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Determination antioxidant activities methanol extracts of bangun-bangun (*Coleus amboinicus* L.) Leaves with DPPH method

Kasta Gurning [1,*]

[1]Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan, Indonesia
*Corresponding author: KG, kastagurning@gmail.com

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Abstract

Bangun-bangun (*Coleus amboinicus* Lour.) leaves is a plant that has various types of active compounds that can be utilized in various fields of health and food. The purpose of this research is to determine the components of the active compound contained in the sample when extract with methanol by maceration method and antioxidant activity test with DPPH method using a UV-Vis spectrophotometer. The value of antioxidant activity is determined by the amount of ability to inhibit 50% concentration of free radicals from DPPH. Phytochemical screening results of methanol extract active compounds contained alkaloids, tannins, flavonoids, terpenoids and steroids, as well as phenolic & polyphenols. The test activity of methanol extract of *Coleus amboinicus* leaves and ascorbic acid in the amount of 38.83 and 4.18 included in the very strong category as antioxidants. The extract of *Coleus amboinicus* leaves has lower activity compared to ascorbic acid and has the potential to be used as a natural antioxidant in preventing and minimizing the effects of free radicals.

Keywords: *Coleus amboinicus* Lour, Antioxidant, Methanol extract, IC50, DPPH

1. Introduction

Free radicals (oxidative stress) is a species that causes damage to biomolecules especially DNA, protein, lipids, and cell function. Free radical species in general is Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Wojcik et al. 2010). Presence of free radicals is causes of degenerative diseases such as arthritis, atherosclerosis, cancer, diabetes, and parkinson's (Rydlewski et al. 2017). Preventing and minimizing the impact of free radicals is needed antioxidants (Huang et al. 2013). Based on the source of antioxidants can be divided that is two category endogenous

and exogenous antioxidants. Endogenous antioxidants are produced in the body while exogenous antioxidants are obtained from outside the body (food/drink). Exogenous antioxidants are divided into synthetic and natural (Sen & Mandal, 2017). Synthetic antioxidants are not a good choice for long-term use because they have adverse health effects, are toxic and carcinogenic (Farahmandfar et al. 2019; Karpińska-Tymoszczyk & Draszanowska, 2019).

Natural antioxidants are an alternative choice as an antidote of free radicals. Aside from counteracting of free radicals, certain diseases and effectively make slows of the aging process and is safer for the body (Huang et al. 2013). Sources of natural antioxidants come from of the various living things such as plants etc. One of the potential plants that can be used as natural antioxidants is bangun- bangun (*Coleus amboinicus* Lour.) leaves. *Coleus amboinicus* leaves reported has few varieties of secondary metabolite bioactive compounds such as essential oils (Gurning, 2015), terpenes, phenolics, flavonoids, esters (El-hawary et al. 2012; Arumugam et al. 2016), tannins, alkaloids, and steroids (Aswini and Girish, 2014). Empirically this plant in the community, especially the bataknese is used as a vegetable and while mothers who are breastfeeding the juice is drunk to increase milk productivity. Based on these descriptions, this research is aims to carry out phytochemical screening and determination of the antioxidant activity value of methanol extracts from of *Coleus amboinicus* leaves by the DPPH method.

2. Methods

2.1 Preparation Sample

Fresh leaves of sample were taken and collected from Parmaksian villages (district of Toba Samosir) without regardless of the age and size of the leaves. The samples are cleaned by washing in running water, drained and dried in an open room that avoids direct contact with sunlight. The sample is pulverized using a blender.

2.2 Preparation of Methanol Extract of Coleus Amboinicus Leaves

950 g of simplicia powder was extracted by maceration method using methanol (p.a) for 2 days at room temperature and stirring occasionally. After 2 days, filtered by using Whatman's paper No. 1, and then the filtrate was concentrated using a vacuum rotary evavorator at 40°C (Bhatt et al. 2013; Asiimwe et al. 2014) to get a crude extract, the residue is remacerate 2 times. Crude methanol extract phytochemical screening and antioxidant activity testing by using DPPH method.

2.3 Phytochemical Screening of Methanol Extract Coleus Amboinicus Leaves

Phytochemical screening from secondary metabolites of methanol extract using standard phytochemical screening methods:

Alkaloids: Crude methanol extract is evaporated to dry boiling waterbath. The residue is dissolved in 2 N HCl. The mixture is filtered and the filtrate is divided into 3

equal parts. The first part drops some Mayers reagents; the second part with the Dragondroffs reagent and the third part with the Wagners reagent. Pay attention to sediments that are formed if orange deposits and brown deposits, indicate the presence of each alkaloids (Vaghasiya et al. 2011).

Tannins: Crude extract added with a few drops of alcoholic $FeCl_3$ reagent. The blue color indicates the presence of tannins (Vaghasiya et al. 2011).

Terpenoids and Steroids: Crude extracts methanol dissolved with dichloromethane are treated with acetic acid anhydride and a few drops of concentrated H_2SO_4 are added through the test tube side forming red-purple showing terpenoids and green-blue showing steroids (Kumar et al. 2013).

Flavonoids with Shinoda-Test: Crude extract methanol was put into a drip plate then a magnesium tape was added and the HCl formed pink showed positive flavonoids (Gul et al. 2017).

Phenolic and Polyphenolic: 1 mL sample crude extract methanol, 2 mL distilled water followed by a few drops of 10% $FeCl_3$. Blue or green formations indicate the presence of phenols formed in brown deposits indicating polyphenols (Ndam et al. 2014).

2.4 Test of antioxidant activity

DPPH is dissolved with methanol (p.a) to get a DPPH concentration of 0.4 mM. Variation concentration of crude methanol extract of *Coleus amboinicus* used 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, as a negative control of DPPH with methanol and ascorbic acid with various concentrations of 2.5 ppm, 3.0 ppm, 3.5 ppm, 4.0 ppm and 4.5 ppm, 4.5 ppm as positive control. The volume of the sample (extract and ascorbic acid) used 250 µL for each variation, then add 1 mL DPPH 0.4 mM and add methanol to the 5 mL limit mark. The mixture was incubated for 30 minutes at room temperature. Absorbance is measured at a maximum wavelength of 515 nm (Kedare and Singh, 2011) by using UV-Vis spectrophotometer. The measurement were replicated 3 times. Calculation of% inhibition by using the equation (Gul et al. 2017):

$$Inhibition(\%) = \left[\frac{A_{Contol} - A_{Sample}}{A_{Control}}\right] x100$$

where A = absorbance; Acontrol = Absorbance without sample and Asample = Absorbance of the sample tested. IC_{50} of the linear regression equation y = bx + a.

3. Results and Discussion

3.1 Preliminary Phytochemical Screening

Phytochemical screening for metabolites secondary from of crude methanol extract *Coleus amboinicus* leaves is shown in Table 1. Phytochemical screening results crude methanol extract of showed contains has variety of secondary metabolites

that are diverse. Besides that, methanol has the ability to find various components of secondary metabolites.

Tabel 1

Phytochemical screening for metabolites secondary of crude methanol extract *Coleus amboinicus* leaves

Secondary Metabolites	Results
Alkaloids	+
Flavonoids	+
Phenolic / polyphenols	+ / +
Coumarin	+
Triterpenoids	+
Steroids	+

3.2 Antioxidant Activity Test

Determination of antioxidant activity by the DPPH method is a very sensitive, simple, relatively faster test compared to other methods (Farahmandfar et al. 2019) and the process is easy and can be done in small samples quantities (Marjoni and Zulfisa, 2017). The principle of determining antioxidant activity with the DPPH method is by electron transfer and radical hydrogen transfer (Marjoni and Zulfisa, 2017). The amount of free radical inhibition is directly proportional to the large concentration of methanol extract of *Coleus amboinicus* leaves (Table 2).

Table 2

Measurement of the antioxidant activity of crude methanol extract of *Coleus amboinicus* leaves

[ ] ppm	Absorbansi			Inhibition (%)			Average
	I	II	III	I	II	III	
Blanko	0.81	0.81	0.80	0	0	0	0
10	0.63	0.63	0.63	22.22	22.22	21.25	21.90
20	0.57	0.57	0.57	29.63	29.63	28.75	29.34
30	0.44	0.44	0.44	45.68	45.68	45.00	45.45
40	0.34	0.34	0.35	58.02	58.02	56.25	57.43
50	0.23	0.23	0.23	71.60	71.60	71.25	71.49

IC_{50} value of the crude methanol extract *Coleus amboinicus* leaves is calculated from the linear regression equation of Fig 1 and obtained 33.83 with a very strong category (Marjoni and Zulfisa, 2017). Phenolic groups, polyphenols and flavonoids are reported to provide strong antioxidant activity in every natural ingredient (Skrovankova et al. 2015). The higher the content of the active compound in natural extracts, the greater the antioxidant activity.

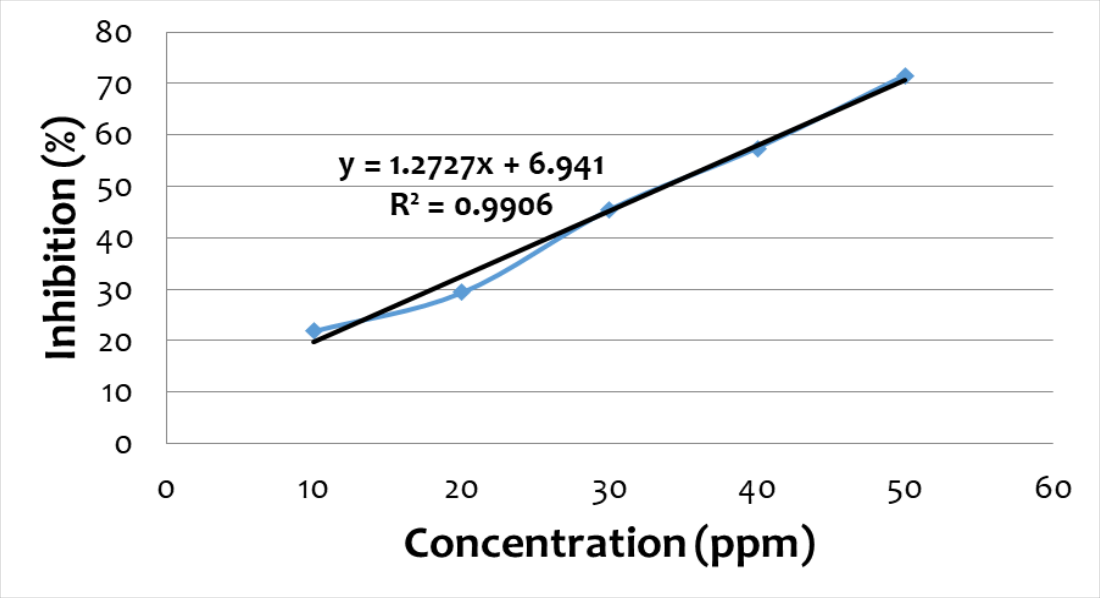

Fig 1. Activity crude methanol extract of *Coleus amboinicus* leaves as antioxidants

The value of the ability to inhibit free radicals from DPPH and measurement of the antioxidant activity of ascorbic acid (vitamin C) is shown in Table 3 and Fig 2. Based on data from Table 3 and Fig 2, IC_{50} value of ascorbic acid is 4.18 and belongs to the very strong category as an antioxidant (Situmeang et al. 2016; Marjoni and Zulfisa, 2017; Simorangkir et al. 2019). Ascorbic acid activity is still stronger than the methanol extract of Coleus amboinicus leaves.

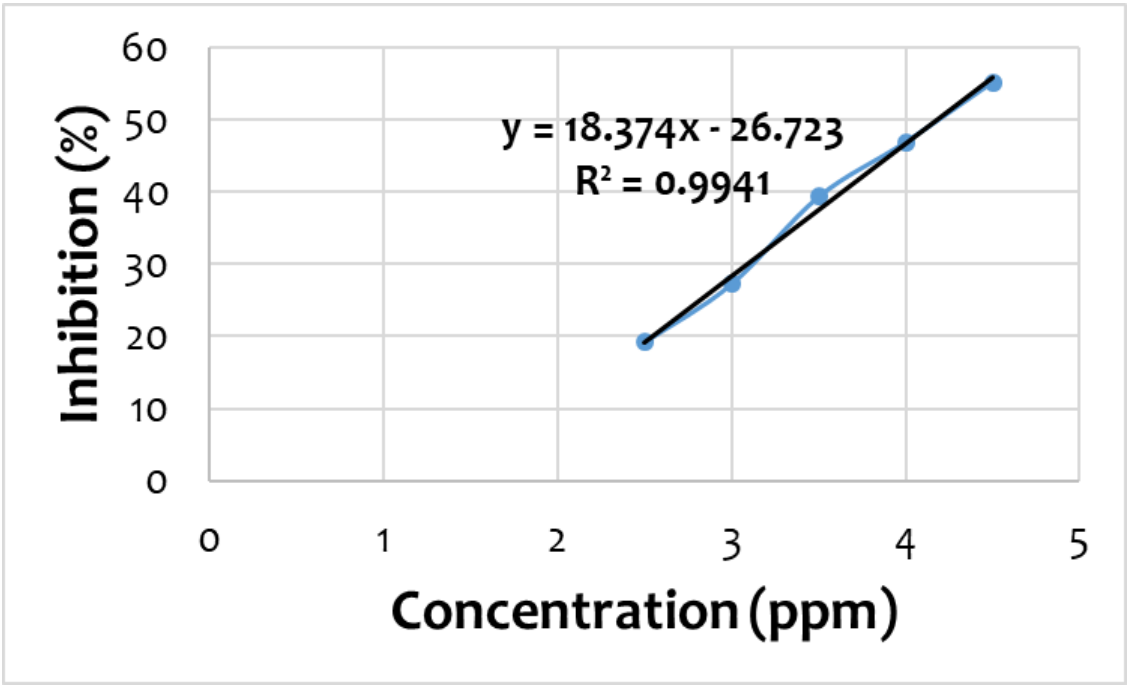

Fig 2. Activity of ascorbic acid as antioxidant

Table 3
Measurement of the antioxidant activity of ascorbic acid as a positive control

[ ] ppm	Absorbansi			%inhibisi			Average
	I	II	III	I	II	III	
Blanko	0.81	0.81	0.80	0	0	0	0
2.5	0.65	0.65	0.65	19.38	19.51	18.63	19.17
3	0.59	0.59	0.58	26.79	27.78	27.13	27.23
3.5	0.50	0.49	0.49	38.77	39.88	39.38	39.34
4	0.43	0.43	0.43	47.04	47.16	46.63	46.94
4.5	0.37	0.36	0.36	54.81	55.56	55.38	55.25

4. Conclusion

Crude methanol extract *Coleus amboinicus* leaves has an IC_{50} value of 33.83 and belongs to the very strong category as an antioxidant.

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