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Comparative evaluation of leaf and seed methanolic extracts obtained from *Sophora tomentosa* Linn. for phytochemical, phenolic content, antioxidant and antibacterial activities

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Abstract *Sophora tomentosa* Linn. (Fabaceae), also known as a yellow necklace pod, is a traditional medicinal plant widely used to treat dysentery and diarrhea. This study evaluated the biological activities of methanolic leaf and seed extracts of *S. tomentosa*. The total phenolic content of methanolic leave (STL) seed (STS) and dechlorophyll of leave extract (DSTL) showed 92.02 ±1.78, 38.48 ± 0.61, and 35.35 ± 2.45 mg GAE/g extract), respectively. For antioxidant activity, DPPH, ABTS and FRAP assay was found in leaves (STL) more than in dechlorophyll of leave extract (DSTL) and seed (STS). The methanolic extracts were assessed for antibacterial activity against eight pathogenic bacterial species. The seed extract showed the most potent antibacterial activity against *Kocuria rhizophila* with 12.85±3.85 mm inhibition zones. For phytochemical evaluation, both leaf and seed extracts exhibited alkaloids and coumarin. The results showed that the leaf extract (STL) had higher antioxidant activity and total phenolic content than seeds and dechlorophyll of leave extract (DSTL). On the other hand, the seed extract presented the most potent antibacterial activity. The current study suggested that both leave and seed extracts of *S. tomentosa* gave the potential source of natural bioactive compounds, which will be further evaluated in other biological activities.

Keywords: Phytochemical, Total phenolic content, Antibacterial, Antioxidant, *Sophora tomentosa* Linn

Introduction

Sophora species have secondary metabolites from different parts showing numerous pharmacological activities (Abd-Alla *et al*., 2021), such as the root extract from *S. viciifolia* (Ao *et al*., 2019). The seed extracts of *S. alopecuroides* have properties of antimicrobial, antioxidant, and enzyme inhibition (Zahra *et al.*, 2021). The *Sophora* genus was rich in chemical compounds. Including their biological activities were identified from different parts and *Sophora* species (Krishna *et al*., 2012; Awwad *et al.*, 2015; Bansode and Salalkar, 2015; Li *et*

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al., 2020). For antioxidant activity, the extracts from *S. japonica* are rich in flavonoids and phenolics, showing high antioxidant capacities (Zhu *et al.,* 2022). Generally, antioxidant activity was correlated with total phenols and flavonoids. *Sophora* species have been reported with antimicrobial activities (Yamaki *et al.,* 1990; Cho *et al.,* 1999; Sato *et al.,* 1995; Sohn *et al.,* 2004; Lin *et al.,* 2019).

Chlorophyll helps plants create their food through photosynthesis. On the other hand, chlorophyll can act as a peroxidation or negatively impact the resulting plant extract (Olatunde *et al.*, 2018). The greenish chlorophyll color might interfere with in vitro biological activities on fluorescence measurements. Therefore, this experiment compared biological activities between the extracted with and without chlorophyll removal.

Sophora tomentosa L. belongs to the Fabaceae family, also known as the yellow necklace pod, a small tree and shrub of up to 3-4 meters. It grows on coastal habitats such as sandy beaches, seashores, and open grassland along the beach. Although *S. tomentosa* is a native medicinal plant used against dysentery, diarrhea and cholera in Southeast Asia, some studies report on the chemical composition of extracts and their biological activities. It has been reported that 17 flavonoids, such as sophoraflavanoneA-E and isosophoranone, are from the root and stem of *S. tomentosa* (Tanaka *et al*., 1997). However, the prenylated flavonoids from the genus *Sophora* like sophoraflavanoneG, sophoflavescenol and alopecuronesA and B, play important roles in their biological properties as antibacterial and cytotoxic activities (Boozari *et al*., 2019). Therefore, this study aimed to investigate the phytochemicals, total phenolic contents, antioxidant, and antibacterial activities of leaf and seed methanolic extracts obtained from *Sophora tomentosa* with and without chlorophyll removal.

Materials and methods

Plant material collection and extraction

Sophora tomentosa (the Thai name is Sara phat phit) leaves were collected from Koh Yao Yai, Phang Nga province and seeds were purchased from the herbal drug store in Nonthaburi province, Thailand. The leaves and seeds were cleaned with tap water, air drying and ground with an electric grinder to obtain a fine powder. The samples were extracted with methanol using maceration for a week. Afterward, the extracts were filtered through filter paper and concentrated in a rotary evaporator to obtain the methanolic extracts of *S. tomentosa* leaves (STL) and seeds (STS).

Remove the chlorophyll from the methanolic leaves extract

The chlorophyll removal efficiency was established following the protocol of Phaisan *et al.* (2020) with some modifications. Briefly, the leaf extract was mixed with palm oil at 1:1 (w/v) for the liquid-liquid extraction method. The mixtures were separated by centrifugation at 4500 rpm for 10 minutes. The top layer without chlorophyll was collected and concentrated in a rotary evaporator to obtain the dechlorophyllized *S. tomentosa* leaves (DSTL).

Phytochemical screening

Phytochemical was determined for the potential presence of alkaloids, tannins, coumarin, and saponins of the LST, SLT and DLST extracts following the method of Evans (2002) and Farnsworth (1966) with some modifications.

Evaluation of total phenolic content

The extract's total phenolic content was evaluated by the Folin- Ciocalteu method, according to Natungnuy and Poeaim (2018). Briefly, 50 µl of the sample was prepared at 1000 µg/ml, and 50 ul of 10% Folin- Ciocalteu was mixed and kept in the dark. Then, 100 µl of 7.5 % (w/v) sodium carbonate was added and incubated for 30 minutes. The absorbance was measured at 765 nm. The total phenolic content was expressed as a milligram of gallic acid equivalents per gram extract (mg GAE/g of extract) using gallic acid as a standard.

Evaluation of antioxidant activities

DPPH and ABTS free radical scavenging assay and Ferric-reducing antioxidant power (FRAP) assay were evaluated using the in-house method (Natungnuy and Poeaim, 2018). For DPPH and ABTS assays were expressed in milligrams of Trolox equivalent per g extract (mg TE/g extract). For FRAP, ascorbic acid was used and constructed as a standard curve, and the result was expressed as milligrams of ascorbic acid equivalent per g extract (mg AAE/g extract).

Evaluation of antibacterial activity

The disc diffusion method has been standardized according to the CLSI adapted in 2012. Eight different strains of bacteria, i.e., *Bacillus subtilis* TISTR

1248, *Bacillus cereus* DMST 5040, *Kocuria rhizophila* ATCC 9341, *Staphylococcus aureus* TISTR 1466, *Staphylococcus epidermidis* TISTR 2141, *Propionibacterium acnes* DMST 14916, *Pseudomonas aeruginosa* TISTR 2370 and *Escherichia coli* TISTR 746 were obtained from the Department of Biology, School of Science, KMITL, Thailand. The inoculum of bacteria was transferred individually to Mueller Hinton Broth (MHB) and incubated. After incubation, the bacteria culture was swabbed on Mueller Hinton Agar (MHA). The extract solutions (3 mg/disc) were loaded on 6 mm in diameter of paper discs, and the disc was dried and placed on the surface of MHA. The plates were incubated at 37°C, 24 hr. Gentamicin and methanol were used as the positive and negative control, respectively. The zone of inhibition was to be measured.

Statistical analysis

All experiments were measured in triplicates and are expressed as means ± standard deviation (SD) using SPSS version 25 statistical software for a one-way analysis of variance (ANOVA).

Results

Phytochemical Screening

For phytochemical screening, alkaloids and tannins are recorded in *S. tomentosa* seeds (STS), leaves (STL), and the dechlorophyllized *S. tomentosa* leaves (DSTL) extract. Coumarins are presented only in the extract of the seeds. Saponins were absent in the leaves and seeds extract. However, saponins can be found in the dechlorophyllized *S. tomentosa* leaves (DSTL), which are presented in Table 1.

Table 1. Phytochemical screening of the methanolic extract of seeds and leaves from *S. tomentosa*

Phytochemical	Name of test/Solution	Extracts		
		STS	STL	DSTL
Alkaloid	Dragendorff's solution	+	+	+
	Mayer's solution	+	+	+
	Wagner's solution	+	+	+
Tannin	Gelatin solution	-	-	-
	Gelatin salt solution	-	-	-
	$FeCl_3$	+	+	+
Coumarin	Coumarin test	+	-	-
Saponin	Froth test	-	-	+

+ = presence, - = absence

Total phenolic content

The total phenolic content of STS, STL, and DSTL extracts was estimated using gallic acid (0-100 μg/ml) as a standard compound. The calibration curve showed y = 0.0033x with an r^2 value of 0.9984, and the values of total phenolic content were expressed as mg GAE/g extract. The total phenolic content of STL, STS and DSTL extract was 92.07 ± 1.80, 38.48 ± 0.610 and 35.35 ± 2.45 mg GAE/g extract, respectively. The total phenolic contents of STL extracts were significantly higher than STS and DSTL extracts, which was insignificant between STS and DSTL extracts ($p < 0.05$).

Antioxidant activities

Three assays estimated antioxidant activity including DPPH, ABTS and FRAP are presented in Table 2. *S. tomentosa* leaves (STL) extract showed the highest antioxidant capacity with values of 20.52±1.71, 76.70±4.64 mgTE/g extract and 55.16±1.94 mgAAE/g extract in DPPH, ABTS and FRAP assays, respectively. The antioxidant capacity of seeds extract (STD) was significantly lower than STL and DSTL extracts. For DPPH and FRAP assays, the dechlorophyllized *S. tomentosa* leaves (DSTL) presented insignificantly lower antioxidant capacity than the STL, with values of 19.02±0.83 mgTE/g extract and 54.48±0.47 mgAAE/g extract. However, in ABTS assays, the DSTL presented significantly lower antioxidant capacity than the STL, with values of 65.90±2.43 mgTE/g extract.

Table 2. Antioxidant activities of the methanolic extracts from *S. tomentosa* leaves and seeds

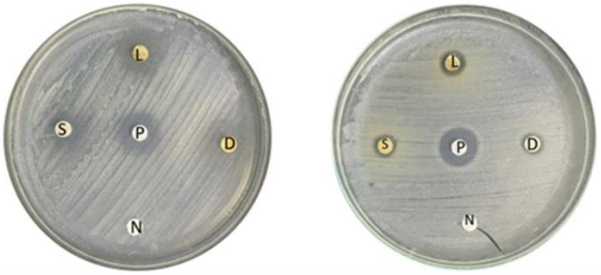
Methanolic extracts	Antioxidant capacity		
	DPPH assay (mg TE/g extract)	ABTS assay (mg TE/g extract)	FRAP assay (mg AAE/g extract)
STS	11.40^{b} ±3.17	56.40^{c} ±3.84	38.80^{b} ±2.82
STL	20.52^{a} ±1.71	76.70^{a} ±4.64	55.16^{a} ±1.94
DSTL	19.02^{a} ±0.83	65.90^{b} ±2.43	54.48^{a} ±0.47

The results are expressed as mean ±SD. The letters a-c within the same column indicate the statistical significance at $p<0.05$.

Antibacterial activity

Antibacterial activity of the methanolic extracts from *S. tomentosa* leaves (STL) and seeds (STS) extract, as well as the dechlorophyllized *S. tomentosa* leaves (DSTL), was done using *B. subtilis*, *B. cereus*, *K. rhizophila*, *S. aureus*,

S. epidermidis, *P. acnes* as gram-positive bacteria and *P. aeruginosa* and *E. coli* as gram-negative bacteria. The antibacterial activity of each extract was determined by the disc diffusion method, which used gentamicin 10 ug/ml as a positive control. The result revealed three extracts (3 mg/disc) presented antibacterial activity only with *K. rhizophila* and *B. cereus*, as shown in Figure 2 and Table 3. On the other hand, its did not inhibit *B. subtilis*, *S. aureus*, *S. epidermidis*, *P. acnes*, *P. aeruginosa* and *E. coli*. However, DSTL extract (8.65 ±1.62 and 7.37 ± 0.59 mm) showed lower antibacterial activity than STL extract (12.70 ±0.93 and 8.06 ±0.10 mm) for *K. rhizophila* and *B. cereus*, respectively.



Kocuria rhizophila *Bacillus cereus*

Figure 1. Antibacterial activity of the methanolic extracts from *S. tomentosa* leaves (STL), seeds (STS) extract and the dechlorophyllized *S. tomentosa* leaves (DSTL) by disc diffusion method. P: positive control, N: Negative control, S: STS, L: STL and D: DSTL

Table 3. The inhibition zone of the methanolic extracts prepared from *S. tomentosa* leaves (STL), seeds (STS) extract and the dechlorophyllized *S. tomentosa* leaves (DSTL) (3 mg/disc) and gentamicin (10 µg/disc)

Extracts	Inhibition zone (mm)	
	Kocuria rhizophila	***Bacillus cereus***
STS	$12.44^{ab} \pm 2.81$	$9.87^{b} \pm 0.32$
STL	$12.70^{ab} \pm 0.93$	$8.06^{c} \pm 0.10$
DSTL	$8.65^{b} \pm 1.62$	$7.37^{c} \pm 0.59$
Gentamicin	$15.06^{a} \pm 0.42$	$15.20^{a} \pm 1.03$

The results are expressed as mean ±SD; the letters a-c within the same column indicate the statistical significance at $p<0.05$

Discussion

The literature survey found that all plants have a considerable proportion of important phytochemicals and secondary metabolites. Several plant species of *Sophora* have been used in traditional medicine. Different parts of *Sophora*

species have secondary metabolites showing numerous pharmacological activities, such as *S. alopecuroides*, *S. flavescens*, *S. japonica*, *S. secundiflora*, and *S. tetraptera* (Krishna *et al*., 2012; Boozari *et al*., 2019; Abd-Alla *et al*., 2021). *Sophora tomentosa*, whose local name is Sara phat phit, is one of the species that has not been previously reported for its biological activities of methanolic extract. Therefore, the study focused on the leaves and seeds extraction for phytochemical activity, total phenolic contents, and antioxidant and antibacterial activities. However, leaves presented a greenish-dark color which interfered with the resulting analysis. So, leaves were applied to remove chlorophyll using palm oil by liquid-liquid extraction. In this study, the results of the alkaloid test using Dragendorff, Mayer and Wagner reagents, alkaloids were represented in three extraction samples. All extracts exhibited tannin only on the $FeCl_3$. Coumarins are presented only in the extract of the seeds. Saponins were absent in the leaves and seeds extract. However, the dechlorophyllized *S. tomentosa* leaves (DSTL) extract presented saponin on the froth test. The previous research has shown the percentage of secondary metabolites among *Sophora* species extract like alkaloids, tannin, chromones, coumarins, sterols, isoflavonoids, flavonols, saponins, and stilbene oligomers (Awwad *et al.*, 2015; Bansode and Salalkar, 2015; Li *et al.*, 2020). Alkaloids are found primarily in plants such as in *Sophora* species Ex. Sophovicine A-C from *S. davidii*, epilamprolobin-N-oxide from *S. tomentosa* (Abd-Alla *et al*., 2021).

According to the results of this study, the methanolic extracts from STS, STL and DSTL extracts of *S. tomentosa* are composed of the phenolic compound that STL extract revealed a higher total phenolic content (92.07 ± 1.80 mg GAE/g extract) than STS and DSTL extracts (38.48 ± 0.610 and 35.35 ± 2.45 mg GAE/g extract). Our results are consistent with results from a previous study that the extract from flowers of *S. japonica* has total phenolic content of 84.23±4.69 mg GAE/g extract (Zheng *et al.*, 2018). In agreeing with Chang *et al*. (2019), who reported that the total phenolic content in the leaves extract of *S. tomentosa* had total phenolics containing 61.21±0.08 mg GAE/g extract.

Generally, total phenolic content and antioxidant activity are correlated because phenolics react with various free radicals (Zeb, 2020). This study determined the antioxidant capacities of the sample extracts. It found that STL extract has the effect of the best antioxidant activity, followed by DSTL extract and STS extract, respectively. The extracts showed a strong correlation between phenolic content and antioxidant activities. These results agree with previous reports that *S. japonica* had a high antioxidant capacity because it has a high phenolic and flavonoid content (Zhu *et al.*, 2022). In DPPH and FRAP assays,

the dechlorophyllized *S. tomentosa* leaves (DSTL) presented insignificantly lower antioxidant capacity than the STL. However, in ABTS assays, the DSTL presented lower antioxidant activity than the STL. In this study, the dechlorophyllized *S. tomentosa* leaves found that the antioxidant capacity was reduced. According to the results of Benjakul and Tagrida (2020), it has been reported the total phenolic content, and antioxidant activity of *Piper sarmentosum* leaf extracts that removed chlorophyll were decreased. In contrast, Phaisan *et al*. (2020) reported using oils for dechlorophyllization, and the result indicated palm oil and soybean oil gave high antioxidant activity. So, the result of the antioxidant activity of this study is implicated in their total phenolic content.

In this study, the methanolic extract from the seed of *S. tomentosa* (STS) presented the highest inhibited zone of *K. rhizohila* and *B. cereus.* On the other hand, its did not inhibit *B. subtilis*, *S. aureus*, *S. epidermidis*, *P. acnes*, *P. aeruginosa* and *E. coli*. These findings are inconsistent with those previously reported that *Sophora* species have been reported with antimicrobial activities against both gram-positive and gram-negative (Yamaki *et al.,* 1990; Cho *et al.,* 1999; Sato *et al.,* 1995; Sohn *et al.,* 2004; Lin *et al*., 2019). Several compounds in *S. flavescens* have been shown against *Staphylococcus aureus* and *Streptococcus mutans* (Yamaki *et al.,* 1990). The methanol extract from *S. flavescens* showed growth inhibition against gram-positive bacteria such as *Bacillus subtilis* and *S. aureus* and gram-negative bacteria such as *P. aeruginosa* (Cho *et al*., 1999). In addition, bioactive compounds such as flavanone isolated from *S. exigua* inhibited the growth of 21 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) (Sato *et al.,* 1995). The extract of *S. viciifolia* had higher antioxidant and antimicrobial activity potential (Lin *et al.,* 2019). The extracts with chlorophyll removal exhibited antibacterial activity lower than those without chlorophyll removal. However, our result follows that coumarin is related to antibacterial activity.

This study reported on the biological activity of the methanolic extract of seed and leaves from *S. tomentosa* and the effect of chlorophyll removal from leaves. The results showed that the extraction without chlorophyll removal is not related to the potential of biological activity. The antioxidant activity is implicated in their total phenolic content. *S. tomentosa* leaves extract showed high antioxidant capacity. In addition, coumarins and antibacterial activity are correlated. The methanolic extract from the seeds of *S. tomentosa* presented the highest inhibited zone of *K. rhizohila* and *B. cereus.* Further studies should be promoted and needed to evaluate the potential of biological activity and identify the constituent of their bioactive compounds from both seeds and leaves of *S. tomentosa*.

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