International Journal of Agricultural Technology 2024 Vol. 20(4):1527-1544
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Investigating plant growth and germination inhibition of extract from *Leucaena leucocephala* (Lamk.) de Wit

Quy, N. T.[1], Ngan, N. L.[1], Tram, N. N.[2] and Men, T. T.[2*]

[1]Institute of Food and Biotechnology, Can Tho University, Can Tho City, Vietnam; [2]College of Natural Sciences, Can Tho University, Can Tho City, Vietnam.

Quy, N. T., Ngan, N. L., Tram, N. N. and Men, T. T. (2024). Investigating plant growth and germination inhibition of extract from *Leucaena leucocephala* (Lamk.) de Wit. International Journal of Agricultural Technology 20(4):1527-1544.

Abstract The utilization of chemically derived herbicides has caused many negative impacts, it is essential to investigate some biosynthetic products, being an alternative for inhibiting weeds. The inhibition of *Leucaena leucocephala* (Lamk.) de Wit. extract on plant germination and growth is reported. Many components belonging to the allelochemical group were found in distinctive parts' extract of *L. leucocephala*, mostly detected in leaves with flavonoid 292.7 mg QE/g extract and phenolic 397.6 mg GA/g extract. The findings proved that the extract inhibited the plant germination and growth in various test concentrations, being the highest point at the concentration of 20 mg/mL extract. In this condition, the inhibitory effects regarding each parameter were 70% (seed germination), 91.46% (root length), 69.89% (stem length), 78.73% (fresh weight), and 49.53% (dry weight). Furthermore, this study also revealed the inhibitory concentration of 50% (IC_{50}) of the extract on germination, starting the inhibition at 17.40 mg/mL. The content of photosynthetic pigments and water uptake decreased gradually after 7-day treatment, suggesting that the ethanol extract from *L. leucocephala* influenced these biological processes which directly caused the plant growth inhibition.

Keywords: Allelopathy, *Leucaena leucocephala,* Germination and growth inhibition, *Raphanus sativus* L.

Introduction

Weeds are one of the most prevalent causes resulting in the decline of agriculture both in productivity and quality (Kubiak *et al.*, 2022). To be specific, weeds have demolished over 34% of the crops (Gharde *et al*., 2018) and its consequences are anticipated at more than 100 billion US dollars. These plants can quickly absorb scarce natural resources like light, water, nutrients from the soil, and space. Because of characteristics similar to their deep root structure, tolerance to frost and drought, and high nutrient usage efficiency, they can reproduce more quickly than cultivated plants. Furthermore, weeds

[*]**Corresponding Author:** Men, T. T.; **Email:** ttmen@ctu.edu.vn

can release compounds that are allelopathic into the soil, which in turn promotes the growth of pests and crop pathogens. Due to these characteristics, they can compete with arable crops, which frequently results in lower agricultural yields and higher cultivation expenses (Trognitz *et al*., 2016).

In recent years, an increasing number of synthetic herbicides have been used to manipulate undesirable vegetation in agriculture (Gharde *et al*., 2018). Although there was much effectiveness recorded in controlling weeds, these chemically derived herbicides had serious side effects including the surge of weed resistance, changes in some physiological characteristics and contamination of soil as well as threats to other non-target organisms surrounding (Khang *et al*., 2023; Kubiak *et al*., 2022). In addition, the herbicides also negatively affected the environment and human health, which not only caused the direct influences but also existed some kinds of residues detrimental to soil and surrounding organisms, should be considerable (Jugulam and Shyam, 2019). Even though there were a multitude of measures based on physics and plant physiology had been applied to manage the growth of weed such as soil solarization, mowing, flaming, transplant, contributing to improve the features of soil as well as environmental conditions to limit the weed's growth. Furthermore, in some fields, farmers used a method called "Cover crop". It was assumed that utilizing cover crops around the boundaries of fields and in between rows could help control weeds and promote the growth of another crop in place of weeds. The kind of soil, the surrounding environment, and the ornamental crop would all influence the choice of cover crop. However, in this case, herbicides were still be used, sprayed on the cover crop to turn it into a nonliving mulch, or it could be left as a living mulch that is regularly mowed to reduce competition (Hasan *et al*., 2021). Hence, finding an alternative or biological method is essential for the development of safe and sustainable agriculture.

For several plant species, allelopathy is a biological phenomenon in which the plants evaporate or decompose plant tissue, release bioactive substances into the surrounding environment through root exudates, or leach from their aerial or underground segments. The substances that are released into the environment have the potential to either stimulate or impede the growth and development of other creatures, specifically weeds, other plants, animals, and microbes (Isin Ozkan *et al*., 2023). The allelochemicals could have adverse influences on the growth of other surrounding plant species (Xuan *et al*., 2005). Some preceded research proved that it was effective to use the extract from species having inhibitory compounds and their growth inhibitory components against weeds in the application of plant protection (Scavo *et al*., 2020; Soltys *et al*., 2013). As natural products, allelochemicals are capable of being used as

commercial herbicides for controlling and limiting the consequences of weeds in agroecosystems.

Leucaena leucocephala (Lam.) de Wit, a plant species belonging to the Fabaceae family, is an indigenous species in the Southern Mexico and Central America area (Soltys *et al*., 2013). According to the Global Invasive Species Database, *L. leucocephala* is at the top of 100 of the world's invasive alien species, meaning that it is an aggressive colonizer that harms native plant communities, particularly for species living in oceanic areas. The great invasive potential of *L. leucocephala* might come from its allelopathy (Olckers, 2011). The research of Chou and Kuo, (1986) illustrated that a significant number of allelopathic compounds found in many body parts of *L. leucocephala*, which were able to generally impact the germination and growth of the adjoining plants; however, the other physiological activities have not been explained yet.

This investigation conducted the bioassays associated with germination and growth of *L. leucocephala* extract which inhibits the growth of *Raphanus sativus* L. by qualification of the chemical compounds contained and quantification of total flavonoid and phenolic contents. Furthermore, the influences on photosynthesis and water absorption of *R. sativus* affected by *L. leucocephala* extract were examined to clearly characterize its growth inhibitory ability on other plant species.

Materials and methods

Reagents

Ethanol 96%, quercetin - 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4, sodium nitrite ($NaNO_2$) (Merck - Germany), aluminum chloride $AlCl_3$, sodium hydroxide NaOH (Merck – Germany), gallic acid - 3,4,5-trihydroxybenzoid acid (China), sodium carbonate (Na_2CO_3) (China), Folin – Ciocalteu's Phenol Reagent (Merck – Germany), acetone 80%, Tuong An Cooking Oil (Vietnam).

Plant materials

There were several parts of *L. leucocephala* (leaves, stems and fruits) collected at Can Tho University. Subsequently, they were dried by a drying machine at around 50℃. The dried samples were pulverized to a fine powder using a grinding machine.

Seeds of *Raphanus sativus* L. were purchased from Phu Nong Variation Company, which was used for testing in experiments.

Preparation extracts of L. leucocephala

The dry powder (leaves, stems and fruits) was soaked in ethanol 96% for 3 days to extract phytochemical constituents. Then, the sample was filtered through cloth and filter paper, after which the ethanol extract was collected. Each sample extracted from each part was replicated three times. The sample was put into the rotary evaporator to evaporate the solvent and get the plant extract.

Qualitative determination of chemical components

The chemical components of the ethanol extract were determined based on the principles of colored-forming reaction according to the description of previous research (Prashant *et al*., 2011; Sofowora, 1996). Many particular reactions were depicted in detail (Table 1).

Table 1. Determination of chemical compositions

Composition	Experimental performance	Observation of indicator
Alkaloid	2 mL extract + 3 - 4 drops of Mayer's reagent	White or yellow precipitation
Flavonoid	1 mL extract + 1 mL Pb $(CH_3COO)_2$ (10%)	Yellow precipitation
Saponin	1 mL extract + 5 mL distilled water + 3-4 drops of ethanol. Shaking and stabilizing for 15 min.	The column of white foam still remained static after stabilizing for 15 min.
Terpenoid	2 mL extract + 2 mL chloroform + 2-3 drops of concentrated H_2SO_4	Jade green color
Coumarin	2 mL extract + 3 mL NaOH 10%	Yellow color
Quinone	2 mL extract + 2-3 drops of concentrated HCl	Green color
Phenol	2 mL extract + 2 mL distilled water + 2-3 drops of $FeCl_3$ (5%)	Dark green or orange-red precipitation
Tannin	2 mL extract + 5 drops of Gelatin 1%	White precipitation

Quantification of total phenolic content

The total content of phenolic was determined by the method of (Dewanto *et al*., 2002). In this method, the Folin-Ciocalteu (F-C) reagent was used for quantification of total phenolic. Folin-Ciocalteu is a mixture of phosphomolybdate and phosphotungstate, having yellow reductors that are oxidized with the presence of phenolic to form the blue complex phosphotungstic – phosphomolybdenum. This complex was absorbed greatly at the wavelength of 756 nm. The total phenolic content was quantified according to the standard curve of gallic acid.

The ethanol extract of *L. leucocephala* was prepared as per the aforementioned description. 250 µL of plant extract diluted at testing concentrations was shaded well with distilled water, at which point, 250 µL of Folin reagent was added, shaded, and stood for 5 min. Then, 250 µL Na_2CO_3 10% was combined, shaded carefully and incubated at 40℃ for 30 min. The sample measured the absorbance at the wavelength of 765 nm. Gallic acid was used as a positive control. The experiments were triplicated.

Quantification of total flavonoid content

The total flavonoid content in this study was determined by the Aluminum chloride colorimetric method according to the description of (Bag *et al*., 2014). The basis for the aluminum chloride ($AlCl_3$) colorimetric approach is that $AlCl_3$ combines with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols to generate acid-stable complexes. These complexes have strong absorbance at the wavelength of 510 nm. The total content of flavonoids was quantified based on the standard calibration curve of quercetin.

The extract of *L. leucocephala* was diluted with ethanol at the concentration of 10 mg/mL, which was followed by an ultrasound-assisted technique to facilitate the dilution of extraction. 200 µL of plant extract diluted at testing concentrations was shaded well with distilled water. Then, 40 µL $NaNO_2$ 5% was added and stabilized for 5 min before adding 40 µL $AlCl_3$ 10%, followed by shaking and maintaining for 6 min. After that, 400 µL NaOH 1M and 120 µL distilled water were added and shaded carefully. The sample was measured absorbance at the wavelength of 510 nm. Quercetin was used as a positive control. The experiments were triplicated.

Germination and growth inhibition assays

The herbicidal assays were assessed by using the protocol described in the preceding research (Xuan *et al*., 2003). The sample solutions (4 mL in different concentrations including 5, 10, 15, and 20 mg/mL) were put in each Petri dish which was already loaded with the filter paper. The control treatment did not use any extract, using 1 mL methanol solvent instead. The Petri dishes were placed in a fume cupboard for 2 h to allow the methanol to evaporate completely, leaving only the extract on the filters. After methanol evaporated, a total of 10 healthy seeds of *R. sativus* were placed and added 1 mL of distilled water. All dishes were put in the conditions having natural light, 12/12 h day/night with a temperature of 25℃. After 5 days, germination rate, shoot

height, root length, fresh weight, and dry weight were evaluated. The percentages of germination, shoot, root, fresh weight, and dry weight over the control were determined as the inhibitory percentage (%). Each experiment was replicated three times.

Quantification of photosynthetic pigments

The amount of chlorophyll was quantified following the modified protocol of Wellburn (1994). To be specific, 1 g of leaves was ground and combined with 5 mL acetone 80% (v/v) in a test tube. Paper was used for wrapping the test tube to prevent the evaporation of acetone. This experiment was conducted with the plant extract at a concentration of 15 mg/mL. All the experiments were replicated three times. Methanol was used for control. After 15 min, the extract in the test tube was collected to determine the amount of chlorophyll.

The total amount of chlorophyll a, b, and carotenoid were calculated by the formula of Wellburn (1994) with some modifications as follows:

$C_a = (12{,}21 \times A_{663,2} - 2{,}81 \times A_{646,8}) \times 25$ *µg/gFW*

$C_b = (20{,}13 \times A_{646,8} - 5{,}03 \times A_{663,2}) \times 25$ *µg/gFW*

$C_{a+b} = ((1000 \times A_{470} - 3{,}27 \times X_a - 104 \times X_b)/198) \times 25$ *µg/gFW*

Note:

C_a: the amount of chlorophyll a in leaves (µg/g fresh leaves).

C_b: the amount of chlorophyll b in leaves (µg/g fresh leaves).

C_{a+b}: the amount of carotenoid (carotene and xanthophyll) in leaves (µg/g fresh leaves).

$A_{663,2}$; $A_{646,8}$; A_{470}: the values measured by spectrophotometer UV-VIS at the wavelength of 663.2, 646.8, and 470 nm, in turns.

Evaluation of water absorption

The water absorption was evaluated by changes in water level, growth, and some characteristics associated with the color of roots, stems, and leaves. The experiment was conducted in a test tube having 5 mL of *L. leucocephala* extract (15 mg/mL), which was triplicated. Distilled water was used for the control experiment. The plant samples were fresh, had a green color, and the similar in size to each other. After that, 1 mL of cooking oil was added to limit the water evaporation. The level of water was observed and evaluated after the treatment time of 4 days.

The amount of water absorption (%) was determined by the formula:

Absorption = ((Height of initial water level - Height of final water level)/Height of initial water level) *100

Statistical analysis

All data were collected in Microsoft Excel 2013. Minitab 16.0 was used for analysis of variance (ANOVA) and standard deviation (SD). Means were compared by using Tukey's test.

Results

Chemical compositions

The qualitative investigation of the chemical composition of the ethanol extract are displayed in Table 2. The results presented that *L. leucocephala*'s extract had the presence of alkaloid, flavonoid, saponin, terpenoid, coumarin, quinone, phenolic, and tannin. However, in each specific body part of the plant, there was differed between their components.

Table 2. Chemical components of ethanol extract from *L. leucocephala*

Chemical	Stems	Leaves	Fruits
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	-	-	+
Terpenoid	-	+	-
Coumarin	+	+	+
Quinone	-	+	+
Phenolic	+	+	+
Tannin	+	+	+

Note: (+): present, (-): absent

Leaves and fruits had the most chemical compositions (7 out of 8 compounds), which was greater than that of stems (Table 2). The components found in stem extract lacked saponin, terpenoid, and quinone compared to other parts. Furthermore, there were few chemicals just appearing in particular parts, typically saponin which was found in fruit extract, and terpenoid present in leaf extract. Hence, fruits and leaves were the two parts of *L. leucocephala* containing most of the bioactive components.

Total contents of phenolic and flavonoid

The total phenolic content of the extract was quantified based on the standard curve equation of gallic acid. The total flavonoid content of *L.*

leucocephala ethanol extract was determined and grounded on the standard calibration curve of quercetin. The two aforementioned standard curves were constructed from absorbance values at each particular wavelength. The total contents of phenolic and flavonoid were illustrated in the Table 3.

Table 3. The total contents of phenolic (mg GA/g extract) and flavonoid (mg QE/g extract) from different parts of *L. leucocephala*

Part	Total content of phenolic (mg GA/g extract)	Total content of flavonoid (mg QE/g extract)
Fruits	131.2 ± 2.81^{b}	106.2 ± 1.94^{b}
Stems	62.86 ± 1.14^{c}	119.1 ± 2.62^{b}
Leaves	397.6 ± 3.30^{a}	292.7 ± 5.66^{a}

Note: The data expressed in this table were means ± standard deviation. Values followed by different letters are significantly difference at 5% (Tukey's test).

The total contents of phenolic and flavonoid were determined by analysis of absorbance. The total phenolic content was distinguished in various parts. Result expressed that the content of phenolic found in fruits, stems and leaves extract were 131.2, 62.86 and 397.6 mg GA/g extract (Table 3). Leaf is the part containing the highest amount of phenolic, which was three-fold and over six times greater than that of fruit and stem, in turns. The total flavonoid contained in 1 g of extract was different in each part, which was 106.2, 119.1 and 292.7 mg QE/g in fruits, stems and leaves, respectively (Table 3).

Inhibitory effect of L. leucocephala extract

The inhibition of *L. leucocephala* extract on *R. sativus* seed germination was recorded after 5 days within in-vitro conditions.

The inhibitory impacts on *R. sativus* germination of *L. leucocephala* extract increased proportionally to its concentrations, which was significantly different compared to the control (Table 4). Three parts of the plant, including fruits, stems, and leaves depicted the highest inhibition at the concentration of 20 mg/mL, which were 53.33%, 40%, and 70%, respectively. Specifically, the leaf extract with the concentration of 20 mg/mL showed the greatest inhibitory level in comparison with others. Furthermore, the treatment of leaf extract evaluated after 10 days recorded the surge of inhibition percentage, which increased by six times compared to the figure in day 5. The result was relevant to the previous qualification. The total contents of flavonoid and phenolic were recorded with the highest figure observed in leaves, suggesting that leaves can be the part containing most of the bioactive compounds.

Table 4. Inhibitory effects (%) of *L. leucocephala* extract on *R. sativus* seed germination

	Treatment (mg/mL)	Inhibition percentage (%)	IC_{50} (mg/mL)
Control		0^{d}	-
Fruits	5	3.33 ± 3.33^{d}	19.62
	10	6.67 ± 3.33^{cd}	
	15	23.33 ± 3.33^{bcd}	
	20	53.33 ± 8.82^{ab}	
Stems	5	3.33 ± 3.33^{d}	24.09
	10	6.67 ± 6.67^{cd}	
	15	23.33 ± 8.82^{bcd}	
	20	40 ± 5.77^{abc}	
Leaves	5	3.33 ± 3.33^{d}	17.40
	10	20 ± 5.77^{bc}	
	15	46.67 ± 8.82^{bcd}	
	20	70 ± 15.3^{a}	

Note: The control was conducted without using plant extract. The data expressed in table were three replicates of means ± standard deviation. Values followed by the different letters are significantly different in statistical analysis at 5% (Tukey's test).

The linear equation was constructed to describe the correlation between concentrations of extract and inhibitory percentage on germination. The equation $y = ax + b$, with $y = 50\%$ and x was the inhibitory concentration of 50% (IC_{50}). Hence, the IC_{50} on seed germination of plant extract was determined as demonstration of the Table 4.

Being grounded on the aforementioned linear equation, the IC_{50} values of each kind of plant extract were different. There was an inverse proportion between IC_{50} and the inhibitory capability of the plant extract, meaning that in case the IC_{50} is high, the inhibitory effect is low. The fruit extract inhibited 50% of seed germination at the concentration of 19.62 mg/mL while the figure for stem extract was 24.09 mg/mL. It could be elucidated that the fruit extract was more effective than the stem extract in inhibiting seed germination. More importantly, the lowest IC_{50} value was recorded in leaves extract, 17.40 mg/mL, illustrating its own highest inhibition on germination.

The results delineated that the *L. leucocephala* extract influenced the growth of test species in in-vitro conditions (Table 5). The effect of growth inhibition using plant extract followed an upward trend of testing concentrations, which was statistically different compared to the control. The inhibitory effect rose when the concentration of treatments increased. Specifically, the concentration of 20 mg/mL significantly inhibited the growth of *R. sativus*, illustrated by the highest data of inhibition percentage observed in

each criterion including 91.46%, 69.89%, 78.73% and 49.53% in root length, stem length, fresh weight and dry weight, respectively. The leaf extract showed the greatest inhibitory effects whereas the extract from stems had the lowest inhibition among the three parts' extract.

Table 5. Growth inhibition percentage (%) of *L. leucocephala* extract after 5-day treatment

Treatment (mg/mL)		Inhibition percentage (%)			
		Root	**Stem**	**Fresh weight**	**Dry weight**
Control		0^{i}	0^{f}	0^{g}	0^{d}
Fruits	**5**	73.58 ± 0.86^{gh}	43.38 ± 0.43^{de}	33.61 ± 5.33^{ef}	3.88 ± 6.14^{d}
	10	85.27 ± 1.20^{cde}	47.87 ± 0.75^{cd}	41.47 ± 3.74^{def}	5.82 ± 1.94^{d}
	15	88.56 ± 0.54^{abc}	47.52 ± 1.47^{cd}	43.00 ± 1.42^{de}	8.59 ± 0.58^{d}
	20	90.63 ± 0.84^{ab}	65.51 ± 1.77^{ab}	67.53 ± 2.22^{ab}	38.95 ± 1.78^{ab}
Stems	**5**	77.07 ± 0.71^{fg}	32.04 ± 0.97^{e}	45.09 ± 1.30^{de}	0.32 ± 2.44^{d}
	10	82.76 ± 0.57^{de}	57.50 ± 5.15^{bc}	47.23 ± 1.20^{cd}	5.28 ± 5.56^{d}
	15	84.76 ± 0.48^{cde}	61.78 ± 3.96^{ab}	45.27 ± 4.45^{de}	7.42 ± 2.61^{d}
	20	85.93 ± 1.39^{bcd}	66.21 ± 1.52^{ab}	59.23 ± 1.62^{bc}	26.86 ± 4.35^{bc}
Leaves	**5**	69.95 ± 1.64^{h}	39.69 ± 4.11^{de}	29.22 ± 1.62^{f}	5.85 ± 3.79^{d}
	10	80.52 ± 1.53^{ef}	56.41 ± 2.20^{bc}	46.29 ± 2.78^{d}	11.23 ± 3.81^{cd}
	15	86.56 ± 1.10^{bcd}	60.17 ± 0.94^{ab}	71.58 ± 0.54^{ab}	30.92 ± 3.44^{b}
	20	91.46 ± 0.41^{a}	69.89 ± 1.19^{a}	78.73 ± 0.84^{a}	49.53 ± 5.82^{a}

Note: The control was conducted without using plant extract. The data expressed in table were three replicates of means ± standard deviation. Values followed by the different letters are significantly different in statistical analysis at 5% (Tukey's test).

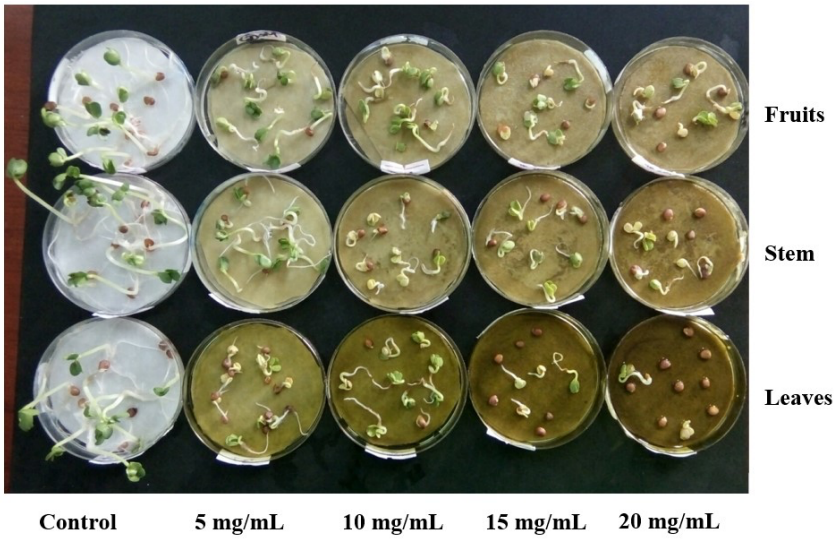

Figure 1. The seeds of *R. sativus* with treatments of different concentrations of extract *L. leucocephala* after 5 days

Influence of L. leucocephala extract on photosynthesis

Table 6. The average amount of photosynthetic pigments of *R. sativus* after 7-day treatment (µg/g fresh leaves)

Pigment	Chlorophyll a	Chlorophyll b	Carotenoid
Control	26.44 ± 0.28^{a}	21.19 ± 0.33^{c}	8.88 ± 0.16^{g}
Fruits extract	23.09 ± 0.49^{b}	16.19 ± 0.11^{f}	4.21 ± 0.11^{h}
Stems extract	19.79 ± 0.16^{d}	19.21 ± 0.11^{d}	4.11 ± 0.09^{h}
Leaves extract	16.45 ± 0.18^{f}	18.04 ± 0.11^{e}	3.11 ± 0.06^{h}

Note: The control was conducted without using plant extract. The data expressed in table were three replicates of means ± standard deviation. Values followed by the different letters are significantly different in statistical analysis at 5% (Tukey's test).

The decline of main photosynthetic pigments consisting of chlorophyll a, b, and carotenoid recorded in Table 6 which delineated the inhibition of *L. leucocephala* extract on photosynthesis. To be specific, the amount of these pigments decreased in the treatment of 15 mg/mL, which was significantly different in statistics in comparison with the control. The leaves treatment expressed the greatest inhibition, meaning that the content of chlorophyll a and carotenoid were the lowest figures, were 16.45 µg/g fresh leaves and 3.11 µg/g fresh leaves. On the other hand, chlorophyll b was inhibited dramatically by the fruit extract, which was 16.19 µg/g fresh leaves. The photosynthetic pigment content was depicted in Figure 2, describing the different inhibitory effects of each part's extract on each particular pigment.

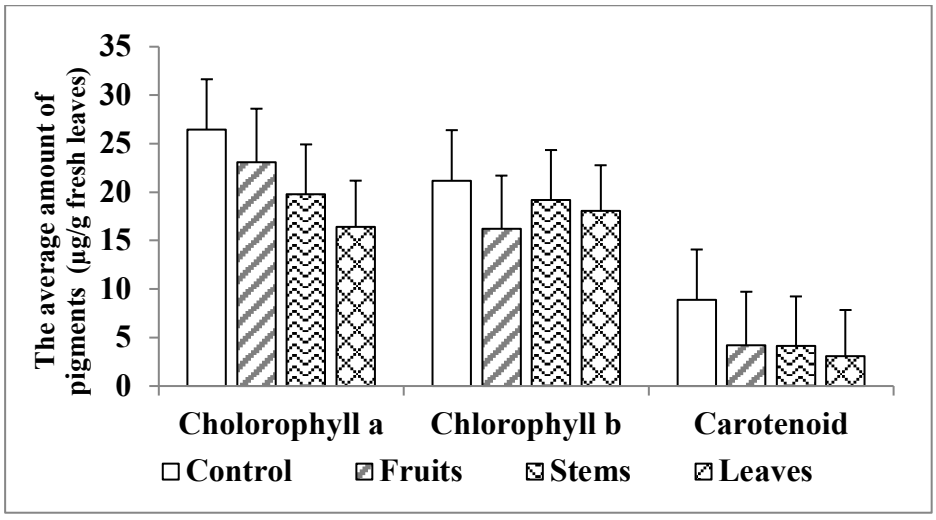

Figure 2. The photosynthetic pigment contents in treatments

Influence of L. leucocephala extract on water uptake

It recorded after 4 days of the survey showed that the extract from the fruit, stem, and leaves of *L. leucocephala* effectively inhibited water absorption of *R. sativus* (Table 7). In the control treatment, water was absorbed by the roots and transported by the xylem from the bottom up, therefore the water level height decreased significantly. The extracts showed that the water uptake of the plant was inhibited and had a statistically significant difference compared to the control. The inhibition effect of the fruit, stem, and leaf extracts on water absorption was 5%, 1.111%, and 0.550%, respectively. The leaf treatment showed the highest inhibitory effect compared to the fruit and stem treatments. Extracts of all parts of *L. leucocephala* affected the water absorption of *R. sativus*, this might be explained that allelochemicals cause water stress by inhibiting water use for long periods, leading to lack of water absorption by the plant as well as other mineral ions, making the plant would be died.

Table 7. Inhibitory effect (%) of *L. leucocephala* extract on water uptake

Treatment (mg/mL)	Water uptake percentage (%)
Control	9.375 ± 0.36^{a}
Fruits extract	5.000 ± 1.67^{b}
Stems extract	1.111 ± 0.556^{bc}
Leaves extract	0.550 ± 0.550^{c}

Note: The control was conducted without using plant extract. The data expressed in the table were three replicates of means ± standard deviation. Values followed by the different letters are significantly different in statistical analysis at 5% (Tukey's test).

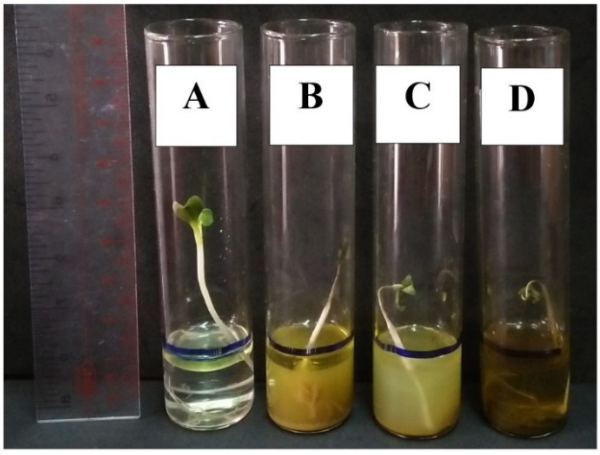

Figure 3. Water absorption of *R. sativus* in different treatments after 4 days. A: the control without using extract; B: Treatment with fruit extract, C: Treatment with stems extract, D: Treatment with leaves extract

The water flow was uptaken by the roots, being a means of transporting dissolved minerals to the root surface for absorption, the extract was absorbed and transported by the roots, which moved from roots to stems and leaves. However, this absorption was clearly reduced in the fruit, stem, and leaves, respectively. Therefore, the phenomenon observed in the roots and stems of *R. sativus* in the treatments recorded a change in color from white to the color of the extract from *L. leucocephala,* and some insoluble substances still remained around the roots. In the leaf extract treatment, the inhibitory effect was highest. In addition, the stem of *R. sativus* was rotted in half of the upper part. The leaves of *R. sativus* were dried, and curling, appearing black spots on the entire leaves, which seemed to be wilted and rotten (Figure 3).

Discussion

The chemical compounds of ethanol extract from *L. leucocephala* recorded in this research were similar to the result of (Elbanoby *et al.,* 2022), which detected some phytochemicals belonging to flavonoid, phenolic groups and others in some parts. Many phytochemicals found in this study are allelochemicals, secondary metabolites having great bioactivity existing in certain plants. Each kind of allelochemical could be found in specific plant tissues, which is able to express antioxidant production or antibacterial activity, helping plants to resist the pathogenic penetration (Sood *et al*., 2021). The preceding studies convinced that allelochemicals could be associated with the plant immune system, which largely contributed to preventing the invasion of harmful factors to host plants and played a role as signaling molecules stimulating the immunogenic response in some specific conditions (Kong *et al*., 2019; Pélissier *et al*., 2021). Therefore, the qualification of phytochemicals was evidence to determine the plant bioactivity.

As a matter of contention, this could be because many environmental factors remarkably influenced the synthesis, metabolism as well as concentration of chemical components, especially the climatic conditions and soil properties were much considered (Bibi *et al*., 2022). Another study of Dong *et al*. (2011) investigated the correlation between environmental conditions (altitude) and total flavonoid content. It was proved that the level of altitude and the content of flavonoids was proportional, meaning that the altitude affected the synthesis of secondary metabolites. Following the same pattern, the formation of phytochemicals during the temperature stress, which was a testament to self-protection in plants. In the case of phenolic content, the figures were almost high or tended to increase within the condition of low temperature (Ghasemi *et al*., 2011). Besides environmental conditions, the

solvent used for extraction also contributed to the difference of chemical content, which was characterized by the discrepancy of total flavonoid content from *L. leucocephala* ethanol 50% extract recorded in the study of Kim and In. (2017). The investigation of Chaurasia, (2015) proved that the content of phenolic having in methanol extract from *L. leucocephala* was 1.55 mg/g, lower than the case of ethanol.

The present research revealed that *L. leucocephala* extract considerably decreased the germination and seedling growth of *R. sativus*, demonstrating the inhibitory ability of plant species. The allelochemicals contained in *L. leucocephala* extract were the principal reason contributing to the inhibition. In many previous studies, phenolics, a bioactive compound in plant extract, were greatly recognized in the allelopathic potential (Djurdjevic *et al*., 2004; Lorenzo *et al*., 2010). Many mechanisms engaged in the inhibition of seed germination and growth were studied, typically the disruption of respiration occurring in mitochondria. The allelochemicals directly affect a chain of reactions including many important steps including glycolysis, the Krebs cycle, electron transport, and oxidative phosphorylation, which causes some interference in the mitochondrial membrane (Hussain *et al*., 2011). It was assumed that the germination ability declined caused of the impact of the allelopathy phenomenon on plants due to its inhibitory activity of chemicals. Fundamentally, as the extracts' concentration increased, the rate of seed germination decreased, suggesting a quantitative relationship between the extracts' concentration and seed germination. Germination is an initial stage of plant growth, controlling the germination therefore it has the potential to manipulate and prevent the growth of weeds as well as other harmful species, decreasing many negative impacts and improving the crop yield.

In the research of Dai *et al*. (2022), three plants, Shanghai green, wheat, and barnyard grass, which were tested for germination potential and growth of seed, and the aqueous extract from the leaves of *Flaveria bidentis* showed significant inhibitory effects on each of these parameters. The inhibition also increased proportionally with concentration. The germination potential and seed growth of the three plants were significantly different from the control at a concentration of 25 mg/mL. The three plants' germination was most strongly inhibited over 50% at a dose of 100 mg/mL while in our study, the extract from *L. leucocephala* fruits and leaves recorded the percentage of germination inhibition higher than 50% when the concentration of extract was 20 mg/ml, especially the inhibitory effect on seedling was greater than 90%. It was assumed that the inhibitory influence on plant germination and growth of *L. leucocephala* was quite high compared to other plant extracts.

The allelochemicals diffused in the environment which caused influences on the germination and growth of surrounding plants, especially the rise of root and shoot. Many mechanisms engaged in the allelopathy were studied, particularly causing changes in the micro and ultra-structure of cells. Inhibition of cell division and elongation as well as the effect on the plant growth regulator system, which were closely associated with seed germination and plant growth (Cheng and Cheng, 2015). The inhibition of mitosis stemmed from bioactive compounds and was explained by the decline of root length. There were some investigations reported that the *Datura stramonium* L. extract inhibited the growth of lateral root, typically decreased the length, halted the cell division of root apex and resulted in the blastogenesis, chromosomal aberration index, and micronucleus index found in soybean (Cai and Mu, 2012). Furthermore, in some higher concentrations of treatments, it was observed that the primary root elongation was significantly inhibited and the length and density of root hair considerably decreased. The results in this study were in accordance with the preceding research, which evaluated the effect of *L. leucocephala* extract on the root growth of *R. sativus*, suggesting that at distinctive concentrations of extract, the root length declined (from 5.1 to 4.5 cm) compared to the control without using the extract (5.9 cm). In addition, other research investigating the extract of *Eucalyptus* convinced that the germination and growth of *Eleusine corocana* Gaertn cv. AKP-2 was influenced, which reduced the length of the lateral root and stem while increasing the concentrations of treatment (Retnoningrum and Setiawan, 2021). Yang *et al*. (2008) proposed that the phenolic content in allelochemicals was able to activate the activity of indoleacetic acid (IAA) oxidase and repress the reaction of pyruvic oxime dioxygenase (POD) with IAA, linked to gibberellic acid (GA_3) or IAA to interfere the endogenous hormones activities.

Allelochemicals influence many biological and physiological processes, including photosynthesis and water uptake (Gniazdowska and Bogatek, 2005). In terms of photosynthesis, allelochemicals principally inhibited or damaged the synthesis machinery of decomposition of photosynthetic pigments. As a result, the content of photosynthetic pigments declined, leading to the block of energy and electron transformation, which alleviates the synthesis of ATP, and enzyme activity and inhibits photosynthesis, especially PSII (Wang *et al*., 2014). This can be because allelochemicals induced water stress, the roots had difficulty in transporting water and minerals to the stem and leaves, leading to wilting of the leaves due to lack of water and mineral ions. It was proved that the allelochemicals could influence water absorption in plant roots through a long-period inhibition of water usage. There were a number of mechanisms studied that the activities of Na+/K+-ATPase associated with the uptake and

transport of ions at the cell plasma membrane were inhibited, suppressing the cellular absorption of these kinds of ions (Cheng and Cheng, 2015).

Acknowledgements

The author would like to offer particular thanks to Department of Biology, College of Natural Sciences, Can Tho University for supporting in equipment and facilities during the period of study.

References

Bag, G. C., Devi, P. G. and Bhaigyabati, T. (2014). Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley. International Journal of Phamarceutical Sciences, 154-159.

Bibi, N., Shah, M. H., Khan, N., Al-Hashimi, A., Elshikh, M. S., Iqbal, A., Ahmad, S. and Abbasi, A. M. (2022). Variations in Total Phenolic, Total Flavonoid Contents, and Free Radicals' Scavenging Potential of Onion Varieties Planted under Diverse Environmental Conditions. Plants, 11.

Cai, S. L. and Mu, X. Q. (2012). Allelopathic potential of aqueous leaf extracts of Datura stramonium L. on seed germination, seedling growth and root anatomy of Glycine max (L.) Merrill. Allelopathy Journal, 30:235-245.

Chaurasia, S. (2015). Evaluation of Total Phenolic, Flavonoid Contents and Antioxidant Activity of Acokanthera oppositifolia and *Leucaena leucocephala*. International Journal of Pharmacognosy and Phytochemical Research, 7:175-180.

Cheng, F. and Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Frontiers in Plant Science, 6.

Chou, C.-H. and Kuo, Y.-L. (1986). Allelopathic research of subtropical vegetation in Taiwan. Journal of Chemical Ecology, 12:1431-1448.

Dai, L., Wu, L., Zhou, X., Jian, Z., Meng, L. and Xu, G. (2022). Effects of water extracts of Flaveria bidentis on the seed germination and seedling growth of three plants. Scientific Reports, 12.

Dewanto, V., Xianzhong, W., Adom, K. K. and Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, 50:3010-3014.

Djurdjevic, L., Dinic, A., Pavlovic, P., Mitrovic, M., Karadzic, B. and Tesevic, V. (2004). Allelopathic potential of *Allium ursinum* L. Biochemical Systematics and Ecology, 32: 533-544.

Dong, J., Ma, X., Wei, Q., Peng, S. and Zhang, S. (2011). Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of Eucommia ulmoides. Industrial Crops and Products, 34:1607-1614.

Elbanoby, N. E., El-Settawy, A. A. A., Mohamed, A. A. and Salem, M. Z. M. (2022). Phytochemicals derived from *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) biomass and their antimicrobial and antioxidant activities: HPLC analysis of extracts. Biomass Conversion and Biorefinery, DOI:10.1007/s13399-022-03420-1

Gharde, Y., Singh, P. K., Dubey, R. P. and Gupta, P. K. (2018). Assessment of yield and economic losses in agriculture due to weeds in India. Crop Protection, 107:12-18.

Ghasemi, K., Ebrahimzadeh, M. A., Ghasemi, Y., Ehteshamnia, A., Nabavi, S. M., Fazel Nabavi, S., Ali Ebrahimzadeh, M. and Pourmorad, F. (2011). Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. Article in Journal of Medicinal Plants Research, 5:1128-1133.

Gniazdowska, A. and Bogatek, R. (2005). Allelopathic interactions between plants. Multi-site action of allelochemicals. Acta Physiologiae Plantarum, 27:395-407.

Hasan, M., Mokhtar, A. S., Rosli, A. M., Hamdan, H., Motmainna, M. and Ahmad-Hamdani, M. S. (2021). Weed control efficacy and crop-weed selectivity of a new bioherbicide weedlock. Agronomy, 11.

Hussain, M. I., González, L. and Reigosa, M. J. (2011). Allelopathic potential of Acacia melanoxylon on the germination and root growth of native species. Weed Biology and Management, 11:18-28.

Isin Ozkan, T. G., Appiah, K. S., Akalin, E. and Fujii, Y. (2023). Plant Growth Inhibitory Activity of Hibiscus sabdariffa Calyx and the Phytotoxicity of Hydroxycitric Acid Lactone. Agronomy, 13.

Jugulam, M. and Shyam, C. (2019). Non-target-site resistance to herbicides: Recent developments. In *Plants* (Vol. 8, Issue 10). MDPI AG.

Khang, D. T., Quy, T. N., Dam, N. P., Tuan, N. T., Men, T. T., Van Ay, N. and Thuy, N. P. (2023). Isolation and purification of potential weed inhibitors from *Mimosa pigra* L. Heliyon, 9.

Kim, D. C. and In, M. J. (2017). Antioxidative ability of ethanol extract from the leaves of *Leucaena leucocephala* (Lam.) de wit. Journal of Applied Biological Chemistry, 60:18-190.

Kong, C. H., Xuan, T. D., Khanh, T. D., Tran, H. D. and Trung, N. T. (2019). Allelochemicals and Signaling Chemicals in Plants. Molecules, 24.

Kubiak, A., Wolna-Maruwka, A., Niewiadomska, A. and Pilarska, A. A. (2022). The Problem of Weed Infestation of Agricultural Plantations vs. the Assumptions of the European Biodiversity Strategy. Agronomy, 12.

Lorenzo, P., Pazos-Malvido, E., Reigosa, M. J. and González, L. (2010). Differential responses to allelopathic compounds released by the invasive Acacia dealbata Link (Mimosaceae) indicate stimulation of its own seed. Australian Journal of Botany, 58:546-553.

Olckers, T. (2011). Biological control of *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) in South Africa: A tale of opportunism, seed feeders and unanswered questions. African Entomology, 19:356-365.

Pélissier, R., Violle, C. and Morel, J. B. (2021). Plant immunity: Good fences make good neighbors? Current Opinion in Plant Biology, 62:102045.

Prashant, T., Bimlesh, K., Mandeep, K., Gurpreet, K. and Harleen, K. (2011). Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencial, 1:98-106.

Retnoningrum, M. D. and Setiawan, M. A. (2021). Concentration Effect of Ethanol Extract Pinus merkusii Leaves Litter on Zea mays L. Seed Germination. Journal of Physics: Conference Series, 1783:012003.

Scavo, A., Pandino, G., Restuccia, A. and Mauromicale, G. (2020). Leaf extracts of cultivated cardoon as potential bioherbicide. Scientia Horticulturae, 261:109024.

Sofowora, A. (1996). Research on Medicinal Plants and Traditional Medicine in Africa. *The* Journal of Alternative and Complementary Medicine, 2:365-372.

Soltys, D., Krasuska, U., Bogatek, R. and Agnieszka, G. (2013). Allelochemicals as Bioherbicides — Present and Perspectives. In A. J. Price & J. A. Kelton (Eds.), Herbicides (p. Ch. 20). IntechOpen.

Sood, M., Kumar, V. and Rawal, R. (2021). 8 - Seed biopriming a novel method to control seed borne diseases of crops. In S. Jogaiah (Ed.), Biocontrol Agents and Secondary Metabolites, pp.181-223. Woodhead Publishing.

Trognitz, F., Hackl, E., Widhalm, S. and Sessitsch, A. (2016). The role of plant-microbiome interactions in weed establishment and control. FEMS Microbiology Ecology, 92. Oxford University Press.

Wang, C.-M., Chen, H.-T., Li, T.-C., Weng, J.-H., Jhan, Y.-L., Lin, S.-X. and Chou, C.-H. (2014). The Role of Pentacyclic Triterpenoids in the Allelopathic Effects of *Alstonia scholaris*. Journal of Chemical Ecology, 40:90-98.

Wellburn, A. R. (1994). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal of Plant Physiology, 144:307-313.

Xuan, T. D., Tawata, S., Khanh, T. D. and Chung, I. M. (2005). Decomposition of Allelopathic Plants in Soil. Journal of Agronomy and Crop Science, 191:162-171.

Xuan, T. D., Tsuzuki, E., Terao, H., Matsuo, M., Khanh, T. D., Murayama, S. and Hong, N. H. U. U. (2003). Alfalfa, rice by-products and their incorporation for weed control in rice. Weed Biology and Management, 3:137-144.

Yang, G. Q., Wan, F. H., Liu, W. X. and Guo, J. Y. (2008). Influence of two allelochemicals from Ageratina adenophora Sprengel on ABA, IAA, and ZR contents in roots of upland rice seedlings. Allelopathy Journal, 21:253-262.

(Received: 13 December 2023, Revised: 1 July 2024, Accepted: 2 July 2024)