The antioxidant activity and plant growth inhibitory activity of purple Dioscorea alata flour

Ratnaningsih1,*, S Suzuki2 and Y Fujii2

1 Indonesian Center for Agricultural Postharvest Research and Development, Jl. Tentara Pelajar no.12 Cimanggu, Bogor 16114 – Indonesia
2 Department of International Environmental and Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo 183-8509 – Japan

Email: ratnaningsih@pertanian.go.id

Abstract. Dioscorea alata, an indigenous Indonesian food crop, contains a lot of bioactive compounds. In addition, this yams tuber can be processed into flour using such kind of method to expand its utilization, make more affordable and extend the shelf life. The objective of this research was to study the antioxidant activity and its plant growth inhibitory activity of purple D. alata flour. The experiment results showed that antioxidant activities and plant growth activities of D. alata flour significantly correlated with its bioactive compounds.

1. Introduction

D. alata was reported rich of chemical compounds, such as saponins [1], cinnamic acid [2], sinapic acid, ferulic acid [3], catechin [4], quercetin, anthocyanin [3, 5] and inulin [6]. Phytochemicals compounds are contained within tuber, related to its antioxidant activity. Recent researches have focused on polyphenolic compounds, as the main responsible for antioxidant activity.

The antioxidant activity of D. alata has already reported. Flesh tuber of D. alata was reported providing 0.58 – 0.86 mg GAE g-1 reducing power activity and 0.22 – 0.73 mg TE g-1 DPPH radical-scavenging activity [7]. Five cultivars of the Philippine’s D. alata were reported higher values. The EC50 values were reported 3.3 – 14.8 mg ml-1 for DPPH scavenging activity, 9.5 – 31.7 mg ml-1 for reducing power activity, and 21.9 – 34.0 mg ml-1 for iron chelating capacity. These yams also provided total antioxidant activity 92.4 to 95.6 at 50 mg sample per mL methanol [8].

Information about the antioxidant activity of D. alata is important for the further expansion of this product and its product derivative utilization. It can be developed to be good natural antioxidant sources. Investigation to know more about the potency of this tuber is still needed, not only for food purposes, but also for other purposes.

Other potency of D. alata, because it reported rich of chemical compounds, is on allelopathic activity. Allelopathic activity is an activity of allelo-chemical on plant that influences the surrounding, such as on inhibition or stimulation other or same species around it, like suppressing weed growth [9]. This allelo-chemical can be developed to be natural herbicide, one of many important products which safety implemented to support the recently issues of sustainable agriculture.

The allelopathic activity on D. alata flour has not been reported yet, neither did on the antioxidant activity, especially associated with anti-browning inhibition treatment application. Therefore, the
objective of this study was to identify the antioxidant activity and plant growth inhibitory activity of
D. alata flour related with application of anti-browning treatment.

2. Materials and methods
The research has been conducted from January to July 2014 at the Laboratory of Biological production
and resources science – Tokyo University of Agriculture and Technology. The materials were used
consist of two local cultivar of D. alata tuber origin of Indonesia and chemicals for analysis. The
equipments were used in this research such as oven, dry miller, and analytical equipment.

2.1. Samples
Two local cultivars (Kulonprogo and Malang) of indigenous Indonesian D. alata tubers were used.
These yams were harvested, washed, hand peeled, sliced, and soaked on three submersion solution
treatments (water, Na-bisulfite 0.2%, and ascorbic acid 0.1%) for 20 minutes. Then samples were
dried at 55°C for 10 hours and dish milled through a 60-mesh degree. The resulting flours were stored
at sealed plastic and keep on -22°C until analysis.

2.2. Antioxidant activity measurement
The scavenging activity of D. alata extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical
scavenger were analyzed using Hsu et al. [10] method. Aliquots of 1 ml extract at concentration
ranging from 0 – 10 mg ml⁻¹ and 5 ml of freshly prepared 0.1 mM DPPH in 80% methanolic solutions
were thoroughly mixed, and kept for 50 minutes in the dark. The absorbance of the reaction mixture at
517 nm was measured on spectrophotometer. 80% Methanol solution was used as the blank.

\[
\text{DPPH scavenging effect (\%)} = \frac{(A_0-A_1)}{A_0} \times 100\%, \text{ where: } A_0 \text{ is the absorbance of the control and } A_1 \text{ is the absorbance in the presence of the extract.}
\]

EC₅₀ value is the sample extract concentration at 50% inhibition activity. EC₅₀ were determined by
interpolation using plotting the extract concentration versus the DPPH scavenging effect.

2.3. Plant inhibitory activity measurement
Plant growth inhibitory activity was analysed using sandwich method [9, 11]. A total of 10 mg, 25 mg,
50 mg, 100 mg and 150 mg D. alata flour was placed into two wells of two dish of six-well multi-dish
plastic plates, with two wells other as control. Agar flour with a gelling temperature of 30-31°C was
used as the growth media (0.75% w v⁻¹). The first layer of agar (5 ml) was applied with a pipette, as a
result, the D. alata flour arose to the surface of agar. After gelatinization, the second layer of agar (5
ml) was applied on the top. Five seeds of the test plant, lettuce were seeded on the gelatinized surface
of each well. The multi-dish was covered by plastic tape, labeled, and incubated in the dark room at
20°C for three days. The length of the hypocotyls and radicles of the lettuce seedlings were measured
on the third day. These data were used to calculate the percentage elongation compared to the control.

\[
\text{Inhibition (\%)} = \frac{(\text{Control length} - \text{length of seed germination of sample})}{\text{(Control length)}} \times 100.
\]

2.4. Statistical analysis
The data were analyzed by the analysis of variance (ANOVA) and the significant differences among
means were determined by Duncan’s multiple range tests (P<0.05) using SPSS 18.0.

3. Results and discussions

3.1. Antioxidant activity
The DPPH scavenging effect (\%) of D. alata crude extract on range concentration from 1 to 25 mg ml⁻¹
have shown at Figure 1. Kulonprogo has higher DPPH scavenging effect (\%) than Malang on each
treatment. On Kulonprogo, D. alata flour with Na-bisulfite treatment provided crude extract with
highest DPPH scavenging effect (\%) than ascorbic acid and water treatment, whereas on Malang
water treatment is the lowest.
EC50 (mg ml⁻¹) of the *D. alata* flour crude extract can be seen at Figure 2. EC50 was the concentration (mg ml⁻¹) of *D. alata* crude extract that required on decreasing the initial DPPH concentration by 50%. The lowest value of EC50 was the most effective concentration. EC50 of *D. alata* was ranged from 2.55 to 8.70 mg ml⁻¹. The lowest EC50 was *Kulonprogo* with Na-bisulfite treatment (2.55 mg ml⁻¹), while the EC50 of ascorbic acid and water treatment were 2.91 mg ml⁻¹, and 3.05 mg ml⁻¹ respectively, no significantly different values. The highest was *Malang* with water treatment (8.70 mg ml⁻¹) that had significantly different values with Na-bisulfite and ascorbic acid. Na-bisulfite and ascorbic acid treatment on *Malang* showed no significantly different values, 4.56 and 4.83 mg ml⁻¹ respectively.

![Figure 1. DPPH scavenging effect (%) of *D. alata* flour.](image)

![Figure 2. EC50 (mg ml⁻¹) of *D. alata* flour [12].](image)

**3.2. Correlation between antioxidant activity (EC50) with total anthocyanins, phenolics, and flavonoids content**

Antioxidant activity of *D. alata* flour had negative correlation with total anthocyanins, phenolics, and flavonoids content, Figure 3 and Figure 4. EC50 of *D. alata* flour antioxidant activity significantly correlated at 0.01 level with total anthocyanins content (r = -0.602), total phenolics content...
(r = -0.940), and flavonoids content (r = -0.938). Higher values of total anthocyanins, phenolics, and flavonoids content provided lower EC$_{50}$ values. It because EC$_{50}$ was concentration (mg ml$^{-1}$) of $D. alata$ crude extracts that required on decreasing the initial DPPH concentration by 50%. So, the lowest value of EC$_{50}$ was the most effective concentration.

![Graph](image1.png)

**Figure 3.** Correlation between antioxidative activity (EC$_{50}$) with total anthocyanins content and total phenolics content on $D. alata$ flour.

![Graph](image2.png)

**Figure 4.** Correlation between antioxidant activity (EC$_{50}$) with flavonoids on $D. alata$ flour.

3.3. Plant growth inhibitory activity

10 mg $D. alata$ flour tended to promote the hypocotyls growth for both Kulonprogo and Malang cultivar. However, weight of 50, 100 and 150 mg tended to inhibit the hypocotyls growth, **Figure 5.** The higher inhibitions were given by higher mass of $D. alata$ flour. EC$_{50}$ values on hypocotyls growth inhibition for Kulonprogo were 28.7, 18.6, and 13.2 mg ml$^{-1}$; and for Malang were 18.8, 18.8, 13.0 mg ml$^{-1}$ for water, Na-bisulfite, and ascorbic acid treatment respectively. For hypocotyls inhibition growth, ascorbic acid treatment provided $D. alata$ flour with strongest inhibition, on both Kulonprogo and Malang cultivar.
*D. alata* flour tended to inhibit the radicles growth for both *Kulonprogo* and *Malang* cultivar, Figure 6. The higher inhibitions were given by higher mass of *D. alata* flour. EC_{50} values on radicles growth inhibition for *Kulonprogo* were 3.53, 3.97, 2.25 mg ml^{-1}; and for Malang were 2.7, 4.38, 1.79 mg ml^{-1} for water, Na-bisulfite, and ascorbic acid treatment respectively. For radicles inhibition growth, ascorbic acid treatment provided *D. alata* flour with strongest inhibition, on both *Kulonprogo* and *Malang* cultivar.

Ascorbic acid provided strongest inhibition on hypocotyls and radicles growth for both cultivars, its maybe related to function of ascorbic acid as antioxidant. Ascorbic acid could maintain the allelochemical inside the *D. alata* flour.

![Figure 5. Hypocotyl inhibition (%) at 10 mg, 50 mg, 100 mg, and 150 mg.](image)

![Figure 6. Radicle inhibition (%) at 10 mg, 25 mgg, 50 mg, 100 mg and 150 mg.](image)
Figure 7. Hypocotyl inhibition (%) on different weight (g) of *D. alata* flour (a) Kulonprogo, (b) Malang.
4. Conclusions
EC50 of anti-oxidant activity of D. alata, 2.55 to 8.70 mg ml⁻¹, significantly correlated with total anthocyanins, phenolics, and flavonoids content. D. alata flour has a tendency to inhibit the growth of radicles and hypocotyls of lettuce, but small amount of D. alata flour tended to promote the hypocotyls growth. Plant growth inhibitory activities were not correlated with the anti-oxidative activity.

Acknowledgement
The authors wish to express our gratitude to Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture Republic of Indonesia for providing the scholarship and research funding.
References
[1] Lasztity R, Hidvegi M and Bata A 1998 Saponin in food Food Reviews Int. 14 371–90
[2] Martin FW and Rubert R 1976 J. Agric. Food Chem. 24 67–70
[3] Fang Z, Wu D, Yu D, Ye X, Liu D and Chen J 2011 Food Chem. 128 943–8
[4] Ozo O N, Caygill J C and Coursey D G 1984 Phytochem. 23 329–31
[5] Champagne A, Hilbert G, Legendre L and Lebot V 2011 J. Food Compos. Anal. 24 315–25
[6] Winarti S, Harmayanti E and Nurismanto R 2011 Agritech. 31 378–83 [in bahasa]
[7] Chung Y C, Chiang B H, Wei J H, Wang C K, Chen P C and Hsu C K 2008 Int. J. Food Sci. Tech. 43 859–64
[8] Cornago D F, Rumbaoa R G and Geronimo I M 2011 Philippine J. Sci. 140 145–52
[9] Fujii Y, Shibuya T, Nakatani K, Itani T, Hiradate S and Parvez MM 2004 Weed Biol. Manag. 4 19–23
[10] Hsu C L, Chen W, Weng Y M and Tseng C Y 2003 Food Chem. 83 85–92
[11] Fujii Y, Parvez S S, Parvez M M, Ohmae Y and Iida O 2003 Weed Biol. Manag. 3 233–41
[12] Ratnaningsih, Richana N, Suzuki S, Fujii Y 2018 Indones. J. Chem. 18 656–63