Original Article

Sesbania javanica Miq leaf extracts increase Balb/c 3T3 fibroblast cell migration

Extratos de folhas de *Sesbania javanica* Miq aumentam a migração de células de fibroblastos Balb/c 3T3

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Abstract

Sesbania javanica Miq. is widely distributed in canals throughout Thailand and its flowers are commonly consumed in Thailand. *Sesbania javanica* floral extracts have previously been demonstrated to have antimutagenic activity, but information on the bioactivity and beneficial properties of other plant parts, such as the leaf, remains limited. In this study, the induction of cell proliferation and wound-healing activity of DMSO extracts from flowers and leaves of *S. javanica* was evaluated. A high concentration (1:25 and 1:50 dilutions) of all extracts was cytotoxic to Balb/c 3T3 fibroblast cells. A low concentration (1:500 and 1:1000 dilutions) of extracts A, B, and C (extracted from leaves) significantly promoted cell viability after treatment of the fibroblast cells for 24 or 48 h. The 1:500 and 1:1000 dilutions of extracts B and C were selected for a scratch assay to assess their wound-healing activity and significantly decreased the wound area after treatment for 24 h. These findings provide evidence that leaf extracts from *S. javanica* have potential utility for the treatment of wounds.

Keywords: phytochemical, wound healing, Sesbania javanica, herbs, extracts.

Resumo

Sesbania javanica Miq. é amplamente distribuído em canais da Tailândia e suas flores são comumente consumidas no país. Extratos florais de *Sesbania javanica* já demonstraram atividade antimutagênica, mas informações sobre a bioatividade e propriedades benéficas de outras partes da planta, como a folha, permanecem limitadas. Neste estudo, avaliaram-se a indução da proliferação celular e a atividade cicatrizante de extratos de DMSO de flores e folhas de *S. javanica*. Uma alta concentração (1:25 e 1:50 diluições) de todos os extratos foi citotóxica para células de fibroblastos Balb/c 3T3. Já uma baixa concentração (1:500 e 1:1000 diluições) de extratos A, B, e C (extraído das folhas) promoveu significativamente a viabilidade celular após o tratamento das células fibroblásticas por 24 ou 48 horas. As diluições de 1:500 e 1:1000 dos extratos B e C foram selecionadas para um ensaio de raspagem para avaliar sua atividade cicatrizante e diminuíram significativamente a área da ferida após o tratamento por 24 horas. Esses resultados fornecem evidências de que extratos de folhas de *S. javanica* têm potencial para o tratamento de feridas.

Palavras-chave: fitoquímico, cicatrização de feridas, Sesbania javanica, ervas, extratos.

1. Introduction

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Wounds are one type of skin injury caused by physical, chemical, or thermal damage. The healing process is immediately activated after wounding to regenerate the tissue. The three phases of wound healing comprise inflammation, proliferation, and remodelling (Darby et al., 2014). Many studies have shown that plant extracts contain a variety of bioactivities beneficial for human health, including wound healing, anti-inflammatory, antioxidant, antimicrobial, and antitumor properties (Hussain et al., 2015; Rawiwan et al., 2019). During the proliferative phase of wound healing, fibroblasts assume a critical role at the wound site by facilitating wound contraction and promoting the restoration of tissue integrity (Thiruvoth et al., 2015). Fibroblasts are the predominant cells involved in this phase and are responsible for the synthesis and secretion of essential components, such as collagens and glycosaminoglycans. These components contribute to the formation of new granulation tissue, which subsequently undergoes remodelling into mature dermal tissue (Sharifi et al., 2013). Several traditional Thai medicinal plants are utilized for their wound-healing properties. Ethanolic extracts from three Thai medicinal herbs, namely,

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Garcinia mangostana L., Glycyrrhiza glabra L., and Nigella sativa L., promote wound healing (Siriwattanasatorn et al., 2020). Recently, ethanolic extracts from *Derris scandens* (Roxb.) Benth. stems were demonstrated to have *in vitro* wound-healing activity on human skin fibroblast cells (Somwong and Kamkaen, 2022). An ethanolic leaf extract from *Sesbania grandiflora* (L.) Poir., one of the Thai plants commonly named Khae ban, applied in 2% or 4% (w/w) ointment form induced a significant acceleration in wound healing compared with the control group (Sree et al., 2017). An extract from the bark of the Khae ban plant using 95% ethanol has potential utility as a cosmeceutical product or as a treatment for mouth ulcers (Satsue et al., 2019).

Sesbania javanica Miq. (S. javanica) is commonly known in Thai as "Sano" or "Sano Kin Dok". The species is a member of the Fabaceae and is especially common in Asian countries, including India, Bangladesh, Sri Lanka, Indonesia (Java), Malaysia, Papua New Guinea, the Philippines, Cambodia, Laos, Myanmar, Vietnam, South China, Tai-wan, and Thailand (Lim, 2014). Sesbania javanica is widely distributed in rainy season, especially throughout canals in Thailand. In Thai traditional medicine, various parts of S. javanica, including the leaves, flowers, and bark, have been used for centuries to treat a host of health conditions. The plant produces numerous bioactive compounds, such as flavonoids, phenolic compounds, and other phytochemicals, which contribute to its medicinal properties, including antiinflammatory for treatment of insect bites, detoxification, alleviation of stomach discomfort, intestinal abscess healing, and relief of internal fever and thirst (Krasaekoopt and Kongkarnchanatip, 2005). The flowers of S. javanica contain pigments that are beneficial for enhancing the color of egg yolks (Kijparkorn et al., 2010). The antimutagenic activity of S. javanica flowers has been previously reported in relation to the high content of flavonoids (especially quercetin 3-2G-rhamnosylrutinoside) (Tangvarasittichai et al., 2005). Sesbania javanica flowers and leaves also contain several phytochemical compounds (prunetin, genistein, and 4-hydroxycinnamic acid) that have antioxidant activities (Looedsaksaesakul, 2007).

In this study, the bioactivity of dimethyl sulfoxide (DMSO) extracts from flowers and leaves of *Sesbania javanica* was assessed at different concentrations of the crude extract. Specifically, the potential of the extracts for *in vitro* cytotoxicity, induction of cell proliferation, and wound-healing activity was investigated using mouse fibroblast cell cultures.

2. Material and Methods

2.1. Plant material preparation

Leaves and flowers of *Sesbania javanica* were collected from the Tha Ruea, Nakhon Luang, and Bang Pahan districts, Ayutthaya province, Thailand (Table 1 and Figure 1). The samples were cleaned with water, dried for 24 h, and then ground into a fine powder. The powder was stored in an airtight container in the dark in a desiccant cabinet with relative humidity of 45%.

2.2. Sample extraction

Two grams of each ground sample of leaves or flowers were extracted with 9.9% DMSO at the ratio of 1:10. The samples were extracted by soaking in a glass bottle sealed with a lid with shaking at 100 rpm for 24 h. The extraction step was repeated twice. The extract solution was filtered to separate the coarse sediments with a thin white cloth and then filtered with Whatman No. 1 filter paper. The filtered solutions were centrifuged at 10,000 g for 10 min. The supernatants were stored at 4°C for use in the following assays.

2.3. Cell culture conditions

A fibroblast cell line, Balb/c 3T3, was obtained from Asst. Prof. Dr. Danupon Nantajit then was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin cultured at 37 °C in a 5% CO2-humidified atmosphere. When the cells attained approximately 80% confluency, the cell lines were subcultured. The culture plates were maintained in a 5% CO2-humidified incubator at 37 °C.

2.4. Cell viability assay

After subculture, culture medium supplemented with different concentrations of the *S. javanica* extracts (1:25, 1:50, 1:100, 1:500, or 1:1000) was placed in each well for PrestoBlue[™] analysis. The PrestoBlue[™] reagent is quickly reduced by metabolically active cells to the fluorescent molecule, resorufin, thereby providing a quantitative measure of cell viability and cytotoxicity. After seeding of cells for the PrestoBlue[™] assay and incubation for the required treatment period, 10 µL PrestoBlue[™] reagent (Molecular Probes, Invitrogen Corp., USA) was added to each well and incubated at 37 °C for 30 min. The fluorescence was measured at the

No.	Sample ID	Plant part	Collection site (District)	Drying process
1	А	Leaf	Nakhon Luang	Shade-dried
2	В	Leaf	Tha Ruea	Sun-dried
3	С	Leaf	Bang Pahan	Sun-dried
4	D	Flower	Tha Ruea	Sun-dried
5	E	Leaf	Bang Pahan	Shade-dried
6	F	Flower	Bang Pahan	Sun-dried

Table 1. Provenance, plant part, and drying process used for Sesbania javanica samples.



Figure 1. The image showing raw material of flower (a), leaf (b), Shade-dried leaf and Sun-dried leaf from Sesbania javanica.

wavelengths 560 and 590 nm using an EnSight Multimode Plate Reader (PerkinElmer Inc., USA), and was expressed as the percentage cell viability of the control.

2.5. Wound-healing assay

Balb/c 3T3 cells were seeded in six-well plates and incubated until they attained confluency. Scratches through the cells were performed using a sterile 200 µL pipette tip, then the cells were washed twice with PBS to remove loose cells and debris. The cells were treated for 24 h with extracts B and C at 1:500 and 1:1000 dilutions. The cell-free region was observed using a light microscope (Eclipse Ts2R-FL; Nikon Instruments, Tokyo, Japan). The cell-free area was measured using NIS-Elements AR software (Nikon) with the Wound Healing function, General Analysis, of the program.

2.6. Statistical analysis

The experimental results are reported as the mean ± standard deviation. The cell proliferation data and wound healing analysis were statistically analysed with GraphPad Prism® version 9.00 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Statistical significance was determined using one-way ANOVA and Dunnett's multiple comparisons test (p-value < 0.05).

3. Result

3.1. Cell proliferation

In this context, the extracts derived from *S. javanica* were investigated for their potential to enhance cell proliferation

and promote wound healing. The extracts of S. javanica at dilutions ranging from 1:25 to 1:1000 had varying effects on the proliferation of Balb/c 3T3 cells (obtained from ATCC (Manassas, VA). High concentrations of the extract and solvent (DMSO) were cytotoxic, with cell viabilities ranging from 30.29% to 56.88% (1:25 dilution) and 40.40% to 84.88% (1:50 dilution) (Table 2). Low concentrations of the extracts A, B, and C with 1:500 and 1:1000 dilutions promoted cell proliferation, resulting in cell viabilities of 141.84% to 142.41% (Extract A), 138.28% to 142.41% (Extract B), and 138.27% to 180.27% (Extract C). In contrast, the solvent control (DMSO at 1:500 and 1:1000 dilutions) resulted in cell viabilities (99.11% to 102.27%) lower than those observed with extracts A, B, and C (Figure 2a). The results obtained after treatment with the S. javanica extracts for 48 h were consistent with those for 24 h treatment (Figure 2b). Treatment with extracts A, B, and C at low concentrations (1:500 and 1:1000 dilutions) resulted in increased cell viabilities of 144.8% to 145.18% (Extract A), 135.21% to 148.34% (Extract B), and 118.68% to 130.65% (Extract C) (Table 3). Based on these findings, extracts B and C at 1:500 and 1:1000 dilutions were selected for subsequent evaluation in a wound-healing assay.

3.2. Wound-healing assay

Cell migration is a critical process in wound healing, and thus exploring the wound-healing potential of extracts from *S. javanica* is of great interest. To evaluate the efficacy of the extracts, a scratch assay was used to create an artificial wounded monolayer to assess fibroblast cell migration (Figure 3). Given that extracts B and C were demonstrated to have notable cell proliferation effects, these extracts were selected for the cell migration assay to evaluate their impact

Dilation	DMSO	Extract	A	Extract	в	Extract	c	Extract	D	Extract 1	(1)	Extract	н
DIULIOI	(Mean±SD)	(Mean ± SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value
1:25	56.88 ± 2.32	31.82 ± 5.06	0.0049	30.29 ± 4.69	0.0054	34.51 ± 4.92	0.0336	37.03 ± 6.31	0.0857	33.67 ± 3.58	0.0239	32.97 ± 4.88	0.0178
1:50	79.57 ± 4.21	40.40 ± 5.53	<0.0001	84.88 ± 21.4	0.9843	101.57 ± 17.30	0.0209	62.81 ± 14.83	0.1608	87.51 ± 15.75	0.893	51.41 ± 14.02	0.001
1:100	100.81 ± 2.78	108.01 ± 6.07	0.9305	120.77 ± 12.59	0.0499	142.43 ± 1.79	<0.0001	103.61 ± 2.0	0.9995	121.19 ± 7.28	0.0422	95.65 ± 1.35	0.9864
1:500	99.11 ± 2.0	142.41 ± 13.25	<0.0001	142.41 ± 13.32	<0.0001	180.27 ± 15.45	<0.0001	102.83 ± 10.45	7790.0	109.6 ± 7.43	0.6936	108.99 ± 8.89	0.7494
1:1000	102.27 ± 3.11	141.84 ± 15.38	<0.0001	138.28 ± 13.22	<0.0001	138.27 ± 15.70	<0.0001	101.16 ± 9.26	>0.9999	108.64 ± 4.14	0.9718	101.61 ± 0.01	>0.9999
The percentage (of cell viability com	pared to untreated (controls. SD: 5	Standard Deviation.									

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Dilution	DMSO	Extract	A	Extract	В	Extract	С	Extract	D	Extract	E	Extract	н
עווחטוו	(Mean±SD)	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean ± SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value	(Mean±SD)	<i>p</i> -value
1:25	28.67 ± 1.85	17.10 ± 3.43	0.8072	17.39 ± 3.56	0.8289	27.41 ± 14.19	>0.9999	25.66 ± 7.19	>0.9999	18.16 ± 2.0	0.8783	18.51 ± 1.5	0.8973
1:50	51.19 ± 3.93	31.53 ± 13.37	0.0886	54.08 ±10.88	0.9996	56.07 ± 24.11	0.9927	49.18 ± 10.12	>0.9999	49.41 ± 5.38	>0.9999	31.05 ±7.87	0.0747
1:100	86.65 ± 10.94	77.01 ± 4.53	0.8173	102.96 ± 3.42	0.2476	82.9±3.53	0.9975	87.28 ± 11.19	0.9801	87.81 ± 7.29	>0.9999	60.34 ± 12.16	0.1154
1:500	105.84 ± 2.73	144.8 ± 16.42	<0.0001	148.34 ± 13.12	<0.0001	130.65 ± 22.82	0.0052	112.88 ± 2.83	0.9355	114.04 ± 5.35	0.8746	103.91 ± 2.43	>0.9999
1:1000	92.65 ± 4.47	145.18 ± 17.29	<0.0001	135.21 ± 17.06	<0.0001	118.68 ± 13.27	0.0066	108.16 ± 1.39	0.3051	114.45 ± 3.65	0.0403	101.16 ± 4.45	0.8896
The percentage	of cell viability con	npared to untreate	d controls.										

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Table 2. Percentage fibroblast cell proliferation (an indicator of cell viability) in response to treatment for 24 h with extracts from Sesbania javanica leaves and flowers.

Figure 2. The effects of DMSO extracts from *Sesbania javanica* leaves and flowers on Balb/c 3T3 cell viability and wound healing. (**a**, **b**) Effects of DMSO extracts on cell viability measured using the PrestoBlue viability assay. The cells were grown in the medium and treated with 1:25, 1:50, 1:100, 1:500, or 1:1000 dilutions of extract A, B, C, D, E, or F for 24 hr (**a**) and 48 hr (**b**).

Figure 3. Effect of *Sesbania javanica* extracts B and C on cell migration in a wound-healing assay (a-f). Images were captured after treatment for 24 h with the extracts after an artificial wound in the monolayer was created. The cell-free shaded area represents the wound area. Cell migration into the cell-free area represents wound closure. (g) Percentage wound area after treatment with extracts B and C at dilutions 1:500 and 1:1000 for 24 h. Balb/c 3T3 cells were grown in medium until confluency prior to conducting the wound-healing assay. Each data point and error bar represent the mean \pm SE of three independent experiments and are expressed relative to the control. In (g), extracts B, and C were significantly different compared with the untreated control. *** $p \le 0.001$, **** $p \le 0.0001$, ns: no significant difference.

Extract and		Wound area (%)		Mean + SD	n-value
dilution	Experiment 1	Experiment 2	Experiment 3	Mean ± SD	<i>p</i> -value
Untreated control	100.00	100.00	100.00		
DMSO 1:1000	100.60	101.13	100.12	100 ± 0.50	>0.9999
Extract B 1:500	59.23	76.15	61.21	65.53 ± 9.25	<0.0001
Extract C 1:500	73.34	76.20	77.94	75.83 ± 2.32	0.0006
Extract B 1:1000	64.53	66.11	68.64	66.42 ± 2.07	<0.0001
Extract C 1:1000	74.45	64.87	82.10	73.81 ± 8.63	0.0003

Table 4. Percentage wound area in response to treatment with Sesbania javanica extracts B and C at two dilutions.

Results are presented from three independent experiments. The percentage of wound area compared to untreated controls.

on wound healing. The extracts B and C at both dilutions 1:500 and 1:1000 resulted in a significant decrease in the percentage wound area. Wound closure was measured as the percentage area of the initial wound area. The wound area was 65.53% and 66.42% under treatment with Extract B at dilutions 1:500 and 1:1000, respectively, and 75.83% and 73.81% in response to treatment with Extract C at dilutions 1:500 and 1:1,000, respectively (Table 4 and Figure 3h).

4. Discussion

In the context of tissue damage, a wound refers to any type of injury to the skin resulting from physical, chemical, electrical, or thermal damage. In response to wounding, the body's natural healing process is immediately activated to regenerate the damaged tissue. This healing process involves three distinct phases, namely, inflammation, proliferation, and remodelling, which are complex processes that involve the cooperation of multiple cells and molecules to repair the damaged tissue (Darby et al., 2014). Fibroblasts are a type of cell that plays a crucial role in the wound-healing process. These cells differentiate into myofibroblasts, which are activated by mediators and migrate to the wound site (Singer and Clark, 1999). In the present study, we used Balb/c 3T3 cells, which represent one type of fibroblast cell, that have been previously used to analyse the effects of various chemicals or plant extracts with biological activities, especially concerning medical applications (Chaiwaree et al., 2022; Lertphadungkit et al., 2020; Siriyong et al., 2020). The major flavonoid glycoside in DMSO extracts from S. javanica flowers has been identified and shown to have antimutagenic activity (Tangvarasittichai et al., 2005). In the present experiment, DMSO extracts from flowers and leaves of S. javanica contained bioactive compounds, which might include flavonoid glycoside compounds consistent with the previous report (Tangvarasittichai et al., 2005). Moreover, extracts from S. javanica flowers also have antioxidant and anti-glucosidase activities (Thummajitsakul et al., 2022). In addition, a previous investigation using S. javanica flower extracts demonstrated antioxidant activity with potential utility in the pharmaceutical and food industries (Manickam et al., 2011). In the present study, three of the six extracts demonstrated significant induction of fibroblast cell proliferation (extracts A, B, and C) after treatment

for 24 or 48 h. Only low concentrations of the extracts (1:500 and 1:1000 dilutions) had this effect, whereas high concentrations (1:25 and 1:50 dilutions) were cytotoxic to the fibroblast cells, which might be caused by the DMSO concentration in the extracts. In a recent study of ethanolic extracts from eight Thai medicinal herbs, the extracts from Garcinia mangostana, Glycyrrhiza glabra, and Nigella sativa promoted cell proliferation and accelerated wound recovery at a low concentration. Other reports have shown that extracts from plant leaves have bioactivity, including anti-inflammatory or wound-healing properties (Nayak et al., 2009; Sharma et al., 2014). A crude extract from the Thai medicinal plant Dioscorea bulbifera L. at a concentration of 1-3 µg/ml induced cell proliferation and cell migration of human dermal fibroblasts (Chaniad et al., 2020). In the current study, extracts B and C promoted cell proliferation and cell migration in a wound-healing assay in comparison with the control. Thus, leaf extracts from S. javanica showed potential for wound healing of fibroblast cells. Dried S. *javanica* flower powder has been reported to produce Thai sponge cake with improved health properties and an acceptable appearance, odor, taste, and texture when 20% (w/w) of the powder was used (Weenuttranon et al., 2022). Ethanolic extracts from *S. javanica* flower samples (ESJ1 and ESJ2) and dye samples (PESJ1 and PESJ2) contained significantly elevated contents of total phenolic compounds and total flavonoids. In addition, these ethanolic extracts had superior antioxidant activity and α-glucosidase inhibitory activity compared with the corresponding aqueous extracts. These results emphasize the potential of ethanolic extraction as a more efficient method for obtaining extracts enriched with bioactive constituents (Thummajitsakul et al., 2022).

5. Conclusion

In conclusion, information is limited on the bioactivity of *S. javanica* extracts, especially from different parts of the plant other than the flower. The present results indicated that DMSO extracts from leaves of *S. javanica* may be effective for wound treatment and support their utility in Thai traditional medicine. Identification of the important bioactive compounds in the crude extract from *S. javanica* leaves is required in a future study.

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