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Antioxidant activity and total phenolic contentof ethyl acetate extract and fractions of *Lantana camara* L.leaf

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ABSTRACT

The ethyl acetate extract of Lantana camara L. was subjected in investigation of its antioxidant properties by DPPH method. The concentrated ethyl acetate extract of Lantana camara L. was column chromatographed by separation gradien polarity (SGP) method using silica gel as the stationary phase where is hexane, ethyl acetate and methanol selected as the mobile phases. Seven fractions were obtained, each fractions among them were subjected in investigation of its antioxidant properties and total phenolic content. This studies demonstrated that IC_{50} value of Lantana camara L. extractwas found to be 36.18 mg/L with a total phenolic content was 2419.6 GAE. And IC_{50} value and total phenolic content of each fractions were found to be: Fraction A (132.62 mg/L and237.8 GAE), Fraction B (113.51 mg/L and589.4 GAE), Fraction C (85.23 mg/L and995.5 GAE), Fraction D (81.26 mg/L and1041 GAE), Fraction E (24.83 mg/L and3156 GAE), Fraction F (83.50 mg/L and1037.8 GAE), and Fraction G (806.71 mg/L). Based on the IC_{50}, extract ethyl acetate and Fraction Eare highly active antioxidants, fraction C, D, and Fare active antioxidant, fraction A and B moderate antioxidant and fraction G is not active antioxidant. Antioxidant activity is influenced by total phenolic with correlation number was 98.14%.

Keywords: *Lantana camara* L., antioxidant, DPPH, total phenolic content, IC_{50}.

INTRODUCTION

Lantana camara L. is one of plant that has been use traditionally in Asia and South America regions to cure skin disease, guts problem, tetanus, malaria and tumor [1]. The extract has been known to have antimicrobial, antifungal, insecticidal, antibacterial and antidiabetic activities [2-7]. The extract of the *Lantana camara* L. leaves is very toxic with LC_{50} value of hexane, ethyl acetate and methanol extracts are 34 µg/mL, 27 µg/mL, dan 133 µg/mL respectively[8].

Lantana camara L. has phenolic compounds content that acts as antioxidant that is spread out in leaves and roots and the IC_{50} value of the methanol extract is 40.32 mg/L. The other research also reported that *Lantana camara* L. plant is positive to contain phenolics, flavonoids, triterpenoids, steroids and saponine. So in this research antioxidant activity and total phenolic content tests will be done to the extract and ethyl acetate column fractions of *Lantana camara* L. leaves.

MATERIALS AND METHODS

2.1 The chemicals, instruments and tools

The sample is a ethyl acetate extract of *Lantana camara* L. leaves. The chemicals that have been used are: distillated technical solution (hexane, ethyl acetate, methanol), aluminium foil, reactants for phytochemical tests, silicagel(0.063 – 0.200 mm), KLT plate, 1,1-*diphenyl*-2-*picrylhydrazyl*(DPPH), galic acid, Folin-Ciocalteu reagents, sodium carbonate and distillated water.

The instrument and equipment that have been used are distillation equipment, oven, chromatography column (diameter 2.5 cm, 60 cm long), spectrophotometer, chamber, capillary pipes, UV lamps (254 nm and 365 nm) and other glasswares that are commonly used in organic chemistry laboratory.

2.2 Research procedure

Phytochemical sample profile tests

Ethyl actetae extract is poured into reaction tube and dilutes with chloroform and distillated water, each 5 ml, shaken well then let the solution forms 2 layers of chloroform-water and separate the bottom layer (chloroform) by decantation to the other reaction tube. Chloroform layer is used for triterpenoid and steroid compounds measurement. The water layer is used for flavonoid, saponine and phenolic tests

a. Flavonoid test

The water layer that has been decantated is taken for 1 mL and poured in mini reaction tube and then poured with conc. hydrochloride acid and some magnesium powder. The orange to red coloring gave the sample positively contain flavonoid.

b. Phenolic test

The upper layer is taken for 1mL and poured in mini reaction tube. Then in that is poured Fe(III) chloride solution. Phenolic characteristics are complexed with Fe(III) chloride that gives blue or dark purple colors.

c. Saponine tests

Water layer is taken for 1 mL and poured into reaction tube. Then it is shaken vigorously in tube, the formation of bubbles that aren't vanished for 5 minutes by adding drops of conc hydrochloride acid shows that there is indication of saponine in there.

d. Triterpenoids and steroids test

Chloroform layer is dropped on 3 hole drop plates. Plate 1 is added with conc. sulfuric acid, playe 2 is added conc sulfuric acid amd acetic anhydride and plate 3 is added chloroform layer only as standard. Red or reddish purple colouring gives the sample is positive containing Triterpenoids and green or bluish green gives the sample is positive containing steroid.

e. Alkaloid test

Sample is grinded in mortar with a little grain of sand and 10 mL chloroform. Then is added with 10 mL solution of chloroform-ammonia and filtered. Filtrate that is received is added with sulfuric acid. Acid layer is then separated and then added with Meyer reactant. Indication of alcaloid is marked with the formation of white sediment.

f. Coumarine test

Water layer is poured into reaction tube then added with methanol and then is heated. The filtrate is dropped in KLT plate and then is elucidated with fixed eluen ratio. The elution product is then observed below UV light (254 nm and 365 nm). Blue fluorescence signed that the elution product is positively containing coumarin.

Antioxidant activity test of ethyl acetate extracts

a. Synthesis of DPPH solution

4 mg DPPH is scaled and then mixed with 100 ml methanol until the limit mark so that DPPH solution is synthesized.

b. Synthesis of test solution
Test solution is synthesized by mixing 10 mg ethyl acetate extracts with 10 ml methanol so that the solution concentration is 1000mg/L. Then from the test solution, 5 concentration variation is made by dilution method. Concentration variations are 10, 15, 20, 25, and 30 mg/L respectively.

c. Synthesis of Negative control solution
Negative control is made by pouring 1 ml methanol into vial and DPPH solution 0.1 mM 2,5 mL is added. After that each solution 0,2 ml is taken by micropipette and then is added into micro plate 96 wall. Then they are waited for 30 minutes after DPPH 0.1ml solution is added. This work is done in dark room.

d. Antioxidant activity test
1mL from each concentration variation of test solution is added into vials and then is added with 2.5 mL DPPH 0.1 mM. After that each solution 0.2 ml is taken by micropipette and then is added into micro plate 96 wall. Then they are waited for 30 minutes after DPPH 0.1ml solution is added. This work is done in dark room. After that absorbance is measured from each test solution's concentration and negative control for wavelength 517 nm. From the absorbance that is received, percentage of inhibition is calculated with following formula:

$$\% \text{ inhibition} = \frac{\text{Anegativecontrol} - \text{A sample}}{\text{Anegativecontrol}} \text{ x } 100\%$$

A = absorbance

Total phenolic Content test of ethyl acetate extract
a. Synthesis of Standard solution
Standard solution is made by mixing 10 mg galic acid and 10 ml methanol, so that concentration 1000mg/L is achieved. Variation concentration of the standards is made by taking 0.1, 0.2, 0.4, 0.6,and 0.8 mL from standard solution and then added into volumetric flask 10mL. To each variation is added 0.5 mL Folin-Ciocalteu reagent and then it is waited for 5 minute. After that 1 mL sodium carbonate solution is added and is diluted with distillated water until limit mark. The mixture is waited for 2 hours. And then absorbance of the mixture is measured in wavelength 765nm. From the absorbance value, calibration curve is made and regression formula is obtained from standard solution.

b. Synthesis of test solution
Standard solution is made by mixing 10 mg ethyl acetate extract with 10 ml methanol, so that concentration 1000mg/L is achieved. 0.5 mL is taken from the solution and then added into volumetric flask 10mL. To each volumetric flask is added 0.5 mL Folin-Ciocalteu reagent and then is waited for 5 minute. After that 1 mL sodium carbonate 20% solution is added and is diluted with distillated water until limit mark. The mixture is waited for 2 hours. And then absorbance of the mixture is measured in wavelength 765nm. Total phenolic concentration for each test solutions is measured from the regression formula of standard solution calibration curve

Separation by column chromatography
Ethyl acetate extract is separated by column chromatography by SGP method (*step gradient polarity*). The product is held in vial and tested with KLT plate, and then the elucidation product is observed in 254 and 365nm UV lights. From the KLT result, elucidation products that have same node pattern is mixed into one fraction.

RESULTS AND DISCUSSION

Phytochemical sample profile
Ethyl acetate extracts of *Lantana camara* L. is positive containing triterpenoids, steroids, coumarines and phenolics.

The result of antioxidant activity of ethyl acetate extract
Antioxidant activity test toward ethyl acetate extracts are done by measuring the absorbance of ethyl acetate extract for each concentration variation. From the absorbance measurement, IC_{50} value of ethyl acetate extract is 36.18 mg/L. This shows that ethyl acetate extract has antioxidant activity that is very active. According to Jun *et.al* (2003), antioxidant activity is considered very active if the value of $IC_{50}$is less than 50 mg/L, active if the value is 50-100

mg/L, moderate if the value is 101-250 mg/L, and weak if the value is 250-500 mg/L and nonactive if the value of IC_{50} is greater than 500 mg/L. From the result advanced separation are needed toward ethyl acetate extracts.

Separation by column chromatography

The product of column chromatography are 180 vials, which each vials are monitored with KLT by increment interval of 10 amd monitored below UV light with wavelength 254 nm and 365 nm. This is done to group the vials with same Rf and same node patterns. This mixing result is done and 7 simpler fractions are obtained. To each of the fraction antioxidant activity test and total phenolic Content test are done.

Antioxidant activity for each column fractions

Antioxidant Activity test is done to all column fractions that are obtained from the chromatography column separation. The result of antioxidant activity test result for all column fractions are shown in Table 1.

Table 1: The result of antioxidant activity test of column chromatography elucidation products (λ = 517 nm)

Fraction	IC_{50} (mg/L)
A	132.62
B	113.51
C	85.23
D	81.26
E	24.83
F	83.50
G	806.71

Based on Table 1, fraction Eare highly active antioxidants, fraction C, D, and Fare active antioxidant, fraction A and B moderate antioxidant and fraction G is not active antioxidant.

Total phenolic Content test of ethyl acetate extract and column fractions

Determination of total phenolic content aimed in strengthening the data and observations about antioxidant activity. This is because that phenolic group act as antioxidants active side in the compound. Phenolic compounds have a unique structure and properties, which have one or more hydroxyl groups attached to one or more aromatic benzene rings, so that these compounds can be oxidized. Ability to form a stable phenoxy radicals in the oxidation process cause these compounds are widely used as an antioxidant. Thus the fraction G obtained from the elution column chromatography testing was not performed because fraction G is inactive antioxidants

The results of the measurement of total phenolic contents are listed in Table 2.

Table 2: Total phenolic Content in ethyl acetate extract and fractions A-F of leaves of *Lantana camara* L. (λ = 765 nm)

No	Sample	Total phenolic content (mg/L GAE)
1	Extract	2419.6
2	Fraction A	237.8
3	FractionB	589.4
4	FractionC	995,4
5	Fraction D	1041
6	FractionE	3156
7	FractionF	1037.8

Based on the received data, the total amount of phenolic from fraction E is highest than any other fraction. This is came from the antioxidant activity of the fraction E is very high (Table 3.1). The larger amount of phenolic in the sample higher antioxidant activity because the amount of phenolics in the sample is really significant toward antioxidant activity test.

The correlation between antioxidant activity to the phenolic total content.

The correlation between antioxidant activity with the total phenolic content can be seen in Figure 1. Based on the graphic, it is known that the determinant value (r^2) between antioxidant activity with total phenolic content is 0.9631 and the correlation value (r) is 0.9814. From the data above we can conclude that 98.14% of total phenolic content is influenced antioxidant activity of the extract and ethyl acetate fractions of *Lantana camara* L.

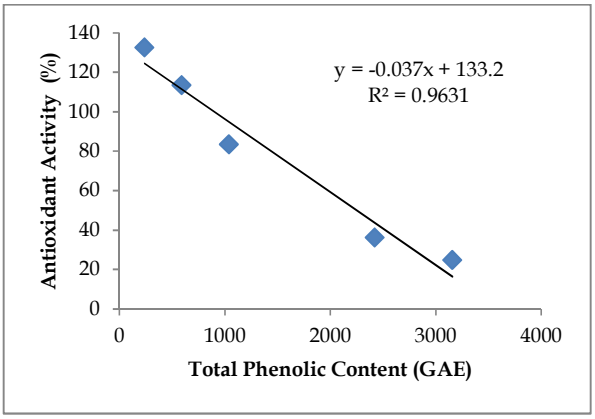

Figure 1: The correlation of antioxidant activity with total phenolic content from extract and active antioxidant fractions of *Lantana camara* L.

CONCLUSION

Conclusion

The current study shows that:

a. The ethyl acetate extract of *Lantana camara* L. containing phenolic compounds, triterpenes, steroids, saponine and coumarine.

b. IC_{50} value and total phenolic content ofextract of ethyl acetate and each fractions were found to be: Extract of ethyl acetate (36.18 mg/Land 2419.6 GAE), Fraction A (132.62 mg/L and237.8 GAE), Fraction B (113.51 mg/L and589.4 GAE), Fraction C (85.23 mg/L and995.5 GAE), Fraction D (81.26 mg/L and1041 GAE), Fraction E (24.83 mg/L and3156 GAE), Fraction F (83.50 mg/L and1037.8 GAE), and Fraksi G (806.71 mg/L). Based on the IC_{50}, extract ethyl acetate and Fraction Eare highly active antioxidants, fraction C, D, and Fare active antioxidant, fraction A and B have moderate antioxidant and fraction G is not active antioxidant.

c. Antioxidant activity of extract and fractions ethyl acetate of *Lantana camara* L. leaves is directly proportional with the total phenolic content of extract and fractions with correlation index of 98.14 %.

Advices

Some advices for next research would be as following:

a. There is need to isolate the secondary metabolites compounds of antioxidant active fractions from ethyl acetate extracts.

b. Structure characterization usingUV-Vis, FTIR, ^{1}H-NMR, and^{13}C-NMR of isolated compounds is needed to achieve the molecular structure of isolated compounds.

c. Do another bioactivity tests to the isolated compounds.

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