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# Fatty acids from itchgrass (*Rottboellia cochinchinensis*) and their herbicidal activity

**A. Bundit[1], K. Yamada[2], H. Shigemori[2] and T. Pornprom***
Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen,
Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand;
E. Mail: agrtpp@ku.ac.th, tosapon.p@ku.ac.th, apirat.b@cmu.ac.th,
eak_agron@hotmail.com, yamada.kosumi.gn@u.tsukuba.ac.jp,
shigemori.hideyuk.fn@u.tsukuba.ac.jp.

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## ABSTRACT

There is increasing attention towards phytotoxic substances from plants as they offer an alternative biological approach to control weeds. Itchgrass (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) is used as mulching material for weed control in vegetable fields and is suspected to release allelochemicals. Thus, we conducted this research to find the phytotoxic compounds from itchgrass shoot. Their phytotoxic activities were assayed *in-vitro* on the shoot and root growth of lettuce (*Lactuca sativa* L.). Two active substances were isolated from *n*-hexane crude extracts of itchgrass shoots and identified as linoleic acid (9,12-octadecadienoic acid) and linolenic acid (9,12,15-octadecatrienoic acid) by $^{1}H$ and $^{13}C$ nuclear magnetic resonance spectral analyses. In addition, the average individual inhibition from each concentrations of two active compounds inhibited shoot growth of lettuce seedlings by 16.7 % and 38.8 % and root growth by 34.1 % and 58.8 %, respectively. These results indicated that the two phytotoxic compounds: linoleic acid and linolenic acid may contribute to the phytotoxic activity of itchgrass.

**Key words:** Allelochemicals, allelopathy, fatty acids, herbicidal activity, itchgrass, linoleic acid, linolenic acid, *Rottboellia cochinchinensis*.

## INTRODUCTION

Allelopathy is a biological phenomenon involving chemical interactions between living organisms in the ecosystem, which influences the growth and development of plants through the release of allelochemicals. These allelochemicals may be beneficial for weed control (10,14,20). Higher plants produces the allelochemicals that stimulate or inhibit the growth of other plants (25). Allelochemicals are released into the soil rhizosphere through volatilization, decomposition of residues and root exudates (27). The rhizomes of Johnson grass (*Sorghum halepense*) release 5-allelopathic phenolic compounds (*p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, ethyl *p*-hydroxybenzoate, tricin and diosmetin) through root exudates at different developmental stages (12). The allelopathic activity of rice (*Oryza sativa* L. cv. Koshihikari) is the inducible defence mechanism through chemical-mediated plant interactions between rice and barnyard grass (*Echinochloa crus-galli* (L.) Beauv), providing a competitive advantage for rice to suppress the barnyard grass growths (15). Thus, allelopathy may be an environmental friendly strategy for weed control (10), which could reduce herbicide use in sustainable agricultural systems.

*Correspondence author, [1]Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand, [2]Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan.

The structure elucidation of allelochemicals is important to understand the biological plant interactions in ecosystem or field conditions. Based on the structure, the allelochemicals are classified as (a) water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; (b) simple unsaturated lactones; (c) long-chain fatty acids and polyacetylenes; (d) quinines (benzoquinone, anthraquinone and complex quinines); (e) phenolics; (f) cinnamic acid and its derivatives; (g) coumarins; (h) flavonoids; (i) tannins; (j) steroids and terpenoids (sesquiterpene lactones, diterpenes and triterpenoids) (19). A better understanding of allelopathic activity is necessary to effectively utilize it in weed management (24,29). For example, allelochemicals released from the allelopathic rice plant roots inhibits the neighbouring plant species (10). Sunn hemp intercrop suppresses the weeds more effectively than other intercrops and polythene mulching in cotton fields (4). Many allelochemicals (such as syringaldehyde and acetosyringone) were isolated and identified from the aqueous methanolic extracts of *Eleocharis atropurpurea* (31). Therefore, the identification of allelochemicals and their biological activity from allelopathic plants will provide a basic information to decide the optimum weed management programme in cropping systems.

Itchgrass (*Rottboellia cochinchinensis* (Lour.) W. D. Clayton) is an aggressive weed and wildly distributed in tropical and subtropical regions (17). It affects the growth and yield of maize, sugar cane and orchards in Thailand (23). It is an allelopathic plant (5,7,23) and has been used as mulch for weed control in vegetable production in farmer's fields in Chaehom-Lampang area, Northern Thailand, where traditional weed control is practised (Fig. 1A).

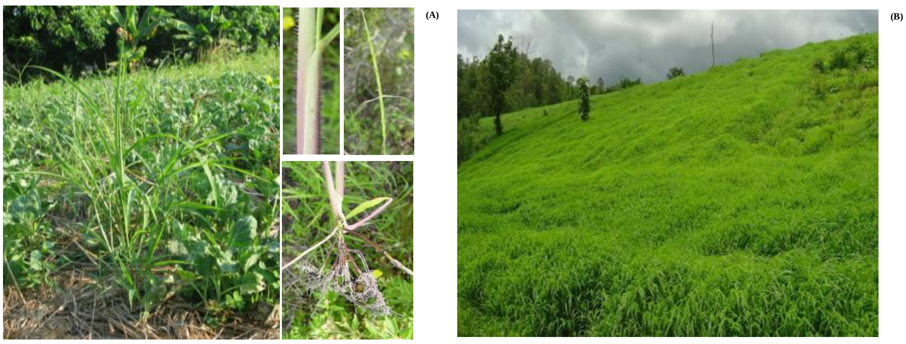


Figure 1. Itchgrass plant (A) in farmer fields (B) in the Chaehom-Lampang area in northern Thailand.

Farmers sow the itchgrass seed in rainy season, it is harvested at maturity and biomass is spread as mulch on soil surface before vegetable cultivation in the growing season (October - January). The itchgrass mulch remarkably reduces weeds density in the vegetable fields (5,6,23). It is suggested that itchgrass produces and releases allelochemicals which controls the weeds (Fig. 1B). Recently, Bundit *et al*. (7) reported that *trans-p*-coumaric acid was isolated and identified from the aqueous methanol extracts of itchgrass. However, the allelopathic activity of donor plant is related to release of many allelochemicals affecting the receiver plant (13,31). Thus this study aimed to isolate and identify all potential allelochemicals in the shoots of itchgrass. This information will improve the understanding of the allelopathic potential of itchgrass for weed control.

# MATERIALS AND METHODS

## I. Plant material

Itchgrass was harvested after physiological maturity from a farmer's field village Ban Sa subdistrict, Chaehom district of Lampang Province, Northern Thailand (18°41'43.5"N 99°34'22.8"E). The experiments were conducted from 2019 to 2020. The aerial plant parts were cut into 1 to 2 cm pieces and dried in an oven at 40 °C for one week. The dried samples were ground into a powder, passed through a 0.5 mm sieve and stored in a plastic bottle at -30°C. Lettuce (*Lactuca sativa* L.) was selected as the test species for the bioassay.

## II. Extraction

One kilogram of itchgrass powder was extracted with *n*-hexane (5 L) and kept at room temperature (25 °C) for 3 days. During the extraction period, it was sonicated every day for 1.0 h and filtered through filter paper. The filtrates were concentrated by a rotary evaporator and kept at –30°C.

## III. Purification and identification

The isolation and purification procedures are shown in Fig. 2. Silica gel (15 g, 60 N, 40 to 100 µm, Kanto Chemical Co., Inc., Tokyo, Japan) was suspended using chloroform

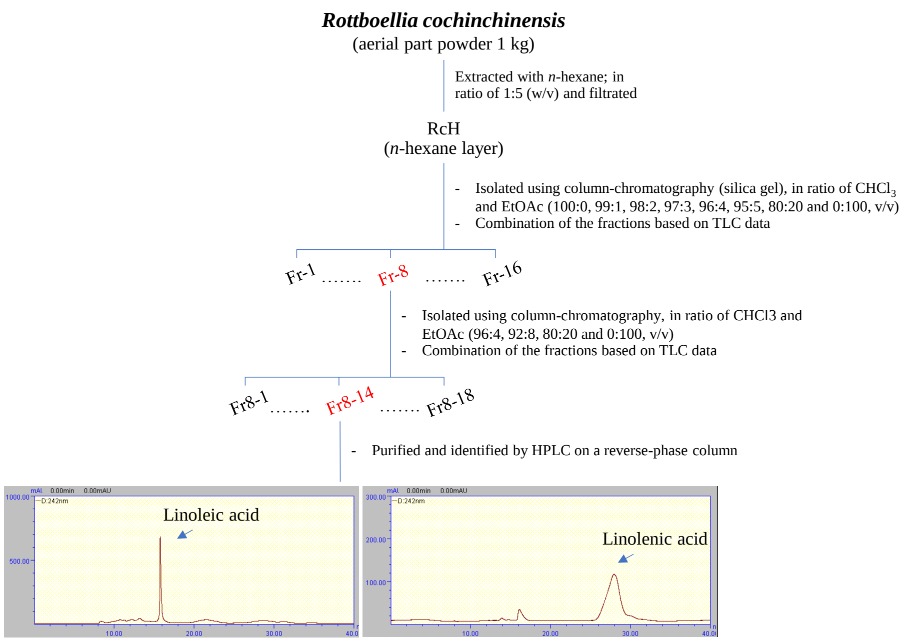


Figure 2. Isolation and purification scheme of linoleic acid and linolenic acid from itchgrass.

and placed into a glass column (1×25 cm). The *n*-hexane crude extract (0.5 g) was diluted with 0.5 mL of *n*-hexane before being subjected to a glass column and eluted with chloroform/ethyl acetate (50 mL per step; 100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 80:20 and 0:100, v/v) mixture. Each fraction (10 mL) was evaporated and then diluted with *n*-hexane before used in thin-layer chromatography (TLC). The TLC spots of each fraction combination were seen under ultraviolet light. Sixteen fractions (Fr-1 to Fr-16) were obtained and subjected to bioassay as described above. The fractions that showed strong growth inhibition were further purified. In this study, Fraction 8 (Fr-8; yielding 83.8 mg) showed obvious activity (Fig. 3A). Fr-8 was then subjected to another silica gel column and eluted with a chloroform/ethyl acetate mixture [96:4 (100 mL), 92:8 (50 mL), 80:20 (50 mL) and 0:100, v/v]. Each fraction (10 mL) was evaporated and then applied to TLC. After combining the fractions based on TLC data, 18-fractions (Fr8-1 to Fr8-18) were obtained and subjected to bioassay. Among the fractions, fractions 8-14 (Fr8-14; yielding 23.2 mg) showed stronger inhibition than others (Fig. 3B). Therefore, Fr8-14 was further purified and identified by high-performance liquid chromatography (condition: acetonitrile:water, 70:30 (v/v); column oven 45°C; 0.8 mL $min^{-1}$; detected at 242 nm) on a reverse-phase column (TSKgel ODS-80Ts, 10 µm, diameter 7.8x300 mm, Tosoh Corporation, Tokyo, Japan). The active peaks with retention times of 15.8 and 28.3 min were identified by comparing with standard substances (linoleic acid and linolenic acid standards were purchased from Wako Pure Chemical Ind. Co., Osaka, Japan). In addition, the chemical structures of the isolated compounds were interpreted by $^{1}H$ and $^{13}C$ nuclear magnetic resonance (Avance 500, Bruker, Billerica, MA, USA) in chloroform-d.

**IV. Bioassay**

The inhibitory effects of *n*-hexane crude extracts and standard substances (linoleic acid and linolenic acid) were investigated in bioassay tests on lettuce seedlings. The crude extracts and standard substances were dissolved in *n*-hexane and diluted to 0.75, 1.50 and 7.50 mg $mL^{-1}$ concentrations for the extracts and to 0.2 and 0.4 mol $L^{-1}$ for the standards.

Lettuce seeds were soaked in distilled water at 25 °C for 24 h. The Petri dishes (33 mm dia) were lined with filter paper and then 0.5 mL of sample solution dissolved in *n*-hexane was added to Petri dish. The corresponding pure solvent was used as the control. After the solvent was completely volatilized in fume hood at room temperature, 0.5 mL distilled water was added. Five germinated seeds (radicle length was approximately 2 mm or less) were put on each moistened filter paper. Petri dishes were placed in the incubator (25 °C) for 5 days. Shoot and root lengths of lettuce seedlings were measured.

**V. Statistical analysis**

Each isolated fraction from the isolation steps was investigated through a bioassay test and compared with a control treatment. The experiment was conducted as a completely randomized design with four replicates. The percentage of inhibition or stimulation was calculated using the equation:

$$\text{Inhibition (-) or Stimulation (+) \%} = [(\frac{D1}{D2}) - 1] \times 100 ;$$

Where, D1: Treatment response, D2: Control response. Data were analyzed by the least significant difference (LSD) test.

## RESULTS AND DISCUSSION

### Allelopathic effects of *n*-hexane crude extracts of itchgrass

The *n*-hexane crude extracts at 7.50 mg $mL^{-1}$ inhibited the shoot and root growth in lettuce seedlings by 22.2 and 89.9 % than control, respectively. Conversely, the lower concentrations at 0.75 and 1.50 mg $mL^{-1}$ stimulated the shoot growth by 34.8 and 33.0 %, and root growth by71.4 and 18.1 %, respectively (Table 1). Tanaka *et al.* (29) concluded that hexane extracts from *Cryptomeria japonica* leaves inhibited the radicle and hypocotyl growth of *Robinia pseudoacacia* and *Trifolium repens*. The active components of *C. japonica* leaves were extracted more effectively with a low-polarity solvent (hexane) than with a high-polarity solvent (methanol). However, Perveen *et al.* (26) reported that the phytotoxic effects of hexane extracts from *Celosia argentea* leaves were slightly inhibitory on the germination and early seedling growth of *Lepidium sativum*. Thus, the allelopathic effects from plant extracts depended on the polarity property of allelochemicals that could be extracted by different organic solvents. Interestingly, *n*-hexane crude extracts were inhibitory to both the shoot and root growth in lettuce seedlings at the highest concentration, whereas they stimulated growth at lower concentration, suggesting a hormetic dose-response (9,11), which is characteristic for many phytotoxins. Therefore, hormetic or biphasic response patterns are characterized by stimulation at low doses and inhibition at higher doses (1,3). For example, the allelopathic effects of *Ocimum basilicum* extracts on the germination and growth of test plant seedlings is a characteristic of hormesis (22). The essential oil from the aerial parts of *Rhynchosia minima* showed significant inhibition of *Dactyloctenium aegyptium* and *Rumex dentatus* germination, while the radicle length of *D. aegyptium* seedlings was stimulated (2). Furthermore, allelochemicals from *Carthamus tinctorius* root exudates shows hormesis effects on germination and growth of various weed species (28). These results suggest that *n*-hexane crude extracts of itchgrass contained candidate substance(s) that can be used for weed control.

Table 1. Effects of *n*-hexane crude extracts on the growth of lettuce seedlings

| Concentration (mg/mL) | Inhibitory or stimulatory effects ( %) | |
|---|---|---|
| | Shoot growth | Root growth |
| 0.00 | 0.0 ± 0.00 b[1] | 0.0 ± 0.00 b |
| 0.75 | +34.8 ± 1.13 c | +71.4 ± 7.14 c |
| 1.50 | +33.0 ± 2.38 c | +18.1 ± 10.14 b |
| 7.50 | -22.2 ± 1.90 a | -89.9 ± 0.43 a |
| $LSD_{0.01}$ | 7.03 | 26.80 |
| Significance | ** | ** |

[1]Means with the same letter are not significantly different and compared using LSD test at $P < 0.01$. (-) = inhibition (%) and (+) = stimulation (%).

Further bioassay guided fractionation showed that Fr-8 was most inhibitory than other fractions, inhibiting shoot and root growths of lettuce seedlings by 24.0 % and 32.3 %, respectively (Fig. 3A). Thus Fr-8 was used for further fractionation. The fraction Fr8-14 were the only fractions inhibiting the shoot growth of lettuce seedling by > 40 %, thus allelochemicals contained in this fraction did not inhibit the root growth (Fig. 3B).

**Identification of biologically active substances in Fr8-14**

Two compounds (compounds 1 and 2) were isolated by comparing the linoleic and linolenic acid standards through high-performance liquid chromatography. The chromatogram of Fr8-14 is shown in Fig. 4A. In addition, compounds 1 and 2 were analyzed by $^{1}H$ and $^{13}C$ nuclear magnetic resonance and identified as linoleic acid (9,12-octadecadienoic acid, Fig. 5A) and linolenic acid (9,12,15-octadecatrienoic acid, Fig. 5B), respectively. The $^{1}H$ and $^{13}C$ nuclear magnetic resonance data of compounds 1 and 2 are interpreted in Table 2.

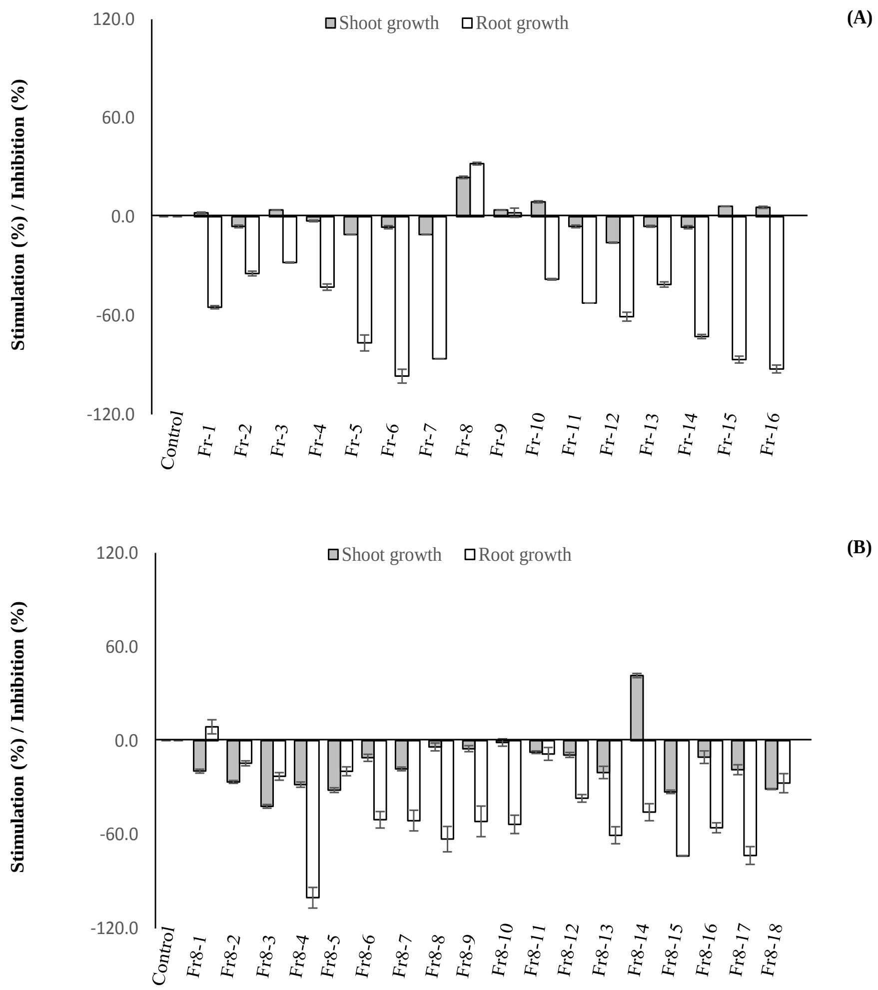


Figure 3. Inhibitory effect of the fractions from isolated-1 (A) and isolated-2 (B) steps on lettuce seedlings growth and the (+) value of means is an inhibitory effects and (-) value of means is a stimulatory effects.

Table 2. $^1$H and $^{13}$C NMR data of compounds 1 and 2 in chloroform-d

| Position | Compound 1 | | Compound 2 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | - | 179.7 | - | 179.7 |
| 2 | 2.35 (2H, m) | 33.97 | 2.35 (2H, m) | 34.0 |
| 3 | 1.63 (2H, m) | 24.65 | 1.63 (2H, m) | 24.7 |
| 4 | 1.32 (2H, m) | 29.01 | 1.32 (2H, m) | 19.8 |
| 5 | 1.32 (2H, m) | 29.13 | 1.32 (2H, m) | 29.4 |
| 6 | 1.32 (2H, m) | 29.06 | 1.32 (2H, m) | 29.3 |
| 7 | 1.32 (2H, m) | 29.69 | 1.32 (2H, m) | 29.2 |
| 8 | 2.05 (2H, t, *J* = 6.0 Hz) | 27.19 | 2.04 (2H, m) | 27.2 |
| 9 | 5.36 (1H, m) | 130.24 | 5.36 (1H, m) | 132.0 |
| 10 | 5.36 (1H, m) | 128.28 | 5.36 (1H, m) | 130.2 |
| 11 | 2.77 (2H, t, *J* = 6.0 Hz) | 25.61 | 2.81 (2H, t, *J* = 6.0 Hz) | 25.6 |
| 12 | 5.36 (1H, m) | 128.24 | 5.36 (1H, m) | 128.1 |
| 13 | 5.36 (1H, m) | 130.21 | 5.36 (1H, m) | 127.9 |
| 14 | 2.05 (2H, t, *J* = 6.0 Hz) | 27.17 | 2.81 (2H, t, *J* = 6.0 Hz) | 25.5 |
| 15 | 1.32 (2H, m) | 29.31 | 5.36 (1H, m) | 127.7 |
| 16 | 1.32 (2H, m) | 31.92 | 5.36 (1H, m) | 127.1 |
| 17 | 1.32 (2H, m) | 22.56 | 2.04 (2H, m) | 20.5 |
| 18 | 0.89 (3H, t, *J* = 7.2 Hz) | 14.1 | 0.98 (3H, t, *J* = 7.2 Hz) | 14.3 |

The spectral $^1$H and $^{13}$C nuclear magnetic resonance data of compounds 1 and 2 were identical to those in the literature (16,21). According to the report by Li et al. (19), long-chain fatty acids can also be classified as allelochemicals. Overall, the results showed that the retention time in the high-performance liquid chromatography chromatogram between the isolated compounds and standard substances correlated well (Fig. 4A-C).

**Phytotoxic activity of linoleic acid and linolenic acid on the growth of lettuce seedlings**

Commercially available linoleic acid and linolenic acid at different concentrations were subjected to bioassays and their phytotoxic activities as allelochemicals in itchgrass were assessed. Linoleic acid and linolenic acid inhibited shoot growth by 16.7 % and 38.8 % and root growth by 34.1 % and 58.8 % in lettuce seedlings, respectively (Fig. 6A). Linolenic acid was more inhibitory to both shoot and root growth than linoleic acid. Furthermore, the inhibitory effects of linoleic acid and linolenic acid were dose-dependent. Linoleic acid and linolenic acid at the highest concentration (0.4 mol $L^{-1}$) inhibited shoot and root growth by 51.7 and 74.8 %, respectively (Fig. 6B). Buta (8), isolated and identified the linoleic acid from *Sesbania punicea* seeds and it was found to be dominant plant growth inhibitor. In contrast, linolenic acid induced jasmonate biosynthesis in plant cells, leading to the suppression of mitosis (32). Thus, linolenic acid may inhibit the plant growth by an indirect inhibition mechanism. Furthermore Bundit *et al.* (7), isolated the *trans-p*-coumaric acid from methanolic crude extracts of itchgrass. Therefore, all allelochemicals (linoleic acid, linolenic acid and *trans-p*-coumaric acid), may play important roles in the allelopathy of itchgrass.

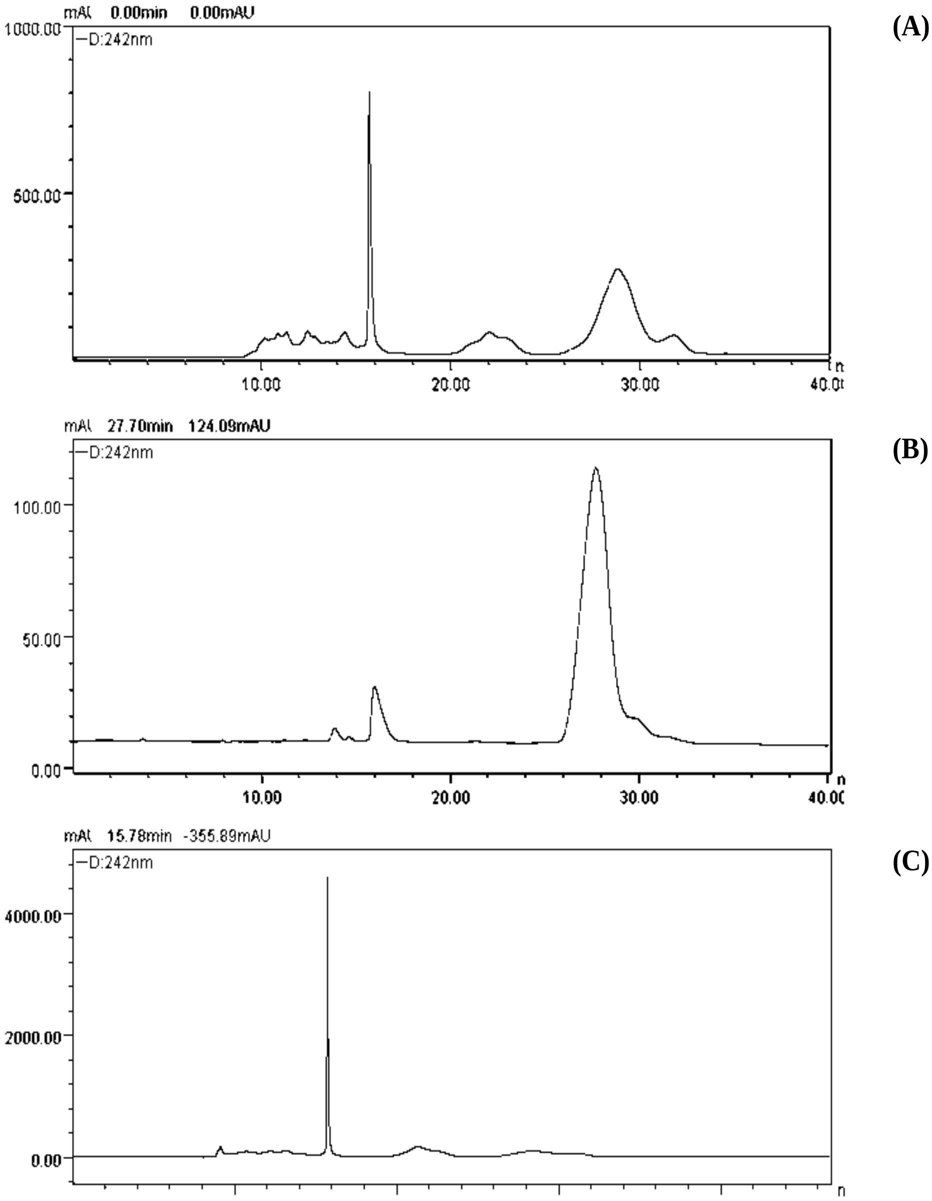


Figure 4. HPLC chromatogram of the Fr8-14 fraction (A), authentic linoleic acid (B) and authentic linolenic acid (C).

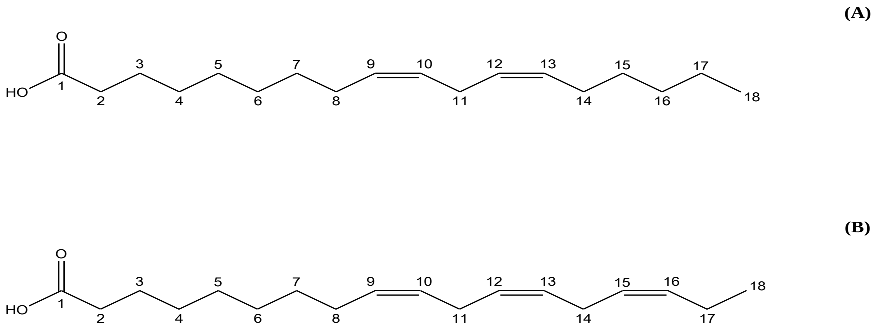


Figure 5. Chemical structure of linoleic acid (A) and linolenic acid (B).

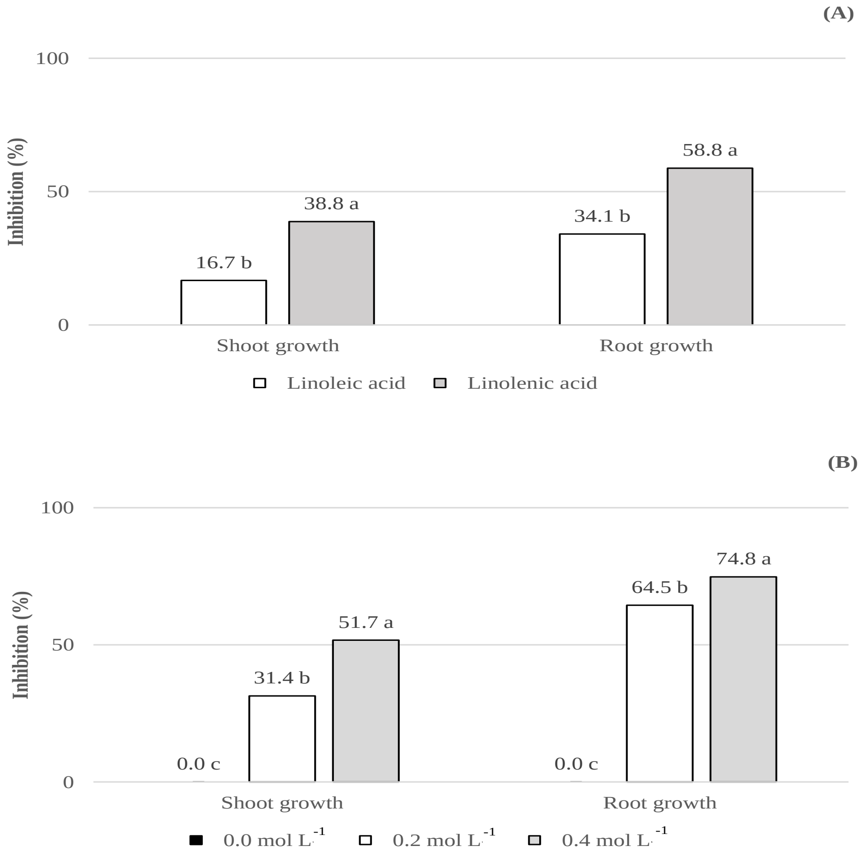


Figure 6. Comparison of the average individual inhibitory effects from each concentrations (A) and different concentrations (B) of commercially available fatty acids on shoot and root growth of lettuce seedlings. Means was compared by using LSD test at $P < 0.01$ and the (+) value of means is an inhibitory effects.

Traditional weed control using the allelopathic potential of itchgrass has been practised through mulching in vegetable fields (23). In this study, linoleic acid and linolenic acid were extracted, isolated and identified from the aerial parts of itchgrass as potential allelochemicals. These compounds had significant allelopathic effects on the seedling growth of lettuce. Although the phytotoxic activities of these compounds were moderate compared to that of *trans*-*p*-coumaric acid (7), this is the first report demonstrating that the two fatty acids in itchgrass showed plant growth inhibitor activity. However, the mechanism of their action should be further studied. In addition, further study is also necessary to demonstrate that the allelochemicals are present in sufficient concentrations to suppress the growth of weeds under field conditions.

One of the major challenges in agrochemical research is managing weeds with less dependence on synthetic herbicides use (14,23,31). Phytotoxic substances from plants have drawn attention as they provide an alternative biological approach to controlling weeds. Thus, it is expected that allelopathic plants can be developed as a new strategy for weed management in agricultural systems. Although mulching material has physical effects on suppressing weeds unfound explored other phytotoxic compounds in the aerial part of itchgrass. The results revealed linoleic acid (9,12-octadecadienoic acid) and linolenic acid (9,12,15-octadecatrienoic acid). These phytotoxic compounds played a part in the phytotoxic activity of itchgrass. In addition, the fundamental information obtained here will provide some ideas for crop breeding programmes, especially in Poaceae family, with allelopathic plants. This strategy for weed control can also contribute to reducing the use of synthetic herbicides. Therefore, understanding the target site and physiological mechanism of allelochemicals in allelopathic plants will lead to a precision strategy for weed management in sustainable agriculture.

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest** : The Authors declare no conflict of Interest.

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