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Phytotoxicity of *Passiflora incarnata* extracts on germination and growth of *Hordeum vulgare* and *Raphanus sativus*

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ABSTRACT

Phytotoxic chemicals are of interest because they represent potentially novel pharmacophores possessing herbicidal activity that may target new molecular receptor sites. This study aimed to evaluate the phytotoxic activity of *Passiflora incarnata* extracts against the monocotyledonous and dicotyledonous indicator test species barley and radish. Continuous exposure to total aqueous extracts of *P. incarnata* inhibited the barley germination at the lowest tested concentration (1.25% w/v), whereas 10% w/v concentration was required for radish. Aqueous fractions from sequential-solvent partitioning showed reduced seed germination inhibitory activity in both test species relative to total aqueous extracts. A 14-day growth assay showed that 24 h pre-/post-germination exposure to total aqueous extracts had no effect on dry weights of either species. However, 47% leaf-bleaching was observed in the 24 h post-germination exposure in barley. GC-MS analysis of solvent extracts identified phytol and palmitic acid as the major components. LC-ESI-MS/MS analysis of aqueous fractions tentatively identified ten compounds: phenylalanine, leucine, and tryptophan, swertisin, vitexin, saponarin, isoschaftoside, orientin, lucenin-2, and eugenol.

Keywords: Barley, germination, isoschaftoside, lucenin-2, palmitic acid, *Passiflora incarnata,* Passionflower, radish, saponarin, vitexin

INTRODUCTION

Plants that produce phytotoxins are potentially applicable towards weed management, either through traditional agricultural methods (i.e. direct applications of plant material) or as potential sources of useful phytochemicals (3). These phytochemicals are of interest because they represent potentially novel pharmacophores possessing herbicidal activity that may target undiscovered molecular receptor sites (7). With the ever-increasing development of herbicide resistance in weedy plant species, the need for novel synthetic leads that can function as herbicides is of continued and pressing importance.

An example of commercially successful herbicides derived from a plant source is the triketone class (e.g. sulcotrione, mesotrione, tembotrione) (18). This class of herbicides is derived from the phytochemical leptospermone, isolated from *Callistemon*

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citrinus (red bottlebrush), which served as the synthetic lead for the subsequent structure activity relationship optimization (4). The original phytochemical and a number of the subsequent analogues target a previously untargeted molecular site: *p*-hydroxy-phenylpyruvate dioxygenase, which results in selective leaf-bleaching of dicotyledonous plants (5,6,10).

Passiflora incarnata L. represents a widely distributed temperate member of the Passionflower genus. It has traditionally been studied for its anxiolytic and sedative effects, most often to treat insomnia and restlessness (9,12). However, in a study investigating the phytotoxic potential of several medicinal plants as applied toward rice paddy weed control, direct application of whole *P. incarnata* plant material was strongly phytotoxic, with the conclusion that the phytochemical composition of this plant should be further elucidated (17). Other studies have identified multiple phytotoxins also identified from *P. incarnata* (8,19). For example, isoschaftoside from *Desmodium uncinatum* inhibited *Striga hermonthica* germination at concentrations of 1 mg/L (15). Lucenin-2, saponarin, and vitexin extracted from mosses inhibited radish seed germination at concentrations of 10 mg/L (1). In addition, Vaid et al. (20) demonstrated an inhibition of both germination and growth in *Bidens pilosa* and *Cassia occidentalis* when exposed to eugenol. This study aimed to (i) characterize the phytotoxic activity of *P. incarnata* extracts using two standard indicator test species, barley (*Hordeum vulgare* cv. 'Conlon') and radish (*Raphanus sativus* var. oleiferus), using germination and growth bioassays; (ii) identify the major components of fractionated *P. incarnata* extracts via gas chromatography-mass spectrometry (GC-MS) and Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS/MS).

MATERIALS AND METHODS

Chemicals

All solvents [hexanes (H303-4), chloroform (C297-4), ethyl acetate (E196-4), acetone (A929-4,) acetonitrile (A996-4)] used for extraction were obtained from Fisher Scientific, OPTIMA grade. Coumarin (MP Biomedicals, LLC., Solon, OH), palmitic acid (Sigma-Aldrich, St. Louis, MO), and phytol (SAFC Supply Solutions, St. Louis, MO) were purchased from the indicated companies.

Biological material

Passiflora incarnata was grown under unmodified field conditions in Coffee county Tennessee, USA. Two years after planting, the leaves were harvested during August 2009, dried at 22°C for 24 h and coarsely ground. The dried harvested plant material was then stored, light protected, at room temperature under dehumidified conditions until use. *Artemisia tridentata* was used as a positive control in this study (11, 14) (Wholistic Botanicals, UT, USA). Barley and radish seeds (Johnny's Selected Seeds, Winslow, ME, USA) were used as the test species in the germination and growth bioassays.

Total aqueous extract bioassay

An initial 10% (w/v) solution of *P. incarnata* and de-ionized water was prepared by static-maceration in the dark at 22±2ºC for 24 h, followed by decantation from the plant material to obtain an aqueous extract. Dilutions with de-ionized water were used to prepare 5, 2.5, 1.25 and 0.625% concentrations to determine possible dose-dependent activity. Replicates consisted of 15 seeds of the test species placed on a Whatman® No. 1 filter paper-lined 100 mm Petri dish, sealed with Parafilm®, and dark incubated for seven days at 25±2ºC. Each treatment was replicated six times and each filter disk within a treatment received 5 mL of one of the following treatment solutions: 10, 5, 2.5, 1.25, or 0.625% aqueous *P. incarnata* solution, negative control (de-ionized water), positive control (20% w/v aqueous extract of *A. tridentata*), 138 mOsm/Kg osmotic control and 6.4 pH control (which were the averaged osmolarity and pH of a 10% w/v *P. incarnata* extract). After a 7-day incubation period, plates were inspected for seed germination, with germination defined as any seed having radicle elongation greater than 1 mm.

Pre-/Post-germination exposure bioassay

Barley and radish seeds, using the previously described germination conditions employing only de-ionized water, were treated with 5 mL of a 10% w/v total aqueous extract of *P. incarnata* for 24 h, either at day 1 (pre-germination) or at day 5 (post-germination). Identically treated de-ionized water controls were tested simultaneously for each species. After 24 h individual seeds were planted in 6 x 6 x 8 cm (LxWxD) plastic cells filled with 12 g of horticulture vermiculite. Each of the treatment groups (35 replicates each) were grouped into treatment trays for watering. Trays were then placed in a 76 x 122 cm growth chamber and illuminated by eight 122 cm, 3,150 lumen, fluorescent lights. Lights were distanced approximately 20 cm from the trays with a 16h:8h (light:dark) photoperiod. Each tray initially received 3 L of water, with an additional 1.2 L of water every five days, along with physical rotation to insure even light treatment amongst the trays. After 14-days of growth, the total plant material was harvested and growth media removed by gently water dipping until no visible vermiculite adhered to the plant roots. Plant material was then individually placed into pre-weighed test tubes and oven dried at 80°C until dry, then weighed to determine dry plant mass.

Sequential-solvent extract bioassay

A six step sequential-solvent extraction using solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone, ethanol (95%), and de-ionized water) was performed using macerated *P. incarnata* plant material. Each step in the sequential extraction was performed in the dark at room temperature. Six g initial plant material was used and sequentially extracted in 120 mL of each solvent. Each extraction period was 24 h, followed by decantation, and 24 h to allow for solvent evaporation under a fume hood before the processed plant material was introduced to the next solvent.

Each of the extracts obtained was equally divided amongst 12 Whatman® No. 1 filter papers and the solvent evaporated for 24 h under a fume hood. Treated filter papers were then used to conduct germination experiments on radish and barley (6-replicates each). Each extract infused filter paper was individually plated in 100 mm disposable Petri dishes, receiving 5 mL of de-ionized water to produce a final concentration of 10% w/v of *P. incarnata* leaf/de-ionized water, prior to being sealed with Parafilm® and dark

incubated for seven days at 25±2ºC. Identically treated controls with six replicates were run for each solvent. After the seven-day incubation period plates were inspected for germination, with germination being defined as any seed having radicle elongation greater than 1 mm. Mean germination inhibition rates for each treatment were compared to their respective solvent control.

GC-MS analysis of sequential-solvent extracts

GC-MS analysis of non-aqueous solvent extracts was done using a Hewlett Packard® model 5890 GC coupled to a Hewlett Packard® 5970 series Mass Selective Detector. The carrier gas was helium (7.7 psi) and the data were obtained on a Zebron™ ZB-5 column (0.25 x 30 mm, 0.25 µm). The GC oven temperature program was 50°C for 4 min (including a 3 min solvent hold to protect the filament lifespan), with a 25°C per min increase to 310°C, followed by a 15 min hold. The injector and detector temperatures were set at 320°C and 280°C respectively, with the mass range scanned from 50-550 amu. A 1 µl aliquot of 10% w/v extract was injected splitless into the GC-MS and constituents were identified by utilizing Agilent Technologies Enhanced ChemStation coupled with the National Institute of Standards & Technology (NIST, USA) MS Search 2.0.

Tentatively identified compounds were verified with their commercially available standards, by both retention time and mass spectrometric output, to confirm their identification. The ethyl acetate extract was used as a representative extract and was spiked with a coumarin standard (5.4 mg/L) to determine the approximate concentrations of identified compounds by comparing areas under the curves.

LC-ESI-MS/MS analysis of sequential-solvent aqueous extract

LC-ESI-MS/MS analysis was performed on sequential-solvent aqueous extracts using a Thermo Scientific® Exactive Plus Orbitrap LC-ESI-MS/MS. The mass spectrometer was calibrated by analysis of a standard test mixture and the subsequent analysis was performed using this initial mass calibration. The system was equipped with a Phenomenex Kinetex™ C18 (2.1 x 50 mm, 1.7 µm) column. Mobile phase A was 0.1% v/v aqueous formic acid, mobile phase B was acetonitrile. The chromatography gradient employed was A/B (95/5) to A/B (20/80) over 6.3 min, held at A/B (20/80) for 1 min, to A/B (1/99) over 1 min, held at A/B (1/99) for 1 min, returning to A/B (95/5) over 1 min, followed by 2 min re-equilibration. The column temperature was ambient, with a sample injection volume of 1 µL and a flow rate of 300 µL/min. Analysis was performed in full scan mode with positive electrospray ionization, with the ionization source conditions as follows: spray voltage: 3800 V; sheath gas: 30 au; capillary temperature: 300ºC; heater temperature: 300ºC. Collected full scan MS data was fragmented via higher-energy C-trap dissociation (HCD) with full scan resolution set at 50,000 and analyzed mass range of 90-1500 amu.

Tentative identifications of major peaks were made using the obtained liquid chromatographic and mass spectrometric data by comparing with the calculated fragment product masses (HighChem Mass Frontier 6.0) in conjunction with Thermo Scientific XCalibur™ processing software. The sequential-solvent aqueous fraction extract was spiked with a coumarin standard (10 mg/L) to determine the approximate concentrations of tentatively identified compounds by comparing peak intensities.

Statistical analysis

Data analyses were performed using the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software package. Germination data (percent) were arcsine transformed to achieve normality prior to analysis. Data were analyzed using a single-factor analysis of variance (ANOVA) to determine if mean germination inhibition differed among treatment groups ($\alpha = 0.05$). If differences were found, Tukey-Kramer minimum significant difference (MSD) analyses were performed *post hoc* to further discern which treatment group means differed from one another at a 0.05 level of significance. Both the pre-/post-germination exposure dry mass data and the sequential-solvent extract germination data were analyzed by unpaired t-test comparing the treatments to their respective negative/solvent control. Mean inhibition of treatment groups as percent of their negative control was calculated for each treatment for tabular display.

RESULTS

Total aqueous extract bioassay

Treatments for both barley and radish were compared to their negative control (de-ionized water) only, as both the pH and osmotic controls were not different from the negative controls. Analyses of treatment groups indicated that aqueous extracts of *P. incarnata* inhibited seed germination in both barley (ANOVA; $F_{(9,44)}$=21.67; p<0.0001) and radish (ANOVA; $F_{(9,44)}$=55.54; p<0.0001) relative to negative controls. Tukey's MSD indicated that only the 10% concentration inhibited germination in both test species, while the 5, 2.5, and 1.25% extracts also demonstrated activity in barley (Fig. 1).

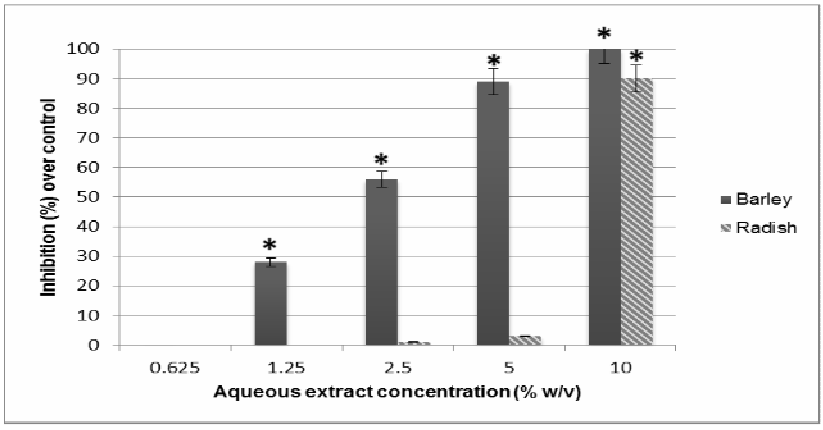


Figure 1. Mean inhibitory effects of *P. incarnata* total aqueous extracts on germination of test species in bioassay, error bars = standard error of the mean, (*) indicates critical mean difference ($\alpha = 0.05$).

Pre-/Post-germination exposure bioassay

There were no differences when pre-germination treatments were compared to their respective controls for barley ($T_{(68)}$=0.55; p=0.58) or radish ($T_{(68)}$=0.17; p=0.86).

Similarly, no differences were observed for post-germination treatments for barley ($T_{(68)}$=0.49; p=0.62) or radish ($T_{(68)}$=1.32; p=0.19), when compared to their respective controls. However, with the post-germination exposure, leaf-bleaching was observed in 47% of the treated barley plants, with leaf-bleaching being defined as chlorosis the width of the leaf and greater than 1 cm in length.

Sequential-solvent extract bioassay

The water extract strongly inhibited germination of barley and radish ($\alpha = 0.05$). The hexane, chloroform and ethyl acetate extracts also showed marginal inhibition of barley germination ($\alpha < 0.1$) (Fig. 2).

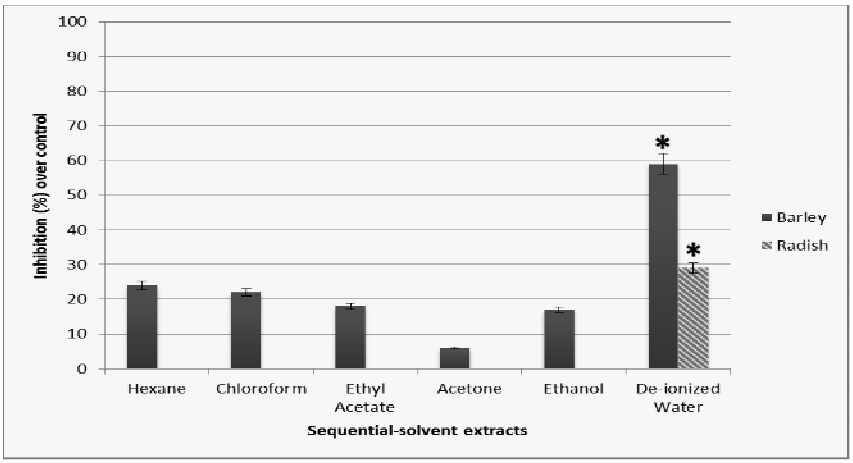
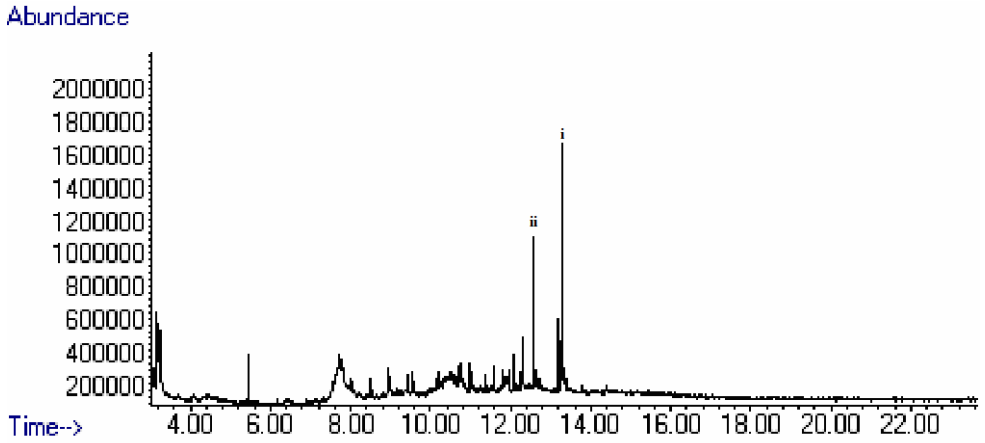


Figure 2. Mean inhibitory effects of *P. incarnata* sequential-solvent extracts on germination of test species in bioassay, error bars = standard error of the mean, (*) indicates critical mean difference ($\alpha = 0.05$).

GC-MS analysis of sequential-solvent extracts

The hexane, chloroform, ethyl acetate, and ethanol extracts were each initially analyzed by GC-MS. However, hexane and ethanol did not produce peaks different from background and were concentrated by room-temperature atmospheric evaporation, to produce a more concentrated solution (3X) for further analysis. The major component in the hexane, chloroform, and ethyl acetate extracts (retention time of 12.09 min) was phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) (highest probability of 81-85%). Additionally, both hexane and ethanol extracts possessed peaks at approximately 13.18 min and their MS outputs were also tentatively identified as phytol (74-79% probability). The retention time of analytical grade phytol dissolved in ethanol was 13.18 min. The ethanol extract possessed a peak at 12.57 min whose MS output was tentatively identified as palmitic acid, which was verified by comparing retention times and MS to that of palmitic acid standard (Fig. 3 and Table 1).



Figue 3. Gas chromatogram from ethanolic extract of *Passiflora incarnata*; Peaks of standard verified substances: (i) 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (ii) hexadecanoic acid.

Table 1. Molecular weight, retention time, major peaks, and common name of the GC-MS analysis of the standard verified compounds from *Passiflora incarnata*

Compound	M.W.	Retention Time (min)	Major Peaks (*m/z*)	Common name
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	13.18	71, 43, 57, 81, 123, 95	Phytol
Hexadecanoic acid	256	12.57	73, 60, 129, 97, 51	Palmitic acid

LC-ESI-MS/MS analysis of sequential-solvent aqueous extract

LC-ESI-MS/MS analysis of the sequential-solvent aqueous fraction, which was the most active in our assays, tentatively identified 10-compounds (Table 2). Compound concentrations were calculated by comparing individual relative intensities to the coumarin spike control.

Compound 1 (Rt = 0.86) had a *m/z* parent ion peak at $[M+H]^+$ 166.08580 (Fig. 4). The fragmentation of compound 1 gave prominent *m/z* product ion peaks at $[M+H]^+$ 120.08108 and $[M+H]^+$ 103.05465. These peaks likely correspond to the fragmentation of a hydroxymethanone moiety and then that of an amine group. From this fragmentation pattern the compound was tentatively identified as the amino acid phenylalanine.

Compound 2 (Rt = 0.61) had a *m/z* parent ion peak at $[M+H]^+$ 132.10170 (Fig. 4). The fragmentation of compound 2 gave prominent *m/z* product ion peaks at $[M+H]^+$ 100.07614 and $[M+H]^+$ 95.04960. These peaks likely correspond to the fragmentation of a methanol group and then that of an amine plus a hydroxy group. From this fragmentation pattern the compound was tentatively identified as the amino acid leucine.

Table 2. LC-ESI-MS/MS data, tentative structure, retention time (Rt), protonated molecule (m/z) for peak, relative intensity, calculated concentration from spiked sample, and detected fragments of constituents found in fractionated-aqueous extract of *Passiflora incarnata*

#	Tentative Structure	Rt (min)	$[M+H]^+$ (m/z)	Relative Intensity	Calculated Concentration (mg/L)	MS^2
1	Phenylalanine	0.86	166.08580	833232787	1091	120.08108, 103.05465
2	Leucine	0.61	132.10170	518120940	679	100.07614, 95.04960
3	Tryptophan	1.46	205.09650	407835653	534	188.07051, 159.09160, 144.08069
4	Swertisin	4.27	447.12799	47304102	61	429.11749, 411.10706, 297.06064
5	Vitexin	4.19	433.11261	46306458	60	343.08102, 283.05988
6	Saponarin	4.05	595.16528	25589559	34	415.10205
7	Isoschaftoside	3.91	565.15503	18894885	24	547.14471, 529.13403, 431.09753
8	Orientin	3.97	449.10800	13877011	18	413.08676, 329.06555, 299.05505
9	Lucenin-2	3.85	611.16089	4568487	6	581.15057, 461.10809
10	Eugenol	0.84	165.09052	208211	0.3	149.05966, 120.05672, 107.04946

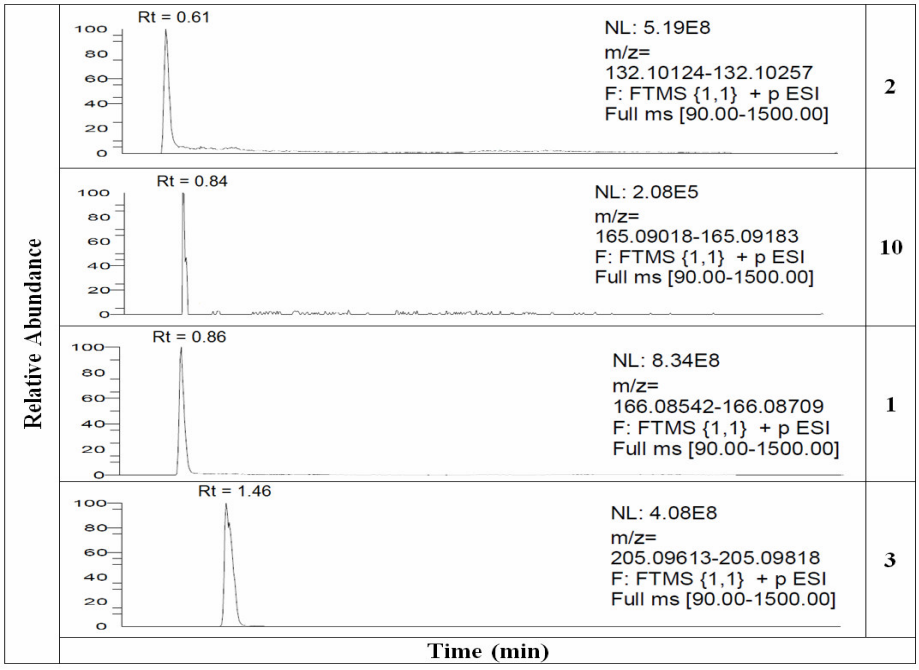


Figure 4. LC-ESI-MS/MS chromatograms of proposed compounds from fractionated aqueous extracts of *Passiflora incarnata,* arranged by retention time, numbers in the right column correspond to compounds in Table 2.

Compound 3 (Rt = 1.46) had a *m/z* parent ion peak at $[M+H]^+$ 205.09650 (Fig. 4). The fragmentation of compound 3 gave prominent *m/z* product ion peaks at $[M+H]^+$ 188.07051, $[M+H]^+$ 159.09160, and $[M+H]^+$ 144.08069. These peaks likely correspond to the fragmentation of a hydroxymethanone moiety, an amine group, and then both groups, respectively. From this fragmentation pattern the compound was tentatively identified as the amino acid tryptophan.

Compound 4 (Rt = 4.27) had a *m/z* parent ion peak at $[M+H]^+$ 447.12799 (Fig. 5). The fragmentation of compound 4 gave prominent *m/z* product ion peaks at $[M+H]^+$ 429.11749, $[M+H]^+$ 411.10706, and $[M+H]^+$ 297.06064. These peaks likely correspond to the fragmentation of a single hydroxy group, two hydroxy groups, and then that of a 1,5-anhydrohexitol moiety. From this fragmentation pattern the compound was tentatively identified as the flavonoid swertisin.

Compound 5 (Rt = 4.19) had a *m/z* parent ion peak at $[M+H]^+$ 433.11261 (Fig. 5). The fragmentation of compound 5 gave prominent *m/z* product ion peaks at $[M+H]^+$ 343.08102 and $[M+H]^+$ 283.05988. These peaks likely correspond to the partial fragmentation of a 1,5-anhydrohexitol moiety and then that of a flavonoid core pyran ring in addition to an adjoining phenol moiety, respectively. From this fragmentation pattern the compound was tentatively identified as the flavonoid vitexin.

Compound 6 (Rt = 4.05) had a *m/z* parent ion peak at $[M+H]^+$ 595.16528 (Fig. 5). The fragmentation of compound 6 gave a prominent *m/z* product ion peak at $[M+H]^+$ 415.10205 which likely represents the complete fragmentation of a hexopyranose moiety. This compound was tentatively identified as the flavonoid saponarin.

Compound 7 (Rt = 3.91) had a *m/z* parent ion peak at $[M+H]^+$ 565.15503 (Fig. 5). The fragmentation of compound 7 gave prominent *m/z* product ion peaks at $[M+H]^+$ 547.14471, $[M+H]^+$ 529.13403, and $[M+H]^+$ 431.09753. These peaks likely correspond to the fragmentation of a single hydroxy group, two hydroxy groups, and lastly that of a 3,4,5-trihydroxypyran moiety. From this fragmentation pattern the compound was tentatively identified as the flavonoid isoschaftoside.

Compound 8 (Rt = 3.97) had a *m/z* parent ion peak at $[M+H]^+$ 449.10800 (Fig. 5). The fragmentation of compound 8 gave prominent *m/z* product ion peaks at $[M+H]^+$ 413.08676, $[M+H]^+$ 329.06555, and $[M+H]^+$ 299.05505. These peaks likely correspond to the fragmentation of two hydroxy groups, a 1,5-anhydrohexitol moiety, and that of a flavonoid core pyran ring in addition to an adjoining catechol moiety. From this fragmentation pattern the compound was tentatively identified as the flavonoid orientin.

Compound 9 (Rt = 3.85) had a *m/z* parent ion peak at $[M+H]^+$ 611.16089 (Fig. 5). The fragmentation of compound 9 gave prominent *m/z* product ion peaks at $[M+H]^+$ 581.15057 and $[M+H]^+$ 461.10809. These peaks likely correspond to the fragmentation of a methanol group and a 1,5-anhydrohexitol moiety. From this fragmentation pattern the compound was tentatively identified as the flavonoid lucenin-2.

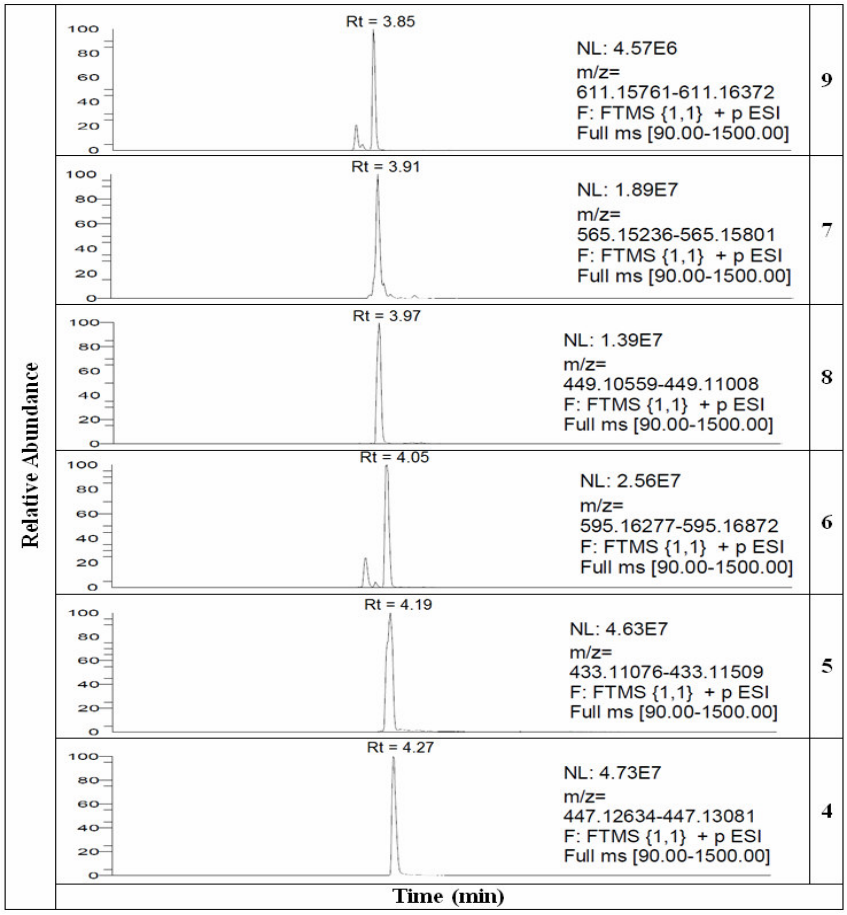


Figure 5. LC-ESI-MS/MS chromatograms of proposed compounds from fractionated aqueous extracts of *Passiflora incarnata,* arranged by retention time, numbers in the right column correspond to compounds in Table 2.

Compound 10 (Rt = 0.84) had a *m/z* parent ion peak at $[M+H]^+$ 165.09052 (Fig. 4). The fragmentation of compound 10 gave prominent *m/z* product ion peaks at $[M+H]^+$ 149.05966, $[M+H]^+$ 120.05672, and $[M+H]^+$ 107.04946. These peaks likely correspond to the fragmentation of a hydroxy group, a ethylene group, and a propene group. From this fragmentation pattern the compound was tentatively identified as the phenylpropene eugenol.

DISCUSSION

The phytotoxic activity of the total aqueous *P. incarnata* extracts were greater in barley (monocotyledonous indicator test species), than in radish (dicotyledonous indicator

test species). Previous research (17) reported 100% germination inhibition of radish by total aqueous extracts of *P. incarnata* at concentration of both 30 and 5% w/v. However, in this study a 10% w/v extract solution of *P. incarnata* was required to inhibit the germination of radish by 90%. This discrepancy in activity in radish may be the result of a chemotypic difference in the *P. incarnata* material used, as this has previously been reported (21).

Only the aqueous extract fraction of the *P. incarnata* extract retained significant germination inhibition activity in both barley and radish, but the activity was reduced from that of the non-fractionated total aqueous extract. The initial activity of the total aqueous extract was likely the result from the combined activity of several compounds, as is often the case in environmental phytotoxicity, which were reduced/removed from the sequential-solvent aqueous extracts.

GC-MS analysis revealed that phytol was the major component in the hexane, chloroform, ethyl acetate, and ethanol extracts. The retention time of phytol standard (13.18 min) matched that of the peak observed in the hexane and the ethanol extracts. However, hexane, chloroform, and ethyl acetate extracts each possessed a peak at 12.09 min with the same fragmentation pattern suggesting that it may be an analogue of phytol. Both phytol and palmitic acid have been previously identified from *P. incaranta* extracts (2,8), and palmitic acid is known to inhibit seed germination (16).

LC-ESI-MS/MS analysis of the sequential-solvent fractionated aqueous extract tentatively identified ten compounds (Table 2). By far the most abundant were the amino acids phenylalanine, leucine, and tryptophan. The remaining seven compounds were the flavonoids swertisin, vitexin, saponarin, isoschaftoside, orientin, lucenin-2, and the phenylpropene eugenol. These compounds have been previously identified in *P. incarnata* extracts (8, 13, 19) and some of these (vitexin, saponarin, and lucenin-2) inhibit radish seed germination and root elongation (1). Additionally, both eugenol and isoschaftoside have been reported to inhibit seed germination (15, 20).

The components in the complex mixture were tentatively identified by LC-ESI-MS/MS coupled fractionation. Five of these known phytotoxins were purified from the aqueous extract and one was identified in the solvent fraction, suggesting that the activity in this extract may be due to numerous active phytotoxins rather than being the activity of a single compound. In addition, both the causative agent(s) and the long-term implications of the observed leaf-bleaching deserve further study. *Passiflora incarnata* may provide a source of novel allelochemicals capable of disrupting carotenoid biosynthesis and function, in addition to its observed seed germination inhibitory activity.

In conclusion, *P. incarnata* exerts its phytotoxic effects on barley and radish through seed germination inhibition via continuous exposure to highly polar (i.e., aqueous) extracts. However, the dry weights of the test species, both pre- and post-germination 24 h exposure, were not affected when harvested after 14 days of growth. Nonetheless, 47% leaf-bleaching was observed in the post-germination treated barley group. The observed germination inhibition activity was likely the result of a number of compounds, as activity was reduced during sequential solvent extraction once the effects of the total aqueous extract and sequential solvent aqueous extract were compared. Further research into the phytotoxic composition of *P. incarnata* is warranted.

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DISCLAIMER

The contents of this paper do not necessarily reflect nor should they be construed to reflect any position, official policy of, or endorsement by the U.S. government, U.S. Food and Drug Administration, or any subsidiaries.

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