Bulgarian Journal of Agricultural Science, 28 (No 6) 2022, 1080–1087

Allelopathic effects of *Spondias pinnata* (L.f.) Kurz leaf extracts

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Abstract

Kyaw, E. H. & Kato-Noguchi, H. (2022). Allelopathic effects of *Spondias pinnata* (L.f.) Kurz leaf extracts. *Bulg. J. Agric. Sci., 28 (6)*, 1080–1087

Spondias pinnata (L.f.) Kurz (Anacardiaceae), a deciduous tree, is cultivated in home gardens in tropical southern Asia. The fruits, leaves, and flowers of *S. pinnata* are edible and its various parts have been traditionally utilized in folk medicine. Although it possesses a diverse range of pharmacological properties and several bioactive substances from the plant have been well reported, the allelopathic effects of *S. pinnata* have not yet been documented. Hence, in this study, we aim to explore the allelopathy of *S. pinnata* for weed control. Extracts of *S. pinnata* inhibited the germination and seedling growth of *Lepidium sativum* L., *Lactuca sativa* L., *Echinochloa crus-galli* (L.) P. Beauv., and *Lolium multiflorum* Lam. Significant reductions in the germination and seedling growth were observed as the extract concentration increased. The concentrations needed for 50% inhibition (I_{50}) values of the test plant roots and shoots were 2.2 – 6.3 and 4.3 – 30.6 mg D.W. equivalent extract/mL, respectively. The *S. pinnata* extracts were purified through different chromatography steps, and an inhibitory substance (SPL – 1) was isolated. These results indicate that the *S. pinnata* extracts had an allelopathic effect, which may be caused by SPL – 1. The SPL – 1 could be released into the soil under *S. pinnata* trees through the decomposition of fallen leaves and possibly acts as a plant growth inhibitory substance. Therefore, the allelopathy of *S. pinnata* is potentially useful for weed management in an eco-friendly way.

Keywords: Allelopathy; Inhibitory substance; *Spondias pinnata*; Weed control

Introduction

Weeds are serious biotic threats that adversely affect the growth, development, and yield of cultivated plants (Farooq et al., 2013). While conventional agriculture relies mainly on chemicals for weed control, alternative biological methods might be an essential tool to reduce weed infestation in an eco-friendly way (Głąb et al., 2017). Hence, plants with allelopathic potential have been investigated as an ecological approach for sustainable weed management (Singh et al., 2001; Appiah et al., 2015; Nornasuha & Ismail, 2017; Poonpaiboonpipat et al., 2021). Allelopathy is a mechanism of plant interference mediated by allelochemicals; under certain conditions, these allelochemicals are released into the environment to inhibit the growth and germination of neighboring plants (Cheng & Cheng, 2015; Tahir et al., 2020). The allelopathic plants could be utilized in different ways, for instance, (i) sowing as cover crops, (ii) directly applying extracts as natural herbicides, or (iii) isolating and identifying their active substances and applying those identified substances as a tool for bio-herbicide development (Nornasuha & Ismail, 2017; Mekky et al., 2019). Accordingly, a number of plants, including tree, crop, and weed species, possess allelopathic potential, and a variety of active substances have been isolated from different tree spe-

cies and identified as allelochemicals. Matuda et al. (2021) reported that two allelopathic substances, (+)-rhododendrol and 9-*epi*-blumenol C, isolated from the fossil tree species *Metasequoia glyptostroboides* Hu et W.C. Cheng inhibited the growth of *Lepidium sativum* L. and *Lolium multiflorum* Lam. In addition, the shoots and roots of *Vulpia myuros* (L.) C.C. Gmel. and *L. sativum* are significantly suppressed by methyl gallate (an identified allelochemical) from mango, *Mangifera indica* L. (Suzuki et al., 2017). It has also been reported that five characterized allelochemicals — xanthyletin, chalepensin, ammirin, chalepin, and pellitorine — extracted from the rutaceous tree species *Stauranthus perforates* Liebm. significantly inhibit the growth of *Amaranthus hypochondriacus* L. and *E. crus-galli* (Anaya et al., 2005).

The genus *Spondias* belongs to the family Anacardiaceae; it comprises 17 species, of which 10 are native to tropical Asia and 7 are confined to the Neotropics (Tianlu & Barfod, 2008; Rymbai et al., 2016). Members of *Spondias* have been widely used in folk medicine to treat various ailments (Sameh et al., 2019). Moreover, diverse pharmacological properties are reported for *Spondias*, including antioxidant, cytotoxic, anti-inflammatory, hepatoprotective, and antidementia (Sameh et al., 2019). Notably, some *Spondias* species such as *S. dulcis* (Raihan et al., 2019), *S. mombin*, and *S. purpurea* (Morikawa et al., 2012) are documented to inhibit the growth of tested plants and exhibit allelopathic activities. Therefore, species of the genus *Spondias* possess a broad range of biological activities.

Spondias pinnata (Linn. f.) Kurz, known as hog plum or wild mango, is a deciduous tree, growing up to 40 m tall (Sujarwo et al., 2017). The tree is naturally established in South-East Asian countries, including Myanmar, Indonesia, Laos, Bhutan, and Thailand (Bora et al., 2014). Its leaves are compound and imparipinnate (30–45 cm long) with pairs of leaflets. It has small, sessile, glabrous greenish white flowers attached to its panicles at the end of the branches. The fruit of *S. pinnata* is ellipsoid to elliptic-ovoid drupe, yellowish orange at maturity, and edible (Bora et al., 2014; Sujarwo & Keim, 2019). In some areas of tropical southern Asia, *S. pinnata* is cultivated in home gardens, and its leaves, flowers, and fruits are consumed locally (Xu et al., 2004; Elfrida et al., 2020). Acharyya et al. (2011) and Sujarwo et al. (2015) reported that various parts of *S. pinnata* have been traditionally utilized as a remedy for diabetes mellitus, muscular rheumatism, earache, and sore throat, and also for detoxification and the relief of swelling and pain. The extracts and essential oil of *S. pinnata* show antimicrobial activity against different microbial strains such as *Streptococcus mutans*, *Salmonella bacteria*, and *Bacillus subtilis* (Muhammad et al., 2011; Chai et al., 2013; Manik et al., 2013). Three active compounds — furfural, α-terpineol, and γ-terpineol — were identified as antimicrobial substances (Chai et al., 2013). Extracts of *S. pinnata* have also exhibited an antibacterial effect on tested bacterial strains, and 7-hydroxy-6-methoxy coumarin (scopoletin) was characterized as an antibacterial substance (Muhammad, 2015). Additionally, antioxidant (Jain et al., 2014; Sujarwo et al., 2017), anticancer (Mondal et al., 2021), antidiabetic (Sutradhar et al., 2018), and anti-inflammatory properties (Li et al., 2020) of *S. pinnata* have been studied in human cells and animal models. Although several biological activities of *S. pinnata* have been reported, to date, there is no relevant information about its allelopathic potential. Thus, our experiment aimed to investigate the allelopathy of *S. pinnata*.

Materials and Methods

Plant materials

Spondias pinnata leaves were collected from Zeyarthiri township, Nay Pyi Taw, Myanmar (19 °83′ 67″ N; 96 ° 27′ 21″ E), during August 2019. The leaves were washed two times with tap water, then dried in a shaded area and kept at +2°C until extraction. Two dicotyledonous plants (*Lepidium sativum* L. and *Lactuca sativa* L.) and two monocotyledonous plants (*Echinochloa crus-galli* (L.) P. Beauv. and *Lolium multiflorum* Lam.) were used as test plant species to evaluate the biological activity of *S. pinnata*.

Preparation of Spondias pinnata extracts

Dried *S. pinnata* leaves (100 g) were chopped and extracted with 500 mL of 70% (v/v) aqueous methanol for 48 h. The extract was then filtered using a single sheet of filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residue was re-extracted with the same amount of methanol for 24 h and filtered. The two filtrates were mixed and concentrated at 40°C using a rotary evaporator.

Germination bioassay

The concentrated *S. pinnata* extract was diluted with methanol to prepare six assay concentrations of 1, 3, 10, 30, 100, and 300 mg dry weight (D.W.), which were added to a sheet of filter paper (No. 2) in 28 mm Petri dishes. The methanol was evaporated in a laminar flow cabinet followed by adding 0.6 mL of a 0.05% (v/v) aqueous solution of polyoxyethylene sorbitanmonolaurate (Tween 20; Nacalai, Kyoto, Japan). Ten seeds of *Lepidium sativum* L., *Lactuca sativa* L., *Echinochloa crus-galli* (L.) P. Beauv., and *Lolium multiflorum* Lam. were arranged on the filter paper in the Petri dishes and germinated in the dark at 25°C. Germinated seeds were counted at 12 h intervals up to 96 h (the time when the germination became constant), and were considered to have germinated when the

radical emerged from the seed coat. Control Petri dishes were also prepared in each experiment using only Tween 20 without the *S. pinnata* extracts. The germination % compared with control was determined using the equation according to Islam and Kato-Noguchi (2013).

$$\text{Germination (\% of control)} = \frac{G_T}{G_o} \times 100,$$

where G_T = number of germinated seeds with *S. pinnata* extracts at each measurement time, and G_o = number of germinated seeds without *S. pinnata* extracts at the same measurement times.

Growth bioassay

The extracts and the Petri dishes were prepared as mentioned above. Ten seeds of *L. sativa* or *L. sativum*, or ten germinated seeds of *E. crus-galli* or *L. multiflorum* (germinated in the dark at 25°C for 48 h) were arranged on the filter paper in the Petri dishes. Control treatments were also provided for the growth bioassay. Finally, the shoot and root length of the test plant seedlings were measured after 48 h incubation in the dark at 25°C.

Separation of the Spondias pinnata extracts

The leaf extracts of *S. pinnata* were concentrated to produce an aqueous residue. The pH of the residue was then adjusted to 7.0 with phosphate buffer (1 M) and partitioned five times with ethyl acetate (same volume of aqueous residue). The obtained ethyl acetate fraction was chromatographed on silica gel (60 g, silica gel 60, 70-230 mesh; Nacalai Tesque, Kyoto, Japan) and eluted with 20, 30, 40, 50, 60, 70, and 80% ethyl acetate in *n*-hexane (150 mL each), ethyl acetate (150 mL), and methanol (300 mL) to obtain nine fractions (F_1 to F_9). Significant inhibitory activity was observed in F_6 (the fraction eluted with 70% ethyl acetate in *n*-hexane). After evaporation of this active fraction, the residue was purified using Sephadex LH-20 (100 g; GE Healthcare Bio-Sciences, Uppsala, Sweden) chromatography, and eluted with 20, 40, 60, and 80% (v/v) aqueous methanol (150 mL each) and methanol (300 mL) to furnish five fractions (F_1 to F_5). The active fraction was eluted with 40% aqueous methanol and evaporated to dryness. The residue of the active fraction was further separated using a reverse-phase C_{18} cartridge (1.2 × 6.5 cm; YMC, Kyoto, Japan). The cartridges were eluted with 20, 40, 60, and 80% (v/v) aqueous methanol (15 mL each) and methanol (30 mL) to obtain five fractions (F_1 to F_5). The active fraction was obtained by elution with 40% aqueous methanol and evaporated to dryness. The residue was finally purified using reverse-phase HPLC (500 × 10 mm I.D. ODS AQ-325; YMC Ltd.) at a flow rate of 1.5 mL/min with 40% aqueous methanol and detected at wavelength 220 nm and 40°C. The activity of all the fractions in each separation step was determined by *L. sativum* bioassay.

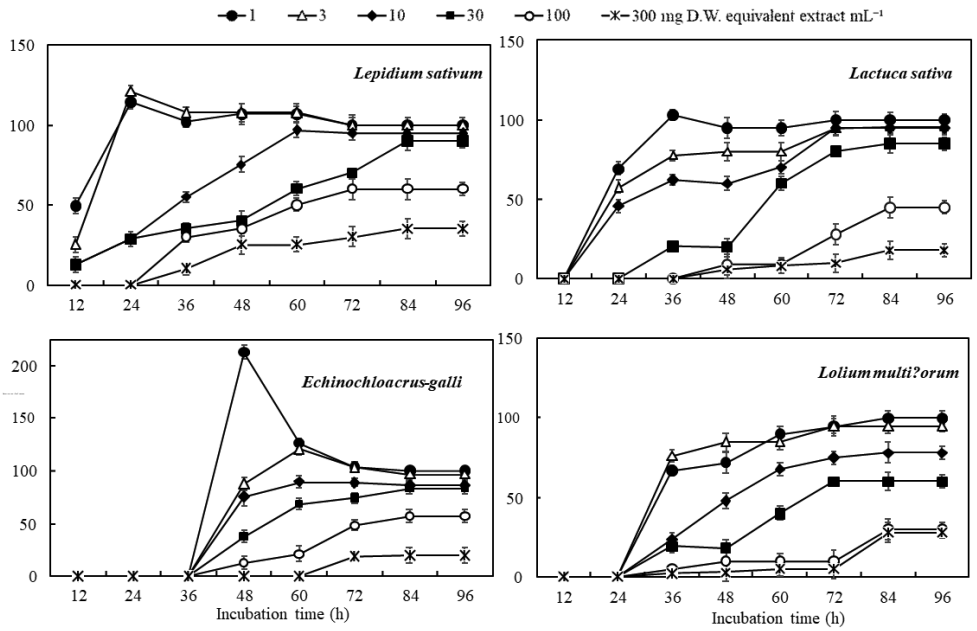

Fig. 1. Effect of *Spondias pinnata* extracts on the germination of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum* at 12 h intervals for four days. The bars for each experiment express mean ± SE with three replicates (n = 30)

Statistical analysis

The data were statistically analyzed using SPSS version 16.0 with one-way ANOVA, and post hoc analysis using Tukey's test. The student *t*-test was used when two data groups were compared. The concentrations needed for 50% inhibition (I_{50}) values of the test plants were analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA).

Results

Allelopathic effect of the Spondias pinnata extracts

The *S. pinnata* extracts suppressed the germination of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum* at concentrations greater than 30 mg D.W. equivalent extract/mL (Figure 1). Germination was significantly suppressed (> 50% that of control) at the concentration of 100 mg D.W. equivalent *S. pinnata* extract/mL for *L. multiflorum*, and 300 mg D.W. equivalent *S. pinnata* extract/mL for *L. sativum*, *L. sativa*, and *E. crus-galli*.

The *S. pinnata* extracts also significantly suppressed the shoot and root growth of the four test plant species at concentrations ≥ 3 – 10 mg D.W. equivalent extract/mL (Figure 2 a, b). The concentration of 10 mg D. W. equivalent *S. pinnata* extract/mL restricted the shoot growth of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum* by 30.4, 39.4, 65.5, and 29.8% of control shoot growth, respectively, and restricted the root growth of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum* by 23.5, 25.3, 15.5, and 22.3% of control root growth, respectively.

The extracts obtained from 300 mg of *S. pinnata* completely inhibited the shoot and root growth of *L. sativum*, *L. sativa*, and *L. multiflorum*, whereas the extracts inhibited the shoots and roots of *E. crus-galli* by 20.1 and 0.38% of control growth, respectively. The I_{50} values of the *S. pinnata* extracts against the seedling growth of the four test plant species varied from 2.2 to 30.0 mg D.W. equivalent extract/mL (Table 1).

Separation of an active substance from the S. pinnata extracts

The *S. pinnata* extracts were separated into ethyl acetate and aqueous fractions. Both the ethyl acetate and aqueous fractions exhibited a concentration-dependent inhibitory effect on *L. sativum* (Figure 3 a, b); however, the greater inhibitory effect was found with the ethyl acetate fraction. Thus, the ethyl acetate fraction was further separated using a silica gel column.

The fraction eluted with 70% ethyl acetate in *n*-hexane (F_6) had the greatest inhibitory activity (Figure 4).

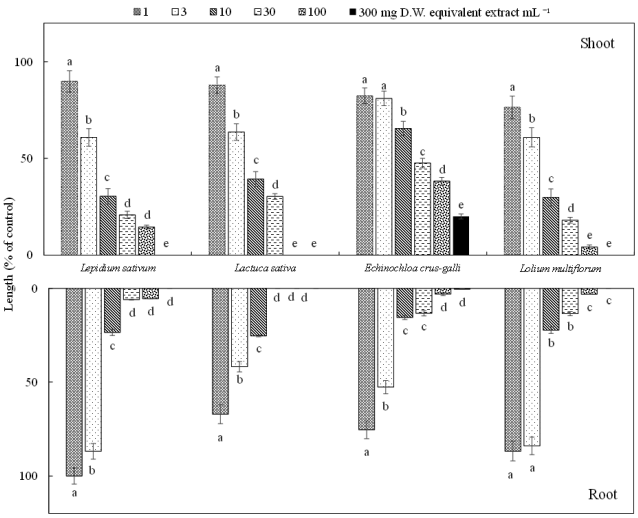

Fig. 2. (a) Effect of *Spondias pinnata* extracts on the shoot and root growth of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum*. The bars for each experiment express mean ± SE with six replicates (n = 60). Different letters in each category indicate significant differences among the treatments (one-way ANOVA, Tukey's HSD, $p < 0.05$

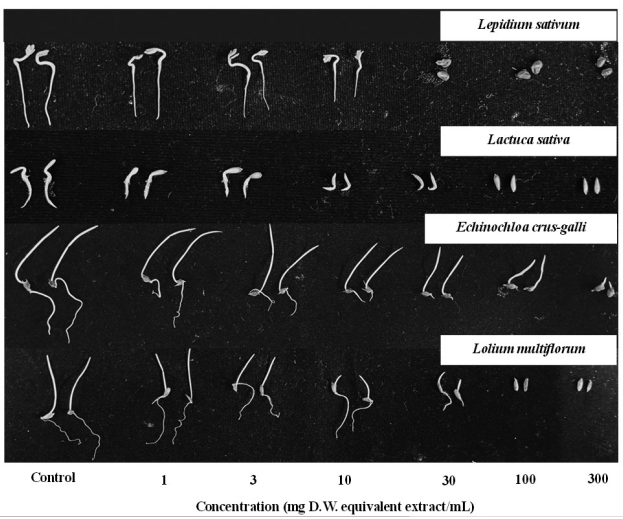

Fig. 2. (b) Effect of *Spondias pinnata* extracts on the growth of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum*

Therefore, F_6 was purified using Sephadex LH-20 and C_{18} Sep-Pak cartridges, and finally an active substance (SPL – 1) was isolated using high-performance liquid chromatography (HPLC) at the retention time of 122 – 128 min (Figure 5). The roots and shoots of *L. sativum* were restricted by

Table 1. The concentration required for 50% inhibition (I_{50}) of the shoots and roots of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum* by the *Spondias pinnata* extracts

Test plant species	I_{50} (mg D.W. equivalent extract/mL)		Level of significance
	Shoot	Root	
Cress	5.45	6.33	*
Lettuce	6.73	2.19	**
Barnyard grass	30.65	2.97	***
Italian Ryegrass	4.34	5.96	**

The I_{50} values were determined using logistic regression analysis. The significant differences between the I_{50} values of the shoot and root growth are denoted by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Student *t*-test)

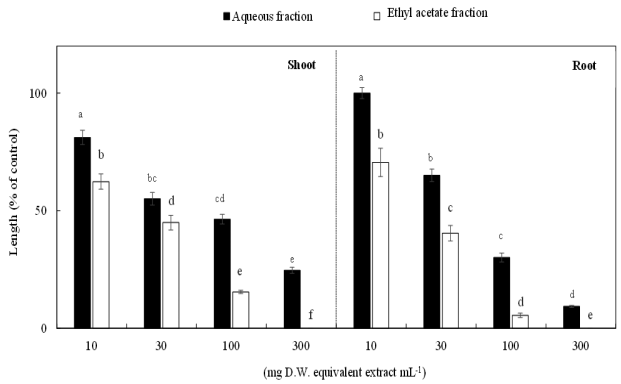

Fig. 3. (a) The effect of the aqueous and ethyl acetate fractions obtained from *Spondias pinnata* extracts on the shoot and root growth of *L. sativum*. The bars for each experiment express mean ± SE with three replicates (n = 30). Different letters indicate significant differences among the treatments (Tukey's HSD, $p < 0.05$)

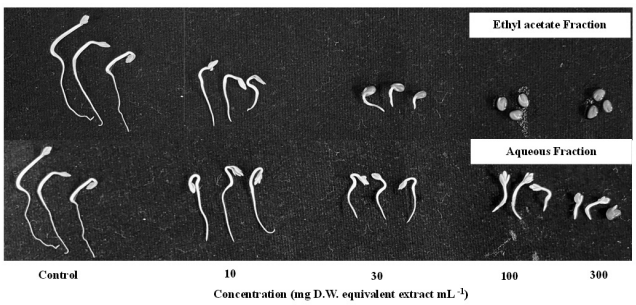

Fig. 3. (b) Effect of ethyl acetate and aqueous fractions obtained from *Spondias pinnata* extracts on the growth of *L. sativum*

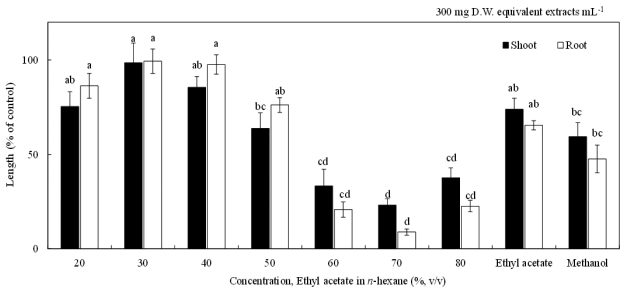

Fig. 4. Effect of the fractions obtained from a silica gel column of *Spondias pinnata* extracts on the shoot and root growth of *L. sativum*. The test plant was exposed to a concentration equivalent to the extract obtained from 300 mg D.W. of *Spondias pinnata* leaf mL^{-1}. The bars for each experiment express mean ± SE with three replicates (n = 30)

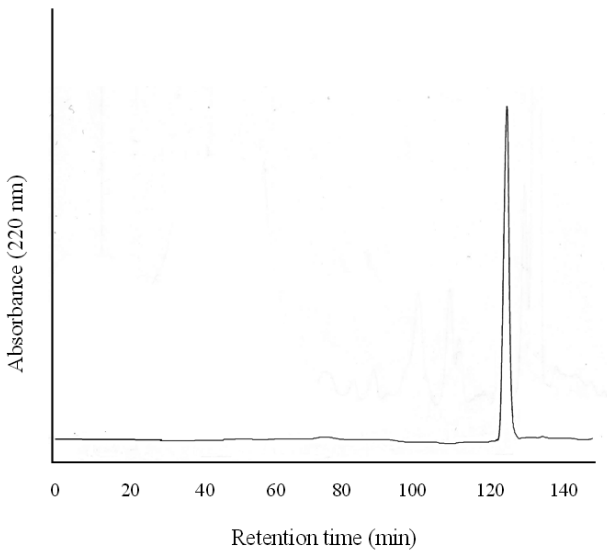

Fig. 5. Chromatogram of an inhibitory substance obtained using reverse-phase HPLC

the active substance (SPL – 1) to 30.0 and 26.2% of control growth, respectively.

Discussion

The *S. pinnata* extracts suppressed the germination and seedling growth of both the dicotyledonous species (*L. sativum* and *L. sativa*) and monocotyledonous weed species (*E. crus-galli* and *L. multiflorum*) (Figure 1 and Figure 2 a, b).

Significant reductions in germination were observed as the extract concentration increased. Such dose-dependent responses in germination of test plants by *Melissa officinalis* L., *Leonurus sibiricus* L., and *Sphenoclea zeylanica* Gaertn. were reported by Kato-Noguchi (2003), Islam & Kato-Noguchi (2014), and Krumsri et al. (2020), respectively. The growth suppressive activity of the *S. pinnata* extracts against the seedling growth of the test plant species also depended on the extract concentrations. Similar findings for extracts of *Nephrolepis cordifolia* (L.) C. Presl (Boonmee et al., 2020), *Leucas cephalotes* (Roth) Spreng. (Lun & Kato-Noguchi, 2021), and *Conocarpus erectus* L. (Alsharekh et al., 2022) resulted in an increase in growth inhibition of several test plant species. Moreover, many studies have reported that allelochemicals isolated from plant extracts inhibited the germination and seedling growth of a number of target plant species in a concentration-dependent manner (Baruah et al., 1994, Miranda et al., 2015, Bari et al., 2019, Krumsri et al., 2022, Moh et al., 2022). Therefore, the growth suppressive properties of the *S. pinnata* extracts in this study suggest that the extracts may have allelopathic activity and possess allelopathic substances with inhibitory activity.

The I_{50} values for the test plant species varied, indicating that the inhibitory effect of the *S. pinnata* extracts was also species dependent (Table 1). Similarly, extracts of *Capparis spinosa* L. (Ladhari et al., 2013), *Melilotus indicus* (Mousavi et al., 2013), *Garcinia xanthochymus* Hook. f. ex T. Anderson (Rob & Kato-Noguchi, 2019), and *Dregea volubilis* (L.f.) Benth. ex Hook.f. (Kyaw & Kato-Noguchi, 2022) have species-specific effects against common weed and crop species. Kobayashi (2004) and Sodaeizadeh et al. (2009) have documented that the difference in sensitivities of test plant species to plant extracts might be due to the different biochemical and physiological nature of each species. Bioassay-directed fractionations of the *S. pinnata* extracts resulted in the separation of an active substance with growth inhibitory properties. However, further investigation is necessary to identify the isolated inhibitory substance from the *S. pinnata* extracts.

Conclusion

The *S. pinnata* extracts exhibited an allelopathic effect on the germination and seedling growth of *L. sativum*, *L. sativa*, *E. crus-galli*, and *L. multiflorum*. An inhibitory substance (SPL – 1) was isolated from the *S. pinnata* extracts, and it might be responsible for the allelopathy of the extracts. SPL – 1 may be released into the soil through the decomposition of the fallen leaves of the *S. pinnata* tree and may inhibit the germination and growth of undergrowth plants, both weed and crop species. Therefore, the present study suggests that *S. pinnata* leaves might be useful as a soil-additive material for controlling weeds in sustainable agriculture. Further field research should be carried out to confirm the allelopathy of *S. pinnata*.

Acknowledgments

We acknowledge the Government of Japan for providing a scholarship (MEXT) to conduct this research. We also thank to Professor Dennis Murphy for editing the English of the manuscript.

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Received: **July, 28, 2022**; *Accepted*: **November, 01, 2022**; *Published*: **December, 2022**