Research Article

# *In Vitro* antioxidant activity of *garden* Croton (*Codiaeum variegatum* (L.) rumph. Ex A.Juss.) and phytochemical analysis using gas chromatography–mass spectrometry

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**ABSTRACT**

**Introduction:** Garden Croton (Codiaeum variegatum (L.) Rumph. ex A.Juss.) is an easily grown plant, has a various shape and colorful leaves which usually used as an ornamental plant. Objectives: The aims of the research were to determine the antioxidant activity of croton and its phytochemical content. The crude drug was extracted using 96% ethanol . **Methods:** Antioxidant activity was performed using DPPH free radical scavenging method and qualitative analysis was determined using GC-MS. **Results:** Based on the in vitro antioxidant test using DPPH, IC50 of croton extract was 797.61 ppm while the IC50 of ascorbic acid was 8.97 ppm. Based on analysis of the chemical compound of croton using GC-MS, it obtained 10 compounds with percent of quality ( degree of similarity ) average > 90%. The main compounds were pentadecadien-1-ol 34.15% and hexadecanoic acid 21.14%. **Conclusions:** Croton leaves extract was categorized as low antioxidant activity and contained (6Z,9Z) pentadecadien-1-ol and hexadecanoic acid as the primary chemical content.

**KEY WORDS:** Antioxidant, *Codiaeum Variegatum*, Garden Croton, Gas chromatography–mass spectrometry

## INTRODUCTION

Oxidative stress, which is induced by oxygen radicals, is found as the primary cause of illness, especially the degenerative diseases such as cardiovascular, cancer, and hypercholesterolemia.[1,2] Therefore, an exogenous antioxidant is required to maintain sufficient antioxidant level in our body to balance oxygen radicals.[3] Plants are a source of exogenous antioxidant because they produce it as a protection to oxidative stress from sunbeam and oxygen.[4] Many plants including fruit and vegetables were known to have potential antioxidant activity against free radicals. It is associated with their natural product content particularly phenolic, flavonoids, tannins, Vitamin C, tocopherol, and β-carotene.[1,5] In this regard, interest in searching new antioxidant source from plants is significantly increased to improve human health and to substitute synthetic antioxidant, which had carcinogenesis side effect.[6,5]

Garden croton (*Codiaeum Variegatum*) is involved as Euphorbiaceae family[2]. It is grown natively in South Asia, Southeast Asia, and Pacific region [7] In Indonesia, it is usually used as ornamental plant. Its leaves have a variety of colors and shapes, which make it potential to be tested as an antioxidant.[8,9] There were many compounds isolated from various *C. variegatum* including alkaloids glaucine, Oxyglaucine , hemiargyrine ( CAS RN : 210101-98-7) and then two diterpenoids, entrachyloban-3-one and 18-OH-trachyloban-3-one. [2] Root decoction of *C. variegatum* is traditionally used for gastric ulcers. The recent research showed that *C. variegatum* extract had a pharmacological activity against influenza virus and cytotoxic to brian shrimp lethality bioassay[9]. The previous research about antioxidant activity of *C. variegatum*. Spirale and Royal showed that the plants had potent antioxidant activity.[9] In this research, we evaluated *C. variegatum* (L.) Rumph. Ex A.Juss. leaf extract as

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**Table 1: Data of concentration, absorbance, percentage inhibition, and $IC_{50}$ of extract and ascorbic acid**

| Sample | Concentration ppm (x) | Absorbance | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| Garden Croton Extracts (*Codiaeum variegatum* (L.) Rumph. ex A.Juss.) | 800 | 0.5190 | 50.61 | 797.61 µg/ml |
| | 1000 | 0.4750 | 54.79 | |
| | 1200 | 0.4118 | 60.81 | |
| | 1400 | 0.3354 | 68.08 | |
| | 1600 | 0.2973 | 71.71 | |
| Ascorbic Acid | 4 | 0.7899 | 24.83 | 8,97 µg/ml |
| | 6 | 0.7191 | 31.57 | |
| | 8 | 0.5886 | 43.98 | |
| | 10 | 0.4536 | 56.83 | |

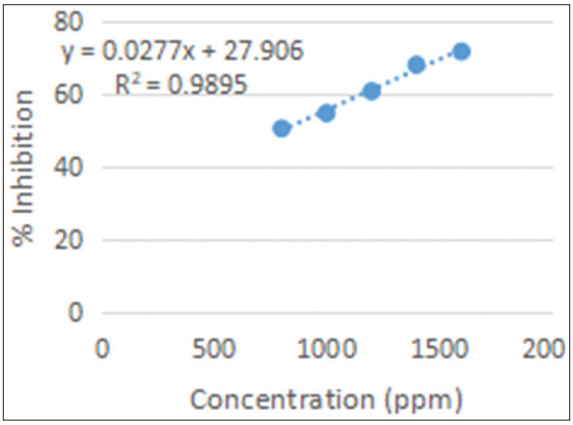


**Figure 1:** Calibration curve of extract

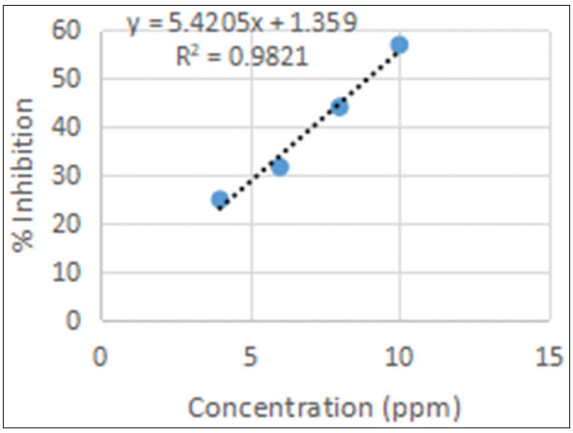


**Figure 2:** Calibration curve of ascorbic acid

antioxidant and its phytochemical content using gas chromatography–mass spectrometry (GC-MS).

## MATERIALS AND METHODS

### Materials

Aquadest,$MgSO_4$,$FeCl_3$,HCl,amylalcohol,96%ethanol, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were used.

### Sample Preparation

Garden croton leaves were collected from Balitro (Badan Penelitian Obat Tradisional) Bogor, West Java, and determined in LIPI Cibinong as *C. variegatum* (L.) Rumph. Ex A.Juss. Leaves are dried in an oven at temperature of 30°C and then grinded.

### Sample Extraction

Garden croton leaves were extracted using maceration by 96% ethanol during 3 × 24 h.

### Flavonoid Test

A total of 0.5 g of concentrated extract was dissolved in 1 mL of 96% ethanol. 0.5 g of magnesium *P* powder, 3 drops of concentrated HCl acid, and 1 ml of amyl alcohol were added into extract and then shaked vigorously; positive results of flavonoids are indicated by the formation of pink or magenta-red color.[10]

### DPPH Assay

Antioxidant activity test was used ascorbic acid as a positive control and methanol as blank. The maximum wavelength of 100 ppm DPPH main solution was measured by UV-Visible spechtrophotometer in the wavelength range of 400-800 nm. Based on the optimization, DPPH solution had maximum wavelength 517 nm with absorbance 1.0508. Various concentration of garden croton leaves extract and ascorbic acid as shown in Table 1 was measured by UV-visible spechtrophotometer to find the absorbance. Then it will be calibrated into calibration curve as shown in figure 1 and figure 2 to calculate the IC50 of garden croton leaves extract and ascorbic acid as comparison. Garden Croton leaves extract had IC50 797.61µg/ ml, while ascorbic acid had IC50 8.97 µg/ml.

### Phytochemical Analysis by GC-MS

The pythochemical content of garden croton leaves was analyzed using GC-MS. Based on table 2, there were 12 volatile compounds in garden croton leaves with 10 compounds had a degree of similarity in more than 90%. The compound with the highest content in croton leaves were (6Z, 9Z) -6,9-Pentadecadiene-1-ol with a content of 34.65% and Hexadecanoic acid 21.14%.[11]

### Phytochemical Analysis using GC-MS

The GC-MS analysis was carried out by Turbo Matrix 650 ATD (perkin Elmer, USA). The column was heated

**Table 2: Phytochemical analysis of compounds in garden croton using GC-MS**

| Retention Time | Quality | Compound | Concentration (%) | Retention Time | Quality | Compound | Concentration (%) |
|---|---|---|---|---|---|---|---|
| 3.494 | 38 | 1,3-cyclopentanedione | 1.43 | 29.123 | 99 | hexadecanoic acid | 21.14 |
| 6.038 | 27 | 2,5-anhydro-1,6-dideoxyhexo-3,4-diulose | 2.2 | 29.689 | 99 | Methyl linoleate | 3.55 |
| 8.169 | 94 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4hpyran-4-one | 10.81 | 29.813 | 90 | Phytol | 4.37 |
| 8.203 | 95 | 4H-pyrani-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl | 4.74 | 30.199 | 96 | (6Z, 9Z)-6,9-pentadecadien-1-OL | 34.65 |
| 11.782 | 90 | Furancarboxaldehyde, 5-(hydroxymethyl) | 4.06 | 30.475 | 95 | Cis-vaccenic acid | 1.8 |
| 11.899 | 90 | 2-Furanearboxaldehyde, 5-(hydroxymethyl) | 3.65 | 32.943 | 86 | Beta-monoolein | 1.5 |

GC-MS: Gas chromatography–mass spectrometry

at 110 0C as initial temperature and maintanance its temperature for 4 minutes. At the end of the periods, the temperature rose to 280 0C with rate of increase 5 0C/ minutes. Column VF-WAXms (30m x 0.25 mm x 1.00 µm) was used in injection port that was set up at 250 0C with helium flow rate 1 ml/minutes. The mass molecule was detected by mass spectroscopy (MS) electron ionization mode with a mass range of 35-400 amu, scanning speed of 0.6 s. Total GC running time was 56 minutes.

## RESULTS AND DISCUSSION

Ethanol is categorized as a polar solvent because it has dielectric constant more than 15[7]. It is usually used as a solvent for extraction caused by its characteristic, volatile and less toxic than other organic solvents. In this experiment, one-kilogram crude drug of garden croton is extracted using 96% ethanol to produce 15,72% extract yield. Garden croton contains flavonoid. It is proved by the formation of orange color in the extract after addition of reagents.

### DPPH Assay

Antioxidant activity test was used ascorbic acid as a positive control and methanol as blank. The maximum wavelength of 100 ppm DPPH main solution was measured using UV-Visible spechtrophotometer in the wavelength range of 400-800 nm. Based on the optimization, DPPH solution had maximum wavelength 517 nm with absorbance 1.0508. Various concentration of garden croton leaves extract and ascorbic acid as shown in Table 1 was measured by UV-visible spechtrophotometer to find the absorbance. Then it will be calibrated into calibration curve as shown in figure 1 and figure 2 to calculate the IC50 of garden croton leaves extract and ascorbic acid as comparation. Garden Croton leaves extract had IC50 797.61µg/ ml, while ascorbic acidhad IC50 8.97 µg/ml.

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## CONCLUSIONS

Garden croton leaf extract had low antioxidant activity because it had $IC_{50}$ > 200 µg/ml. There were 12 compounds in garden croton which were analyzed using GC-MS. (6Z, 9Z) pentadecadien-1-ol was an abundant compound in garden croton with a concentration of 34.65%.

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