

Phytochemical characterization and bioherbicide potential of *Duranta erecta* L.

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ABSTRACT

We characterized the chemical compounds present in the extract of dry leaves from *Duranta erecta* L. using ESI-MSn mass spectrometry and assessed their phytotoxic potential on *Lactuca sativa* L.. The extraction process involved exhaustive extraction with 50% ethanol, and the resulting extracts were subsequently dried through lyophilization. Solutions of 2.5, 5, 10, 20, and 40 mg/mL concentrations were prepared from the dried extracts to investigate their allelopathic effects on *L. sativa*, focusing on germination, germination speed, root elongation, shoot length, and fresh biomass. Statistical analysis was performed using Analysis of Variance, and means were compared using the Scott-Knott test ($p < 0.05$). The ESI-MSn analysis identified a total of 20 compounds in the extract, with 12 of them being reported for the first time in *Duranta* sp. Our findings suggest that *D. erecta* possesses allelopathic potential, as evidenced by its cytotoxicity, mitodepressive effects, and phytotoxicity on germination and morphological parameters in the bioassay conducted with *L. sativa*.

Keywords: Allelopathic effect index, Allelopathy, Bioherbicide, Cytotoxicity, Golden dewdrop, Germination, *Lactuca sativa*, Phytochemicals, Phytotoxicity, Seedling growth.

INTRODUCTION

The genus *Duranta* spp. belongs to the Verbenaceae family and comprises a total of 73 species, although only 32 of them are officially recognized (54). Among these species, *Duranta erecta* (synonym *Duranta repens* L.) stands out as a woody shrub, typically reaching heights of 1.5 to 3 meters. Its distinct golden yellow leaves make it a popular choice for ornamental gardening (59) and landscaping (Fig. 1). Additionally, its rapid growth rate and adaptability have led to its frequent use as live fences (53).

Duranta erecta is native to the regions stretching from Mexico to South America and throughout the Caribbean. Due to its versatility and hardiness, it is extensively cultivated in tropical and subtropical areas (27). However, it is essential to note that in some regions, such as China, Australia, and South Africa, this species is considered invasive (3). This genus holds great promise as a source of phytochemicals, with many of these compounds being involved in allelopathic and cytotoxic processes. The key constituents of *Duranta* spp. include iridoid glycosides (durantosides), phenylethanoid glycosides (acteoside and isoacteoside), and other chemical groups such as alkaloids, flavonoids, flavonoid glycosides, saponins, steroids, iridoids, triterpenes, and coumarins. These compounds exhibit significant potential for various scientific applications.

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Figure 1. Population of *Duranta erecta* L. A: On main campus, Federal University of Alfnas-MG, Brazil. B: Branches of *D. erecta*, discarded branches (left) collected branches (right).

Biochemical interactions between plants occur through the release of signaling substances (produced via secondary metabolism) into the environment, which can positively or negatively influence neighboring plants (4). When these substances affect the growth and development of other plants and organisms, they are referred to as allelochemicals (38). These allelochemicals directly or indirectly interfere with several physiological processes, including CO₂ assimilation, photosynthesis, nutrient absorption, cell division, reduction in chlorophyll content, inhibition of protein synthesis, stomatal opening, and electron transport (30). Their versatility allows for specific targeting of unique sites in recipient plants, making them valuable tools for developing next-generation herbicides with a safer and more sustainable toxicological and environmental profile (40).

Identifying phytochemicals in plants with allelopathic potential has emerged as an alternative in weed management (16). With a global demand for low-impact agricultural products (50), there is considerable interest in discovering new compounds. Therefore, this study aims to characterize the metabolites present in the leaves of *Duranta erecta* and quantify their phytotoxic, genotoxic, and allelopathic potential in bioassays with *Lactuca sativa*.

MATERIAL AND METHODS

I. Sample collection and preparation

The *Duranta erecta* material was collected from the southern region of Minas Gerais State, specifically from Alfnas city, near the Federal University of Alfnas (S21° 25' 09.7", WO 45° 56' 55.8"), at an altitude of 835 m. The region experiences an annual precipitation of 112 mm, and temperatures range between 16.5 to 26 °C. The study was conducted between September 2020 and February 2022. Exsiccates of the collected specimens were deposited in the Herbarium of the university. Flowering and fruiting branches were not collected (Fig. 1B), and only leaves in the vegetative phase were collected.

Following collection, the leaves were dried in an oven at 45 °C until a constant weight was achieved, and then ground in a Mill (Skymesen-TA04). The resulting powder was analyzed using an electromagnetic sieve shaker (Bertel®). The hydroethanolic extract (50%)

was obtained through an exhaustive extraction process using a stainless steel percolator (12) and later concentrated in a rotary evaporator at 40 °C under a pressure of 400 mmHg. After that, the extract was dried using lyophilization (lyophilizer – Liotop L101) and then dissolved in water to prepare concentrations of 2.5, 5, 10, 20, and 40 mg/mL. The effects of the 40 mg/mL concentration were studied on pH (Analion, PM608) and osmotic potential (measured using an osmometer from Advanced Instruments, Inc., model 3320). The osmotic potential was expressed in mOsmol.Kg-1 and converted to MPa.

II. Phytochemicals characterization

The dry extract was subjected to analysis using the LTQ XL Thermo Scientific Linear 2D Mass Spectrometer. Spectra were obtained through direct flow injection (FIA) and analyzed using electrospray ionization (ESI). Multi-stage fragmentation (MS2, MS3) was conducted in an ion-trap interface (IT). Both negative and positive modes were selected for generating and analyzing first-order mass spectra (MS), as well as for other multistage experiments (MSn). The acquisition range was m/z 50-2000, with two or more scanning events carried out simultaneously on the LTQ XL mass spectrometer (8,11).

III. Phytotoxicity assays

The bioassays were conducted in 70 mm Petri dishes, each containing two Germitest® paper sheets moistened with 3 mL of extract solution at concentrations of 2.5, 5, 10, 20, and 40 mg/mL. Distilled water was used as a control. In each Petri dish, 30 seeds of *Lactuca sativa* L. 'Babá de Verão' (ISLA PAK Sementes Ltda) were evenly sown on the filter papers. The dishes were placed in a BOD germination chamber at 25 °C with a 12-hour photoperiod for 7 days.

The Physiological Germination (PG) was recorded at 24 hours, and Final Germination (FG) was recorded at the end of the 168-hour experiment. The Germination Speed Index (GSI) was calculated according to adaptations (23) and counted at intervals of 4 hours until 48 hours and at 12-hour intervals until 168 hours. The chosen observation intervals were based on the rapid germination of 'Babá de Verão' (47). The allelopathic effect index (RI) corresponds to negative values for inhibition and positive for stimulation,

Where, -1: Maximum inhibition, and +1: Maximum stimulation. The RI was calculated at 24-h intervals as under:

$$RI = 1 - C/T \quad (T \geq C) \quad \text{or} \quad RI = T/C - 1 \quad (T < C),$$

Where, C: Control germination speed and T: Treatment germination speed.

$$\text{For germination speed (GS), we used: } GS (\%) = \frac{\sum (Gt/D)}{\sum (Gc/D)} \times 100$$

Where Gt: Number of seeds germinated daily from the treatment and Gc: Number of seeds germinated daily from the control, D: the number of corresponding days (9,60).

From each replicate, 10 seedlings were randomly selected, and their Root elongation (RE), shoot length (SL), and fresh biomass (FB) were measured. Root elongation and shoot length were measured using a digital caliper (DIGIMESS® 150mm), while fresh biomass was obtained following the procedure described by Amâncio *et al.* (4).

IV. Cytotoxicity assays

The *L. sativa* roots exposed to the treatments were collected, fixed in Carnoy 3:1 (ethyl alcohol: acetic acid P.A.), and stored at $-18 \pm 1^\circ\text{C}$. The leaf blades were crushed

following the procedure described by Pereira *et al.*, (42). To determine the mitotic index (MI), 6000 cells were analyzed for each treatment. The calculation was done as follows:

$$MI = (NCM/TNC) \times 100$$

Where: NCM: Number of cells in mitosis and TNC: Total number of cells analyzed (4). The Data was collected up to 72 h after start of experiment.

V. Statistical analysis

The experimental design was a completely randomized design (CRD), with one treatment and six concentrations (1x6), and each treatment was replicated three times. The obtained means were subjected to analysis of variance using the Scott-Knott test at a significance level of 5%. The statistical analysis was performed using the Sisvar software version 5.6 (17).

RESULTS AND DISCUSSION

Physicochemical characterization of the extract

At the application of the 40 mg/mL concentration, the pH and osmotic potential were measured at 5.2 and -0.22 MPa, respectively. These results indicated that the inhibitory effects observed in the bioassays were due to the presence of allelochemical substances. Notably, the values for pH and osmotic potential fell within the tolerance limit (20,56). Osmotic potential variations close to -0.2 MPa did not significantly affect the final germination of *L. sativa*, whereas values greater than -0.3 MPa resulted in a substantial 77% inhibition of seed germination (4,20).

The compounds identified in the dry extract are presented in Table 1, along with their corresponding fragments observed during multiple-stage analysis. A total of twenty compounds were identified based on their mass and charge ratio, and their fragmentation mechanisms were consistent with existing literature. Impressively, twelve of these compounds (1, 2, 4, 5, 13, 14, 15, 16, 17, 18, 19 and 20) were reported for the first time in *Duranta sp.* The complete scanning spectra of the ions in both negative and positive modes can be found in Fig. 2 and Fig. 3, respectively. The chemical structures of these compounds are elucidated in Fig. 4.

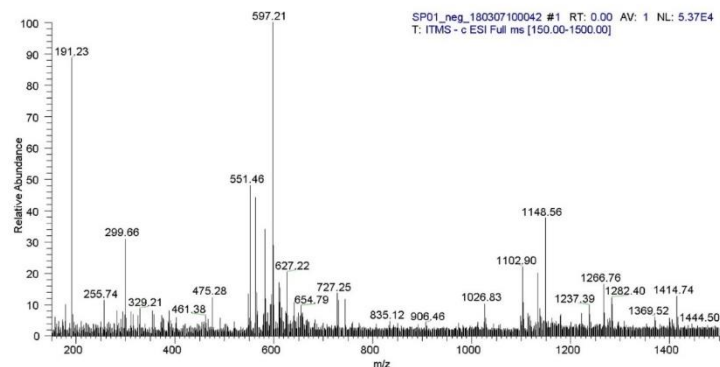


Figure 2. Full scan digital spectrometry (FIA-ESI-IT-MS) obtained in negative ion mode from *D. erecta* L. leaves.

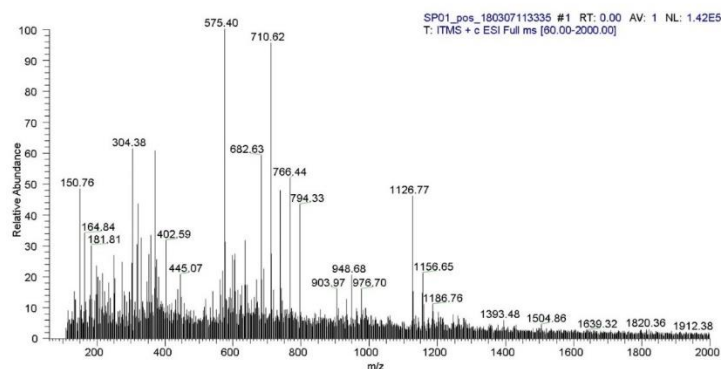


Figure 3. Full scan digital spectrometry (FIA-ESI-IT-MS) obtained in positive ion mode from *D. erecta* L. leaves.

Table 1. Main metabolites identified in the dry extract of *Duranta erecta* L. by mass spectrometry, in negative and positive ionic mode.

Substance	[M-H]	MSn	Class	Reference
[M-H]⁻				
1	Quinic acid	191	173, 145, 101	Phenolic acid (48)
2	Pinocembrine	255	211	Flavonoid (32)
3	Diosmetin	299	283, 227	Flavonoid (25)
4	Betuletol	329	314, 285	Flavonoid (13)
5	Cheffouxanthone	396	353, 296	Xanthone (29)
6	β -Sitosterol	414	386, 372, 324	Steroid (2)
7	Duranterectoside B	551	533, 403, 385, 371, 241	Iridoid (55)
8	Duranterectoside C	567	523, 387, 241	Iridoid (55)
9	Duranterectoside A	597	551, 385, 295, 223	Iridoid (55)
10	Durantinin IV	1005	977, 802, 729, 592, 391	Saponin (3)
11	Durantinin V	1219	1129, 955, 867	Saponin (3)
[M-H]⁺				
12	Durantol	298	280, 266, 254	Steroid (52)
13	Cephalezomine H	304	285, 106, 102	Alkaloid (36)
14	Neoclerodanendiolide	343	342, 327, 313, 297	Diterpene (26)
15	Tetrahydrostefabine	371	355	Alkaloid (41)
16	Triacetyl-d-glucopyranosyl-tetrahydroxyurone	575	543, 427, 413, 395, 265, 247	Flavonoid (58)
17	Pinoresinol diglucopyranoside	683	664, 621, 609, 412, 310	Lignan (44)
18	Bruceantinoside A	711	692, 607, 579, 531, 429, 338	Coumarin (61)
19	Peonidin 3-O-caffeoyl-O-rhamnosylglycoside-5-O-glycoside	933	915, 901, 859, 753, 724, 656, 597, 575, 516, 333	Anthocyanidin (18)
20	Watterose H	1127	1051, 915, 723, 691, 575	Oligomer (28)

[M-H]: Ionic mass/load ratio; (MSn): Multi-stage fragmentation; [M-H]⁻: Negative ionic mode; [M-H]⁺: Positive ionic mode.

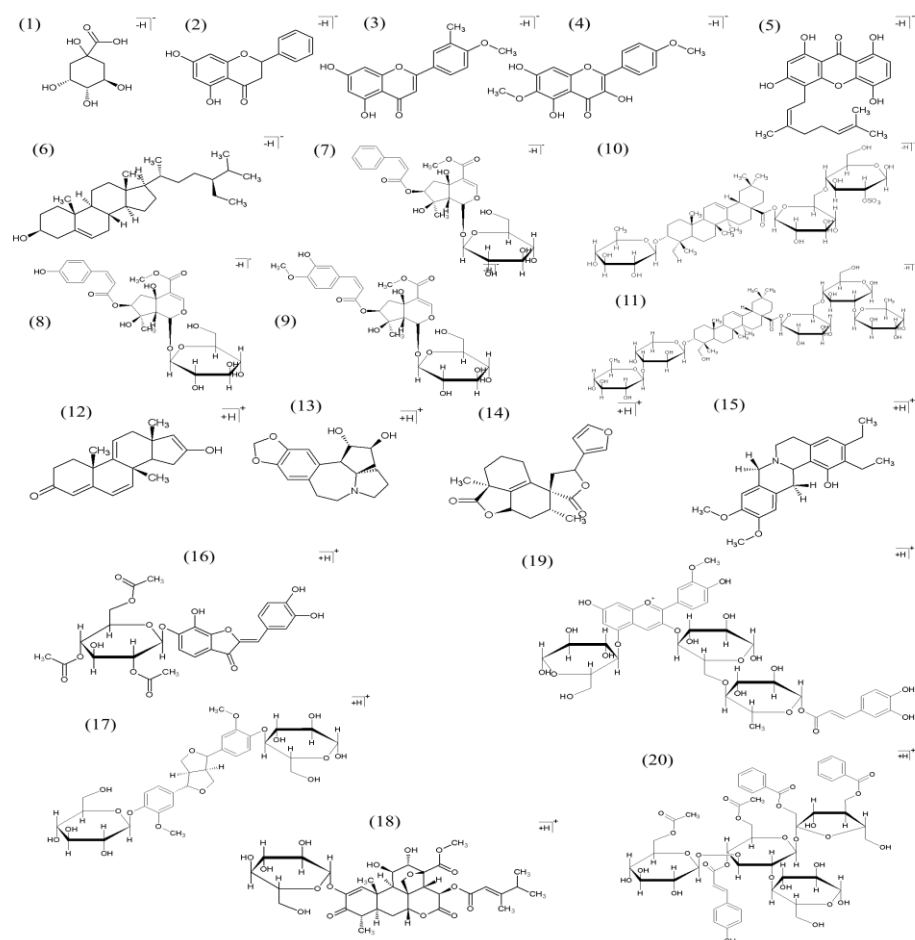


Figure 4. Chemical structures of *D. erecta* L. leaves. (1) Ácido quínico; (2) Pinocembrina; (3) Diosmetina; (4) Betuleto; (5) Cheffouxanthone; (6) β -Sitosterol; (7) Duranterectosídeo B; (8) Duranterectosídeo C; (9) Duranterectosídeo A; (10) Durantanin IV; (11) Durantanin V; (12) Durantol; (13) Cefalezomina H; (14) Neoclerodanendiólido; (15) Tetraidrostefabina; (16) Triacetil-D-glicopiranosil-tetraidroxiaurona; (17) Pinoresinoldiglicopyranosídeo; (18) Bruceantinosídeo A; (19) Peonidina 3-O-cafeoil-O-ramnosilglicosídeo-5-O-glicosídeo; (20) Watterose H

Germination parameters

The germination capacity of *L. sativa* exposed to the dry *D. erecta* extract showed concentration-dependent inhibitory effects for Physiological germination (Fig. 5A), with a significant effect ($p < 0.05$) even at the lowest concentration. For Final germination (Fig. 5B), the 40 mg/mL concentration caused an inhibition of 21.8%. Germination speed

index also displayed concentration-dependent effects (Fig. 5C), with all concentrations delaying the germination vigor and survival of *L. sativa* seedlings.

The allelopathic effects index (Fig. 5D) exhibited stimulatory effects at 2.5 mg/mL. However, at other concentrations, the allelopathic effects were inhibitory, with the maximum inhibition (-0.55) observed at 40 mg/mL.

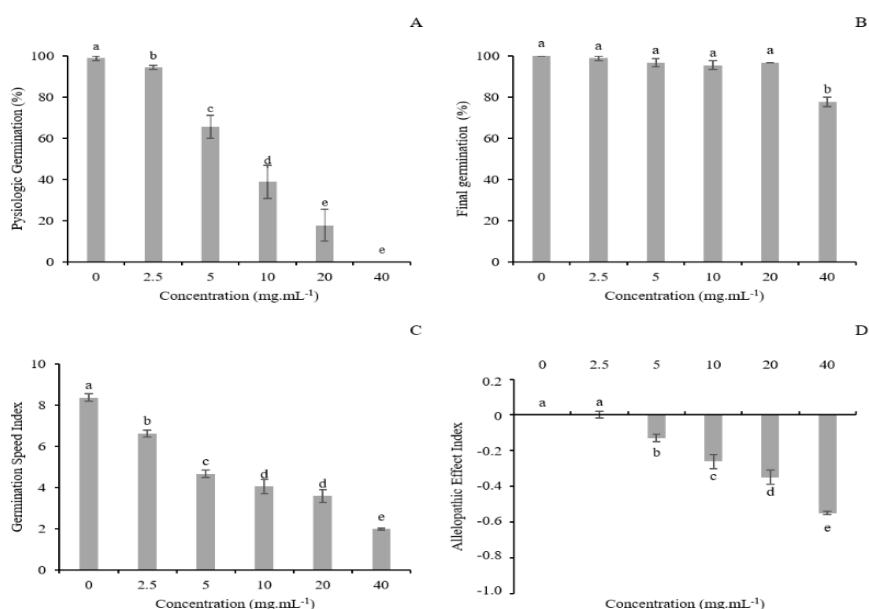


Figure 5. Effects of *D. erecta* extracts concentrations on germination parameters of *L. sativa*. A : Physiologic germination (PG) at 24 h, B : Final germination at 168 h (FG), C : Germination Speed Index (GSI), D : Allelopathic Effect Index (RI). Same letters do not differ by the Scott-Knott test at 5 % significance.

Tur *et al.* (56) observed that *Duranta repens* leaf extract concentrations proportionally reduced the germination speed index of seeds in two bioassays used, with no significant effect on the final germination of *L. sativa*. However, for *L. esculentum*, final germination was significantly reduced. Additionally, the aqueous extract of *D. repens* had bioprotective effects on *S. lycopersicum* seedlings, positively affecting their survival and germination under saline conditions (39).

Borella *et al.* (10) reported inhibition in the allelopathic effect index of radish seeds at all concentrations of *Trema micrantha* leaf extracts, while *Solidago canadensis* leaf extracts showed both inhibitory and stimulatory effects depending on the concentration used on *L. sativa* (57).

Among the compounds identified in the dry extract, Diosmetin (3) may be directly linked to the inhibition observed in the germination bioassay. It has been shown to be the

cause of *Avena fatua* L. allelopathic activity, which lowers the germination rate and growth of *Triticum aestivum* L. seedlings (33). On the other hand, β -Sitosterol (6), present in the ethyl acetate extract of *Oryza sativa* bark, showed no inhibitory activity on the germination of *Leptochloa chinensis* L., *Amaranthus retroflexus* L., or *Cyperus difformis* L. (15). The allelopathic effect often manifests not only in germination percentage but also in root elongation, germination speed, and allelopathic effect indices, indicating that cytogenetic and/or ecophysiological events may be influencing germination and the initial growth of the bioassay (5,31).

The use of *L. sativa* 'Babá de Verão' proved to be an efficient model to evaluate allelopathic effects due to its high responsiveness and homogeneity in the evaluated parameters, particularly for rapid germination, where the control treatment reached 98% of Physiological germination within 24 hours, as reported by (23,38,47).

Morphological parameters

Regarding the morphological parameters of *L. sativa* seedlings, the root length (Fig. 6A) showed statistically significant differences from the control at all concentrations ($p < 0.05$). Starting from 2.5 mg/mL, there was a reduction of 90% in root length. Similarly, the shoot length of *L. sativa* (Fig. 6A) was reduced by 66.2% at the 40 mg/mL concentration. The anomalous formation of the radicle (Fig. 7) at all concentrations further demonstrated the activity of phytotoxic compounds, with a greater impact on the root system than on germination. Fig. 6B depicts the inhibitory effects of the dry extract on the fresh biomass of *L. sativa* seedlings, showing the same concentration-dependent behavior observed for root length.

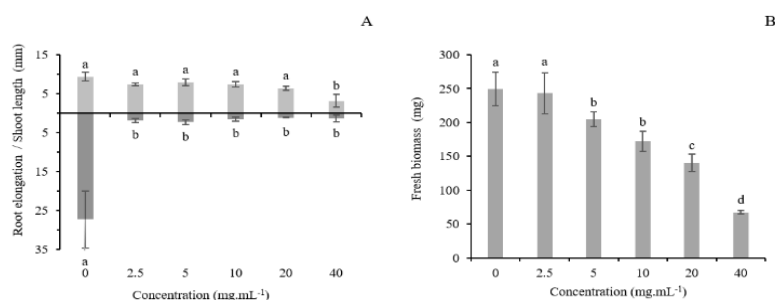


Figure 6. Effects of *D. erecta* extracts concentrations on morphological parameters of *L. sativa*. A : Root elongation (RE) / Shoot length (SL), B : Fresh biomass (FB). Same letters do not differ by the Scott-Knott test at 5% significance.

The results for root elongation are consistent with those obtained by Tur *et al.* (56), who reported inhibitory effects of *D. repens* leaf extracts in bioassays with *L. esculentum*. The reduction in root elongation may be related to the presence of saponins called durantanins. Hiradate (24) isolated durantanin I, II, and III from *D. repens* leaves and found that these substances, when applied to *Brassica juncea*, had an inhibitory effect on root elongation. Saponins (10 and 11), characterized as durantanin IV and V, have shown cytotoxic effects against various tumor cell lines and lethality against *Artemia salina* (3). Due to their structural similarity to those described by Hiradate (24), these compounds may be responsible for the reduction in root elongation in *L. sativa*.

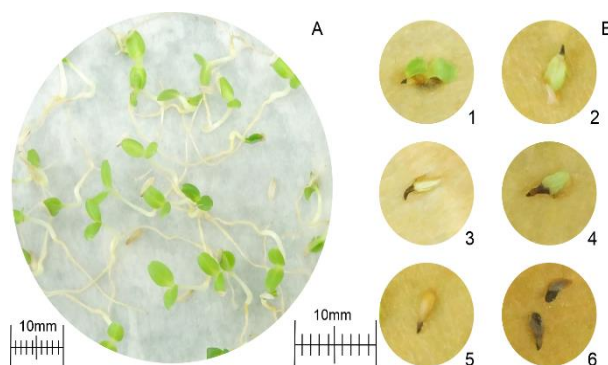


Figure 7. Morphological characteristics observed in *L. Sativa* seedlings at 168 h. A : Control treatment; B : Seedlings exposed to dry *D. erecta* L. extracts at different concentrations, respectively: 1, 2.5 mg.mL⁻¹; 2, 5 mg.mL⁻¹; 3, 10 mg.mL⁻¹; 4, 20 mg.mL⁻¹; 5 and 6, 40 mg.mL⁻¹.

Other chemical compounds identified with activity similar to durantins might act synergistically and potentiate the phytotoxic effects. The acetylated forms of duranterectosides (7,8,9) were evaluated by Ayeb-Zakhama *et al.* (6) as potent allelochemicals in the inhibition of root and shoot growth of *L. sativa*. Allelopathic action was also observed in other iridoids according to Abd-Elgawad *et al.* (1). Pinoresinol is reported as an inhibitor of wheat coleoptile elongation by Fuentes-Gandara (19). This effect may also occur in the presence of its derivative Pinoresinol diglucopyranoside (17). Durantol (12) is another possible candidate for the inhibitory action on the root, as it has been shown to cause growth inhibition of sorghum downy mildew germ tubes by damaging membrane integrity (52).

The concentration-dependent effects observed for root elongation in aqueous and hydroethanolic leaf extracts of different plant species often lead to an anomalous development of the root system, causing delays and deformations in the shoot development of the bioassays (4,38), and resulting in changes in the biomass of the bioassay. Quinic acid (1) is one of the compounds with a potential influence on biomass gain. This compound, found in *Ambrosia artemisiifolia* and *Xanthium strumarium*, directly inhibits the biomass, plant height, and pigment content of invasive plants (48). Among phenolic acids, different allelopathic modes of action are described, which interfere with hormonal activity, membrane permeability, and synthesis of organic compounds (30).

Cytogenetic parameters

The evaluation of the cytogenotoxic effects on *Lactuca sativa* root meristems revealed a suppressive effect at all tested concentrations. The mitotic index (Fig. 8) decreased significantly from 2.5 mg/mL, resulting in a 70% inhibition of cell division, indicating the high toxicity of the compounds present in *D. erecta* extract. This was evident from the significant reduction in the frequency of metaphase cells and an increase in condensed nuclei.

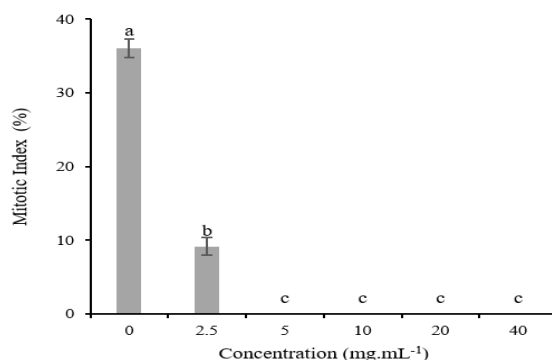


Figure 8. Effects of *D. erecta* extracts concentrations on mitotic Index (MI) of *L. sativa*. Same letters do not differ by the Scott-Knott test at 5 % significance.

The mitodepressor effects observed, with cell division inhibition evident at all concentrations, indicate the cytotoxic action of the examined extract (14,31). The root appears to be particularly susceptible to the influence of allelochemicals, as its growth is directly linked to cell division. Inhibition of cell division disrupts its ability to develop normally, as supported by the data obtained from the mitotic index. Previous studies on leaf extracts have also shown a correlation between inhibitory effects on target plant cytogenetics and impaired primary root development (4,5,14,37,38).

This study detected several classes of allelochemical compounds, such as phenolic compounds, alkaloids, coumarins, steroids, and flavonoids, all of which display cytotoxic effects. For instance, quinic acid (1), present in *Tectona grandis* leaves, has been established as a potent cytotoxic agent (21). Similarly, derivatives of betuletol (4) exhibit cytotoxic effects on the HL-60 cell line, with implications in the induction of apoptosis (45). Additionally, research on alkaloids from *Stephania pierrei* has shown that all alkaloids belonging to the tetrahydroprotoberberine group, including tetrahydrostefabine (15), enhance cytotoxic and antimalarial activity (34).

Morita *et al.* (35) have identified a cluster of alkaloids termed Cephalezomines, among which Cephalezomine H (13) exhibits notable cytotoxic activity against lymphoma and carcinoma cells. The presence of these alkaloids potentially contributes to the enhancement of cytotoxic effects, with these metabolites demonstrating significant cytotoxicity. Moreover, purine alkaloids have also been shown to possess remarkable inhibitory effects on the formation of cell colonies derived from lettuce protoplasts (49).

The coumarin identified as Bruceantinoside A (18) exhibits a moderate level of cytotoxicity against the growth of two cancer cell lines (61). Some coumarins, as reported by Govêa *et al.* (22), have the capability to reduce cell division and, consequently, the mitotic index of *L. sativa* seedlings. On the other hand, β -Sitosterol (6) demonstrates low lethal potential in terms of genotoxic activity and is not considered genotoxic and/or cytotoxic in animal models. However, this substance does increase caspase activity, leading to apoptosis and inhibition of cell proliferation (46).

In reviews conducted by Bagatini *et al.* and Silveira *et al.* (7,51), plant bioassays are highlighted as the primary screening method for assessing the toxicity of compounds derived

from plant extracts and environmental samples, particularly concerning genotoxicity and mutagenesis, before proceeding to animal bioassays.

The wide array of substances identified in *D. erecta*, known for their allelopathic and cytogenotoxic properties, suggests that these compounds may act independently or synergistically, thereby inducing phytocytotoxic effects on various parameters of plant growth and development.

CONCLUSIONS

In the leaves of *Duranta erecta* L., numerous phytochemicals with phytotoxic potential have been characterized and may be associated with the observed reduction in evaluated parameters in *Lactuca sativa*. Despite the minimal impact on the germination of *Lactuca sativa*, the dry extract of *D. erecta* L. significantly diminished the germination vigor. Furthermore, the extract exerted inhibitory effects on later stages of plant development, specifically impeding root and shoot growth and leading to a reduction in seedling mass. As a result, the extract derived from *D. erecta* leaves demonstrates bioherbicide potential through its capacity to decrease germinative, morphological, and cytological parameters in *L. sativa*.

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CONFLICT OF INTEREST

The authors have not disclosed any competing interests. We declare that all authors of this Ms have made substantial contributions. We have not excluded any author that substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

AUTHORS CONTRIBUTION

J.V.B. Calvelli: Investigation, Formal analysis, Data Curation, Writing - Review & Editing, Visualization, Project administration; **V.M. Betelli:** Investigation, Writing - Original Draft, Visualization; **D.V.B. Braga:** Investigation; **R.G. Bastos:** Formal analysis, Data Curation; **A.R. Cunha Neto:** Writing - Review & Editing; **W. Vilegas, M.J.D. Silva and M.A. da Silva:** Resources; **G.A. da Silva and S. Barbosa:** Writing - Review & Editing, Supervision, Funding acquisition, Project administration

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