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Phytochemical Contents of Torbangun (*Coleus amboinicus* Lour) from Fractionation of Pressurized Liquid Extraction

(Kandungan Fitokimia Torbangun (*Coleus amboinicus* Lour) Hasil Fraksinasi Ekstraksi Cairan Bertekanan)

Farida Laila[1,2]*, Dedi Fardiaz[1], Nancy Dewi Yuliana[1], Muhammad Rizal Martua Damanik[3], Fitriya Nur Annisa Dewi[4]

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ABSTRACT

***Coleus amboinicus* (Lour) (torbangun) has been used traditionally as a breast milk stimulant, flavoring agent in many cuisines, and reported to possess many pharmacological values. The aim of this study was to explore the utility of the Pressurized Liquid Extraction (PLE) technique to identify the phytochemical contents of torbangun. In this study, total phenolic content and flavonoid in torbangun were determined using spectrophotometric method. The GC-MS analysis was used to identify the chemical constituents of torbangun, which was sequentially extracted with a wide range of solvent or solvent mixture from nonpolar-moderately polar and finally polar solvent. Total phenolic and flavonoid contents in the methanol extract of torbangun were 42.17±2.96 mg GAE/g and 11.20±0.58 mg QE/g, respectively. The identified chemical constituents in torbangun were sugar, hydrocarbon, ketone, terpenes, phenolics, fatty acid, fatty alcohol, steroids, alkaloids, and others, whereas the dominant constituents were phenolic compound, alkane, and sugar. In conclusion, this study demonstrated the effectiveness and rapid extraction of the PLE technique. Many valuable compounds in torbangun were extracted using gradual composition of solvent and were able to identify certain compounds in different polarities of solvents that are important in functional food preparation, pharmaceutical, and metabolomics research.**

Keywords: bioactive, extract, nontoxic, plant, solvent

ABSTRAK

***Coleus amboinicus* (Lour) (torbangun) telah digunakan secara tradisional sebagai stimulan ASI, flavor dalam sejumlah masakan, serta dilaporkan memiliki nilai farmakologi yang cukup tinggi. Tujuan penelitian ini adalah untuk mengeksplorasi teknik ekstraksi cairan bertekanan atau *Pressurized Liquid Extraction* (PLE) dalam mengidentifikasi kandungan fitokimia dalam torbangun. Kadar fenolik total dan flavonoid dalam torbangun ditentukan menggunakan metode spektrofotometri. Analisis GC-MS digunakan untuk mengidentifikasi senyawa kimia dalam torbangun yang berasal dari hasil ekstraksi teknik PLE secara bertahap menggunakan pelarut dan campuran pelarut dari pelarut nonpolar, semipolar, dan terakhir pelarut polar. Kadar fenolik total dan flavonoid yang diperoleh dari ekstrak torbangun dalam metanol secara berturut-turut adalah 42,17±2,96 mg GAE/g dan 11,20±0,58 mg QE/g. Kandungan senyawa kimia yang teridentifikasi dalam torbangun adalah gula, hidrokarbon, keton, terpena, fenolat, asam lemak, alkohol lemak, steroid, alkaloid, dan lain-lain, dengan kandungan utama berupa senyawa fenolik, alkana, dan gula. Hasil penelitian ini menunjukkan bahwa PLE dapat mengekstraksi torbangun dengan lebih efektif dan cepat. Sejumlah senyawa penting berhasil terekstraksi menggunakan komposisi pelarut secara bertahap dan mampu mengidentifikasi senyawa aktif tertentu dalam berbagai tingkat polaritas pelarut. Hasil penelitian ini dapat digunakan pada penelitian pangan fungsional, farmasi, dan metabolomik.**

Kata kunci: bioaktif, ekstrak, nontoksik, tumbuhan, pelaru

INTRODUCTION

Coleus amboinicus (Lour) with a synonym name *Plectranthus amboinicus* is a plant belongs to the Lamiaceae family. Bataknese people in Indonesia call this plant torbangun and use it traditionally as a breast milk stimulant (a lactagogue) (Damanik 2009). In Caribbean cuisine, the leaves are well-known as a flavoring agent in meat and bean dishes (NParks Flora & Fauna Web 2013). This plant has been reported to possess many bioactive compounds that provide pharmacological and toxicological effects in humans and animals. Amalia & Damanik (2018) reported torbangun leaves powder in capsules supplementation was able to reduce cholesterol level and systolic blood pressure in male subjects with hyper cholesterol. Pharmacological activities of this plant mainly

[1] Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, IPB Darmaga Campus, Bogor 16680
[2] School of Vocational Studies, IPB University, Jl. Kumbang No 14, Bogor 16151
[3] Department of Community Nutrition, Faculty of Human Ecology, IPB University, IPB Darmaga Campus, Bogor 16680
[4] Primate Research Center, Bogor Agricultural University, Jl. Lodaya II/5, Bogor 16151
* Correspondence Writer: Email: flaila.safire@gmail.com

antioxidant activity (El-hawary *et al.* 2012; Hemalatha *et al.* 2016), antiproliperative activity against many cancer cell lines (El-hawary *et al.* 2012; Bhatt *et al.* 2013; Hemalatha *et al.* 2016; Yulianto *et al.* 2016), antihyperglycemic, antihyperlipidemic (Suryowati *et al.* 2015), anticonvulsant (Majumder & Bhattacharjee 2013), as well as antidiuretic and anti-inflammatory (El-hawary *et al.* 2012).

The extraction technique plays a crucial role in the study of active compounds in the plant materials. The basic principle of extraction is the use of a liquid solvent to extract certain chemical compounds from solid or liquid matrices. A number of variables like the nature of the substrate, temperature, pressure, solvent system, and extraction time would affect the extraction efficiency (Mustafa & Turner 2011). A number of new non-conventional techniques that can reduce operating time and toxic organic solvent have been developed in recent years (Azmir *et al.* 2013).

Pressurized liquid extraction (PLE) or Accelerated Solvent Extraction (ASE) is a nonconventional extraction technique using high pressure and temperature. This method has been successfully used to extract plant samples rich in metabolites for metabolomics analysis (Kellogg *et al.* 2017) and neutraceutical metabolite from food and herbal plants (Mustafa & Turner 2011). High pressure will keep organic solvent still in the liquid phase even in elevated temperatures to ensure the effectivity of penetration and solubility of the solute in the solvent (Kellogg *et al.* 2017). The elevated temperature will reduce the viscosity of solvent and increased the diffusion rate of the analyte into the solvent (Mustafa & Turner 2011)

Phytochemical content of torbangun in different solvents or crude extracts using conventional extraction technique have been previously reported (Suryowati *et al.* 2015). Generally, conventional extraction uses toxic solvents to extract more components in the plant. A good extraction technique is needed to get as many active compounds as possible. Due to the very high content of compounds in plants that have diverse physical and polarity properties, it is not possible to use only one type of solvent. The aim of this study was to identify chemical compounds of torbangun that can be extracted utilizing gradient solvents by PLE technique and the possibility to obtain a phytochemical-rich extract for the functional food preparation and pharmaceutical research.

MATERIALS AND METHODS

Sample Solution Preparation for Total Phenolic and Flavonoid Content

Coleus amboinicus leaves were collected from Bogor Agricultural University Teaching Farm, Cikabayan Indonesia. The plant was freeze-dried for 48 hours and ground using *Knife Mill Grindomix GM 200* at 1000 rpm for 20 s. A 1.00 g of plant powder was macerated in 50 mL of methanol for 24 hours. The solution was then filtered and the solid particles were centrifuged several times using 10 mL of methanol until the green color of the leaves fade. The filtrate was diluted using methanol until 250.0 mL.

Determination of Total Phenolic Content (Amar *et al.* 2017 with Modification)

A 0.30 mL of sample solution was added with 6.00 mL of Na_2CO_3 (1%w/v in water) (Merck, Germany) and allowed to stand for 2 minutes at room temperature. A 1.5 mL of Folin Ciocalteau reagent was then added and the solution was incubated for 30 minutes in the darkroom. The calibration curve for the total phenolic compound was made using gallic acid (Sigma Aldrich, Singapore) at concentrations of 0; 100; 125; 150; 175; and 200 mg/L solution. The absorbance of the solution was then measured using visible spectrophotometer *Genesys 30* (ThermoFisher Scientific USA) at λ 750 nm. Total phenol content is expressed as a mean of mg gallic acid equivalent (GAE) per g dry sample ± SD (n=4).

Determination of Total Flavonoid Content (Amar *et al.* 2017 with Modification)

A 10.00 mL of the sample solution was added with 1.00 mL $AlCl_3$ (10%w/v in water) and adjusted until 25.00 mL in measuring flask using acetic acid (5%w/v in water). The solution was then incubated for 30 minutes. The standard series of quercetin (Sigma Aldrich, Singapore) was made at concentrations of 0.00; 2.50; 5.00; 7.50; 10.00; and 12.50 µg/mL in 50.00 mL of measuring flask. 10.0 mL of each standard solution was taken and given the same treatment as the sample. The solution was measured using visible spectrophotometer *Genesys* 30 (ThermoFisher Scientific USA) at λ 425 nm. The total flavonoid content in the sample is expressed in mg quercetin equivalent (QE) per g dry sample± SD (n=4).

Fractionation using Pressurized Liquid Extraction Technique

The raw material of torbangun was fractionated using a pressurized liquid extraction technique with an *Accelerated Solvent Extractor system* (Dionex ASE 350). The chemical profiles of all the fractions were then analyzed using GC-MS (Agilent model 6890). A 1.000 g of the leaves powder was mixed with sea sand (Methanol washed 425–850 µM (20–35 mesh, Waco Japan) and placed into 22 mL stainless steel extractor cell. The samples were extracted automatically using the combination of three solvents (hexane:acetone: water) with the arrangement of gradient composition as shown in Table 1. The extraction was performed under condition of 1500 psi pressure and 50°C temperature. Extraction time for every combination of solvent was 25 minutes until 11 fractions were collected. For solvent evaporation, a centrifugal evaporator was employed.

Table 1 Solvent composition of PLE fractionation

Fraction codes	Solvent composition mixture (%)		
	hexane	Acetone	water
1	100	0	0
2	80	20	0
3	60	40	0
4	40	60	0
5	20	80	0
6	0	100	0
7	0	80	20
8	0	60	40
9	0	40	60
10	0	20	80
11	0	0	100

GC-MS Analysis of Fractions from PLE Technique

The profile of chemical constituents in each fraction was determined using GC-MS (Agilent model 6890) with a mass selective detector. Capillary column model number: Agilent 19091J-411 HP-5, 5% phenyl methyl siloxane (ℓ x d x t = 15.0 m x 320.00 μm x 0.25 μm), operating at max temperature of 325°C and run time 25 minutes. Helium was used as a carrier gas at 20 $mL.min^{-1}$.

Data Analysis

The concentration of phenolic and flavonoids contents were calculated from the calibration plot. The regression equation and coefficient of determination (r^2) were calculated using Microsoft Excel 2010. Interpretation of GC-MS chromatogram was based on mass spectral matching using the National Institute of Standards and Technology (NIST) Mass Spectral Library.

RESULT AND DISCUSSION

Phenolic and Flavonoid Contents

The preliminary step of this study was to estimate total phenolic and flavonoid in torbangun. The therapeutic value of a plant is due to the presence of various bioactive constituents such as phenolic compounds, alkaloids, fatty acids, and steroids. The most abundant secondary metabolites in the plant are phenolic compounds with the most common classes are phenolic acid, flavonoids, and tannin (Dai & Mumper 2010). The two subclasses of phenolic acid are hydroxybenzoic acid and hydrocinnamic acid. The most common compound of hydroxybenzoic acid is gallic acid and the most common hydroxycinnamic acid are p-coumaric, caffeic, and ferulic acid (Ls *et al.* 2016).

The methanol extract of torbangun exhibited high total phenolic and flavonoid contents each with a concentration of 42.17 ± 2.96 mg GAE/g and 11.20 ± 0.58 mg QE/g of dried plant, respectively. The result suggests that the biological activities of torbangun are likely due to the high concentrations of phenolic and flavonoid in the plant.

Total phenolic and flavonoid contents in C. *amboinius* or its synonym (P. *amboinicus*) have been reported in other studies. P *amboinicus* grown in Egypt show total phenolic contents in stems, leaves, and roots are 9.6, 8.4, and 9.4 mg GAE/g, respectively (El-hawary *et al.* 2012). The study by Shubha & Bhatt (2015) reported that total phenolic and flavonoid contents of P. *amboinicus* were 49.91 mg GAE/g and 26.6 mg RE/g, respectively. The differences in plant parts, climate, environmental conditions of plant growth, extraction technique, and solvent types are potentially responsible for the variation in phenolic and flavonoid contents in plant samples.

Chemical Profiles of Torbangun using GC-MS

In this study, the chemical constituents of torbangun were fractionated using gradual composition ranging in the polarity of solvent or solvent mixtures from nonpolar to moderately polar and finally polar solvents. The aim was to achieve a wide range of compounds and analyzed the presence of certain compounds in a different type of solvent or solvent mixture. The spectrum of GC-MS and identified chemical compounds in every fraction is presented in Supplementary Data. The chemical constituents identified in this study were sugar, hydrocarbon, ketone, terpenes, phenolics, fatty acid, fatty alcohol, steroids, alkaloids, and others with the most dominant constituents were phenolic compound, alkane, and sugars.

From the results of the chemical constituents presented in all fractions, we selected the compounds which responsible for therapeutic value and their abundance in different fractions were observed. Figure 1 and Table 2 showed selected bioactive compounds, and caffeic acid as the major compound identified in all fractions, with the most abundance was at 6^{th} fraction (100% acetone) with 51.22% of peak area. The result also showed a compound with a high % peak at retention time of 17.432 in almost all fractions which identified by NIST Database as 3-Cyclohexene-1-ethanol, alpha.-ethenyl-alpha, 3-dimethyl-6-(1 methylethylidene). Unfortunately, the percent similarity with the literature was only 49–58%. Therefore, further analysis to isolate this compound is needed to identify the exact compound.

Many compounds with low concentrations were extracted only in certain solvent/solvent mixtures. Alpha-amyrin, geranic acid, and stigmasterol were only present in the 1^{st} fraction (100% hexane). The rate of extraction of analyte at low concentration is more affected by the rate of mass transfer than by analyte concentration. Therefore, to ensure solvation and the release of the analyte, the right chemical properties of solven should be chosen (Mustafa and Turner 2011).

Five major terpenes identified from GC-MS using PLE were α-amyrin, dehydroabietic acid, geranic acid, isopimaric acid, and thymol. Alpha amyrin was only present in the 1^{st} fraction (100% hexane).This

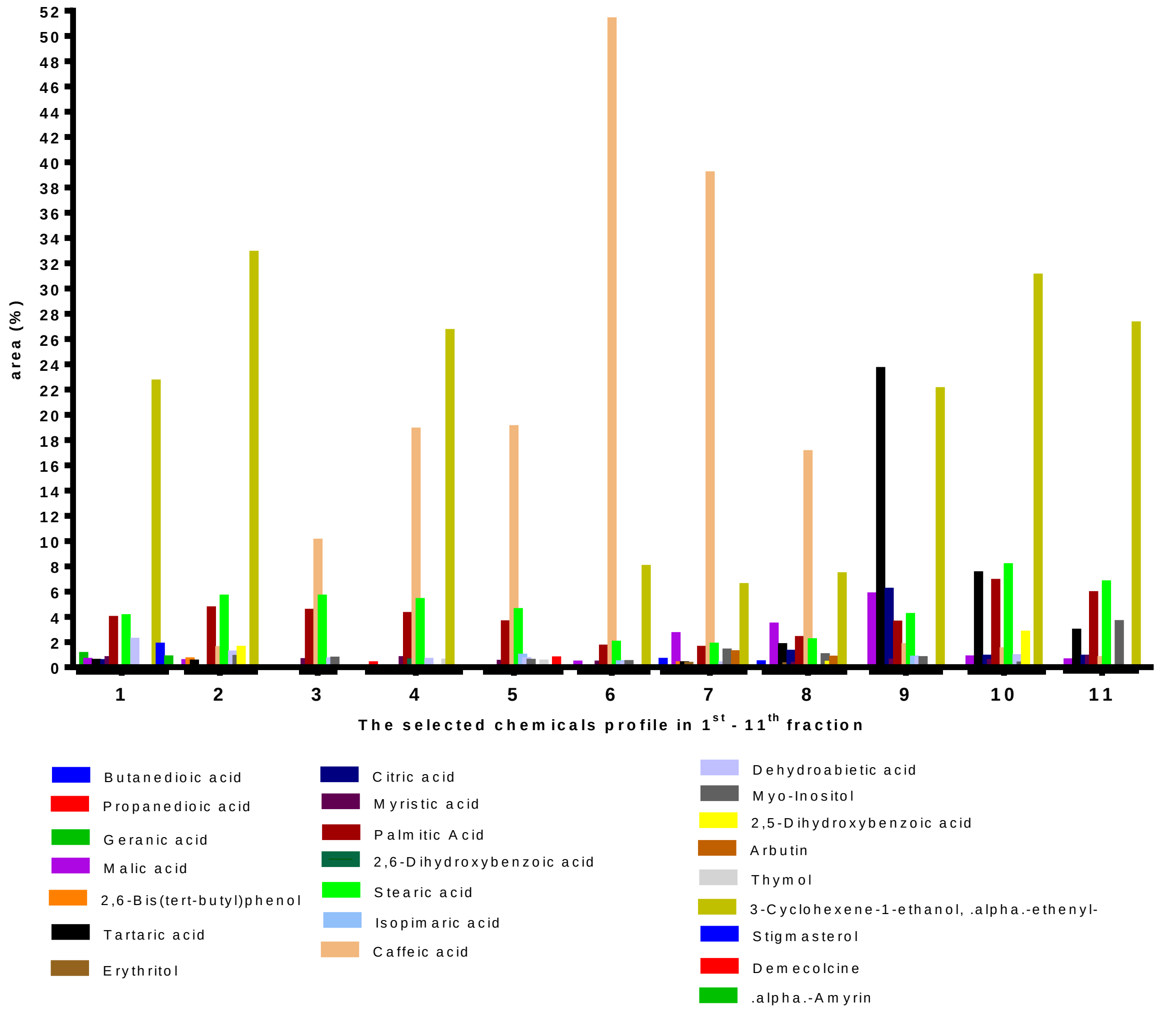

Figure 1 The phytochemicals content of selected compounds in eleven fractions of torbangun extract.

Table 2 Selected phytochemical compounds in torbangun in every fraction of different fractions

Retention time (min)	Name	Chemical Formula	Chemical Classes	Area (%) in the 1st Fraction until 11th Fraction										
				1	2	3	4	5	6	7	8	9	10	11
4.79	Propanedioic acid	$C_3H_4O_4$	dicarboxylic acid	-	-	-	0.18	-	-	-	-	-	-	-
5.362	Butanedioic acid	$C_4H_6O_4$	dicarboxylic acid	-	-	-	-	-	-	0.46	0.26	-	-	-
6.505	Geranic acid	$C_{10}H_{16}O_2$	acyclic monoterpenoids	0.92	-	-	-	-	-	-	-	-	-	-
6.682	Malic acid	$C_4H_6O_5$	alpha-hydroxy acid	0.46	0.35	-	-	-	0.24	2.49	3.25	5.65	0.65	0.41
7.179	2,6-Bis(tert-butyl)phenol	$C_{14}H_{22}O$	phenolic compound	-	0.5	-	-	-	-	0.18	-	-	-	-
7.842	Tartaric acid	$C_4H_6O_6$	dicarboxylic acid	0.38	0.33	-	-	-	-	0.18	1.63	23.5	7.31	2.77
8.38	Erythritol	$C_4H_{10}O_4$	sugar alcohol	-	-	-	-	-	-	0.15	0.1	-	-	-
9.191	Citric acid	$C_6H_8O_7$	tricarboxylic acid	0.35	-	-	-	-	-	-	1.09	6.02	0.7	0.71
9.443	Myristic acid	$C_{14}H_{28}O_2$	saturated long fatty acid	0.6	-	0.43	0.59	0.3	0.25	-	0.15	0.39	0.35	0.69
10.763	Palmitic Acid	$C_{16}H_{32}O_2$	saturated long fatty acid	3.77	4.53	4.35	4.08	3.42	1.51	1.4	2.18	3.4	6.72	5.74
11.088	2,6-Dihydroxybenzoic acid	$C_7H_6O_4$	poliphenol phenolic acid	-	-	-	0.42	-	-	-	-	-	-	-
11.329	Caffeic acid	$C_9H_8O_4$	polyphenol phenolic acid	-	1.38	9.9	18.7	18.9	51.2	39	16.9	1.62	1.3	0.6
11.98	Stearic acid	$C_{18}H_{36}O_2$	long chain fatty acid	3.92	5.46	5.47	5.2	4.39	1.81	1.67	2.01	4.02	7.96	6.59
12.74	Isopimaric acid	$C_{20}H_{30}O_2$	diterpenoids	-	-	-	-	0.79	0.26	-	-	0.63	-	-
12.963	Dehydroabietic acid	$C_{20}H_{28}O_2$	diterpenoids	2.05	1.04	0.49	0.45	0.52	0.19	0.18	-	0.57	0.75	-
13.015	Myo-Inositol	$C_6H_{12}O_6$	sugar alcohol	-	0.68	0.55	-	0.37	0.28	1.19	0.82	0.6	0.17	3.44
13.512	2,5-Dihydroxybenzoic acid	$C_7H_6O_4$	polyphenol phenolic acid	-	1.4	-	-	-	-	-	0.23	-	2.61	-
13.763	Arbutin	$C_{12}H_{16}O_7$	phenolic glycoside	-	-	-	-	-	-	1.06	0.63	-	-	-
15.421	Thymol	$C_{10}H_{14}O$	monoterpene phenol	-	-	-	0.39	0.32	-	-	-	-	-	-
17.432	3-Cyclohexene-1-ethanol, .alpha.-ethenyl-	$C_{15}H_{24}O$	-	22.5	32.7	-	26.5	-	7.82	6.39	7.23	21.9	30.9	27.1
18.758	Stigmasterol	$C_{29}H_{48}O$	steroid	1.67	-	-	-	-	-	-	-	-	-	-
18.856	Demecolcine	$C_{21}H_{25}NO_5$	alkaloid	-	-	-	-	0.57	-	-	-	-	-	-
20.387	.alpha.-Amyrin	$C_{30}H_{50}O$	Pentacyclic trierpenes	0.65	-	-	-	-	-	-	-	-	-	-

compound has antihyperglycemic, hypolipidemic, and effective for drug development in diabetes and atherosclerosis (De Le. Silva *et al.* 2012). Dehydroabietic acid or callitrisic acid were present in the 2nd, 8th, and 10th fractions with the highest peak was at the 10th fraction. Dehydroabietic acid has the potential for the treatment of obesity and metabolic syndrome. Plasma glucose, insulin, and TG levels were decreased in KK-Ay mice treated with DHA. The production of the proinflammatory mediator was also inhibited by DHA (NCATS 2018). Geranic acid is monoterpenoid and also polysaturated acid. This compound is an antifungal agent and a melanin synthesis inhibitor (Kim *et al.* 2016). Isopimaric acid is a tricyclic diterpenoid with many biological and pharmaceutical properties such as antimicrobial, antiviral, and anti-inflammatory activities. This compound also a useful agent in the therapy of various diseases associated with both the central nervous system and smooth muscle system. Thymol is a monoterpene used as a stabilizer in pharmaceutical preparations and has been used as antiseptic, antibacterial, and antifungal to reduce and prevent plaque and gingivitis (NCATS 2018).

The result demonstrated a variety of phenolic compounds with different concentration levels were present in most of the fractions. Phenolic compounds are chemical with an aromatic ring bearing one or more hydroxyl groups and range from simple phenols to complex, high molecular compounds (Balasundram *et al*. 2006). The phenolic compounds identified in torbangun included caffeic acid (the 2nd and 11th fractions), 2,5-dihydroxy benzoic acid (the 2nd, 8th, and 10th fractions), 2,6-Bis(tert-butyl)phenol (the 2ndand 7th fractions), 2,6-hydroxybenzoic acid (the 4th fraction) and arbutin (the 7th & 8th fractions).

Caffeic acid is a polyphenol of a hydroxycinnamic acid derivative. This compound has been reported to show the activities of antioxidant, anti-inflammatory, antithrombosis, antihypertensive, antiviral, and antitumor (Kim *et al.* 2016). The highest concentration of caffeic acid from this study was in moderately polar and more polar fractions. This is likely due to the fact that carbohydrates and/or lipoidal materials were eliminated with a non-polar fraction during the sequential extraction using PLE. The crude extract of the plant usually contains large amounts of sugar and/or lipoidal materials which cause a low concentration of extracted phenolic compounds (Dai & Mumper 2010).

The phenolic compound, 2,5-dihydroxy benzoic acid or gentisic acid has many activities such as antioxidant, anti-inflammatory, and antirheumatic properties (Kim *et al.* 2016). The antioxidant property of this compound is exerted by its phenoxyl group. This chemical also has been used as a skin whitening agent and to treat skin pigmentary disorders by influencing the synthesis of melanin through inhibition of melanosomal tyrosinase activity (NCATS 2018). The phenolic compound, 2,6-bis(tert-butyl)phenol has a role as an antioxidant (Kim *et al.* 2016). Arbutin is beta-D-glucopyranoside of hydroquinone has been reported as a skin whitening ingredient. In vitro study also showed that the compound elicits anti-inflammatory activity (NCATS 2018).

Palmitic acid and stearic acid were the most abundant long fatty acid in torbangun. These compounds were present in all fractions. Interestingly, both compounds showed a similar trend with the highest percent peak was at the most polar fractions (the 10th and 11th fractions). This result showed that eventhough palmitic acid and stearic acid are non-polar compounds, water as a polar solvent is able to extract these compounds in high temperatures. Therefore, it is possible to use more polar water to extract nonpolar compounds with a faster extraction using the PLE technique.

The other important compounds identified in torbangun were stigmasterol, democolcine, Myo-inositol, and erythritol. Stigmasterol is the steroid derivative identified in the torbangun and only present in the 1st fraction (100% hexane). This compound is a phytosterol that has a vital role in cellular processes like cellular differentiation and proliferation, maintaining fluidity in the membrane and also temperature adaptation of the membrane (Ivanov *et al.* 2018). Democolcine is an alkaloid identified in torbangun (the 5th fraction). This compound is a secondary amino compound having potential as antimitotic and antineoplastic activities (NCATS 2018). Myo-inositol is a sugar alcohol which presents in almost all of the fractions. This compound has potential chemopreventive properties, lowering blood sugar levels, antioxidant, and anti-inflammatory (Kim *et al.* 2016). Erythritol is a four-carbon sugar used as a low-calorie sweetener and has been approved by the Food and Drug Administrations of the United States of America and EU. This compound has been investigated as a food supplement to treat endothelial function related to Type II diabetes and has also been studied for the prevention of gingivitis and carries (NCATS 2018).

Phytoconstituents of C.*amboinicus* or P.*amboinicus* using different techniques and different parts of the plant have been characterized in many studies. A study of Bhatt *et al.* (2013) reported the major constituent of stem of P.a*mboinicus* in methanol extract were rosmarinic acid, caffeic acid, rutin, gallic acid, quercetin, and p-coumaric acid. The other study of Hemalatha *et al.* (2016) which analyzed ethanolic extract of P.*amboinicus* leaves by GC-MS, reported the major components were n-Hexadecanoic acid, thymol, 9-octadecenal (z), 10-heneicosane (c,t), and phytol. El-hawary *et al.* (2012) also reported the major components in stems and roots of ethyl acetate extract of P.*amboinicus* or P.*amboinicus* grown in Egypt using UPLC-MS were caffeic acid, eriodyctiol, rosmarinic acid, coumaric acid, chrysoeriol, and quercetin. Shubha & Bhatt (2015) which analyzed the leaves using HPLC also reported that polyphenols and sugars were the dominant constituents and found chlorogenic acid, coumaric acid, and caffeic acid in appreciable concentrations.

The PLE technique using sequential extraction performed in this study was used to concentrate and obtain phytochemical-rich fraction and also to identify the existence of certain bioactive compounds in different types of solvent. The nature of the solvent is an important factor in extracting certain bioactive compounds. Methanol and water are appropriate solvents to extract hydrophilic compounds like polyphenol which have several hydroxyl groups. The extraction efficiency in the PLE technique is also affected by temperature. The study of Hawthore and Miller showed that dielectric constant (polarity) of water decreased substantially during the extraction at high temperature. Viscosity and surface tension also decreased and diffusivity characteristic was faster in higher temperatures (Mustafa & Turner 2011). Therefore, the advantage of this technique is that we can use a nonharmful solvent like water to extract polar, moderately polar, and non-polar organic compounds. Kellogg *et al.* (2017) compared the conventional extraction maceration with Accelerated Solvent extraction (ASE) from green tea for the metabolomic analysis purpose. This study reported that extraction techniques produce similar profiles of metabolites, but ASE extraction results in higher catechin yields, better repeatability, and shorter sample preparation time.

CONCLUSION

Coleus amboinicus (Lour) exhibited high total phenolic and flavonoid contents with many types of bioactive compounds. This study demonstrated a rapid and efficient extraction of the plant and reduced the solvent requirement using the PLE technique. The fractionation using different types of solvent or solvent mixture is useful to select an appropriate solvent that can maximize the compound extraction and to identify phytochemical constituents for further research.

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