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Assessment of Relationship between Antioxidant Activity, Toxicity and Phenol Content of *Cayratia trifolia* Ethanolic Extract

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ABSTRACT

Introduction: *Cayratia trifolia* is a plant that has the potential as a natural antioxidant. The use of natural antioxidant need to be known its toxicity and phenol content. As the limited knowledge of the antioxidant properties and phenolic content of many plant species used as traditional plant medicine. Phenolic molecules and flavonoids are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals.
Objective: this study aims to assess relationship between the antioxidant activity, toxicity and phenol content of ethanol extract, n-hexane fraction, chloroform fraction and leaf ethanol fraction and *Cayratia trifolia* stems.
Methods: The antioxidant activity test used the DPPH (2,2-diphenyl-1-pikril-hidrazil) method the total phenol content used the Folin-Ciocalteu method and the toxicity test used the method Brine Shrimp Lethality Test.
Results: The results of this study indicate that the highest % yield is at leaf ethanol fraction 24.52%. The phytochemical screening showed that the extract and ethanol fraction leaves and stems contain alkaloid compounds, flavonoids, polyphenols and steroids. N-hexane fraction and Chloroform leaves and stems contain polyphenol and steroid compounds. There is antioxidant activity in the ethanol fraction of *Cayratia trifolia* rods has an IC_{50} value of 58.4 ppm and the highest total phenol content which is 56.2 µg / mL. The toxicity test showed that the chloroform and ethanol fraction was stem had the highest toxicity activity with an LC_{50} value of 92.4 ppm.
Conclusion: This indicates that the ethanol fraction is active as an antioxidant and the chloroform fraction of the stem is active as an anti cancer.

Keywords: Antioxidant, *Cayratia trifolia*, phenol, toxicity

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INTRODUCTION

Cayratia trifolia is one of tropical plant included in Vitaceae family and including types of wild plants which are easily found in the forest, especially in riverside area. *Cayratia trifolia* parts which is often used by the community, namely the fruit, stems and leaves. *Cayratia trifolia* leaves, empirically, it has been used by society for herbal drinks for women who finished giving birth and ulcers. *Cayratia trifolia* has the same family as grape leaves purple (*Vitis vinifera* L). Research on *C. trifolia* has been conducted by several researchers, namely *C. trifolia* contains flavonoids, tannins, saponins, triterpenoids, anthraquinones and alkaloids which has the potential as an antioxidant[1,2,3]. The previous study reported that petroleum ether extract of *C. trifolia* leaves (Linn) has potential as an anti-inflammatory[4].

Research on the group of leaves and stem chemical compounds of seven species of *Cayratia* spp (*C. anemonifolia*, *C. auriculata*, *C. carnosa*, *C. elongata*, *C. planicaulis*, *C. pedata* and *C. trilobata*) ie has a chemical content of carbohydrates, lignin, steroids, sterols, polyphenols, flavonoids, glycosides[5,6]. The previous study reported several parts of *C. trifolia* and Cayratian species, it is suspected that the leaves and stems are also *C. trifolia* potential as an antioxidant and anticancer. However, until now there is no reference about the total phenol content, the toxicity test and antioxidant activity of the leaves and stems *C. trifolia*. Therefore, it needs to be done phytochemical screening testing, total phenol determination, antioxidant activity, and toxicity test of extracts and fractions of *C. trifolia* leaves and stems.

MATERIALS AND METHODS

Preparation of Sample

Cayratia trifolia samples obtained from one of wild gardens in Bangkalan regency, Madura Island cleaned and determined the species at the Herbarium Bogoriense Biology Research Center-LIPI Bogor. Then separated between the stems and leaves, then sample dried and mashed with using a blender.

Extraction and Sample Partition

Cayratia trifolia leaf and stems powder macerated with ethanol for 3x24 hours at temperature room. The extract was then filtered in order obtained a separate filtrate from the residue. The ethanol extract was then partitioned using solvents with a degree of polarity the different are n-hexane and the chloroform, then concentrated use the evaporator to obtain concentrated extract from each fraction.

Phytochemical Analysis

Identify the chemical content in the extract carried out on compounds[7]:

a. Steroids / triterpenoids

A total of 1 ml of extract solution was added with the Lieberman-Burchard reagent. Existence steroid compounds are characterized by a green or blue color and triterpenoids to result in red or violet color.

b. Alkaloids

A total of 3 ml of extract solution was added with 1 ml of 2 N HCl, and 6 ml of distilled water, then heat for 2 minutes, then cooled and filtered. After that filtrate was checked for presence alkaloid compounds with Dragendorff reagent, Mayer and Wagner.

c. Polyphenols

The extract solution was added with 2 drops of reagent iron (III) chloride 1%. Phenolic compounds will produce blue, green, red, purple or black colors.

d. Flavonoids

A total of 2 ml of extract solution was added a little magnesium powder and 2 ml HCl 2N. Flavonoid compounds deliver orange, yellow or red colors.

Determination of the total phenol content[8].

Determination of the total phenol content was carried out with the Folin-Ciocalteu method. A total of 0.5 mL of a 0.05% extract solution of the ethanol extract, ethanol fraction, chloroform fraction and n-hexane fraction and mixed with 0.75 mL of reagent Folin-Ciocalteu 10% and 2 mL Na_2CO_3 (2% w / v). Then the mixture is homogenized with a vortex for 15 seconds and heated at a temperature of 45^oC for 15 minutes. The absorbance of the sample was measured at λ max 720 nm using a UV-Vis spectrophotometer. The standard curve for tannic acid was defined with using a linear regression equation, that is states the relationship between tannic acid concentrations (0, 20, 40, 60, 80, 100 ppm) are expressed as the X axis with magnitude the absorbance results of the reaction of tannic acid with Folin-Ciocalteu reagent stated as the Y axis.

Antioxidant activity test by DPPH method[9].

A total of 1 mL of the solution of ethanol extract and the respective fractions (ethanol, chloroform and n-hexane) with concentrations of 50, 100, 150, 200, and 250 µg / ml were mixed with 2 mL of 0.002% DPPH solution, then let the mixture sit for 30 minutes. then the absorption was measured at length wave 525 nm. As a positive control used vitamin C (concentrations 2, 4, 6, 8 and 10 ppm). The inhibitory strength was calculated using a formula: % inhibition = (A0-A1)/A0 X 100

Toxicity test using the Brine Shrimp Lethality Test (BSLT)[10].

a. Preparation of shrimp larvae and basic solution

The container was divided in two parts, filled with water sea as much as 1 liter. Eggs of *Artemia salina* inserted in the closed bulkhead and one partition was left open, then given a light above the part open to attract *Artemia salina* prawns towards the part where the light was exposed so that they separate from the shell. Eggs of *Artemia salina* will hatch into larvae within 48 hours and used for the test toxicity of the ethanol extract, n-hexane fraction, chloroform fraction and ethanol fraction leave and stems of *C. trifolia*. Two thousand ppm of basic solution was prepared with dissolve as much as 0.5 g of the extract ethanol, n-hexane fraction, and chloroform fraction and ethanol fraction of leaves and stems of *C. trifolia*. Dissolved in 25 mL of sea water, plus 3 drops of DMSO, then diluted to three kinds of concentrations, 0, 10, 100, and 1000 ppm.

b. Toxicity test

A total of 10 shrimp was put in in each filled vial ethanol extract solution, n-hexane fraction, fraction chloroform and leaf and stem ethanol fraction *C. trifolia* with each concentration of 1000, 100 and 10 ppm in three repetitions. Bottle vials each containing 5 mL (sea water, sample and 10 shrimp), one as a control. After 24 hours, the number of shrimps that died was observed for each concentration. Research data were processed and presented in tabular form and graphs. The data from the toxicity test were analyzed by using probit analysis SPSS version 20.0 to find out the LC_{50} value.

RESULTS

Extraction

Extraction of leaves and stems of *C. trifolia* was carried out with the maceration method using an ethanol. Result of leaf and stems maceration of *C. trifolia* with ethanol obtained yields of about 9.81% and 9.57% (Fig. 1). The partition results from leaf and stem extracts of *C. trifolia* obtained three fractions each (Table 1).

Table 1. Yield of fraction of n-hexane, Chloroform and Ethanol Leaves and Stems of *C. trifolia*

Fraction	Condensed Mass (gr)	Yields (%)
n-hexane leaves	4.312	10.78
Chloroform leaves	6.944	17.36
Ethanol leaves	9.808	24.52
n-hexane stems	2.104	0.42
Chloroform stems	5.77	11.54
Ethanol stems	9.575	19.15

* initial mass of leaf fraction: 40 grams * initial mass of stem fraction: 50 grams

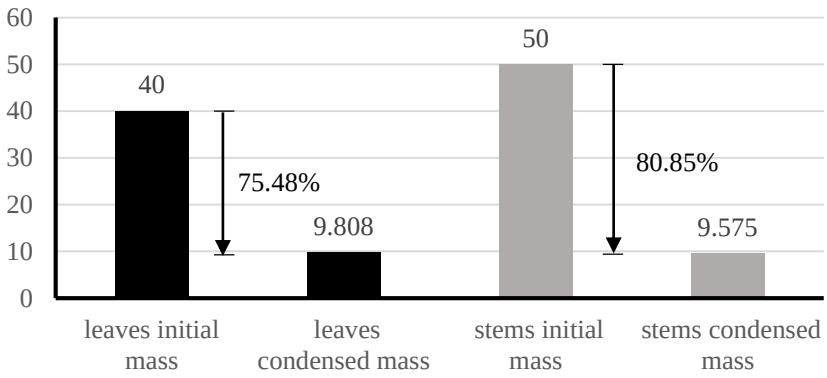

Figure 1. The yield comparison of leaves and stems fraction of *C. trifolia* after extraction through maceration with ethanol

Phytochemical Test

The phytochemical test was carried out as a qualitative preliminary test to find out chemical content. The screening phytochemicals in various fractions in the *C. trifolia* leaves and stems seen several compound groups (Table 2).

Table 2. The screening phytochemicals in various fractions in the *C. trifolia* leaves and stems.

Sample	Compound Groups Test			
	Alkaloids	Flavonoids	Polyphenols	Steroids
Ethanol extract of leaves	+	+	+	+
n-hexane fraction of leaves	-	-	+	+
Chloroform fraction of leaves	-	-	+	+
Ethanol fraction of leaves	+	+	+	+
Ethanol extract of stems	+	+	+	+
n-hexane fraction of stems	-	-	+	+
Chloroform fraction of stems	+	-	+	+
Ethanol fraction of stems	+	+	+	-

Note: **+** there was a change in color**; -** there was no color change

Determination of Total Phenol Content

Determination of the total of phenol content with the Folin-Ciocalteu method was carried out based on Folin-Ciocalteu's reagent capabilities oxidizes the hydroxyl (OH) from phenol group compounds. Phenolic compounds reducing the phosphomolybdate phosphotungstate in Folin-Ciocalteu forms the molybdenum blue.

Table 3. Content of Total Phenol of Leaf and Stems of *C. trifolia*

Extract/Fraction	Total Phenol (µg/mL)
Ethanol extract of leaves	23.0
n-hexane fraction of leaves	11.6
Chloroform fraction of leaves	38.2
Ethanol fraction of leaves	42.4
Ethanol extract of stems	31.5
n-hexane fraction of stems	13.6
Chloroform fraction of stems	21.8
Ethanol fraction of stems	56.2

Test of Antioxidant Activity by Method DPPH (2,2-diphenyl-1-picryl-hidrazil)

The results show that in the stem ethanol fraction sample has the smallest IC_{50} value compared to the sample other, but still has more IC_{50} value large compared to the IC_{50} value of vitamin C (Table 4).

Table 4. Antioxidant Activity Test by DPPH Method

Extract/Fraction	IC_{50} (ppm)
Ethanol extract of leaves	163.7
n-hexane fraction of leaves	582.2
Chloroform fraction of leaves	128.4
Ethanol fraction of leaves	122.6
Ethanol extract of stems	156.8
n-hexane fraction of stems	515.9
Chloroform fraction of stems	236.0
Ethanol fraction of stems	58.4
Vitamin C	8

Table 5. The Relationship between Antioxidants and Total Phenol Content

Extract/Fraction	Total Phenol (µg/ml)	IC_{50} (ppm)
Ethanol extract of leaves	23.0	163.7
n-hexane fraction of leaves	11.6	582.2
Chloroform fraction of leaves	38.2	128.4
Ethanol fraction of leaves	42.4	122.6
Ethanol extract of stems	31.5	156.8
n-hexane fraction of stems	13.6	515.9
Chloroform fraction of stems	21.8	236.0
Ethanol fraction of stems	56.2	58.4

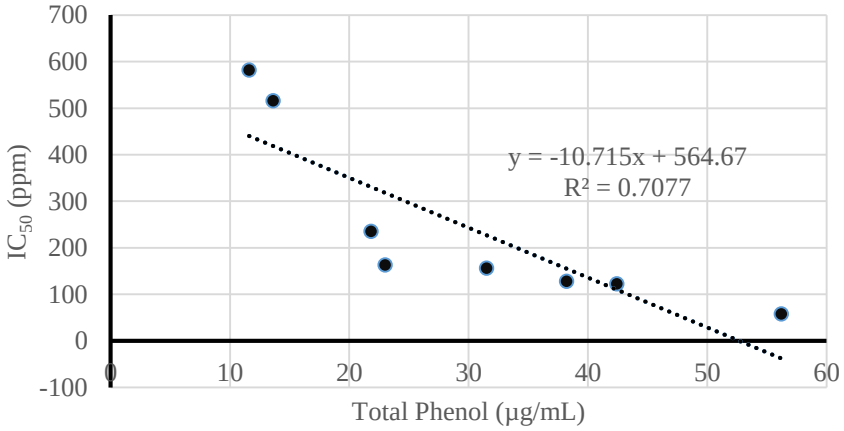

Figure 2. The Relationship between antioxidants activity and total of phenol content of each extract and fraction of *C. trifolia*

Toxicity Test with Brine Shrimp Lethality Test (BSLT)

The Brine Shrimp Lethaly Test (BSLT) was carried out to determine the toxicity activity of a natural material samples. This method is an initial test used to determine anticancer activity based on ability a sample to kill shrimp larvae (*Artemia salina*)[11].

Table 6. LC_{50} of Leaves and Stems of *C. trifolia*

Extract/Fraction	LC_{50} (ppm)
Ethanol extract of leaves	654.5
n-hexane fraction of leaves	1196.4
Chloroform fraction of leaves	241.6

Ethanol fraction of leaves	1165.4
Ethanol extract of stems	643.2
n-hexane fraction of stems	990.2
Chloroform fraction of stems	92.4
Ethanol fraction of stems	92.4

DISCUSSION

The maceration method using an ethanol damage walls cells of leaves and stems of *C. trifolia* so that the compounds are polar or nonpolar can be dissolved in ethanol. During the process there is a process diffusion[12]. Table 1 shows that in the fraction of ethanol leaves and stems of *C. trifolia* has value the highest yield compared to the fraction of chloroform and n-hexane fraction. This condition because during the sample maceration process of *C. trifolia* leaves have more surface area large compared to stems of *C. trifolia* and the water content in the leaves is less so that the weight shrinkage on the *C. trifolia* leaves smaller[13].

The phytochemical test was carried out as a qualitative preliminary test to find out chemical content. Table 2 showed that almost all samples contain a class of compounds of polyphenols and steroids. Compound group of flavonoids exist only in extracts and fractions ethanol. This is because of the compound group of flavonoids are found in solvents that are polar, namely the extract and fraction of ethanol. There is a class of alkaloid compounds in ethanol extract, ethanol fraction and fraction of chloroform stem. Phytochemical extract screening of petroleum ether of *C. trifolia* contains class of flavonoid compounds, steroids, tannins and terpenoids[1]. According to the previous study[14], extract ethanol leaves, stems and roots *C. trifolia* contains alkaloid compounds, flavonoids, terpenoids, tannins, and saponins.

Analyses of total phenol content of *C. trifolia* indicates that more phenolic compounds much extracted in the stem ethanol fraction of *C. trifolia* than semi-polar and non-polar fractions. The highest total phenol content was found in the stem ethanol fraction was 56.2 µg / ml (Table 3). This can be happened because of group compounds phenol is polar or semi-polar[15].

The stem ethanol fraction sample has the smallest IC_{50} value compared to the sample other, but still has more IC_{50} value large compared to the IC_{50} value of vitamin C (Table 4). Ethanol fraction has a smaller IC_{50} value compared to other extracts because of the presence of flavonoid compounds that play a role as an antioxidant[16]. Antioxidant activity test by method DPPH was carried out to determine capacity of sample activity to inhibit DPPH radical stabilized by donating hydrogen atom. Samples that have activity antioxidants will reduce DPPH to DPPH-H[17]. Reduction takes place marked with a purple change to yellow. The IC_{50} value is considered a good indicator for the antioxidant efficiency of the compounds pure or extract. According to the previous study[15] that the smaller the IC_{50} value means higher in antioxidant activity.

Based on the statistical analysis, analysis of variants ($P < 0.05$) between concentration and % inhibition has a significant difference. Antioxidant activity is known to have relationship with total phenol content (Fig. 2). It was shown that the stem ethanol fraction which has a total content the highest phenol was 56.2 µg / ml and shows the lowest IC_{50} value, namely 58.4 ppm (Table 5). The lower the IC_{50} value then the higher the total phenol content, so that antioxidant activity is directly proportional to total phenol, the higher the phenol content in a material the higher it is its activity as an antioxidant[18]. Based on the statistical analysis by analysis of variants showed that IC_{50} value and total phenol content has significant difference with the values confidence 0.852 means the effect of the content of total phenol on antioxidant activity 85.2% whereas 14.8% is influenced by other compounds.

The LC_{50} value is the concentration of a chemical compounds that can kill 50% test organisms[11]. Result showed that the stems chloroform fraction of *C. trifolia* are more toxic than another fraction of this can be seen from the LC_{50} value the smallest was 92.4 ppm (Table 6). This matter based on the previous study[11] that extract toxic if the LC_{50} value <1000 mg / ml. The more toxic extract is the chloroform fraction and ethanol fraction of stems of *C. trifolia* due to *C. trifolia* stems have a high mortality rate bigger than the *C. trifolia* leaves in various factions, apart from that because of influence of secondary metabolite compounds contained namely polyphenols, alkaloids, flavonoids and steroids that kill shrimp larvae.

CONCLUSION

In conclusion, the highest total phenol content was in the stem ethanol fraction. The highest antioxidant activity was the stem ethanol fraction. The most toxic LC_{50} was the stem ethanol fraction and stem chloroform fraction. Phytochemical screening indicated the compounds play a role in antioxidant activity estimated from the flavonoid group and compounds that play a role in activity cytotoxic was from the alkaloid group and steroids. The greater the total phenol content, the greater the antioxidants activity.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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