Total phenolic content, total flavonoid content, and antioxidant activity of leaves, twigs, and inflorescences of *Stachytarpheta jamaicensis* (L.) Vahl

Chaowalit Monton¹*, Kanchala Pongpichayarisir¹, Phatcharin Gomenake¹, Sukanya Settharaksa¹, Natawat Chankana²

¹ Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand.
² Sun Herb Thai Chinese Manufacturing, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand.

* Corresponding Author: Tel. +66(0)29972222 ext. 4911; E-mail address: chaowalit@rsu.ac.th

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**Introduction**

*Stachytarpheta jamaicensis* (L.) Vahl is an herbal medicinal plant in the family of Verbenaceae. Many publications report its pharmacological effects such as antimicrobial, antifungal, antioxidation, antiinflammation, antinociceptive, antidiarrheal, antihypertensive, antidiyslipidemia activities, etc.¹ Several chemical constituents of the plant are reported. Saponins, tannins, and flavonoids are found in a water extract of *S. jamaicensis* leaves.² Leaf extract also contains alkaloids, carbohydrates, glycosides, phenolics, proteins, quinone, steroids, and terpenoids.¹ Water and ethanol extracts of stems contain phenolic compounds and tannins, alkaloids, glycosides, phytoestrogen, and saponins.³ The stem also contains carbohydrates, flavonoids, phenols, proteins, and terpenoids.¹ Phenolic compounds are secondary metabolites in plants. They are typically found in fruits, vegetables, cereals, and beverages. They have good antioxidant activity perhaps via deactivation of reactive oxygen species or reactive nitrogen species. The epidemiological data suggests that they may have beneficial effects against numerous chronic diseases and degenerative diseases.⁴ Flavonoids are a subtype of phenolic compounds, which are the most studied group of the subtypes.⁴ Flavonoids are known as one of the most potent antioxidants from plants. They comprise six main classes including flavones, flavonols, flavanones, flavanols, anthocyanidins, and isoflavones.⁵ An antioxidant is important for the prevention of several free radical-related diseases. Interest has increased extensively in investigating naturally occurring antioxidants for use as dietary or medicinal resources.¹ The aim of this work was to determine the total phenolic content and total flavonoid content in *S. jamaicensis*. The antioxidant activity was determined using two assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Their linear correlations with total phenolic content and total flavonoid content were also investigated.

**Methods**

**Plant sample and extraction:** *S. jamaicensis* was harvested in April 2017 from Buached District, Surin Province, Thailand. The plant sample was authenticated by Chair Prof. Dr. Nijsiri Ruangrungsi. Plant sample was deposited at the Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University; voucher specimen no. CM-SJ001-1-4-2017. Leaves, twigs, and inflorescences of *S. jamaicensis* were dried at 50 °C for 24 h. They were pulverized and passed through a 40-mesh sieve. Each plant part (20 g) was added into 500-mL Erlenmeyer flask and extraction solvent (200 mL) was added. Three extraction solvent systems were used: water, 50% ethanol, and 95% ethanol. The mixture was sonicated for 30 min and filtered. The marc was re-extracted two times using 100 mL of solvent. Three parts of the filtrate were pooled and dried. The samples were coded as L, T, and I for leaves, twigs, and inflorescences, and 0, 50, and 95 for water, 50% ethanol, and 95% ethanol, respectively.

**Determination of total phenolic content:** The Folin-Ciocalteu method was performed for the total phenolic content determination.⁶ A stock solution of gallic acid monohydrate (1 mg/mL) aqueous
solution was prepared. It was diluted into five concentrations: 25, 50, 100, 150, and 200 µg/mL. The extract in a concentration of 2.5 mg/mL was also prepared. The test sample (12.5 µL) and water (50 µL) were added to 96-well plate (n=3). Folin & Ciocalteu’s phenol reagent (12.5 µL) was then added. The mixture was stored in the dark at room temperature for 6 min. The 7% sodium carbonate (125 µL) and water (100 µL) were added. The mixture was stored in the dark at room temperature for 90 min. The absorbance of the mixture was measured at 760 nm using a microplate reader. The total phenolic content of plant extract was calculated from the calibration curve of gallic acid monohydrate.

**Determination of total flavonoid content:** The 5 mg/mL concentration of extract was prepared. A stock solution of (+)-catechin hydrate (1 mg/mL) was prepared using ethanol as solvent. It was diluted into five concentrations: 15.625, 31.25, 62.5, 125, and 250 µg/mL. The method for determination of total flavonoid content was modified from Chen and Li. The test sample (25 µL) and water (125 µL) were added to 96-well plate (n=3). Sodium nitrite 5% aqueous solution (10 µL) was then added. The mixture was kept in the dark at room temperature for 6 min. The 10% aluminium chloride hexahydrate aqueous solution (15 µL) was added and the obtained mixture was kept in the dark at room temperature for 90 min. Then, 1 M sodium hydroxide (50 µL) was added and mixed. The absorbance of the mixture was measured at 510 nm. The total flavonoid content of plant extract was calculated from the calibration curve of (+)-catechin hydrate.

**DPPH radical scavenging assay:** The six concentrations of plant extract were prepared: 100, 250, 500, 1000, 2500, and 5000 µg/mL. Gallic acid monohydrate 1-10 µg/mL was prepared. The method described by Brand-Williams et al. was modified in this work. Each sample (100 µL) was added to 96-well plate (n=3). The 0.2 mM of DPPH ethanolic solution (100 µL) was added and mixed. The mixture was stored in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. The percent inhibition was calculated from the absorbance of the test sample (A_t) and the absorbance of the control (A_c) as Equation 1.

\[
\%\text{Inhibition} = \left(\frac{A_t - A_c}{A_c}\right) \times 100 \quad \text{Eq.1}
\]

The inhibition curve for concentrations of the extract versus percent inhibition was constructed. The half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the inhibition curve.

**FRAP assay:** FRAP reagent was prepared as follows: 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (2.5 mL), 20 mM ferric (III) chloride hexahydrate (2.5 mL), and 300 mM acetate buffer pH 3.6 (25 mL) were mixed, incubated at 37 °C for 30 min then cooled to room temperature. The six concentrations of plant extract were prepared: 100, 250, 500, 1000, 2500, and 5000 µg/mL. Ferrous (II) sulfate heptahydrate 1-200 µg/mL was prepared. The methods described by Benzie and Strain and Wong et al. were modified in this work. The sample (30 µL) was added to 96-well plate (n=3). Then, FRAP reagent (270 µL) was added. The mixture was stored in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 595 nm. The percent inhibition was calculated from the absorbance of the test sample (A_t) and the absorbance of the control (A_c) as Equation 2.

\[
\%\text{Inhibition} = \left(\frac{A_t - A_c}{A_c}\right) \times 100 \quad \text{Eq.2}
\]

The inhibition curve for concentrations of the extract versus percent inhibition was constructed. The IC_{50} was calculated from the equation of the inhibition curve.

**Results**

The extraction yield of L0, L50, L95, T0, T50, T95, I0, I50, and I95 were 22.95, 26.82, 11.60, 8.70, 11.65, 7.94, 12.59, 15.59, and 9.10%, respectively. Figure 1 presents total phenolic content and total flavonoid content of *S. jamaicensis*. Leaves extracted with 50% ethanol and twigs extracted with water had the highest and the lowest total phenolic content, respectively, as was the case for total flavonoid content. In addition, total phenolic content varied with regard to plant parts and extraction solvent; leaves had total phenolic content higher than inflorescences and twigs, respectively, for the three extraction solvent systems. Similar variation was observed for total flavonoid content, except that twigs extracted by 95% ethanol had higher flavonoid content compared to leaves and inflorescences. Accordingly, solvent type is an important variable for determination of total phenolic and total flavonoid content. Extraction of *S. jamaicensis* using 50% ethanol provided the highest total phenolic content for all plant parts. However, total flavonoid content was highest when extracted using 50% ethanol and 95% ethanol compared to water. The high degree of linear correlation between total phenolic content and total flavonoid content (R = 0.822, p = 0.007) was found due to flavonoid is a subclass of a phenolic compound so that high flavonoid content provided high phenolic content as well. IC_{50} values of *S. jamaicensis* extract obtained from DPPH radical scavenging assay and FRAP assay are shown in Figure 2. IC_{50} value from two assays was high in water extract, indicating that it had low antioxidant activity. According to DPPH radical scavenging assay, twigs extracted using water and 95% ethanol had the highest IC_{50} (827.08±73.24 µg/mL) and the lowest IC_{50} (27.06±0.91 µg/mL), respectively. The
positive control, gallic acid monohydrate, revealed IC$_{50}$ of 2.03±0.05 µg/mL. In the case of FRAP assay, twigs extracted using water and leaves extracted using 50% ethanol had the highest IC$_{50}$ (445.69±17.68 µg/mL) and the lowest IC$_{50}$ (62.24±0.47 µg/mL), respectively. The positive control, ferrous (II) sulfate heptahydrate, had IC$_{50}$ of 11.15±0.07 µg/mL. The antioxidant activity of S. jamaicensis was high in 50% ethanol and 95% ethanol extracts, which corresponded to the high total phenolic content and total flavonoid content. The IC$_{50}$ value obtained from DPPH radical scavenging assay and FRAP assay exhibited strong linear correlation (R = 0.968, p < 0.0001).

![Figure 1](image1.png)

**Figure 1** Total phenolic content (a) and total flavonoid content (b) of different parts of *S. jamaicensis* extracted using different solvents.

High linear correlations between IC$_{50}$ obtained from two assays for total content of phenols and flavonoid were also observed. We found that when total phenolic content and total flavonoid content increased, IC$_{50}$ decreased due to the potent antioxidant property of phenolic compound and its subclass. The R values between four pairs of data: total phenolic content vs. IC$_{50}$ from DPPH radical scavenging assay, total flavonoid content vs. IC$_{50}$ from DPPH radical scavenging assay, total phenolic content vs. IC$_{50}$ from FRAP assay, and total flavonoid content vs. IC$_{50}$ from FRAP assay were -0.859, -0.833, -0.854, and -0.772, with p-value of 0.003, 0.005, 0.003, and 0.015, respectively.

**Discussion**

The result that ethanol could extract more phenolic and flavonoid content in this work was similar to a report for *Limnophila aromatica*. Furthermore, Muruzović et al. found that ethanol extraction of phenolic compound yields results close to water extraction; however, ethanol extraction yields the highest content of total flavonoid from *Agrimonia eupatoria*. Sivarajanani et al. reported the IC$_{50}$ of *S. jamaicensis* leaf extract from DPPH radical scavenging as 164 µg/mL, which was close to our results of 185.41±7.64 µg/mL. However, the IC$_{50}$ value of leaves with 95% ethanol extraction from FRAP assay in our work was very low compared to previous report: 66.74±0.37 µg/mL vs 495 µg/mL. Do et al. reported that the ethanol extract of *Limnophila aromatica* had the highest antioxidant activity due to the high total phenolic content and total flavonoid content. El-Haskoury et al. reported that antioxidant capacity of *Ceratonia siliqua* honeys correlated with total phenolic content and total flavonoid content. Lin et al. also reported that *Cyclea gracillima* extract with a high content of polyphenolic compound offered remarkable antioxidant activity in six assays. The correlations between the content of the two compounds and antioxidant activity were also reported in the previous publications. Lou et al. found positive correlation between DPPH scavenging potency and total phenolic content of *Citrus mitis* (R = 0.689, p < 0.01) as well as total flavonoid content (R= 0.491, p < 0.05). The linear correlation between DPPH radical scavenging activity and total phenolic content of *Melilotus albus* and *Dorycnium herbaceum* of R = 0.84 was similar to total flavonoid content where R = 0.65. The correlations between content of phenolic and flavonoid compound and DPPH radical scavenging activity were also investigated by Muruzović et al. Moreover, high correlation between antioxidant activity and total...
phenolic content of Ruta chalepensis was also reported. The positive linear correlation between total phenolic content and DPPH radical scavenging activity was observed ($R = 0.85$), while DPPH radical scavenging activity and flavonoid content had a negative linear correlation ($R = -0.09$).

**Conclusions**

Leaves extracted using 50% ethanol and twigs extracted using water had the highest and the lowest total phenolic content, respectively. This is true as well for total flavonoid content. The highest antioxidant activity was found in twigs extracted using 95% ethanol and leaves extracted using 50% ethanol for DPPH radical scavenging assay and FRAP assay, respectively. A high correlation coefficient was found when total phenolic content, total flavonoid content, and antioxidant activity were compared.

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**References**