Phenolic acids as plant growth inhibitors from *Tridax procumbens* L.

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**Abstract.** This study examined inhibitory effects of phenolic acids from *Tridax procumbens* L. on germination and growth of *Raphanus sativus* (radish). Four phenolic acids from this plant, namely benzoic acid, ellagic acid, vanillin, and ferulic acid were identified and quantified from a fraction of ethyl acetate extract by high performance liquid chromatography. These phenolic acids were subjected to germination and growth assays. Vanillin was the major constituents (364.689 µg/mg fraction) of ethyl acetate extract of this plant followed by benzoic, ellagic, and ferulic acids (69.888, 17.589, and 3.590 µg/mg fraction, respectively). In germination and growth assays, benzoic acid presented the most inhibitory effects (IC₅₀ of germination = 5.148 mM) on radish germination and growth. This compound decreased root elongation and shoot growth with IC₅₀ values of 0.947 mM and 3.452 mM respectively. This study revealed that *T. procumbens* possessed phenolic acids that can be utilized as plant growth inhibitors. Benzoic acid might play a role in the phytotoxicity of this plant. However, further trials on evaluating how this plant releases benzoic acid in soil, and examining the influence on more plants in its vicinity are needed to clarify potential use of this plant in weed control of agricultural practice.

1. Introduction

*Tridax procumbens* L. is an invasive plant species and belongs to Asteracea family. It is widely spread in more than 80 countries in the world. This plant commonly known as “coat button” or “tridax daisy” (USA) or “gletang” (Indonesia) [1]. *T. procumbens* has been reported to be a weed for more than 30 crops [2]. In some countries, it has been used as a traditional medicine. In India, *T. procumbens* was used for wound healing [3], hepatoprotective [4], anti-inflammatory [5], and antimicrobial agents [6]. In Guatemala and Togo, this plant has been used to treat gastrointestinal disorders, protozoal infections, and liver diseases [7-8].

Primary studies conducted on *T. procumbens* have focused on its pharmaceutical properties. Extracts of this plant showed antioxidant [9], antidiabetic [10], anti-inflammatory [11], and antihyperglycemic activities [12]. For pest control, extract of this plant was reported to have vigorous larvicidal activities against *Anopheles subpictus* & *Culex tritaeniorhynchus* [13].

Allelopathic activity of *T. procumbens* has been studied sporadically. The inhibitory effects of this plant on some indicator plants such as *Raniceps ranninus*, *Sorghum bicolor*, *Lactuca sativa*, and *Alium cepa* have been reported [14,15], but the identification of allelopathic substances has not been yet.
clarified. Additionally, Mecina et al. [16] noted that flavonoid compounds of this plant detrimentally affected mitosis and mutagens on cell division.

In our previous study, we screened and fractionated allelopathic substances of *T. procumbens* using difference polarities of solvent system in a column chromatography. We found that F1 fraction, a fraction isolated from ethyl acetate extract, gave the most inhibitory effects on the indicator plant (*Raphanus sativus*). This fraction decreased chlorophylls and carotenoids contents of *R. sativus* and increased of malondialdehyde (MDA), a cell-membrane responding mechanism against oxidative stresses. The chromatography-mass spectrophotometry (GC-MS) revealed that the major compounds of F1 fraction was phenolic compounds [17]. However, which phenolic compound that responsible to allelopathic activity of this plant is still remain unknown. This study therefore, was conducted (1) to identify and quantify of phenolic compounds form F1 fraction of *T. procumbens* by high performance liquid chromatography (HPLC) analysis, and (2) to examine inhibitory effects of phenolics identified form F1 fraction of *T. procumbens* on germination and growth of *Raphanus sativus* (radish).

2. Material and methods

2.1. Chemicals

Standard phenolics including benzoic, caffeic, cinnamic, chlorogenic, ellagic, ferulic, gallic, protocatechuic, p-coumaric, p-hydroxybenzoic, sinapic, syringic, and vanillic acid as well as rutin, catechol, and vanillin were purchased from Kanto Chemical Inc. Methanol and acetic acid for HPLC analysis were obtained from Sigma-Aldrich.

2.2. Plant material and F1 fraction

The plant materials of *T. procumbens* were collected in Subang, Indonesia (6°33'56.0"S 107°44'54.9"E) in 2016. The materials were authenticated in Herbarium Bogoriense, Botany Division, Research Center for Biology, Indonesian Institute of Sciences, Indonesia. The species voucher (no. PPBC161210) was dried and sterilized before deposited at Laboratory of Plant Physiology and Biochemistry, Graduate School for International Development and Cooperation (IDEC), Hiroshima University, Higashi-Hiroshima, Japan. Seeds of radish (*R. sativus*) were purchased commercially (Sakata Seed Corporation, Yokohama, Japan).

An amount of 2.3 kg of *T. procumbens* powder was immersed in 16 L of methanol, evaporated by vacuum evaporator, and fractionated using hexane, chloroform, ethyl acetate, and water to get 1.99 g of ethyl acetate extract. This extract was then fractionated by using column chromatography with chloroform and methanol as solvent system to get several fractions. F1 fraction, the most inhibitory fraction obtained from column chromatography using CHCl₃ as solvent system [17], was then subjected for identification and quantification phenolics contents by HPLC analysis.

2.3. Identification and quantification of phenolic compounds by high performance liquid chromatography (HPLC)

Phenolic acids identification and quantification were performed by HPLC with UV detection followed previous method [18]. The phenolic composition of F1 fraction was determined at 254 nm by using HPLC system (LC-Net II/ADC, UV-2075 Plus and PU-2089 Plus, Jasco, Tokyo, Japan). The column was RPC18 (Jasco, Tokyo, Japan) with 250.0 mm in length, 4.6 mm internal diameter, and 5.0 µm in thickness. The mobile phase was methanol 99.8% (solvent A) and 0.1% acid acetic (v/v) (solvent B). A gradient elution was run with 1 mL/min flow-rate using the following time gradients: 5% A (0 - 5 min), 20% A (5 - 10 min), 50% A (10 - 20 min), 80% A (20-30 min), 100% A (30 - 50 min), 5% A (50-60 min). Phenolic standards (0.01 - 0.1 mg/mL) and extracts (1 mg/mL) were injected to HPLC with an amount of 5 µL.
2.4. Germination and growth bioassay
The germination and growth assay was conducted by filter paper method based on previous study [17]. The radish seeds were sterilized with sodium hypochlorite (5 %) for 10 minutes then rinsed with distilled water several times. Ten seeds were sowed in each 12 well-plate (22.1 mm diameter × 35 mm height) in a growth chamber (Biotron NC system, Nippon Medical & Chemical Instrument, Co. Ltd, Osaka, Japan). The photoperiod was set up at day/night 12/12 h with 25/23 °C. The test solution (300 µL) was added to each well lined with filter papers. The methanol in the wells was allowed to evaporate within 6 h at room temperature, and an aliquot of 300 µL distilled water was added to each well. An additional volume of 100 µL distilled water was added subsequently at the 2nd, 3rd, and 4th days. Methanol added with distilled water using similar protocol mentioned above was used as controls. This bioassay was replicated three times. Germination percentages and root and shoot lengths over the controls were expressed as the percentage of inhibition. Concentrations in reducing 50% of germination, shoot height, and root length (IC50) were also calculated.

2.5. Statistical analysis
All data was analyzed using the Minitab 16.2.3 (copyright © 2012 Minitab Inc., Philadelphia, USA). The means and standard deviations of test samples were calculated. One-way ANOVA was used to analyze the data. The mean differences were determined by using Fisher’s test with the confidence level of 95% (p <0.05).

3. Results and discussion
3.1. Identification and quantification of phenolic compounds by HPLC
Phenolic compounds of F1 fraction were identified by comparing retention time of fifteen standards of phenolic acids separated by HPLC (Figure 1). The results showed that four phenolic acids including vanillin, ferulic, benzoic, and ellagic acids were detected in F1 fraction. The amounts of phenolic acid identified by HPLC is shown in Figure 2. Vanillin was the major constituents (364.689 µg/mg fraction) followed by benzoic, ellagic, and ferulic acids (69.888, 17.589, and 3.590 µg/mg fraction, respectively). Phenolic compounds are a class of common allelochemicals in the ecosystem. As phytotoxic compound, vanillin was also found in dehulled rice [19], Biddens pilosa [20], and Imperata cylindrical [21]. While benzoic acid was identified in several plants such as Azadirachta indica [22], Cucumis sativus [23], and Ageratum conyzoides [24]. Ferrulic acid and ellagic acid were detected in Lupinus albus [25], and Xanthium strumarium and Avena fatua [26], respectively.

![Figure 1. Phenolic acids of F1 fraction](image-url)
3.2. Effects of phenolic acids of T. procumbens on germination and growth of R. sativus

The inhibitory effects of phenolic acids identified from F1 fraction of T. procumbens are shown in Table 1. Benzoic acid showed the most inhibitory effects on germination and growth of R. sativus followed by vanillin, ferulic, and ellagic acids. At concentration of 1.0 mg/mL benzoic acid totally inhibited R. sativus. Inhibition concentrations (IC$_{50}$) of this compound are presented in Figure 3. In this study, benzoic acid gave more suppression on root elongation than germination and shoot growth of radish.

Benzoic acid was reported to be a strong allelochemicals against several indicator plants. Politycka [27] reported that this compound decreased phenols glycosylation and phenyl-ß-glucosyltransferase (PGT) activities, a cell membrane permeability responding mechanism against oxidative stresses. Li et. al. [28] noted that benzoic acid induced inhibition of eutrema wasabi root elongation up to 81.1% after a 7-day treatment. The root cells were irregularly arranged and organelle structures were severely damaged. Another study incubated cucumber seedlings in solutions containing derivatives of benzoic and cinnamic acids, and the results showed that leaf transpiration, stomatal conductance, and the intercellular CO$_2$ concentrations were all decreased [29].

4. Conclusion

Four phenolic compounds including benzoic, ellagic, and ferulic acids, and vanillin were identified in F1 fraction, the most inhibitory fraction of T. procumbens isolated from ethyl acetate extract. Vanillin was the major components (364.689 µg/mg fraction) followed by benzoic, ellagic, and ferulic acids. In the same dose, benzoic acid gave the most inhibitory effects on germination and growth of R. sativus compared the others phenolic acids detected. Benzoic acid may play a role in the allelopathic property of this plant. However, interaction among phenolic acids detected on different recipient plants and release of benzoic acid from this plant should be evaluated to better understand their phytotoxic properties.
Table 1. Effect of phenolics from *T. procumbens* on germination and growth of *R. sativus*

| Treatments          | Concentration (mg/mL) | Germination (%) | Root length (%) | Shoot height (%) |
|---------------------|-----------------------|-----------------|-----------------|-----------------|
| Vanilin             |                       |                 |                 |                 |
| 1                   | 26.67 ± 11.55<sup>b</sup> | 50.32 ± 24.47<sup>bc</sup> | 43.04 ± 9.79<sup>b</sup> |
| 0.5                 | 3.33 ± 5.77<sup>d</sup>  | 48.05 ± 17.21<sup>bc</sup> | 36.52 ± 3.98<sup>bc</sup> |
| 0.25                | 0.00 ± 0.00<sup>d</sup>  | 30.19 ± 10.41<sup>c</sup> | 33.04 ± 11.09<sup>bc</sup> |
| Ferrulic acid       |                       |                 |                 |                 |
| 1                   | 0.00 ± 0.00<sup>d</sup>  | 68.51 ± 6.26<sup>b</sup>  | 30.38 ± 32.93<sup>bc</sup> |
| 0.5                 | 0.00 ± 0.00<sup>d</sup>  | 47.05 ± 16.59<sup>bc</sup> | 23.04 ± 10.35<sup>bc</sup> |
| 0.25                | 0.00 ± 0.00<sup>d</sup>  | 44.16 ± 7.31<sup>bc</sup>  | 18.69 ± 1.99<sup>bc</sup> |
| Benzoic acid        |                       |                 |                 |                 |
| 1                   | 100.00 ± 0.00<sup>a</sup> | 100.00 ± 0.00<sup>a</sup> | 100.00 ± 0.00<sup>a</sup> |
| 0.5                 | 23.33 ± 32.15<sup>bc</sup> | 70.78 ± 12.20<sup>b</sup>  | 43.04 ± 32.01<sup>b</sup> |
| 0.25                | 6.67 ± 5.77<sup>cd</sup>  | 66.23 ± 7.38<sup>b</sup>  | 38.69 ± 13.80<sup>bc</sup> |
| Ellagic acid        |                       |                 |                 |                 |
| 1                   | 0.00 ± 0.00<sup>d</sup>  | (+) 43.18 ± 8.66<sup>d</sup> | 13.07 ± 9.50<sup>c</sup> |
| 0.5                 | 0.00 ± 0.00<sup>d</sup>  | (+) 54.36 ± 29.74<sup>d</sup> | (+) 26.18 ± 15.23<sup>d</sup> |
| 0.25                | 0.00 ± 0.00<sup>d</sup>  | (+) 43.50 ± 24.47<sup>d</sup> | (+) 17.39 ± 9.13<sup>d</sup> |

Data were presented as a mean ± standard deviation. Means within a column followed by similar letter are not significantly different by a Fisher’s test (p<0.05). (+) = promote.

Figure 3. Inhibition concentration (IC<sub>50</sub>) of benzoic acid on germination and growth of *R. sativus*. Bar values are mean ± SD (standard deviation) (n=3). Different letters on the bars indicated significance difference by a Fisher’s test at p < 0.05.

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References
[1] Holm L, Doll J, Holm E, Pancho J and Herberger J 1997 *World Weeds: Natural Histories and Distribution* (New York: John Wiley and Sons, Inc.)
[2] Randall R P 2012. *A Global Compendium of Weeds* 2nd Ed. (Western Australia: Department of Agriculture and Food)
[3] Talekar Y, Das B, Paul T, Talekar D Y, Apte K and Parab P B 2012 Asian J. Pharm. Clin. Res. 5 3–7.

[4] Ravikumar V, Shivashangari K S and Devaki T 2005 J. Ethnopharmacol. 101 55–60. doi:10.1016/j.jep.2005.03.019

[5] Jachak S M, Gautam R, Selvam C, Madhan H, Srivastava A and Khan T 2011 Fitoterapia 82 173–177 doi:10.1016/j.fitote.2010.08.016

[6] Pandey M, Pandey A, Kumar R, Pathak A and Dikshit A 2016. Int. Curr. Pharm. J. 5 22–26

[7] Cáceres A, López B., González S, Berger I, Tada I and Maki J 1998 J. Ethnopharmacol. 62 195–202 doi:10.1016/S0378-8741(98)00140-8

[8] Kpodar M S, Karou S D, Katawa G, Anani K, Gbekley H E, Adjrah Y, Tchacondo T, Batawila K and Simpore J 2016 J. Ethnopharmacol. 181 263–273 doi:10.1016/j.jep.2015.12.051

[9] Zhang J, Yuan K E, Zhou W E N-L G Z, Zhou J I A N and Yang P I N G 2012 Asian J. Chem. 24 58–62

[10] Desai G S, Desai S V, Gavaskar R S, Mulabagal V, Wu Y and Mathews S T 2015 Phytther. Res. 29 1404–1411

[11] Margaret I, Srinivasa R P and Kaiser J 1998 Phyther. Res. 12 285–287

[12] Pareek H, Sharma S, Khajja B S, Jain K and Jain G 2009 BMC Complement. Altern. Med. 9 48 doi:10.1186/1472-6882-9-48

[13] Kamaraj C, Bagavan A, Elango G, Zahir A A, Rajakumar G, Marimuthu S, Santhoshkumar T and Rahuman A A 2011 Indian J. Med. Res. 134 101–106 doi:10.1007/s00436-008-1306-8

[14] Nurul A M B, Nornasuha Y and Ismail B S 2016 AIP Conference Proceedings 1784 pp 1–8 doi:10.1063/1.4966877

[15] Mecina G, Montenoti M, Moraes V, Silva L and Goncalves R 2016 Res. J. Med. Plant 10 120–126 doi:10.3923/rjmp.2016.120.126

[16] Mecina G, Santos V, Andrade A, Dokkedal A, Saldanha L, Silva L and Silva R 2016b South African J. Bot. 102 130–136 doi:10.1016/j.sajb.2015.05.032

[17] Andriana Y, Xuan T D, Quan N Van and Quy T N 2018 Allelopathy Journal doi:10.26651/allelo.j./2018-43-2-1143

[18] Tuyen P T, Khanh D T, Ha P T T, Hai T N, Elzaawely A A and Xuan T D 2016 Int. Lett. Nat. Sci. 54 85–90 doi:10.18052/www.scipress.com/ILNS.54.85

[19] Khanh D T, Hoang Anh L, Thi P, Ha T, Tuyen P T, Quan N Van, Minh L T, Quan N T, Minh T N, Xuan T D, Khanh T D and Trung K H 2016 Int. Lett. Nat. Sci. 58 1–10 doi:10.18052/www.scipress.com/ILNS.58.1

[20] Deba F, Xuan T D, Yasuda M and Tawata S 2007 Weed Biol. Manag. 7 77–83. doi:10.1111/j.1445-6664.2007.00239.x

[21] Xuan T D, Toyama T, Fukuta M, Khanh T D and Tawata S 2009 J. Agric. Food Chem. 57 9448–9453. doi:10.1021/jf902310j

[22] Xuan T D, Tsuzuki E, Hiroyuki T, Mitsuhiko M, Khanh T D and Chung I M 2004 Crop Prot. 23 335–345. doi:10.1016/j.cropro.2003.09.004

[23] Yu J Q and Matsui Y 1994 J. Chem. Ecol. 20 21–31

[24] Xuan T D, Shinkichi T, Hong N H, Khanh T D and Min C I 2004a Crop Prot. 23 915–922 doi:10.1016/j.cropro.2004.02.005

[25] Stalikas C D 2007 J. Sep. Sci. 30 3268–3295. doi:10.1002/jssc.200700261

[26] Qasem J R and Foy C L 2001 J. Crop Prod. 4 43–95 doi:10.1300/J144v04n02

[27] Politycka B 1997 Acta Physiol. Plant. 19 311–317

[28] Li H-H, Inoue M, Nishimura H, Mizutani J and Tszuzuki E 1993 J. Chem. Ecol. 19 1775–1787

[29] Yu JQ, Ye S F, Zhang M F and Hu WH 2003 Biochem. Syst. Ecol. 31 129–139