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Research Note

Bioherbicidal Activity of *Medinilla magnifica* Lindl. Leaf Extract

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The bioherbicidal activity of kapa-kapa (*Medinilla magnifica* Lindl.) was studied for the first time. Phenolics from its leaves were extracted with 7:7:6 methanol-acetone-water (MAW). The phenolics of the crude extract were quantified using the Folin-Ciocalteu method and values of 71.86 mg GA·g^{-1} extract for total phenolics and 29.58 mg QUE·g^{-1} extract for total flavonoids were obtained. The crude extract was subjected to acid hydrolysis and the bioherbicidal activity of the resulting hydrolysate was determined. Lettuce seed germination assay was done and the median lethal dose (LD_{50}) of the extract was determined as 64.69 ppm. This relatively low LD_{50} value shows that it has great potentials for use against weeds. The extract inhibited the growth of *Echinochloa crus-galli*, *Cyperus iria*, and *Ludwigia hyssopifolia*, with *E. crus-galli* showing the greatest sensitivity among the three species. Total chlorophyll content of soybean was reduced by the extract. This suggests that the acid-hydrolyzed extract of *M. magnifica* decreased chlorophyll production, resulting in reduced biomass of the test weeds.

Key Words: bioherbicide, *Cyperus iria*, *Echinochloa crus-galli*, flavonoids, *Ludwigia hyssopifolia*, *Medinilla magnifica*, phenolics

Abbreviations: AHE – acid-hydrolyzed extract, GA – gallic acid, LD50 – median lethal dose, MAW – methanol-acetone-water, QUE – quercetin

INTRODUCTION

Weeds account for a large percentage of reduction in crop yield worldwide due to competition for water, light, and mineral nutrients (Robbins et al. 1952). In a study conducted in Indonesia, relative yield losses of rice averaged 50%, and actual yield losses were higher in wet season and transplanted rice plants (Zoschke 1990). In this study, reduction in yield was mainly due to grasses and sedges. In another study done in Brazil, grain yield reduction in corn due to lack of weeding reached 38% (Silva et al. 2009). Oad et al. (2007) concluded from their study that weeds can cause 24–39.95% economic yield loss of wheat crop depending on weed density.

Majority of terrestrial weeds are categorized into grasses, sedges, and broadleaves (Ross and Lembi 2009). It is crucial to know the category of a weed before attempting to plan its control (Zimdahl 2013). For instance, lipid biosynthesis inhibitors, which are specific for grasses, do not affect dicots and non-grass monocots (Monaco et al. 2002). The herbicide 2,4-D amine is suitable for the control of broadleaves but not of grasses and sedges (Newman 2002).

Although herbicides protect crops from yield reduction, they can have harmful effects to the environment by being toxic to non-target organisms as well as contaminating soil and water (Aktar et al. 2009). Due to these problems concerning use of herbicides, biorational alternatives are being considered for weed control. With allelochemicals such as phenolic compounds present in the leaves, flowers, roots, seeds and stems of plants, allelopathy has great potential as a biorational alternative (Weston 1996). Reigosa et al. (1999) tested the effect of six phenolic compounds (ferulic acid, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-vanillin) on germination of various

Fig. 1. Reproductive shoot of *Medinilla magnifica* Lindl.

weeds. At 10 mM, these compounds inhibit the germination of the tested weeds although lower concentrations either have no effect or are stimulatory.

Search for new herbicides is still needed mainly due to problems regarding increase of weed resistance to existing herbicides (Kraehmer 2012). For instance, glyphosate, which is the most common herbicide, led to the selection of glyphosate-resistant weeds (Van Bruggen et al. 2018). Moreover, this broad-spectrum herbicide can affect non-target honeybees by reducing their sensitivity to nectar reward and impairing their associative learning (Herbert et al. 2014).

Medinilla magnifica Lindl. is a Philippine endemic plant distinguished from closely related species by its fairly large pink inflorescences with showy bracts (Regalado 1995). This species is popularly grown in gardens as ornamental plant. A photograph of its reproductive shoot is shown in Fig. 1. Rayos et al. (2016) analyzed the leaf phenolics of different species of *Medinilla* by two-dimensional paper chromatography, and *M. magnifica* is among the 18 species included in the study. Casteele et al. (1981) studied the phenolic compounds found in this species and detected simple phenols, flavonoids, hydrolysable tannins, and condensed tannins. However, the effect of these compounds present in the leaves of these plants on living systems such as other plants has not yet been investigated.

This study aimed to quantify the phenolic compounds in the leaves of *M. magnifica* and evaluate its bioherbicidal properties.

MATERIALS AND METHODS

Collection and Preparation of the Plant Sample

About 100 g of fresh leaves (old leaves from lateral branches) of *M. magnifica* were collected from plants growing in the University of the Philippines Land Grant in Siniloan, Laguna. A voucher specimen of the plant with Accession No. 256230 was deposited at the Philippine National Herbarium. The leaves were washed and oven-dried at 40 °C to approximately 5% moisture content.

Extraction of Phenolics

Twenty grams of dried *M. magnifica* leaves were extracted with 7:7:6 (v/v/v) methanol-acetone-water solution (MAW) with recurrent stirring. The resulting extract was collected through vacuum filtration using filter paper. The chlorophylls and carotenoids were removed through liquid-liquid extraction performed using equal amounts of petroleum ether. The aqueous layer was collected and washed with petroleum ether until the organic layer became clear. MAW was removed from the aqueous layer at 45 °C *in vacuo*, until approximately 90% of the solvent has evaporated. The resulting extract was placed in an amber vial and stored in a refrigerator at 5 °C.

Quantification of Phenolics

The total phenolics of the unhydrolyzed extract was determined using Folin-Ciocalteu reagent. In succession, 200 µL of the extract was mixed together with 0.5 mL of Folin-Ciocalteu reagent (1:1) and 3 mL of 10% Na_2CO_3 in a vortex mixer. The resulting solution was maintained at 28 °C for about 15 min. The mixture was diluted using 10 mL deionized distilled water. UV-visible spectrophotometry was employed in determining the absorbance of the solution at 725 nm. Gallic acid (GA) and quercetin (QUE) were used as standards for phenolics and flavonoids, respectively.

Acid Hydrolysis

One gram of the crude extract was dissolved in a hydrolysis reagent composed of 10 mL concentrated HCl, 40 mL distilled water and 50 mL methanol. The solution was heated at 90 °C in a reflux set-up for 4 h. The phenolic compounds were extracted using liquid-

liquid extraction with ethyl acetate as the solvent. The obtained organic layer containing the phenolic compounds was concentrated in vacuo at 45°C with the use of a rotary evaporator.

The resulting solution was stored as acid-hydrolyzed extract and stored in the refrigerator at 5°C.

Lettuce Seed Germination Bioassay

To determine the bioactivity of the acid-hydrolyzed extract (AHE), lettuce seed germination inhibition bioassay was done following the method of Iyer and Viswanathan (2012). A setup was prepared per concentration of the extracts, with concentrations of 20, 40, 60, 80 and 100 ppm. For each filter paper disk, 3 mL of the extract of each concentration was placed in petri dishes prepared with filter paper and 30 lettuce (*Lactuca sativa*) seeds were sown in each dish. Percent germination inhibition was determined after 2 d, indicated by 2 mm root growth. Three replicates were used per extract with water in the control. The computation for % inhibition is shown as follows:

$$\% \text{ inhibition} = [1-(\text{total number of germinated seeds})/(\text{total number of germinated in control})] \times 100$$

Pot Assay

For the pot assay based on the method of Salamanez et al. (2015), three weeds were used: *Echinochloa crus-galli* (representing grasses), *Cyperus iria* (representing sedges), and *Ludwigia hyssopifolia* (representing broadleaves). Seeds of each weed were sown at a depth of 1–2 cm in plastic pots containing fertilized soil inside a greenhouse. The plants were treated and watered every day. On the fourth day after seedling emergence, they were transferred and thinned out to one plant per container and placed under natural light. Three replicates were maintained per treatment.

Extracts with concentrations of 200, 400, 600, 800 and 1000 ppm were prepared. These solutions (10 mL of each) were applied separately to the weeds (through root absorption) upon reaching the two-leaf stage of growth. For each solution, a non-ionic surfactant, 5% Tween 20, was added. After a week of application, the shoots of the plants were measured. Relative heights were measured and % inhibition values were calculated.

$$\% \text{ inhibition} = [(\text{height\_untreated} - \text{height\_treated})/\text{height\_untreated}] \times 100$$

Statistical analysis was used to compare the means correlating the effect of the AHE to the weed species. GraphPad Prism v7.0 was used to perform one-way ANOVA with Tukey's multiple range test at 95% confidence interval.

Chlorophyll Content Determination

To determine the effect of the AHE on chlorophyll synthesis, the following experiment modified from Einhellig and Rasmussen (1979) was done. Soybeans (*Glycine max* L.) were germinated for 8 d. Upon germination, the seeds were individually transplanted to 80 mL light-free plastic vials containing Hoagland's culture solution. The Hoagland's solution used was of two-thirds strength with twice the formula Fe. The seedlings were transferred to similar nutrient solutions containing the appropriate extracts after 3–4 d of acclimatization. The seedlings were separately treated with extract concentrations of 200, 400, 600, 800 and 1000 ppm. Nutrient solutions containing the extracts were added to the setup throughout the 6-d period when necessary.

Upon harvest, chlorophyll was extracted from the two unifoliate leaves of each soybean. The leaves were immersed in 30 mL of 95% ethanol for 24 h. The ethanol-chlorophyll solutions were decanted and the leaves were soaked again in a similar manner with fresh aliquots of 95% ethanol. The solutions were combined and added with water to a final volume of 100 mL. The solutions were kept in the dark at room temperature. The absorbances of the chlorophyll extracts were determined at 665 and 649 nm using a UV-Vis spectrophotometer. Chlorophyll content was calculated using the formulas:

$$(\mu\text{g Chlorophyll a})/(\text{mL solution}) = (13.70)(A665\text{ nm}) - (5.76)(A649\text{ nm})$$

$$(\mu\text{g Chlorophyll b})/(\text{mL solution}) = (25.80)(A649\text{ nm}) - (7.60)(A665\text{ nm})$$

Unifoliate leaf dry weights were used to obtain µg chlorophyll/mg dry weight and the ratio of chlorophyll a and b were also determined.

RESULTS AND DISCUSSION

Total Phenolics

In quantification of the total phenolic compounds present in the leaves of *Medinilla magnifica*, the unhydrolyzed extract was subjected to the Folin-Ciocalteu method. The values were expressed as mg GA·g^{-1} extract as this unit estimates the total phenolics present in the sample. For the quantification of total flavonoids, the unit mg QUE·g^{-1} extract was used.

The values obtained for total phenolics and total flavonoids were 71.86 ± 1.35 mg GA·g^{-1} extract and 29.58 ± 0.55 mg QUE·g^{-1} extract, respectively. These values are similar to those reported by Castelee et al. (1981) which showed that the distribution of the compounds they

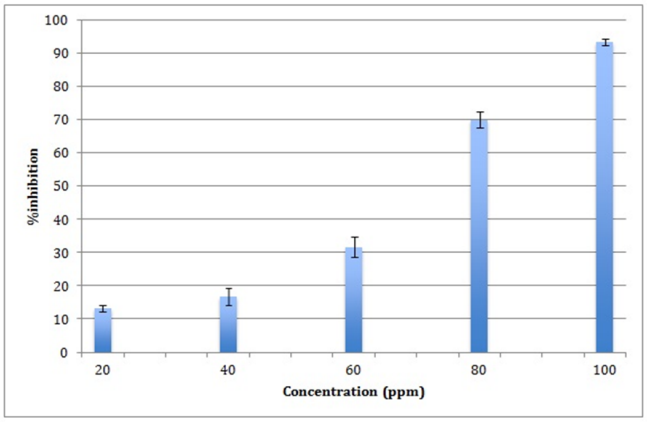

Fig. 2. Dose-dependent response of lettuce on increasing concentration of acid-hydrolyzed extract (AHE) of *Medinilla magnifica* leaves.

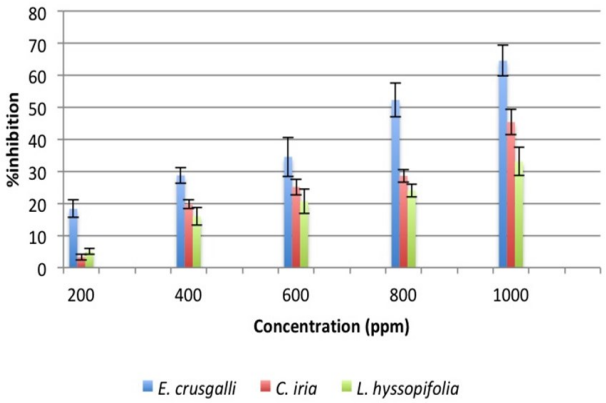

Fig. 3. Effect of increasing concentrations of acid-hydrolyzed extract (AHE) of *Medinilla magnifica* leaves on the growth of selected weeds.

obtained in *M. magnifica* were 19% and 5% for phenolics and flavonoids, respectively. These values showed that the total amount of phenolics present in the leaves of the plant is approximately three times more than that of the total amount of flavonoids.

Lettuce Seed Germination Bioassay

In determining the effect of the AHE of *M. magnifica* on seed germination, lettuce (*Lactuca sativa*) seeds were used. Lettuce seed germination bioassay is a simple, inexpensive, and rapid test that provides valid statistical correlations necessary in testing desired bioactivities (Iyer and Viswanathan 2012).

In a previous study by Waqas et al. (2013) where lettuce seed germination assay was also used, bioactive metabolites from fungal epiphytes of *Helianthus annuus, Capsicum annuum,* and *Cucumis sativus* were tested. Concentrations of 100, 500, and 1000 ppm were used for testing the dose-dependent response of lettuce seeds. However, in the current study, increasing concentrations (20, 40, 60, 80 and 100 ppm) of AHE were used to determine its inhibitory effects on lettuce seeds. Low concentrations of the extract were used because complete inhibition was observed at 200 ppm. The seeds were considered to have germinated when 2-mm radicle can be observed after 2 d of incubation (Haughland and Brandsaeter 1996). The results of the bioassay showed that percent inhibition increased as the concentration of the extract increased (Fig. 2). The dose response curve of the assay showed a median lethal dose (LD_{50}) value of 64.69 ppm extract. These findings show that the AHE of *M. magnifica* can be potentially commercialized for herbicidal use.

Pot Assay

In this bioassay, three different weed species each representing grasses (*Echinochloa crus-galli*), sedges (*Cyperus iria*), and broadleaves (*Ludwigia hyssopifolia*) were tested treated with increasing concentrations of AHE. These three selected weeds are among the most commonly found in rice fields in Asia (Caton et al. 2010).

Statistical analysis of the effect of AHE showed greater inhibition on the growth of the grass, *Echinochloa crus-galli*, with a 64–65% reduction in height at 1000 ppm (Fig. 3). The sedge, *Cyperus iria*, showed lower sensitivity with 45% height reduction at 1000 ppm while the broadleaf, *Ludwigia hyssopifolia*, showed the least effect of the extract with only 33% height reduction at 1000 ppm.

Chlorophyll Content Determination

As AHE concentration increased, the chlorophyll content of soybean decreased (Fig. 4). A gradual decrease was observed up to 800 ppm, where there was a change in slope going to 1,000 ppm due to a drastic decrease in the chlorophyll content of the sample. The decrease in chlorophyll content indicates that a probable mechanism of action of AHE is photosystem inhibition. Herbicides classified as bleachers or carotenoid pigment inhibitors prevent carotenoid formation followed by chlorophyll loss (Ross and Lembi 2009) because carotenoids prevent photo-oxidation of chlorophyll pigments. This is another possible mechanism of action of AHE. Yang et al. (2004) investigated the effect of three allelopathic phenolic compounds (*o*-hydroxyphenyl acetic acid, ferulic acid, and *p*-coumaric acid) on the chlorophyllase activity of rice leaf. The chlorophyll content was decreased while the chlorophyllide content was increased by increasing phenolic concentrations. The study showed that the three phenolics may affect the biosynthetic and degradative pathways of chlorophyll. This is also possible for the AHE of *M. magnifica*. However, further investigation must be done to prove this.

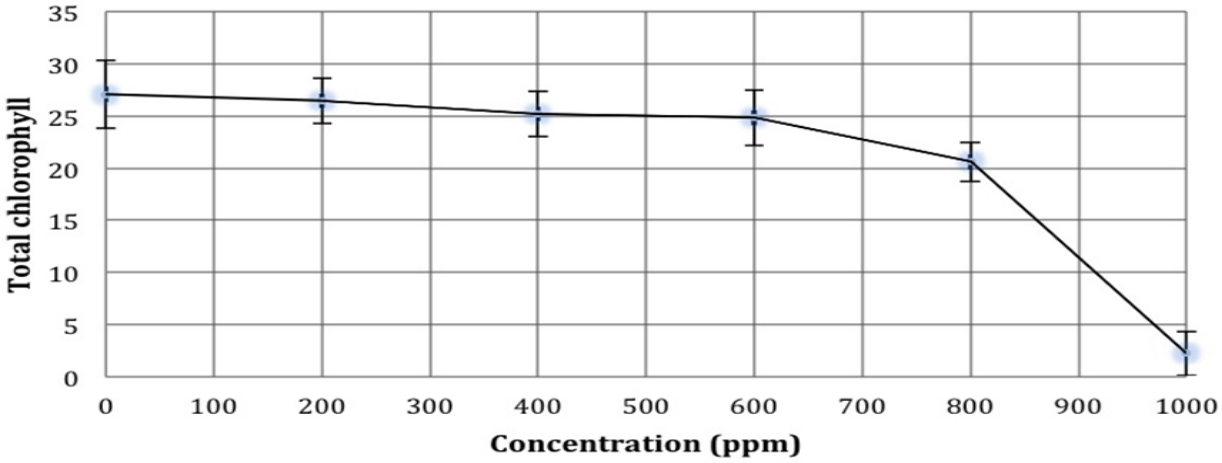

Fig. 4. Effect of increasing concentrations of acid-hydrolyzed extract (AHE) of Medinilla magnifica leaves on the chlorophyll content of soybean.

CONCLUSION

Our results demonstrated that AHE of *M. magnifica* showed significant herbicidal activity. With a relatively low LD_{50} value of 64.69 ppm, it has great potential for commercial use for agriculture. This can be attributed to the fairly high amount of phenolic compounds in the extract. Although this study suggests that photosystem inhibition is a possible mechanism of action of the extract, further studies must be done to determine the basis of its toxicity to weeds.

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