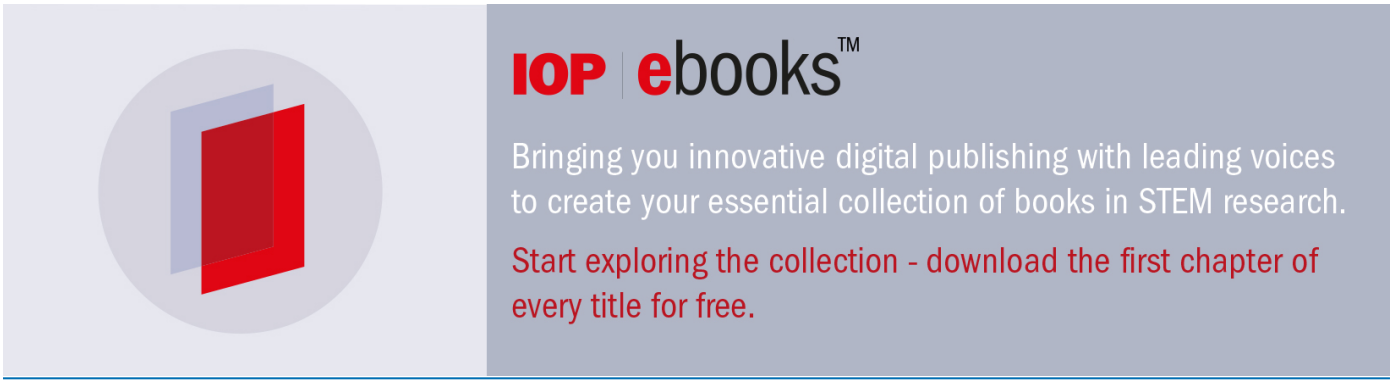
The antioxidant activity and plant growth inhibitory activity of purple *Dioscorea alata* flour



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

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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 EC_{50} 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 Januari 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.

DPPH scavenging effect (%) = $(A_0–A_1)/A_0 \times 100\%$, where: A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the extract.

EC_{50} value is the sample extract concentration at 50% inhibition activity. EC_{50} 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^o 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^o 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.
Inhibition (%) = (Control length- length of seed germination of sample)/(Control length) ×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.

EC_{50} (mg ml^{-1}) of the *D. alata* flour crude extract can be seen at Figure 2. EC_{50} 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 EC_{50} was the most effective concentration. EC_{50} of *D. alata* was ranged from 2.55 to 8.70 mg ml^{-1}. The lowest EC_{50} was *Kulonprogo* with Na-bisulfite treatment (2.55 mg ml^{-1}), while the EC_{50} 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.

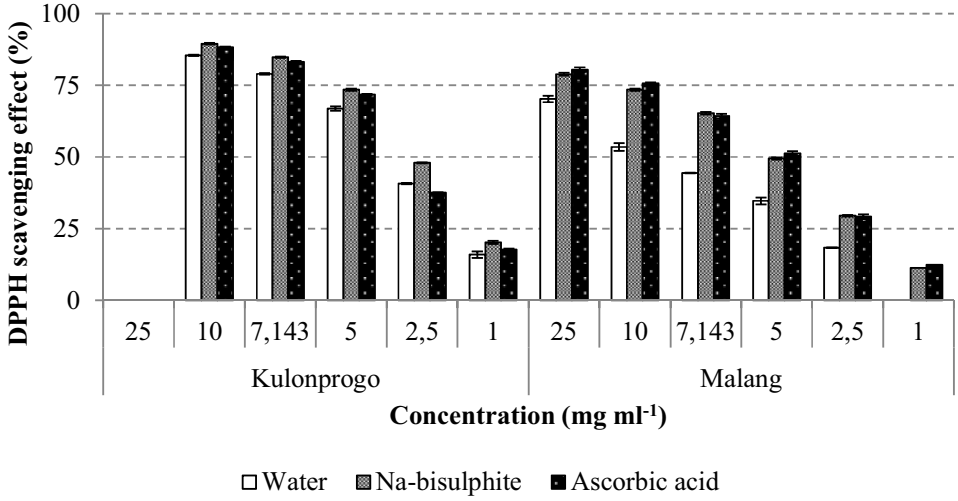


Figure 1. DPPH scavenging effect (%) of *D. alata* flour.

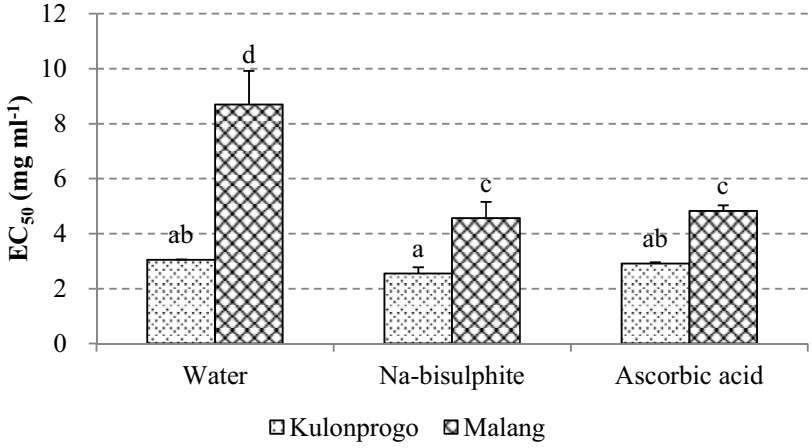


Figure 2. EC_{50} (mg ml^{-1}) of *D. alata* flour [12].

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. EC_{50} 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.

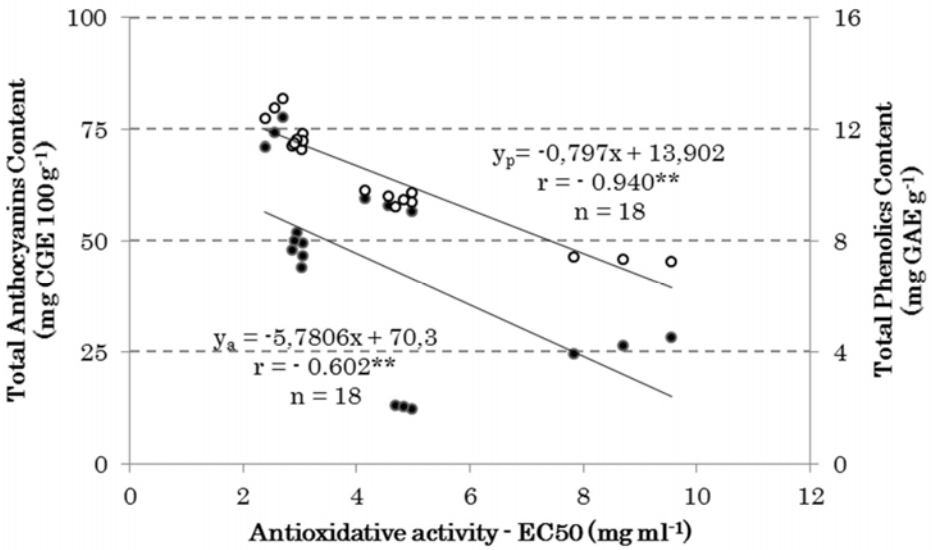


Figure 3. Correlation between antioxidative activity (EC_{50}) with total anthocyanins content and total phenolics content on *D. alata* flour.

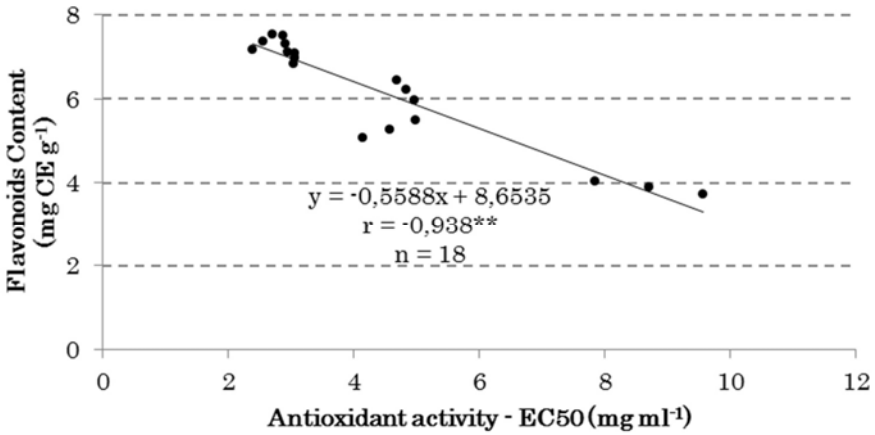


Figure 4. Correlation between antioxidant activity (EC_{50}) with flavonoids on *D. alata* flour.

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.

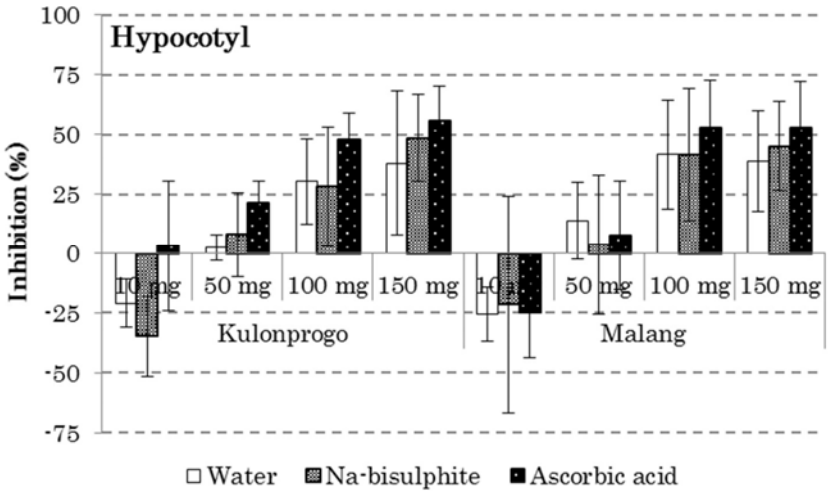


Figure 5. Hypocotyl inhibition (%) at 10 mg, 50 mg, 100 mg, and 150 mg.

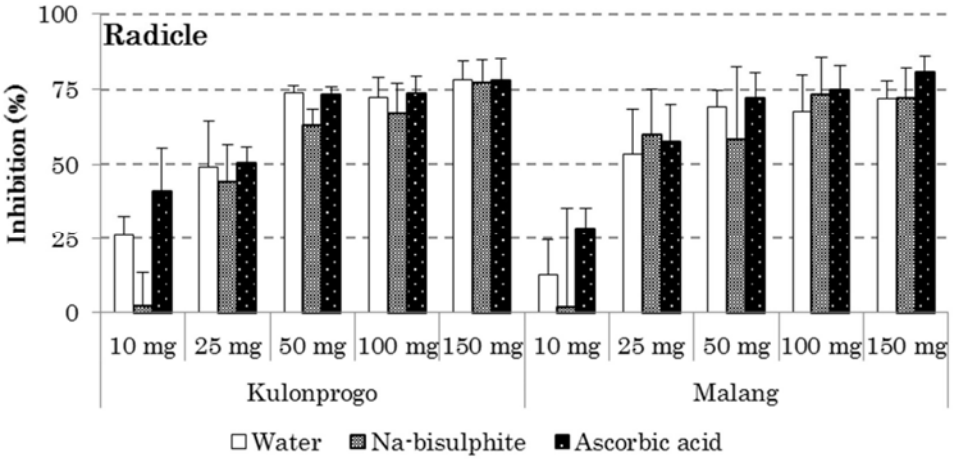
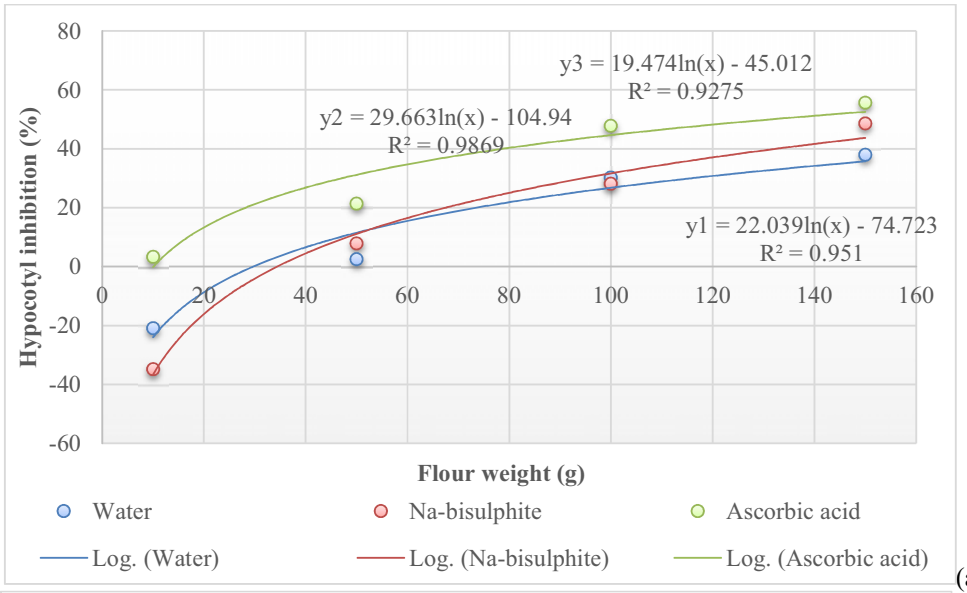
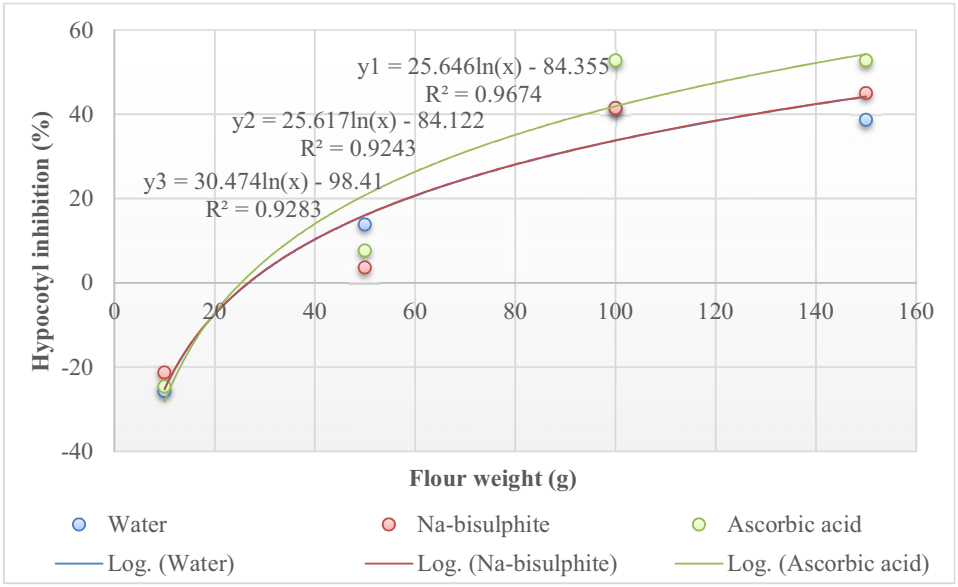


Figure 6. Radicle inhibition (%) at 10 mg, 25 mgg, 50 mg, 100 mg and 150 mg.

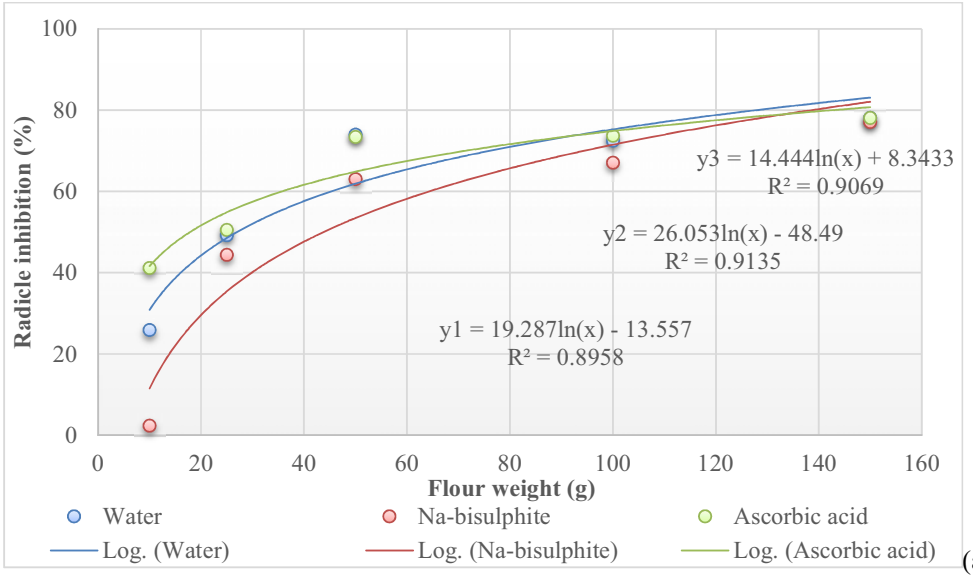


(a)

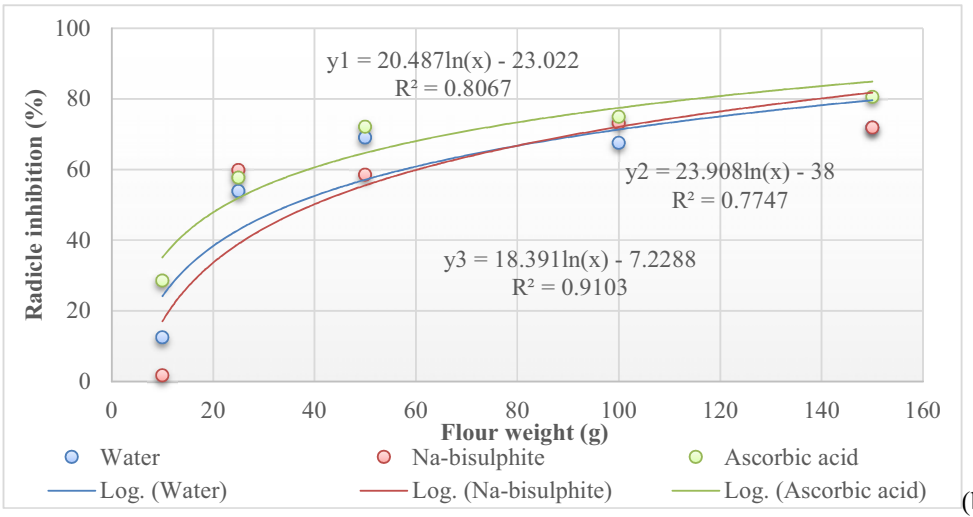


(b)

Figure 7. Hypocotyl inhibition (%) on different weight (g) of *D. alata* flour (a) *Kulonprogo*, (b) *Malang*.



(a)



(b)

Figure 8. Radicle inhibition (%) on different weight (g) of *D. alata* flour (a) *Kulonprogo*, (b) *Malang*.

4. Conclusions

EC^{50} 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

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