BioLink : Jurnal Biologi Lingkungan, Industri dan Kesehatan, Vol. 7 (1) August (2020)
ISSN: 2356- 458X (print) ISSN: 2550-1305 (online)
DOI: 10.31289/biolink.v7i1.3732

BioLink
Jurnal Biologi Lingkungan, Industri, Kesehatan

Available online http://ojs.uma.ac.id/index.php/biolink

IDENTIFICATION OF SECONDARY METABOLIC AND TEST OF ACTIVITY ETHYL ACETATE FRACTION OF BANGUN- BANGUN (COLEUS AMBOINICUS LOUR.) LEAVES AS ANTIOXIDANT

Kasta Gurning

Departement Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Indonesia

Submited : 13-02-2020; Accepted : 21-04-2020: Published : 10-08-2020

Corresponding author: E-mail: kastagurning@gmail.com

Abstract

Antioxidants have an important role in delaying the oxidation process and preventing the occurrence of degenerative diseases in the body. Antioxidants consist of synthetic and natural; the use of synthetic antioxidants in a relatively long period is hazardous so that the use of natural antioxidants is considered more potential and safe for the body. The process of extracting secondary metabolites from the leaves of bangun-bangun (Coleus amboinicus L.*) is carried out by the maceration method with methanol, then partitioned in stages with n-hexane, chloroform, and ethyl acetate. This research intends to identify secondary metabolites and test antioxidant activity from the ethyl acetate fraction with the DPPH method. Phytochemical screening results of ethyl acetate fraction showed the different secondary metabolite groups such as alkaloids, phenolics & polyphenols, flavonoids, coumarin, and triterpenoids. Antioxidant activity (IC_{50}) obtained is 64.97 with a healthy category, so that the potential to be used as a natural antioxidant.*

Keywords*: Antioxidants, Phytochemical Screening, Bangun-bangun Leaves, and* IC_{50}

How to Cite: *Gurning, K. (2020). Identification of secondary metabolic and test of activity ethyl acetate fraction of bangun- bangun (Coleus amboinicus lour.) Leaves as antioxidant, BioLink: Jurnal Biologi Lingkungan, Industri dan Kesehatan, Vol.7 (1): Hal. 117-122*

INTRODUCTION

Antioxidants have an essential part in delaying the process of oxidation in food and preventing degenerative diseases for health caused by free radicals (Rani, 2017; Akbarirad et al., 2015). Neurodegenerative diseases caused include coronary heart disease, stroke, diabetes, and cancer (Serlahwaty et al., 2011). The free radicals are unstable atomic/molecular species because they have one or more unpaired electrons (Mbah et al., 2019). Minimizing the influence of free radicals requires an antioxidant. Antioxidants can be divided into two, such as synthetic and natural. The use of synthetic antioxidants is hazardous for health because it has side effects. The side effects caused by using synthetic antioxidants in a long period such as liver swelling, affecting enzymes, fatal bleeding in the pleural cavity, and drops of the epididymis and pancreas (Andarwulan et al., 1996). The use of natural antioxidants is safer and beneficial for health and has much lower toxicity (Biswas et al., 2017).

Bangun-bangun leaves have a variety of bioactive compounds that can be tested for their activity. *Coleus amboinicus* L. has 76 volatile compounds and 30 non-volatile compounds such as the monoterpenoid, diterpenoid, triterpenoid, sesquiterpenoid, phenolic, flavonoid, alcohol, and aldehyde esters (Arumugam et al., 2016; Riyanto et al, 2020). which has antioxidant activity, diarrhea, flatulence, constipation, cough medicine, chronic asthma, bronchitis, liver-kidney, antibacterial malaria, cytotoxic, anti-inflammatory, anti-diabetic, analgesic, immunomodulatory, stimulate breast milk production (laktogogue) and hepatoprotective (Damanik et al., 2017).

MATERIALS AND METHODS

Preparation of samples *Coleus amboinicus* Lour.

Fresh leaves samples were taken and collected from the Sub-district of Parmaksian (Toba Samosir district). The sample is cleaned and dried in an open room, which avoids direct contact with sunlight. The sample is pulverized by using a blender; the powder is continued for extraction. This research was carried out in the Pharmacy laboratory at STIKes Senior Medan in January-February 2020.

Preparation and extraction process of *Coleus amboinicus* Lour.

950 g of *Coleus amboinicus* Lour. Simplicia powder, maceration using methanol (p.a) for 2 days. After 2 days, it was filtered using Whatman No. 1 paper.

Then the filtrate was concentrated using a vacuum rotary evaporator to obtain a crude extract, and the residue was two remunerated. The crude extract is continued by partitioning gradually using n-hexane (p.a), chloroform (p.a), and ethyl acetate solvents. The ethyl acetate fraction was concentrated, and a viscous extract was obtained. The viscous ethyl acetate extract was continued to phytochemical screening and testing its antioxidant activity.

Screening of phytochemical ethyl acetate fraction

Secondary metabolite compounds analyzed ethyl acetate fraction by standard phytochemical methods (Kumar et al., 2013; Gul et al., 2017).

Test of antioxidant activity with the DPPH method

Antioxidant activity of the ethyl acetate fraction is carried out in vitro. 2,2, diphenyl-1-picrylhydrazyl (DPPH) is used as a source of free radicals. DPPH was dissolved with methanol (p.a) at a concentration of 0.4 mM and incubated for 30 minutes at room temperature. Absorbance was measured at the maximum wavelength of DPPH in methanol 515 nm (Kedare & Singh, 2011) using a UV-Vis spectrophotometer. Variations concentration of ethyl acetate fraction *Coleus amboinicus* Lour., which used 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm and methanol with DPPH as negative control and ascorbic acid with variations in the concentration of 2.5 ppm, 3.0 ppm, 3.5 ppm, 4.0 ppm, and 4.5 ppm. All of the measurements were carried out with three times replication. Calculation % inhibition against a reduction of free radical concentration using by the following equation (Gul et al., 2017):

$$\text{Inhibition } (\%) = \frac{[Abs_{control} - Abs_{sample\ test}}{Abs_{control}} x\ 100$$

Where abs = absorbance; Abs control = total radical activity without the sample and abs test sample = activity in the presence of the test composition. IC_{50} of the linear regression equation y = bx + a.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The weight of ethyl acetate fraction is *Coleus amboinicus* Lour., obtained by 4.43±0.02 g. Results of phytochemical screening ethyl acetate fraction showed different secondary metabolite compounds (Table 1).

Table 1. Secondary screening metabolites of ethyl acetate *Coleus amboinicus* Lour.

Secondary Metabolites	Reagent	ethyl acetate
Alkaloids	Mayer	+
	Dragenddroff	+
	Wagner	+
Flavonoids	Shinoda test	+
Phenolic and polyphenols	$FeCl_3$ 5% in ethanol	+
Coumarin	KOH 5% in ethanol	+
Triterpenoids	Liebermann Bouchard	+
Steroids		-

Antioxidants activity test

Scavenging activity of free radical from ethyl acetate fraction Coleus amboinicus Lour. Is testing by the DPPH method. Ascorbic acid is used as a positive control and methanol as a solvent. The high value of antioxidants that support assert as inhibition concentrations of 50 (IC_{50}). The short of measuring antioxidant activity was carried out by adding 250 µL of concentration variation in each 5 mL volumetric flask according to the concentration label. The extract flask is added 1 mL DPPH 0.4 mL, and methanol is added to the 5 mL limit mark and then incubated for 30 minutes. The absorbance measurements were performed at a maximum wavelength of 515 nm with a UV-Vis spectrophotometer (Table 2 and Table 3).

Table 2. Measurement of antioxidant activity of ethyl acetate fraction by DPPH method

[ ] ppm	Absorbance			Inhibition (%)			Average
	I	II	III	I	II	III	
Blanko	0.79	0.79	0.79				
10	0.72	0.72	0.72	8.86	8.86	8.86	8.86
20	0.69	0.68	0.69	12.66	13.92	12.66	13.08
30	0.59	0.58	0.59	25.32	26.58	25.32	25.74
40	0.55	0.55	0.55	30.38	30.38	30.38	30.38
50	0.49	0.49	0.48	37.97	37.97	39.24	38.40

Table 3. Measurement of antioxidant activity of ascorbic acid by DPPH method

[ ] ppm	Absorbance			Inhibition (%)			Average
	I	II	III	I	II	III	
Blanko	0.79	0.79	0.79				
2.5	0.61	0.62	0.61	22.78	21.52	22.78	22.36
3.0	0.54	0.54	0.54	31.65	31.65	31.65	31.65
3.5	0.46	0.45	0.45	41.77	43.04	43.04	42.62
4.0	0.39	0.39	0.39	50.63	50.63	50.63	50.63
4.5	0.33	0.32	0.32	58.23	59.49	59.49	59.07

The measurement results for each concentration variations showed that the increase in concentration was directly proportional to the increased ability to inhibit free radicals from DPPH. The higher the concentration of the fraction

and assets of Coleus amboinicus Lour., the higher its ability to inhibit free radicals.

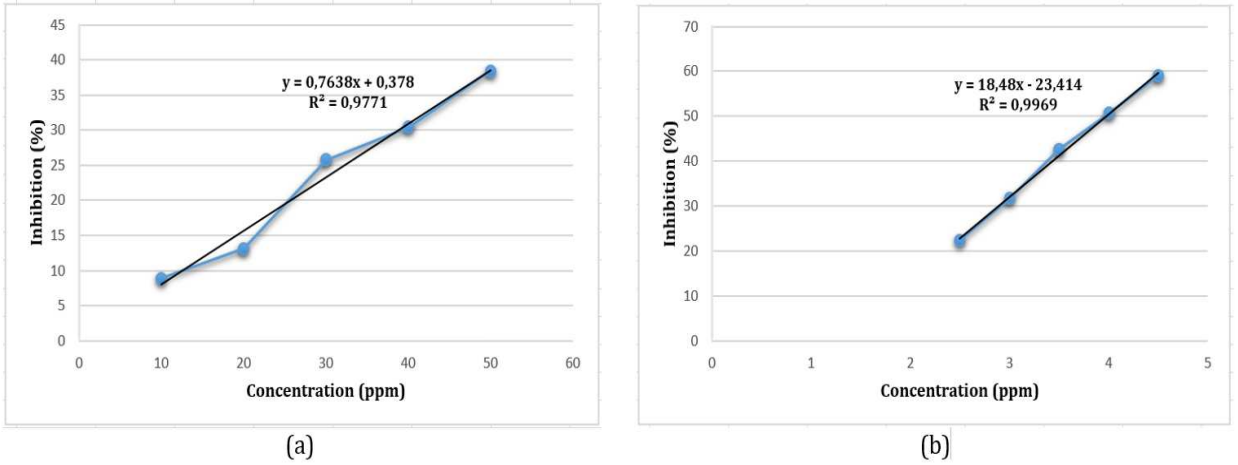

Figure 1. Antioxidant activity of (a) ethyl acetate *Coleus amboinicus* Lour fraction and (b) ascorbic acid

The IC_{50} value of the linear regression y= 0.7638x + 0,378; R^2= 0,9771 in Figure 1a and 1b was 64.97 with a strong category and antioxidant activity of ascorbic acid as a positive control with linear regression y = 18.48x - 23.414; R^2 = 0.9969 obtained IC_{50} value of 3.97 with a very strong category (Nihlati et al., 2008). The ability of strong antioxidant activity of ethyl acetate *Coleus amboinicus* Lour., fraction due to the presence of active compounds of phenolic & polyphenol and flavonoid groups. Phenolic & polyphenol groups and flavonoids are reported to be responsible as strong antioxidants in every natural ingredient (Skrovankova et al., 2015).

CONCLUSION

The ethyl acetate of Coleus amboinicus Lour fraction has a variety of secondary metabolites (alkaloids, flavonoids, phenolic and polyphenols, coumarin, and triterpenoids). It shows antioxidant activity with IC_{50} 64.97 with a healthy category.

REFERENCES

Akbarirad, H., Ardabili, A.G., Kazemeini, S.M & Khaneghah, A.M. (2015). An overview of some of the important sources of natural antioxidants. International Food Research Journal. 23(3): 928-933.

Andarwulan, N., Wijaya, C.H & Chayono, D.T. Antioxidant Activity of Betle Leaf (Piper betle L.). Buletin Teknologi dan Industri Pangan. 7(1): 29-37.

Arumugam, G., Swamy, M.K & Sinniah, U.R. (2016). Plectranthus amboinicus (Lour.) Spreng: Botanical, Phytochemical, Pharmacological and Nutritional Significance. Molecules, 21(4): 1-26.

Biswas, N.N., Acharzo, A.K., Anamika, S., Khushi, S & Bokshi, B. (2017). Screening of Natural Bioactive Metabolites and Investigation of Antioxidant, Antimicrobial, Antihyperglycemic, Neuropharmacological, and Cytotoxicity Potentials of Litsea polyantha Juss. Ethanolic Root Extract. Evidence-Based Complementary and Alternative Medicine. 1-11.

Damanik, R.M., Kustiyah, L., Hanafi, M & Iwansyah, A.C. (2017). Evaluation Lactogenic Activity of Ethyl Acetate Fraction of Torbangun (Coleus amboinicus

L.) Leaves. IOP Conf. Series: Earth and Environmental Science, 101: 1-10.

Gul, R., Jan, S.U., Fardullah, S., Sherani, S & Jahan, Nusrat. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. Hindawi The Scientific World Journal. 1-7.

Kadare, S.B & Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 48(4): 412-422.

Kadum, H., Hamid, A.A., Abas, F., Ramlii, N.S., Mohammed, A.K.S., Muhialdin, B.J & Jaafar, A.H. (2019). Bioactive Compounds Responsible for Antioxidant Activity of Different Varieties of Date (Phoenix dactylifera L.) Elucidated by 1 H- NMR Based Metabolomics. International Journal of Food Properties. 22(1): 462-476.

Kumar, R.S., Venkateshwar, C., Samuel, G & Rao, S.G. (2013). Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celastrus emarginata (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. International Journal of Engineering Science Invention. 2(8): 65-70.

Mbah, C.J., Orabueze, I & Okorie, N.H. (2019). Antioxidants Properties of Natural and Synthetic Chemical Compounds: Therapeutic Effects on Biological Systems. Acta Scientific Pharmaceutical Sciences, 3(6): 28-42.

Nihlati, A.P., Rohman, A & Hertiani, T. (2008). Daya antioksidan ekstrak etanol rimpang temu kunci (Boesenbergia pandurata) Roxb. Schelecth dengan metode penangkapan radikal DPPH. J. Traditional Medicines. 13(45): 136-144.

Rani, K. (2017). Role of antioxidants in the prevention of diseases. Journal of Applied Biotechnology & Bioengineering. 4(1): 495-496.

Riyanto, Nasution, J.,Saragih, W., & Saragih, W. (2020). Analysis Of Potentials Bangun-Bangun (*Coleus amboinicus*) and Belimbing Wuluh (*Averhoa bilimbi*) Plants, As Antimicrobial Material. *Biospecies*, *13*(1), 37–45.

Serlahwaty, D., Sugiastuti, S & Ningrum, R.Z. (2011). Antioxidant Activity of Water and Ethanol Extract of Green Betle Leaf (Piper betle L.) and Red Betle Leaf (Piper cf. fragile Benth.) using DPPH Free Radical Inhibition Method. Jurnal Ilmu Farmasi Indonesia. 9(2): 143-146.

Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T & Sochor, J. (2015). Bioactive Compounds and Antioxidant Activity in Different Types of Berries. Int J Mol Sci. 16(10): 24673-24706.