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Full Length Article

Inhibition of *Echinochloa crus-galli* using Bioactive Components from the Stems and Leaves of *Camellia oleifera*

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Abstract

Five fractions (named A_1, A_2, A_3, A_4 and A_5, which obtained by the sequential solvent extraction and purification, see Fig. 1) were isolated from the methanol extracts of the stems and leaves of *Camellia oleifera* Abel. The inhibitory activities of these fractions against *Echinochloa crusgalli* L. were determined, and the biochemistry and physiology of A_1 against barnyardgrass was investigated. The results indicated that the inhibition activity of A_1 was significantly higher than that of the other four tested fractions. When treated prior to seed germination using the cup culture method, the concentration for 50% reduced activity (IC_{50}) of seed germination, shoot length, radical growth and fresh weight were 0.0034, 0.0011, 0.0005 and 0.0021 g/mL, respectively, against barnyardgrass, while treatment after seed germination resulted in IC_{50} values of 0.0017, 0.005 and 0.0017 g/mL, respectively. In addition, the results indicated that the root activity, total amino acid contents and α-amylase and total amylase activities in barnyardgrass decreased with increasing A_1 concentrations. The purified B_{21} fraction was identified as gallic acid using ^{1}H NMR and MS analyses. These results suggest that the fractions and active compounds isolated from the methanolic extract of the stems and leaves of *C. oleifera* showed potent weed control activity and might have potential as a botanical pesticide for the regulation of plant growth.

Keywords: Botanical herbicides; Gallic acid; *Echinochloa crusgalli* (L.) Beauv.; *Camellia oleifera*; Phytotoxicity

Introduction

Echinochloa crusgalli (L.) Beauv is a prolific annual grass weed that is widely distributed in temperate and tropical regions of rice culture worldwide (Maun and Barrett, 1986; Marambe and Amarasinghe, 2002). In 61 countries, this weed has been reported as competing with crops for nutrients, water and light (Barkley *et al*., 1980) resulting in a decrease in crop yield and quality to varying degrees. Thus, synthetic herbicides are used to control weeds, achieving labour-saving effects and economic benefits. However, the indiscriminate use of herbicides has increased the number of herbicide-resistant weeds, potentially causing serious local and global contamination and health problems (Solomon *et al*., 1996; Ecobichon, 2000; Masia *et al*., 2015). Thus, weed management systems need biological solutions to minimize the environmental impact through the development of the natural herbicides to replace traditional agrochemicals. The suppressive effects of allelochemicals can occasionally be effectively used to control biological pests and weeds (Tesio *et al*., 2011; Wang *et al*., 2013). Therefore, the most environmentally acceptable and sustainable approach to weed control is allelopathy, in which natural products are directly utilized as herbicides or indirectly used for herbicide discovery. Based on the fact that allelopathic activity has been the most common approach to the selection of sources of natural products for the discovery of potential herbicides and most biologically active natural products are water soluble (Duke *et al*., 2000). We observed that the inhibition activities of *Camellia oleifera* Abel water extract against barnyardgrass were significantly higher than that of the other 20 species of plants belonging to the 12 families tested (Zhong *et al*., 2008), while screening natural products high herbicidal activity. This species has been demonstrated to have potential as a natural fungicide (Lee and Yen, 2006; Hu *et al*., 2012; Jin and Ning, 2012), antioxidant (Chang and Shyu, 2007; Chen *et al*., 2010; Zhang *et al*., 2010; Ye *et al*., 2013; Feng *et al*., 2014) or insecticide (Wei *et al*., 2005; Potter *et al*., 2010). However, little is known about the phytotoxicity of *C. oleifera* extracts for the sustainable management of weeds. The objectives of the present study were to examine the inhibitory effects of *C. oleifera* on the seed germination and seedling growth of barnyardgrass and to identify potential allelochemicals in this plant.

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Materials and Methods

Materials and Chemicals

The stems and leaves of *C. oleifera* were collected from the Arboretum of South China Agricultural University in Guangzhou, China. This species was widely distributed in South China and was cultivated purposefully there, no chemicals used in the whole growth period. When this large shrub or small tree reached a height of about 20 feet with thin, upright, multiple trunks and branches, the stems and leaves were taken, washed, and dried at 50℃, ground into a fine powder, and subsequently sifted through an 80-mesh sieve.

E. crusgalli seeds were collected from Xiangyin County (Hunan Province, China) and cleaned, desiccated and stored in paper envelopes until further use.

Ascorbic acid, ninhydrin, N, N-dimethylformamide (DMF) and methylene blue were purchased from Bio Science & Technology (Shanghai, China). Maltose, sucrose and soluble starch were obtained from Lizhudongfeng Biotechnology (Shanghai, China). Polyamide and polyamide film were purchased from Sijiashenhua (Taizhou, China). Sephadex LH-20 was purchased from Pharmacia (Peapack, USA). All reagents, including 3,5-dinitrosalicylic acid (DNS), citric acid and sodium citrate, were of analytical grade.

Extraction and Isolation

The dried aerial parts of *C. oleifera* were extracted three times with CH_3OH (V/W=10) under RT (>72 h each) and subsequently filtered using a filter paper. The extract was evaporated under reduced pressure to remove the methanol. The methanol extract (10 g) was dissolved in methanol (40%, 400 mL) and extracted 3 times with ethyl acetate (300 mL/times). Next, the ethyl acetate extract was obtained from ethyl acetate solution extracted using an ethyl acetate extract-water mixture (1:3, v/v). The extraction procedure is summarized in Fig. 1.

The ethyl acetate extract (5 g) was loaded onto a polyamide and eluted using a CH_3OH-H_2O gradient (from 3:1 to 8:2), followed by methanol and acetone. Using a capillary, a small spot of solution containing the sample was applied to a plate, a small amount of an appropriate solvent (CH_3OH: H_2O=8:2) was poured into the container, then the TLC plate was placed in the chamber. The plate was visualized by using ultraviolet light at 365 nm. According to the R_f value of each spot, resulting in five fractions, named $A_1 \sim A_5$.

Identification of Efficient Compound in B_{21} Fraction

The A_1 fraction, dissolved in methanol, was loaded onto a Sephadex LH-20 gel column and the column was eluted with methanol at a flow rate of 20 mL/h. The eluents for B_{21} were pooled and lyophilized.

B_{21} dissolved in methanol was identified through ^1H NMR analysis, using CH_3OH or C_5D_5N as a solvent and TMS as the internal standard, and subsequent MS analysis.

Seed Germination and Growth Bioassay

E. crusgalli seeds were surface-sterilized with 2.5% NaOCl for 10 min, followed by washing with abundant distilled water. Small vibrating glass beads were added to obtain a 20 mL volume in a 100 mL glass beaker (cup culture method), and one filter paper was placed on top of the glass beads to achieve a flat surface (the paper size was the same with the inner diameter of the beaker). A total of 20 seeds of robust barnyardgrass were placed on filter paper, and the substances were separated at different concentrations (10 mL). The glass beakers were placed in a growth chamber maintained at 28℃ under a 14 h light: 10 h dark photoperiod for 7 days. The control was added to an equal amount of water or solvent. The assays were arranged in a complete randomized block design with three replications, including controls. According to Areco (Areco *et al.*, 2014), germination was considered when the radical protruded 2 mm. After 7 days, the number of germinated seeds was counted, and the root and shoot lengths and fresh weight of the germinated seedlings were determined.

The unanimously germinated barnyardgrass seeds were selected for use in the growth bioassay and other processes were conducted as described above. After 7 days, the root and shoot lengths and seedling fresh weight were determined.

Determination of the Root Systems Activities

After the barnyardgrass seeds germinated, the seedlings were treated with A_1 fraction at concentrations of 0.005, 0.0025, 0.00125, 0.001 and 0.0005 g/mL, respectively. The experiment was conducted in a complete randomized block design with three replicates. After 7 days of culture, approximately 40 roots from each sample were frozen in liquid nitrogen for 1 min and stored at −80℃ for the root activity assay. The total absorption area of the root and the active absorption area of the fresh root samples were determined using the methylene blue dye method (Zhang, 1998).

With methylene blue concentration as the abscissa and the absorbance value at 660 nm as the vertical coordinate, a standard regression line equation was generated using the equation y=1.7643x + 0.0341 (r=0.9978).

Determination of Amino Acid Contents

The ninhydrin colorimetric method was used to determine the amino acid contents. Based on the generation of a coloured product, resulting from the formation of Ruhemann's purple complex, a standard regression line equation y=0.0045x+0.0049 (r = 0.9945) was acquired, with

amino nitrogen (μg) as the horizontal axis and the absorbance value at 610 nm as the vertical axis.

The barnyardgrass seedlings were cultured and treated using the same method as used for the determination of the root systems activities. The data were obtained at 10 d after A_1 fraction treatment. The fresh leaves of barnyardgrass were cut into pieces and ground to a fine powder, and 0.5 g of the powder was extracted with boiling distilled water (10 mL), followed by cooling and filtration using filter paper.

This experiment was performed as previously described by Chen *et al.* (Chen *et al.*, 2009) with some modifications. In a 25 mL volumetric flask, 1 mL of each water extraction solution was added, followed by the addition of 1 mL of 1/22 mol/L citric acid buffer solution (pH 5.0) and 1 mL of 1% ninhydrin ethanol solution. The mixtures in the volumetric flasks were incubated in an 80℃ water bath for 15 min. The probes were rapidly cooled in cold water and adjusted to 25 mL with water. After standing for 10 min, the absorbance values of the blue-purple products were measured against a reagent blank (an equal volume of water was carried throughout the procedure). Each experiment was performed in triplicate. The amino acid contents were expressed as milligrams per 1 gram of fresh matter (mg/g) for each of the treated plants.

Amylase Activity Assays

The procedure was conducted using 2 mL of maltose standard solution at different concentrations and 2 mL of 1% 3,5-dinitrosalicylic acid solution (3% sodium potassium tartrate and 0.2 mol/L NaOH), followed by boiling in a water bath for 5 min. The reaction mixture was cooled and diluted with 21 mL of distilled water, and the absorbance was measured at 520 nm. Using the maltose contents as the abscissa and the absorbance values as the vertical coordinates, a standard regression line equation was generated using the equation y=0.2335x + 0.0491 (r = 0.9968).

The barnyardgrass seedlings were collected after culture for 10 days for in vivo and in vitro research. The enzymatic assay was performed as previously described by Bernfeld (Bernfeld, 1955) with slight modifications. The α-amylase (0.5 g seedlings) was extracted with 50 mL of 0.5% NaCl at 30℃ with shaking. The suspension was centrifuged at 5000 g for 10 min and subsequently filtered (5℃). Dextrinization was performed using a mixture of 2 mL of 1% (w/v) starch solution, 1 mL of 0.1 M citric acid/sodium phosphate solution (pH 5.6) and 3 mL of distilled water (40 ℃ for 5 min). As a control, 4 mL of 0.4 mol/L NaOH was used. Subsequently, 1 mL of enzyme extract (β-amylase activity passivated at 70℃ for 15 min for the α-amylase assay) was added, and after 5 min, the reaction was terminated with the addition of 4 mL of 0.4 mol/L NaOH. The hydrolysis was determined using 2 mL of enzyme solution and 2 mL of 1% 3,5-dinitrosalicylic acid solution (3% sodium potassium tartrate and 0.2 mol/L NaOH), followed by boiling in a water bath for 5 min. The reaction mixture was cooled and diluted with 21 mL of distilled water, and the absorbance was measured at 520 nm. The maltose contents were determined using a calibration curve with a maltose standard. The absorbance was read at 520 nm. Amylase activity (y) was represented as:

$$y = \frac{a-b}{W*V2} * V1$$

Where: a = the maltose contents in each treated seedling, b = the maltose contents in the control, W = the sample weight (g), V1= the total volume of sample diluted, and V2 = the volume of the sample determined (mL).

Statistical Analyses

The statistical analysis was performed using SAS software (Cary, NC, USA) or SigmaPlot 10.0 software (Systat Co. USA). Statistically significant differences were considered at $P \leq 0.05$ (Duncan's Multiple Range Test, DMRT).

Results

Isolation and Phytotoxic Activity of the Five Bioactive Fractions

Based on the results of pre-bioassays, the extraction rates of *C. oleifera* dry powder extracted with methanol were the highest (8.37%) among the different solvents tested (data unpublished), and the phytotoxicity of total methanolic extracts against barnyardgrass was the most effective. After extraction with ethyl acetate, the main active ingredient was detected in the ethyl acetate phase. Because ethyl acetate has a relatively strong polarity, the ethyl acetate solution was extracted using distilled water (ethyl acetate extract:water = 1:3) before loading onto the polyamide. Five fractions (A_1, A_2, A_3, A_4 and A_5) were isolated from the stems and leaves of *C. oleifera* through polyamide chromatography and TLC profiling (Fig. 1). The R_f (retention factor) values for A_1, A_2, A_3, A_4 and A_5 were approximately 0.53, 0.44, 0.28, 0.19 and 0.14, respectively.

A severe impact of A_1, A_2, A_3, A_4 and A_5 on the root length and fresh weight of barnyardgrass seedlings was observed. ANOVA indicated that the difference in the inhibition effects on the fresh weight of barnyardgrass among the four fractions (A_1, A_2, A_3 and A_4) was significant, with the exception of a non-significant difference on the root length (Fig. 2). The inhibition rates of the A_1 fraction on the shoot length, root length and fresh weight of barnyard grass were 39.01%, 97.49% and 63.71%, respectively and the A_2, inhibition rates were 27.22%, 97.21% and 52.37%, respectively. These results showed that the inhibitory activity of A_1 was significantly higher than that of the four other tested fractions (Fig. 2).

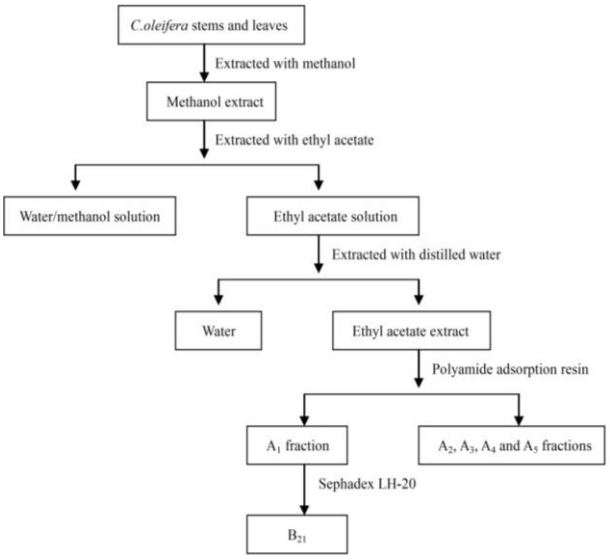

Fig. 1: Flow chart for the extraction and fractionation of the 5 fractions from the stems and leaves of *C. oleifera*

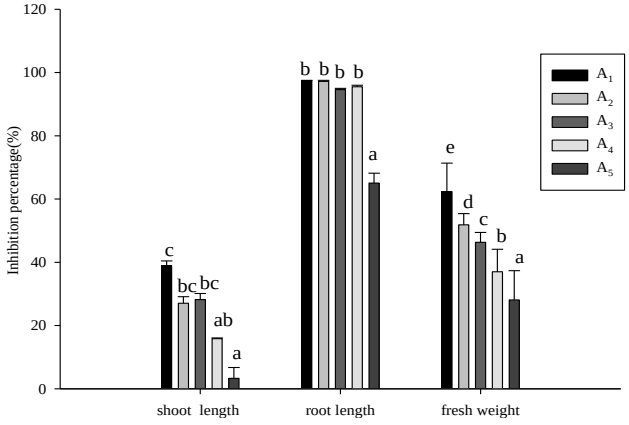

Fig. 2: Inhibition (%) of barnyardgrass treated with fractions A_1, A_2, A_3, A_4 and A_5 from the stems and leaves of *Camellia oleifera* (values of the bars represent the means ±SE (n= 3). Different letters show significant differences at the 5% probability level. Each treatment concentration was 0.001 g·mL^{-1})

Identification of Efficient Compound of B_{21} Fraction

B_{21} was obtained from A_1 purified using gel column chromatography, and this fraction was dissolved in methanol and used to identify the chemical structure of B_{21}.

Only one aromatic protonated molecule signal (δ 7.04, s) was observed in the ^{1}H NMR spectrum, and predominant fragment ions were observed at m/z 171[M + H]$^+$, 153 [M + H – H_2O]$^+$, 127 [M + H – H_2O – CHCH]$^+$ and 109 [M + H – H_2O – CHCH – H_2O]$^+$ using atmospheric pressure chemical ionization mass spectra (APCIMS). Compared with the gallic acid standard, the TLC profiles were the same, confirming that B_{21} was gallic acid (Fig. 3).

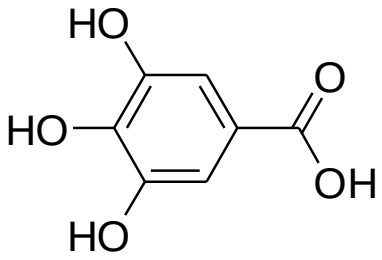

Fig. 3: The chemical structure of gallic acid [^{1}H NMR (400 MHz, CD_3OD), δ 7.04(2H, s); APCIMS *m/z*, 171[M + H]$^+$ (25)]

A_1 Fraction Herbicidal Activity

The pre-emergence bioassays revealed a dose-dependent response of barnyardgrass to the inhibition of seed germination, and root and shoot growth. The A_1 fraction showed the strongest inhibitory activity against root growth, with an IC_{50} (50% inhibition) value of 0.0005 g/mL; however, seed germination inhibition was relatively weak. A_1 fractions inhibited barnyardgrass seed germination and growth, and the sensitivity of the tested barnyardgrass targets to A_1 was root length> stem length> fresh weight> germination rate. In addition, post-emergence bioassays confirmed that the reduced root and shoot growth observed in pre-emergence bioassays primarily reflected growth inhibition, rather than germination delay (Table 1).

Impact of A_1 Fraction on Root Activity

We observed that the A_1 fraction showed its greatest effect at lower concentrations on the active uptake area of root systems. At the lowest concentration of 0.0005 g/mL, the A_1 fraction reduced root activity 53.77%. Barnyardgrass root activity was reduced 91.50% after treatment with A_1 at 0.005 g/mL. The IC_{50} value for the inhibition of the active absorption area was 0.0004 g/mL (Fig. 4a), consistent with the observation that the IC_{50} values for root growth were lowest under seed germination and growth bioassay.

Effect of A_1 Fraction on Amino Acids in the Leaves of Barnyardgrass

Measuring the amino acid content in leaves reveals the nitrogen uptake and assimilation capacity of barnyardgrass under exogenous substances stress conditions. The results showed that A_1 fraction reduced the total amino acid contents in the leaves of barnyardgrass (Fig. 4b). When the A_1 concentration was 0.001 g/mL, the amino acid biosynthesis was significantly inhibited, and nitrogen ratios for the A_1 concentration at 0.005, 0.0025, 0.00125 and 0.001 g/mL were 0.12, 0.17, 0.19 and 0.37, respectively.

Table 1: Inhibition activity of A_1 from the stems and leaves of *C. oleifera* against barnyardgrass

Treatment stages	Tested targets	Regression equation for inhibition	Correlation coefficient	IC_{50} ($g \cdot mL^{-1}$)	95% Confidence Limit ($g \cdot mL^{-1}$)
Pre-emergence treatment	Germination rate (%)	y = 7.5226＋1.0209x	0.9582	0.0034	0.0017~0.0067
	Shoot length (cm)	y = 9.0205＋1.3670x	0.9720	0.0011	0.0008~0.0017
	Root length (cm)	y = 16.7796＋3.6080x	0.9882	0.0005	0.0004~0.0007
	Fresh weight (g)	y = 7.4611＋0.9208x	0.9809	0.0021	0.0012~0.0037
Post-emergence treatment	Shoot length (cm)	y = 7.4281＋0.8791x	0.9676	0.0017	0.0010~0.0030
	Root length (cm)	y = 12.6727＋2.3138x	0.9958	0.0005	0.0003~0.0008
	Fresh weight(g)	y =8.0135＋1.0929x	0.9849	0.0017	0.0011~0.0027

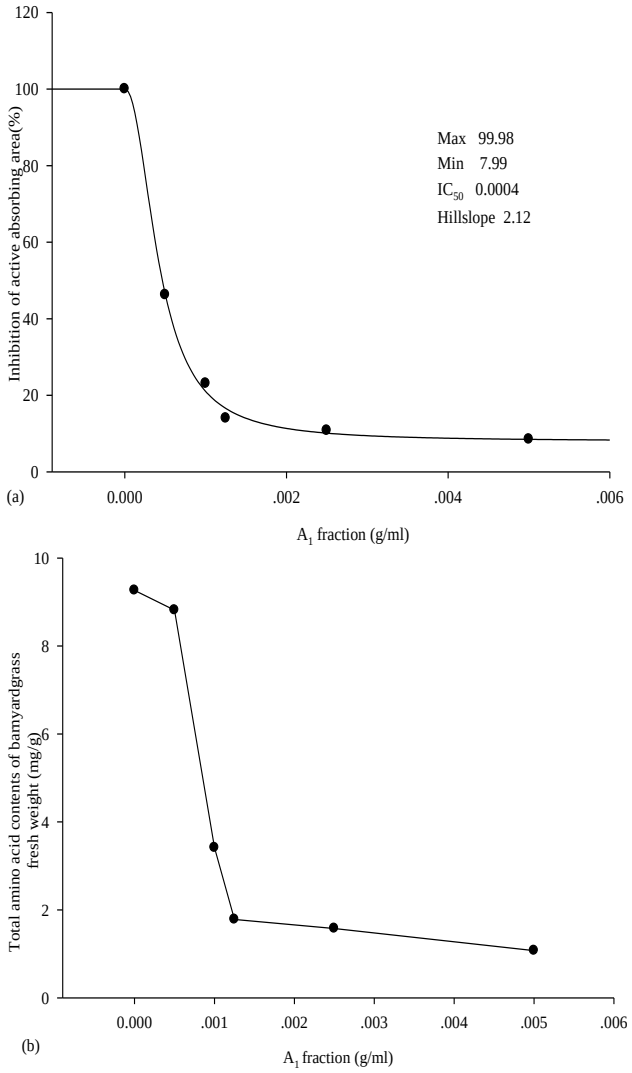

Fig. 4: The impact of A_1 fraction on root systems and the total amino acid contents in the leaves of barnyardgrass

Effect of A_1 Fraction on Amylase Activity

The results of the present study showed that the A_1 fraction significantly affected amylase activity *in vitro*. The A_1 fraction, at concentrations of 0.0001 and 0.001 g/mL, inhibited the α-amylase activity in barnyardgrass 42.16% and 60.00%, respectively, and at the same concentrations, inhibited total amylase activity in barnyardgrass 65.81% and 67.33%, respectively (Table 2).

The effects of the A_1 fraction on amylase activity *in vivo* were consistent with the results obtained *in vitro*. The A_1 fraction induced the highest effect on α-amylase with increasing concentration, however, no significant difference on total amylase was observed among treatments (Table 2 and 3).

Thus, the activities of α-amylase showed little difference at lower concentrations *in vitro and vivo*, but total amylase showed a significant difference compared with the control. Increasing concentrations of the A_1 fraction significantly inhibited both α-amylase and total amylase activity. These results showed that the A_1 fraction interfered with the performance of amylases during the seedling growth stage of barnyardgrass, resulting in a decrease in the maltose contents and the inhibition of enzymes.

Discussion

When studying the herbicidal activity of cineole derivatives against annual ryegrass and radish, Barton *et al.* (2014) pointed out that reduced root and shoot growth observed in pre-emergence herbicidal bioassays were due to post-emergence activity rather than delayed germination (Barton *et al*., 2014). A_1 fraction mainly exhibited post-emergence activity in our tests, which is consistent with Barton' findings. Other studies also have shown that the post-emergence application of these extracts had a greater inhibitory effect on the seedlings than the pre-emergence application (Uddin *et al*., 2012; Voltarelli *et al*., 2012). Though these extracts came from different species, similar allelopathic affects might be associated with same or similar compounds (such as phenolic compounds) from different species. Since A_1 is a starting fraction for separation of B_{21}. Thus we can assume that A_1 fraction could contain more compounds than gallic acid, which was identified in B_{21}. Gallic acid is a phenolic compound, identified in the extracts of *Carum carvi* (Marichali *et al*., 2014), *Mangifera indica* Linn. (old mango leaves) (Saleem *et al*., 2013), *Ailanthus altissima* (Mill.) Swingle (Albouchi *et al*., 2013), *Calamintha nepeta* L. (Savi) (Araniti *et al*., 2013), *Peganum harmala* L. (Sodaeizadeh *et al*., 2009),

Table 2: Effect of A_1 fraction on amylase activity *in vitro*. The maltose content values indicated within a column with different letters are significantly different at the P = 0.05 level (DMRT). The data are presented as the means of three repetitions (mean±SE)

Treatment ($g \cdot mL^{-1}$)	Maltose content for α - amylase (mg)	α - amylase activity (maltose mg/g/5 min)	Inhibition rate (α - amylase activity %)	Maltose content for total amylase (mg)	Total amylase activity (maltose mg/g/5 min)	Inhibition rate (total amylase activity %)
0.001	0.6193±0.01b	0.3961	60.00	0.5713±0.06b	4.3737	67.33
0.0001	0.6351±0.01ab	0.5728	42.16	0.5889±0.05b	4.5771	65.81
CK	0.6714±0.02a	0.9904	—	1.2946±0.17a	13.3887	—

Table 3: Effect of A_1 fraction on amylase activity *in vivo*

Treatment ($g \cdot mL^{-1}$)	Maltose content for α - amylase (mg)	α - amylase activity (maltose g/g/5 min)	Inhibition rate (%) of α - amylase activity	Treatment (g/mL)	Maltose content for total amylase (mg)	Total amylase activity (maltose mg/g/5 min)	Inhibition rate (%) of total amylase activity
0.0025	0.6107±0.03b	2.9443	42.27	0.005	0.6257±0.02b	4.0203	53.93
0.00125	0.6865±0.03ab	3.8919	30.30	0.00125	0.6368±0.02b	4.1595	52.33
0.001	0.7156±0.03ab	4.2559	23.78	0.0005	0.7002±0.03b	4.9518	43.25
CK	0.8218±0.12a	5.5835	—	CK	1.0021±0.04a	8.7259	—

Myrcia guianensis (Souza Filho *et al*., 2006), *Fagopyrum esculentum* Moench. (buckwheat) (Iqbal *et al*., 2003), *Acacia confuse* (Chou *et al*., 1998) and *Ageratum conyzoides* L. (Xuan *et al*., 2004). These phenolic compounds contain a variety of active ingredients that exhibit different herbicidal activities. These compounds also exhibit the same properties, namely, the concentration-dependent allelopathic effects of the chemical substances (Souza Filho *et al*., 2006).

Plant roots are specialized organs for the uptake and transport of water and nutrients, A_1 fraction can effectively reduce the total uptake area and active uptake area of the root systems which are important physiological features to enhance the growth and development of plants (Li *et al*., 2000). And the variation in root activity was closely associated with plant senescence and improving root nutrition and root activity at earlier growth stages could delay leaf senescence (Wei *et al*., 2004). So it's inhibition to root activity also can promote seedlings senescence and accelerate plant to death. On the other hand, Nitrogen (N) is an essential macronutrient, generally absorbed as either nitrate (NO_3^-) or ammonium (NH^{4+}) in plant roots. Gaseous nitrogen pollutants, such as N dioxide (NO_2), are also absorbed by plant surfaces and assimilated via the NO_3^- assimilation pathway (Hu *et al*., 2014), Inhibition of A_1 fraction to amino acid content disturbed the barnyardgrass's metabolism of substances and energy. The research results suggest that the A_1 fraction decreases both root activity and N acquisition, thus affecting root growth and the development of barnyardgrass.

Amylases catalyse the hydrolysis of the a-D-(1,4)-glucan linkage in glycogen and other related carbohydrates (Strobl *et al*., 1998; Diwan and Shenoy, 2001; Zibaee *et al*., 2008). The α-amylase inhibitors show potential for crop protection (Franco *et al*., 2002) and are attractive candidates for the control of weeds, which are highly dependent on starch as an energy source. We safely speculated that inhibition of A_1 fraction to amylase activity is closely related to phenolic compounds (The B_{21} separated from A_1 fraction was identified as gallic acid, a phenolic compound.). Reportedly, phenolic acids play a significant role in the inhibition activity of α-amylase and amyloglucosidase, thereby delaying starch hydrolysis (Adisakwattana *et al*., 2011; Kandil *et al*., 2012; Quesille-Villalobos *et al*., 2013; Nouri *et al*., 2014). However, seaweed extracts (contained high levels of phenolic compounds) showed the weak inhibition of α -amylase (Tong *et al*., 2014), likely associated with the type of phenolic acid. Gallic acid also increases the concentration of reactive oxygen species (ROS; with consequent oxidative stress) in plant cells (Golisz *et al*., 2008). Notably, multiple target sites are a common characteristic of many potent allelochemicals (Leu *et al*., 2002). Therefore, the mechanisms underlying the effects of gallic acid on barnyardgrass need to be studied further.

Conclusion

The inhibition of barnyardgrass activity through several fractions derived from the methanol extracts of the stems and leaves of *C. oleifera* was evaluated. The results showed that the A_1 fraction had the highest herbicidal activity. A_1 exhibited dose-dependent inhibition activity and post-emergence herbicidal activity against barnyardgrass. The B_{21} separated from A_1 fraction was identified as gallic acid, which strongly inhibited the root activity of barnyardgrass and greatly decreased the activity of α-amylase, total amylase and the synthesis of amino acids in barnyardgrass seedlings. Thus, we conclude that *C. oleifera* is an allelopathic plant, whose stem and leaf extracts show potential as botanical herbicides for weed control in paddy fields. The identification of other phytotoxic compounds is currently in progress.

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