

# *Dalbergia brasiliensis* Vogel extracts *in vitro* antioxidant activity and phytotoxic effect in *Lactuca sativa* and *Allium cepa*

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**Abstract:** Based on medicinal use of *Dalbergia* species, the present study aimed to evaluate *D. brasiliensis* leaves and barks crude extracts and fractions' antioxidant and phytotoxic potential, employing allelopathic essays and 3 antioxidant essays: Phosphomolybdenum complex reduction, DPPH free radical scavenging and TBARS (lipid peroxidation). Concerning Phosphomolybdenum complex reduction potential, leaves chloroform fraction (LCF) and barks ethyl acetate fraction (BEAF) overcame rutin's reduction potential in 90% and 23%. DPPH essay revealed leaves ethyl acetate fraction (LEAF) antioxidant potential (IC<sub>50</sub> de 40,629 µg/mL), given standards ascorbic acid (IC<sub>50</sub> = 4,503 µg/mL) and rutin (IC<sub>50</sub> = 7,330 µg/mL) activities. TBARS essay demonstrated that leaves hexane fraction (LHF) (51%) and barks ethyl acetate fraction (BEAF) (41%) stand out when compared to BHT antioxidant activity (54%). Regarding phytotoxicity, the lowest crude extracts and fractions concentration (250 µg/mL) employed were able to inhibit *L. sativa* and *A. cepa* primary root initial growth, specially leaves hexane fraction (LHF) and leaves chloroform fraction (LCF). *Dalbergia brasiliensis* fractions might function as stress inhibitors in oxidant systems and alter meristem cellular division, due to present substances' activity.

**Keywords:** Allelopathy, primary root inhibition, antioxidant effect, oxidative stress

## INTRODUCTION

A wide range of plants produce compounds, which, in certain amounts, may provoke toxic effects to surrounding plant species (Harborne, 1994). Allelopathy defines plant interactions employing phytotoxins, once donor plant negatively affects target plant, providing advantage to donor plant (Dayan & Duke, 2009). Secondary metabolites are able to bring on beneficial and harmful effects, depending on some characteristics such as amount, chemical properties and present functional groups (Barbosa et al., 2005; Goldfarb et al., 2009). Also, are considered as greatly responsible for allelopathic effects observed in nature, since these compounds are important agents against natural predators and ultraviolet radiation, including simple phenolic and

heteroside compounds, coumarins, xanthans, tannins, flavonoids, saponins, alkaloids and sulfur compounds (Haida et al., 2010; Jácome et al., 2004).

*Dalbergia* genus (Fabaceae), comprehends shrubs, creepers and small to medium sized trees, widely distributed in tropical and subtropical regions. Many *Dalbergia* species are employed in Chinese traditional medicine (Carvalho, 2004) and present confirmed biological properties like analgesia, anti-inflammatory, antihelminthic, antioxidant, antimicrobial, antiulcerogenic, antitumoral, larvicidal, among others. Genus chemical characteristics are flavonoids, isoflavonoids, proanthocyanidins, xanthonoids, neoflavonoids, glycosides, cinamoil phenols, benzophenones and rotenoids (Vasudeva et al., 2009; Saha et al., 2013).

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*Dalbergia brasiliensis*, popularly known as jacarandá, caroaba-brava, caviúna or marmeleiro, is distributed among Cerrado regions and Brazilian Atlantic Forest (Carvalho, 2004). *Dalbergia brasiliensis* research scarcity along *Dalbergia* genus known biological and pharmacological potential, encouraged antioxidant activity evaluation by three methods (phosphomolybdenum complex, TBARS and DPPH) and allelopathic potential evaluation on *Lactuca sativa* e *Allium cepa* inicial growth.

## MATERIAL AND METHODS

### Plant material collection

*D. brasiliensis* leaves and barks were collected in Curitiba-Paraná (Brazil) 25°26'48.0''S; 49°20'51.2''W. Plant material taxonomic identification was determined at Municipal Botanic Museum Herbarium (Curitiba) and kept at room temperature for 10 days, until dehydration. Dried plant material was stored in plastic bags, protected from light and heat.

### Crude Ethanolic Extract and Fractions

Leaves and barks crude ethanolic extracts were obtained in Soxhlet apparatus, employing ethanol 96° GL, approximately at 78°C. Crude ethanolic extracts were vacuum filtered (Büchner) and concentrated in rotary evaporator (reduced pressure, 78°C, 150 rpm). Crude ethanolic extracts were fractioned by liquid-liquid partition in modified Soxhlet apparatus (Carvalho et al., 2009) employing solvents in increasing polarity scale, obtaining leaves' and barks' hexane, chloroform and ethyl acetate fractions. Crude ethanolic extracts and fractions were evaporated (water bath, 50° C) and stored in cool dry place.

### Antioxidant Activity Evaluation

Leaves and barks crude ethanolic extracts and fractions had *in vitro* antioxidant potential evaluation determined employing three different methods.

- *Phosphomolybdenum complex reduction*

Samples' ability to reduce phosphomolybdenum complex were evaluated according to Prieto *et al.* (1999). Samples were diluted in metanol (200 µg/mL). Reagent solution (complex solution) containing sodium phosphate 0,1M solution (28 mL), ammonium molybdate 0.03M solution (12 mL) and sulfuric acid 3M solution (20 mL) had it final volume adjusted to 100 mL. Sample solution was added (0.3

mL) to reagent solution (3 mL) and test tubes were kept sealed in water bath (95° C) for 90 minutes. After cooling, solutions were analyzed in spectrophotometer (Shimadzu, UV 1601) at 695 nm.

Results showed samples' Relative Antioxidant Activity (RAA%) when compared to standards (ascorbic acid and rutin) by following equation:

$$RAA\% = \frac{Abs\ sample - Abs\ blank}{Abs\ standard - Abs\ blank} \times 100$$

- *Free Radical Scavenging [2,2-diphenyl-1-picrylhydrazyl (DPPH)]*

Samples' DPPH scavenging potential were evaluated according to Mensor *et al.* (2001). Samples and standards (rutin and ascorbic acid) methanolic solutions were obtained (15 to 125 µg/mL, except hexane fractions: 100 e 450 µg/mL). To each solution (2.5 mL), DPPH methanolic solution (0.03 mmol/mL) was added (1 mL). Blank samples (2.5 mL of sample solution and 1 mL of methanol) were also evaluated. After thirty minutes, samples, standards and blank samples were analyzed in spectrophotometer (518 nm, which corresponds to maximum DDPH absorption). Samples' ability to scavenge DPPH was determined as following:

$$\%DPPH\ inhibition = 100 - \frac{(Abs\ sample - Abs\ blank) \times 100}{Abs\ blank}$$

Samples IC<sub>50</sub> (50% of inhibitory concentration) were determined by linear regression, establishing an y=ax+b equation, for comparing purposes.

- *Thiobarbituric Acid Reactive Substances (TBARS)*

Samples' ability to inhibit lipid peroxidation were evaluated according to adapted Moraes *et al.* (2006). Samples were diluted in methanol (1000 ppm) and butylated hydroxytoluene (BHT) was employed as standard. Filtered egg yolk (5%) in sodium dodecyl sulfate solution (0.55%) was employed as lipid source.

Each sample (1000 µL) was added to distilled water (400 µL) and lipid solution (500 µL). Afterwards, to each tube: chlorine 2.2-azo-bis-acid (ABAP) 0.035% solution (50 µL), 1.5 mL of acetic acid (20%, pH 3.5) and thiobarbituric acid (TBA) solution (0.4%) were added. Tubes were kept sealed in water bath (95° C) for an hour. After cooling, n-butanol (1.5 mL) was added and well mixed, aiming to extract lipid material. Supernatants were collected after centrifugation (3000 rpm, 5 minutes) and analyzed in

spectrophotometer (532 nm). Antioxidant Index (AI%) was determined by following equation:

$$AI\% = 1 - \left( \frac{Abs\ sample}{Abs\ standard} \right) \times 100$$

### **Allelopathic activity evaluation**

#### *Germination and growth essays*

*D. brasiliensis* samples' allelopathic effects were evaluated on lettuce (*Lactuca sativa*) and onion (*Allium cepa*) germination and growth. Extracts and fractions solutions (DMSO 1%) were prepared in 250 µg/mL, 500 µg/mL and 1000 µg/mL concentrations. To previously autoclave sterilized (120° C, 20 minutes) Petri dishes (9.0 cm diameter) containing Whatman filter paper (n° 1,0), samples solutions (5 mL) were added. Samples and control (sample absence) solutions were buffered by MES (2-morpholine ethanesulfonic acid) (10 mM), adjusting pH (6.0) by adding KOH 0.1 N (Macias et al., 2000).

Each Petri dish was randomly sown (30 seedlings) with *Lactuca sativa* and *Allium cepa*, separately in quadruplicates, as described in Brasil (2009). Sown Petri dishes were placed in BOD germination chamber (relative humidity ± 80% and constant temperature: 25° C for lettuce and 15° C for onion). Lettuce seedlings were incubated in the dark and onion seedlings in 12 hour photoperiod, as suggested in Brasil (2009).

Germination bioassays followed Macias *et al.* (2000) methodology. Data collection was performed daily for seven days, evaluating radicle protusion (minimum length 2.0 mm). Essay conclusion was attested when germination became nule for three consecutive days. Speed Germination Index (SGI) was determined according to Maguire (1962), as shown on following equation:

$$SGI = \frac{N\ germinated\ seedlings}{N\ days\ of\ germination} + \frac{N\ germinated\ seedlings}{N\ days\ of\ germination} + \dots$$

Growth bioassays followed Barnes et al. (1987) and Macias et al. (2000) described methods. After seven days of radicle protusion, root and hypocotyl/coleoptile were measured over millimeter paper (10 seedlings from each Petri dish).

### **RESULTS AND DISCUSSION**

Essays performed on *D. brasiliensis* extracts and fractions, demonstrated fractions' antioxidant activity varied between *in vitro* methods (Table 1).

Concerning reduction potential, evaluated through phosphomolybdenum complex essay, LCF, BCF and BEAF overcame rutin's activity in 90%, 5% and 23.8%, respectively. LEAF nealy achieved rutin's activity, reaching 93% of rutin's antioxidant activity. Free radical scavenging (DPPH) essay revealed higher antioxidant potential for LCF (IC<sub>50</sub> =42.343 µg mL) and LEAF (IC<sub>50</sub> = 40.629 µg mL). Extracts and fractions are complex compound blends, therefore rarely will show similar activity as standards ascorbic acid (IC<sub>50</sub> = 4.503 µg mL) and rutin (IC<sub>50</sub> = 7.330 µg mL). TBARS essay demonstrated LHF activity (51%) as similar to standard BHT (54%). Data revealed species' antioxidant potential through different mechanisms, confirming *Dalbergia* medicinal use.

Free radicals or reactive oxygen species are generated by exogenous chemical products or endogenous metabolic processes and are able to oxidize biomolecules, such as nucleic acids, proteins, lipid and DNA. These molecules may initiate different degenerative illnesses like neurological disturbs, cancer, emphysema, cirrhosis, arthritis, atherosclerosis, among others (Halliwell, 2007; Sies, 1997).

Mammals natural antioxidant mechanisms are not always efficient, therefore antioxidant compounds intake becomes an alternative supply, pointing lower rates of antioxidant-related diseases (Antolovich et al., 2002). Supplement ou tea intake protects organism against free radical effects (Szent-Giörgyi, 1963). Many medicinal plants showed antioxidant activity throught pharmacological traditional methods for the past two decades (Kasote et al., 2013). Knowing that, the present study aimed evaluating *D. brasiliensis* antioxidant activity, since other genus species are traditionally known for its antioxidant properties.

Etnopharmacological and nutraceutical *in vitro* investigation often employ antioxidant evaluation methods to track and verify plants and its phytochemicals antioxidant potentials. Still, many times, plant extracts oxidant mechanism are difficult to comprehend. Therefore, the present study evaluated antioxidant activity employing methods based on antioxidant mechanism, like DNA damage prevention, lipid peroxidation inhibition or ammonium molibdate reduction (Antolovich et al., 2002).

Table 1. *D. brasiliensis* effects on antioxidant activity essays

Sample	Phosphomolybdenum		DPPH	TBARS
	Rutin RAA (%)	Ascorbic Acid RAA (%)	IC <sub>50</sub> (µg/mL) x± dp	AI(%)
LCEE	45,78	11,67	72,476±1,0293	30,16
LHF	102,81	26,22	165,602±2,1437	51,57
LCF	190	48,46	42,343±1,1240	43,21
LEAF	93,75	23,91	40,629±0,1384	36,88
BCEE	50,93	13,23	48,303±0,6064	34,12
BHF	73,98	17,6	273,868±2,1608	33,95
BCF	105,01	24,57	43,572±1,0178	34,7
BEAF	123,82	28,98	47,310±0,1298	41,87
Ascorbic Acid	-	-	4,503±0,0507	-
Rutin	-	-	7,330±0,1168	-
BHT	-	-	-	54,03%

Note: LCEE: leaves crude ethanolic extract; LHF: leaves hexane fraction; LFC: leaves chloroform fraction; LEAF: leaves ethyl acetate fraction; BCEE: barks crude ethanolic extract; BHF: barks hexane fraction; BCF: barks chloroform fraction; BEAF: barks ethyl acetate fraction;

Antioxidant activity essays are performed in medicinal plants in order to evaluate free radical elimination potential, which leads to understanding plants' role in oxidative stress minimizing, as well as related illnesses physiopathology. In this aspect, it is important to state *D. brasiliensis* phytotoxic activity

provided by its antioxidant characteristics and interferes in other plants' growth.

Results on lettuce and onion initial growth show *D. brasiliensis* allelopathic potential on seedlings, as growth parameters evaluated on laboratory were altered. All samples in every concentration were able to inhibit lettuce germination period. LHF and BHF provided accentuated delay, when compared to other samples. BAEF provided similar effect to BHF, as shown on Table 2.

Table 2. *D. brasiliensis* effects on *L. sativa* laboratory germination speed index

<i>Lactuca sativa</i> Germination Speed Index (GSI)		
Crude ethanolic extracts		
n = 4 repetitions +-	Leaves	Barks
Control	83.33±2.19	98.00±0.66
1000 µg/mL	69.48±3.18*	47.58 ±1.17*
500 µg/mL	74.24±2.23*	49.16±1.26*
250 µg/mL	87.3±3.45*	48.81±2.65*
Hexane Fractions		
n = 4 repetitions	Leaves	Barks

Control	83.33±2.19	98.00±0.66
1000 µg/mL	44.63±2.47*	47.45±1.17*
500 µg/mL	47.54±1.28*	47.80±2.37*
250 µg/mL	49.54±1.12*	48.33±1.22*
<b>Chloroform Fractions</b>		
<b>n = 4 repetitions</b>	<b>Leaves</b>	<b>Barks</b>
Control	83.33±2.19	98.00±0.66
1000 µg/mL	61.66±1.33*	48.88 ±1.17*
500 µg/mL	57.77±2.37*	47.70±1.26*
250 µg/mL	64.72±1.89*	47.62±2.65*
<b>Ethyl acetate Fractions</b>		
<b>n = 4 repetitions</b>	<b>Leaves</b>	<b>Barks</b>
Control	83.33±2.19	98.00±0.66
1000 µg/mL	72.36±2.47*	48.56±1.17*
500 µg/mL	83.95±1.28 <sup>NS</sup>	48.26±2.37*
250 µg/mL	84.44±1.12 <sup>NS</sup>	47.22±1.22*

<sup>1</sup>Mean ± standart deviation. \*Treatment mean differs significantly (P < 0.05) when compared to control mean on Dunnet test. <sup>NS</sup> Treatment mean does not differ significantly when compared to control mean.

Hormesis effect was observed in onion seedling treated with LCEE and LHF, revealing inhibitory activity in 250 µg.ml<sup>-1</sup> concentration (75.91 e 74.43, respectively). LCF and LEAF boosted onion seedlings germination speed in all concentrations in a

non dose-dependent mean. All BCEE and BEAF concentrations inhibited onion seedlings germination speed and LHF and LCF (both in 500 and 1000 µg.ml<sup>-1</sup>) provided most damages, revealing selective mechanism to monocotyledons (Table 3).

Table 3. *D. brasiliensis* effects on *A. cepa* laboratory Germination Speed Index

<i>Allium cepa</i> Germination Speed Index (GSI)		
Crude Ethanolic Extracts		
n = 4 repetitions +-	Leaves	Barks
Control	41.73±1.84	64.67±1.15
1000 µg/mL	45.80±1.23*	40.45 ±1.12*
500 µg/mL	66.82±1.56*	46.33±1.00*
250 µg/mL	75.91±1.07*	34.54±1.07*
Hexane Fractions		
n = 4 repetitions	Leaves	Barks
Control	41.73±1.84	64.67±1.15
1000 µg/mL	62.19±1.33*	33.84±1.05*
500 µg/mL	58.39±1.17*	33.77±1.12*
250 µg/mL	74.43±1.13*	47.29±1.00*
Chloroform Fractions		
n = 4 repetitions	Leaves	Barks
Control	41.73±1.84	64.67±1.15
1000 µg/mL	75.07±1.33*	37.60 ±1.00*
500 µg/mL	68.63±2.37*	35.58±1.12*
250 µg/mL	76.23±1.89*	43.20±1.05*

Ethyl Acetate Fractions		
n = 4 repetitions	Leaves	Barks
Control	41.87±1.13	64.67±1.15
1000 µg/mL	74.26±1.17*	48.03±1.05*
500 µg/mL	72.86±1.33*	42.43±1.13*
250 µg/mL	84.44±1.32*	41.48±1.15*

<sup>1</sup>Mean ± standart deviation. \*Treatment mean differs significantly (P < 0.05) when compared to control mean on Dunnet test. <sup>NS</sup> Treatment mean does not differ significantly when compared to control mean.

Concerning primary root, samples' 250 µg/ml concentrations were able to affect lettuce root growth, highlighting LCF (24%) and BCEE (17%). Inhibitory effect was also observed in LHF (500 µg/ml: 11.1% and 1000 µg/ml: 11.44%) while LEAF (1000 µg/ml) boosted root growth in 16.15% (Figure 1A and B). BEAF 1000 µg/ml concentration also showed primary root growth inhibition in *L. sativa* in 11% (Figure 1B).

Hormesis effect was also observed in onion, revealing inhibitory activity in crude ethanolic extracts 250 µg/ml concentrations. Fractions showed similar activity, especially LHF (46%) and LHF (42%). Although inhibitory effect was also observed in barks crude ethanolic extracts, was considered minor when compared to leaves extracts and fractions (Figure 1C and D).

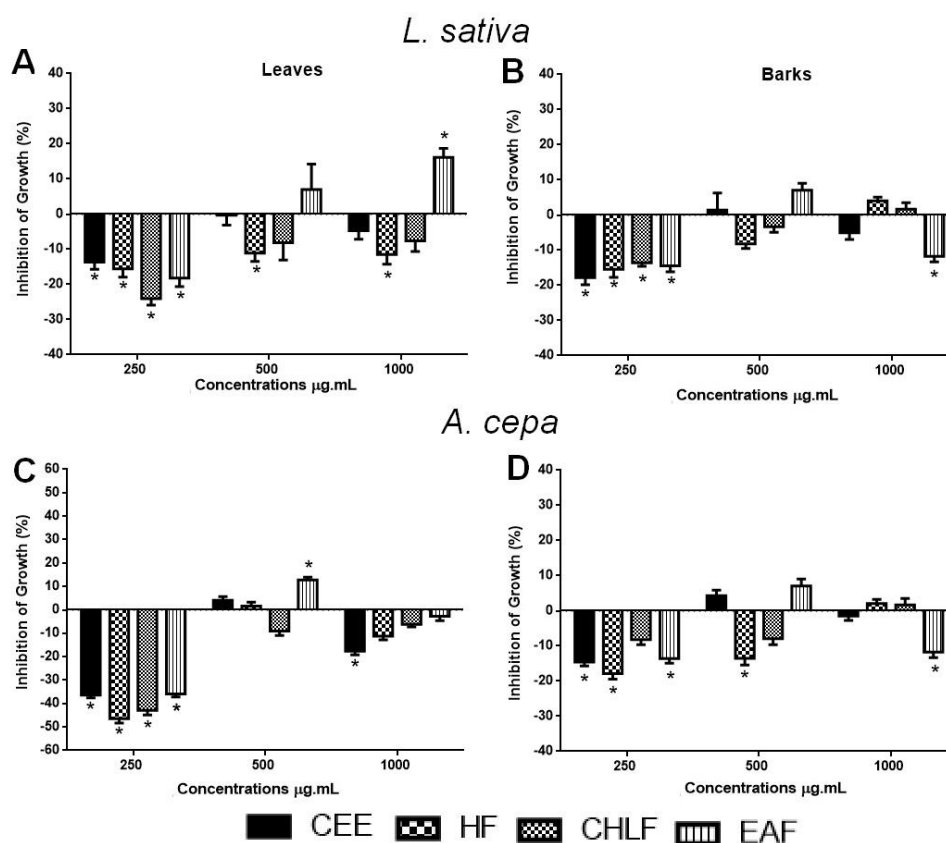


Figure 1. Effects of Crude ethanolic extract (CEE), Hexane fraction (HF), Chloroform fraction (CF) and Ethyl acetate fraction (EAF) of *D. brasiliensis*, on growth the primary root in leaves (A), barks (B) of *L. sativa*, and growth the primary root in leaves (C), barks (D) of *A. cepa*. Data expressed in percentage compared to the control. \*Statistically different from the control treatment (Dunnett Test, p<0.05).

Concerning hypocotyl growth in *L. sativa*, inhibitory effect was also observed in LHF (16.8%), LEAF (11.5%), BHF (12.7%) and BEAF (12.8%) at 250 µg/ml concentration, revealing phytotoxic activity is not related to extracts concentrations (Figure 2A and B). In *A. cepa*, 250 µg/ml concentration provided coleoptile inhibition: LCEE (38.4%), LHF (42%) and

LEAF (35.8%), LEAF 500 µg/ml concentration inhibit growth in 46%. BCEE and BCF at 500 µg/ml concentration provoked coleoptiles elongation in 19% and 58%. All fractions in 250 µg/ml concentration inhibit coleoptiles growth, especially HF (35%) and CF (36%) when compared to control.

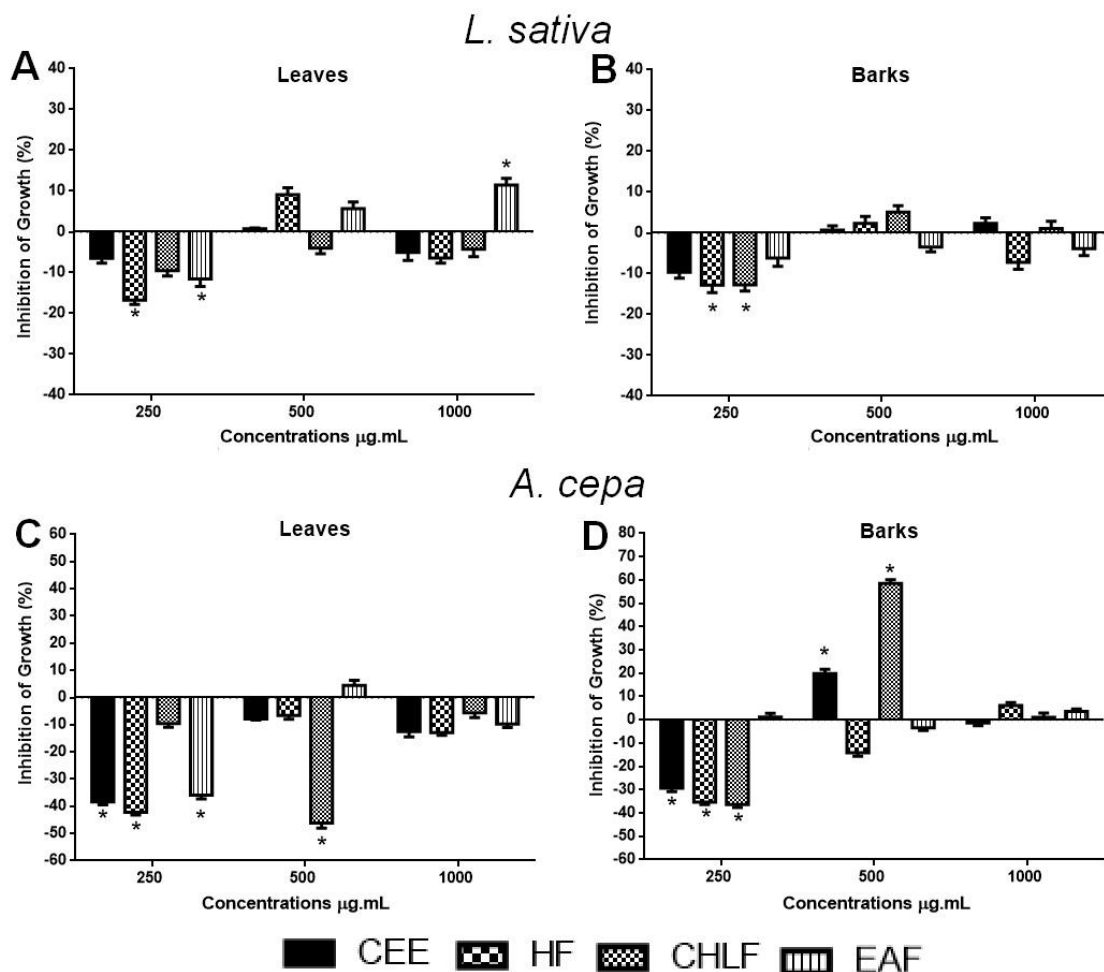


Figure 2. Effects of Crude ethanolic extract (CEE), Hexane fraction (HF), Chloroform fraction (CF) and Ethyl acetate fraction (EAF) of *D. brasiliensis*, on growth the Hypocotyl in leaves (A), barks (B) of *L. sativa*, and growth the coleoptyl in leaves (C), barks (D) of *A. cepa*. Data expressed in percentage compared to the control. \*Statistically different from the control treatment (Dunnnett Test,  $p < 0.05$ ).

Lipophilic fractions (hexane and chloroform fractions) results might be related to its revealed antioxidant profile. Allelopathic studies have demonstrated that lipidic substances like terpenes may affect cell proliferation and DNA synthesis on plant meristems (Nishida et al., 2005), once mitosis get altered, leading to rampant increase on dividing cells, damaging tubulins, resulting in polyploids nucleus (Hallak et al., 1999; Sanchez-Moreiras et al., 2008).

*L. sativa* and *A. cepa* root growth were most affected than hypocotyls and coleoptiles. Baziramakenga et al., (1995) emphasizes that, besides root contact with extracts, root tissues are more permeable to allelochemicals which may alter tissues' initial growth (Nishida et al., 2005), damaging root metabolic activities and cellular division on root extremities that might lead to dry weight reduction (Singh et al., 2003).

Over the present investigation, negative impact observed on discussed parameters is possibly due to allelochemicals large amounts, which might, significantly or not, have raised stress levels on *L. sativa* e *A. cepa*. Then, in line with plant activity studies strategies' on evaluating antioxidant and phytotoxic potential, it is advisable to combine *in vivo* antioxidant assays and toxicity evaluation.

### CONFLICTS OF INTEREST

The authors declared no conflict of interest.

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