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The principal component analysis of antioxidant activities of Songgak, a traditional herbal drink in West Nusa Tenggara, Indonesia

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Abstract

Songgak is a traditional herbal tea of the Sasak Tribe in Lombok Island, Indonesia, formulated to strengthen people's stamina. Songgak is composed of seven kinds of roasted spices: *Piper nigrum* L., *Coriandrum sativum* L., *Piper retrofractum* Vahl, *Myristica fragrans* Houtt, *Syzygium aromaticum*, *Elaeocarpus grandiflorus*, and *Helicteres isora*. This study aimed to assess the benefits of Songgak and its constituent spices by evaluating their antioxidant activities, total phenolic content (TPC) and flavonoid content (TPC). Antioxidant activity tests were performed on the water extract of Songgak, ethanolic extract of Songgak, and ethanolic extract of seven spices of Songgak including 2,2′-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, Trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP) assay. The antioxidant activity using DPPH method showed that the water extract of Songgak has a strong categorisation of antioxidant activity with DPPH IC_{50} value of 63.78±4.29 µg/mL. The constituent species of Songgak with the strongest antioxidant activity was *S. aromaticum*. Total phenolic content (TPC) and total flavonoid content (TFC) of Songgak tea were 26.85±4.29 mg GAE/g and 6.99±0.29 mg RE/gram, respectively. The correlation between TPC, TFC, and antioxidant activities was determined using principal component analysis (PCA). The loading plot showed that TPC and TFC were strongly correlated with the antioxidant activity (FRAP method) of Songgak tea and its constituent spices. These results revealed that the presence of flavonoid and phenolic compounds in the seven herbal medicinal plants were responsible for the antioxidant properties of Songgak as a healthy herbal drink. Therefore, consuming Songgak every day can be beneficial for preventing various diseases involving free radicals.

1. Introduction

Spices have different definitions of names depending on the region of origin, the composition, shape, condition of the material, and utilization of the spices. Spices can be flowers, leaves, seeds, stems, bark, and roots containing the main compounds of essential oils with various properties that people have believed for generations (Putri and Fibrianto, 2018). In some studies on degenerative diseases, the herb is reported to be used in cancer prevention, diabetes mellitus management, and the prevention and therapy of Alzheimer's disease (Kaefer and Milner, 2008; Mirmosayyeb *et al*., 2017; Seetaloo *et al*., 2019). However, more research is needed regarding the content of compounds and the efficacy of spices to determine appropriate intervention strategies to achieve maximum benefits from herbs and spices without causing adverse consequences (Kaefer and Milner, 2008).

In the community of Sasak tribe, Lombok-West Nusa Tenggara (NTB), spices are always needed in very diverse types. In addition to being used as a seasoning in cooking, spices are also made into a potion to increase stamina called "Songgak". Songgak is made from various types of spices that are roasted and ground into

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powder and consumed by brewing with warm water. The types of spices used to make Songgak were usually *sang* or pepper (*Piper nigrum* L.), coriander (*Coriandrum sativum* L.), *sebie gawah* or javanese chili (*Piper retrofractum* Vahl), *pale* or nutmeg pulp (*Myristica fragrans*), cloves (*Syzygium aromaticum*), *anyang* (*Elaeocarpus grandiflorus*) and *lilit* or woody fruit (*Helicteres isora*) that give the effect of a warm feeling on the body.

Sasak tribe on Lombok Island (Figure 1), Indonesia, who consumes Songgak, claimed that Songgak has properties to improve body fitness and lose weight. These effects can be caused by the various pharmacological effects of each spice used to make Songgak. These spices have antioxidant potential, hypolipidemia, and hypo cholesterol effects, antidiabetics, antibacterial, anticancer, antiobesity, and hepatoprotective (Jaiswal *et al*., 2009; Kim *et al*., 2011; Zarai *et al*., 2013; Cortés-Rojas *et al*., 2014; Kumar and Singh, 2014; Li *et al*., 2020). The antioxidant content in a herbal ingredient is essential to reduce free radicals that cause oxidative stress. Oxidative stress causes impaired biological functions, such as various degenerative diseases (Yuslianti, 2018).

Figure 1. Sasak tribe on Lombok Island.

All constituent spices of Songgak were reported to have antioxidant activity. Li *et al*. (2020) reported that the essential oil of white pepper (*Piper nigrum* L.) showed the highest antioxidant activity at a concentration of 10 mg/mL of 57.59% using the DPPH method. The main compounds in white pepper are piperine and piperic acid (Zarai *et al*., 2013). Coriander, nutmeg, *lilit*, and Javanese chilli were reported to have antioxidant activities, especially in protecting the body from attacks by reactive oxygen species (ROS) (Jaiswal *et al*., 2009; Kumar and Singh, 2014; Wei *et al*., 2019).

Songgak herbal tea can potentially be used as one of the therapies to prevent non-communicable diseases and degenerative diseases. Songgak is a drink made from seven spices, empirically considered a stamina-enhancing drink by the people of the Sasak tribe, Lombok-West Nusa Tenggara, Indonesia. Singh *et al*. (2017) reported that the antioxidant compound can enhance the human body's stamina and prevent various diseases. However, research on Songgak physicochemical properties and antioxidant activity has never been reported. Thus, as a basis for the development of Songgak in the prevention and therapy of diseases, the study aimed to analyze antioxidant activity using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method, Trolox equivalent antioxidant capacity (TEAC), and Ferric reducing antioxidant power (FRAP), total phenolic content and total flavonoids content. The principal component analysis was applied to describe the correlation of TPC and TFC in seven spices on the antioxidant activity of Songgak herbal tea.

2. Materials and methods

2.1 Samples and reagents

The sample in this study was Songgak, a herbal drink and seven spices of herb used by the Sasak tribe; *sang* or pepper (*Piper nigrum* L.), coriander (*Coriandrum sativum* L.), *sebie gawah* or Javanese chilli (*Piper retrofractum* Vahl), *pale* or nutmeg pulp (*Myristica fragrans*), cloves (*Syzygium aromaticum*), anyang (*Elaeocarpus grandiflorus*) and *lilit* or ules wood fruit (*Helicteres isora*) collected in Sukarara Village, Jonggat District, Central Lombok Regency, NTB. The reagents used were obtained from Merck (Darmstadt, Germany): ethanol, Folin-Ciocalteau phenol reagent (FCR), acetic acid, $AlCl_3$, $NaCO_3$, $FeCl_3.6H_2O$, $FeSO_4 \cdot 7H_2O$, and L (+)-ascorbic acid. Other reagents purchased from Sigma (Aldrich, USA) include 2,2′-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzotiazolin-6-sulfonic acid) (ABTS), 2,4,6-Tris(2 -pyridyl)-s -triazine (TPTZ), Trolox, gallic acid, quercetin, and rutin.

2.2 Preparation of the powder of Songgak and its constituent spices

The Songgak and its seven constituent spices were roasted using the traditional method. In the Sasak tribe, traditionally, the sample was roasted on a frying pan using low heat until the sample blackened. All samples were grounded and then powdered. The powdered samples were sieved with mesh 40.

2.3 Preparation of water extract of Songgak

The powder (40 g) of Songgak was extracted using 200 mL of boiling water. Extraction was carried out three times with a total of 500 mL of water (according to how the drink was served, 8 g brewed with 100 mL boiling water). The extract was stirred thoroughly and stirred every 10 mins until the water cooled. The material was allowed to settle and was filtered. The extract was dried in the oven (Binder FED 400) at 70°C until a constant weight was reached.

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2.4 Preparation of ethanol extract of Songgak and its constituent spices

The powders of Songgak and its seven spices were extracted with 90% ethanol (1:10) by a maceration procedure. Each 40 g of sample was extracted using 200 mL of ethanol. All extract was stirred well every 6 hrs for 48 hrs and then filtered. Extraction was carried out three times with a total ethanol of 500 mL. The ethanol extract of Songgak and its constituent spices was concentrated using a vacuum rotary evaporator (Buchi) and then dried in the oven (Binder FED 400) at 40°C until a constant weight was reached.

2.5 2,2'-diphenyl-2-picrylhydrazyl assay

Measurement of the antioxidant activity of the 2,2'-diphenyl-2-picrylhydrazyl (DPPH) was carried out as described by Li *et al*. (2020). A solution of Songgak with a concentration of 10; 5; 2.5; 1; 0.5; 0.1 and 0.01 mg/mL was made with ethanol. To each 3 mL of Songgak and its seven spices solution, 2.7 mL of 0.2 mM DPPH was added, shaken vigorously and incubated for 30 mins in a dark room. The absorbance was measured at a wavelength of 517 nm using a spectrometer (Multiskan Sky-Thermo Scientific, Finland). L (+)-ascorbic acid was a positive control. Each sample was repeated 3 times. The formula used to calculate the percentage of DPPH radical scavenging activity was as follows:

$$\%DPPH\ radical\ scavenging\ activity = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100\%$$

2.6 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) value reflected the reduction ability of the test material. FRAP reagent was made by mixing acetate buffer (300 mM; pH 3.6), a solution of 10 mM 2,4,6-Tris(2-Pyridyl)-S-Triazine (TPTZ) in 40-mM HCl, and 20-mM $FeCl_3.6H_2O$ with a volume ratio of 10: 1: 1. The $FeSO_4.7H_2O$ solution was used to produce a standard curve with a final concentration of 100–1000 µM/mL. In a 2 mL micro-tube, 1350 µL of FRAP reagent was mixed with 150 µL of the sample extract or standard antioxidant quercetin. The mixture was then incubated for 30 mins at 37°C, and the absorbance was read at a wavelength of 595 nm using a spectrometer (Multiskan Sky-Thermo Scientific, Finland). The FRAP value was calculated as M Fe^{2+}/g of the sample using a standard curve of ferrous sulphate (Widodo *et al*., 2020).

2.7 Trolox equivalent antioxidant capacity assay

The solution of radical cation of ABTS ($ABTS^{\bullet+}$) was made by dissolving 38.4 mg of ABTS in 10 mL of 2.5 mM $K_2S_2O_8$ solution and stored in a dark at room temperature for 12-16 hrs. The solution was diluted with methanol to obtain an absorbance of 0.70±0.02 at a wavelength of 743 nm. The sample reaction was carried out with a mixture of 50 µL of the sample and a solution of 1950 µL $ABTS^{\bullet+}$. The absorbances of the samples were measured at 743 nm using a spectrometer (Multiskan Sky-Thermo Scientific, Finland). For control absorbance, the $ABTS^{\bullet+}$ solution was replaced with 2.5 mM of $K_2S_2O_8$ diluted using methanol of the same volume as the dilution factor of the $ABTS^{\bullet+}$ solution. Linear regression of Trolox as standard was produced by making a concentration circuit of 0 - 45 µM. Trolox equivalent antioxidant capacity (TEAC) values were expressed as the Molar Equivalent of Trolox per gram of extract (M TE/g) (Widodo *et al*., 2020).

2.8 Total phenolic content

A total of 40 µL of the sample was mixed with 360 µL deionized H_2O and 100 µL Folin-ciocalteu reagent and left for 2 mins. The reaction was mixed homogeneously with 500 µL of 10% $CaCO_3$ solution and left for 20 mins in the incubator at 40°C. The absorbance of the test solution was measured at 732 nm using a spectrometer (Multiskan Sky-Thermo Scientific, Finland). The gallic acid used as a standard reference was made with methanol dilution series of 0; 5; 10; 15; 20; and 25 µg/mL to produce linear regression to determine the phenolic content of the extract by plotting the sample absorbance. Total phenolic content was expressed as mg gallic acid equivalent/g extract (mg GAE/g) (Widodo *et al*., 2020).

2.9 Total flavonoid content

The sample was diluted with methanol to obtain a concentration of 1250 µg/mL (w/v). The mixed reaction consisted of 100 µL of the sample, 250 µL of acetate buffer (pH 3.8), 150 µL of 0.1 M solutions of Al_2Cl_3 (replaced with 150 µL methanol for blanks), 350 µL of ultra-pure deionized H_2O, and adjusted with methanol to obtain a volume of 1250 µL. The test solution was placed into the incubator for 30 mins at 35°C. A sample volume of 150 µL was pipetted into a 96-wellplate. The absorbance of the sample was measured using a spectrometer (Multiskan Sky-Thermo Scientific, Finland) at 398 nm. Rutin was used as a standard reference through the manufacture of linear regression using a concentration series of 0-90 µg/mL. The total flavonoid content (TFC) was expressed as the rutin equivalent/g of extract (mg RE/g) (Widodo *et al*., 2020).

2.10 Statistical analysis

All the data obtained through three replications were represented as mean ± standard deviation (SD). All data were analyzed using the Windows software of Statistical Package for Social Sciences (SPSS, version 22). The

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method to analyze all data was using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD). The correlation between TPC, TFC, and antioxidant activity was determined using chemometrics analysis. The chemometrics analysis was obtained from Principal Component Analysis (PCA). The PCA was performed using Minitab version 17 (Minitab Ins., USA).

3. Results and discussion

3.1 Yield of extract

The extraction of Songgak and its components showed a different yield of extract depending on the solvent used. Songgak water extract has the highest percentage of yield extract (48.15±1.37%) (Figure 2). Meanwhile, the Songgak ethanol extract (Songgak-EtOH) has a small percentage of yield extract (10.07±0.45%). It showed that water was better able to extract the components of the compounds in the Songgak. The yield of seven spices extracted using ethanol in Songgak differed (7.39–45.01%). Water can extract almost all components in the plant (lipophilic and hydrophilic) (Sasidharan *et al*., 2010). Thus, the process of serving Songgak using water can maximize the dissolution of the compounds in Songgak.

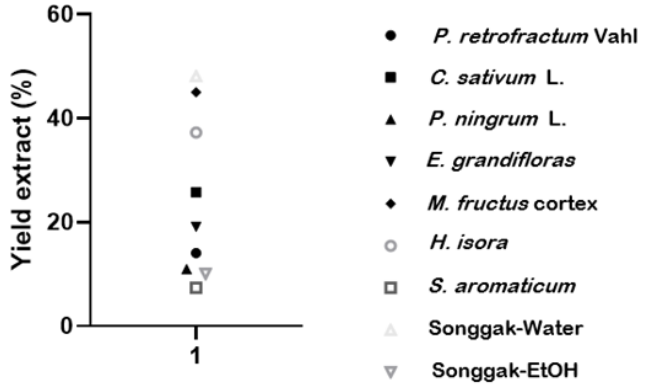

Figure 2. The percentage yield extract of Songgak and its seven constituent spices.

3.2 Total phenolic and total flavonoid content

TPC and TFC usually have a close relationship with the antioxidant activity of a plant sample (Permatasari *et al*., 2019). The TPC of Songgak and its components are shown in Figure 3a. The highest TPC was *S. aromaticum,* with a value of 270.79±12.03 mg GAE/g extract, and was significantly different from the other sample (p<0.05). Its value was classified as high TPC (Yang *et al*., 2015). The TPC of the water and ethanol extract of Songgak was ten times smaller than *S. aromaticum.* This assumption was made since the results showed that the other six components of Songgak have TPC values 3-30 times lower than *S. aromaticum.*

Most of the flavonoid compounds are contained in plants. Flavonoid compounds were reported to contribute to antioxidant activity (Irnawati *et al*., 2022). TFC in Songgak and its components were analyzed using the $AlCl_3$ method. The results showed the highest TFC in *S. aromaticum* (84.77±2.14 mg RE/g) which was significantly different from the other sample (Figure 3b). The other sample has a TFC range from 0.81 - 19.67 mg RE/g. The mixture of seven spices causes the flavonoid content in Songgak to be relatively small (6.99±0.29 mg RE/g).

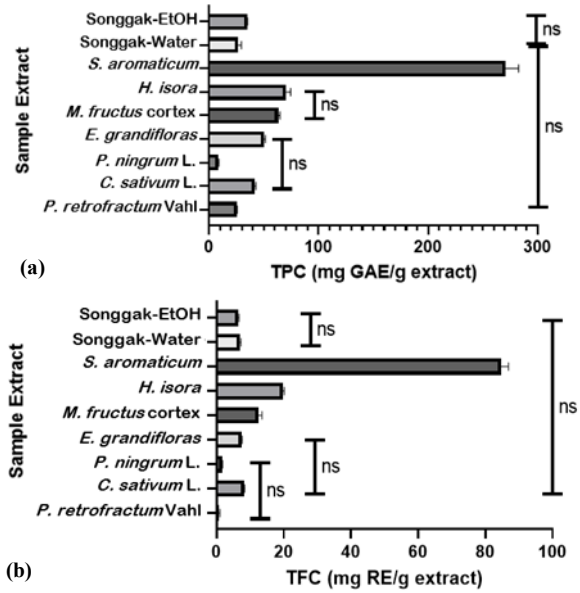

Figure 3. (a) Total phenolic and (b) total flavonoid content of Songgak and its constituent spices.

3.3 2,2'-diphenyl-2-picrylhydrazyl assay

The antioxidant activity of Songgak and its components tested using the DPPH method was expressed as IC_{50}. The smaller the IC_{50} value, the higher the antioxidant activity of the sample. *S. aromaticum* has the highest antioxidant activity (Table 1), which was the same (p>0.05) as the antioxidant activity of ascorbic acid as a control positive. The antioxidant activity of *S. aromaticum* was classified as very strong (<50 µg/mL), followed by *H. isora* and *M. fragrans*. Likewise, *S. aromaticum* was rich in polyphenol compounds such as eugenol, which caused its antioxidant activity to be higher than many other fruits, vegetables, and spices (Cortés-Rojas *et al*., 2014). In addition, *P. ningrum* has the smallest antioxidant activity and was classified as the weak category activity (>150 µg/mL). Measurement of the antioxidant activity of the DPPH method in white pepper (*P. ningrum*) extract obtained with supercritical CO_2 and hydro distillation showed IC_{50} were 103.28 µg/mL (moderate) and 316.27 µg/mL (weak), respectively (Zahin *et al*., 2021). Measurement of the antioxidant

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activity of Javanese chili peppers (*Piper retrofractum* Vahl) included moderate criteria with IC_{50} of 133.14±2.36 µg/mL. Musthapa and Gumilar (2021) revealed that *P. retrofractum* has IC_{50} 825 µg/mL, lower than the results of this study because the solvent used was a nonpolar solvent. The antioxidant compound was extracted in a polar solvent (Zazouli *et al*., 2016).

The IC_{50} of the other constituent spices of Songgak ranged from 41.13-400.17 µg/mL, and the composition of *S. aromaticum* in the Songgak was as much as 1/6 part. The results indicated the cause of the antioxidant activity of the water extract of Songgak was ten times smaller than *S. aromaticum.* However, the antioxidant of the water extract of Songgak was classified as having a strong antioxidant activity (50-100 µg/mL) (Manurung *et al*., 2017). Thus, Songgak ingredients that have strong to very strong antioxidant activity contributed to providing strong antioxidant activity to Songgak drinks. The antioxidant activity of Songgak was close to butylated hydroxytoluene (BHT), the potent synthetic antioxidant (Yang *et al*., 2008). Meanwhile, the antioxidant activity of the ethanol extract of Songgak was categorized as moderate (>100 µg/mL). The results showed that serving Songgak tea using water was acceptable.

3.4 Trolox equivalent antioxidant capacity

TEAC was the method to determine the antioxidant activities using ABTS radical as a free radical. *S. aromaticum* has the highest TEAC, with 2641.11±19.17 mM TE/g extract. El-Maati *et al*. (2016) study reported that the antioxidant activity of ethanol and water extract of *S. aromaticum* using DPPH and TEAC (ABTS) methods, as well as total phenolic levels and total flavonoids, showed excellent results. *P. ningrum* was the lowest antioxidant activity, the same as antioxidant testing using the DPPH method. The principle of TEAC and DPPH methods was the same, proton transfer to stabilize the free radical (Shah and Modi, 2015), thus, the level of antioxidant capacity was the same. The capacity of the water extract of Songgak to stabilize the radical of ABTS was ten times smaller than *S. aromaticum* (Table 1). The occurrence was because the seven spices in Songgak have TEAC that ranges from weak to very strong (57.65-2641.11 mM TE/g). The antioxidant activity of the ethanol extract of Songgak in the TEAC method was smaller than the water extract of Songgak but not significantly different ($p>0.05$).

3.5 Ferric reducing antioxidant power

In the FRAP method, the determination of the antioxidant activity of a sample uses the total reduction indicator of Fe^{3+} to Fe^{2+}. The greater the total value of Fe^{3+} reduction, the greater the antioxidant activity of the sample. As the positive control, quercetin has a higher total Fe^{3+} reduction, but *S. aromaticum* revealed a more potent reducing power than other samples (Table 1). *E. grandiflorus* was the second sample with potent reduction power or antioxidant activity. However, *H. isora* was the second sample with potent antioxidant activity in the TEAC method. TEAC and FRAP have different antioxidant mechanisms. The antioxidant activity of the TEAC method is based on hydrogen atom transfer (HAT). Meanwhile, the FRAP method is nonradical-based on electron transfer (ET) (Zhong and Shahidi, 2015).

The water extract of Songgak has eleven times smaller antioxidant activity (7.29±0.12 M Fe(II)/g) than *S. aromaticum.* The results were caused by the seven constituent spices of Songgak having an extensive range of Fe^{3+} reduction (1.90-82.28 M Fe(II)/g). In addition, the value of Fe^{3+} in the water extract of Songgak was similar ($p>0.05$) to the ethanol extract of Songgak, *E. grandiflorus, M. fragrans, H. isora, C. sativum* and *P. retrofractum* Vahl (Table 1). In the research of Mahaldar *et al*. (2020), ethanol extract of *P. retrofractum* was investigated for antioxidant and hepatoprotective activity. The reducing antioxidant power of other *Terminalia chebula* showed potent activity to reduce the toxic iron level and oxidative stress in mice liver (Sarkar *et al*., 2012). The antioxidant activity using the reducing power of Songgak and its constituent spices might act as hepatoprotective agents.

3.6 Correlation of total phenolic content, total flavonoid content, and antioxidant activity using principle component analysis

PCA was used to see the correlation between antioxidant activity, total phenolic and flavonoid content of Songgak, and its seven constituent spices. The PCA showed the eigenvalue of PC1 and PC2 was 3.4077 and 1.3336, respectively. PC1 and PC2 contributed 68.2% and 26.7% to all variables, respectively. The total of PC1 and PC2 was more than 90%, showing all data has been represented. The score plot (Figure 4a) showed that *P. ningrum* L., *P. retrofractum* Vahl, *C. sativum* L. have the same characteristics of TPC, TFC, and antioxidant activity albeit catagorized as weak. Meanwhile, water and ethanol extract of Songgak, *E. grandiflorus, M. fragrans*, and *H. isora* have the same antioxidant activity and total phenolic and flavonoid content, which was strong. However, *S. aromaticum* has different characteristics from all the samples.

The angle vector in the loading plot (Figure 4b) between antioxidant activity using the DPPH method was >90°C with TPC and TFC. It showed that they did not correlate. The TPC and TFC did not correlate with

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Table 1. Antioxidant activity of Songgak and its components.

Samples	Antioxidant Activity		
	IC_{50} DPPH (µg/mL)	TEAC (mM TE/g)	FRAP (M Fe(II)/g)
Piper retrofractum Vahl	133.14±2.36 [a]	308.06±11.19[a]	5.31±0.08[a]
Coriandrum sativum L.	85.99±4.71[b]	504.85±13.96[b]	9.53±0.10[b]
Piper ningrum L.	400.17±11.08[c]	57.65±1.19[c]	1.90±0.06[c]
Elaeocarpus grandiflorus	67.37±2.97[d]	572.17±49.50[d]	10.64±0.12 [b]
Myristica fragrans	43.57±0.98[e]	591.90±12.11[d]	8.90±0.15 [b]
Helisteres isora	41.13±2.24[e]	665.23±16.49[e]	9.88±0.14 [b]
Sysygium aromaticum	5.65±0.11[f]	2641.11±19.17[f]	82.28±2.26[d]
Water extract of Songgak	63.78±4.29[d]	266.27±11.66[a,g]	7.29±0.12[a,b]
Ethanol extract of Songgak	101.18±3.87[g]	244.89±10.60[g]	5.95±0.16[a]
Ascorbic Acid	2.41±0.01 [f]	-	-
Quercetin	-	-	138.32±2.21[c]

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) using Tukey's HSD test.

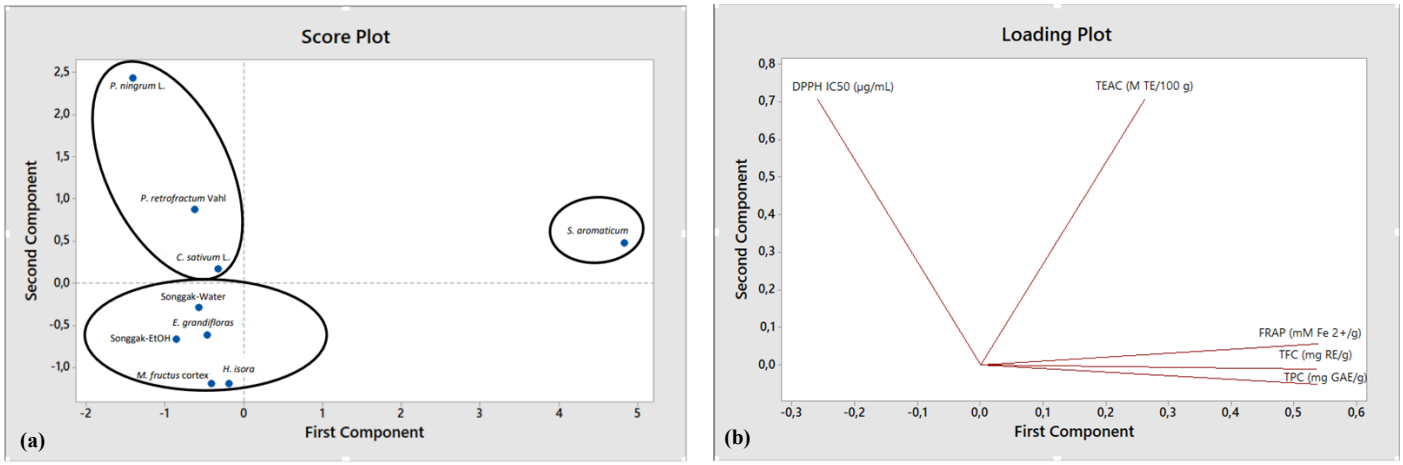

Figure 4. Principal component analysis of antioxidant activities, TPC, and TFC of Songgak and its constituent spices. (a) Score plot; (b) Loading plot.

the value of DPPH IC_{50}. The results indicated the antioxidant mechanism of phenolic and flavonoid compounds in Songgak and its seven constituent spices was not through the hydrogen atom transfer. In addition, the TEAC has a higher correlation with TPC and TFC than DPPH. Although the mechanisms of DPPH and ABTS are the same, ABTS is able to detect the antioxidant activity of lipophilic compounds such as hesperetin 7-O-glucosyl-6"-O-laurat that cannot be detected using the DPPH method. However, the FRAP antioxidant activity method is strongly correlated with TPC and TFC. The study of Yang *et al*. (2015) showed that FRAP and TFC in the plant have a higher correlation than DPPH and ABTS. The result of the present study indicated a mechanism of antioxidant activity of phenolic and flavonoid compounds in Songgak and its constituent spices was by electron transfer.

4. Conclusion

Seven constituent spices in Songgak were identified as containing phenolic and flavonoid compounds. PCA analysis showed that these compounds contributed to the antioxidant activity of Songgak through the electron transfer process. The seven constituent spices contained in the herbal drink Songgak have antioxidant activity from relatively weak to very strong, which were analyzed using different methods. The antioxidant activity of Songgak was classified in the strong category. Therefore, consuming Songgak every day can be beneficial for preventing various diseases involving free radicals.

Conflict of interest

The authors declare no conflict of interest.

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