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Efficacy of *Ochna integerrima* (Lour.) Merr leaf extracts against seedling growth of six important plants

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Abstract

Ochna integerrima (Lour.) Merr, a deciduous shrub in the family Ochnaceae, is a great source of active compounds and is used as a medicinal plant in tropical Asia, Africa, and America. Previous studies have focused on the pharmacological properties of *O. integerrima*; however, the allelopathic potential of this plant has not yet been identified. Therefore, this study was carried out with completely randomized design to determine the allelopathic potential of *O. integerrima* leaf extracts in six concentrations against the seedlings growth of dicots plants [garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), and alfalfa (*Medicago sativa* L.)] and monocot plants [Italian ryegrass (*Lolium multiflorum* Lam.), timothy (*Phleum pratense* L.), and barnyard grass (*Echinochloa crusgalli* (L.) Beauv.)]. The results showed that the leaf extracts inhibited the seedling growth of all the test plants at concentrations ≥10 mg dry weight (D.W.) equivalent extract mL^{-1}, except for the barnyard grass shoots. The concentrations needed for 50% inhibition (I_{50} values) of the shoot and root growth of the test plants were in the ranges 1.97–48.01 and 1.18–9.64 mg D.W. equivalent extract mL^{-1}, respectively. The I_{50} values showed the roots of all the tested plants were more susceptible to the extracts than their shoots, except alfalfa. These findings suggested that the leaf extracts of *O. integerrima* may possess allelopathic substances which could be used as a source of bio-herbicide for reducing the weed burden in crop fields. Thus, more research is needed to isolate and identify the allelopathic substances in *O. integerrima* in order to produce bio-herbicide for weed management.

Keywords: *Ochna integerrima* (Lour.) Merr, deciduous shrub, allelopathic potential, allelopathic substances, medicinal plant.
Abbreviations: mg D.W. _ milligrams dry weight, mL^{-1} _ per milliliter, I_{50} _ concentration needed for 50% inhibition.

Introduction

Weeds are plants found in vegetable and crop fields, where they can cause notable losses in yields, greater than 50% for a few crops, if left uncontrolled (Muñoz et al., 2020). Nowadays, herbicides are frequently used to control weeds because they are simple to use and act quickly against target plants. However, the heavy use of synthetic herbicides has resulted in harmful effects on human health, environmental pollution, and an increase in the number of herbicide-resistant weeds (Chauhan et al., 2018; Hasanuzzaman et al., 2020; Hussain et al., 2021; Matzrafi et al., 2021). Therefore, researchers are looking for innovative weed control methods that can ensure biosafety and eco-sustainability (Jabran and Farooq, 2013; Zeng, 2014; Bajwa et al., 2019).

In recent decades, many researchers have been aiming for sustainable agriculture by using natural weed control methods. Allelopathy is a biological phenomenon in which plants produce secondary metabolites (allelochemicals) that interfere with the germination, development, survival, and reproduction of other plants (Rice, 1984). These allelochemicals may hinder the growth of nearby plants and organisms (Yuan et al., 2018; Nawaz et al., 2019; Hussain, 2020). According to numerous studies, medicinal herbs are a rich source of secondary metabolites and possess allelopathic potential (Chevallier, 1996; Wink, 2010). Islam and Kato-Noguchi (2014) described medicinal herbs having more bioactive compounds than other plants. Among 195 medicinal plants in Cambodia, 58 were shown to have substantial allelopathic effects on lettuce root elongation (Sothearith et al., 2020). Aniya et al. (2020) examined the allelopathic capacity of 50 medicinal herbs from China and discovered that the fruit extracts of *Illicium verum* Hook. f considerably decreased lettuce radicle growth and hypocotyls. Because of the strong inhibitory effect of 14 therapeutic plants from northern Thailand, Suwitchayanon et al. (2017) suggested that *Cymbopogon nardus* and *Piper retrofractum* are potential candidates for producing natural herbicides. *Ochna serrulata* also showed a toxic effect against *Artemia salina* and a probable allelopathic effect against lettuce germination (Colla et al., 2011). Mecina et al. (2014) discovered that aqueous extracts of *Ouratea spectabilis* (Mart. ex Engl.) Engl.

(Ochnaceae) considerably suppressed lettuce germination by 53.34% in contrast to control. In addition, several studies have found that most medicinal herbs have significant allelopathic properties that limit the growth of test plants with varying levels of inhibition (Islam et al., 2018). Therefore, medicinal herbs that possess allelopathic activities are one of the alternative approaches to weed management (Bhadoria, 2011). *Ochna* is a genus with 85 species of evergreen trees, shrubs, and shrublets (Verdcourt, 2005). Many phytochemical investigations have revealed the genus to be a plentiful source of flavonoids, phenolic compounds, terpenoids, glycosides, steroids, saponins, and fatty acids (Messanga et al., 1992; Kalenga et al., 2021). Many species in this genus have been used in medicine to treat cancer, diabetes, obesity, cardiovascular disease, typhoid, fever, malaria, snake bites, and menstrual pain (Abdullahi et al., 2010; Bandi et al., 2012; Ndoile and Heerden, 2018). In Myanmar, two species of *Ochna* are known: *O. integerrima* and *O. pumila* (Buch.-Ham. ex DC.). The first species, *O. integerrima*, is synonymous with *Ochna fruticulosa* and is locally recognized as Indaing-seni (Kanis, 1968). Its leaves are ovate-oblong, flowers are blight yellow with 5 petals, and has drupaceous fruits (Trang et al., 2018) (fig 1). This plant is widespread in Myanmar, Bangladesh, Cambodia, Southeast China, Hainan, Laos, Indonesia, Malaya, Thailand, and Vietnam. The bark is used to make a digestive tonic, and its roots and leaves are reputed to possess anthelmintic, antidysenteric, and antipyretic properties (Perry, 1980). This plant possesses bioflavonoids and flavonoid glycosides (Ichino et al., 2006). Moreover, extracts of the leaves and twigs contain anti-HIV-1 flavonoid glycosides (Reutrakul et al., 2007), and extracts of the stem bark possess potent antibacterial properties (Seephonkai et al., 2021). Many researchers have explored *O. integerrima* from a pharmacological point of view; however, no study has yet described the allelopathic potential of this plant. Hence, the current study was done to evaluate the allelopathic potential of *O. integerrima* extracts against six test plants under laboratory conditions.

Results

All the tested plants were subjected to six concentrations of *O. integerrima* leaf extracts, which had different growth inhibitory effects on the growth of the test plant seedlings (Fig. 2 and 3). Except for the shoot growth of barnyard grass, the seedling growth of all the tested plants was inhibited by more than 50% with 30 mg D.W. equivalent extract mL^{-1}, compared with control. The extract concentration of 100 mg D.W. equivalent extract mL^{-1} completely suppressed the shoot growth of the dicot plants (cress, lettuce, and alfalfa), while the shoot growth of the monocot plants (barnyard grass, Italian ryegrass, and timothy) was inhibited by 33.1, 2.2, and 7.1%, respectively, compared with control. At the same concentration (100 mg D.W. equivalent extract mL^{-1}), the root growth of cress, lettuce, Italian ryegrass, and timothy was fully suppressed at the similar dose, whereas the root growth of alfalfa and barnyard grass was inhibited by 2.9 and 1.7%, respectively. At the concentration of 300 mg D.W. equivalent extract mL^{-1}, the shoot and root development of all the tested plants was totally suppressed except for barnyard grass and timothy shoots. In addition, a negative correlation (r) was found between the extract concentration and seedling growth: $r = -0.66$ to -0.89 for shoot growth and $r = -0.65$ to -0.93 for root growth (Table 1). This result revealed that the higher the dosage of *O. integerrima* extract, the greater the inhibition of seedling development of the tested plants.

The I_{50} values were in the ranges 1.97–48.01 and 1.18–9.64 mg D.W. equivalent extract mL^{-1} for the shoot and root growth, respectively (Fig 4). Furthermore, the alfalfa shoots and timothy roots were the most susceptible to the extracts, whereas the barnyard grass shoots and roots were the least sensitive. The I_{50} values were 4.40, 7.26, 48.01, 6.40, and 4.62 mg D.W. equivalent extract mL^{-1} for the shoots of cress, lettuce, alfalfa, barnyard grass, Italian ryegrass, and timothy, which were greater than those for their roots ($P \leq 0.05$) at 1.35, 2.54, 9.64, 3.31, and 1.18 mg D.W. equivalent extract mL^{-1}, respectively.

Discussion

Our results indicate that the leaf extracts of *O. integerrima* had significant allelopathic activities and inhibited the seedling growth of the dicot plants (lettuce, cress, alfalfa) and monocot plants (barnyard grass, Italian ryegrass, and timothy) in a concentration-dependent manner (Fig 2 and 3). These results are in accordance with those of other studies: Kyaw and Kato-Noguchi (2020) noted that increasing concentrations of *Acacia pennata* (L.) leaf extracts inhibited the seedling growth of six test plants; Laizer et al. (2021) reported that higher concentrations of *Sphaeranthus suaveolens* extracts resulted in greater inhibition of the germination of common bean and rice; Poonpaiboonpipat et al. (2021) also stated that the highest concentration of *Chromolaena odorata* (L.) leaf extract showed the greatest inhibitory effect on amaranthus and barnyard grass.

According to the I_{50} values, the alfalfa shoots and timothy roots exhibited the most sensitivity to the *O. integerrima* extracts compared with the other tested plants, and barnyard grass seedling growth showed the least sensitivity to the leaf extracts (Fig 4). These results are similar to other studies (Ida et al., 2020; Krumsri et al., 2020), which reported that the I_{50} values of *Cyathea lepifera* and *Dalbergia cochinchinensis* showed the greatest inhibitory activity against the shoot growth of cress and the seedling growth of lettuce, and the least inhibitory activity against the seedling development of barnyard grass. These different inhibitory activities of the extracts against the tested plant species may be related to the physiological and biochemical characteristics of each species (Sodaeizadeh et al., 2009). Furthermore, the I_{50} values in this study revealed that, with the exception of alfalfa, the roots of all the tested plants were more sensitive to the extracts than the shoots. This result may be due to higher root tissue permeability compared with the shoot tissue because the root tissue emerges first during germination and has direct contact with the allelopathic substances (Turk et al., 2005; Ercoli et al., 2007; Ladhari et al., 2013; Gulzar et al., 2016). Furthermore, Islam and Kato-Noguchi (2013) revealed that the roots of the plants they tested were more sensitive to the extracts of five *Labiatae* plants than their shoots. Our results are similar to the findings of other researchers (Shafiqul and Kato-Noguchi, 2016; Bari and Kato-Noguchi, 2017; Hossen et al., 2020;

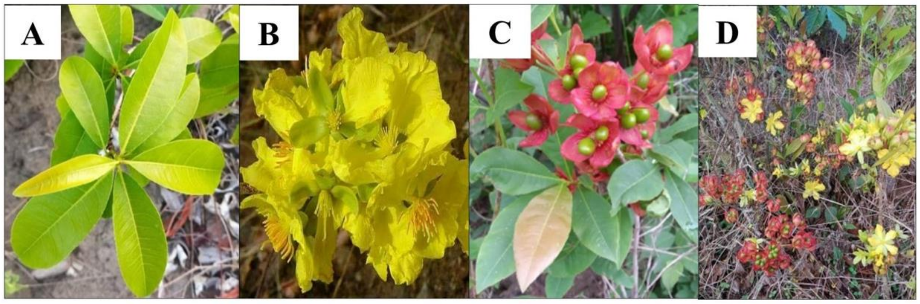

Fig 1. (A) Leaf, (B) flower, (C) fruit and (D) whole plant of *O. integerrima.*

Table 1. Correlation Coefficient (r) between *O. integerrima* extract concentration and seedlings growth (shoot and root growth) of six test plants.

Test plants		Correlation coefficient (r)	
		Shoot	Root
Dicots	Cress	– 0.81**	– 0.85**
	Alfalfa	– 0.66**	– 0.65**
	Lettuce	– 0.89**	– 0.93**
Monocots	Barnyard grass	– 0.75**	– 0.84**
	Italian ryegrass	– 0.77**	– 0.84**
	Timothy	– 0.75**	– 0.76**

Correlation coefficient (r); asterisks represent statistical significance at **P < 0.01 by the two-tailed Pearson correlation test.

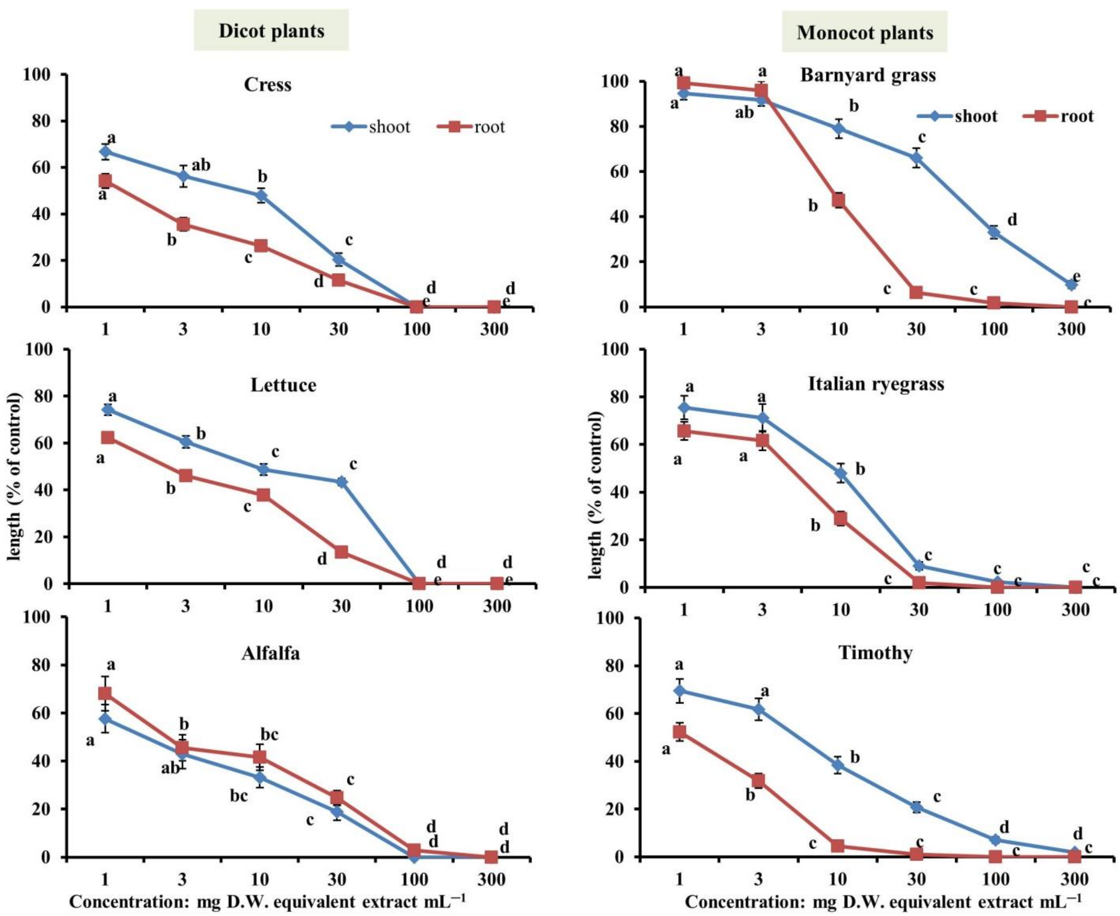

Fig 2. Effect of *O. integerrima* leaf extracts on the seedling growth of six test plant species with six concentrations. The bars on each experiment show mean ± SE with six replications (n=60). The different letters indicate significant difference according to Tukey's HSD test (P < 0.05).

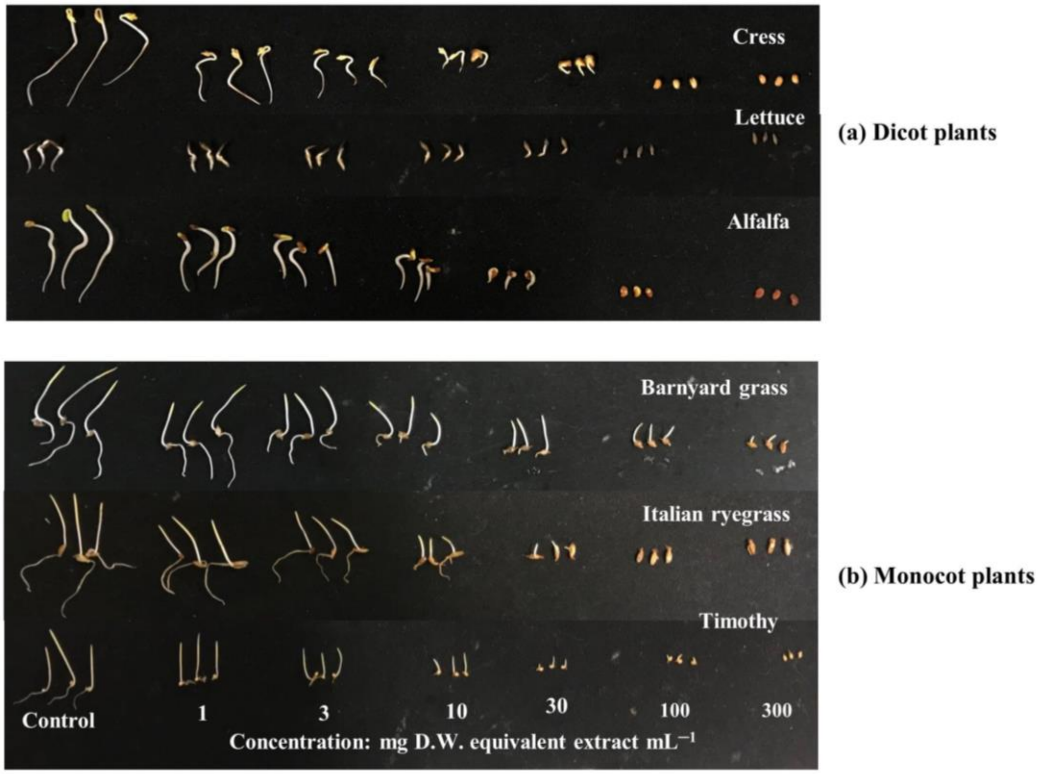

Fig 3. Effect of *O. integerrima* extracts on seedling growth of six test plant species at six concentrations (after 48h).

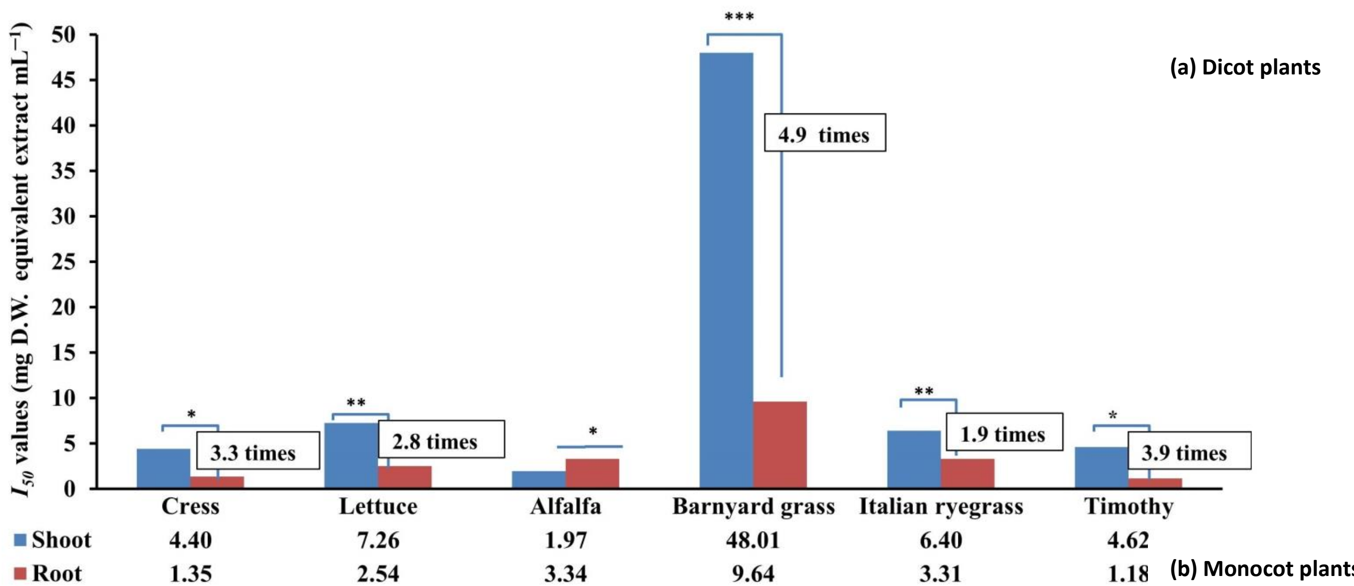

Fig 4. Concentration of *O. integerrima* leaf extracts required for 50% inhibition (I_{50} values) of the shoot and root growth of six test plant species. The significant differences between I_{50} values of shoot and root growth are indicated by asterisks: *P < 0.05, **P < 0.01, and ***P < 0.001 (paired *t* -test).

Mousavi et al., 2021), who indicated that the inhibitory activities depended on the extract concentration of different plants and varied with the tested plant species. Therefore, the results of this study suggested that the leaf extracts of *O. integerrima* may possess inhibitory activities and may include allelopathic substances which could be used as a source of bio-herbicide for reducing the weed burden in crop fields. Accordingly, *O. integerrima* is a potential candidate for characterization of allelopathic substances and its extracts could be used as a bio-herbicide for weed management.

Materials and methods

Plant materials

The *O. integerrima* leaves were gathered from Khin-U Township, Shwe Bo district, Sagaing Division Region, Myanmar (22°49′4″ N and 95°48′12″ E) during July–August 2020. The collected leaves were dehydrated in the shade. The samples were chopped into small pieces and ground to a fine powder using an electric grinder. This powder was kept in airtight plastic bags and retained in a refrigerator at 2°C until extraction. To assess the allelopathic potential of *O. integerrima*, six test plants [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), barnyard grass (*Echinochloa crusgalli* (L.) Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)] were selected. Alfalfa, cress, lettuce, and timothy were chosen for their known seedling behavior, and barnyard grass and Italian ryegrass were selected because they are found in cultivated fields (Kato-Noguchi et al., 2016).

Extraction of the O. integerrima leaves

The leaf powder (250 g) of *O. integerrima* was extracted with 2000 mL of 70% (v/v) aqueous methanol and retained in a sealed beaker for 48 h. The extract was then filtered using a single layer of filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residue was extracted again with an equal amount of methanol for 24 h and filtered. To get the crude extracts, the two residues were combined and evaporated at 40°C using a vacuum rotary evaporator.

Bioassay preparation

The crude extracts of *O. integerrima* were dissolved and diluted with methanol. Aliquots of the leaf extracts at six concentrations of 1, 3, 10, 30, 100, and 300 mg D.W. equivalent extract mL^{-1} were applied to sheets of filter paper (No. 2) in 28 mm Petri dishes. The methanol in the Petri dishes was then evaporated in a laminar flow cabinet. Subsequently, 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan) was applied to all the Petri dishes. Germinated seeds (n=10) of the monocot test plants and seeds (n=10) of the dicot test plants were placed on the filter paper in each Petri dish. For the control treatment, an aqueous solution of Tween 20 was applied to the seeds. All the test plants were incubated in the dark in a growth chamber at 25°C for 48 h. The seedling length of all the tested plants was measured after the 48 h incubation period. The following formula was used to calculate seedling length percentage:

$\text{Percentage of seedling length (\%)} = \frac{\text{lengeh of treated seedling}}{\text{length of control seedling}} \times 100$ (Krumsri et al., 2019).

Statistical analysis

All bioassay tests were performed using a completely randomized design with six replications and treatments: 1, 3, 10, 30, 100 and 300 mg dry weigh equivalent extract mL^{-1}. Data in each experiment were analyzed using the software SPSS version 16.0 followed by Tukey's HSD test at the 0.05 probability level. The paired *t*-test was used to compare the concentration needed for 50% inhibition (I_{50} value) of the shoot and root development. The correlation coefficient (r) between extract concentration and shoot and root length of the test plants was determined using a two-tailed Pearson correlation test. The I_{50} data were examined using GraphPad Prism 6.0. (GraphPad Software, Inc., La Jolla, California, USA).

Conclusion

The aqueous methanol extracts of *O. integerrima* exhibited an inhibitory effect on the seedling development of six test plants in a concentration- and species-dependent manner. The results of this investigation indicate that the leaf extracts of *O. integerrima* may contain allelopathic substances. Therefore, *O. integerrima* could be used for allelopathic compound isolation and identification, as well as a source of bio-herbicide for weed management.

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