EVALUATION OF ANTIOXIDANT ACTIVITIES FROM ETHYL ACETATE FRACTION OF CURRY LEAF USING DPPH METHOD

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Abstract: Curry Plant (*Murraya koenigii* (Linn.) Spreng) is one of the plants that thrive in Indonesia. Curry contains secondary metabolites of alkaloids and flavonoids that have the potential as antioxidants. Antioxidants can reduce the activity of free radicals that can cause degenerative diseases such as cancer, coronary heart disease, and premature ageing of body cells by donating the protons to free radical compounds. This study aimed to determine the antioxidant activity and identify the active compound class of ethyl acetate fraction from curry leaves. Separation of secondary metabolites of ethyl acetate extracts was carried out by column chromatography method, which obtained 5 different fractions. The antioxidant activity of fractions A, B, C, D, and E was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with variations concentrations of 25, 50, 100, 200, and 400 ppm, and ascorbic acid was used as a comparison which measured at a wavelength of 517 nm. The results showed that fractions A and E have weak antioxidant activities, while fractions B, C, and D showed moderate antioxidant activities. The highest antioxidant activity was found in fraction B with an IC_{50} value of 341.38 ppm, where the fraction showed the presence of secondary metabolites of alkaloids and terpenoids.

Keywords: *Murraya koenigii*, Curry Leaf, Antioxidant, DPPH, Ethyl Acetate Extract.

Abstrak: Tanaman kari ((*Murraya koenigii* (Linn.) Spreng) merupakan salah satu tanaman yang tersebar di Indonesia. Kari mengandung senyawa metabolit sekunder alkaloid dan flavonoid yang memiliki potensi sebagai antioksidan. Antioksidan dapat mengurangi aktivitas radikal bebas yang dapat menyebabkan penyakit degeneratif seperti kanker, penyakit jantung coroner dan penuaan sel dengan cara menyumbangkan proton pada senyawa radikal bebas. Tujuan dari penelitian ini adalah untuk menentukan aktivitas antioksidan dan mengidentifikasi kelas senyawa aktif fraksi etil asetat dari daun kari. Pemisahan metabolit sekunder dari ekstrak etil asetat dilakukan menggunakan metode kromatografi kolom dengan menghasilkan 5 fraksi yang berbeda. Aktivitas antioksidan dari fraksi A, B, C ,D ,dan E dilakukan menggunakan metode DPPH (1,1-diphenil-2-pikrilhydrazil) dengan variasi konsentrasi 25, 50, 100, 200, dan 400 ppm dan asam askorbat digunakan sebagai perbandingan yang diukur pada panjang gelombang 517 nm. Hasil menunjukkan bahwa fraksi A dan E memiliki aktivitas antioksidan yang lemah, sedangkan fraksi B, C, dan D menunjukkan aktivitas antioksidan sedang. Aktivitas antioksidan yang paling tinggi ditemukan pada fraksi B dengan IC_{50} 341,38 ppm. Fraksi tersebut menunjukkan adanya kandungan senyawa metabolit sekunder alkaloid dan terpenoid.

Kata kunci: *Murraya koenigii*, Daun Kari, Antioksidan, DPPH, Ekstrak Etil Asetat.

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Introduction

Indonesia is a tropical country rich in flora diversity, both as a source of medicine, protective plants, or food. One of the plants that thrive in Indonesia is *Murraya koenigii* (Linn.) Spreng). Commonly, this plant is known as a curry leaf or *temurui* as a local name in Aceh, Indonesia (Fachraniah *et al.*, 2012). The leaves are used as traditional vegetables, especially used in Indian cooking for flavouring foodstuffs. The majority of Acehnese people use this plant as spices in a local culinary because it can give a distinctive flavor and aroma to the food (Safrizal *et al*., 2017). Traditionally, these *temurui* leaves are also used as herbs, condiments and treat various ailments such as cure dysentery, diabetes mellitus, and stimulants (Bhandari, 2012).

Based on chemotaxonomy literature, *M. koenigii* plants contain many metabolite compounds such as alkaloids, terpenoids, phenolics, flavonoids, carbohydrates, protein, and amino acids. The secondary metabolites could serve many biological activities. Research on *M. koenigii* as bioactivity has been widely studied and reportedly active as antioxidant, hypoglycemic, hepatoprotective, anti-inflammatory, antidiabetic, anticarcinogenic, and antimicrobial (Rahayu *et al*., 2019; Ningappa *et al*., 2010; Mahipal and Pawar, 2017; Ghasemzadeh *et al*., 2014).

Free radicals are unstable molecules that have one or more unpaired electrons in their outer shell. Free radicals can damage proteins, carbohydrates, fats, and Deoxyribose Nucleic Acid (DNA), which can cause various dangerous diseases such as cancer, coronary heart disease, diabetes, and cell ageing. But in certain circumstances, such as exposure to radiation rays, smoking habits, and unhealthy lifestyles, can trigger the emergence of free radical molecules in the body. The number of antioxidants produced by the body cannot neutralize all free radical molecules. Therefore, the body needs antioxidant compounds from outside the body. One source of natural antioxidants is *M. koenigii* leaves (Khaira, 2010). Antioxidants are compounds that can neutralize free radicals by protecting the body's proteins, cells, tissues, and organs. Based on research, antioxidants have been proven to prevent premature ageing (anti-ageing), prevent heart disease, prevent various types of cancer, prevent blindness and increase immunity (Nugroho, 2015).

In Indonesia, especially in Aceh, research on the potential of *M. koenigii* as the antioxidant agent has still very limited. Based on pharmacology review, *M. koenigii* can be potentially active as an antioxidant. The results of this study are

expected to contribute to the medicinal to develop *M. koenigii* is a natural source of antioxidant agents. They can be widely used as a safe source of antioxidants.

Procedures

Preparation of Sample

M. koenigii (L.) Spreng leaves were collected from Langsa City, Aceh (Indonesia). The samples were separated from the leaf stalks and then air-dried for 7 days. The air-dried plant material was powdered by using a blender. The powder was used for the extraction of active constituents of the plant material.

The air-dried leaves (1.0 Kg) of plant materials were ground and extracted with increasing polarity of n-hexane, ethyl acetate, and methanol by maceration method for 3 x 24 hours. The maceration was repeated until the filtrate is clear. The extracts solution was filtered and evaporated by a rotary evaporator to give the crude extract.

Separation of Compounds Using Column Chromatography

The separation process was carried out to obtain the chemical compound fraction from ethyl acetate extract of *M. koenigii* leaves, an antioxidant (Wati *et al*., 2017). The process of separating chemical compounds used column chromatography. The ethyl acetate extract was subjected to column chromatography over silica gel and eluted with an increasing polarity solvent system of hexane, ethyl acetate, and methanol. The separation of samples yielded 5 fractions that continued to antioxidant test using DPPH assay.

DPPH Assay

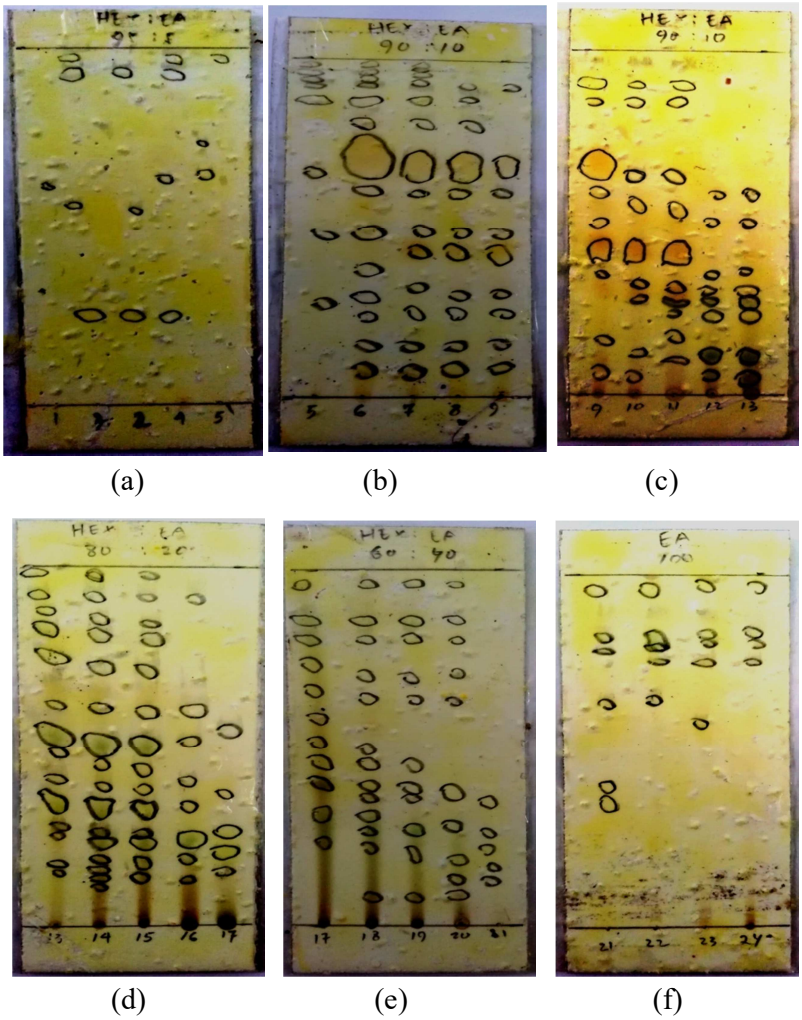
Determination of antioxidant activity was used in the DPPH method. DPPH testing has been considered a standard colorimetric method and is easy to estimate antioxidant properties by assessing the free radical scavenging capacity of antioxidants. This method is based on reducing DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. Briefly, a 0.4 mM solution of DPPH in methanol was prepared, and 1 mL of this solution was added each to 250 µL, 500 µL, 1000 µL, 2000 µL, and 4000 µL of the solution of fractions ethyl acetate in methanol at different concentration (25, 50,100, 200, 400 ppm). The volume is adjusted to 5 mL with methanol. The mixtures were shaken with a vortex mixer and allowed to stand at room temperature for 30 minutes. After that, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (1240 Shimadzu). Ascorbic acid was used as the reference. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula. DPPH scavenging effect (% inhibition) = {(A0 – A1)/A0)*100} Where A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of all of the fractions samples and reference. The antioxidant activity was expressed as

IC_{50}, defined as the amount of antioxidant needed to decrease the initial free-radical concentration by 50%. The IC_{50} values can be calculated using a linear regression equation of y = bx + a between the concentration of the test solution (x) with the percentage of antioxidant activity (y) and analyzed descriptively (Halimatussakdiah *et al*., 2020).

Result and Discussion

Separation Compound using Column Chromatography

Ethyl acetate extract of *M. koenigii* was separated by column chromatography to compare variations in the concentration of n-hexane, ethyl acetate, and methanol solvents. This process produced several fractions of the compound. These fractions were monitored using TLC to find out which groups of fractions have the same stain pattern. Monitoring results produced five fractions, namely fractions A, B, C, D, and E. All fractions could be seen in Figure 1 the same stain pattern was tabulated in Table 1.



(a) (b) (c)

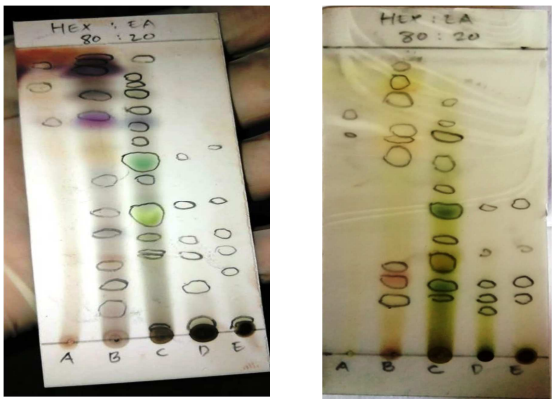
(d) (e) (f)

Figure 1. TLC Pattern of Ethyl Acetate Fraction of *M. koenigi* with solvent system (a) n-Hexane : Ethyl Acetate (95:5)% (b) n-Hexane Compound Fraction (90:10)% (c) n-Hexane : Ethyl Acetate (90:10)% (d) n-Hexane : Ethyl Acetate (80:20)% (e) n-Hexane : Ethyl Acetate (60:40)% (f) Ethyl Acetate 100%.

Table 1. Classification of *M. koenigii* Ethyl Acetate Fraction Groups

Fraction Group	Fraction
A	1-5
B	6-11
C	12-15
D	16-20
E	21-24

The five groups of fractions were monitored by the staining pattern with two different specific tests: terpenoid and flavonoid tests by TLC plate burning and alkaloid tests using Dragendorff reagents. It was used to see the pattern of stains that were not previously detected in irradiation with UV lamps. The results of monitoring the stain patterns of the five fractions could be seen in Figure 2.



(a) (b)

Figure 2. Patterns Test of Five Fraction Groups (a) Terpenoid and Flavonoid Specific Tests (b) Alkaloid Specific Tests.

In Figure 2. there were differences in the stain luminescence of the five fractions formed in the terpenoid and flavonoid tests and the alkaloid test. Burn test using 5% H_2SO_4 as a reagent was used to detect the presence of terpenoid and flavonoid compounds. In the test results, it could be seen that fraction B showed a purple stain glow and a brown stain glow which is assumed to be a glow stain from a group of terpenoid compounds. Fraction C was yellow, green, and light purple stains where yellow color stains are considered to be stain colors from the flavonoid compound group, and light purple strains were assumed to stain from terpenoid compounds. There were no stain color in fractions A, D, and E.

Dragendorff reagent was used to detect classes of alkaloids. The results showed that fraction B was a glowing brown stain, which was assumed to be a glow stain from the alkaloid compound group. Fraction C has a greenish-brown glow which was believed to be a luminous stain from a class of alkaloid compounds, but different types. Fraction D has a green stain but not too prominent, and it was assumed that the alkaloid compound group in that fraction was minor. Whereas in the fractions A and E, there was no luminous color of the stain that forms.

Specific tests on this TLC could be used to predict the class of compounds from a sample. Specific group test result on the ethyl acetate fraction of *M. koenigii* leaves was tabulated in Table 2.

Table 2. Compound Group presenced in *M. koenigii* Ethyl Acetate Fraction Groups

Fraction Group	Compound Groups
A	-
B	alkaloids and terpenoids
C	alkaloids, terpenoids, and flavonoids
D	alkaloids
E	-

Research of phytochemistry test from ethyl acetate fractions was not done before. Still, a phytochemistry test from *M. koenigii* leaves extract has been done many times in previous research that showed the same compound group. Alkaloids are the majority of compounds that presences in this sample. Leaves of *M. koenigii* contained alkaloids and tannins (Salwe et al., 2017), alkaloids, terpenoids and flavonoids (Patil, 2018), alkaloids, saponins, tannins, and flavonoids (Widyanti et al., 2019), alkaloids and terpenoids (Mustanir et al., 2019), alkaloids (Samantha *el al*., 2018) and terpenoids and polyphenols (Tan *et al*., 2017)

Antioxidant Assay Using DPPH Method

In this study, all five fractions from ethyl acetate of *M. koenigii* (L) Spreng leaves were evaluated for antioxidant activity. Ascorbic acid was a positive control against free radicals and showed an IC_{50} value of 7.24 µg/mL. The antioxidant of the fractions was assayed at various concentrations of 25, 50, 100, 200, and 400 µg/mL. The absorbance was measured at 517 nm using a UV-VIS spectrophotometer and expressed in IC_{50} values as showed in Figure 3 and summarized in Table 3.

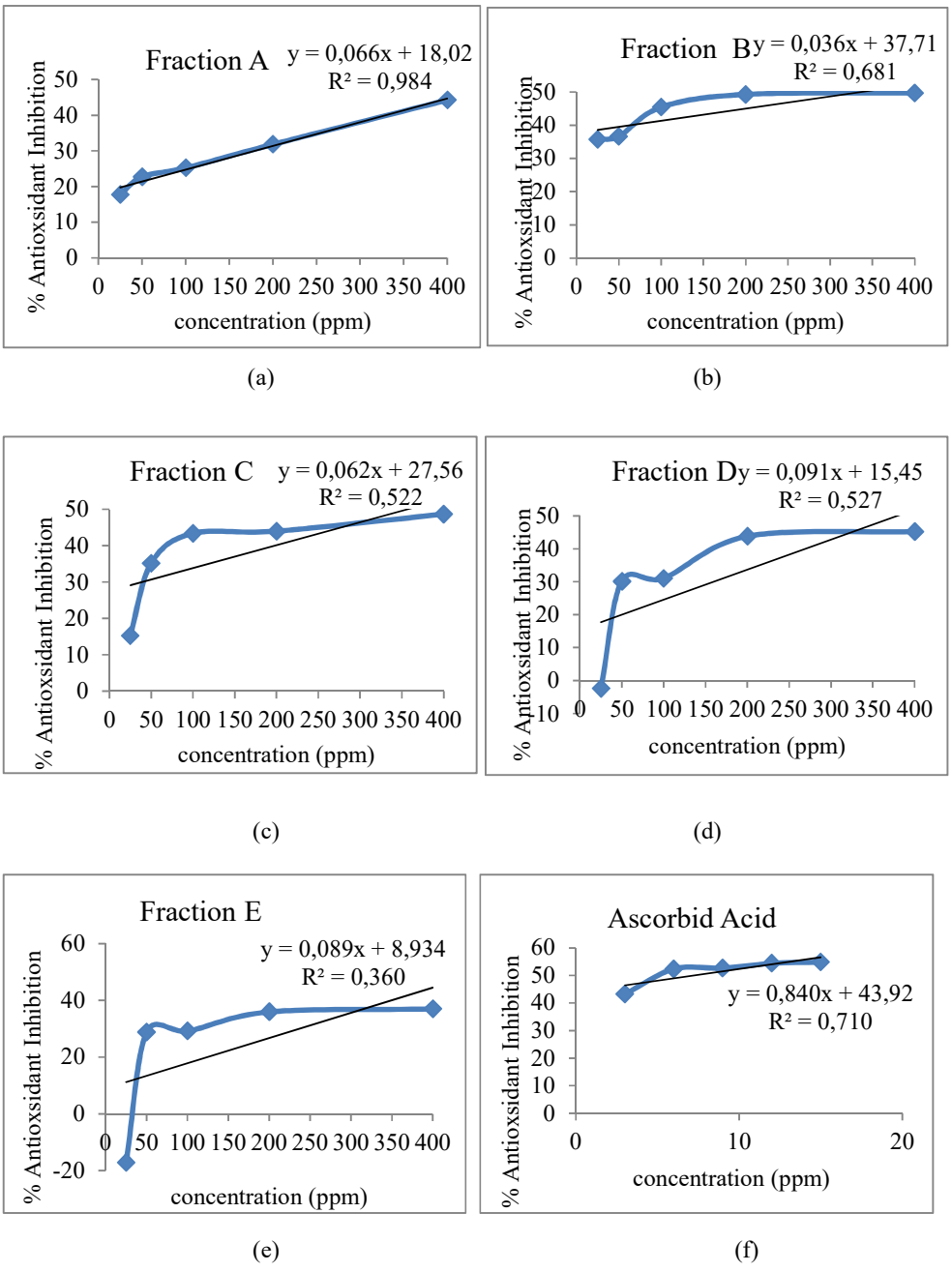


Figure 3. Graph % Antioxidant Inhibition Vs. Concentration of Ethyl Acetate Fractions of *M. koengii* Leaves and Ascorbic Acid (a) Fraction A (b) Fraction B (c) Fraction C (d) Fraction D (e) Fraction E (f) Ascorbic Acid.

Table 3. Antioxidant Activity of Ethyl Acetate Fractions of *M. koenigii* Leaves against Free-Radicals

Sample	IC_{50} (µg/mL)	Intensity
A	484,54	Weak
B	341,38	Moderate
C	361,93	Moderate
D	379,67	Moderate
E	461,41	Weak
Ascorbic Acid	7,24	Very Strong

Figure 3 and Table 3 showed that fractions B, C, and D have higher antioxidant activity than fractions A and E. These differences in antioxidant activity occur because the antioxidant compounds contained in each fraction were different. Fractions A and E have weak antioxidant intensity. It was assumed that there are no compounds that have potential as antioxidants in infractions A and fraction E. Fractions B, C, and D have IC_{50} values in moderate antioxidant intensity. A compound with very strong or antioxidant solid properties where the IC_{50} values obtained are <150 or 150-300 µg/mL (Mangkasa *et al*., 2018), so the antioxidant compounds measured in this study were less active still have potential as antioxidants. The high IC_{50} value in the sample due to the fact that the sample was a fraction, not yet a pure compound. Therefore, the compounds contained in these fractions may have stronger free radical scavenger activity than their fractions.

All fractions showed that fraction B is very good based on antioxidant activity because the IC_{50} is the lowest. IC_{50} showed the potentiality of antioxidant activity. The lower IC_{50} values displayed good antioxidant activity. This antioxidant activity of fraction B from ethyl acetate fraction of *M. koenigii* leaves is contributed by secondary metabolites in the plant that can reduce free radicals. The compounds that reduce free radicals are thought to be terpenoid and alkaloid compounds.

Research of antioxidant activities from ethyl acetate fraction was not yet done before. But, compared with previous research, antioxidant activity from methanol extract of *M. koenigii* leaves (polar) showed strong activity, ethyl acetate extract (semipolar) showed as moderate, and petroleum eter extract (nonpolar) showed the lowest activity (Dahlia *et al.,* (2017). Another research also showed a similar result where chloroform extract of *M. koenigii* leaves (polar) showed good antioxidant activity compare with hexane extract (nonpolar) that showed low antioxidant activity (Ng et al., 2018).

This research was used ethyl acetate extract that has been fractionated with column chromatography. Generally, the ethyl acetate fraction showed moderate antioxidant activities in accordance with previous research that showed antioxidant activities from semipolar extract as moderate.

Conclusions

Based on the findings, it could be concluded that the content of *M. koenigii* (Linn.) Spreng leaves were alkaloids, terpenoids, saponins, flavonoids, and tannins. The separation of compounds using the chromatography method produces 5 fractions with different antioxidant values. Fraction A and E have low antioxidant activity, and Fraction B, C, and D show moderate antioxidant activity. The highest antioxidant activity was obtained in fraction B with an IC_{50} value of 341.38 ppm. Based on the results of stains on TLC, fraction B contain flavonoids and terpenoids compound groups.

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