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Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics

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ABSTRACT

Liver disease has become one of the major health problems in the world, and the death rate is going rapidly to increase. Oxidative stress plays a crucial role in the emerging, development, and the progression of liver diseases. Ethnobotanical research has an undoubted profound impact on the development of numerous new drugs. The aim of this research, therefore, was to examine the antioxidant activities of 14 selected plants used for treating liver diseases by traditional healers of Indonesia ethnicities and to classify these plants using chemometrics of principal component analysis (PCA). The extraction using methanol as the solvent was performed with two stages maceration. Total phenolic and flavonoid compounds were determined by Folin–Ciocalteau and $AlCl_3$ method, respectively, whereas antioxidant activity was estimated using 2,2′-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP) assay. Among 19 methanol extracts of 14 plants, the leaves of *Baccaurea racemosa*, *Macaranga subpeltata*, and *Piper* sp. showed the highest antioxidant properties. The phenolic content correlated with TEAC, FRAP, and DPPH radical scavenging activity, while flavonoid did not significantly affect these antioxidant activity methods. PCA successfully classified the plant samples using the variables of antioxidant activities and phenolic-flavonoid contents. The selected plants have promising antioxidant properties which support their utilization for either liver diseases medication or oxidative stress-related diseases prevention.

INTRODUCTION

Liver diseases become one of the major world health problems, and the death rate caused by this disease is rising rapidly in the US over the last decade (Reinberg, 2018). There are various liver diseases, including metabolic disorder (Wilson's disease and hemochromatosis), infection (virus, bacteria, fungi, and amoeba), liver cirrhosis, liver cancer, autoimmune, and xenobiotic substances (Anand and Lal, 2016). A comprehensive data about the amount of each kind of liver diseases in Indonesia is still limited except for hepatitis which its prevalence increases two times during 5 years (0.2% in 2013 and 0.4% in 2018) (NiHRD RI, 2019).

Regardless of the liver diseases causal, inflammation is considered as the main causes of progression of the disease chronicity (Marcellin and Kutala, 2018). Oxidants affect all stages of the inflammatory; moreover, the undoubted evidences emphasize that oxidative stress plays a crucial role in the development of inflammation (Lugrin *et al*., 2014). Stress oxidative induced by various factors contributes to the initiation and progression of liver diseases. A condition in which this disturbance continues to occur resulting in severe liver diseases. Extra-hepatic organs damages, such as brain impairment and kidney failure, are among other

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consequences of systemic oxidative stress arising during liver diseases (Li *et al*., 2015).

The external exposures (environmental pollutants/toxicant and irradiation), the aging process, pathological incidents, and other factors are unavoidable events, leading to oxidative stress (Pandey and Rizvi, 2012). Therefore, maintaining a balanced state of oxidant and antioxidant to prevent oxidative stress is a crucial part of reasonable health maintenance (Sochor *et al*., 2010). Plants produce various phytochemicals which have a significant source of exogenous antioxidant as dietary intake (Chen *et al*., 2016), flavonoids, and phenolic compound are a reputable plant-derived antioxidant (Khoddami *et al*., 2013). Ethnobotanical/ethnopharmacological research has valuable roles in the pharmacological studies (McClatchey *et al*., 2009), even in discovering novel drugs (de Albuquerque, 2010; Ntie-Kang *et al*., 2013).

National Institute Health Research and Development Republic of Indonesia (NiHRD RI) has compiled enormous valuable metadata via "The Community Based Exploration of Local Knowledge of Ethno-medicine and Medicinal Plant in Indonesia" known as RISTOJA held in 2012, 2015, and 2017 (Widodo *et al*., 2019). According to that research, there were about 381 taxonomically identified species of plants had been used for liver disease medication by Indonesian ethnic traditional healer (IETH). Among these, three most frequently used plants for medication, this disease, were *Bambusa vulgaris* Schrad, *Curcuma longa* L., and *Curcuma zanthorrhiza* Roxb which taken apart as the main ingredient of 52, 42, and 41 potions by 42, 34, and 33 IETHs throughout Indonesia, respectively. Those plants are well-studied and known their reputable antioxidant properties. This present study, therefore, was intended to evaluate antioxidant activities of some selected medicinal plants used for liver diseases medication by IETHs from the metadata and its correlation with phenolics and flavonoid contents.

MATERIAL AND METHODS

Materials

Plant samples were collected from the Province of Aceh [*Piper* sp., *Poikilospermum erectum* (Blanco) Merr.], Central Java [*Baccaurea racemosa* (Reinw. Ex Blume) Müll.Arg., *Chromolaena odorata* (L.) R.M.King & H.Rob.., *Litsea glutinosa* (Lour.) C.B.Rob., *Nenga pumila* (Blume) H.Wendl., *Paspalum conjugatum* P.J.Bergius., *Scaevola taccada* (Gaertn.), *Ipomoea pes-caprae* (L.) R. Br.], Yogyakarta [*Intsia bijuga* (Colebr.) Kuntze, *Peronema canescens* Jack], and East Java (*Stellaria vestita* Kurz, *Macaranga subpeltata* K.Schum. & Lauterb, *Capparis sepiaria* L.). These plants were authenticated in MPTMRDC—NiHRD RI. Methanol, Folin-Ciocalteau's phenol reagent (FCR), Acetic acid, $AlCl_3$, $NaCO_3$, $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, L-(+)-ascorbic acid were obtained from E. Merck (Darmstadt, Germany). 2,2′-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Trolox, Gallic acid, Quercetin, and Rutin were purchased from Sigma (Aldrich, USA). All materials used were analytical grade.

Preparation of methanolic extract

Preparation of methanolic extract was performed according to Mistriyani *et al*. (2018). Sample plants were washed, drained, oven-dried (40°C), grounded, and sieved (60 mesh). Finely powdered plant materials were macerated using methanol (1:10 w/v) as extracting solvent. The samples were mixed with 60% volume of solvent for 3 days (stirred every 6 hours), the solids were filtered and re-macerated using 40% volume of solvent for 3 days. Extracts were concentrated using vacuum rotary evaporator (Buchi) and then oven-dried (40°C). The methanolic extracts were then subjected to evaluation of antioxidant activities, determination of phenolics content and flavonoid contents.

Quantification of total phenolic contents

Total phenolic contents (TPC) of methanol extracts of plant samples was measured using calibrated UV-Vis spectrophotometer (Multiskan Sky—Thermo Scientific, USA) as described by Cicco *et al*. (2009) with slight modifications. The final condition of the test solution: 40 mg extract, 4% methanol, 10% FCR, and 5% $CaCO_3$. A 40 µl of plant extract (1 mg/ml; 1 mg dissolved in 1 ml methanol) was mixed with 360 µl of distilled water and 100 µl of FCR, and the solution was shaken and left for 2 minutes. The reaction was neutralized using 500 µl of 10% $CaCO_3$ and mixed until homogeneous. The mixture was incubated for 20 minutes at 40°C. A 150-µl test solution was included in the microplate, and the absorbance was measured at wavelength 732 nm. The TPC is expressed as mg gallic acid equivalent/g of the extract through linear regression prepared from gallic acid standards at various concentrations (0, 5, 10, 15, 20, and 25 µg/ml).

Quantification of total flavonoid contents

Total flavonoid content (TFC) was measured spectrophotometrically according to Li *et al*. (2013) with slight modification. The reaction mixture consisted of 100 µl extract [1,250 µg/ml (w/v)], 150 µl solution of 0.1-M $AlCl_3$ (blank without $AlCl_3$ and replaced with methanol 150 µl), 350-µl ultra-pure dH_2O, 250-µl acetate buffer (pH 3.8), and added with methanol up to a total volume of 1,250 µl. The test solution was incubated at 35°C for 30 minutes. TFC is expressed as rutin equivalents per g extract (mg RE/g) via generating a standard curve with a series concentrations of 0–100 µg/ml of rutin (*x*-axis) against absorbance (*y*-axis). A 150 µl of solution was pipetted into the microplate, and the absorbance was measured with a UV-Vis spectrophotometer at wavelength 398 nm.

DPPH radical scavenging activity

2,2′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the sample extracts was determined according to Sharma and Bhat (2009) with a slight modification. The reaction was prepared to 1.0 ml total volume with final concentrations of methanol extract (0–100 µg/ml) and DPPH (70 µM). Absorbance at 515 nm was measured after 25–60 minutes (based on prior determined operating time). The percentage inhibition activity was calculated using $[(A_0 - A_1)/A_0] \times 100$, where A_0: the absorbance of the control and A_1: the absorbance of the extract or standard. The inhibition curves were generated and IC_{50} values were calculated. L-(+)-ascorbic acid (Vitamin C) with final concentration 0–5 µg/ml was used as the standard antioxidant.

Trolox equivalent antioxidant capacity assay

The assay was performed according to Dong *et al*. (2015) as follows: a fresh ATBS·+ working solution was made

by dissolving 38.4 mg of ABTS in 10 ml 2.5 mM $K_2S_2O_8$, mixed completely and kept in the dark (room temperature; 12–16 hours). The solution was then diluted with methanol to obtain 0.70 ± 0.02 absorbance. A standard curve was generated by making a series with concentrations of 0–45 mM of Trolox. Ten mg plant extracts were dissolved in 1 ml methanol, sonicated (15 minutes), and diluted to get 100 µg/ml.

Ferric reducing antioxidant power assay

The reducing ability of the plant extracts was estimated using the ferric reducing antioxidant power (FRAP) assay according to Benzie and Strain (1996) with minor modifications. The FRAP reagent was produced by mixing acetate buffer (300 mM; pH 3.6), 10 mM TPTZ solution in 40-mM HCl, and 20-mM $FeCl_3 \cdot 6H_2O$ solution in a volume ratio of 10:1:1 prepared by daily basis preparation. $FeSO_4 \cdot 7H_2O$ was used to generate a standard curve using final concentration 100–1,000 µM/ml. The reaction mixture in a microtube 2 ml contained 1,350 µl of FRAP reagent and 150 µl methanolic extract sample or standard antioxidants [Quercetin, L-(+)-ascorbic acid] solution. The tube was then incubated for 30 minutes at 37°C, and the absorbance was recorded at 595 nm. The FRAP value was calculated as M Fe^{2+}/g of sample using ferric chloride standard curve.

Data analysis

Antioxidant assays using 2,2′-diphenyl-1-picrylhydrazyl (DDPH) scavenging activity, trolox equivalent antioxidant capacity (TEAC), and FRAP, as well as the determination of phenolics and flavonoid contents were performed in triplicates. All data were expressed as mean ± standard deviation and processed using Excel (Microsoft Inc., USA). The chemometrics of principal component analysis (PCA) was performed using Minitab version 17 (Minitab Inc., USA).

RESULTS AND DISCUSSION

Oxidative stress is considered as a causative factor of the emergence and development of degenerative disorders. It represents a physiological imbalance of the homeostatic system, which may be due to either an abnormal elevation of reactive oxygen species (ROS) or deficiencies in antioxidant defense systems. The liver is the most exposed organ of ROS, implies that oxidative stress often initiates liver damage; therefore antioxidant has a crucial role in preventing the onset of liver disease. Ethnomedicinal research allows obtaining many plants potential to be explored as antioxidants. Many plants used for treating liver disease were reported to have antioxidant activities (Widodo *et al*., 2009); therefore, it is necessary to confirm the antioxidant activities of selected medicinal plants *in vitro* using reliable methods such as radical scavenging activity and reducing power.

DDPH free radical scavenging is one of the accurate and the most frequently employed assay for evaluating antioxidant activity. The test was based on the bleaching of the violet solution of a stable free radical DPPH in methanol into pale yellow (Nur Alam *et al*., 2012). DPPH free radical in methanol has the maximum absorbance at 515 nm. The higher the radical-scavenging activity of the extract, the lower the absorbance value at 515 nm. The kinetic reaction between samples analyzed with DPPH free radical was monitored as a function of time to determine the operating time. Figure 1 revealed the relationship between absorbance changes (*y*-axis) as a function of reaction time, revealing that operating time was in about 30–90 minutes depending on plant samples, as indicated that the absorbance value was stable at these operating times (Table 1). DPPH radical scavenging activity was expressed by inhibitory concentration

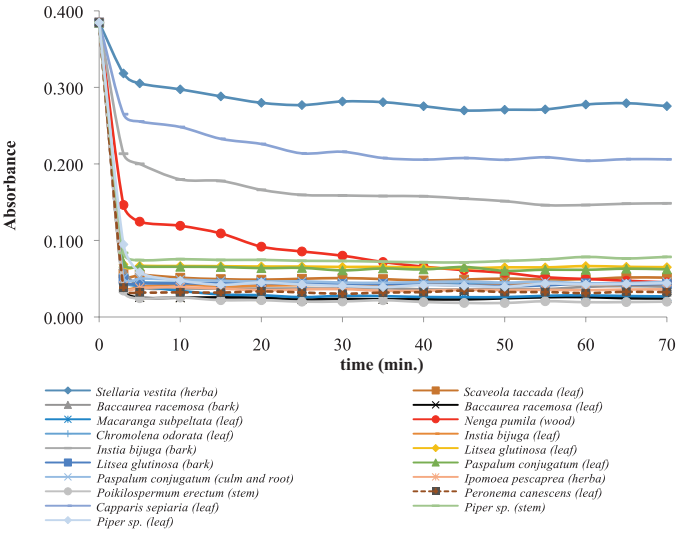

Figure 1. Time course of scavenging of DPPH free radical by methanolic extract of the plant samples. The reaction mixture contained DPPH (70 mM) and methanolic plant extracts (100 µg/ml). The absorbance was measured at 515 nm.

Table 1. IC_{50} value of DDPH radical scavenging of methanolic crude extract of medicinal plants used for liver diseases medication along with reference sample [vitamin C or L-(+)-ascorbic acid].

Plants	Part of plant	Operating time (minute)	IC_{50} ± SD (µg/ml)
Stellaria vestita	Herba	40	115.128 ± 2.931
Baccaurea racemosa	Leaf	40	4.298 ± 0.306
Baccaurea racemosa	Bark	40	10.627 ± 0.996
Ipomoea pes-caprae	Herba	35	12.932 ± 0.489
Scaveola taccada	Leaf	60	23.718 ± 1.223
Nenga pumila	Stem	90	26.690 ± 1.991
Chromolaena odorata	Leaf	30	13.782 ± 0.321
Instia bijuga	Leaf	35	8.484 ± 0.242
Instia bijuga	Bark	35	71.345 ± 2.730
Litsea glutinosa	Leaf	30	14.006 ± 0.679
Litsea glutinosa	Bark	45	15.298 ± 0.777
Paspalum conjugatum	Leaf	30	9.414 ± 0.858
Paspalum conjugatum	Culm and root	35	10.482 ± 0.465
Macaranga subpeltata	Leaf	30	4.139 ± 0.150
Poikilospermum erectum	Stem	60	4.860 ± 0.380
Peronema canescens	Leaf	35	9.389 ± 0.679
Capparis sepiaria	Leaf	35	39.393 ± 2.942
Piper sp.	Leaf	15	5.728 ± 0.461
Piper sp.	Stem	60	17.341 ± 0.977
L-(+)-ascorbic acid	Reference sample	30	2.097 ± 0.078

(IC_{50}), defined as the concentration of plant sample extracts necessary to scavenge 50% of DPPH radical, calculated by an equation generated from linear regression. Among plant samples evaluated, *S. vestita* demonstrated the lowest scavenging capacity against DPPH, as showed by its highest IC_{50} value. This plant is endemic to the high mountainous area of Semeru and is only used by a the IETH of Tengger ethnic in the East Jawa. In addition, the highest DPPH antiradical activity was found in the methanolic extract of *M. subpeltata*, as indicated by the lowest IC_{50} value. In general, the IC_{50} of leaves have lower values than those of barks of the studied plants (Table 1). The IC_{50} values of samples were higher than those of positive control; therefore, fractionation of extract was suggested to get fraction with more active antiradicals.

TEAC method, also known as ABTS [2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay, was based on the measurement of color loss of ABTS·+ having blue-green color due to the presence of an antioxidant. Plant samples containing antioxidant compounds would reduce ABTS·+ to ABTS and decolorize it at wavelength 743 nm. Therefore, the decrease in absorbance of ABTS·+ could be used as an indication of antioxidant activity using this method. The standard calibration curve is constructed using Trolox, as a consequence, the antioxidant activity was expressed as TEAC values (in mM), calculated from a standard curve of Trolox (Seeram *et al*., 2006). Table 2 compiled TEAC values of 14 plant samples. The higher the TEAC value, the more active the plant sample as an antioxidant. Among these, the methanolic extract of *B. racemosa* leaves revealed the highest antioxidant activities using TEAC method, i.e., 354.88 ± 0.55 Trolox Equivalent (µM TE/100 µg). This result was in agreement with that of DPPH radical scavenging activities.

FRAP method was based on the capability of antioxidants to reduce the absorption of complex of Fe^{3+} ions and TPTZ due to the formation of Fe^{2+} ions at low pH at 595 nm (Benzie and Strain, 1996). FRAP value was obtained by comparing the changes of absorbance values in the sample mixture with those obtained from the increased concentrations of Fe^{3+}. FRAP values were expressed as M of Fe2+ equivalents per g sample. Table 3 compiled FRAP values of plant samples in which the methanolic extracts of leaves of *B. racemosa* and *Piper* sp. showed the highest FRAP values, namely, 900.18 ± 15.41 and 899.17 ± 17.41, respectively. The FRAP assay is simple, quick, and reproducible to evaluate antioxidant capacity, in addition, it has a direct relation with phenolics content (Parameswari and Suriyavathana, 2012).

From 19 methanolic extracts of 14 plant samples, the leaves of *B. racemose*, *M. subpeltata*, and *Piper* sp. showed the highest antioxidant properties, as evaluated by DPPH radical scavenging assay, FRAP, and TEAC. Therefore, these methanolic extracts of three plants extracts will be subjected to further fractionation and *in vivo* antioxidant assay.

Classification of the plant samples using principal component analysis

Classification of the methanolic extracts of leaf, bark, and herb of plant samples was carried out using chemometrics of PCA, one of the unsupervised pattern recognition techniques (Che Man *et al*., 2011). The variables used were IC_{50} values of DPPH radical scavenging activity, TEAC, FRAP value, TPCs, and TFCs. Figure 2 revealed the score plot of PCA of 19 methanolic

Table 2. TEAC of methanolic crude extract of medicinal plants used for liver diseases medication.

Plants	Part of plant	TEAC ± SD (µM TE/100 µg)
Stellaria vestita	Herba	16.31 ± 0.27
Baccaurea racemosa	Leaf	354.88 ± 0.55
Baccaurea racemosa	Bark	135.52 ± 0.26
Ipomoea pes-caprae	Herba	76.14 ± 0.14
Scaveola taccada	Leaf	44.19 ± 0.14
Nenga pumila	Stem	26.16 ± 0.34
Chromolaena odorata	Leaf	142.79 ± 3.15
Instia bijuga	Leaf	148.23 ± 0.05
Instia bijuga	Bark	188.02 ± 0.25
Litsea glutinosa	Leaf	122.09 ± 0.05
Litsea glutinosa	Bark	165.21 ± 0.32
Paspalum conjugatum	Leaf	145.03 ± 0.29
Paspalum conjugatum	Culm and root	128.09 ± 0.50
Macaranga subpeltata	Leaf	297.59 ± 0.94
Poikilospermum erectum	Stem	310.23 ± 5.02
Peronema canescens	Leaf	120.28 ± 0.12
Capparis sepiaria	Leaf	53.71 ± 0.03
Piper sp.	Leaf	271.27 ± 1.59
Piper sp.	Stem	133.04 ± 0.89

Table 3. FRAP of methanolic crude extract of medicinal plants used for liver diseases medication along with reference sample [L-(+)-ascorbic acid].

Plants	Part of plant	FRAP value ± SD (mM Fe^{2+}/10 mg)
Stellaria vestita	Herba	50.32 ± 0.51
Baccaurea racemosa	Leaf	900.18 ± 15.41
Baccaurea racemosa	Bark	325.79 ± 18.85
Ipomoea pes-caprae	Herb	355.52 ± 5.42
Scaveola taccada	Leaf	44.19 ± 0.14
Nenga pumila	Stem	207.46 ± 2.39
Chromolaena odorata	Leaf	394.75 ± 7.14
Instia bijuga	Leaf	338.31 ± 1.27
Instia bijuga	Bark	368.19 ± 21.38
Litsea glutinosa	Leaf	282.15 ± 11.66
Litsea glutinosa	Bark	410.48 ± 12.01
Paspalum conjugatum	Leaf	385.13 ± 24.74
Paspalum conjugatum	Culm and root	389.69 ± 4.64
Macaranga subpeltata	Leaf	601.46 ± 16.90
Poikilospermum erectum	Stem	613.75 ± 7.75
Peronema canescens	Leaf	266.33 ± 20.87
Capparis sepiaria	Leaf	66.82 ± 0.54
Piper sp.	Leaf	899.17 ± 17.41
Piper sp.	Stem	315.73 ± 7.81
L-(+)-ascorbic acid	Reference samples	2,031.88 ± 51.74
Quercetin	Reference sample	3,185.94 ± 42.55

extracts of plant samples (leaf, bark, and herba), as shown in Table 1 for samples identification. Score plot of PCA was used for the classification among plant samples and it represented the sample projections expressed by the first principle component (PC1) which accounting the most variation in variables and second principle component (PC2) accounting the second largest variation of data variables. The eigenvalue indicated that PC1, PC2, and PC3 contributed to variable variations of 64.5%, 22.0%, and 11.1%, respectively, therefore, 97.6% of data variations could be described by three PCs. Based on the score plot, the plant samples could be classified into five groups, as clustered in Figure 2. The closer the score plot value, the closer of variables, therefore, the methanolic extract of leaves (code sample = 13) was closer in terms of antioxidant activities, TPCs, and TFCs with methanolic extract of culm and root of *P. conjugatum* (code sample = 12) and methanolic extract of *C. odorata* leaf (code sample = 7) (Yang *et al*., 2015).

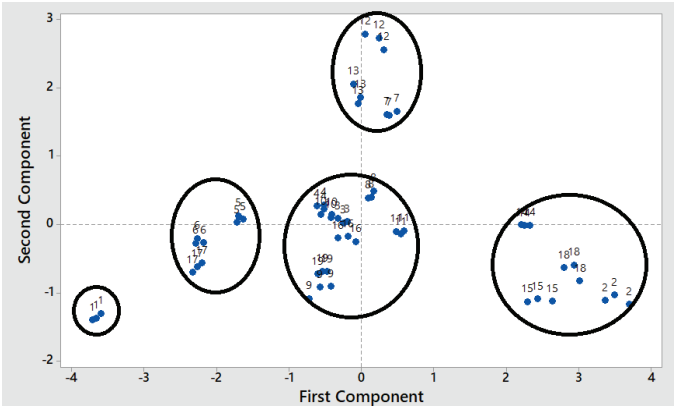

Figure 2. The PCA score plot expressed by first components (PC1) and second component (PC2) of 19 methanolic extract samples of: 1 = *Stellaria vestita* (herba); 2 = *Baccaurea racemosa* (leaf); 3 = *Baccaurea racemosa* (bark); 4 = *Ipomoea pes-caprae* (herba); 5 = *Scaveola taccada* (leaf); 6 = *Nenga pumila* (stem); 7 = *Chromolaena odorata* (leaf); 8 = *Instia bijuga* (leaf); 9 = *Instia bijuga* (bark); 10 = *Litsea glutinosa* (leaf); 11 = *Litsea glutinosa* (bark); 12 = *Paspalum conjugatum* (leaf); 13 = *Paspalum conjugatum* (culm and root); 14 = *Macaranga subpeltata* (leaf); 15 = *Poikilospermum erectum* (stem); 16 = *Peronema canescens* (leaf); 17 = *Capparis sepiaria* (leaf); 18 = *Piper* sp. (leaf); 19 = *Piper* sp. (stem).

In order to evaluate the correlation among variables, loading plot could be used (Fig. 3). Loading plot described how the vectors are pinned from the origin of PC1 = 0 and PC2 = 0. Loading plot was also understood as the weight of each variable to PCs. If two vectors are close, forming a small angle, the two variables are positively correlated. If among variables form an angle about 90°, they are not likely to be correlated, and when two variables are diverge and form a large angle (about 180°), they indicated a negative correlation. Phenolic compounds correlated with FRAP and TEAC positively and correlated negatively with the IC_{50} value of DPPH. The negative correlation between phenolics and IC_{50} indicated the positive correlation between phenolics content and antiradical scavenging activities. This indicated that phenolics compound affected significantly toward all antioxidant activities. In addition, flavonoid compounds do not contribute significantly toward antioxidant activities. Table 4 compiled the Pearson correlation as indicated with the coefficient of correlation (r) values among IC_{50} of DPPH radical scavenging activity, TEAC, FRAP, and TFCs. The higher the r-value, the higher the correlation of variables. Among each variable, the correlation between TEAC and TPC exhibited the highest r-value (0.945). These results were similar to those reported by Katalinic *et al*. (2006) and Thaipong et al. (2006). Figure 4 exhibited the scatter plot for the correlation

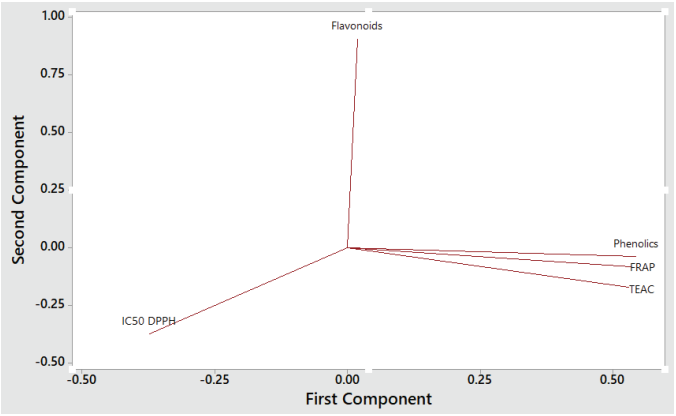

Figure 3. The loading plot of PCA using variables of IC_{50} values of DPPH radical scavenging activity, TEAC, FRAP value, TPCs and TFCs.

Table 4. Pearson correlation among IC_{50} of DPPH radical scavenging activity, TEAC, FRAP, TPCs and TFCs.

Correlation among variables		Coefficient of correlation (r-value)			
		IC_{50} DPPH	TEAC	FRAP	TPC
TEAC	Pearson Correlation	−0.463**			
	Sig. (two-tailed)	(0.000)			
FRAP	Pearson Correlation	−0.516**	0.923**		
	Sig. (two-tailed)	(0.000)	(0.000)		
TPC	Pearson Correlation	−0.562**	0.945**	0.933**	
	Sig. (two-tailed)	(0.000)	(0.000)	(0.000)	
TFC	Pearson Correlation	−0.202	−0.086	−0.006	0.029
	Sig. (two-tailed)	(0.132)	(0.527)	(0.965)	(0.833)

**Correlation is significant at the 0.01 level (two-tailed). The numbers in parentheses are p-values at significant level of 0.01.

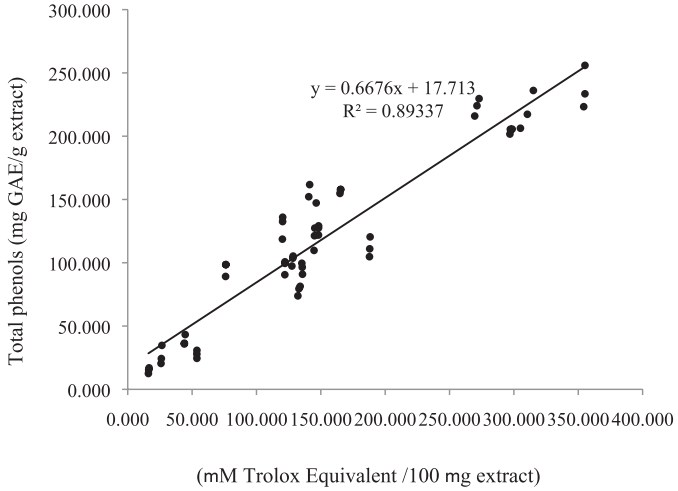

Figure 4. The scatter plot for the correlation between TEAC (*x*-axis) and TPC (*y*-axis).

between TEAC (*x*-axis) and TPC (*y*-axis) with the coefficient of determination (R^2) of 0.8934. This indicated that phenolics contents contributed to 89.34% of TEAC activities of 19 methanolic extracts of plant samples.

CONCLUSION

Among plant samples, the methanolic leaves extracts of *B. racemosa*, *M. subpeltata*, and *Piper* sp. showed the highest antioxidant properties as evaluated by DPPH radical scavenging activity, TEAC, and FRAP. The phenolics contents correlated with antioxidant activities. PCA successfully classified the plant samples according to antioxidant activities phenolic contents, and flavonoid contents. These three plants have promising antioxidant properties which support their utilization to prevent oxidative stress-related diseases including liver disease. A study on chemical compounds and their activity in a biological system is still needed to provide basic data on their use in traditional medicine.

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CONFLICT OF INTERESTS

Author declares that there are no conflicts of interest.

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None.

REFERENCES

Anand K, Lal UR. Hepatitis and medicinal plant: an review. J Pharmacogn Phytochem, 2016; 5(6):408–15.

Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem, 1996; 239(1):70–6.

Che Man YB, Rohman A, Mansor TST. Differentiation of lard from other edible fats and oils by means of Fourier transform infrared spectroscopy and chemometrics. J Am Oil Chem Soc, 2011; 88:187–92.

Chen W, Jia Z, Pan M-H, Babu PVA. Editorial: natural products for the prevention of oxidative stress-related diseases: mechanisms and strategies. Oxid Med Cell Longev, 2016; 4628502.

Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio VA. Reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining Phenolics of plant methanol extracts. Microchem J, 2009; 91:107–10.

de Albuquerque UP. Implications of ethnobotanical studies on bioprospecting strategies of new drugs in semi-arid regions. Open Complement Med J, 2010; 2(2):21–3.

Dong JW, Cai L, Xing Y, Yu J, Ding ZT. Re-evaluation of ABTS•+ assay for total antioxidant capacity of natural products. Nat Prod Commun, 2015; 10(12):2169–72.

Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem, 2006; 94:550–7.

Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules, 2013; 18:2328–75.

Li G, Yu S, Zhou Y-H, Chen Q-F. Spectrophotometric determination of flavonoids content in leaves of *Fagopyrum cymosum* complex. Asian J Chem, 2013; 25(13):7575–8.

Li S, Tan H-Y, Wan N, Zhang Z-J, Lao L, Wong C-W, Feng Y. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci, 2015; 16:26087–124.

Lugrin J, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. Biol Chem, 2014; 395(2):203–30.

Marcellin P, Kutala BK. Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. Liver Int, 2018; 38(1):2–6.

McClatchey WC, Mahady GB, Bennett BC, Shiels L, Savo V. Ethnobotany as a pharmacological research tool and recent developments in CNS-active natural products from ethnobotanical sources. Pharmacol Ther, 2009; 123(2):239–54.

Mistriyani, Riyanto S, Rohman A. Antioxidant activities of Rambutan (*Nephelium lappaceum* L) peel *in vitro*. Food Res, 2018; 2(1):119–23.

National Institute of Health Research and Development (NiHRD) of Republic of Indonesia. Laporan Nasional Riset Kesehatan Dasar 2018. Badan Penelitian dan Pengembangan Kesehatan, 2019 (In Press).

Ntie-Kang F, Lifongo LL, Mbaze LM, Ekwelle N, Owono LCO, Megnassan E, Judson PN, Sippl W, Efange SM. Cameroonian medicinal plants: a bioactivity versus ethnobotanical survey and chemotaxonomic classification. BMC Complement Altern Med, 2013; 13:147.

Nur Alam Md, Bristi NJ, Rafiquzzaman Md. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J, 2013; 21:143–52.

Pandey KB, Rizvi SI. Ferric reducing and radical scavenging activities of selected important polyphenols present in foods. Int J Food Prop, 2012; 15(3):702–8.

Parameswari G, Suriyavathana M. *In-vitro* antioxidant of *Chromolaena odorata* (L.) King & Robinson. IRJP, 2012; 2(3):187–92.

Reinberg S. U.S. deaths from liver disease rising rapidly. 19 July 2018 (HealthDay News). Availabel via https://www.webmd.com/digestive-disorders/news/20180719/us-deaths-from-liver-disease-rising-rapidly#1 (Accessed 25 January 2019).

Seeram NP, Henning SM, Lee R, Niu Y, Scheuller HS, Heber D. Catechin and caffeine contents of green tea dietary supplements and correlation with antioxidant activity. J Agric Food Chem, 2006; 54:1599–603.

Sharma OP, Bhat TK. DPPH antioxidant assay revisited. Food Chem, 2009; 113:1202–5.

Sochor J, Ryvolova M, Krystofova O, Salas P, Hubalek J, Adam V, Trnkova L, Havel L, Beklova M, Zehnale J, Provaznik I, Kizek R. Fully automated spectrometric protocols for determination of antioxidant activity: advantages and disadvantages. Molecules, 2010; 15(12):8618–40.

Thaipong K, Booprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal, 2006; 19:669–75.

Widodo H, Rohman A, Sismindari. Pemanfaatan Tumbuhan Famili *Fabaceae* Untuk Pengobatan Penyakit Liver Oleh Pengobat Tradisional Berbagai Etnis Di Indonesia. Media Penelitian dan Pengembangan Kesehatan, 2019; 29(1):65–88.

Yang M, Shen Q, Li L-Q, Huang Y-Q, Cheung H-Y. Phytochemical profiles, antioxidant activities of functional herb *Abrus cantoniensis* and *Abrus mollis*. Food Chem, 2015; 177:304–12.

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