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**Research paper**

# Antithrombotic and antioxidant effects of *Sophora japonica* flower buds collected in different provinces in Vietnam, in correlation with rutin and other phytochemicals content

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## ABSTRACT

***Context:*** flower buds of *Sophora japonica* L. (SJ), a great source of rutin, have been popular for several pharmacological effects, but limited studies were concerned with the correlation of rutin and the bioactivities of this plant. ***Objective:*** to evaluate the *ex vivo* antithrombotic and *in vitro* antioxidant activities of rutin and SJ extracts. ***Methods:*** the antiaggregatory and anticoagulant activities were investigated on human plasma. The antioxidative effect was determined by different radical scavenging assays. Rutin content, total phenolic content, and flavonoid content were analyzed and correlated with the bioactivities. The results showed that most SJ samples exhibited platelet aggregation inhibitory effects by reducing the maximal aggregation percentage and lowering the aggregation speed. Moreover, these samples were able to prolong the blood coagulation time via the intrinsic and common pathways. Rutin showed a weaker antithrombotic effect than SJ samples, suggesting that components in the plant extracts might possess a synergic inhibitory action on blood clot formation. All samples expressed a strong scavenging ability against both DPPH and ABTS radicals (with $IC_{50}$ lower than 50 μg/mL), but a moderate effect on reducing $H_2O_2$. Pearson correlation analysis revealed that rutin, TFC, and TPC were strongly correlated with the antithrombotic effect of the plant extracts. Meanwhile, rutin content and TFC significantly correlated with $H_2O_2$ assay while TPC was remarkably associated with DPPH and ABTS assays. ***Conclusion:*** the findings in this study provide a reference for exploring *S. japonica* samples in the prevention and treatment of thrombosis- and oxidative stress-related diseases.

***Keywords:*** antioxidant, antithrombotic, phytochemical-activity correlation *rutin*, *Sophora japonica*

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## INTRODUCTION

*Sophora japonica* L. extracts have been widely used in folk medicine throughout Eastern Asia to treat various ailments, including cancers, inflammatory conditions, wounds, bleeding disorders, etc, (Dos Santos et al., 2023; He et al., 2016). The antioxidative effect of this plant has been expressed by scavenging ability against DPPH (Zhang et al., 2024), and ABTS radicals (Abdelhady et al., 2014); the oxygen radical absorbance capacity (ORAC) (Park et al., 2022) or the halting of lipid oxidation within food chains (Mihaylova and Schalow, 2013) Besides that, previous research showed that *S. japonica* and its isolated compounds could effectively prevent the formation of blood clots, or thrombosis, in animal models (Gong et al., 2023; Kim and Yun-Choi, 2008; Mihaylova and Schalow, 2013). In the literature, many scientific reports demonstrated that the oxidative stress caused by an overproduction of reactive oxygen species has been believed to significantly promote thrombus formation (Li et al., 2024; Wang and Zennadi, 2020). Therefore, the use of antioxidants against oxidative stress might have a positive impact on reducing the risk of thrombosis-associated cardiovascular diseases.

Phytochemical analysis of *S. japonica* has shown that this medicinal plant contains various groups of compounds including flavonoids, isoflavonoids, and polysaccharides. Notably, rutin also known as quercetin-3-rutinoside, is the most prevalent and important flavonoid found in *S. japonica* (Guo et al., 2024). Besides rutin, this plant also possessed other potential polyphenols such as quercitrin, quercetin, kaempferol, or hyperoside (Tian et al., 2022). In the literature, many previous studies have demonstrated high contents of rutin as well as total flavonoid and total phenolic compounds in *S. japonica* (Nguyễn Thị Thu Huyền, 2010; Tian et al., 2022). In Vietnam, *S. japonica* commonly known as "*hoa hoè*", is cultivated popularly in many places along the country. Thai Binh province is considered the largest growing area of *S. japonica*, with high rutin contents up to 35.20% (Nguyễn Thị Thu Huyền, 2010), followed by Quang Tri province with 32.49% of rutin and 48.40% of total flavonoid (Le et al., 2024). However, the correlation between those phytoconstituents and the bioactivity of the plant is still limited.

The present study aimed to evaluate the *ex-vivo* antithrombotic effect on human plasma of rutin and *S. japonica* collected in different provinces in Vietnam. The *in vitro* antioxidant activity of those plant samples was also confirmed by several radicals' scavenging abilities. In addition, the amount of rutin, total flavonoid, and phenolic compounds contained in *S. japonica* was determined to seek correlations between the phytochemicals and bioactivities of this plant.

## METHODS

### *Chemicals*

Aspirin, collagen, heparin, and dimethyl sulfoxide (DMSO) were provided by Sigma-Aldrich. Blood clotting reagents including APTT, PT, and TT reagents were obtained from Dade Behring Marburg GmbH (Marburg, Germany). Solvents (pure water and methanol) were purchased from Thermo Fisher. Radicals (1,1-diphenyl- 2-picryhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and $H_2O_2$) and other reagents ($Na_2CO_3$, NaOH, $NaNO_2$, $AlCl_3$, gallic acid, and Folin-Ciocalteu, ascorbic acid, trolox) were provided by Sigma Aldrich.

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***Plant material and extraction***

Voucher specimens were kept at the Department of Life Science, University of Science and Technology of Hanoi. 100 mg of dried powder of each sample was extracted 3 times with 1 mL methanol using sonication for 20 minutes, then centrifuged for 10 minutes at 10000 rpm. The supernatant was stored at 4°C before analysis.

***Blood sampling***

The ethics approval was obtained from the Research Ethics Committee, School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (document number 022020/CN- HĐĐĐ). Healthy adults between the ages of 18 and 25 had blood samples taken. All of the samples were collected while fasting in the morning. None of the donors had any allergies, lipid or carbohydrate metabolism issues, blood coagulation issues, or medication use in the two weeks before the test. Each subject had two milliliters of venous blood extracted, which was then placed in a polypropylene centrifuge tube with 3.8% sodium citrate (9:1 ratio) acting as an anticoagulant. To make platelet-rich plasma (PRP), entire blood was centrifuged for 10 minutes at room temperature at 500 rpm. $(250 \pm 25) \times 10^9$/L was the adjusted platelet count in PRP. After removing the PRP, the leftover material was centrifuged for 10 minutes at 3000 rpm to produce platelet-poor plasma (PPP). After the blood was drawn, all plasma samples were used within three hours.

***Ex vivo antiplatelet aggregation test***

The antiplatelet aggregation studies were performed using the turbidimetric method with some modifications (Le et al., 2022). The mixtures of PRP (450 µL) and plant extracts (50 µL) at final concentrations of 0.125, 0.25, and 0.5 mg/mL in DMSO 0.1% were kept at 37°C for 3 minutes and then the aggregation was activated by adding 1 µL of collagen 1µM. The samples were further incubated at 37°C with constant stirring at 1200 rpm for 5 min and measured for aggregation using an aggregometer (490-2D, USA). DMSO 0.1% and aspirin at 0.1 mg/mL were respectively utilized as the negative and positive controls. The antiplatelet effect was evaluated by the capacity to increase the inhibition percentage of platelet aggregation and/or reduce the aggregation speed (represented by the slope of the aggregation curve). The inhibition percentage of platelet aggregation generated by plant extracts was calculated as follows: % Inhibition = (X – Y)/X x 100, where *X* is the maximum aggregation of the negative control; *Y* is the maximum aggregation of the tested samples.

***Ex vivo anticoagulant assay***

The anticoagulation activity of plant samples was determined by measuring PT (prothrombin time), activated partial prothrombin time (APTT), and thrombin time (TT) using a Sysmex CS-2100i machine (Japan). The mixtures of 50 µL of plant extracts at different concentrations and 450 µL of PPP were prepared and incubated at 37°C for 5 minutes. PT, APTT, and TT reagents were subsequently added to generate the blood coagulation via the extrinsic, intrinsic, and common coagulation pathways, respectively. DMSO 0.1% in plasma was used as the negative control. Heparin was used as the positive control at 0.2 IU/mL for APTT and PT but at 2 IU/mL for PT assay. Both antiplatelet and anticoagulant assays were carried out in triplicates on three different blood samples.

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***DPPH and ABTS assays***

These assays were performed following the method described in the study by Le et al (Le et al., 2022). The *S. japonica* extracts at 100 to 1000 µg/mL and rutin at 5 to 50 µg/mL were prepared in methanol (MeOH). In each well of a 96-well plate, a total of 200 µL mixture composed of 10 µL of the sample and 190 µL of each radical solution were incubated at 37°C for 20 minutes for DPPH and at 25°C in the dark for 10 minutes for ABTS assay. The absorbance of the mixtures was correspondingly recorded at 517 and 734 nm using a microplate spectrophotometer (xMark, Bio-Rad). Ascorbic acid and trolox were used as the positive control for DPPH and ABTS assays. The inhibition against DPPH or ABTS radical was determined as: % Inhibition = 100 - (As/ Ac x100%), where As represents the absorbance of the samples and Ac represents the absorbance of the control containing only DPPH solution.

***$H_2O_2$ assay***

The capacity of *S. japonica* samples to neutralize $H_2O_2$ radicals was evaluated following the method of Merugu et al. (2017) with minor changes. Briefly, 150 µL of various concentrations of the *S. japonica* extracts or pure rutin were incubated with 2.85 mL of hydrogen peroxide solution (20 mM in water) for 10 minutes. The absorbance of the solution was read at 230 nm using a blank solution (MeOH) for baseline correction. Ascorbic acid was used as the positive control. The percentage of scavenged hydrogen peroxide was determined with the equation: $\% I = [(A_c – (A_s - A_0)) / A_c] \times 100$, where $A_c$ represents the control solution's absorbance (hydrogen peroxide solution without extract), $A_0$ represents the absorbance of *S. japonica* samples without hydrogen peroxide, and $A_s$ represents the absorbance of *S. japonica* samples with hydrogen peroxide solution. $IC_{50}$ values, the concentrations of samples that scavenge 50% of the radicals, were determined using the linear curve between %I and concentrations for each sample.

***Quantification of rutin by HPLC-DAD method***

The chromatographic technique was performed using an HPLC system (Ultimate 3000, Thermo Scientific, UK) coupled with a diode array detector (DAD). The methanolic extracts of *S. japonica* samples were diluted into concentrations of 195.31, 390.62, and 781.25 mg/L. 20 µL of each sample were loaded on a BDS Hypersil C18 column (250 x 4.6 mm, 5 µm) at 1.4 mL/min, under a gradient with 1% acetic acid (solvent A) and methanol (solvent B) as follows: 0 - 2 min: 0 - 10% B, 2.1 – 4 min: 30% B, 4.1 – 9 min: 55% B, 9.1 – 10 min: 10% B. Rutin absorbance was measured at 257 nm. The calibration curve of rutin was constructed from 12.5 to 150 mg/L.

***Total flavonoid content***

The aluminum chloride colorimetry assay was utilized to quantify the total flavonoid content of *S. japonica* samples (Le et al., 2024). 50 µL of each *S. japonica* extract was incubated with 10 µL of sodium nitrite solution (5%) for 6 minutes at 25°C. Next, 10 µL of aluminum chloride solution (10%) was mixed, followed by the same incubation period. 80 µL of sodium hydroxide solution (1M) and 50 µL of 30% ethanol were added to the reaction mixture and incubated at 25°C for 15 mins. The absorbance of the solution was read at 520 nm. A calibration curve of rutin (in MeOH) was constructed to determine the total flavonoid content (TFC) expressed as grams of rutin equivalents per 100 grams of the sample (g RE/100g dried extract).

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***Total phenolic content***

This experiment was performed using the Folin-Ciocalteu method (Abdelhady et al., 2014). 10 µL of each *S. japonica* extract was mixed with 95 µL of 10 times diluted Folin-Ciocalteu reagent and rest for 1 minute. Then, 95 µL of $Na_2CO_3$ 6% (in water) was added to the mixture and kept at 40°C for 15 minutes. The absorbance of the blue complex obtained after incubation was measured at 765 nm. A calibration curve of gallic acid (in MeOH) was used to determine the total phenolic content (TPC) expressed as grams of gallic acid equivalents per 100 grams of the dried sample (g GAE/100g sample).

Statistical analysis

***Statistical analysis***

All results were analyzed using the GraphPad 9.5.0 software and represented as the means ± standard deviation (SD). Data were statistically compared using one-way ANOVA tests, and Tukey post-tests. Pearson correlation was analyzed to determine the statistical association between the phytochemicals content and the bioactivities of *S. japonica* samples.

## RESULTS

***Plant material***

Dried flower buds of *Sophora japonica* (*S. japonica*) were harvested at different places in Vietnam in June 2024 and labeled as mentioned in Table 1.

**Table 1.** Labels for *S. japonica* samples collected in different provinces

| Symbol | Date of collection | Collection place | Voucher number |
|---|---|---|---|
| SJ1 | 10/6/2024 | Dien Bien district, Dien Bien province | SJ.DB.01 |
| SJ2 | 05/6/2024 | Vu Thu district, Thai Binh province | SJ.TB.02 |
| SJ3 | 05/6/2024 | Hung Ha district, Thai Binh province | SJ.TB.03 |
| SJ4 | 15/6/2024 | Dien Chau district, Nghe An province | SJ.NA.04 |
| SJ5 | 08/6/2024 | Hai Hau district, Nam Dinh province | SJ.ND.05 |
| SJ6 | 09/6/2024 | Cu Kum district, Dak Lak province | SJ.DL.06 |

***Ex vivo antiplatelet aggregation activity***

The antiaggregatory effect of the samples was expressed by two factors: percentage of inhibition (% I) and reduction of aggregation speed (slope). Figure 1 shows the aggregation curve of rutin in comparison with the control, in which the maximal percentage of aggregation tended to decrease with increasing concentrations of rutin. Therefore, the percentage of inhibition of rutin increased while the speed of aggregation decreased from 0.5 to 2 mg/mL.

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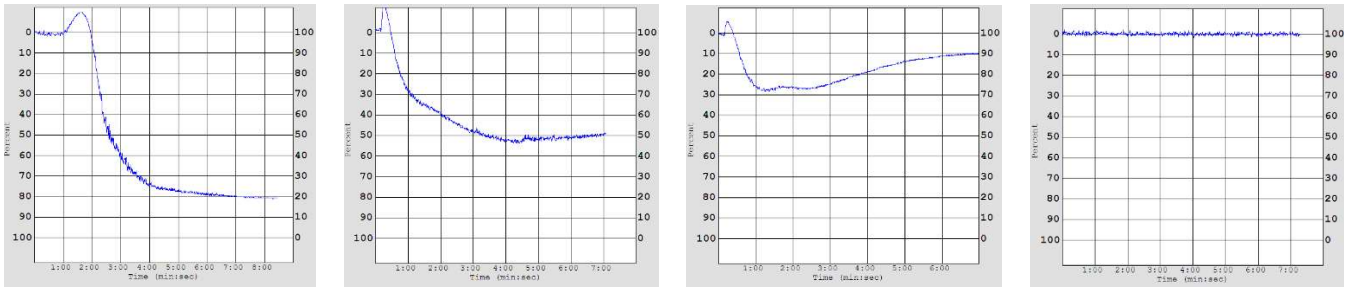

Control (0.1% DMSO) Rutin at 0.5 mg/mL Rutin at 1 mg/mL Rutin at 2 mg/mL

**Figure 1.** Aggregation curve of rutin at different concentrations compared to the negative control

Figure 2 demonstrated that except for SJ3, all SJ samples from 0.25 mg/mL displayed the highest inhibitory effect which is comparable to the positive control used ($p < 0.05$ compared to aspirin). Particularly, samples SJ4, SJ5, and SJ6 showed approximately 100% inhibition at the lowest concentration tested (0.125 mg/mL). Meanwhile, rutin was able to reach 100% I at 2 mg/mL, which is 4 to 8 times more concentrated than the extracts. This finding suggested that components in *S. japonica* might exhibit their synergic effect on the platelet aggregation, so their activity was stronger than rutin itself. Therefore, SJ samples can be considered a promising source to impede platelet aggregation.

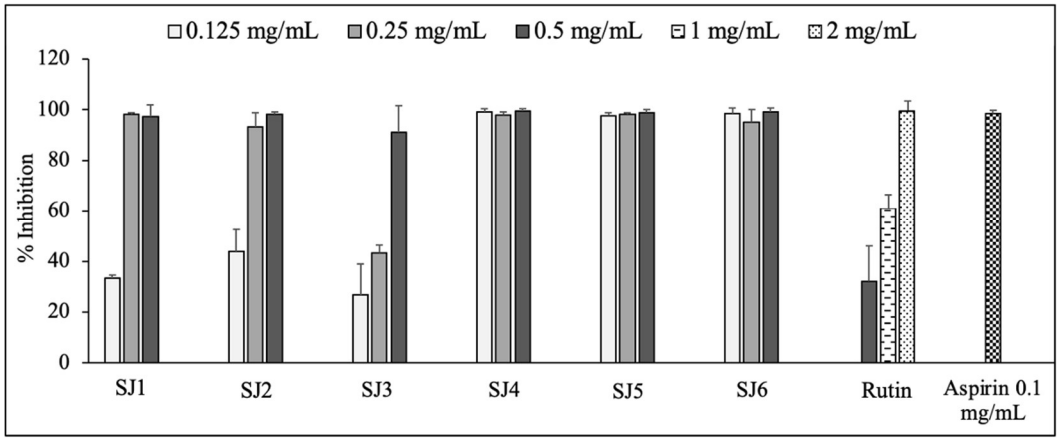


**Figure 2.** Percentage of inhibition on the collagen-induced platelet aggregation

The aggregation speed of most of the samples decreased with increasing concentrations (Figure 3). From 0.25 mg/mL, all SJ samples had slower aggregation velocities in comparison with the control ($p < 0.05$). Particularly, SJ5 expressed remarkably smaller slopes than the control sample at three concentrations tested, and comparable to the effect of aspirin at 0.1 mg/mL. Rutin displayed a dose-dependent effect on lowering the speed of platelet aggregation from 0.5 to 2 mg/mL. However, the activity of rutin was weaker than all SJ samples, indicating that the use of the plant extracts seems to be more beneficial than pure rutin.

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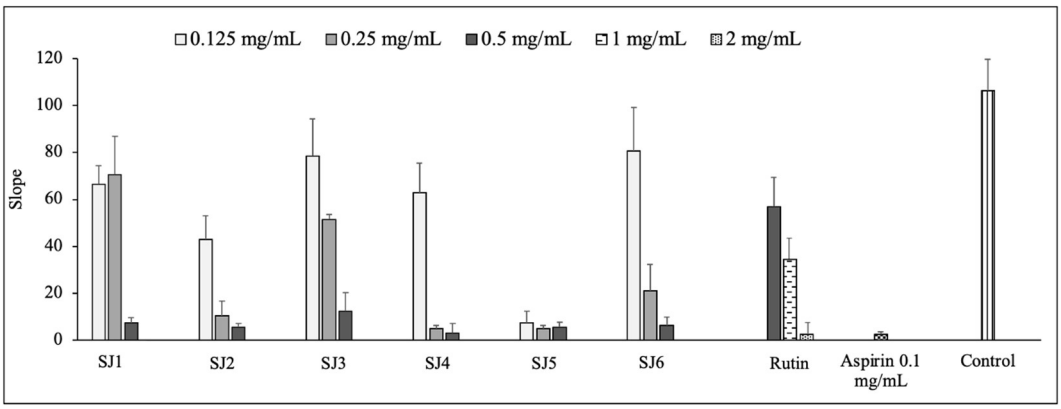


**Figure 3.** Aggregation slope of *S. japonica* samples

***Ex vivo anticoagulant activity***

The results in Table 2 show that most of the *S. japonica* bud flower samples at 1 mg/mL displayed the effect of prolonging the coagulation time *via* the intrinsic coagulation pathway (APTT) and the common coagulation pathway (TT) when compared to DMSO 0.1% ($p < 0.05$). However, all tested samples were not able to prolong the extrinsic coagulation time (PT) regardless of the concentrations used ($p > 0.05$ compared to DMSO 0.1%). Moreover, all plant samples were significantly less effective than the positive control in all 3 coagulation pathways ($p < 0.05$ compared to heparin). Rutin at 0.5 to 2 mg/mL only could extend the time of blood clot formation in the common cascade TT. Like the antiplatelet activity, the use of plant extracts seems to be more effective than rutin in prolonging blood clot formation.

***Antioxidant activity of S. japonica samples and rutin***

According to the results shown in Table 3, all six tested samples expressed a strong scavenging ability against both DPPH and ABTS radicals (with $IC_{50}$ lower than 50 µg/mL), but only a moderate effect against $H_2O_2$ ($IC_{50}$ up to 375.24 µg/mL). Typically, SJ5 displayed a significantly stronger activity than other samples for 3 tested assays ($p < 0.05$). The $IC_{50}$ of SJ5 was 28.29, 29.03, and 89.14 µg/mL respectively for DPPH, ABTS, and $H_2O_2$. However, those values were much higher than the ones of rutin ($p < 0.05$). This means that rutin is a powerful antioxidant agent, which can destroy several radicals.

***Phytochemical analysis***

The results presented in Table 4 demonstrated a significant difference in the amount of rutin obtained in several collection places (ANOVA, $p < 0.05$). Generally, the content of rutin ranged from 18.77% in SJ3 (Hung Ha, Thai Binh) to 31.41 % in SJ5 (Nam Dinh). SJ2 (Vu Thu, Thai Binh) and SJ6 (Dak Lak) had comparable amounts of rutin while SJ1 (Dien Bien) had a similar content of rutin as SJ4 (Nghe An) ($p > 0.05$).

TFC ranged from 18.89 in SJ3 to 31.86 g RE/100 g dried weight in SJ5 while TPC was obtained at much lower values ranging from 4.89 in SJ1 to 9.08 g GAE/100 g in SJ5 (Table 4). It is worth noting that SJ5 had a remarkably more abundant content of rutin, total flavonoid, and total phenolic compounds than other samples ($p < 0.05$).

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**Table 2.** Coagulation time of rutin and *S. japonica* samples

| Samples | | Concentration (mg/mL) | APTT (s) | PT (s) | TT (s) |
|---|---|---|---|---|---|
| SJ1 | | 0.25 | 32.35 ± 2.62 | 12.3 ± 1.83 | 15.7 ± 0.42$^{\#,*}$ |
| | | 0.5 | 33.25 ± 1.48 | 12.4 ± 0.57 | 16.00 ± 0.14$^{\#,*}$ |
| | | 1 | 33.65 ± 0.07$^{\#*}$ | 12.4 ± 0.85 | 17.15 ± 0.78$^{\#,*}$ |
| SJ2 | | 0.25 | 31.75 ± 2.76 | 12.15 ± 1.91 | 15.35 ± 1.2 |
| | | 0.5 | 31.8 ± 0.85 | 12.2 ± 0.99 | 14.8 ± 1.27 |
| | | 1 | 34.45 ± 2.19$^{*}$ | 11.95 ± 0.21 | 17.25 ± 1.63$^{\#,*}$ |
| SJ3 | | 0.25 | 31.9 ± 2.26 | 12.1 ± 1.84 | 15.4 ± 0.71 |
| | | 0.5 | 31.55 ± 0.35 | 12.45 ± 0.49 | 14.95 ± 0.35 |
| | | 1 | 34.55 ± 2.05$^{*}$ | 12.8 ± 1.41 | 17.6 ± 1.13$^{\#,*}$ |
| SJ4 | | 0.25 | 31.85 ± 2.19 | 12.3 ± 1.84 | 15.7 ± 0.28$^{\#,*}$ |
| | | 0.5 | 32.7 ± 0.14 | 13.2 ± 0.00 | 16.6 ± 0.42$^{\#,*}$ |
| | | 1 | 33.9 ± 0.00$^{\#*}$ | 12.65 ± 1.34 | 18.1 ± 0.28$^{\#,*}$ |
| SJ5 | | 0.25 | 31.7 ± 2.12 | 12.25 ± 1.77 | 15.8 ± 0.71$^{\#,*}$ |
| | | 0.5 | 33.85 ± 1.48 | 13.45 ± 0.21 | 16.8 ± 0.42$^{\#,*}$ |
| | | 1 | 36.35 ± 1.63$^{\#*}$ | 12.15 ± 0.49 | 16.9 ± 1.84$^{\#,*}$ |
| SJ6 | | 0.25 | 31.85 ± 1.48 | 12.15 ± 1.63 | 15.4 ± 0.42$^{\#,*}$ |
| | | 0.5 | 30.8 ± 3.54 | 12.25 ± 1.63 | 15.3 ± 0.28$^{\#,*}$ |
| | | 1 | 34.55 ± 0.35$^{\#*}$ | 12.5 ± 1.27 | 16.5 ± 1.41$^{\#,*}$ |
| Rutin | | 0.5 | 32.95 ± 4.87 | 11.90 ± 0.28 | 16.40 ± 0.71$^{\#,*}$ |
| | | 1 | 33.60 ± 3.96 | 11.95 ± 0.21 | 16.75 ±0.63$^{\#,*}$ |
| | | 2 | 33.55 ± 5.02 | 12.05 ± 0.35 | 16.65 ± 0.35$^{\#,*}$ |
| Negative control | | 0.1% DMSO | 30.45 ± 1.78 | 11.6 ± 1.43 | 13.63 ± 0.8 |
| Positive control | | Heparin | 47.01 ± 1.90 | 31.21 ± 0.82 | ± 1.30 |

$^{\#}$ and $^{*}$ refer to the significant differences between SJ samples and the negative control and positive control, respectively ($p < 0.05$).

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**Table 3.** Antioxidant activity of *S. japonica* samples

| Sample | DPPH ($IC_{50}$, µg/mL) | ABTS ($IC_{50}$, µg/mL) | $H_2O_2$ ($IC_{50}$, µg/mL) |
|---|---|---|---|
| SJ1 | $49.87 \pm 0.99^{a}$ | $48.95 \pm 1.26^{a}$ | $302.52 \pm 28.05^{a}$ |
| SJ2 | $44.90 \pm 3.59^{b}$ | $42.15 \pm 2.05^{b}$ | $322.59 \pm 18.87^{a}$ |
| SJ3 | $47.27 \pm 1.06^{b}$ | $45.54 \pm 1.75^{b}$ | $375.24 \pm 29.85^{b}$ |
| SJ4 | $37.11 \pm 2.29^{c}$ | $33.22 \pm 1.21^{c}$ | $312.89 \pm 25.21^{a}$ |
| SJ5 | $28.29 \pm 0.96^{d}$ | $29.03 \pm 1.02^{d}$ | $89.14 \pm 5.27^{c}$ |
| SJ6 | $42.39 \pm 0.70^{b}$ | $36.62 \pm 1.72^{b}$ | $334.38 \pm 15.41^{a}$ |
| Rutin | $12.52 \pm 0.51^{**}$ | $12.63 \pm 0.23^{**}$ | $\pm 6.54^{**}$ |
| Positive control | $10.32 \pm 1.07^{***}$ | $8.29 \pm 0.37^{***}$ | $30.52 \pm 2.15^{***}$ |

Different superscript letters mean considerable differences among SJ samples ($p < 0.05$). ** and *** refers to significant differences compared to rutin ($p < 0.01$) and positive control ($p < 0.001$).

**Table 4.** Chemical composition of *S. japonica* samples

| Sample | Rutin content (g /100g DW) | TFC (g RE/100g DW) | TPC (g GAE/100g DW) |
|---|---|---|---|
| SJ1 | $20.93 \pm 1.04^{a}$ | $21.59 \pm 0.42^{a}$ | $4.89 \pm 0.03^{a}$ |
| SJ2 | $23.18 \pm 0.84^{b}$ | $23.66 \pm 0.52^{b}$ | $5.39 \pm 0.18^{b}$ |
| SJ3 | $18.77 \pm 0.95^{c}$ | $18.89 \pm 0.84^{c}$ | $5.75 \pm 0.22^{b}$ |
| SJ4 | $20.52 \pm 0.91^{a}$ | $21.52 \pm 0.78^{a}$ | $8.01 \pm 0.24^{c}$ |
| SJ5 | $31.41 \pm 1.23^{d}$ | $31.86 \pm 0.79^{d}$ | $9.08 \pm 0.47^{d}$ |
| SJ6 | $22.19 \pm 0.55^{b}$ | $23.12 \pm 0.43^{b}$ | $6.88 \pm 0.35^{e}$ |

DW: dried weight of *S. japonica* samples, RE: rutin equivalent, GAE: gallic acid equivalent, TFC: total flavonoid content, TPC: total polyphenol content. Different superscript letters refer to significant differences among samples in each column

The Pearson correlation analysis indicated that rutin content was highly associated with TFC ($R^2 = 0.95$, $p < 0.001$) but not in agreement with TPC ($R^2 = 0.42$, $p > 0.05$) (Figure 4). Moreover, there is an insignificant difference between rutin content and TFC (Table 4), meaning that rutin accounted for most of the flavonoid compounds obtained in *S. japonica.*

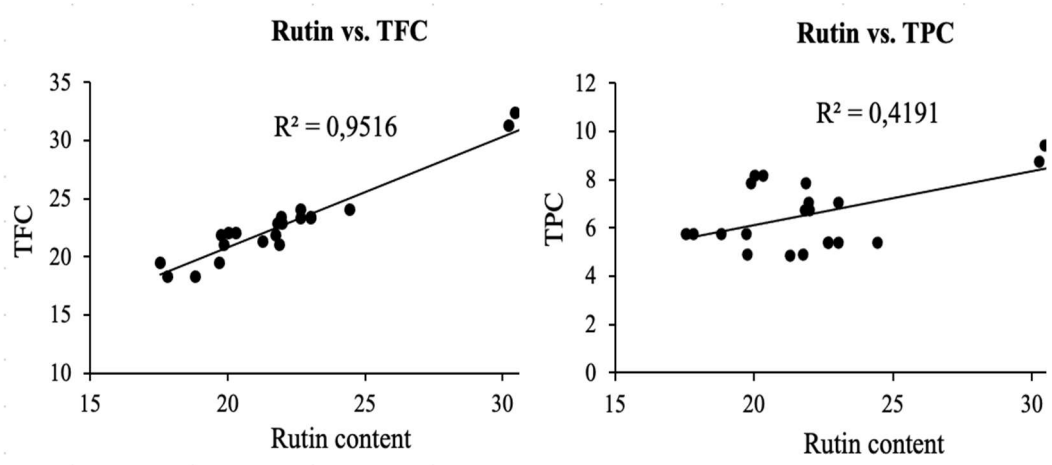


**Figure 4.** Correlation between rutin content and TFC, TPC


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***Correlation between phytochemicals and antithrombotic effect***

The relationship between the amount of rutin, TFC, and TPC and the antithrombotic effect of SJ samples collected in different provinces in Vietnam was determined using Pearson correlation analysis for the first time. In terms of the antiplatelet effect, results shown in Table 5 indicate that the rutin content, TFC, and TPC values were significantly and negatively correlated with the slope ($p < 0.05$). This finding means that increasing rutin, TFC, and TPC probably decreased the slope of the aggregation curve, so lowered the platelet aggregation speed. On the contrary, those factors were not considerably associated with the percentage of inhibition ($p > 0.05$), suggesting that other types of compounds might contribute to the inhibitory effect of SJ samples. In terms of the anticoagulant activity, the rutin content, TFC, and TPC were strongly correlated with both APTT and TT factors with high Pearson correlation coefficients and low p-values.

**Table 5.** Correlation between rutin content, TFC, TPC, and antithrombotic effect of SJ samples

| | Antiplatelet effect | | | | Anticoagulant effect | | | |
|---|---|---|---|---|---|---|---|---|
| | %I | | Slope | | APTT | | TT | |
| | R | p-value | R | p-value | R | p-value | R | p-value |
| Rutin content | 0.51 | 0.91 | - 0.70 | $4.86 \times 10^{-8}$ | 0.89 | $2.12 \times 10^{-6}$ | 0.73 | 0.002 |
| TFC | 0.51 | 0.92 | - 0.71 | $4.01 \times 10^{-8}$ | 0.89 | $2.52 \times 10^{-6}$ | 0.74 | 0.001 |
| TPC | 0.52 | 0.99 | - 0.72 | $5.06 \times 10^{-8}$ | 0.85 | $2.15 \times 10^{-5}$ | 0.78 | 0.0004 |

***Correlation between phytochemicals and antioxidant effect***

The obtained results in Table 6 indicated that both rutin content and TFC were only significantly correlated with the $H_2O_2$ assay. It means that an increase in rutin content in *S. japonica* samples certainly leads to a stronger scavenging ability against $H_2O_2$. In contrast, a significant correlation between TPC and both DPPH and ABTS assay indicates that an increase in TPC will lead to a more potent antioxidative effect of the *S. japonica* sample against those two radicals.

**Table 6.** Correlation between rutin content, TFC, TPC, and antioxidant activity of SJ samples

| | DPPH ($IC_{50}$, μg/mL) | | ABTS ($IC_{50}$, μg/mL) | | $H_2O_2$ ($IC_{50}$, μg/mL) | |
|---|---|---|---|---|---|---|
| | R | p-value | R | p-value | R | p-value |
| Rutin content | - 0.78 | 0.06 | - 0.69 | 0.12 | - 0.92 | 0.008 |
| TFC | - 0.79 | 0.06 | - 0.72 | 0.11 | - 0.92 | 0.009 |
| TPC | - 0.97 | 0.002 | - 0.94 | 0.005 | - 0.67 | 0.15 |

## DISCUSSION

*Sophora japonica* flower buds have widely been investigated for several pharmacological activities, in which the antioxidative and anti-cardiovascular effects are two of the most highlighted ones (He et al., 2016; Hn and Cl, 2010). The phytochemical analysis of this plant revealed the main phenolic constituents such as rutin, quercetin, kaempferol, japonicasins A, or genistein (Gong et al., 2023). Among those compounds, rutin has been considered the most important marker for quality control of *S. japonica* flower buds, due to its large amount and significant contribution to the

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various effects of the plant (Nguyen et al., 2024). The present research evaluated and compared the antithrombotic and antioxidant activities between rutin and the plant extracts, then correlated with rutin content in *S. japonica* samples.

The results showed that rutin displayed a weaker antithrombotic activity than the plant extracts. This suggests a synergetic effect between rutin and other components in *S. japonica*. In the study of Kim *et al.*, rutin was found to be less effective than other compounds isolated from *S. japonica* such as biochanin A, irisolidone, genistein, or tectoridin (Kim and Yun-Choi, 2008). However, the content of those components seems to be much lower than rutin, therefore, they could act synergically to make a greater antiplatelet effect of *S. japonica* compared to rutin. Moreover, a few studies demonstrated that rutin exhibited anticoagulant activity via APTT and PT pathways (Choi et al., 2015, 2021). The current study added evidence proving that this compound was also able to prolong blood coagulation via the common pathway.

Rutin, total phenolic, and flavonoid content were found to be significantly correlated with the antiaggregatory and anticoagulant activities for the first time. This result signifies that an increase in phenolic compounds in general could certainly prevent blood clot formation and then decrease the risk of cardiovascular diseases.
Unlike the antithrombotic effect, rutin was found to be significantly stronger than *S. japonica* extracts. Together with its high abundance, rutin greatly contributed to the potent antioxidative effect of this plant. In this experiment, *S. japonica* samples and rutin were tested for their scavenging capacity against $H_2O_2$ for the first time. Unfortunately, most of *S. japonica* samples did not express a potent effect, except for SJ5 with an $IC_{50}$ value lower than 100 μg/mL. This result of SJ5 is remarkably better than many plant extracts investigated in the study of Keser et al. (Keser et al., 2012), or Fernando et al. (Fernando and Soysa, 2015), but similar to the effect of black tea *Camellia sinensis* (Fernando and Soysa, 2015). The correlation analysis indicated that besides rutin found as the most abundant flavonoid in *S. japonica* flower buds, other phenolic components might also contribute to the significant antioxidative activity of this plant. In a recent study, Tian et al. demonstrated an accumulation of several polyphenols such as quercitrin, quercetin, kaempferol, or hyperoside in *S. japonica* samples (Tian et al., 2022). These compounds have been proven to be strong antioxidant agents (Bangar et al., 2023), so their presence can probably contribute to the antioxidative effect of the plant.

Rutin levels varied from 18.77 to 31.41%, which is similar to samples originating from China, also a large *S. japonica* cultivating country (Liao et al., 2015; Peng et al., 2018). Although soil and regional factors can greatly affect rutin content, both countries Vietnam and China are considered potential sources for providing *S. japonica* at high quality.

## CONCLUSION

In the current study, flower buds of *Sophora japonica* collected in different provinces in Vietnam expressed potential antithrombotic and antioxidant activities. Moreover, those samples contained high amounts of rutin, total flavonoids, and phenolic compounds. Interestingly, there are significant correlations between those phytochemicals and bioactivities of *S. japonica* samples. In detail, rutin, TFC, and TPC were strongly correlated with the capacity to decelerate the aggregation speed and prolong the coagulation time of the plant extracts. In terms of the antioxidant effect, rutin and TFC were remarkably correlated with $H_2O_2$ scavenging assay while TPC was considerably associated with DPPH and ABTS assays. The findings in this research provide evidence to justify that *S. japonica* is a promising source rich in bioactive

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compounds for preventing and treating thrombosis and oxidative stress-related diseases. Furthermore, the result of different growing locations is useful for selecting supplying sources of raw materials, served for developing functional food products of high quality from *S. japonica*.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper's content.

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## DECLARATION OF HONOR

We declare on our honor that our research are not fake and make up.

## AI ASSISTANCE DISCLOSURE

The authors used [ChatGPT/GPT-5] to improve the clarity and readability of the manuscript. The authors carefully reviewed and edited the content to ensure accuracy and take full responsibility for the final text.

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