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ISOLATION AND CHARACTERIZATION OF ANTIOXIDANT COMPOUNDS OF BANGUN-BANGUN (*Coleus amboinicus*, L.) LEAVES FROM NORTH SUMATERA, INDONESIA

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ABSTRACT

Coleus amboinicus, Lour has been successfully isolated and characterized active isolates from the ethyl acetate fraction and tested its antioxidant activity. Isolation was carried out by maceration method with methanol followed by stratified fractionation with *n*-hexane, chloroform and ethyl acetate. The each fraction was tested for antioxidant potential by DPPH method at a wavelength of 515 nm and the ethyl acetate fraction gave a better antioxidant potential then it's purified. Purification using by gravitational column chromatography with the stationary phase silica gel 60 and the mobile phase ratio of *n*-hexane: ethyl acetate with polarity are gradually increased. Isolates were characterized by UV, FT-IR, NMR, and GC-MS spectrophotometry. Antioxidant activity (IC₅₀) test obtained 386.46. The molecular formula is C₂₂H₂₈O₆ isolate with the name of the chemical compound 16-hydroxy-7 α -acetoxyroleanone (syn. 16-hydroxy-7-O-acetylhorninone).

Keywords: Antioxidant, *Coleus amboinicus*, DPPH, Ethyl acetate, and Stratified fractionation

INTRODUCTION

The potential of plants in recent times is become the center of attention of researchers in a variety of uses. This is because in addition to the nutritional content, this plants also have secondary metabolites (bioactive compounds) that is potential to be developed for various of natural treatments.^{1,2,3} One of the plants that can be utilized is bangun - bangun (the name of the Bataknese area, North Sumatera Province, Indonesia). The bangun -bangun plant with the latin name is *Coleus amboinicus*, L., with the synonym *Plectranthus amboinicus* (Lour) Spreng., and *Coleus aromaticus*, Benth.^{4,5,6} Traditionally, people in north Sumatera Indonesia consumes leaves as vegetables and believed it's able to increase the productivity of breast milk when breastfeeding.⁷ The leaves of *C. amboinicus* is reported contain alkaloids, carbohydrates, glycosides, proteins, flavonoids, tannins, phenolics and terpenoids⁸ and has essential oils.⁹ *C. amboinicus* has potential as an antioxidant, diuretic, analgesic, prevent cancer, antitumor, antivertigo, immunostimulant, anti-inflammatory, anti-infertilization, hypocholesterolemic, hypotensive.⁷ The various of degenerative diseases that arise are very closely related to the presence of free radicals. Free radicals is the most reactive species. The presence of free radicals can be minimized by the effects of antioxidant compounds. *C. amboinicus* can be used as a potential herbal medicine which is active as a natural antioxidant. Antioxidants that are natural are considered safer than synthetic antioxidants. Based on the description, the purpose of this research was to isolate potential compounds from *C. amboinicus* leaves fraction as an antioxidants

EXPERIMENTAL

Chemicals

The chemicals used were methanol pa, *n*-hexane pa, pa ethylacetate, chloroform (Merck), acetone (Merck), NH₄OH (Merck), NaOH (Merck), dragendorff (Merck), anhydrous acetic acid (Merck), ethanol (Merck), FeCl₃ (Merck), Mg powder (Merck), HCl (pa), KOH (Merck), sulfuric acid (Merck), GF256 silica gel TLC plate (Merck), silica gel 60 (kieselgel 0.063-0.0200 mm) (Merck), aquabides and DPPH (Merck). Instrumentation used UV-Vis spectrometers (spectronic 3000, Genesis 10), FT-IRKBr spectrophotometry (Shimadzu IR Prestige-21), NMR spectrometers (JEOL spectrometers), GC-MS-QP2010S Shimadzu and Electrothermal 9100.

Preparation and Process of the Extract Plant

The *C. amboinicus* was originated from the village of Sosor Ladang, sub-district Parmaksian, North Sumatera Province, Indonesia and this plant identification is carried out at the botanist at the Faculty of Biology, Universitas Gadjah Mada, Indonesia (No.0437/S.Tb/V/2013). The *C. amboinicus* was cleaned by the running water and then dried in an open room (avoid direct contact with sunlight). The sample was mashed with an electric blender and obtained a weight of 950 g. *C. amboinicus* powder was extracted by maceration with methanol for 48 hours, then filtered and concentrated with a rotary vacuum evaporator at 50°C. The residue was reminerated and repeated two times. The crude extract of methanol obtained is partitioned in stages starting with *n*-hexane, chloroform and ethyl acetate solvents with two replications each. Clarifying the boundary plane at the time of fractionation is done to add sufficient distilled water. The ethyl acetate fraction resulting from the partition is continue to the purification stage.

Antioxidant activity test

The Each extraction and fraction is teste² for antioxidant activity by using DPPH method (2,2-diphenyl-1-picrylhydrazyl) with varying concentrations of 10 ppm, 20 ppm, 33 ppm, 40 ppm, and 50 ppm. The antioxidant activity test of isolates used the variations in the concentration of 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm. 250 µL of each concentration and 1 mL DPPH 0.4 mM then added exactly 5 mL methanol, then incubated for 30 minutes in a closed state. Blank (negative control) 1 mL DPPH 0.4 mM added 4 mL methanol without extract and incubated for 30 minutes.¹⁰ Absorbance was measured at a wavelength of 515 nm with a visible spectrophotometer.^{11,12} Determination of free radical inhibition with the following equation:

$$\% \text{Inhibition} = \left[\frac{A_0 - A_T}{A_0} \right] \times 100$$

where, A₀ = Absorbance blank and A_T = Absorbance of the test sample. % inhibition is plotted against concentration and get a regression equation linear is obtained to calculate the inhibitory concentration (IC₅₀). IC₅₀ is expressed as the amount of an antioxidant extract that reacts with an initial DPPH radical of 50%.^{13,14}

Purification

The purification by using silica gel 60 as a stationary phase by gravity column chromatography. The gravity column used was 1 cm in diameter with 40 cm in height. 2 g of ethyl acetate fraction is mixed evenly with 0.5 g of silica gel. The column were filled with 3 g silica gel and compacted with *n*-hexane. Purification by using the mobile phase between *n*-hexane: ethyl acetate and polarity are increased gradually. The selection of eluents as mobile phases is based on identifying the better separation in thin layer chromatography (TLC) under ultraviolet light and visible light at wavelengths (λ) of 254 nm and 366 nm. The eluate was collected in vial of bottles with each a volume of 10 mL and then evaporate. The isolates were analyzed by using UV-Vis, FT-IR, ¹H & ¹³C-NMR, GC-MS spectrophotometry and tested as antioxidant activity.

RESULTS AND DISCUSSION

Sample Preparation

Weight percentages of each extract and fraction leaves of *C. amboinicus* powder and crew antioxidant activity were presented in Table-1.

Table-1: The percentage of extract and fraction of weight of powder and IC₅₀ from *C. amboinicus* leaves

	Crude Methanol Extract	Fraction n-hexane	Fraction CHCl ₃	Fraction EtOAc	Fraction Residue
Powder (950.0 g)	58.44 g (6.13%)	25.63 g (2.69%)	20.94 g (2.20%)	4.43 g (0.47%)	7.45 g (0.78%)
IC ₅₀ Early	34.73±0.10*	-	167.06±1.10*	65.85±0.75 ^a	-

Description: * The results are expressed as mean ± SD, n = 3 and ^a a purified fraction

Isolate from the Purification of Ethyl Acetate Faction

The eluate was collected and in vial bottle number 5 a crystal was obtained in the eluent *n*-hexane (10): ethyl acetate (1) ratio. The crystals (isolates) obtained weighing 50 mg (1.13%) were yellow, vase-shaped, and tapered (Fig.-1).



Fig.-1: Isolate

Characterization and Analysis of Isolate Structures

The characterization of isolates by measuring melting points 214-215°C. The isolate was completely dissolved with methanol, ethyl acetate, and chloroform. Characterization using various instruments was presented in Table-2.

Table-2: Characterization of isolates with various instruments

UV spectrophotometry		FT-IR _(KBr)		GC-MS		
Uptake λ (nm)	Absorbansi	Wave Number (cm ⁻¹)	Interpretation of functional groups	Retention time/t _R (minutes)	%Area	MS (m/z)
210	Methanol (solvent)	3425 and 3387	-OH	7.316	100	390
274	Isolated	2394	C-H aliphatic			
		1728	C = O esters			
		1234	C-O methoxy			
		1643	C = C alkenes			

The results of the proton analysis with ^1H -NMR obtained 28 types of protons and carbon analysis with ^{13}C -NMR there were 22 types of carbon. Structural analysis followed by 2D-NMR (HMBC) was shown in Table-3. Molecular structure of the results of NMR spectra analysis was shown in Fig.-2.

Table-3: Chemical shifts and HMBC isolates

Carbon position	δ_c (ppm)	δ_H (ppm)	HMBC
1	38,54	2,62-2,66 <i>tt</i> , ($^3J_{CH}$ 5,2 Hz; 4,6 Hz)	2, 3, 10, 9, 5, 20
2	24,36	1,47-1,50 <i>tt</i> , ($^3J_{CH}$ 5,2 Hz; 4,6 Hz)	1, 3, 19, 5
3	42,45	1,57 <i>t</i> (2H, $J = 3,85$ Hz)	4, 18, 2, 19
4	33,86		
5	49,93	1,83-1,87 <i>tt</i> (1H, $^3J_{CH}$ 5,2 Hz; 4,6 Hz)	1, 6, 2, 10, 9
6	21,69	1,78 <i>s</i> (2 H, melebar)	5, 8, 7, 10, 9
7	68,93	5,66 <i>d</i> (1H)	5, 8, 9, 1', 14
8	137,27		
9	150,09		
10	38,81		
11	183,47		
12	151,08		
13	124,86		
14	185,94		
15	20,03	3,17 <i>m</i> (1H)	16, 17, 13, 12, 14
16	67,24	4,32 <i>s</i> (widened) (2H)	15, 17, 13
17	19,15	1,20 <i>d</i> , (3H, $J = 12,03$ Hz)	16, 15, 13
18	33,72	1,24 <i>d</i> , (6 H, $J = 5$ Hz)	3, 4, 5, 19
19	24,01		2, 3, 4, 5, 18
20	19,90	0,94 <i>s</i> (3H)	10, 1, 5, 9
1'	169,77		
2'	21,13	2,05 <i>s</i> (3H)	1'
at -OH C-12 at -OH C-16		7,21 <i>s</i> brs	11, 12, 13

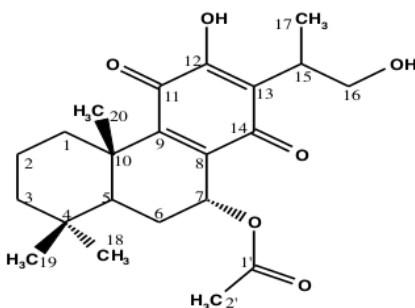


Fig.-2: Molecular structure of isolates (16-hydroxy-7 α -acetoxyroyleanone syn. 16-hydroxy-7-O-acetylhorninone)

The GC-MS analysis results showed a molecular weight of 390 m/z, prediction of the fragmentation pattern of the isolate with the molecular formula of $C_{22}H_{28}O_6$ isolate with the name of the chemical compound 16-hydroxy-7 α -acetoxyroyleanone (Fig.-3).

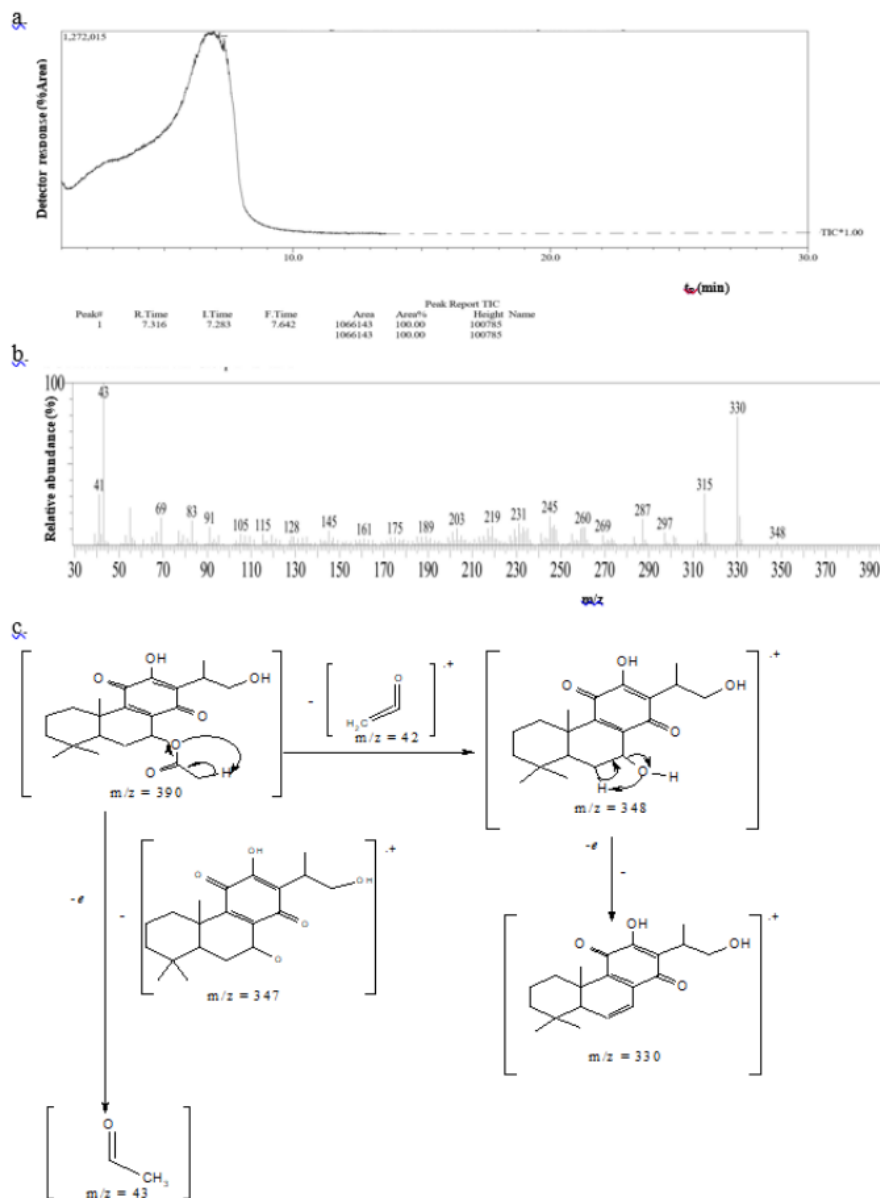


Fig.-3: a. chromatogram on GC; b. MS spectrum; and c. fragmentation pattern of 16-hydroxy-7 α -acetoxyroyleanone structure

Isolate (16-hydroxy-7 α -acetoxyroyleanone) Antioxidant Activity Test

The analysis antioxidant activity of the isolates was carried out by using the DPPH method. This method uses a measurement process that is easy, fast and has a good level of sensitivity to the determination of antioxidant activity. Increasing the concentration of isolates showed an increase in percent reduction of free radicals from DPPH. The percentage inhibition of free radicals and IC₅₀ activity of the isolate was shown in Fig.-4.

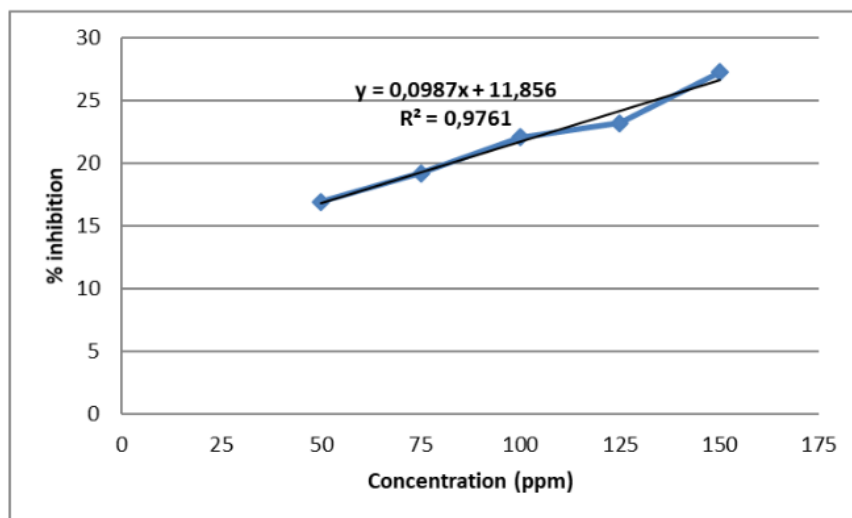


Fig.-4: Antioxidant activity of 16-hydroxy-7 α -acetoxyroyleanone

The antioxidant activity was expressed by the ability of isolates to reduce 50% of free radicals sourced from DPPH. The value of antioxidant activity (IC₅₀) of isolates was 386.46. The principle of reducing free radicals from DPPH by involving proton and electron transports from isolates to free radicals to form a neutral DPPH-H compound that is not reactive.¹⁵

CONCLUSION

The *C. amboinicus* leaf extraction with various methanol, chloroform and ethyl acetate solvents showed antioxidant activity which was tested by DPPH method. Isolate isolated from ethyl acetate fraction showed IC₅₀ 386.46 which had weaker activity than the extract. The molecular formula is C₂₂H₂₈O₆ isolate with the name of the chemical compound 16-hydroxy-7 α -acetoxyroyleanone (syn. 16-hydroxy-7-O-acetylhorninone).

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