CURRENT BIOCHEMISTRY

Article

Influence of Maggot Residue Fertilizer on Phenolic and Flavonoid Content and Antioxidant Activity of Red Betel (*Piper crocatum* Ruiz & Pav.)

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Abstract: *Red betel (Piper crocatum Ruiz & Pav.) contained many active compounds that played a role in the treatment of degenerative diseases, such as phenolics and flavonoids. This research aimed to determine the effect of maggot manure application on the growth, total phenolic content, flavonoid content, and antioxidant activity of red betel plants. The total phenolic and flavonoid content were measured using colorimetric methods with Folin-Ciocalteu and aluminum chloride reagents. Antioxidant activity was measured using the DPPH and FRAP methods. The results showed that treatment with Dose 3 (1 maggot manure: 7 planting media) had the highest content of active compounds compared to other treatments, at 166.41 ± 4.50 mg GAE/g for phenolics, 47.59 ± 1.33 mg QE/g for flavonoids, 272.39 ± 15.49 ppm for IC50 value, and 545.27 ± 12.05 µM TE/g for antioxidant capacity. The highest plant growth, leaf count, wet weight, and dry weight were obtained from the negative control treatment, at 115.41 cm, 20.08 leaves, 19.03 grams wet weight, and 3.85 grams dry weight, respectively. The usage of maggot manure can increase the production of active compounds such as phenolics and flavonoids and enhance the antioxidant activity of red betel plants. However, this was in contrast to the growth of the plants when maggot manure was added.*

Keywords: antioxidant, flavonoid, phenolics, maggot, red betel

Abstrak: Sirih merah (*Piper crocatum* Ruiz & Pav.) mengandung banyak senyawa aktif yang berperan dalam pengobatan penyakit degeneratif, seperti fenolik dan flavonoid. Penelitian ini bertujuan menentukan pengaruh pemberian pupuk bekas maggot terhadap pertumbuhan, kadar total fenolik, flavonoid, dan aktivitas antioksidan tanaman sirih merah. Kadar total fenolik dan flavonoid diukur menggunakan metode kolorimetri dengan reagen *folin-ciocalteu* dan alumunium klorida. Aktivitas antioksidan diukur menggunakan metode DPPH dan FRAP. Hasil penelitian menunjukkan perlakuan dosis 3 (1 pupuk kasgot : 7 media tanam) memiliki kandungan senyawa aktif tertinggi dibandingkan dengan perlakuan lainnya, yaitu sebesar 166,41 ± 4,50 mg GAE/gr untuk fenolik, 47,59 ± 1,33 mg QE/gr untuk flavonoid, 272,39 ± 15,49 ppm untuk nilai IC50, dan 545,27 ± 12,05 µM TE/gr untuk kapasitas antioksidan. Pertumbuhan tinggi tanaman, jumlah daun, bobot basah, dan bobot kering tertinggi diperoleh dari perlakuan kontrol negatif, yaitu sebesar 115,41 cm, 20,08 lembar daun, 19,03 gram bobot basah, dan 3,85 gram bobot kering. Penggunaan pupuk kasgot mampu meningkatkan produksi senyawa aktif berupa fenolik dan flavonoid serta dapat meningkatkan aktivitas antioksidan tanaman sirih merah. Namun, hal tersebut berbanding terbalik dengan pertumbuhan tanamannya yang terhambat ketika ditambahkan pupuk kasgot.

Kata kunci: antioksidan, fenolik, flavonoid, sirih merah, maggot

Citation: Safithri M, Andrianto D, Nasution FH. 2025. Influence of maggot residue fertilizer on phenolic and flavonoid content and antioxidant activity of red betel (*Piper crocatum* Ruiz & Pav.). 12(1): 36-46.

Received: September 24, 2025
Revised: February 10, 2026
Accepted: June 23, 2026
Published: June 30, 2025

pISSN: 2355-7877
eISSN: 2355-7931

1. Introduction

Indonesia is an agrarian country endowed with a wide diversity of plant species. Among these, medicinal plants are particularly valued and have long been utilized in traditional medicine, with knowledge transmitted across generations. As noted by Shosan (2014), the majority of drugs intended for human use are derived from plants, either in their simple forms

or as extracts and mixtures. Red betel (*Piper crocatum* Ruiz & Pav.) is one such medicinal plant that contains a variety of secondary metabolites with therapeutic potential, including flavonoids, tannins, and alkaloids (Januarti *et al.*, 2019). Flavonoids function as potent antioxidants capable of neutralizing free radicals, tannins are traditionally employed to treat diarrhea and hemorrhoids due to their protein-precipitating properties (Tonahi *et al.*, 2018), and alkaloids serve as antibacterial agents through their antimicrobial activity (Anggarini *et al.*, 2019). In addition, red betel has been reported to contain bio-phenolic compounds such as hydroxychavicol, eugenol, chavibetol, and piperol, which exhibit antimutagenic and antiproliferative effects, particularly against prostate cancer, and are capable of eliminating malignant cells (Zulhairini *et al.*, 2018). The diverse pharmacological properties of red betel underscore the importance of enhancing its potential by stimulating the production of secondary metabolites and bioactive compounds, given their considerable relevance to human health.

Plant growth and metabolism are strongly influenced by the type of fertilizer applied. Although chemical fertilizers are widely used in Indonesia due to their ability to promote rapid growth, excessive and continuous use often leads to nutrient imbalances, degradation of soil structure, and reductions in microbial diversity, which in turn adversely affect crop quality and yield (Murnita & Taher, 2021). To address these challenges, the use of organic fertilizers has emerged as a sustainable alternative, one example being maggot residue fertilizer (kasgot).

Maggots, the larvae of the black soldier fly (*Hermetia illucens*), are commonly employed for bioconversion of organic waste. Their residue, known as kasgot, can be utilized as an organic fertilizer rich in essential nutrients such as nitrogen, phosphorus, and potassium, which are critical for comprehensive plant growth including stems, roots, branches, and leaves (Muhadat, 2021). Previous studies have shown that kasgot enhances the growth of *Brassica rapa*, improving parameters such as plant height, leaf number, leaf width, and fresh biomass (Fauzi *et al.*, 2022). However, its influence on the biosynthesis of secondary metabolites and the activity of bioactive compounds remains poorly understood.

Therefore, this study aimed to determine the effect of maggot residue fertilizer on the production of secondary metabolites and bioactive compounds in red betel, with a particular focus on total phenolic and flavonoid contents, antioxidant activity, and their relationship to plant growth performance, to support the optimal cultivation of this medicinal plant.

2. Materials and Methods

2.1. Materials

The equipment used in this study included 35 cm diameter pots, gloves, a hand shovel, a digital balance, plastic containers, an oven, a blender, standard glassware, a shaking incubator, a rotary evaporator, micropipettes, vial bottles, 96-well plates, a microplate reader, micropipette tips, and filter paper.

The materials used were soil, cow manure, 20 kg of maggot residue fertilizer (kasgot), 48 red betel (*Piper crocatum* Ruiz & Pav.) seedlings, 70% ethanol, analytical grade ethanol, 10% $AlCl_3$ solution, 1 M potassium acetate (CH_3COOK), distilled water, quercetin, 10% Folin–Ciocalteu reagent, 7.5% sodium carbonate (Na_2CO_3), acetate buffer (pH 3.6), 10 mM TPTZ, 40 mM HCl, 20 mM $FeCl_3$, 125 μM DPPH solution, and Trolox standard.

2.2. Red Betel Cultivation

Red betel seedlings, one month old, were obtained from the Biofarmaka Experimental Garden. The seedlings were cultivated in pots containing a growth medium composed of soil, cow manure, and rice husks in a ratio of 2:2:1. The planting medium was watered with clean water until evenly moist.

At two months of age, when the root systems were well established, the red betel plants were subjected to four treatments, each with four replicates and three experimental units, resulting in a total of 48 plants. The treatments consisted of three doses of kasgot fertilizer incorporated into 6 kg of planting medium, and one negative control group.

Dose 1 (1:13) — 461 g kasgot per 6 kg medium
Dose 2 (1:9) — 666 g kasgot per 6 kg medium
Dose 3 (1:7) — 857 g kasgot per 6 kg medium

Negative Control — growth medium only, without kasgot
The maggot residue fertilizer used in this study was supplied by PT Biomagg Indonesia.

2.3. Red Betel Maintenance

The maintenance of red betel plants consisted of watering and weeding. Watering was performed three times daily to maintain optimal soil moisture. Weeding was conducted once per week to remove grasses and other unwanted plants that could interfere with growth.

2.4. Red Betel Harvesting

Leaves of red betel were harvested after four months, when each pot contained approximately 15–20 leaves. Harvesting was carried out in the morning to facilitate the drying process, as afternoon harvesting may hinder proper dehydration. Leaves were collected from the lower parts of the plant upwards using sterilized cutting tools to minimize contamination.

2.5. Preparation of Red Betel Simplicia

The harvested red betel leaves were separated from foreign materials or debris, thoroughly washed, and cut into smaller pieces. The samples were then dried in an oven at 55 °C until fully desiccated and brittle. Dried leaves were pulverized using a blender, sieved through a 60-mesh filter, and stored in airtight containers for further analysis.

2.6. Determination of Moisture Content in Simplicia

Porcelain crucibles were cleaned, dried, and placed in an oven at 105 °C for 30 minutes. The empty crucibles were weighed to obtain their baseline mass. Approximately 1 g of powdered simplicia was added to each crucible, and the combined weight of crucible and sample was recorded before drying. Samples were then oven-dried at 105 °C for 3 hours and weighed immediately after drying. Moisture content was determined in triplicate and calculated using the following equation:

$$\text{Moisture Content (\%)} = \frac{B - C}{B - A} \times 100$$

where:
A = weight of empty crucible (g)
B = weight of crucible + sample before drying (g)
C = weight of crucible + sample after drying (g)

W_1 is the weight of the sample before drying and W_2 is the weight after drying.

2.7. Sample Extraction

Red betel simplicia was macerated with 70% ethanol at a ratio of 1:4 (w/v, simplicia:solvent). The maceration was performed five times, each for 24 h, using a shaking incubator at 130 rpm at room temperature. After each cycle, the mixture was filtered through filter paper, and all filtrates were combined. The solvent was evaporated under reduced pressure at 50 °C using a rotary evaporator to obtain a viscous extract.

The extraction yield was calculated as:

$$\text{Yield (\%)} = \frac{\text{Final extract weight}}{\text{Initial sample weight x (1-moisture content)}} \times 100$$

2.8. Determination of Total Phenolic Content

A total of 100 mg red betel extract was dissolved in 10 mL of ethanol. An aliquot of 20 µL was mixed with 120 µL of 10% Folin–Ciocalteu reagent and incubated for 5 min. Subsequently, 80 µL of 7.5% Na_2CO_3 was added, and the mixture was incubated for 30 min. Absorbance was measured at 750 nm using a microplate reader.

Phenolic concentration was determined by comparison with a gallic acid calibration curve (0, 50, 75, 100, 150, 200, and 250 ppm). Results were expressed as mg gallic acid equivalents (GAE) per gram of extract and calculated using the following formula:

$$C = \frac{c \times V}{m} \times FP$$

where:
C = total phenolic content (mg GAE/g)
c = phenolic concentration of the sample (mg/L)
V = volume of extract (mL)
m = weight of extract (g)
FP = dilution factor

2.9. Determination of Total Flavonoid Content

A total of 100 mg red betel extract was dissolved in 10 mL of ethanol. An aliquot of 10 µL was mixed with 60 µL ethanol, 10 µL of 10% $AlCl_3$, 10 µL of 1 M potassium acetate (CH_3COOK), and 120 µL distilled water in a 96-well plate. The mixture was homogenized, incubated for 30 min, and the absorbance measured at 415 nm using a microplate reader.

Flavonoid concentration was determined by comparison with a quercetin standard curve (0, 20, 40, 60, 80, 100, 150, 250, and 350 ppm). Results were expressed as mg quercetin equivalents (QE) per gram of extract and calculated using the following formula:

$$C = \frac{c \times V}{m} \times FP$$

where:
C = total flavonoid content (mg QE/g)
c = flavonoid concentration of the sample (mg/L)
V = volume of extract (mL)
m = weight of extract (g)
FP = dilution factor

2.10. Antioxidant Activity Assay by DPPH Method

To determine the antioxidant activity of red betel extract, 250 mg of extract was dissolved in 25 mL of ethanol to prepare a stock solution at a concentration of 10,000 ppm. Test solutions were prepared by serial dilution to obtain concentrations of 10, 100, 200, 300, 400, 500, 600, 700, and 800 ppm.

From each test solution, 50 µL was mixed with 100 µL of 125 µM DPPH solution in a 96-well plate. The mixtures were incubated in the dark for 30 minutes, and absorbance was measured at 520 nm using a microplate reader. Trolox was used as the positive control at concentrations of 0, 10, 20, 30, 40, and 50 µM.

The antioxidant activity was expressed as the half-maximal inhibitory concentration (IC_{50}), calculated from regression analysis of percentage inhibition versus extract concentration. IC_{50} values of red betel extracts were compared with those of the Trolox standard.

2.11. Antioxidant Activity Assay by FRAP Method

The antioxidant activity was also evaluated using the ferric reducing antioxidant power (FRAP) assay. FRAP reagent was prepared by mixing 10 mL acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ solution in 40 mM HCl, and 1 mL of 20 mM $FeCl_3$ solution. The freshly prepared FRAP reagent is colorless; the appearance of a blue color indicates contamination.

The reagent was incubated for 30 minutes at room temperature before use. A total of 10 µL of diluted red betel extract was mixed with 300 µL of FRAP reagent in a 96-well plate, incubated at 37 °C for 30 minutes, and absorbance was measured at 593 nm.

Antioxidant capacity was calculated based on the linear regression equation obtained from the Trolox calibration curve, and results were expressed as µM Trolox equivalents (TE) per gram of extract.

2.12. Data Analysis

All quantitative data were analyzed using analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences among treatments. Statistical

analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, with significance accepted at $p < 0.05$.

3. Results

3.1. Growth of Red Betel Plants

Plant growth was evaluated using plant height and leaf number, as these parameters are easily observable indicators of vegetative development. As shown in Figures 1 and 2, the application of maggot residue fertilizer (kasgot) significantly affected red betel growth. Control plants (without kasgot) exhibited the highest mean plant height (115.41 cm) and leaf number (20.08 leaves), whereas the lowest growth was recorded in the 1:7 treatment (80.91 cm; 14.16 leaves). Intermediate results were obtained in the 1:9 (88.25 cm; 16.16 leaves) and 1:13 treatments (97.08 cm; 15.83 leaves).

Fresh and dry weights, measured at harvest, followed the same pattern (Figures 3 and 4). The control treatment yielded the highest fresh and dry weights (19.03 g and 3.85 g, respectively), while the 1:7 treatment produced the lowest values (10.91 g and 2.26 g, respectively). The 1:9 treatment resulted in 14.74 g fresh weight and 3.02 g dry weight, while the 1:13 treatment produced 14.47 g and 2.89 g, respectively. These findings indicate that higher doses of kasgot reduced vegetative growth in red betel.

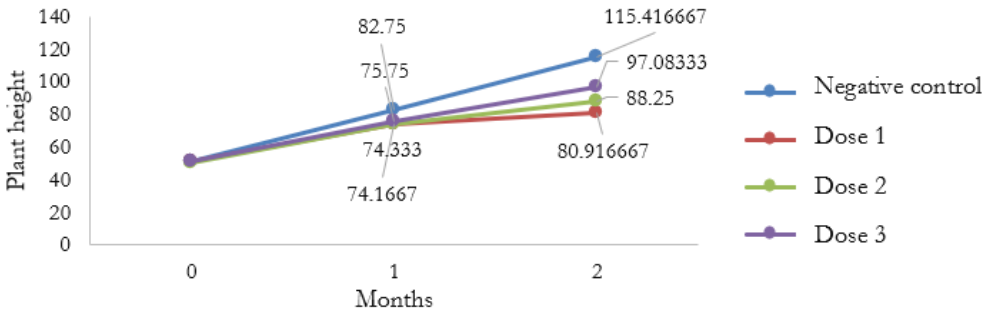

Figure 1. Growth of red betel plants

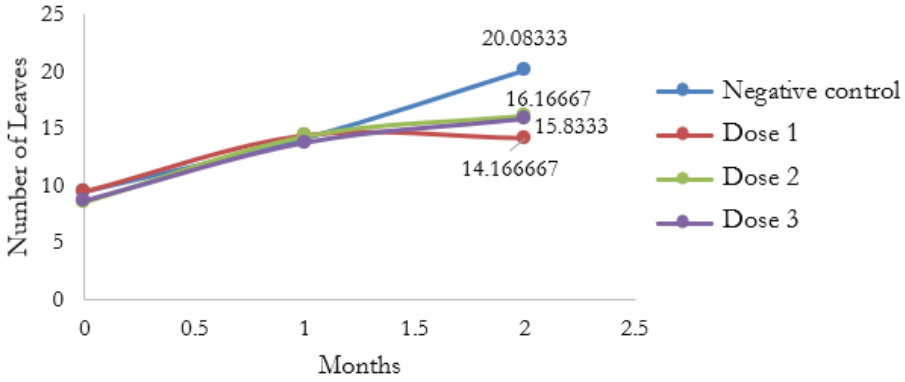

Figure 2. Number of leaves of red betel plants

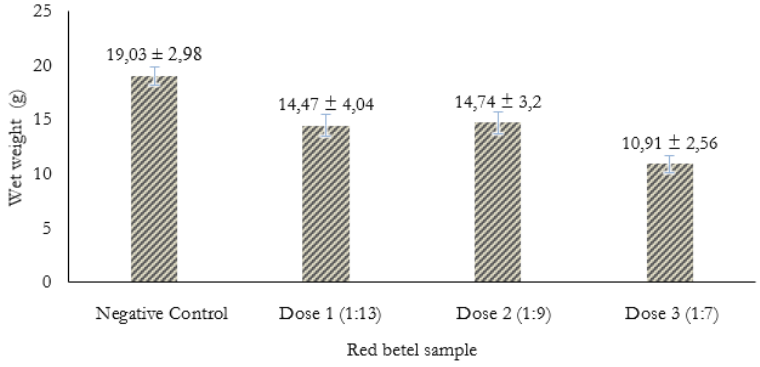

Figure 3. Average wet weight of red betel leaves

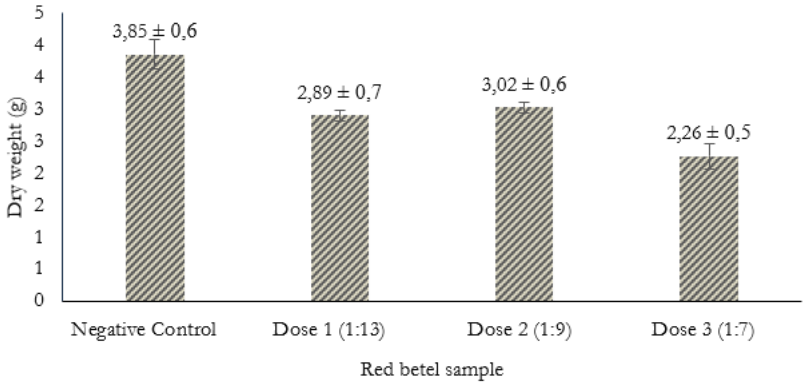

Figure 4. Average dry weight of red betel leaves

3.2. Moisture Content and Extraction Yield

Moisture content of red betel simplicia ranged from 2.67% to 4.33% across treatments (Table 1). ANOVA revealed no significant differences among treatments ($p = 0.596$; $p > 0.05$). All values met the Indonesian Pharmacopoeia standards for simplicia moisture content.

Extraction yields varied with fertilizer treatment. The highest yield was obtained from the 1:13 treatment (15.97%), followed by the control (12.48%). Lower yields were observed for the 1:7 (10.53%) and 1:9 (10.01%) treatments (Table 1).

Table 1. Percentage of moisture content and extraction yield of red betel.

Treatment	Moisture content (%)	Yield (%)
Negative control	2,67 ± 0,57[a]	12,48
Dose 1 (1:13)	4,00 ± 0,0[b]	15,97
Dose 2 (1:9)	3,67 ± 0,57[ab]	10,01
Dose 3 (1:7)	4,33 ± 0,57[b]	10,53

[1] Negative control (without fertilizer), dose 1 (1 maggot residue fertilizer : 13 planting medium), dose 2 (1 maggot residue fertilizer : 9 planting medium), and dose 3 (1 maggot residue fertilizer : 7 planting medium). Values within the same column followed by different letters indicate significant differences ($p < 0.05$). Data are presented as mean ± standard deviation.

3.3. Total Phenolic Content

Total phenolic content was quantified using the Folin–Ciocalteu method, with gallic acid as the standard ($y = 0.0029x - 0.0146$; $R^2 = 0.9976$). Results are shown in Figure 5. The 1:7 treatment produced the highest phenolic content (166.41 ± 4.50 mg GAE/g extract), while the lowest was found in the 1:9 treatment (58.71 ± 0.71 mg GAE/g extract).

ANOVA revealed a significant difference among treatments ($p = 0.001$; $p < 0.05$). Tukey's post hoc test showed no significant differences among the control, 1:9, and 1:13 treatments. However, the 1:7 treatment differed significantly from all other treatments.

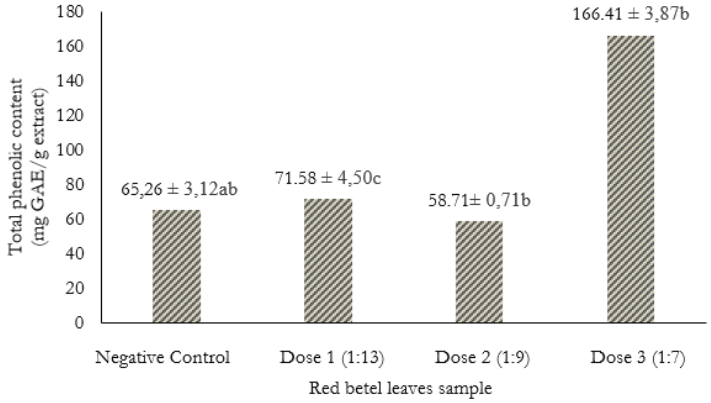

Figure 5. Total phenolic content of red betel leaves extract

3.4. Total Flavonoid Content

Flavonoid levels were determined using the $AlCl_3$ method with quercetin as the standard ($y = 0.0016x + 0.0089$; $R^2 = 0.9961$). As shown in Figure 6, the highest flavonoid content was recorded in the 1:7 treatment (49.82 ± 1.33 mg QE/g extract), while the lowest was observed in the 1:9 treatment (15.57 ± 0.38 mg QE/g extract).

ANOVA followed by Tukey's test revealed significant differences among all treatments ($sig < 0.05$). This indicates that kasgot application markedly influenced flavonoid accumulation in red betel leaves.

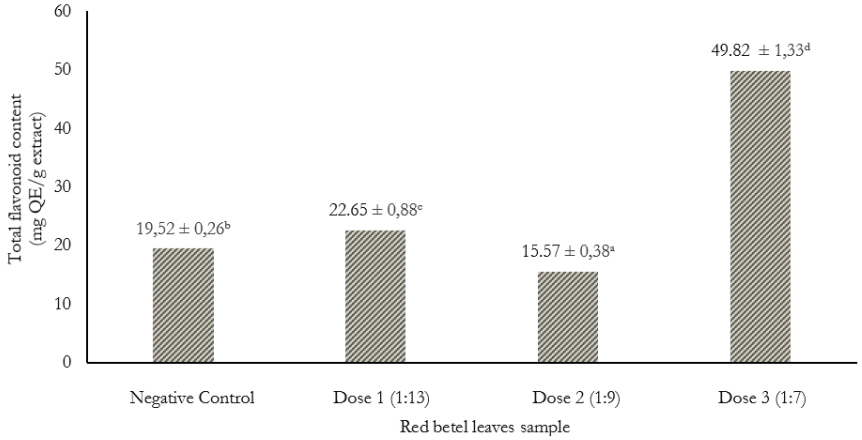

Figure 6. Total flavonoid content of red betel leaves extract

3.5. Antioxidant Activity by DPPH Assay

Antioxidant activity was assessed using the DPPH radical scavenging method. IC_{50} values were determined from linear regression of concentration (x-axis) versus percentage inhibition (y-axis). Smaller IC_{50} values indicate stronger antioxidant activity.

As shown in Figure 7, the strongest antioxidant activity (lowest IC_{50}) was observed in the 1:7 treatment (272.39 ± 0.35 ppm), while the weakest activity was in the 1:9 treatment (898.36 ± 15.49 ppm). ANOVA confirmed significant differences among treatments ($p < 0.05$). Tukey's test further demonstrated that all treatments differed significantly from one another, highlighting a clear dose-dependent effect of kasgot on antioxidant capacity.

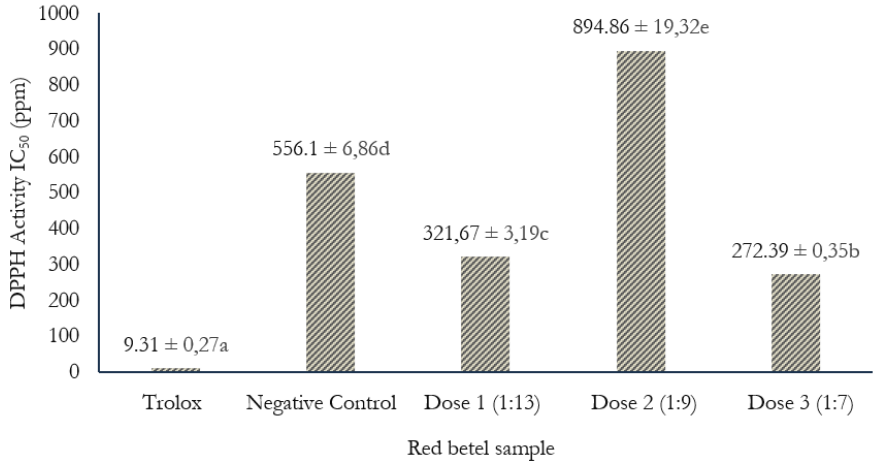

Figure 6. DPPH antioxidant activity of red betel leaves extract

3.6. Antioxidant Activity by FRAP Assay

Antioxidant capacity was also evaluated using the FRAP assay, with Trolox as the standard ($y = 0.0018x – 0.0025$; $R^2 = 0.9934$). Results are presented in Figure 7. The 1:7 treatment yielded the highest FRAP value (545.28 ± 12.05 μM TE/g extract), whereas the lowest was obtained from the 1:9 treatment (229.17 ± 2.88 μM TE/g extract).

ANOVA revealed significant differences among treatments (p = 0.001; p < 0.05). Tukey's test indicated that the control and 1:9 treatments were not significantly different, while both the 1:13 and 1:7 treatments differed significantly from the other groups.

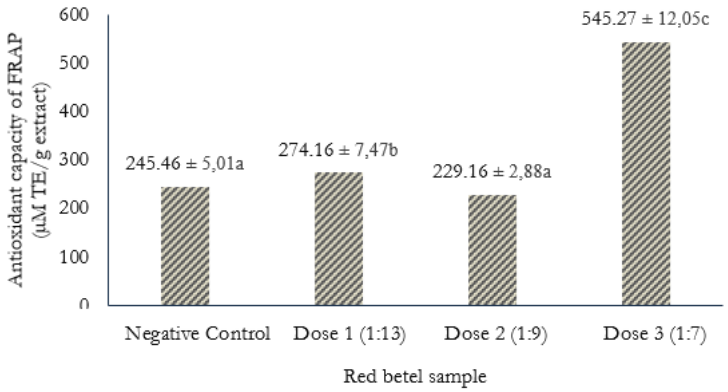

Figure 7. FRAP antioxidant activity of red betel leaves extract

4. Discussion

Growth parameters of red betel (*Piper crocatum*) were measured based on plant height, leaf number, fresh weight, and dry weight. Plant height was measured from the stem above the planting medium to the highest shoot. Growth performance is largely determined by nutrient availability in the planting medium. Nitrogen (N), phosphorus (P), and potassium (K) are the primary macronutrients required for plant development. Nitrogen is essential for protein and nucleic acid synthesis, directly influencing leaf expansion. Potassium regulates several physiological processes, including photosynthesis, carbohydrate accumulation, and carbon dioxide translocation, whereas phosphorus plays a key role in energy transfer through ADP and ATP (Kare *et al.* 2023).

The negative control treatment yielded the tallest plants and the greatest leaf number (115.41 cm and 20.08 leaves, respectively), surpassing all groups treated with maggot residue fertilizer. This superior growth is attributable to balanced nutrient levels from the soil, cattle manure, and husk composition in the medium without additional maggot residue. The cattle manure used contained 37.41% carbon, 1.90% nitrogen, 1.50% P_2O_2, 2.13% K_2O, 1.50% Ca, 0.54% Mg, and a pH of 7.41. As noted by Kare *et al.* (2023), optimal plant growth occurs when nutrients are available in adequate and balanced concentrations, whereas deficiencies or excesses inhibit development.

Application of maggot residue fertilizer appeared to suppress plant growth slightly. The lowest plant height and leaf number were recorded in dose 3 (857 g/6 kg soil, 1:7), suggesting that excess nutrients induced stress conditions. Dose 1 (461 g, 1:13) and dose 2 (666 g, 1:9) showed intermediate growth, with no significant difference between them. Excessive fertilizer application likely increased soil solution concentration, thereby reducing nutrient uptake efficiency. This aligns with Nuryani *et al.* (2019), who reported that excessive fertilization diminishes crop yield. Similarly, Kurniasari *et al.* (2020) observed that the highest fertilizer dose (200 kg/ha) resulted in the lowest shallot plant height (21.93 cm) due to nutrient imbalance, where excess macronutrients interfere with micronutrient absorption. Hamady (2017) further emphasized that excessive phosphate impairs Fe and Zn uptake, while high potassium limits Mg and Cu availability, ultimately stunting growth.

Fresh and dry biomass were also highest in the negative control (19.03 g and 3.85 g, respectively), consistent with its superior height and leaf number. Biomass was directly proportional to vegetative growth. Ghifari (2023) reported that greater leaf number correlates with increased biomass, which is closely linked to nitrogen-driven chlorophyll production. Higher chlorophyll levels enhance photosynthesis, generating energy to support growth hormone activity and leaf initiation (Lima & Yoris 2015). Similarly, Hariyadi *et al.* (2015) showed a positive correlation between plant height and leaf number.

Moisture content is a critical indicator of crude drug quality, as high moisture accelerates microbial growth and degradation (Daud *et al.* 2019). The moisture content of red betel simplicia ranged from 2.67±0.57% to 4.33±0.57% across treatments, all below the 10%

threshold set by SNI 01-3393-1994 (Widyanti *et al.* 2021), ensuring acceptable quality. Safithri *et al.* (2016) previously reported a higher moisture content (8.22%), likely due to differences in drying conditions or cultivation methods.

Extracts were obtained by maceration in 70% ethanol (1:4 ratio), a method that prevents thermal degradation of heat-labile compounds. The highest yield (15.97%) was obtained from dose 1, while the lowest (10.01%) came from dose 2. These results differ from Purnama *et al.* (2023), who reported yields of 12.62–19.42%. Variations may stem from plant age, as older plants synthesize more bioactive metabolites (Gultom *et al.* 2020). Other influential factors include solvent polarity, extraction time, and storage conditions (Yulianti *et al.* 2014).

Phenolic and Flavonoid are key indicators of antioxidant potential in various plant-based products, as they reflect the presence of bioactive compounds capable of scavenging free radicals (Safithri *et al.* 2025). Phenolics, as a major group of antioxidant compounds, include flavonoids, tannins, coumarins, and cinnamic acid derivatives, playing vital roles in oxidative stress mitigation and plant defense. Dhurhania & Novianto (2019) confirmed that phenolic-rich extracts are promising natural antioxidants. In this study, total phenolics were determined using the Folin–Ciocalteu method. The highest phenolic content was observed in dose 3 (166.41 ± 4.50 mg GAE/g), surpassing the control and other treatments. These values were markedly higher than those reported by Simanjuntak (2022) for red betel without maggot residue fertilization (42.38 ± 1.99 mg GAE/g). Supporting this, Safithri et al. (2022) reported a total phenolic content of 162.25 mg GAE/g in extracts and fractions of *P. crocatum*, further highlighting its potential as a rich antioxidant source. The enrichment of phenolics is likely linked to secondary metabolite residues present in maggot fertilizer, which derives from organic waste rich in bioactive compounds such as citrus peels. Maggot residue itself contains high levels of nitrogen (3.28%), phosphorus (3.39%), potassium (9.74%), and beneficial microbes (Fauzi et al. 2021), exceeding the nutrient profile of conventional cattle manure. However, excess nutrients can also impose oxidative stress, leading to the generation of reactive oxygen species (ROS), which in turn stimulate antioxidant defense responses, including phenolic synthesis (Tamas et al. 2017; Dutta et al. 2018).

Flavonoids, a subclass of phenolics, exhibit diverse pharmacological activities including antioxidant, anti-inflammatory, and anticancer effects (Sumiwi & Khoirunnisa 2019). Urrutia *et al.* (2013) demonstrated that *Heterotheca inuloides* flavonoids protect against CCl_4-induced oxidative damage. In this study, flavonoid content was determined via the $AlCl_3$ colorimetric assay using quercetin as a standard. The highest flavonoid concentration was again observed in dose 3 (49.82±1.33 mg QE/g), consistent with phenolic patterns. This aligns with Dewi *et al.* (2019), who noted that higher phenolic levels correspond to higher flavonoid content, as flavonoids are synthesized downstream in the shikimate and acetate–malonate pathways (Liu *et al.* 2021). Similarly, the determination of total flavonoid content was calculated using the standard quercetin curve that the levels of total flavonoids obtained from seven regions ranged from 2.03 to 5.13 mg QE/g, with the highest value observed in Kendari and the lowest in Malang, showing significant differences between regions ($p < 0.05$). These findings indicate that *P. crocatum* from different regions exhibited varied flavonoid responses (Irsal *et al.* 2023).

Antioxidant activity was evaluated using DPPH radical scavenging and FRAP assays. In the DPPH assay, the lowest IC_{50} (highest activity) was obtained from dose 3 (272.39±0.35 ppm), while dose 2 exhibited the weakest activity (898.36±15.49 ppm). Compared with Sifa (2023), who reported an IC_{50} of 1098.99 ppm, the present results suggest stronger radical scavenging activity under maggot residue fertilization. Based on classification (Indrawati *et al.* 2022), IC_{50} values above 200 ppm indicate very weak antioxidant activity, though the trend shows dose 3 improved activity relative to other treatments. In the FRAP assay, dose 3 again yielded the highest antioxidant capacity (545.28±12.05 μM TE/g), exceeding Krisdianty's (2019) value of 433.94 μM TE/g for red betel. This indicates enhanced reducing power in plants treated with maggot residue. Differences in research outcomes may be influenced by several factors, such as the extraction method, plant treatments, or the geographical origin of the plants used. The antioxidant activity values obtained from both the DPPH and FRAP methods were positively correlated with the total phenolic and flavonoid contents in this study, with the highest levels of both compounds observed in the dose 3 treatment (1 maggot residue fertilizer : 7 planting medium). This finding is consistent with the study by Nur *et al.* (2019), which reported that the total flavonoid and phenolic contents of Gmelina arborea leaf

extracts and fractions were directly proportional to their antioxidant activity, with the best results obtained from the ethyl acetate extract and fraction.

The antioxidant activity of a sample is highly dependent on the types of compounds it contains. Phenolic compounds, particularly flavonoids such as flavonols and flavones, play a crucial role as antioxidants because their hydroxyl (-OH) groups are capable of neutralizing free radicals. The ability of flavonoids to scavenge free radicals is also related to their electron-donating capacity. Therefore, phenolic flavonoids are strongly associated with antioxidant activity. The higher the flavonoid and phenolic content in a sample, the greater its ability to counteract free radicals, resulting in stronger antioxidant activity (Nur *et al.* 2019).

In conclusion, maggot residue fertilizer has the potential to be developed as a sustainable organic fertilizer that not only supports plant growth but also enhances the bioactive compound profile of medicinal plants. Optimizing the dosage is essential to balance plant growth and secondary metabolite production. Further research should explore molecular mechanisms underlying stress-induced metabolite biosynthesis and assess the therapeutic efficacy of red betel extracts produced under maggot residue fertilizer treatment.

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