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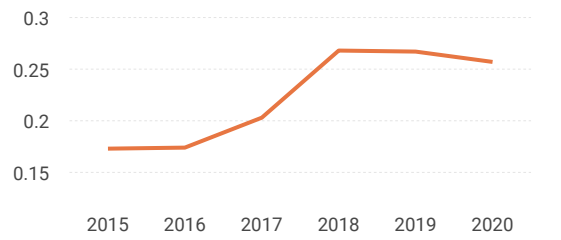

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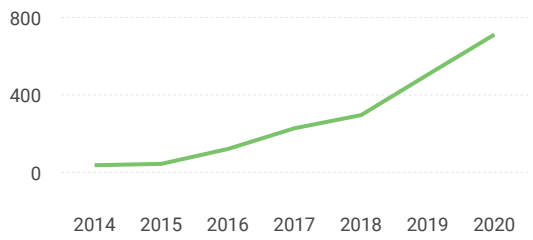

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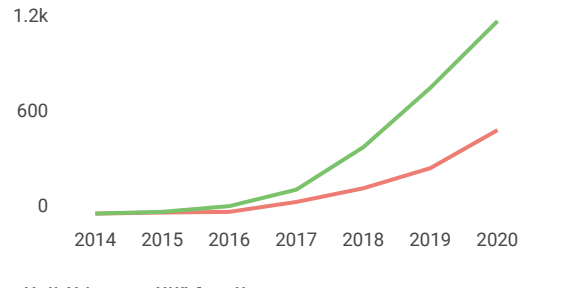

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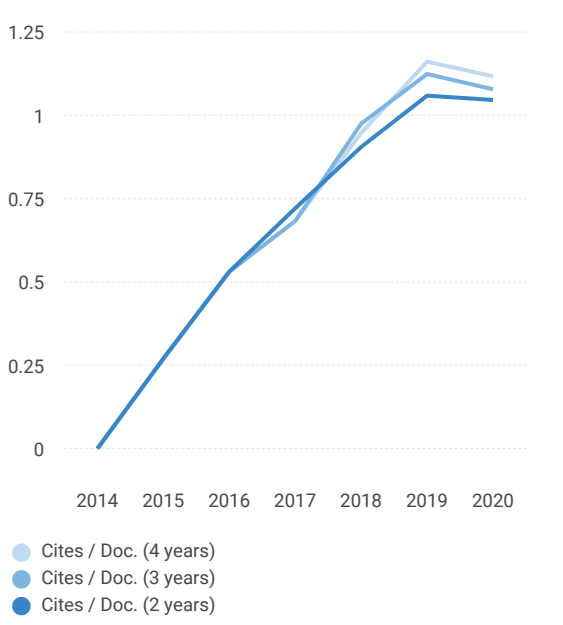

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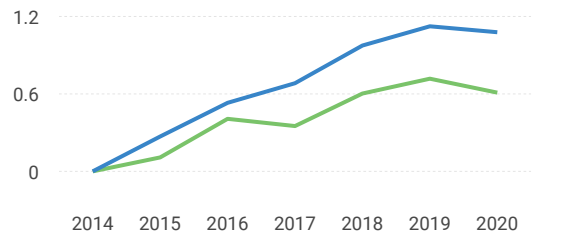

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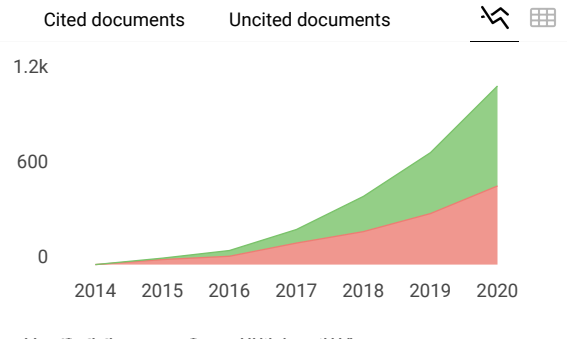

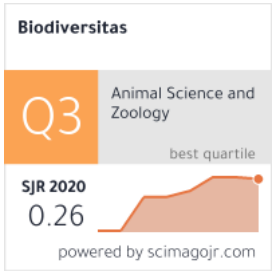

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Antioxidant activity screening of seven Indonesian herbal extract

JOHAN SUKWEENADHI[1], OEKE YUNITA[2], FINNA SETIAWAN[2], KARTINI[2], MAYA THERESA SIAGIAN[2], ANGGREYNI PRATIWI DANDURU[2], CHRISTINA AVANTI[2,♥]

[1]Faculty of Biotechnology, Universitas Surabaya. Jl. Ngagel Jaya Selatan No. 169, Surabaya 60294, East Java, Indonesia
[2]Faculty of Pharmacy, Universitas Surabaya. Jl. Raya Kalirungkut, Surabaya 60293, East Java, Indonesia. Tel.: +62-31-2981110, Fax.: +62-31-298 1113, ♥email: c_avanti@staff.ubaya.ac.id

Manuscript received: 27 February 2020. Revision accepted: 17 April 2020.

Abstract. *Sukweenadi J, Yunita O, Setiawan F, Kartini, Siagian MT, Danduru AP, Avanti C. 2020. Antioxidant Activity Screening of Seven Indonesian Herbal Extract. Biodiversitas 21: 2062-2067.* ***Kumis kucing*** (*Orthosiphon stamineus*), ***pegagan*** (*Centella asiatica*), ***seledri*** (*Apium graveolens*), ***kunyit*** (*Curcuma domestica*), ***temulawak*** (*Curcuma xanthorrhiza*), ***tempuyung*** (*Sonchus arvensis*) and ***meniran*** (*Phyllanthus niruri*) are herbs that commonly used in the Indonesia folk medicine. The constituents that responsible for several important biological activities are phenolic and flavonoid compounds which also possess antioxidant activity. Antioxidant activity of those seven Indonesian herbal extracts was evaluated using DPPH, ABTS and FRAP methods. The extraction was done with the reflux method by using 80% ethanol as a solvent. The total phenol and total flavonoids from each herbal extract were measured using Folin–Ciocalteu reagent and spectrophotometry. Antioxidant activity results by DPPH method on *O. stamineus*, *C. asiatica*, *A. graveolens*, *C. domestica*, *C. xanthorrhiza*, *S. arvensis*, and *P. niruri* showed IC_{50} value at 132; ND; 2221; 361; 538; 1118; and 102 ppm, respectively. Results from ABTS method, showed IC_{50} value at 22; 1199; 169; 100; 82; 143; and 20 ppm respectively. While results from the FRAP method showed that the ethanolic extract of *P. niruri* at a concentration of 20 ppm possesses the strongest antioxidant activity (17.41 ppm AEAC/ppm extract). The content of total phenolic compounds are 22.50; 0.67; 2.16; 11.40; 7.80; 7.22; and 2.62% GAE, while the total flavonoid compounds were 19.88; 6.67; 4.06; 71.02; 34.62; 3.78; and 8.34% QE, respectively. It can be concluded that ethanolic extract of *P. niruri* and *O. stamineus* obtain the highest antioxidant activity based on DPPH, ABTS and FRAP method.

Keywords: Antioxidants, DPPH, ABTS, FRAP

Abbreviations: ABTS: 2,2'-azino-bis: 3-ethylbenzothiazoline-6-sulfonic acid, AEAC: Ascorbic acid equivalent antioxidant capacity, DPPH: 2,2-diphenylpicrylhydrazyl, FRAP: ferric reducing antioxidant power, GAE: Gallic acid equivalent, IC_{50}: half maximal inhibitory concentration, QE: Quercetin equivalent, ROS: reactive oxygen species

INTRODUCTION

A free radical is a relatively unstable molecule having one unpaired electron in its outermost orbit. As it is highly reactive, a free radical is trying to reach a stable state by attracting electron from other molecules or cells in the body. The ability of a free radical molecule to oxidize other substances may cause oxidative damage in the body. A notable example of a free radical is the reactive oxygen species (ROS). ROS can react with and disrupt macromolecules, such as proteins, lipids, and nucleic acids in the human body. If the damage caused by ROS cannot be stopped, it will cause oxidative stress (Schieber and Chandel 2014).

Oxidative stress is an imbalance between free radicals and antioxidants in the body that are triggered by an excess of free radicals and a lack of antioxidants. Oxidative stress can cause oxidative damage starting from cells, tissues, to organs. Oxidative stress also generates accelerated aging (Kunwar and Priyadarsini, 2011). The sources of free radicals are endogenous and exogenous. Endogenous free radicals are produced intracellularly from automatic oxidation or inactivation of small molecules such as mitochondria, whereas exogenous free radicals are obtained from cigarette smoke, environmental pollutants, drugs, organic solvents, and pesticides (Rao et al. 2011).

An antioxidant is a chemical compound that donates an electron to an unpaired free radical, hence reduces the oxidation effect of a free radical. There are numerous compounds from herbs that can be used as natural exogenous antioxidants and clinically proven to be effective as antioxidants (Amorati and Valgimigli 2018). One of the chemical compounds is a phenolic compound, secondary metabolites which protect plants' organs from oxidation. Therefore, the phenolic compound is referred to as a natural antioxidant. In addition to its activity as an antioxidant, a phenolic compound in plants is known to have anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic, and anti-inflammatory properties (Schiavano et al. 2015; Babbar et al. 2015; Cirmi et al. 2017; Hoxha et al. 2015).

Other phytochemicals that possess antioxidant activity are flavonoids. Flavonoids are polyphenolic compounds contained in various plant species and have been useful in maintaining human health. Flavonoids in fruits and vegetables, which are routinely consumed can reduce the risk of cardiovascular disease (Ivey et al. 2017). The distribution and composition of phenolic content are very dependent on maturity, cultivars, horticultural practices,

geographic origin, growing season, post-harvest storage conditions, and processing procedures (Kumar et al. 2019; Yoo and Moon 2016; Kim et al. 2018). Several studies reported that flavonoids affect many pharmacological activities, including antioxidant, anti-cancer, enzyme inhibitor, and anti-inflammatory agents (Aslani and Ghobadi 2016). The biological activities of flavonoids vary greatly, however, the benefit that is almost shared by all groups of flavonoids is the antioxidant activity. The structure of flavonoids is responsible for giving several mechanisms to antioxidant activity, such as radical scavenging and metal ion chelation (Pandey et al. 2012). Some flavonoids also inhibit enzymes that participate in ROS generation (Kumar and Pandey 2013).

Orthosiphon stamineus is usually used as an herbal medicine for gout, rheumatism, hematuria, albuminuria, hypertension, diabetes and diuretics (Alshawsh et al. 2012). *Centella asiatica* possesses various benefits including antibacterial agents, lowering blood sugar levels, lowering blood pressure, reducing liver inflammation, reducing fever, overcoming intestinal worms, increasing appetite, stimulating hair growth, and cleanse the blood (Mala and Tulika 2014). *A. graveolens* is used traditionally, as a booster for digestive enzymes or as an appetite enhancer, lowering blood pressure, diuretics, reducing pain in rheumatism, as well as anti-seizure medications and stomach pain alleviation. *A. graveolens* also has many other benefits, such as a blood purifier, repairing disturbed hormones, and removing uric acid through urine (Ishaq et al. 2016). The compounds contained in *C. domestica* such as curcuminoids and essential oils has an important role as antioxidants, anti-tumor, anti-cancer, anti-microbial, anti-cholesterol, anti-inflammatory. The antioxidant properties of *C. domestica* have been widely accepted as one of the spices with the highest antioxidant activity because they contain curcumin compounds in it. In several countries, including Indonesia, *C. domestica* is used as a treatment for a variety of respiratory disorders such as asthma, allergies, other than that it can be used for liver disorders, anorexia, rheumatism, colds, coughs and wound healing (Hewlings and Kalman 2017). *Curcuma xanthorrhiza* is used to increase appetite, improve digestive function, overcome impaired liver function, reduce joint and bone pain, reduce blood fat, antioxidants, and inhibit blood clotting. Its essential oils are reported as an effective anti-fungal and anti-bacterial agent (Rahmayunita et al. 2016). *Sonchus arvensis* has the potential to overcome hyperuricemia and destroys kidney stones that are known to be caused by the results of active compounds which are antioxidants. It is commonly used to treat bruises by affixing them to bruised parts, hemorrhoids, anti-inflammatory, relieve aches and rheumatism (Seal 2016). *Phyllanthus niruri* has many benefits such as anti-inflammatory, hepatoprotective, increase endurance, antidiarrheal, also as medication for cough, thrush, and heartburn (Twahirwa et al. 2018).

In this study, we determined the total phenolic compounds and total flavonoid compounds (Folin-Ciocalteau method) of seven Indonesian herbal plant extract; there are *O. stamineus*, *C. asiatica*, *A. graveolens*, *C. domestica*, *C. xanthorrhiza*, *S. arvensis*, and *P. niruri*. The morphological appearance of those herbs is shown in Figure 1. All extraction processes were done by the reflux method with 80% ethanol as the solvent. Antioxidant activity test was carried out by using DPPH, ABTS, and FRAP method. The present data would certainly help to explore the potency of the tested Indonesia herbal extract as a potential source of natural antioxidants to be used for pharmaceutical, nutraceutical, and functional food formulations. However, further research is needed to purify and identify the specific compounds that possess antioxidant properties, thus can be developed further on its applications for food and pharmaceutical industries.

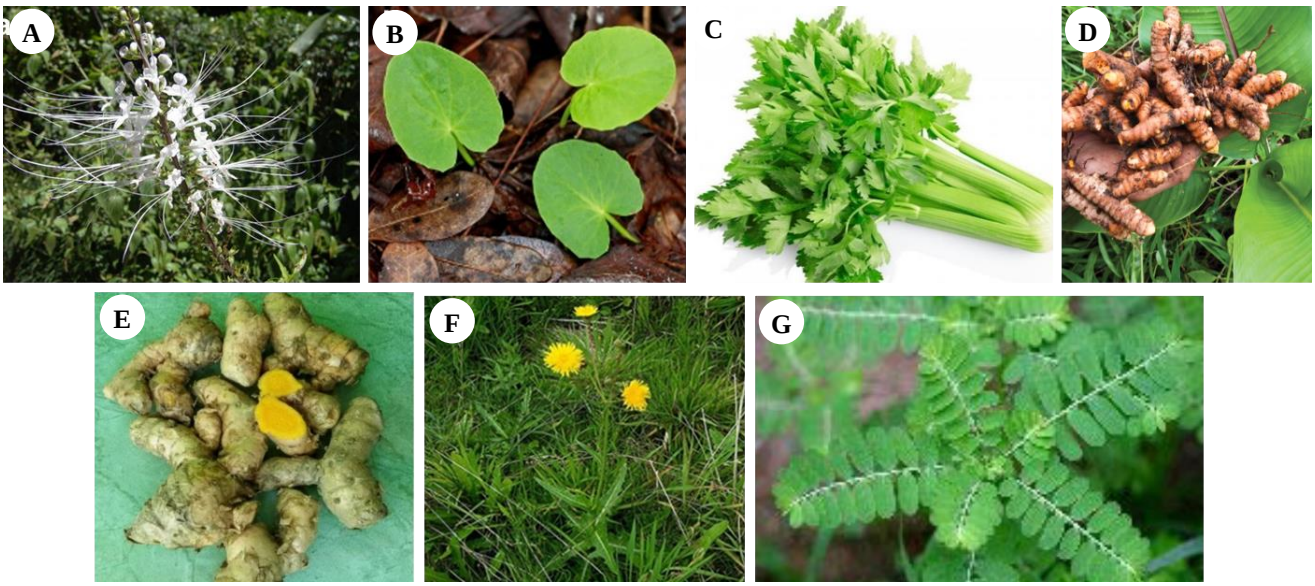

Figure 1. Morphological appearance of seven herbal plants: A. *Orthosiphon stamineus*; B. *Centella asiatica*; C. *Apium graveolens*; D. *Curcuma domestica*; E. *Curcuma xanthorrhiza*; F. *Sonchus arvensis;* G. *Phyllanthus niruri*

MATERIALS AND METHODS

Research materials

Orthosiphon stamineus, *C. asiatica*, *A. graveolens*, *C. domestica*, *C. xanthorrhiza*, *S. arvensis*, and *P. niruri* herbal crude drugs were obtained from the Center for Research and Development of Medicines and Traditional Medicine (*Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional*), Tawangmangu, Central Java, Indonesia.

Extraction method

100 grams of crude herbal powder was added by 200 mL of 80% ethanol before the extraction process using reflux for 3 times 2 hours. Each extract was then evaporated using a rotary evaporator to obtain a viscous ethanol extract (Shi et al. 2005).

Quantification of total phenol and total flavonoid

Standard Curve of Gallic Acid

Twenty-five mg gallic acid was dissolved in 25 mL of distilled water to obtain 1000 ppm of gallic acid solution. This solution was then diluted into five different concentrations (2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm). Each concentration was pipetted as much as 0.5 mL and put into a 10 mL volumetric flask, then 0.5 mL of Folin-Ciocalteu reagent was added in a ratio of 1:1. The flask was shaken until homogeneous for one minute and then allowed to stand. Four mL of 7.5% Na_2CO_3 followed by distilled water until 10.0 mL was added prior to the eight minutes incubation. The absorbance measurements were carried out on a gallic acid solution by using a UV-VIS spectrophotometer at a maximum wavelength of 760 nm (Kamtekar et al. 2014). Three replications were performed on each gallic acid concentration.

Determination of total phenol content

Ten mg of each herbal plant ethanol extract was dissolved in 10 mL of distilled water. Then, 0.5 mL of herbal extract solution was added with 0.5 mL of Folin-Ciocalteu reagent (1:1 ratio), shaken until homogeneous for one minute and allowed to stand. Before the eighth minute incubation, 4 mL of 7.5% Na_2CO_3 was added, followed by distilled water until 10 mL. Then the measurements were carried out on the sample solution using a UV-Vis spectrophotometer with a wavelength of 760 nm. Three replications were performed on each extract.

Standard curve of quercetin

25 mg of quercetin was dissolved in 25 mL of ethanol to obtain a 1000 ppm of quercetin standard solution. The standard solution was diluted into five different concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm). Each solution was pipetted 0.05 mL; 0.1 mL; 0.15 mL; 0.2 mL; 0.25 mL, and put into a 10 mL volumetric flask. Then, 4 mL of distilled water was added, followed by 0.3 mL of 5% $NaNO_2$ reagent addition. After five minutes, 0.3 mL of $AlCl_3$ was added and then 2 mL of 1 M NaOH was added in the sixth minute. The distilled water was added up to 10 mL. The solution was shaken until homogeneous, then absorbance measurements of quercetin standard solutions were carried out by using a UV-Vis spectrophotometer with a wavelength of 415 nm (Kamtekar et al. 2014). Three replications were performed on each concentration.

Determination of total flavonoid content

Ten mg of each herbal plant ethanol extract was dissolved in 10 mL distilled water. 0.3 mL each solution was added with 0.3 mL of 5% $NaNO_2$ reagent (1:1 ratio). After five minutes of incubation, 0.3 mL of $AlCl_3$ was added and in the sixth minute, 2 mL of 1 M NaOH was added. The distilled water was added until the volume of 10 mL was reached. The solution was shaken until homogeneous, then measurements were taken on the sample solution by using a UV-Vis spectrophotometer with a wavelength of 415 nm. Three replications were performed on each extract.

Antioxidant activity test with DPPH method

The DPPH method was conducted according to the previous method reported by Fidrianny et al (2018) with slight modifications. The vitamin C standard solution was prepared by dissolving 5 mg of vitamin C powder in ethanol up to 50 mL volume to obtain a vitamin C solution with a concentration of 100 ppm. The solution was then diluted to obtain five different concentrations (10, 20, 30, 40, and 50 ppm) to create a calibration curve. The sample solution was prepared by dissolving 25 mg herbal extract with ethanol in a 25 mL volumetric flask (1000 ppm). The sample solution was diluted into four concentrations (200, 400, 600, and 800 ppm). Each standard or sample solution was pipetted and the 0.05% of DPPH solution was added with a ratio of 1:3. The mixture was placed into a 96-well microplate reader after being homogeneously shaken. All solutions then were being incubated in a dark place at room temperature for 30 minutes. Free radical scavenging activity was calculated using the following formula:

$$DPPH\ scavenging\ effect\ (\%) = \left[\frac{A_B - A_A}{A_B}\right] \times 100$$

where, A_B = absorbance of DPPH solution (t = 0 min); A_A = absorbance of tested extract solution (t = 30 min). The concentration of extract or standard which exhibited 50% radical scavenging (IC_{50} value) was deduced from the linear regression of concentration versus the percentage of inhibition.

Antioxidant activity test with the ABTS method

The ABTS method was conducted by following the previous method developed by Dasgupta et al. (2015) with slight modification. 19.2 mg of ABTS and 3.31 mg of potassium persulfate were weighed separately. Each of them was then dissolved in 5 mL ethanol to obtain 7 mM ABTS solution and 2.45 mM potassium persulfate solution. In order to make ABTS reagent, the two solutions were mixed and incubated for 12-16 hours in a dark place at

room temperature. 50 mg of vitamin C was dissolved in 100 mL of distilled water (500 ppm) and serial dilution (10, 15, 20, 30, 40, and 50 ppm) was prepared. Ten mg of each plant extract was dissolved in 25 mL of ethanol (400 ppm) and diluted into 50, 100, 150, 200, and 250 ppm. ABTS test was carried out on a 96-well-microplate. The ABTS reagent and sample solution or standard solution (vitamin C) were pipetted in a volume ratio of 1:4, then placed into 96-well-microplate. The absorbance was measured by using a microplate reader at a wavelength of 734 nm. The percentage of inhibition of ABTS oxidation was calculated using the following formula:

$$ABTS\ scavenging\ effect\ (\%) = \left[\frac{A_B - A_A}{A_B}\right] \times 100$$

Where: A_B = absorbance of ABTS reagent; A_A = absorbance of sample or standard. The concentration of extract or standard which exhibited 50% radical scavenging (IC_{50} value) was deduced from the linear regression of concentration versus the percentage of inhibition.

Antioxidant activity test with FRAP method

FRAP solution was prepared by weighing 187 mg sodium acetate trihydrate, 270 mg ferric chloride ($FeCl_3.6H_2O$), and 150 mg TPTZ. Sodium acetate trihydrate powder was added with 16 mL acetic acid (pH 3.6) and dissolved in 250 mL of distilled water, $FeCl_3.6H_2O$ powder was dissolved in 100 mL of distilled water, whereas TPTZ was dissolved in 40 mM HCl up to 50 mL. FRAP reagent was prepared by mixing 25 mL of sodium acetate trihydrate solution, 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution, 2.5 mL of 10 mM TPTZ solution and then adding distilled water up to 100 mL (Hendra et al. 2011). A series concentration of vitamin C (1.25, 2.5, 10, 20, 30, and 40 ppm) was used as standard. The sample solution was prepared by dissolving each extract in ethanol and diluting it to a concentration of 200, 400, 600, and 800 ppm. The FRAP reagent and sample solution or standard solution (vitamin C) were pipetted in a volume ratio of 1:1, then placed into 96-well-microplate. The absorbance was measured by using a microplate reader at a wavelength of 593 nm.

RESULTS AND DISCUSSION

One of the parameters affecting the extract quality is the percentage of extract yield. The yield percentage was obtained by calculating the weight of viscous extract divided by the weight of crude herbs used. The yield percentage of seven Indonesian herbs extract produced from the reflux extraction method using 80% ethanol ranged from 8.76 to 37.50% as shown in Table 1. The reflux method was preferred because it widely used in herbal industries as it is efficient, easy to operate and cost-effective. The extraction solvent is renewed in the extraction, the mass transfer driving force is greater, which leads to a shorter extraction time. The reuse of the solvent in the extraction also decreases the amount of solvent needed (Wang et al. 2013).

Table 2. Total phenol and total flavonoid content of seven herbal extracts

Extract	Total phenol content (%GAE)*	Total flavonoid content (%QE)**
Orthosiphon stamineus	22.50 ± 0.91	19.88 ± 0.91
Centella asiatica	0.67 ± 0.03	4.06 ± 0.27
Apium graveolens	2.16 ± 0.05	6.67 ± 0.31
Curcuma domestica	11.40 ± 0.66	71.02 ± 0.48
Curcuma xanthorrhiza	7.80 ± 0.39	34.62 ± 1.58
Sonchus arvensis	7.22 ± 0.15	3.78 ± 0.01
Phyllanthus niruri	2.62 ± 0.06	8.34 ± 0.74

Note: *GAE= Gallic Acid Equivalent; Linear Regression of Gallic acid standard curve: y = 0.0349 + 0.10095x, with r^2 = 0.9892; **QE= Quercetin Equivalent; Linear Regression of quercetin standard curve: y = 0.0722 + 0.02564x, with r^2=0.9993; Values in the column represent average ± SD of 3 determinations

Table 1. The yield of seven crude drugs after 80% ethanol extraction by reflux

Crude drug	Weight (g)	Extract	
		Weight (g)	Yield (%)
Orthosiphon stamineus	105.20	9.22	8.76
Centella asiatica	101.03	9.33	9.23
Apium graveolens	103.12	33.82	33.80
Curcuma domestica	101.30	28.00	27.64
Curcuma xanthorrhiza	100.30	17.50	17.45
Sonchus arvensis	100.70	17.90	17.78
Phyllanthus niruri	66.90	25.10	37.50

The previous study also reported that the extract yield and the antioxidant activity of herbal extract produced by the reflux method were higher among other methods, regardless of the extraction solvent (Sultana et al. 2009). The extraction efficiency is influenced by various factors such as temperature, solvent type, duration of extraction and solid to liquid ratio, of which the solvent greatly affects the extraction yield and chemical compound of the extracts (Choung et al. 2014). Ethanol was selected since it has a hydroxyl group that is polar and an alkyl group that is nonpolar in its structure, therefore ethanol can extract compounds with various polarities and is easily evaporated. The 80% concentration of ethanol in water is an ideal solution as it was also reported that the aqueous solvent provides better yield than the absolute organic solvent (Sultana et al. 2009; Choung et al. 2014).

Phenolic and flavonoid compounds are generally found in all parts of the plant. This compound is a group of secondary metabolites consists of a large group of polyphenols that can scavenge free radicals and inhibit lipid oxidation (Kamtekar et al. 2014). Therefore, the measurement of phenol and flavonoid contents is important to predict the antioxidant power of each extract. The differences in total phenol and flavonoids content of each plant (Table 2) can be caused by several factors, such as geographical origin, plant maturity, environmental factors (temperature, ultraviolet light, CO_2 levels in the atmosphere), and the solvents used in the extraction

process. As part of phenolic compounds, total flavonoid content should be lower than the total phenolic content. However, some of the results in this study expressed otherwise. Typical phenolic compounds that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. It seems that the total phenolic content measured by the Folin–Ciocalteu procedure does not give a full picture of the quantity of the phenol constituents in the plant extracts, as Folin-Ciocalteu reagent and its gallic acid standard will only determine the phenolic acids. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables (Katsube et al. 2004; Wu et al. 2004).

As the first step of antioxidant activity screening, seven herbal extracts were determined with a free radical scavenging method against stable DPPH (1,1-diphenyl-2-picrylhydrazyl). DPPH is the most commonly used free radical in the antioxidant screening assay. It has an unpaired electron from the nitrogen atom. When a compound or material with the capability to donate hydrogen is reacted with the DPPH, it will transform DPPH into DPPH-H (1,1-diphenyl-2-picrylhydrazyl). According to (Molyneux 2004), a compound is classified as very strong when the IC_{50} value is <50 ppm, strong when the IC_{50} value is 50-100 ppm, moderate when the IC_{50} value is 101-150 ppm, and weak antioxidants when the IC_{50} value is >150 ppm. As shown in Table 3, *P. niruri* and *O. stamineus* extract showed moderate antioxidant activity according to the aforementioned categories.

Table 3. Antioxidant activity of seven herbal extracts determined by DPPH method

Herbal extract	IC_{50} (ppm)
Orthosiphon stamineus	132 ± 6.6
Centella asiatica	ND
Apium graveolens	2221 ± 61.8
Curcuma domestica	361 ± 9.7
Curcuma xanthorriza	538 ± 12.8
Sonchus arvensis	1118 ± 7.1
Phyllanthus niruri	102 ± 1.7
Vitamin C*	31 ± 1.6

Note: * standard compound; ND: not detected; Results are presented as mean ± SD of IC_{50} (inhibitory concentration 50, n = 4)

Table 4. Antioxidant activity of seven 7 herbal extracts determined by ABTS method

Herbal extract	IC_{50} (ppm)
Orthosiphon stamineus	22 ± 2.99
Centella asiatica	1199 ± 4.41
Apium graveolens	169 ± 1.51
Curcuma domestica	100 ± 3.02
Curcuma xanthorriza	82 ± 3.06
Sonchus arvensis	143 ± 1.85
Phyllanthus niruri	20 ± 3.59
Vitamin C*	9 ± 1.90

Note: * standard compound; Results are presented as mean ± SD of IC_{50} (inhibitory concentration 50, n = 4)

Table 5. Antioxidant activity of seven herbal extracts determined by FRAP method

Herbal extract	Concentration (ppm)	Antioxidant activity (ppm AEAC/ ppm extract)
Orthosiphon stamineus	50	6.92±0.34
	100	10.40±0.77
	150	14.38±0.39
	200	16.49±1.03
	250	18.39±0.89
Centella asiatica	200	1.77±0.09
	400	2.42±0.15
	600	2.86±0.13
	800	3.59±0.14
	1000	3.84±0.27
Apium graveolens	200	5.39±0.06
	400	8.36±0.28
	600	8.81±0.29
	800	10.26±0.50
Curcuma domestica	200	18.15±0.60
	400	28.57±1.37
	600	33.88±1.28
	800	47.29±1.19
	1000	50.74±0.68
Curcuma xanthorrhiza	200	5.42±0.16
	400	10.33±0.28
	600	11.99±0.71
	800	14.71±0.53
	1000	16.89±0.46
Sonchus arvensis	200	27.88±0.31
	400	47.63±0.89
	600	54.61±0.36
	800	61.02±1.32
	1000	63.68±0.63
Phyllanthus niruri	20	17.41±0.83
	40	27.50±2.07
	60	42.25±0.18
	80	52.25±2.78
	100	60.10±1.50

Note: AEAC: Ascorbic acid equivalent antioxidant capacity; Results are presented as mean ± SD (n = 4)

The determination of antioxidant activity by the ABTS method was obtained from the oxidation of potassium persulfate with ABTS salt. The principle of the ABTS method is the ability of antioxidant compounds to stabilize free radical compounds by donating proton radicals; it can be seen from the decrease in blue to no color. ABTS testing can be performed for lipophilic or hydrophilic compounds, with observations at a wavelength of 734 nm. According to the results from the ABTS method shown in Table 4, *P. niruri* and *O. stamineus* extract showed strong antioxidant activity.

The determination of antioxidant activity by the FRAP method has several advantages such as fast, easy and simple. This method involves the reduction reaction of the Fe^{3+} complex from tripyridyl-triazine $Fe^{3+\,(TPTZ)}$ to the Fe^{2+} complex, $Fe^{2+\,(TPTZ)}$ which is characterized by a change in color to blue. In the FRAP reagent, there is a mixture of TPTZ, $FeCl_3$ and acetate buffer, so it can be said that the FRAP reagent is a colorless compound of Fe^{3+}-TPTZ. The addition of $FeCl_3$ is used to form Fe^{3+} complex compounds. This method uses pH 3.6 to facilitate the reduction process

(Thaipong et al. 2006). The absorbance value obtained was measured using a microplate reader with a wavelength of 593 nm. Both *P. niruri* and *O. stamineus* extract showed strong antioxidant activity categories according to the FRAP method (Table 5). Consistent results of potential moderate into strong antioxidant activity was shown by *P. niruri* and *O. stamineus* ethanolic extract by DPPH, ABTS, and FRAP method.

In conclusion, based on the results obtained from the determination of antioxidant activity using the DPPH, ABTS, and FRAP method, it can be concluded that the ethanolic extract of *P. niruri* and *O. stamineus* provided the highest antioxidant activity among seven Indonesian herbs involved in this study. Further research is needed to purify and identify the specific compounds that possess antioxidant properties of *P. niruri* and *O. stamineus*, to be applied for adjuvant therapy.

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