International Journal of Agriculture and Biological Sciences- ISSN (2522-6584) July & Aug 2020

August 31, 2020

Effects of Extraction Methods on Allelopathic Activity of *Mimosa pigra* L. Leaf Extract

Author's Details:

Do Tan Khang[1*], Nguyen Van Ay[2], Tran Ngoc Quy[3] Tran Thanh Men[4]

[1]Biotechnology Research and Development Institute, Can Tho University, Can Tho city, 94000, Vietnam;
[2]College of Agriculture, Can Tho University, Can Tho city, 94000, Vietnam; [3]College of Rural Development, Can Tho University, Can Tho city, 94000, Vietnam;
[4]College of Sciences, Can Tho University, Can Tho city, 94000, Vietnam.
* Corresponding author: dtkhang@ctu.edu.vn

Received Date: 27-July-2020 Accepted Date: 19-Aug-2020 Published Date: 31-Aug-2020

Abstract

Giant sensitive plant (Mimosa pigra L.) belongs to Fabacae family, is an invasive species containing biological compounds (allelopathic chemicals) to control other plants. This study was conducted to investigate the effect of different extraction methods on the phenolic, flavonoid, mimosinne contents and evaluation the ability to inhibit germination, growth of radish (Raphanus sativus L.) and leaf mustard (Brassica juncea) from Mimosa pigra leaf extract. The total phenolic content, flavonoid and mimosine concentrations were determined by spectrophotometric measurement, and the ability to inhibit growth was performed in a laboratory, on petri dishes in 5 days. The method of incubation for mimosa leaves in 60 minutes at a temperature of 50°C has the highest phenolic, flavonoid and mimosine concentrations of 6.63 mg, 1.86 mg and 1.89 mg. The 60-minute incubation method inhibited radish with the highest 21.44% of the root length, 38.01% of the shoot height but inhibited the lowest germination rate and dry weight. The mixture of leaf extract 10% and vinegar 0.1% inhibited completely germination, root length, shoot height, and the weight of radish and leaf mustard compared to the control. This result provides scientific literature on the potential ability of this plant to control weeds.

Keywords: *Allelopathy, Brassica juncea, extraction method, Mimosa pigra L., Raphanus sativus L.*

INTRODUCTION

Nowadays, weeds are one of the big problems, causing serious consequences for crops and agriculture in general. Weeds competitive nutrition reducing quality and crop yields, and may be home to pests and host microorganisms. Some contains cyanhydric acid, alkaloids or oxalates, which are toxic to plants. One of the common methods using to eradicate weeds is herbicide. However, herbicides are often chemicals, with a certain shelf life in arable land. Overusing leads to a significant increase in chemical residues in crops and agricultural products, affecting the environment and consumers' health. Therefore, finding new ways to control weeds is especially interested in scientists. In particular, the method using the phenomenon of natural sensory processing of plants (allelopathy) to control harmful plant species is paid attention to research (Khang et al., 2016). Allelopathy is a biological phenomenon in which an organism produces one or more biochemicals, called allelochemicals. These substances affect the germination, growth, survival and reproduction of other organisms. This mechanism is applied to control weeds without adversely affecting the environment as well as human health. However, there are not many reports on this research in the country.

Mimosa pigra L. is considered a wild species that has invaded many parts of Vietnam, posing a serious threat to ecosystems. However, according to recent studies, *M. pigra* contains many compounds such as phenolic, alkaloid, flavonoid, mimosine and steroid (Englert et al., 1995; Okonkwo et al., 2016). According to research by Khang et al. (2019), the extract has the ability to inhibit plants, mainly in the leaves and flowers. In addition, research shows that the mimosa tree contains allelochemicals with high levels in leaves and flowers. These are compounds that inhibit plant growth. Therefore, mimosa can also be a plant with high plant

suppression capacity, thereby taking advantage of natural wild resources, maximizing the potential of this dangerous invasive plant species.

The study aims at examining the effect of extraction methods on the content of natural compounds and the ability to inhibit plant growth from *M. pigra* leaf. The study also aims to identify the leaf extract with the highest inhibitory activity to evaluate the effectiveness of the blending formula on radish and mustard greens.

MATERIALS AND METHODS

Sample collection

Mimosa pigra leaves were collected at Can Tho city. Fresh, whole, unbroken leaves were collected with a weight of 3 kg divided into three portions. Drying leaves at 60°C to constant mass was conducted in an oven for following experiments.

Leaf extract preparation

Six flasks (marked as 1, 2, 3, 4, 5 and 6) containing 100 mL of distilled water were prepared. Leaf powder (10g) were placed into each flask. The mixture was blanchfor 3 minutes at 100°C, then incubate the flasks (1) and (6) with a water heater at the temperature of 50°C for 60 minutes, the beaker (6) was incorporated in an ultrasonic wave. The flasks (2) and (3) was boiled for 1 hour and for 3 hours, respectively. The (4) and (5) flasks was boiled for 1 and 3 hours, respectively and then placed in ultrasonic wave at 50-70 Hz for 20 minute. Then, the extracts were poured into volumetric flasks, and distilled water was added to the 100 ml mark and stored at 5°C for further tests.

Evaluation of the effect of extraction methods on the content of natural compounds from saman leaf extract

Determination of total phenolic content

The total phenolic content was determined by the description of Taga et al. (1984). In particular, Foin-Ciocalteu (F-C) reagent is formed from a mixture of phosphomolybdate and phosphotungstate, after reaction with phenol will form a blue complex of vonfarm and molybdenum. This reaction occurs under alkaline conditions so that electrons are removed from the phenol molecules more easily. The resulting complex had strong spectral absorption at 765 nm.The total phenolic content of leaf extract was determined according to gallic acid standard curve equation (0 - 100 µg/mL).

A volume of 100 µL 10% Folin-Ciocalteu reagent was added into the wells, then 20 µL of extract 100 mg/mL was added and mixed. $Na_2CO_3$7.5% solution (100 µL) was added, mixed well and standed for 30 minutes. The samples were then measured at 765 nm. Each experiment was repeated three times. The gallic acid calibration curve was empoyed to calculate the total phenolic content of the samples.

Determination of total flavonoid content

The total flavonoid content was determined by the description of Zhishen et al. (1999) based on the blue complexing properties of flavonoids with metals ($AlCl_3$). This complex had strong spectral absorption at 510 nm. The total flavonoid content in leaf extract was determined according to the quercetin standard curve equation (0 - 100 µg/mL). A volume of 100 µL $AlCl_3$ solution (2%) was put into the wells, then 100 µL of extracts were added, mixed and incubated at room temperature for 15 minutes before conducting a spectrophotometric measurement at 430 nm. Obtained results were calculated to determine the total flavonoid content in the extracts. The experiment was performed in triplicate.

Determination of total mimosine content

The concentration of mimosine in the extracts was determined by the method of Lalitha et al. (2004). The reaction between diazonium salt and mimosine forms a yellow complex with a spectral absorption at 400 nm.The concentrations of mimosine in leaf extracts were determined according to the mimosine standard curve

equation (0 - 25 µM). A volume of 100 µL of extracts (0.0025 g/mL) was put into the wells, then 50 µL of phosphate buffer solution and 25 µL of reagent mixture solution were added, respectively. The mixtures were kept for 15 minutes before conducting a spectrophotometric measurement at 400 nm. The experiment was performed in triplicate.

Evaluation of the effect of extraction methods on the inhibitory activity of *Mimosa pigra* leaf extract to the germination and growth of radish

Radish seeds were washed and soaked in Javel solution (10%) for about 20 minutes, then picked out and rinsed thoroughly with water (incubation cracked). A volume of 3 mL extracts were put into Petri dishes with filter papers lined and then twenty treated seeds were placed on each Petri dish. The Petri dishes were placed on a tray (some water was added to create moisture condition), covered with food wrap and kept at room temperature. The experiment consisted of seven treatments in triplicate. After 7 days, the plants were recorded including seed germination rate, fresh weight, dry weight, shoot height and root length.

Evaluation of the effect of blending formula on the inhibitory activity of *Mimosa pigra* leaf extract to the germination and growth of radish and mustard greens

The extracts were mixed with vinegar before being sprayed into Petri dishes.The extracts were mixed according to the following formula: vinegar was added to the extracts with concentrations of 0.025%, 0.1% and 0.5%, respectively (SASL, 2001). The effects of mixing formulae on inhibitory activity was evaluated based on seed germination rate, fresh weight, dry weight, shoot height and root length.

Data analysis

Experimental data were processed by Microsoft Excel 2010 software, SPSS software was used to analyze variance ANOVA and DUNCAN test.

RESULTS AND DISCUSSION

Total bioactive compounds content of saman leaf extracts from different extraction methods

Table 1. Total phenolic, flavonoid and mimosine content in *Mimosa pigra* leaf extract.

Experiment	Phenolic	Flavonoid	Mimosine
(1) 60’	$6,63 \pm 0,473^{a}$	$1,86 \pm 0,079^{a}$	$1,89 \pm 0,194^{a}$
(2) 1h	$2,81 \pm 0,100^{c}$	$0,79 \pm 0,058^{d}$	$1,6 \pm 0,141^{ab}$
(3) 3h	$2,70 \pm 0,006^{c}$	$0,74 \pm 0,066^{de}$	$1,70 \pm 0,034^{ab}$
(4) 1h-s	$4,92 \pm 0,608^{b}$	$1,15 \pm 0,116^{c}$	$1,76 \pm 0,033^{a}$
(5) 3h-s	$5,04 \pm 0,292^{b}$	$1,35 \pm 0,157^{b}$	$1,8 \pm 0,037^{a}$
(6) 60’-s	$1,52 \pm 1,155^{d}$	$0,57 \pm 0,036^{e}$	$1,89 \pm 0,194^{a}$

Note: Data is the average of three replicates (Mean ± SD). Numbers with the same following characters are not significant different at 5% through Duncan test.

From this result, it shows that the total phenolic content in the extract of *Mimosa pigra* leaf produced by the 60-minute (1) incubation method has the highest value, followed by boiling for 1 and 3 hours with wave (4; 5), the lowest was 60 minutes incubation method with waveform (6), the difference was statistically significant (Table 1).

The total phenolic content of the extract in (1) was 6.63 mg higher than in *Samanea saman* leaves (Rita et al., 2019) and lower than *Sesbania sesban L*. leaves was 20.98 mg (Kathiresh et al., 2012), similar to extraction method and distilled water solvent. In the study of Khang et al. (2019), the concentration of mimosa leaves in methanol extract is 230.3 mg, which is higher than the extract in this study.

The experiment results of (4), (5) and (2), (3), each pair had no significant difference, so it can be concluded that the time of extraction was different from 1 hour and 3 hours does not affect the total phenolic

content of the extract. (4), (5) were significantly different from (2), (3), which showed that ultrasonic waves increased the phenolic content of mimosa leaves, this result corresponds to the study (Delfan et al. 2018).

Flavonoid content in *Mimosa pigra* leaves in (1) was higher than that in *Samanea saman* leaves with solvents and extraction methods, respectively 1.86 and 1.27 mg, but lower than *Sesbania sesban* leaves is 9.78 mg, and much lower than methanol extract. The results of (4) and (5) were statistically different, showing that extraction time affected flavonoid content. (5) was significantly different from (3), which showed that ultrasonic waves increased the flavonoid content in mimosa leaves for the method of heating. From this result, it is shown that the total mimosisne content in the extract of mimosa leaves produced by extraction method (1), (4) and (5) is higher (6).The mimosine content of this leaf was 1.53% higher than that of *Samanea saman* and *Leucaena leucocephala* leaves and 2.66% of dry weight (Xuan et al., 2006). (2), (3) and (4), (5) were not significantly different, concluded that with the boiling method, the content of mimosine was not affected by the small frequency ultrasound. From the above OD results, the highest phenolic, flavonoid and mimosine (1), (6) levels showed that the combination of ultrasonic waves for the incubation method reduced the phenolic, flavonoid and mimosine in leaf extract.

Effect of extraction methods to inhibition activity of *Mimosa pigra* leaf extract on radish

Table 2: Effect of extraction methods on germination rate, root length, stem length, fresh weight and dry weight of radish

Experiment	Germination rate (%)	Stem length (mm)	Root length (mm)	Fresh weight (g)	Dry weight (g)
C	100 ± 0.0^{a}	19.33 ± 3.7^{ab}	22.76 ± 1.35^{a}	0.760 ± 0.031^{a}	0.259 ± 0.036^{a}
60’	100 ± 0.0^{a}	14.21 ± 0.23^{c}	14.11 ± 0.13^{d}	0.431 ± 0.083^{a}	0.203 ± 0.015^{ab}
1h	86.36 ± 0.0^{ab}	15.15 ± 0.16^{c}	15.0 ± 0.24^{ab}	0.765 ± 0.123^{a}	0.199 ± 0.020^{ab}
3h	56.89 ± 4.1^{c}	16.77 ± 0.8^{bc}	16.10 ± 0.73^{c}	0.600 ± 0.266^{a}	0.135 ± 0.089^{bc}
1h-s	93.18 ± 1.5^{ab}	14.98 ± 0.09^{c}	14.85 ± 0.12^{d}	0.705 ± 0.109^{a}	0.190 ± 0.024^{abc}
3h-s	72.73 ± 3.7^{bc}	15.01 ± 0.10^{c}	14.88 ± 0.08^{d}	0.582 ± 0.182^{a}	0.148 ± 0.039^{bc}
60’-s	40.89 ± 5.0^{c}	19.55 ± 1.50^{a}	18.12 ± 0.83^{b}	0.560 ± 0.495^{a}	0.094 ± 0.088^{c}

Note: Data is the average of three replicates (Mean ± SD). The numbers with the same sequences. the difference is not significant at 5% through Duncan test. In particular, **C**: control (distilled water); **60’** and **60’-s** are treatments (1) and (6) composting with a water bath at 50 ° C for 60 minutes. (6) with ultrasonic waves; **1h** and **3h** are treatments (2) and (3) boil the extract for 1 hour and 3 hours; **1h-s** and **3h-s** are treatments (4) and (5) boil the solution for 1 and 3 hours, respectively using ultrasonic waves. Leaf extracts in all treatments were 10% (0.1 g/mL).

In terms of root length, generally the experiments have statistical differences compared to the control. The differences of (1), (2), (4) and (5) did not make significant difference, inhibiting the highest root length, from 34.23-38.01%, the lowest inhibition of (6) was 20.39%. The (3) inhibited the root length by 34.63%, more than treatment (5) by 29.27%. The development of shoot height was inhibited by most of treatments. The extract of the mimosa leaf from the methods did not affect the fresh weight of radish. The treatments (1), (2), (4) was not different from that in Cin inhibiting dry weight of radish. Treatment (6) inhibited the highest dry weight 63.71% and inhibited the highest germination 59.11%.

The experimental results showed that the incubation method had the best germination rate inhibiting of 59.11% compared to the other methods. The wave-heating method had better inhibition of root length and shoot height than the non-wave heating method. However, the 60-minute incubation method had the best ability to inhibit root length and shoot height. It also had the highest phenolic, flavonoid and mimosine contents.Therefore, this extract method was choose for designing the formula experiment.

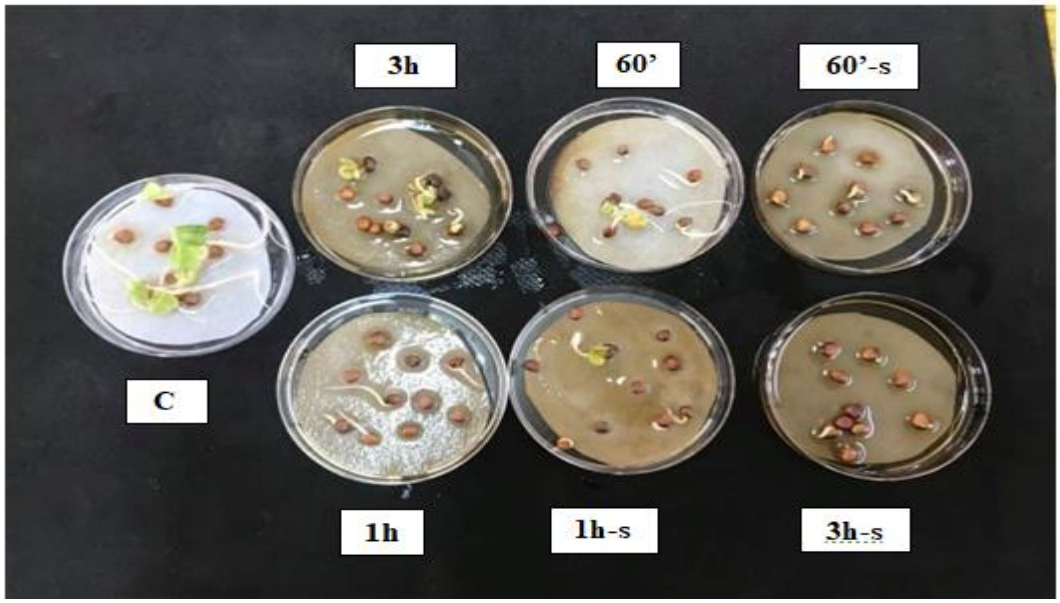

Figure 1. Radish development was affected by the extraction methods of *Mimosa pigra* leaf extract.

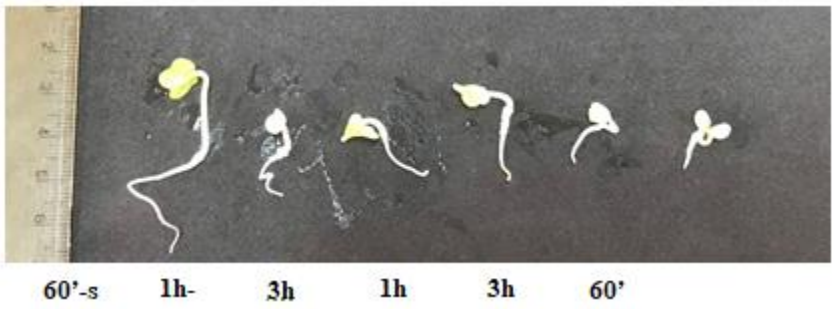

Figure 2. Comparison of stem height and root length of radish in all treatments.

Effect of extract blending formula to inhibiting activity of saman leaf extract on mustard greens

Table 3. Effect of extraction methods on germination rate, root length, stem length and fresh weight on radish

Experiment	G[1h]on	S[1h-s]	R[3h-s]	Fresh weight (g)
ĐC	100±0.00^{a}	17.13±5.95^{a}	22.96±11.66^{ab}	0.713±0.069^{a}
A0.01	66.7± 25.1^{b}	12.7±2.94^{ab}	15.10±8.86^{abc}	0.236±0.080^{bc}
A0.05	66.7± 5.7^{b}	13.6± 1.11^{ab}	29.50± 5.33^{a}	0.262±0.034^{bc}
A0.1	30.0± 10.0^{c}	12.3±6.85^{ab}	14.20±6.23^{abc}	0.116±0.054^{cd}
DA0.01	46.7±37.8^{bc}	16.0±10.71^{a}	26.13±22.96^{a}	0.312±0.193^{b}
DA0.05	43.3±5.7^{bc}	5.13± 1.30^{bc}	5.16 ± 2.53^{bc}	0.212±0.078^{bc}
DA0.1	13.3±5.7c	0.33±0.288^{c}	0.00±0.00^{c}	0.032±0.018^{d}

Note: Data is the average of three replicates (Mean ± SD). The numbers with the same following characters. The difference is not significant at 5% through Duncan test. In particular, **C**: control (distilled water**). A0.01; A0.05; A0.1** is the vinegar treatment with concentration of 0.01, 0.05 and 0.1%, respectively. **DA0.01; DA0.05; DA0.1** is a solution of extract + vinegar with concentration of 0.01, 0.05 and 0.1%, respectively.

Regarding rooting length, the experiment A0.01; DA0.01; A0.05 and A0.1 are not statistically different from C. The DA0.05 and DA0.1 were significantly different from C having the ability to inhibit radish root

length of 82.51 and 100%. DA0.05 and DA0.1 are significantly different from A0.05; A0.1 and C inhibiting the height of the turnip stems were 70.06% and 98.08%. In terms of germination rate and fresh weight. DA0.1 and A0.1 were not significantly different, DA0.1 inhibited 86.7% of the germination rate of radish seeds and 95.5% of fresh weight.

From the experimental results showed that the mixture of extract + vinegar and the vinegar solution at concentrations of 0.05 and 0.1% has the ability to inhibit the germination and the growth of radish. The solution of extract + vinegar at the concentration of 0.05% has the ability to inhibit the root length better than the vinegar solution of 0.05%. and the stem height is at the concentration of 0.1%. This proves that the extract with vinegar has the ability to inhibit radish growth. The higher the concentration. the greater the ability to inhibit germination and growth as evident when compared to the control.

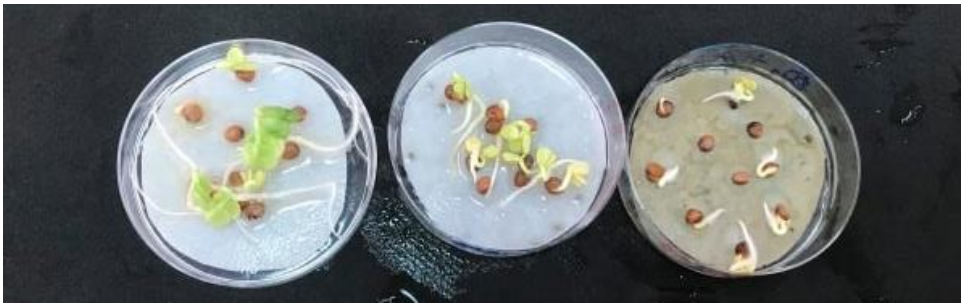

Figure 3. The growth of mustard greens was inhibited after 7 days in control and vinegar treatments at 0.025% concentration

Effect of blending formula on the inhibitory activity of extracts from *Mimosa pigra* leaves to the germination and growth on mustard green

Table 4. Effect of extraction methods on germination rate, root length, stem length, fresh weight and dry weight of mustard green

Experiment	Germination rate (%)	Stem length (mm)	Root length (mm)	Fresh weight (g)
C	100±0.00^{a}	14.53±6.10^{a}	34.9± 12.45^{a}	0.096±0.008^{ab}
A0.01	86.7± 11.5^{a}	16.73±0.41^{a}	18.66±4.35^{b}	0.128±0.036^{a}
A0.05	36.7± 28.8^{b}	5.4±4.77^{bc}	3.63± 3.33^{c}	0.039±0.025^{d}
A0.1	0.0± 0.0^{c}	0.00±0.00^{c}	0.0±0.0^{c}	0.00±0.00^{e}
DA0.01	76.7±20.8^{a}	14.7±3.89^{a}	9.76±2.95^{bc}	0.080±0.029^{bc}
DA0.05	86.7±23.0^{a}	7.9± 0.45^{b}	3.96 ± 0.96^{c}	0.050±0.007^{cd}
DA0.1	0.0±0.0^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}

Note: Data is the average of three replicates (Mean ± SD). The numbers with the same following characters. The difference is not significant at 5% through Duncan test. In particular, **C**: control (distilled water**). A0.01; A0.05; A0.1** is the vinegar treatment with concentration of 0.01. 0.05 and 0.1%. respectively. **DA0.01; DA0.05; DA0.1** is a solution of extract + vinegar with concentration of 0.01. 0.05 and 0.1%. respectively.

In general, the vinegar and extract + vinegar treatments at concentrations of 0.05 and 0.1% all inhibited the germination rate, stem height, root length and fresh weight of leaf mustard. At a concentration of 0.1%, the solutions completely inhibited the root length, stem, germination rate and fresh weight of leaf mustard seeds. At a concentration of 0.05%, the two solutions were not statistically different, but different from the controls, showing that the solutions were capable of inhibiting root length, stem height and mustard weight, but do not inhibit germination. At a concentration of 0.01%, DA0.01 inhibited 72.1% of the root length. NA0.01 was 46.6%. Therefore, it can be concluded that there is no difference in the ability to inhibit the vinegar and extract + vinegar solution.

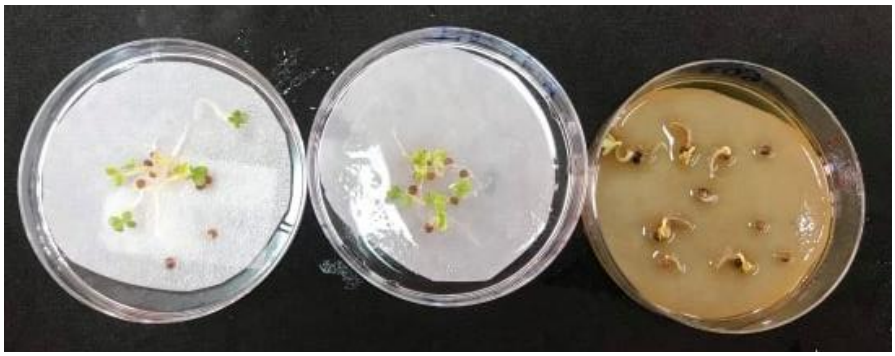

Figure 4. Development of leaf mustard after 5 days in all treatments, respectively water, vinegar 0.05% and leaf extract + vinegar 0.05%.

CONCLUSION

The study showed that the method of incubation of mimosa leaves in 60 minutes at a temperature of 50°C had the highest phenolic, flavonoid and mimosine contens. The 60-minute incubation method has the best inhibition of stem height and root length of radish is 21.44% and 38.01%, but in terms of germination rate and dry weight, the incubation with ultrasonic wave method had the best inhibitory activity. Ultrasonic waves affected the bioactive contents and herbicidal activity. Extracts mixed with vinegar at a concentration of 0.01% had the best ability to inhibit germination and growth of radish and leaf mustard. The results provide a document about the effects of various methods on weed suppression activity of *Mimosa pigra* leaf extract.

REFERENCES

i. *Englert, J., Weniger, B., Lobstein, A., Anton, R., Krempp E., Guillaume, D., and Leroy, Y. 1995. Triterpenoid saponins from Mimosa pigra. Journal of Natural Products, 58(8): 1265-1269.*

ii. *Kathiresh, M., Suganya, P., and Saravanakumar, M. 2012. Bioactive compounds in Sesbania sesban flower and its antioxidant and antimicrobial activity. Journal of Research in Pharmacy, 5(1): 293–390.*

iii. *Kawsar, M.A. 2008. Identification and Quantification of Phenolic Acids in Macrotyloma uniflorum by Reversed Phase-HPLC. American Journal of Plant Physiology, 3: 165-172.*

iv. *Khang, D. T., Tuan, N. T., Men, T. T., Ay, N. V., Thi, N. P. A., and Nhung, H. T. 2019. Phytotoxic effects of aqueous extracts from Mimosa pigra L. on barnyardgrass (Echinochloa crus-galli). International Journal of Agriculture and Biological Sciences, 6: 66-71.*

v. *Khang, D.T., La, A.H., Pham, H.T.H., Phung, T.T., Nguyen, Q.V., Luong, M.T., Luong, M.T., Nguyen, Q.T., Truong, M.N., Tran, X.D., Tran, K.D. and Trung, K.H. 2016. Allelopathic activity of dehulled rice and its allelochemicals on weed germination. International Letters of Natural Sciences, 58: 1-10.*

vi. *Li, Z. H., Wang, Q., Ruan, X., Pan, C. D. and Jiang, D. A. 2010. Phenolics and plantallelopathy. Molecules. 2010(15): 8933 - 8952.*

vii. *Okonkwo, C.J., Njoku, O. U., Okonkwo, T. J. N., Afieroho, O. E., and Proksch, P. 2016. Two new acylated flavonol glycosides from Mimosa pigra L. leaves sub-family Mimosoideae. Future Journal of Pharmaceutical Sciences, 2(2): 71-75.*

vii. *Rita, W., Swantara, I., Astiti Asih, I., and Sinarsih, N. 2018. Antibacterial activity of Samanea saman leaf ethanol extract against Escherichia coli and Staphylococcus aureus and its total flavonoid and phenolic contents. Jurnal Kimia (Journal Of Chemistry), 121-127.*

viii. *Saeheng, S. 2012. Chemical constituents and antioxidant activity of Borussus flabellifer, Elaeis guineensis, Mimosa diplotricha and Mimosa pigra. Medicinal Chemistry & Drug Discovery, 3(1): 52-57.*

ix. *Tanaka, T. 2012. Efficacy of flavonoid for patients with Japanese Cedar Pollinosis. INTECH Open Access Publisher, 382-383.*