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Assessment of Antioxidant Activities, Total Phenolics, and Flavonoids of Different Extracts of *Strobilanthes Schomburgkii* Leaves

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Abstract. The main purpose of this work was to assess the antioxidant activities of the *n*-hexane, ethyl acetate and methanol extracts of *Strobilanthes schomburgkii* leaves collected in Laocai province, Vietnam. Total phenolic and flavonoid contents were determined. The DPPH, H_2O_2 radical scavenging activity, and total antioxidant activity *via* phosphomolybdenum method were investigated. The results showed that the methanol extract concluded a high concentration of phenolics and flavonoids which were 65.42 mg/g and 52.05 mg/g, respectively. There was a significant correlation between total phenolic and flavonoid concentrations and EC_{50} values of different antioxidant assays. The antioxidant properties of the different extracts from *Strobilanthes schomburgkii* leaves were reported for the first time.

Keywords: *Strobilanthes schomburgkii* leaves, flavonoid, phenolic, antioxidant potential

1. Introduction

Strobilanthes Blume is the most species-rich genus of Acanthaceae in Asia with more than 400 species which are widely distributed from India, China, and Southeast Asia, extending to western Afghanistan, northern Japan, and southern New Guinea (Hu *et al.*, 2011). Most species grow in forests with a tropical monsoon climate, and a few species are found in the tropical rainforests of the Malay Peninsula or Borneo (Wood *et al.*, 2017). In Vietnam, the *Strobilanthes* genus has 33 species which are widely distributed from North to South (Tran, 2015). In recent years, three new species have been recorded for the Vietnam flora (Do & Do, 2006; Wood *et al*., 2017; Pham *et al*., 2017; Nguyen *et al*., 2018). Previously, the specie *Strobilanthes schomburgkii* was only recognized as a native species of Thailand but is now also encountered in many countries such as Cambodia, India, and Malaysia (Bui & Do, 2020). In 2020, Bui *et al.* confirmed the plant *Strobilanthes schomburgkii* distribution in diffirent provinces of Vietnam.

In recent years, this plant has been widely grown in the territory of Laocai province and *Strobilanthes schomburgkii* was carried out planting in Thainguyen University, Laocai Campus for botanical research, as well as for study on biochemical composition and biological activities for further use of this plant in various field of food industry.

Despite that *Strobilanthes schomburgkii* is used in food and medicinal applications, and is health-beneficial, a comprehensive research on ochemical ingredient and biological activity of *Strobilanthes schomburgkii* for potential food and pharmaceutical application is still missing. Herein, the purpose of this work was to investigate the antioxidant activities of the *Strobilanthes schomburgkii* in order to confirm its pharmaceutical value. Then, the phytochemical screening and the antioxidant content including flavonoid and phenolics of the different extracts were determined. Reducing power, the DPPH and H_2O_2 scavenging assay were performed. The total antioxidant activity (TAA) was done *via* phosphomolybdenum method.

2. Material and Methods

2.1. Chemicals

All solvents were purchased from Ducgiang Chemical Company (Hanoi, Vietnam) and used without further purification. Distilled water was prepared in Biotechnology Laboratory (Thuyloi University, Hanoi, Viet Nam). Other chemicals used for analysis were obtained from Sigma - Aldrich.

2.2. Material and Method

2.2.1. Material

The plant *Strobilanthes schomburgkii* was collected at Thainguyen University - Laocai campus, Laocai province, Vietnam in January 2021. A specimen sample (SS-01-2021) was kept at the Laboratory of Biotechnology, Faculty of Chemical and Environmental, Thuyloi University, 175 Tay Son, Dong Da, Hanoi, Vietnam.

2.2.2. Preparation of different extracts from the leaves of Strobilanthes schomburgkii

500 g leaves of *Strobilanthes schomburgkii* were oven-dried and ground. The dried ground powder of *Strobilanthes schomburgkii* was extracted with methanol (1.5 L) three-time under ultrasonic wave. Each time was done at 45^{o}C for 45 minutes. The obtained filtrates of three times were combined and concentrated under reduced pressure to create a crude methanol extract that was then partitioned with *n*-hexane, ethyl acetate to yield *n*-hexane extract (H-SS.2021), ethyl acetate extract (E-SS.2021) and the methanol-water layer called methanol extract (M-SS.2021). The dried extracts were weighed for yield calculation, then kept at 2-12^{o}C for further analysis.

2.2.3. Phytochemical screening and the antioxidant and reducing sugar content determination

Phytochemical screening method

The phytochemical screening determination of the various solvent extracts (H-SS.2021, E-SS.2021, and M-SS.2021) was done using the method of Hossain *et al*. (2013).

Determination of reducing sugar content

The reducing sugar concentration of methanol extract (M-SS.2021) was determined using DNS assay as described before. In brief, 1 mL of DNS reagent was added to 3 mL of the M-SS.2021, followed by a thorough mix. 3 mL of deionized water was used instead of extract in the negative control. The mixtures were incubated at 90^{o}C for 15 min to obtain the red-brown color, then cooled to room temperature. Absorbance of the samples was determined at 540 nm against the negative control. Glucose was used as a standard for calibration curve (Jain *et al.*, 2020).

Determination of total phenolic content

The total phenolic concentration of H-SS.2021, E-SS.2021 and M-SS.2021 was estimated according to the method of Ferreira *et al*. (2007). In brief, 1.0 mL of Folin-Ciocalteu's reagent was added to 1.0 mL

of the samples, then incubated for 10 min at 25°C. Then, 1.0 mL of a 35% Na_2CO_3 solution (*w/w*) was added to each sample, and the final volume was brought up to 10 mL with deionized water. Afterward, all samples were incubated 2 hours in the dark, then measured absorbance at 725 nm. Gallic acid was used as a standard for calibration curve. Results were expressed as milligrams of gallic acid equivalence (GAE) per gram of extract.

Estimation of total flavonoid

Flavonoid content was estimated as described before (Chang *et al.*, 2002). In details, 0.5 mL of each extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. All samples were then incubated in the dark for 1 h at room temperature. After incubation, absorbance at 415 nm was recorded for each sample against negative control. Quercetin was used as a standard for calibration curve.

2.2.4. Biological activity assays

Reducing power assay

The reducing power of the extracts was estimated as described before (Oyaizu *et al*., 1986). Briefly, 2.5 mL of extract with various concentrations ranging from 0 to 300 µg/mL were mixed with 2.5 mL of 1% potassium ferricyanide (*w/w*) and 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). The mixture was mixed well and incubated for 30 min at 50°C immediately after incubation, 2.5 mL of 10% TCA was added to each mixture to stop the reaction. Then, the mixture was centrifuged at 3,000 rpm for 15 min. 2.5 mL of the supernatant was collected and transferred to a new tube. To the supernatant, 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride were added. Then, absorbance at 700 nm was measured. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as the standard.

Antioxidant activity in total

The TAA of H-SS.2021, E-SS.2021, and M-SS.2021 was performed using the phosphomolybdenum method (Prieto *et al.*, 1999). In brief, 1 mL of reagent solution was mixed with an aliquot of 0.1 mL of extract with different concentration ranging from 0 to 100 µg/mL). All samples were incubated at 100°C for 1.5 hour, then cooled to 25°C and vortexed. The absorbance of the solutions was recorded at 695 nm against the negative control. The 50% inhibition (EC_{50}) was calculated and ascorbic acid was utilized as standard.

DPPH scavenging assay

The DPPH scavenging assay was done using the method of Choi *et al.* (2014). In detail, 0.2 mL of each extract with various concentrations (6.25-100 µg/mL) was mixed with 0.5 mL of 0.2 mM DPPH dissolved in ethanol. The mixture was incubated for 30 min in the dark at 37°C. After incubation, the absorbance was measured at 517 nm. The EC_{50} of H-SS.2021, E-SS.2021, and M-SS.2021 represents the concentration that causes a 50% inhibition of radical formation.

Hydrogen peroxide scavenging assay

The current scavenging assay was performed using the method of Gülçin *et al.* (2010). The crude extracts H-SS.2021, E-SS.2021 and M-SS.2021 at various concentrations (6.25-100 µg/mL) in 3.4 mL of 0.1M phosphate buffer pH 7.4 were added to 0.6 mL of 43 mM H_2O_2 in 0.1M phosphate buffer pH 7.4. The absorbance of the reaction mixture was recorded at 230 nm against control with phosphate buffer without H_2O_2. The concentration of H-SS.2021, E-SS.2021 and M-SS.2021 necessary to scavenge 50% of the H_2O_2 is the EC_{50} value.

2.3. Statistical analysis

Each determination was performed 3 times. Mean values were presented as mean ± SD. Statistical analysis was done with two-way ANOVA, and Tukey's multiple comparisons test using Prism GraphPad 7.0.

3. Results and Discussion

3.1. Extraction yield of different extracts from Strobilanthes schomburgkii leaves

The percentage yield of different extracts from *Strobilanthes schomburgkii* leaves was shown in Table 1. The percentage of extracts varied from 6.52 ± 0.56 to 19.54 ± 1.19%. The highest yield was given by the methanol extract (19.54 ± 1.19%) whereas the *n*-hexane extract gave the lowest value (6.52 ± 0.56%). The percentage of ethyl acetate residue was 10.14 ± 1.15%. Generally, non-polar and less polar compounds such as the steroid group are concentrated in the *n*-hexane residues. Medium polar compounds are found in the ethyl acetate residues and the polar chemical ingredients will be found in the methanol extracts. So, the phytochemical screening of different extracts from *Strobilanthes schomburgkii* leaves was controlled in the next step of this work.

3.2. Phytochemical screening of different extracts from Strobilanthes schomburgkii

Table 1. The percentage and phytochemical analysis of different extracts of *Strobilanthes schomburgkii* leaves

Percentage and Biochemicals	Inference		
	H-SS.2021	E-SS.2021	M-SS.2021
Extract yield	6.52 ± 0.56	10.14 ± 1.15	19.54 ± 1.19
Phenolics	+	++	++
Flavonoid	+	++	++
Terpenoid	-	+	+
Quinone	-	-	-
Courmarin	-	-	-
Saponin	-	-	-
Steroid	++	+	-
Alkaloid	-	-	-

+: Presence; -: Absence

The results for phytochemical screening of the *n*-hexane, ethyl acetate, and methanol extracts from *Strobilanthes schomburgkii* leaves showed the presence of flavonoids, terpenoids, phenolics, and steroids. Alkaloid, quinone, coumarin, and saponin were not present in the extracts (Table 1). In general, the steroid group was found more in the *n*-hexane residue, less in the ethyl acetate residue, and was an absent in the methanol extracts. Terpenoids were present only in the ethyl acetate and methanol extracts. Flavonoid and the phenolic group were found more in the ethyl acetate and methanol extracts.

3.3. Reducing sugar concentration and the antioxidant content of different extracts from Strobilanthes schomburgkii leaves

Reducing sugar concentration

In food application, the reducing sugars readily interact with amino acids and give rise to Maillard reaction products, which lead to progressive browning and aroma formation. (Chmielewski *et al.*, 2013). Thus, in the context of discovering potential of *Strobilanthes schomburgkii* in the food area, it is important to evaluate the content of reducing sugar in the obtained extract. As result in Table 2, the reducing sugar content was 177.52 ± 1.90 mg/g methanol extract, which means 3.46 ± 0.4% on dried

material. In some provinces of Vietnam, the leaves of this plant are dried and then brewed to drink as black tea. The presence of reducing sugar will play an important role in the food process to create different tea and drinks.

Table 2. Reducing sugar concentration, total phenolic and flavonoid content of different solvent residues from *Strobilanthes schomburgkii* leaves

Chemical composition	H-SS.2021	E-SS.2021	M-SS.2021
Total phenolics (mg GAE/g)	5.99 ± 0.64[a]	28.85 ± 1.37[b]	62.42 ± 2.97[c]
Total flavonoid (mg quercetin equivalent/g	4.04 ± 0.13[a]	23.48 ± 1.52[b]	52.05 ± 2.48[c]
Reducing sugar content, mg/g	-	-	177.52 ± 1.90

H-SS.2021, E-SS.2021, M-SS.2021: n-hexane, ethyl acetate and methanol extracts from *Strobilanthes schomburgkii*, respectively. GAE = gallic acid equivalents; QE = quercetin equivalents; Values are mean ± SD; Different letters within a same row have significant difference by Tukey's test with $p < 0.05$

Antioxidant content

Phenolic compounds are well-known biological active compounds in the phytochemical investigation thanks to the scavenging capacity of the hydroxyl group (Hatano *et al.*, 1989). These ingredients contribute directly to antioxidative action (Duh *et al*., 1999), and also possess notable antioxidant, antimutagenic and anticancer properties (Ahmad *et al*., 1999). Flavonoids are naturally occurring polyphenolic compounds representing one of the most prevalent classes of compounds in vegetables, nuts, fruits, and beverages such as coffee, tea, and red wine (Hertog *et al*., 1993).

Total phenolic and flavonoid content was calculated in gallic acid and quercetin equivalent with the standard curve equation y = 0.0175x - 0.0079 (R^2 = 0.9994) and y = 0.0021x – 0.0137 (R^2 = 0.9934), respectively. The methanol extract showed the highest total phenolic concentration (65.42 ± 2.97 mg/g), whereas the phenolic content of *n*-hexane extract were lower (5.99 ± 0.64 mg/g) (Table 2).
Similarly, the flavonoid content of M-SS.2021 was 52.05 ± 2.48 mg/g which was higher than those of H-SS.2021 and E-SS.2021. The total content of the flavonoid group in E-SS.2021 and H-SS.2021 were 23.48 ± 1.52 and 4.04 ± 0.13 mg/g, respectively. This result is also consistent with the published reports (Jan *et al.*, 2013).

3.4. Biological activity of different solvent extracts of Strobilanthes schomburgkii leaves

3.4.1. Reducing power

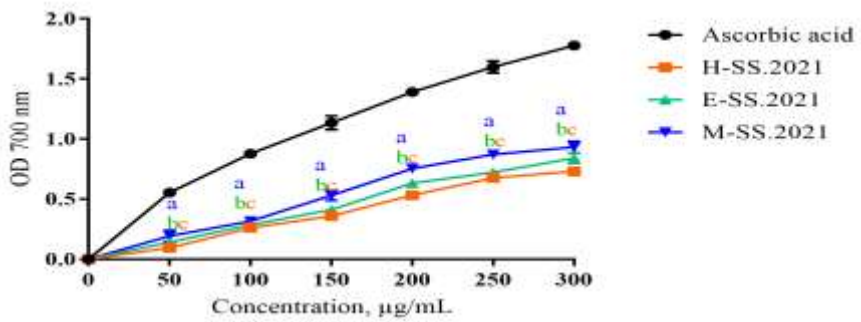

Fig. 1. Reducing power of various extracts of *Strobilanthes schomburgkii* leaves

Different letters above the lines indicate significant differences among samples ($p < 0.05$) by two-way ANOVA, Tukey's test

Figure 1 showed the reducing power of various residues of *Strobilanthes schomburgkii* leaves compared to ascorbic acid. The results indicated that the RPC of H-SS.2021, E-SS.2021 and M-SS.2021 increased with an increase in concentration. The methanol extract M-SS.2021 showed the highest value for RPC with an EC_{50} of 156.05 µg/mL whereas the *n*-hexane residue showed the smallest value of RPC with a an EC_{50} of 191.13 µg/mL. The E-SS.2021 exhibited the medium RPC and the EC_{50} value was 172.12 ± 2.65 µg/mL.

3.4.2. The total antioxidant activity

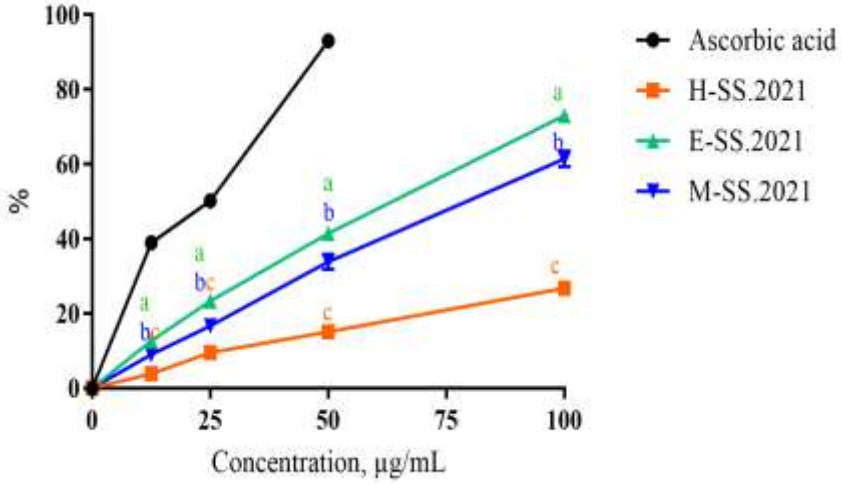

Fig. 2. Total antioxidant activity of various extracts of *Strobilanthes schomburgkii* leaves
Different letters above the lines indicate significant differences among samples ($p < 0.05$) by two-way ANOVA, Tukey's test

Fig. 2 shows the total antioxidant activity of ascorbic acid and different solvent extracts of *Strobilanthes schomburgkii* leaves in the order of ascorbic acid > M-SS.2021 > E-SS.2021 with the EC_{50} values were 23.52, 66.01, and 87.55 µg/mL, respectively. TAA was not exhibited for the H-SS.2021 sample at concentration range 0-100 µg/mL. Ascorbic acid was used as control which was more effective than the experiment plant extracts, with an EC_{50} value of 23.52 µg/mL (Table 3).

Table 3. The EC_{50} values on DPPH radical, H_2O_2 radical, total antioxidant capacity, and reducing power of different solvent extracts from *S. schomburgkii* leaves

Extracts	$EC_{50, µg/mL}$			
	Scavenging ability on DPPH radicals	H_2O_2 scavenging assay	Phosphomolybdate assay	Reducing capacity assay
H-SS.2021	121.61 ± 2.27^{b}	225.63 ± 3.91^{d}	194.27 ± 4.52^{d}	191.13 ± 1.84^{d}
E-SS.2021	103.20 ± 2.06^{b}	64.32 ± 3.10^{b}	87.55 ± 2.23^{b}	172.12 ± 2.65^{b}
M-SS.2021	75.97 ± 1.49^{b}	84.74 ± 4.34^{c}	66.01 ± 1.59^{c}	156.05 ± 2.12^{c}
Ascorbic acid	6.37 ± 0.63^{a}	28.00 ± 0.65^{a}	23.52 ± 1.60^{a}	55.00 ± 1.84^{a}

H-SS.2021, E-SS.2021, M-SS.2021: *n*-hexane, ethyl acetate and methanol extract from *Strobilanthes schomburgkii*, respectively. Values are mean ± SD; Different letters within the same column have a significant difference by Tukey's test with $p < 0.05$

3.4.3. DPPH radical scavenging activity

The DPPH radical scavenging activity is one of the popular assays for testing the antioxidant capacity of medicinal plants and various substances. This method is based on reducing alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant, with the formation of the non-radical form DPPH and in the result, the color of experiment extracts changes from purple to yellow (Gülçin *et al.*, 2010).

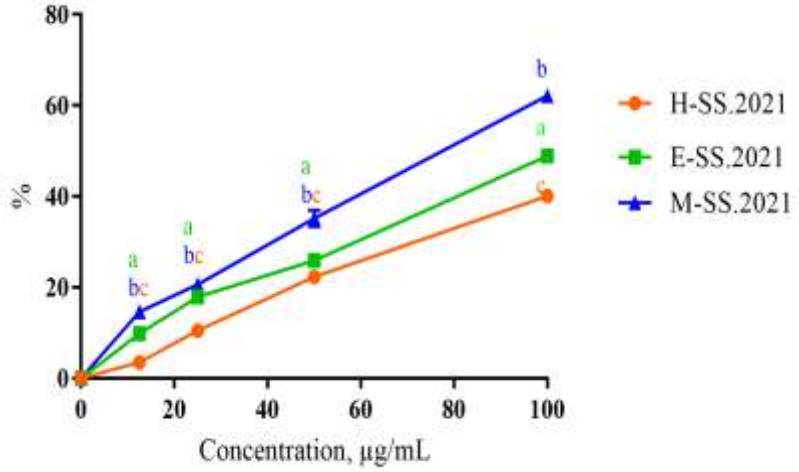

Fig. 3. Scavenging activity of various extracts of *Strobilanthes schomburgkii* leaves on DPPH radical Different letters above the lines indicate significant differences among samples ($p < 0.05$) by two-way ANOVA, Tukey's test

Fig. 3 showed the scavenging activity of standard and the M-SS.2021, E-SS.2021, H-SS.2021 samples on DPPH radical. The scavenging capacity was in the following order ascorbic acid > M-SS.2021 > E-SS.2021 > H-SS.2021. At concentration of 50 µg/mL, the DPPH scavenging capacity values of M-SS.2021, E-SS.2021 and H-SS.2021 were 88.72, 35.19, 25.91 and 22.33%, respectively. As results in Table 3, the DPPH scavenging capacity of methanol residue (75.97 ± 1.49 µg/mL) was more higher than that of the ethyl acetate extract (103.20 ± 2.06 µg/mL). The ethyl acetate extract could be considered as a moderate reagent for DPPH radical scavenging whereas the *n*-hexane extract was not exhibited scavenging DPPH radical (EC_{50} value was higher than 100 µg/mL). In general, the DPPH radical scavenging activity of H-SS.2021, E-SS.2021 and M-SS.2021 samples are in consistent with aboved antioxidant assays. The methanol extract of *S. schomburgkii* leaves could be used as an antioxidant that possesses hydrogen-donating properties.

According to the aboved results about TAA, RPC and DPPH scavenging assays, the methanol extract from *S. schomburgkii* leaves showed the best results related to the higher content of phenolics and flavonoids in this extract. This opinion was also in agreement with what has been reported about the relationship between flavonoid and phenolic contents with antioxidant activity in black garlic and different plant material (Choi *et al*., 2014; Cao *et al.*, 2022).

3.4.4. H_2O_2 radical scavenging activity

As the result shown in Table 3, the scavenging ability of H_2O_2 radicals was in the order ascorbic acid > E-SS.2021 > M-SS.2021 > H-SS.2021. Fig. 4 indicated that the methanol and ethyl acetate extracts showed moderate H_2O_2 scavenging activity with a scavenging ability of 45.31% and 31.65% at 50 µg/mL; 72.18% and 55.01% at 100 µg/mL, respectively. The EC_{50} of H-SS.2021, M-SS.2021, E-SS.2021 were 225.63, 84.73, and 64.32 µg/mL, respectively. Like phosphomolybdate, RPC and ability on DPPH radical assay, the percentage scavenging ability of H_2O_2 radicals increased with the increasing concentration of the extracts. However, a rule contrary to the aforementioned activities was

observed. The ability to scavenge the H_2O_2 radical of ethyl acetate extract was significantly higher than that of the methanol extract ($p < 0.05$). Thus, antioxidant activity is not only related to flavonoid and phenolic contents but also the presence of unknown bioactive compounds which need to be isolated in further work.

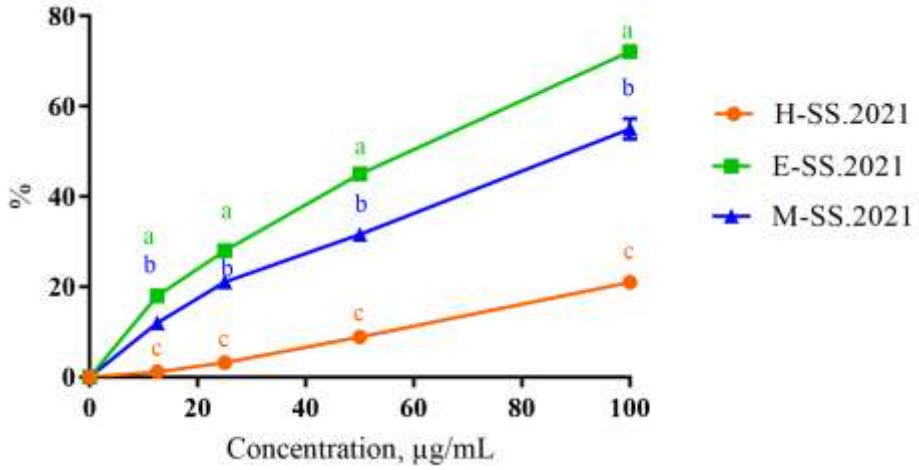

Fig. 4. Scavenging activity of standard and the fractions on DPPH radical
Different letters above the lines indicate significant differences among samples ($p < 0.05$) by two-way ANOVA, Tukey's test

Conclusion

Phytochemical screening for various extracts from *Strobilanthes schomburgkii* leaves collected in Laocai, Vietnam was tested and showed a positive result for steroids, polyphenols, flavonoids and terpenoid. Reducing sugar content was investigated for methanol extract. The antioxidant content determination indicated that the methanol extract included the highest content of flavonoid and phenolics in comparison with *n*-hexane and ethyl acetate residues. Furthermore, *n*-hexane, ethyl acetate, and methanol extracts showed the different antioxidant capacity in the different assays. A significant correlation between total phenolic and flavonoid contents with EC_{50} of RPC, DPPH, phosphomolybdenum assays was observed. So, the leaves of *S. schomburgkii* could be used as a natural antioxidant and recommended to apply in the food or pharmaceutical field. However, the bioactive compounds responsible for the antioxidant activity of ethyl acetate and methanol extracts of *S. schomburgkii* are currently unknown. Therefore, further isolation of chemical ingredients, structure identification, and antioxidant control in living models are required.

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