible to state that the tested treatments have genotoxic potential.

Table 2. Number of *Allium cepa* L. cells analyzed under different conditions of the aqueous extract of *Malva parviflora* L. at the concentration of 5 g L^{-1}, with irregular cells and genotoxic index (GI), in each treatment.

Treatment	Analyzed Cells	Irregular Cells	Genotoxicity Index (%).
T1- NC	4000	15	0.37 a1*
T2- PC	4000	41	1.02 a1
T3- FAPS	4000	21	0.52 a1
T4- FAPWS	4000	19	0.47 a1
T5- DAPS	4000	18	0.45 a1
T6- DAPWS	4000	16	0.42 a1
T7- FRS	4000	37	0.92 a1
T8- FRWS	4000	21	0.55 a1
T9- DRS	4000	18	0.47 a1
T10- DRWS	4000	14	0.35 a1

Source: Elaborated by the authors (2022). T1 = Negative Control (distilled water), T2 = Positive Control (Glyphosate 1.5%), T3 = Fresh aerial part with shade, T4 = Fresh aerial part without shade, T5 = Dry aerial part with shade, T6 = Dry aerial part without shade, T7 = Fresh roots with shade, T8 = Fresh roots without shade, T9 = Dry roots with shade, T10 = Dry roots without shade. *Mediums followed by the same letter do not differ significantly at the 5% level by the Scott-Knott test.

Among the treatments made from roots, T7 (aqueous extract of fresh roots with shading) had the highest GI but showed no significant difference compared to the positive treatment T2 (glyphosate 1.5%) or the negative treatment (T1). The lowest GI and most similar to T1 was T10 (dry roots without shade). The treatments with fresh roots independent of shading, T7 (0.92%) and T8 (0.55%), were the ones that presented the highest GI. These results are similar to Bispo et al. (2021) in their analyses with extracts of aerial part and roots of *Erythrina fusca*, where all concentrations evaluated showed genotoxic potential.

Only T10 had a GI lower than distilled water, not statistically different from it (0.35 and 0.37%, respectively), and it can be said that its consumption is safe. Figure 1 shows several meristematic cells of onion roots in interphase and division. In Figure 1A, there are cells in interphase and one cell in telophase. Figure 1B shows cells in interphase, metaphase, and normal telophase, but also some irregular cells, such as anaphase bridges.

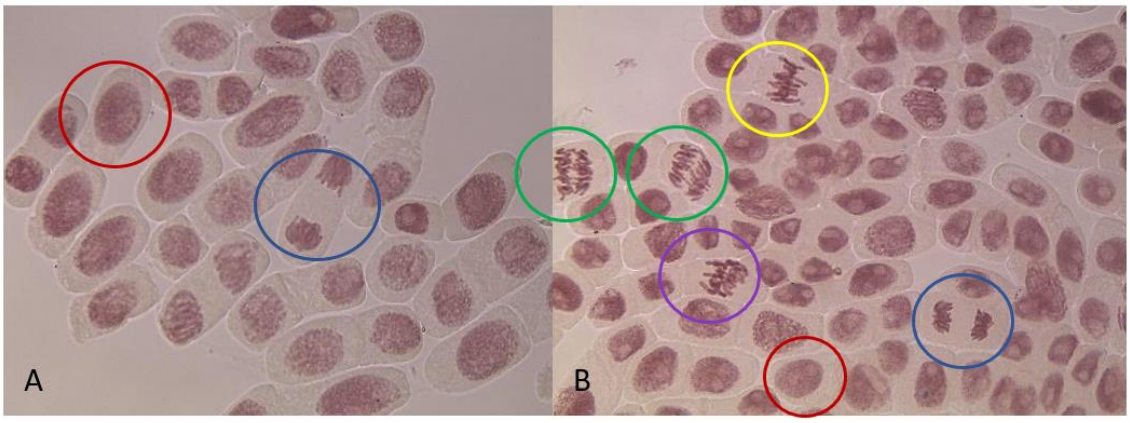

Figure 1. Cells of the meristematic region of *A. cepa* observed in light microscopy at 40x magnification. Red circle - interphase; yellow circle - metaphase; purple circle - irregular metaphase; green circles - irregular anaphase; blue circle - telophase. A - normal cell division; B - cell division with irregularities.
Source: Personal collection of the authors (2022).

Altyar et al. (2022) researched gastric ulcers and mucilage extracted from the leaf and fruit of *M. parviflora* and it was concluded that they are safe, with the lethal dose being above 5 g kg^{-1}. Gastric ulcer is a disease that can arise as a side effect of drugs to treat various diseases (such as anti-inflammatory drugs), takes time to heal, and affects many people around the world, besides disrupting life daily, fatal complications such as gastric bleeding and perforations can occur (Carlotto et al., 2019). Altyar et al. (2022) concluded that the use of *M. parviflora* along with standard drugs significantly reduced ulcer counts, which could have been avoided or be less severe if natural anti-inflammatories (such as the *Malva* species in question) were used from the beginning. In addition, they showed that mucilage from the leaves inhibited 47.99% of chemically induced coughs and was more effective than from the fruits, thus supporting the traditional use of the species' leaves.

El-Naggar et al. (2020) evaluated the prophylactic effect of different concentrations of nanoemulsion of *M. parviflora* leaf extract that combined with yogurt, combats induced ulcerative colitis in rats. This combination can be a healthy food supplement as a good route to natural medicinal products. The results indicate that this nanoemulsion of the species' leaf extract contributed to reducing inflammation and counteracting oxidative stress. Also, Medrano-Jimenez et al. (2019) study on the anti-inflammatory activity of the hydroalcoholic extract of *M. parviflora* leaves on Alzheimer's disease in lean and obese mice showed the suppression of neuroinflammation, which suggests that it can be effectively used to prevent or delay the disease.

The use of the *Allium cepa* test in the initial assessment of cytogenotoxic activity is recommended as it indicates alterations in the cell cycle of *A. cepa*. Therefore, the test can serve as a warning for the population that has the habit of using medicinal plant extracts (Bonciu et al., 2018; Trautenmuller et al., 2023).

The present data indicate numerous benefits of treatments with *M. parviflora*, but there is still a need for further study and further testing.

Conclusion

The aqueous extracts of *Malva parviflora* produced from fresh aerial parts without shading, dry aerial parts independent of shading, and fresh roots without shading showed antiproliferative activity. Most treatments of plants without shading have an antiproliferative effect, thus, this cultivation method enhances cytotoxicity. Although irregularities have been visualized, all treatments are statistically equal, and it is not possible to state that the aqueous extract of *M. parviflora* has genotoxic activity.

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