Majalah Obat Tradisional (Trad. Med. J.), January-April 2026
Vol. 31(1), p 63-71
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 14-12-2024
Revised : 05-07-2025
Accepted : 22-07-2025

The Promising Free Radical Scavenging Activity of Some Fabaceae Flowers

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ABSTRACT

Oxidative stress caused by free radicals is a key contributor to the development of chronic diseases. As a natural defense strategy, the identification of plant-based free radical scavengers has become increasingly important. This study investigates the antioxidant potential of selected flowers from the Fabaceae family, plants which common in Indonesia and contain phenolic, one of the chemicals with antioxidant properties. The objective of this study is to ascertain the total phenolic content and free radical scavenging activity of ethanol extracts of a number of Fabaceae flowers that are frequently found in the neighborhood, including *Arachis pintoi*, *Crotalaria retusa* L., *Crotalaria pallida* Aiton, and *Sesbania grandiflora* L. All samples were extracted with 70% ethanol, which was subsequently analyzed for total phenolic content and free radical scavenging activity by Folin-Ciocalteu and DPPH reagents. As the results, the highest total phenolic content was 62.962 $\pm$ 0.679 mg GAE/g sample (*A. pintoi*), while the lowest one was 23.986 ± 0.613 mg GAE/g sample (*S. grandiflora* L.). In line with the total phenolic compound, the activities of the highest free radical scavenging which is indicated by IC_{50} of those plants is 68.570 ± 1.476 µg/mL (*A. pintoi*), and the lowest value is 203.371 ± 4.706 µg/mL (*S. grandiflora* L.). These findings indicate that among the tested Fabaceae flowers, *A. pintoi* exhibits the strongest antioxidant potential, likely due to its high total phenolic content. This highlights its promise as a natural source of free radical scavengers and supports further exploration for potential therapeutic applications.

Keywords: Antioxidant; Fabaceae; Flower; Free Radical; Phenolic Compound

INTRODUCTION

Free radicals are molecules or molecular fragments that have one or more unpaired electrons (Alfa et al., 2019). Conditions of imbalance free radicals in our body cause oxidative stress that can induce the damage of cells. In order to reduce the negative effects of these free radicals, antioxidants are needed to neutralize those radicals (Sari, 2015). The human body actually produces endogenous antioxidants, but with the increasing number of free radicals entering the body, it necessary needs additional exogenous antioxidants. Unfortunately, synthetic antioxidants that widely used can often cause side effects such as DNA damage (Bouayed & Bohn, 2010). Some examples of synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) have been associated with adverse effects such as liver enlargement, carcinogenic potential, and disruption of endocrine function (Ren et al., 2025; Xu et al., 2021). For instance, BHA has shown tumor-promoting properties in the forestomach of rodents, while TBHQ has been linked to immunotoxicity and oxidative DNA damage in in vitro studies (Esazadeh et al., 2024). This is the reason for the importance of research for finding natural antioxidants.

Phenolic compounds are the largest group of compounds in plants that act as natural antioxidants. This compound has the ability to form a phenoxy radicals that are stable in oxidation reactions (Dhurhania & Novianto, 2019). The content of phenolic compounds is proven to be closely related to the antioxidant activity of the plant (Wardani et al., 2021). One of the plants that are widely found in the community and some of them are widely used empirically as a source of natural antioxidants and medicine is the Fabaceae family (Sukaeningsih et al., 2021). This family generally contains various secondary metabolites such as flavonoids, phenolics, terpenoids, and alkaloids. In more detail, some species of these plants, that are *S. grandiflora* L. Pers., *C. retusa* L., *C. pallida* Aiton, and *A. pintoi* Krapov. & W.C. Greg. have been widely used in the world of traditional medicine without knowing exactly the active substance content. *S. grandiflora* L. Pers. plant, which called as turi in local Indonesian name, has been empirically used to treat coughs, headaches, and sore throats (Sangkal et al., 2021). Meanwhile,

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the genus *Clotaria*, which is locally known as orok-orok, is a plant that grows abundantly in various regions in Indonesia. *C. retusa* L. has empirically been used to treat homoptysis, fever, and as an analgesic (Rouamba, 2018). In the meantime, *C. pallida* Aiton has empirically been used to treat urinary diseases, fever, and swelling of the joints, while the plant *A. pintoi* Krapov. & W.C. Greg. which locally known as kacang hias has been stated in several studies. Those leaves, roots, callus, and seeds have antihelmitic, allelopathic, and antioxidant activities. Furthermore, the Arachis genus is also widely believed to prevent heart disease, type II diabetes, cancer, and alzheimer's (Sousa et al., 2024).

Specifically, *S. grandiflora* L. Pers. is known to contain bioactive compounds such as saponins, tannins, flavonoids, and alkaloids, which exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Mokhtar et al., 2025). *C. retusa* L. and *C. pallida* Aiton contain alkaloids and flavonoids recognized for their antioxidant, antimicrobial, and anticancer properties (Davendra et al., 2012; Islam et al., 2018). Additionally, *A. pintoi* Krapov. & W.C. Greg. is rich in phenolic acids and flavonoids, demonstrating strong antioxidant and anti-inflammatory effects (Thang et al., 2023).

Plant flowers have their own specialty compared to other parts of the plant. This organ is the reproductive part of the plant that also has medicinal properties. Some flowers have pharmacological properties that are different from other parts of the plant or have more diverse metabolites. For example, *Butea monosperma* flowers have the following properties diuretic, astringent, and tonic properties while its roots only have analgesic properties (Gunawardana & Jayasuriya, 2019). There are still many plants having flowers as their main medicinal organ, but they have not been exploited due to lack of awareness of their potential uses (Shubhashree et al., 2017). In addition, flowers also continue to inherit metabolites from other organs as different parts of a plant may have the same metabolites because a plant species has specific biosynthetic pathways to produce certain secondary metabolites (Julianto, 2019).

Based on those descriptions above, it is possible that the flowers of *S. grandiflora* L. Pers., *C. retusa* L., *C. pallida* Aiton, and *A. pintoi* Krapov. & W.C. Greg. also have superior potential than other organs or other plants, considering that other organs in these plants have also proven their ability to treat diseases, especially its activity in capturing free radicals. Not many studies have examined the flowers of the plant. Therefore, a study is needed using a series of studies that prove its activity. One of the things that can be studied is its role in counteracting free radicals which are the source of various health problems where the activity is also closely related to the content of phenolic metabolites. In this study, it will be studied how the free radical capture activity of some Fabaceae family flowers and its relation to its phenolic content.

MATERIALS AND METHODS

Materials

The materials used in this study were sample flowers of *S. grandiflora* L. Pers., *C. retusa* L., *C. pallida* Aiton, and *A. pintoi* Krapov. & W.C. Greg., which were obtained from Yogyakarta, Indonesia. In addition, chemicals such as ethanol 70%, Folin-Ciocalteu reagent, Na_2CO_3, ascorbic acid (Merck®), gallic acid and DPPH (SigmaAldrich®) were also used. Whereas the instruments used in this study were Double Beam Spectrophotometer (Hitachi UH5300), analytical balance (Ohaus® PA214), maceration vessel, vacuum pump (HanDen VP1200), drying cabinet (UD Rekayasa Wangdi, Indonesia), vortex mixer (Thermo Scientific LP88880018), and glassware.

Methods

Sample preparation

Sesbania grandiflora L. Pers. flower samples were harvested from a flower plantation in Sewon, Yogyakarta. The flowers were harvested when the white petals were 6 - 9 cm in size. *C. retusa* L. flowers were harvested from Ngemplak area, Yogyakarta. The flowers were harvested when they were out of bud and had yellow petals. *C. pallida* Aiton flowers are harvested at area which grows in the form of a bush. This flower is also harvested when it comes out of the bud and has yellow petals. Meanwhile, the flowers of *A. pintoi* Krapov. & W.C. Greg. are harvested when the flowers bloom and are yellow in color. Raw materials collection was done manually by taking fresh flower stalks. Samples that have been collected are then determined at the Department of Pharmaceutical Biology, Faculty of Pharmacy UGM with letter's number 1745.15.1/UN1/FA.2/PT/BF/2024 for *C. pallida* and *C. retusa*; number 1716.8.3/UN1/FA.2/PT/BF/2024 for *S. grandiflora* and *A. pintoi*. The flowers that have been collected are then wet sorted by cleaning the flowers from any remaining dirt such as soil, mud, stones and other things that can disrupt the research process (Rachman et al., 2024). Flower that has been obtained is then carried out wet sorting to separate it from dirt or foreign materials. The flower was then dried in a drying cabinet at 50°C for 48 hours and pulverized to a fine degree of 60 mesh.

Extraction

The extraction method used in this research is maceration with 70% ethanol with the ratio of sample and solvent is 1: 5 for 3 days. Remaceration was carried out with a sample and solvent ratio of 1: 3 for 2 days. Both supernatants were put together and evaporated with a rotary evaporator until a thick extract was obtained.

Loss on drying

This analysis is a measurement of the remaining substances after drying process and expressed in percent value. In the test, 500 mg of the extract was weighed precisely and then heated at 105 °C for 30 minutes. After cooling in a desiccator, the sample was weighed again, and the difference between the pre- and post-heating weights was calculated. The sample was reheated and weighed repeatedly until a constant weight was achieved—that is, until two consecutive weighings differed by no more than 0.25 %. This analysis is carried out to provide a maximum limit on the amounts of compounds lost in the drying process. A good requirement for drying shrinkage is less than 10%.

Phenolic total analysis

Determination of total phenolic content was carried out spectrophotometrically with the Folin-Ciocalteu reagent (Andriani & Murtisiwi, 2020). Determining the operating time was carried out by adding of 0.5 mL of a standard solution of 30 μg/mL gallic with 2.5 mL of Folin-Ciocalteu solution (7.5% in water) and leaving for 3 minutes. Next, add by 2 mL of 7.5% Na_2CO_3 and then vortex until homogeneous. The absorbance was read at a wavelength of 765 nm over a period of 15-60 minutes. Measurement of total phenolic content in samples was carried out by adding the extract solution in a certain concentration with Folin-Ciocalteu and Na_2CO_3 solution as the determination of operating time protocol at the maximum wavelength. The total phenolic content in the sample was calculated using the gallic acid standard curve equation obtained previously. When constructing the calibration curve, gallic acid at concentrations of 20, 30, 40, 50, 60, 70, and 80 ppm yielded the equation $Y = 0.0092 X - 0.0456$ (R = 0.9969).

Radical scavenging analysis

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity test method refers to Molyneux (2004) and research conducted by Agustiarini and Wijaya (2022). DPPH stock solution was prepared by dissolving 15.8 mg DPPH with ethanol p.a in a 100 mL volumetric flask to get a solution with a concentration of 0.4 mM and then diluted again with ethanol p.a to achieve a final concentration of 50 μM. A comparison stock solution was prepared by carefully weighing 10 mg of the acid ascorbate, then dissolved in a 100 mL volumetric flask by adding ethanol p.a to get a concentration of 100 μg/mL. The operating time was determined by reacting 2 mL of acid ascorbate 100 μg/mL and 2 mL DPPH 50 μM, and then read the absorbance until a stable absorption value. Determination of the free radical scavenging activity of ascorbic acid was carried out by reacted 2 mL of 50 μM DPPH and 2 mL of 100 μg/mL ascorbic acid in a series concentration solution of 1.0; 1.5; 2.0; and 2.5 μg/mL. From this series of concentrations, the ascorbic acid regression curve was described by the equation $Y = 0.266 X - 0.0027$ (R = 0.9994).

In the samples DPPH assay, mixture of 2 mL of 50 μM DPPH with 2 mL of extract solution at a certain concentration series was incubated in dark place during operating time and read the absorption at the maximum wavelength. The absorbance of a blank (DPPH solution plus ethanol p.a) is measured as a control, and the percent radical-scavenging activity for each concentration is calculated using the formula: $(A_{control} - A_{sample}) / A_{control}) \times 100\%$ (A is absorbance). By plotting percent inhibition against sample concentration, the concentration required to scavenge 50 % of DPPH (IC_{50}) can be interpolated, providing a quantitative measure of the free radical scavenging capacity of the test substance.

Sample testing was carried out by reacting 2 mL of 50 μM DPPH with 2 mL of extract solution at a certain concentration series and then the solution was incubated in dark place during operating time and read the absorption at the maximum wavelength.

Statistical analysis

All assays were repeated three times. Results are expressed as mean ± standard deviation (SD). Analysis of variation and significance of differences were performed by One-way ANOVA followed by post hoc Tukey HSD. Correlation analysis was also performed by

Pearson Correlation Test between total phenolic content and DPPH IC_{50} results. All analyses were performed with a 95% confidence level.

RESULTS

Extract preparation

As we stated in material and method section, we used sample flowers growing in Yogyakarta, Indonesia, as Figure 1. After drying process and pulverization, the sample powder was extracted by ethanol 70% and evaporated. The sample yields and loss on drying analysis are shown in Table I.

Figure 1. The flower sample. *S. grandiflora* (a); *C. retusa* (b); *C. pallida* (c); *A. pintoi* (d)

Determination of phenolic content and free radical scavenging

As we stated in the methodology section, total phenolics and free radical scavenging of extract were determined spectrophotometrically by reacting the phenolic compounds in the sample with the Folin-Ciocalteu reagent for total phenolic determination and DPPH reagent for free radical scavenging. From the experiment results, the operating time required of total phenolic analysis was 45 minutes, while the maximum wavelength obtained was 746 nm. Meanwhile, the operating time for DPPH reaction was 25 minutes, while the maximum wavelenghth was 515 nm. Those two analysis results were shown in Table II and III.

Meanwhile, the comparison standard solution of ascorbic acid used in this study had a value of 1,890 ppm. This result is similar with a reference library of research methods conducted by Agustiarini and Wijaya (2022) who obtained an IC_{50} value for ascorbic acid of 2.058 ppm, and also research by Dewi et al (2019) which obtained the IC_{50} value of ascorbic acid is 1.899 ppm. This data indicated that the free radical capture methodology method used in this research is valid.

DISCUSSION

This study has the main objective of determining the total phenolic content of some Fabaceae plant flowers and its relation to free radical scavenging activity which is analyzed spectrophotometrically. Samples were harvested in same area and same land characteristic, that is grassy regosol soil located near a highway, in order to minimize chemical compound variations in plants which can be affected by differences in soil conditions, environment, and nutrients of plant growth (Rahayu et al., 2021). Flower samples are dried in a drying cabinet at a temperature of 50°C to ensure that the chemical content in the sample is not damaged due to the influence of heat (Ministry of Health, 2017), especially the phenolic compound which is easily oxidized. This drying process also aims to reduce the water content in the sample in order to stop enzymatic reactions degrading chemical compounds in the sample and to prevent microbial growth (Syafrida et al., 2018).

The extraction method of the sample is maceration. This method was chosen because of its convenience and low cost. In addition, this method is carried out without heating so it can reduce disruption of thermolabile chemical contents of natural materials, including phenolic compounds (Susanty & Bachmid, 2016). However, this method also has several disadvantages, for example that it requires a relatively long time and uses a lot of solvent. The maceration process in this research uses 70% ethanol, which is a solvent with good penetration ability and is suitable for dissolving various kinds of plant metabolites. This solvent has broad extraction power, including phenolic compounds, thereby minimizing the risk of compounds in the matrix not being completely extracted (Andriani & Murtisiwi, 2020).

Based on Table I, the obtained extracts have relatively uniform loss on drying, which means that each extract has a uniform solid composition, or in other words, each extract receives the same treatment in solvent evaporation. But on the other hand, each extract has a different yield. It shows that the amount of chemical content that can be extracted in the maceration process varies which is indicated that each flower sample has a different chemical profile. This phenomenon of differences in metabolite profiles, apart from being influenced by external factors, such as differences in growing location, climate and air temperature, is also

Table I. Extraction yield and loss on drying of flower sample extract

No.	Sample	Loss on drying (%)	Yield (%)
1.	*S. grandiflora* (L.) Pers.	9.09	30.18
2.	*C. retusa* L.	8.02	22.54
3.	*C. pallida* Aiton	9.22	17.51
4.	*A. pintoi* Krapov. & W.C. Greg.	9.42	28.31

Table II. Total phenolic content of four Fabaceae flowers

Sample		Total phenolic content (mg GAE/g extract)	Mean of total phenolic content ± SD (mg GAE/g ekstrak)	Coefficient of Variation (%)
***S. grandiflora* (L.) Pers.**	R1	23.397	23.986 ± 0.613[a]	2.554
	R2	24.620		
	R3	23.940		
***C. retusa* L.**	R1	46.783	45.877 ± 1.297[b]	2.827
	R2	46.457		
	R3	44.391		
***C. pallida* Aiton**	R1	52.870	53.304 ± 1.150[c]	2.158
	R2	52.435		
	R3	54.609		
***A. pintoi* Krapov. & W.C. Greg.**	R1	63.641	62.962 ± 0.679[d]	1.079
	R2	62.283		
	R3	62.962		

Different superscript letters [a, b, c, d] mean that the samples have significant differences in total phenolic content.

Table III. DPPH radical scavenging activity of four Fabaceae flowers

Sample		IC_{50} (µg/mL)	Mean of IC_{50} ±SD (µg/mL)	Coefficient of Variation (%)
***S. grandiflora* (L.) Pers.**	R1	204.842	203.371 ± 4.706[a]	2.314
	R2	198.105		
	R3	207.167		
***C. retusa* L.**	R1	96.000	100.832 ± 4.286[b]	4.251
	R2	104.176		
	R3	102.318		
***C. pallida* Aiton**	R1	92.438	90.741 ± 2.416[c]	2.662
	R2	91.811		
	R3	87.976		
***A. pintoi* Krapov. & W.C. Greg.**	R1	66.938	68.570 ± 1.476[d]	2.153
	R2	68.962		
	R3	69.811		

Different superscript letters [a, b, c, d] mean that the samples have significant differences in IC_{50} value. Based on Itam et al. (2021), *S. grandiflora* and *C. retusa* have moderate antioxidant activity, while *C. pallida* and *A. pintoi* have strong antioxidant activity.

influenced by internal plant factors, such as the genetic factors of the plant itself (Toteles et al., 2022). The four samples in this study were different species which makes the yield of extraction results from each sample also different.

In determining the total phenolic content, gallic acid (3,4,5-trihydroxybenzoic acid) is used as a standard solution because this compound is a simple phenolic compound that has three phenolic hydroxy groups which is easy to oxidize and react with the Folin-Ciocalteu reagent (Sam et al., 2016). As we know, Folin-Ciocalteu reagent is a complex reagent composed of acid phosphomolybdate and hetero polyphosphotungstic acid which can oxidize a hydroxyl group. This reaction produces a blue colored phosphomolybdic phosphotungstate complex compound which can be detected with spectrophotometer. The intensity of blue color is proportional to the concentration of formed phenolic ions. The more phenolic content, the more phenolic ions, and the resulting blue color will be more intense. In the analysis of phenolic compounds, Na_2CO_3 is used because phenolic compounds will only react with the Folin-Ciocalteu reagent in an alkaline environment for proton dissociation at phenolic compounds to become phenolic ions (Andriani & Murtisiwi, 2020).

The results of the statistical analysis showed that each sample have total phenolic levels that are significantly different from each other, as shown in Table II. *A. pintoi* flower extract contains the most phenolic compounds compared with other samples by average total phenolic content to 62.962 mg GAE/g sample. Meanwhile, *S. grandiflora* flower extract contains the least phenolic compounds, that is 23.986 mg GAE/g sample even though the yield is the highest. It is indicated that the compound which is dissolved in the extraction process are not only phenolic compound. Other compounds such as flavonoids, saponins and tannins can also be used extracted by ethanol 70%. In several studies, the presence of alkaloids and terpenoids were also found in 70% ethanol extract (Putri & Mahfur, 2023).

The total phenolic content results in this research are better compared to other studies regarding examination of plant flowers. When compared with butterfly pea flowers (*Clitoria ternatea* L.) and rosella flower (*Hibiscus sabdariffa* L.) where both flowers are present very popular as a medicinal plant. Sam et al (2016) reported that total phenolic content of ethanol extract of rosella flowers was 1.85 mg GAE/g extract, while in the *C. ternatea* L. was 19.43 mg GAE/g extract (Andriani & Murtisiwi, 2020).

DPPH is a stable free radical based on the delocalization of spare electrons in the whole molecule which also causes a dark purple color in the dissolved form. This compound is easily dissolved in ethanol and methanol (Molyneux, 2004). When DPPH is reacted with an antioxidant, an electron of the antioxidant compound will bond with the free electrons of the radical (DPPH) to form a non-radical compound diphenylpicrylhydrazine. The reduced DPPH will lose its violet color and become yellow. This color change is related to the number of elesctrons received by DPPH and determines how strong the antioxidant activity of the sample extract is when its absorption is measured by a spectrophotometer.

The results of the statistical analysis showed that each sample have IC_{50} value that are significantly different from each other. As shown in Table III, the results of measuring the free radical scavenging activity for each samples showed that *A. pintoi* flower extract had the smallest IC_{50} value (68.570 ± 1.476 μ/mL) which mean has the strongest free radical scavenging activity compared to other samples. Meanwhile, *S. grandiflora* flower extract had the biggest IC_{50} value (203.371 ± 4.706 μ/mL) which mean has the weakest free radical scavenging activity compared to other samples. Pearson correlation statistical analysis was conducted between total phenolics and IC_{50} giving a Pearson value of -0.976, which means that the two variables have a negative correlation that is close to perfect. The higher the total phenolic content, the lower the IC_{50}, which means that the radical scavenging activity is stronger. However, there are also refracting factors in some non-phenolics compounds may also contribute to free radical scavenging activity (Islam et al., 2018).

The obtained IC_{50} value of sample flowers are better when compared with other research that also examines plant flowers. When compared with butterfly pea flowers (*C. ternatea* L.) and rosella flowers (*H. sabdariffa* L.) where both flowers are very popular as a source of antioxidants, the IC_{50} values of our samples are relatively equal to those two samples (Agustiarini & Wijaya, 2022; Andriani & Murtisiwi, 2020). Apart from that, when compared with the IC_{50} result value of other flowers which has been researched previously, the flower samples of this study are better. Research conducted by Naghiloo et al. (2012) who studied flowers of *Astragalus compactus* Lam. (Fabaceae) reported 237.2 ppm of IC_{50}. Another research which is conducted by Ebrahimzadeh et al. (2009) who studied the flowers of *Sambucus ebulus* L. reported the the IC_{50} value of 228 ppm. Ferula flower which is analyzed by Nabavi et al. (2010) had IC_{50} of 906 ppm. The results above showed that the flower of Fabaceae plant has a high prospective as natural anti oxidant source.

CONCLUSION

Fabaceae plant flowers as we have investigated from *A. pintoi*, *C. retusa*, *C. pallida*, and *S. grandiflora* have a high phenolic content and have exhibited good potential free radical scavenging activity. This finding underlines that these flowers can be used as a reference in the development of natural antioxidants as extra exogenous antioxidants to fight free radicals in the body.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the RTA UGM 2024 (Final Assignment Recognition) Grant with Assignment Letter Number 5286/UN1.P1/PT.01.03/2024 which has funded this research.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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