

Allelopathic herbicidal effects of crude ethanolic extracts of *Veronica persica* (Lour.) Merr. on weeds

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ABSTRACT

We investigated the herbicidal activity of ethanolic extracts of *Veronica persica* (Lour.) Merr. against four weeds (*Echinochloa crusgalli* (L.) Beauv., *Beckmannia syzigachne* (Steud.) Fern., *Leptochloa chinensis* (L.) Nees and *Lindernia procumbens* (Krock.). The chemical constituents of *V. persica* were identified using gas chromatography-mass spectrometry (GC-MS). The possible inhibitory mechanisms of the ethanolic *V. persica* (Lour.) Merr. extracts to *E. crusgalli* (L.) Beauv. were evaluated by comparing the activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes. Lower extract concentrations (50 and 100 µg/mL) were less inhibitory than higher concentrations (150 and 300 µg/mL). The allelopathic herbicidal effects of *V. persica* (Lour.) Merr. on the 4-test weeds followed the order: *L. chinensis* (L.) Nees, *L. procumbens* (Krock.) Philcox, *B. syzigachne* (Steud.) Fern., *E. crusgalli* (L.) Beauv.. Twenty-one compounds were identified in the ethanolic extracts, of which 9,12,15-octadecatrienoic acid (EC₅₀=0.65 µg/mL) was the major component, followed by 4-compounds [9,12-octadecadienoic acid, hexadecanoic acid (EC₅₀=7.13 µg/mL) and decanoic acid (EC₅₀=0.48µg/mL)]. All these compounds were herbicidal. The CAT, POD and SOD activity of *E. crusgalli* (L.) Beauv. leaves increased at lower concentrations of *V. persica* (Lour.) Merr. extracts, but decreased at higher concentrations. It was concluded that *V. persica* (Lour.) Merr. had strong allelopathic herbicidal activity, thus a standard herbicide against weeds can be developed from its major components.

Key words: Allelopathic effects, *Beckmannia syzigachne* (Steud.) Fern., defense enzyme activity, *Echinochloa crusgalli* (L.) Beauv., ethanol extract, GC-MS, germination, herbicidal, *Leptochloa chinensis* (L.) Nees, *Lindernia procumbens* (Krock.) Philcox, *Veronica persica* (Lour.) Merr.

INTRODUCTION

Many plants have shown herbicidal activity against various weed species (6,33). For instance, the *Tagetes erecta* L. concentrated leaf extracts drastically inhibited the seed germination of *Echinochloa crusgalli* (L.) Beauv., inhibiting both α-amylase activity, as well as seedling growth (31). The ethyl fraction of acetate extract *Tridax procumbens* L. was most inhibitory (IC₅₀=0.22 mg/mL) to *Raphanus sativus* L. i.e., possessing the potent plant growth inhibitors (3). However, only few natural products from these plants are effective in field conditions or are presently commercialized (9,13). Hence, screening for natural products from plants exhibiting high herbicidal activities is necessary.

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Ethanol is commonly used to extract bioactive compounds from plants (7,29). The extraction starts when the ethanol penetrates the plant cells and directly affects the cytoplasmic layer, resulting in dissolution of bioactive compounds (26). Ethanol extraction is simple and convenient method to obtain the bio-active compounds from plants (23). For example, the ethanolic extract of *Iva xanthiifolia* Nutt. at low concentration inhibited the germination and seedling growth of *R. sativus* L., *Brassica campestris* L., *B. pekinensis* (Lour.) Rupr, *B. juncea* (L.) Czern. and *B. oleracea* L. (33).



Veronica persica (Lour.) Merr. (family Plantaginaceae), persian speedwell, is widely distributed invasive weed. Its extracts exhibit wide range of bioactivities [antibacterial, anticoagulant, antifungal and antiprotozoal (8,12)]. Its hydroalcoholic extracts have antioxidant, antifungal and anti-inflammatory effects and 13 compounds were identified from the extracts (16). Free phenolic extracts from *V. persica* (Lour.) Merr. significantly inhibited the α -glucosidase (IC_{50} = 532.97 μ g/mL), whereas, the conjugated phenolic extract significantly inhibited the α -amylase (IC_{50} =489.73 μ g/mL) (22).

We aimed to determine the herbicidal activity of ethanolic extracts from *V. persica* (Lour.) Merr. on four weeds: *E. crusgalli* (L.) Beauv., *Beckmannia syzigachne* (Steud.) Fern., *Leptochloa chinensis* (L.) Nees and *Lindernia procumbens* (Krock.) Philcox. The possible mechanisms of inhibition to *E. crusgalli* (L.) Beauv. were studied by comparing the catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities of the ethanolic *V. persica* (Lour.) Merr. extracts. The chemical constituents of ethanolic extracts were analyzed. The results will provide us theoretical basis for developing bioherbicides from *V. persica* (Lour.) Merr.

MATERIALS AND METHODS

I. Plant material collection and preparation

The stems and leaves of *V. persica* (Lour.) Merr. were collected from a yard located at NO. 2 Yuanda St, Changsha, China in May, 2017 (N28°28'20", E113°4'51"; Mean height above sea level 45 m; Annual rainfall 681.6 mm; Maximum and minimum Temp 38 °C and 15 °C). Mature seeds of *E. crusgalli* (L.) Beauv., *B. syzigachne* (Steud.) Fern., *L. chinensis* (L.) Nees and *L. procumbens* (Krock.) Philcox were collected from a paddy field in Gaoqiao village, Changsha, China in September, 2016 (N28°28'53", E113°20'54"). The *E. crusgalli* (L.) Beauv. plants were grown in growth chamber [16 h photoperiod (100-120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 22/18°C light/dark cycle] and its seedlings with 3-leaves were used for enzyme activity experiments.

II. Ethanolic extract (vegetative phase)

Fresh *V. persica* (Lour.) Merr. plants stems and leaves were washed with water to remove soil particles and other dirt during the vegetative period. The ethanolic extracts were prepared by adding 80 L of 100% ethanol to 20 kg stems and leaves of *V. persica* (Lour.) Merr. kept for 24 h, stirring once every 4 h. The residues were re-extracted thrice with the same amount of solvent again for 24 h. The three extracts were combined and the solvent was evaporated on a rotary evaporator and stored at 4°C for GC-MS identification and inhibition tests.

III. Weeds seeds germination and seedling growth

Twenty seeds of each weed were sown separately in 7-cm-dia Petri dishes lined with filter paper, 4 mL water or treatment solution (50, 100, 150 and 300 $\mu\text{g}/\text{mL}$) was added in each petri dish. One mL water was added daily to each petridish to keep the filter paper separated throughout the experimental duration. These Petri dishes were kept in growth chamber [16 h photoperiod (100-120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with a 22/18°C light/dark cycle]. Germination was determined after 7 days. The germination data were analyzed as the proportion of germinated seeds from the total number of seeds in a single Petri dish. The bioassay was repeated thrice.

Six 3-leaf-stage seedlings were planted in 5-cm-dia plastic cup containing 0.5 g agarose with 4 mL water or treatment solution (50, 100, 150 and 300 $\mu\text{g}/\text{mL}$). These plastic cups were kept in growth chamber [16 h photoperiod (100-120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with a 22/18°C light/dark cycle]. The root and leaf (longest) lengths of seedlings were measured after 7 days. The test was repeated in triplicate. Inhibition (%) was calculated as under:

$$\text{Inhibition (\%)} = (\text{Control-Test sample})/\text{Control}\times 100$$

IV. Measurement of defence enzyme activities

E. crusgalli (L.) Beauv. seedlings at 3-leaf-stage were sprayed with 4 mL water and *V. persica* (Lour.) Merr. extract solution at four concentrations (50, 100, 150 and 300 $\mu\text{g}/\text{mL}$). After 7 days, the *E. crusgalli* (L.) Beauv. leaves (0.5 g per treatment) were harvested to measure the enzyme activities (CAT, POD and SOD). The effects of crude extracts on CAT, POD and SOD were determined as per the method of enzyme assay kit (Nanjing Jiancheng Bioengineering Institute, China). The CAT, POD and SOD were determined by measuring the absorbance at 405 nm, 420 nm and 550 nm, respectively. The enzyme activity was defined as 0.001 change in absorbance between 0 and 180 s

under the assay conditions. The results were expressed as relative activity (%) compared to the initial value.

V. GC-MS analyses

V. persica (Lour.) Merr. extract components were identified by GC-MS (ITS-40, GC-ITMS; Finnigan MAT, San Jose, CA, USA). The interface temperature was 280°C and the scan range was 40-450 atomic mass units (AMU). The chromatographic column used for analysing was a capillary column: Elite 5ms (5% phenyl 95% dimethyl- polysiloxane); size, 250 µm; and ID, 0.25 µm 630 m. The stepped temperature program was held at 70°C for 2 min, increased from 70°C to 280°C at 10°C min⁻¹ and held for 5 min. The total run time was 30 min. Helium was used as the carrier gas and injected at 1 mL min⁻¹. The injection volume was 1 µL. The solvent delay was 2 min and was injected in a split ratio of 1:10. The qualitative analysis was carried out on the basis of the MS spectra, which were compared with the spectra of the NIST Research Library, Medicinal and Aromatic Plant and Drug Research Centre (TBAM) Library of Essential Oil Constituents and Wiley GC-MS Library. The relative percentages of the extracts were obtained automatically from the peak areas of the total ion chromatograms (TICs).

VI. Major compounds herbicidal activity

The monomer of hexadecanoic acid and phytol were purchased from Macklin. The monomer of decanoic acid and 9,12,15-Octadecatrienoic acid, methylester (Z,Z,Z) were purchased from Adamas. Indoor toxicity test was done by spraying 4-major compounds (hexadecanoic acid, phytol, decanoic acid, 9,12,15-Octadecatrienoic acid, methylester, (Z,Z,Z) on plant stems and leaves (NY/T 1155.4-2006). Specifically, *E. crusgalli* (L.) Beauv. were grown in pots kept in growth chamber [18-20°C in a 16 h light: 8 h dark cycle with illumination at 100-120 µmol m⁻²s⁻¹]. Each pot (5 cms dia, 5 cms depth) containing nutrients soil, had 15 *E. crusgalli* (L.) Beauv. plants of uniform growth. s The *E. crusgalli* (L.) Beauv. seedlings of 3-leaf-stage were sprayed with 4 mL water and with four major compounds at 0.17, 0.33, 0.83 and 1.5 µg/mL concentrations. Based on the fresh weight data, herbicidal effect was calculated as under:

$$E = 100 \times (C - T) / C$$

Where, E: Herbicidal effect and C: Fresh weight of weeds shoot in control, T : Fresh weights weeds shoot in treatment group.

VII. Statistical analysis

One-way analysis of variance (ANOVA) and the least significant difference (LSD) test were used to determine the differences between the treatment means at a 5% probability level. The statistical analysis were conducted using SPSS 11.5 software (SPSS Inc., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Seeds germination

The ethanolic extracts of *V. persica* (Lour.) Merr. considerably inhibited the seed germination of 4-test weed species (Fig. 1). In control, the seed germination was 80 % in *E. crusgalli* (L.) Beauv., 91.65 % in *B. syzigachne* (Steud.) Fern., 91.65 % in *L. chinensis* (L.) Nees and 95 % in *L. procumbens*. At 150 µg/mL, seed germination was only 10 % in *E. crusgalli* (L.) Beauv., 10 % in *B. syzigachne* (Steud.) Fern., 8.35 % in *L. chinensis* (L.) Nees and 8.35 % in *L. procumbens* (Krock.) Philcox. There was dosage-dependent gradual

reduction with applied *V. persica* (Lour.) Merr. ethanolic extract (Fig. 1). Increasing the dose of *V. persica* (Lour.) Merr. significantly decreased the of *E. crusgalli* (L.) Beauv. ($F=94.57$, $P < 0.001$), *B. syzigachne* (Steud.) Fern. ($F=20.65$, $P < 0.004$), *L. chinensis* (L.) Nees ($F=78.63$, $P < 0.001$) and *L. procumbens* (Krock.) Philcox ($F=44.43$, $P < 0.001$). The lower concentrations of *V. persica* (Lour.) Merr. extracts (50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$) had lower inhibitory activity than higher concentrations (150 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$). Our results are consistent with other allelopathic studies. Among aqueous leaf, root and flower extracts of *Artemisia annua* L. and *Taraxicum officinalis* Weber at 20, 40 and 60% concentration on the germination of maize and wheat in laboratory conditions, 60% concentration of *A. annua* L. had more drastic effects on wheat and maize than lower concentrations (28). Water extracts of *V. persica* (Lour.) Merr. leaves decreased the seed germination by 5-71.9% in *Vigna unguiculata* (L.) Walp., *Ipomoea aquatica* Forsk, *Zea mays* L., *Phaseolus vulgaris* L., *Cucurbita moschata* and *Capsicum annum* Duch cv. Shintosa (30). Some researchers had shown that in bioassay, some allelopathic plants *Rhazya stricta* Decne and *Eucalyptus citriodora* Hook.f. caused 40-75 % inhibition of *Salsola villosa* Delile. ex Schul. and *Sinapis arvensis* L. (2,5). Hence, these studies demonstrated that *V. persica* (Lour.) Merr. ethanolic extraction are very allelopathic to seed germination of test weeds.

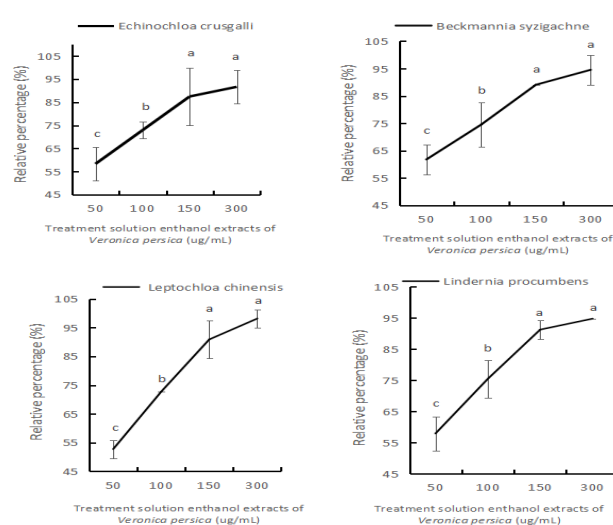


Figure 1. Effects of *Veronica persica* ethanol extracts concentrations on seed germination of four test weed species.

Seedling growth: The ethanolic extracts of *V. persica* (Lour.) Merr. considerably reduced the seedling growth of 4-test weeds (Fig. 2). The 150 $\mu\text{g}/\text{mL}$ caused 48.84%, 62.36%, 68.65% and 56.15% reduction in root length of *E. crusgalli* (L.) Beauv., *B. syzigachne* (Steud.) Fern., *L. chinensis* (L.) Nees and *L. procumbens* (Krock.) Philcox respectively than control, while the reduction in leaf length was 65.18%, 67.21%, 72.61% and 80.43%. At 300 $\mu\text{g}/\text{mL}$, the roots of 4-test weed species showed 59.47%, 71.43%, 77.57%, 72.04% reductions in length than control and the inhibitory percentages in leaf were 71.71%, 76.16%, 84.16% and 85.05%. Based on these results, it was evident that the four weed species exhibited different sensitivities to the *V. persica* (Lour.) Merr. extracts, which corroborates some allelopathic studies (15,20). The allelopathic effects of *V. persica* extracts on the weeds followed the order: *L. chinensis* (L.) Nees > *L. procumbens* (Krock.) Philcox > *B. syzigachne* (Steud.) Fern. > *E. crusgalli* (L.) Beauv.. Other allelopathic studies verified the results. *Poa annua* L. was most sensitive to water extracts of ground *V. persica* (Lour.) Merr., while, *Triticum aestivum* L. and *B. napus* L. were second-most sensitive (32). The resistant and problematic weed *L. chinensis* (L.) Nees in some rice fields is controlled by using ACCase-inhibiting herbicide (21). As the *V. persica* (Lour.) Merr. extracts exhibit high herbicidal activity, these could thus be developed as natural herbicide to control *L. chinensis* (L.) Nees.

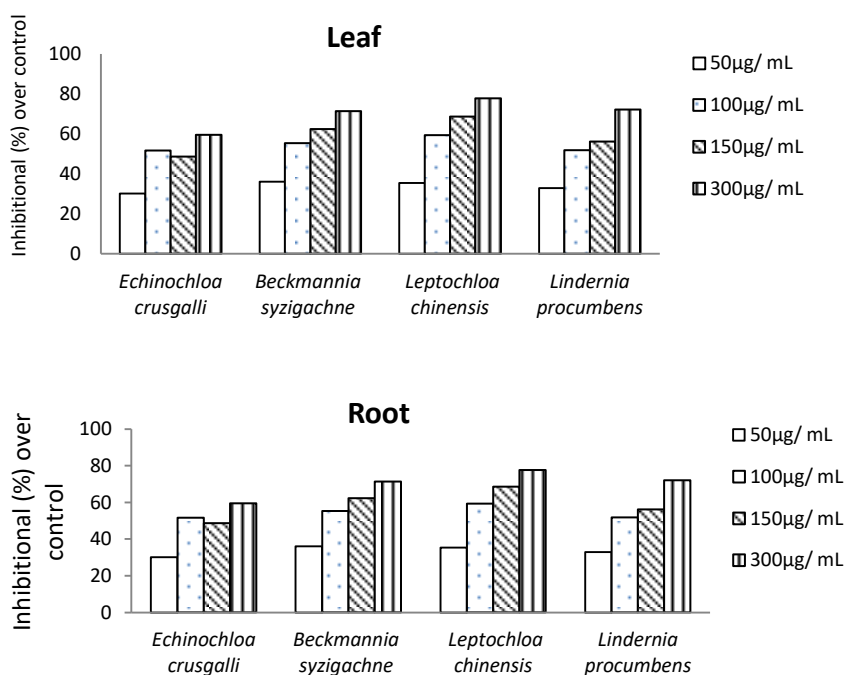


Figure 2. Inhibition effect of *Veronica persica* on root and leaf length of four test weeds over the control.

Antioxidant enzyme activity

The activities of CAT, POD and SOD increased at lower concentrations of extract but decreased at higher concentrations (Fig. 3). For example, from 50 $\mu\text{g}/\text{mL}$ to 150 $\mu\text{g}/\text{mL}$, CAT activity increased from 56.64 U/gFW to 153.49 U/gFW. However, CAT activity decreased to 56.79 U/gFW at 300 $\mu\text{g}/\text{mL}$ ($F = 300.21$, $P < 0.001$). Similar trends were observed for POD and SOD activity. At 150 $\mu\text{g}/\text{mL}$, POD activity reached 344.24 U/gFW, which exceeded its activity in 100 $\mu\text{g}/\text{mL}$ treatment (108.31 U/gFW) ($F = 84.36$, $P < 0.001$). SOD activity at 150 $\mu\text{g}/\text{mL}$ was increased to 222.73 U/gFW, which was 221.49% greater than at 50 $\mu\text{g}/\text{mL}$ ($F = 173.43$, $P < 0.001$).

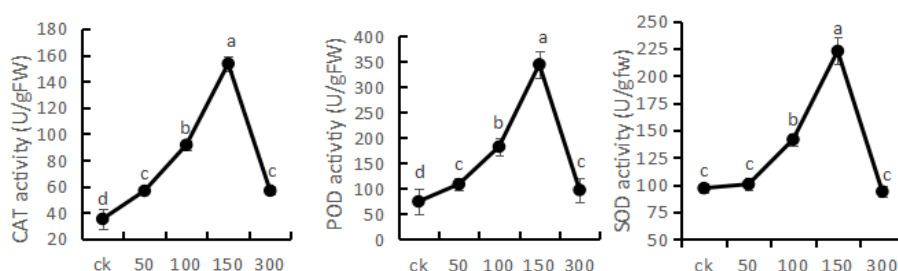


Figure 3. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity of three-leaf *E. crus-galli* seedlings treated with 4 mL water or ethanolic extracts of *V. persica* (50, 100, 150 and 300 $\mu\text{g}/\text{mL}$).

Antioxidant enzymes (CAT, SOD and POD) constitute primary antioxidant defence systems. The production of antioxidant enzymes is one defence mechanism against biotic and abiotic stresses. Studies have reported that antioxidant activity is harmful by-product of biochemical stress in plants, such as responses to ethanol extracts (11,17). *Camellia japonica* L. extracts increased the activity and protein levels of antioxidant enzymes, which may mediate the inhibition of reactive oxygen species (ROS) production (24). The F1 fraction separated from the ethyl acetate extract of *T. procumbens* L. increased the malondialdehyde (MDA) activity, which is a cell-membrane response mechanism against oxidative stress (3). Quercetin reduces or prevents the oxidative stresses induced by ultraviolet A via the activation of antioxidant enzymes and the induction of their expression (14). Additionally, quercetin also exhibited antioxidant and cytoprotective properties against renal ischemia-reperfusion injury by inducing SOD, CAT and glutathione peroxidase expression and activating their activity (18). Thus *E. crusgalli* (L.) Beauv., responds to the *V. persica* (Lour.) Merr. extracts by enhancing the antioxidant enzymes activity. Further studies are required to confirm the manner in which the *V. persica* (Lour.) Merr. extracts affects the antioxidant enzymes.

GC-MS analyses of *Veronica persica* (Lour.) Merr. ethanolic extract

Commercial mass spectral reference libraries were used to identify the components of *V. persica* (Lour.) Merr. extracts from the GC-MS analyses (Fig. 4). The relative percentages of the identified and isolated phytochemicals obtained are given in Table 1.

The major compounds identified included decanoic acid, 2-amino-3-hydroxypropanoic acid, hexadecanoic acid, phytol, 1,2-benzenedicarboxylic acid, dibutyl ester, 9,12-octadecadienoic acid and methylester, (E,E), 9,12,15-octadecatrienoic acid, methylester, (Z,Z,Z). 9,12,15-Octadeca-trienoic acid and methylester, (Z,Z,Z) were dominant and had variable herbicidal activity. Hexadecanoic acid was isolated and identified from the methanol extracts of *Solidago canadensis* L., which inhibited the wheat seedling growth and the inhibitory effect increased with the extract concentrations (34). The inhibitory effects of 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester on shoot length in *Amaranthus viridis* L. (RI =0.92 +/- 0.03) was strongest (25). Octadecatrienoic acid is considered as one

Table 1. Results of the GC-MS analysis of *Veronica persica* ethanolic extracts

Retention time	Compound	Mol. weight	RRI
7.818	Phosphorous acid triphenyl ester	310	52143
8.780	1,2,3-Plopanetriol	92	29518
9.672	Coumarone	118	9736
9.754	3-Hydroxytetrahydrofuran	88	14776
9.901	2,5-Heptadien-4-one, 2,6-dimethyl-	138	13612
11.186	Benfotiamine	466	28852
11.988	1,2-Benzenediol	110	41687
12.138	Benzeneethanol, alpha.,alpha.-dimethyl-	192	6479
12.848	Ethanimidic acid, N-acetyl, -ethylester	129	1279
13.108	2-ethyl-1-hexen-3-yne	108	14954
13.551	Phenol,4-ethenyl-2-methoxy-	150	24843
15.582	cis-2-(7-octynyl)cyclohexanol	280	8828
16.610	4-methylbiphenyl-d(5)	280	5096
17.092	Tetradecanal	362	50057
17.710	Decanoic acid	172	22625
18.812	2-Amino-3-hydroxypropanoic acid	105	38499
19.723	Hexadecanoic acid	256	166607
20.922	Phytol	296	58970
21.586	1,2-Benzenedicarboxylic acid,dibutyl ester	278	77293
22.263	9,12-Octadecadienoic acid, methylester, (E,E)	294	116079
22.664	9,12,15-Octadecatrienoic acid, methylester,(Z,Z,Z)	292	432113

RRI: relative retention indices calculated against n-alkanes,% calculated from the TIC data.

Table 2. Regression formula and relative EC₅₀ of *Echinochloa crusgalli* to major compounds in *Veronica persica* ethanolic extracts

Compounds	Regression formula	Related coefficient	EC ₅₀ (µg/mL)	95% confidence limits
Hexadecanoic acid	Y=4.5634+1.0080x	0.9821	2.7109	1.6521-4.4484
Phytol	Y=4.3333+0.7818x	0.9589	7.1258	2.2972-22.1034
Decanoic acid	Y=5.3879+1.2216x	0.9616	0.4814	0.3454-0.6709
9,12,15-Octadecatrienoic acid, Methylester,(Z,Z,Z)	Y=5.2310+1.2234x	0.9764	0.6474	0.4962-0.8448

Y: barnyard grass fresh biomass (g), x: compounds weight (g).

of the main sources of biologically active compounds (4). It is noted that fatty acid could inhibit the growth of the weeds (9,10,13). The present study confirmed that the potential useful herbicidal agents in *V. persica* (Lour.) Merr. might be useful as natural herbicides to control weeds in the future.

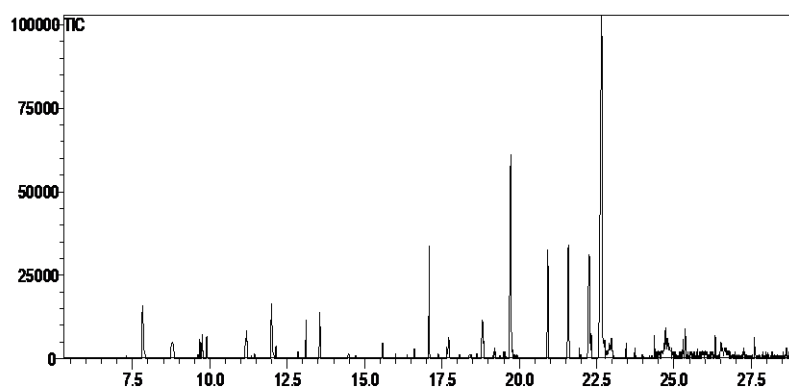


Figure 4. The total ion chromatography of the ethanol extracts of *Veronica persica* from GC/MS chromatogram.

Major compounds herbicidal activity

At 1.5 $\mu\text{g/mL}$, the herbicidal effects were 41.81% in hexadecanoic acid treatment, 29.09% in phytol treatment, 76.95% in decanoic acid treatment and 70.43% in 9,12,15-Octadecatrienoic acid, methylester,(Z,Z,Z) treatment. The EC_{50} values of four major compounds (hexadecanoic acid, phytol, decanoic acid, 9,12,15-Octadecatrienoic acid, methylester,(Z,Z,Z)) for *E. crusgalli* (L.) Beauv. were 2.71, 7.13, 0.48 and 0.65, respectively (Table 2). Four major compounds inhibited the *E. crusgalli* (L.) Beauv. seedlings growth in following order: decanoic acid > 9,12,15-Octadecatrienoic acid > methylester,(Z,Z,Z) hexadecanoic acid > phytol. It was found that decanoic acid was most inhibitory to *E. crusgalli* (L.) Beauv.. Decanoic acid is valuable compound used as precursor for industrial chemicals, pharmaceuticals and biofuels (27). Decanoic acid-based hydrophobic deep eutectic solvents are CO_2 capture agents (19). The fatty acid (C10) is herbicidal compound (10). The present study confirmed decanoic acid would be a potential herbicidal agent.

CONCLUSIONS

Lower ethanolic extract concentrations (50 and 100 $\mu\text{g/mL}$) of *V. persica* (Lour.) Merr. were less inhibitory than higher concentrations (150 and 300 $\mu\text{g/mL}$). The allelopathic herbicidal effects of *V. persica* (Lour.) Merr. on 4-test weeds followed the order: *L. chinensis* (L.) Nees, *L. procumbens* (Krock.) Philcox, *B. syzigachne* (Steud.) Fern., *E. crusgalli* (L.) Beauv. Twenty-one compounds were identified in the ethanolic extracts, of which 4-major herbicidal compounds inhibited the *E. crusgalli* (L.) Beauv. seedlings growth in following order: Decanoic acid ($\text{EC}=0.48$) > 9,12,15-Octadecatrienoic

acid methylester, (Z,Z,Z) (EC=0.65) > Hexadecanoic acid (EC=2.71) > Phytol (EC=7.12). The CAT, POD and SOD activity of *E. crusgalli* (L.) Beauv. leaves increased at lower concentrations (from 50 µg/mL to 150 µg/mL) of *V. persica* (Lour.) Merr. extracts, but decreased at higher concentrations (300 µg/mL). It was concluded that *V. persica* (Lour.) Merr. had strong allelopathic herbicidal activity, thus a standard herbicide for weeds control can be developed from its major components.

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