Potential allelopathic activity of dichloromethane extract from the leaves of talinum paniculatum (jacq.) gaertn against cyperus iria

Kevin C. Salamanez  
*University of the Philippines Los Baños*

Liezel Angelique L. Sabaupan  
*University of the Philippines Los Baños*

Arnoldus M. Mangao  
*University of the Philippines Los Baños*

Evelyn B. Rodriguez  
*University of the Philippines Los Baños*

**Recommended Citation**


https://www.ukdr.uplb.edu.ph/journal-articles/3950

For more information, please contact universitylibrary.uplb@up.edu.ph
POTENTIAL ALLELOPATHIC ACTIVITY OF DICHLOROMETHANE EXTRACT FROM THE LEAVES OF *Talinum paniculatum* (Jacq.) Gaertn AGAINST *Cyperus iria*

Kevin C. Salamanez, Liezel Angelique L. Sabaupan, Arnoldus M. Mangao, Evelyn B. Rodriguez

1 Assistant Professor, Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna
2 BS Chemistry Graduate, Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna
3 Instructor, Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna
4 Retired Professor, Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna

E-mail: kcsalamanez@up.edu.ph (Corresponding author)

Received 18 October 2018
Accepted for publication 15 July 2019

Abstract

This study focused on the isolation, characterization and allelopathic activity assessment of phytotoxic compounds present in the weed *Talinum paniculatum* (Jacq.) Gaertn. The leaves of *T. paniculatum* were sequentially extracted with petroleum ether, dichloromethane, ethyl acetate, and ethanol-water (1:1 v/v). Using the lettuce seed assay, the dichloromethane extract was found to be the most bioactive extract having an IC<sub>50</sub> of 477 ppm. Phytochemical screening of the dichloromethane extract revealed the presence of saponins, steroids, terpenoids, and glycosides. Isolation of the major compound from the extract was carried out using open column chromatography. UV-Vis and IR spectral analyses, melting point determination, phytochemical screening, and chromatographic mobility showed that most active extracts in *T. paniculatum* could be ß-sitosterol. The dose-response whole pot assays revealed that dichloromethane extract of *T. paniculatum* exhibited bioherbicidal potential against *Cyperus iria*, one of the world’s worst weeds of rice.

Keywords: allelopathy, bioherbicide, *Talinum paniculatum*, ß-sitosterol
Introduction

Continuous efforts have been put forth recently to maximize agricultural yields due to increasing world’s population. This has led to heavy use of synthetic pesticides, which notably raised major issues about their adverse effects on the environment, risk in ecological balance and damage to human health. In order to address these concerns, the development of bioherbicides as alternative has become a remarkable focus for researchers and scientists.

One of the strategies leading to the development of natural herbicides is allelopathy. Allelopathy is broadly defined as the effect of one plant (including microorganisms) directly or indirectly to another plant (Rice, 1974). The effect is usually through the release of allelochemicals, which are also known as allelopathins (Soltys, et al., 2013). Allelopathy involves both stimulatory and inhibitory effects of one plant to another plant. The release of allelochemicals that causes stimulatory and/or inhibitory effects is varied (Rice, 1984). According to Putnam (1988), allelochemicals are “nature’s own herbicides.” Allelochemicals are usually secondary metabolites released by plants that have varying effects on other plants (Ferguson, et al., 2013). Allelochemicals affect plants in several ways, the most common of which is growth retardation. Inhibitory effects on seed germination and plant development are also common (Kruse and Strandberg, 2000). The compatibility of some allelochemicals as bioherbicide is due to its similarity in mode of action with synthetic herbicides (Soltys et al., 2013).

Because of the phytotoxic effect of allelochemicals on the growth of weeds, one potential application of allelochemicals in agriculture is its use as selective natural herbicides or bioherbicides. Several studies have been conducted which led to the discovery of some allelochemical compounds that have a potential or are already being used as bioherbicides. Natural herbicides are favored over synthetic herbicides because of their relatively safer effects on human health and on the environment. This is because most natural herbicides are water-soluble and non-halogenated (Bhowmik, 2003). The call for the discovery and improvement of natural herbicides is increasing because of the increasing number of weeds evolving resistance to herbicides. Because of this dilemma, it is important to discover more allelochemicals that can counteract the weeds or even better, improve the discovered allelochemicals in weed management by chemical interferences. The classes of compounds that are established to show allelopathic activities include alkaloids, benzoazinones, cyanogenic compounds, coumarins, flavonoids, polyacetylene and terpenes (Putnam, 1988).

Talinum paniculatum (Jacq.) Gaertn (Figure 1), commonly known as fameflower, is a vegetable crop that belongs to the Portulacaceae family. The plant grows in hot and humid weather which makes it a common plant in the Philippines. It grows in humid and tropical
regions and has a high tolerance for drought due to its high water content. It can be sown, planted or collected from certain areas. Studies regarding its pharmacological and antimicrobial activities have been published. In other countries, the plant is consumed mainly for its therapeutic properties. According to phytochemical studies, the plant is known to contain bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins and tannins (Ogbonnaya and Chinedum, 2013).

It has been observed that Talinum paniculatum is ubiquitous, has a fast method of propagation and relatively dominates the land where it grows. Because of its persistence, it can be invasive and hence, it becomes a weed. The observation regarding its spread in a particular area led to the inference that the plant has potential allelopathic activity. (Amorim et al., 2014). To date, there is no published literature regarding the allelopathic potential of Talinum paniculatum. Hence, this study focused on the investigation on the potential allelopathic compounds of Talinum paniculatum.

This study focused on the investigation of the potential allelopathic effect of Talinum paniculatum against Cyperus iria, one of the world’s worst weeds of rice. Specifically, the objectives were: 1) to obtain organic extracts from the leaves of Talinum paniculatum via sequential extraction and determine the most active extract by means of lettuce seed germination assay; 2) to isolate a compound from the most active extract via column chromatography; 3) to subject the isolated compound to spectral analyses such as UV-Vis and IR spectroscopy and determine its melting point; and 4) to determine the bioherbicidal activity of the most active extract on Echinochloa crusgalli and Cyperus iria via pot assay experiment.

Figure 1. Talinum paniculatum (Jacq.) Gaertn.

Methodology

Sample Collection, Authentication and Preparation

Whole plants of Talinum paniculatum (Jacq.) Gaertn were collected from fallow areas around the vicinity of the Institute of Chemistry, University of the Philippines Los Baños, College, Laguna (14.1640555, 121.2420492). The plant samples were authenticated at the Botanical Herbarium of the Museum of Natural History, University of the Philippines Los Baños. The whole plant samples were air-dried and oven-dried at 40°C for 48 hrs. The oven-dried samples were grounded into powder and passed through 40 mesh sieve. The soil where the plants were grown was submitted for analysis (% moisture, pH, CEC, iron content and % organic matter and available sulfur content) to Analytical Service Laboratory.
Bioassay-guided Extraction

Whole plants of *Talinum paniculatum* (Jacq.) Gaertn were powdered and reduced to fine particles prior to extraction. Sequential extraction with increasing polarity was carried out. The extracting solvents to be used were hexane, dichloromethane, ethyl acetate and ethanol-water (80:20). Dried powder weighing about 25 g was immersed in 50mL of the respective solvent for 24 hours at 27°C. Each of the extract was evaporated to dryness using a rotary evaporator. The dried fractions were stored in their respective containers at 4°C until use.

To determine the most active extract, the bioassay method used by Omezzine et al. (2012) was followed, with slight modifications. Seeds of romaine lettuce (scientific name) were used because these seeds were reported to be highly sensitive to allelochemicals (Vyvyan, 2002). The concentrated organic extracts were dissolved in methanol at 1000 ppm to estimate its seed germination inhibiting property. Two controls were prepared – methanol and water. Filter paper placed in a Petri dish was soaked separately with each of the organic extract, water and methanol. The solvent was evaporated at 27°C. Distilled water (5 mL) was then added and 30 soaked seeds of romaine lettuce was placed in the Petri dish and was left to germinate for 2 days. The germination was determined by counting the number of germinated seeds after 2 days. Seeds with shoots measuring up to 2 mm were considered to have germinated (Omezzine et al., 2011). Three replicates were done for each organic extract. The extract that inhibited the most number of seeds was further studied.

Percent seed germination was calculated using the following equation (Chung et al. (2002)):

\[
\% \text{ seed germination} = \left( \frac{\text{no. of seeds germinated}}{\text{total no. of seeds}} \right) \times 100
\]

Phytochemical Screening of Dichloromethane Extract

*Test for tannins:* About 0.5 g of the dried powdered sample was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. The appearance of brownish green or a blue-black coloration indicates the presence of tannins.

*Test for phlobatannins:* About 5 mL of aqueous extract was boiled with 1% hydrochloric acid. The deposition of a red precipitate indicates the presence of phlobatannins.

*Test for saponin:* About 2 g of the powdered sample was boiled in 20 mL of distilled water and was then filtered. Ten milliliters of the filtrate with 5 mL distilled water was shaken vigorously for a stable persistent froth. The mixture was combined with 3 drops of olive oil and was shaken vigorously. The formation of
emulsion indicates the presence of saponin.

**Test for flavonoids:** About 1 g of the powdered plant sample with 10 mL ethyl acetate was heated over steam for 3 minutes. The mixture was filtered and 4 mL of the filtrate was added with dilute ammonia solution and was shaken. The appearance of a yellow color indicates a positive test for flavonoids.

**Test for steroids:** Approximately 0.5 g of dried powdered plant was mixed with 10 mL of chloroform and the mixture was filtered. One milliliter of acetic anhydride was added to the filtrate followed by the careful addition of a few drops of concentrated sulfuric acid to form a layer. A positive indication of steroids is the presence of blue/green color.

**Test for terpenoids:** Five milliliters of aqueous extract were mixed with 2 mL chloroform. Three milliliters of concentrated sulfuric acid were added carefully to form a layer. The presence of terpenoids is indicated by the reddish brown coloration of the interface.

**Test for alkaloids:** Five millilitres of an aqueous extract was obtained and was added with 2 mL of 2 N HCl. The mixture was heated with stirring in a water bath for 10 minutes. The cooled solution was filtered and was added with a few drops of Dragendorff’s reagent. A reddish-brown precipitate is a positive indication of alkaloids.

**Test for glycosides (Keller-Kiliani test):** About 2 mL of aqueous extract of the sample was obtained and added with 1 mL glacial acetic acid, few drops of FeCl3 and few drops of concentrated sulfuric acid. A positive indication of the presence of glycosides is the presence of green/blue precipitate.

**Test for amino acids (ninhydrin test):** Five to six drops of ninhydrin reagent were added to 2 mL of aqueous extract of the sample. The mixture was heated in boiling water bath for about 5 minutes. Appearance of purple color indicates the presence of amino acids.

**Test for proteins (Biuret test):** Five to six drops of 5% NaOH and 5-7 drops of 1% Cu(SO4)2 were added to 2 mL of aqueous extract. The appearance of a purple color indicates the presence of proteins.

**Purification of Dichloromethane Extract**

Initial determination of the composition of dichloromethane extract was first made by its development in a 5 cm x 2 cm silica gel TLC plate using 1:1 (v/v) dichloromethane-ethyl acetate as developing solvent. Sulfuric acid (20% in methanol) was sprayed for visualization. Prior to extraction with dichloromethane, the *Talinum paniculatum* leaves were extracted twice over 48 hours in order to remove chlorophyll.

To isolate the components, the concentrated bioactive extract in T. paniculatum was then chromatographed on a silica gel column (5 cm x 15 cm) using varying proportions of dichloromethane-ethyl acetate. The purity of bioactive fraction was determined using Thin Layer
Chromatography (TLC). Further purification using silica gel column chromatography was performed as needed.

The major phenolic compounds in T. paniculatum were isolated using preparative Thin Layer Chromatography. The purified fraction was kept in freezer to induce crystallization and then subjected to characterization tests.

Characterization Tests of the Isolate

The purified isolate that showed the highest bioherbicidal activity was characterized by Shimadzu UV Mini 1240 Scanning Spectrophotometer, Shimadzu IR Prestige-21 Fourier Transform Infrared Spectrophotometer equipped with Single Reflection ATR Accessory and Fischer-Jahns melting point apparatus.

Dose-response Assay

This bioassay is based on the method of Salamanze et al. (2015). Seeds of Cyperus iria were germinated on trays. At 4 days after seeding, the seedlings were transplanted on nursery (each square: 3 cm x 3 cm) tray filled with sterilized soil. They were watered and allowed to equilibrate with the soil for one day. Ten milliliters of different concentrations (100, 200, 500 and 1000 ppm) of each of the purified isolate were added to the soil. The tray was placed in open sunlight for 8 hours for seven days. Each of the pots was watered with tap water daily to maintain the soil moisture level capacity. At 7 days after transplanting, the shoot height of each plant was measured and the % shoot height increase relative to the control was calculated. Three replicates were made for each concentration. The EC50 was determined using the log-logistic curve.

Statistical Analysis

GraphPad Prism v5.0 (GraphPad Software, Inc. CA USA) was used for the statistical data analysis of the pot assay. Tukey’s multiple range test at 95% confidence interval was performed to determine the statistical significance at P≤0.05.

Results and Discussion

Soil Analysis of the Plant’s Source Location

The parameters of soil where sample plants were obtained were compared with the literature acceptable values (Table 1). The acceptable values for % moisture, pH, cation exchange capacity (CEC), iron content and % organic matter (OM) and available sulfur content were satisfied by the soil sample where plants were obtained.

Table 1. Analysis of soil where sample plants were obtained.

<table>
<thead>
<tr>
<th>Soil parameter</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>21.21 ± 0.31</td>
</tr>
<tr>
<td>pH</td>
<td>7.59 ± 0.00</td>
</tr>
<tr>
<td>CEC, cmol/kg</td>
<td>25.6 ± 0.0</td>
</tr>
<tr>
<td>Fe, mg/kg</td>
<td>2.10 ± 0.00</td>
</tr>
<tr>
<td>OM, %</td>
<td>4.59 ± 0.05</td>
</tr>
<tr>
<td>Available S, mg/kg</td>
<td>58.7 ± 4.0</td>
</tr>
</tbody>
</table>

Isolation of Bioactive Extract from Talinum paniculatum

Sequential extraction revealed that high % yields were obtained in petroleum ether
(7.95%) and dichloromethane (7.84%) (Table 2). The possibility of co-extracting compounds is reduced using sequential extraction, hence decreasing interferences during analysis (Kettle, 2014).

Table 2. Percent yield of each organic extract.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mass of extract, g</th>
<th>Percent yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>7.9456</td>
<td>7.95</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>7.8369</td>
<td>7.84</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7.0012</td>
<td>7.00</td>
</tr>
<tr>
<td>Ethanol:water (1:1 v/v)</td>
<td>6.8375</td>
<td>6.84</td>
</tr>
</tbody>
</table>

Phytochemical Screening of Dichloromethane Extract

The phytochemical screening revealed the presence of saponin, steroids, terpenoids and glycosides in the dichloromethane extract (Table 3). The phytochemical screening confirmed the presence of some known class of allelochemicals such as terpenoids and saponins. Plants belonging to the family Talinum were reported to be rich in crude protein, total lipids, essential oils, cardiac glycosides, flavonoids, and polyphenols. Studies also revealed the presence of essential nutrients and soluble fibers and vitamins (Swarna and Ravindhran, 2013). A phytochemical screening on chloroform extract of Talinum triangulare performed by Swarna and Ravindhran (2013) also revealed the presence of saponins and steroids. Filho (2010) reported that compounds such as campesterol, β-sitosterol, and stigmasterol can be extracted from the leaves of Talinum paniculatum using hexane, ethyl acetate and methanol as extracting solvents. Moreover, Talinum paniculatum was used as source of saponins, flavonoids, tannins, triterpenes, sterols and polyphenols that are commonly used for pharmaceutical purposes (Manuhara et al, 2015).

Table 3. Phytochemical screening of the most active extract of T. paniculatum.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Observation</th>
<th>Result (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Absence of brown-green/blue-black color</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>Formation of emulsion</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Absence of yellow color</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>Presence of bluish green solution</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Reddish-brown interface</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Absence of cream ppt</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Presence of green ppt</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: present (+), absent (-)

After development and visualization of the spots in thin-layer chromatography, four spots in the dichloromethane extract appeared (Figure 2). The Rf of the four spots are 0.12, 0.28, 0.64, and 0.96, respectively (Table 4).

Figure 2. TLC photograph (a) and schematic representation (b) of thin-layer chromatogram of the dichloromethane extract of T. Paniculatum developed in 1:1 dichloromethane-ethyl acetate.
Table 4. $R_f$ values and observations on the chromatogram of dichloromethane extract of *T. Paniculatum* developed in 1:1 dichloromethane-ethyl acetate.

<table>
<thead>
<tr>
<th>Spot</th>
<th>$R_f$</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>Grayish purple</td>
</tr>
<tr>
<td>2</td>
<td>0.28</td>
<td>Reddish-brown</td>
</tr>
<tr>
<td>3</td>
<td>0.64</td>
<td>Grayish purple</td>
</tr>
<tr>
<td>4</td>
<td>0.96</td>
<td>Intense dark brown</td>
</tr>
</tbody>
</table>

From the sample load of 1.40 g in column chromatography, 0.0943 g of isolate was obtained which constituted 6.74 % of the extract. The fractions collected were subjected to TLC to determine their purity (Figure 3). Upon removal of the solvent, the isolate obtained was a waxy solid. The $R_f$ of the isolate is 0.48. Upon charring, the spot is grayish purple.

The peaks shown are comparable to those of phytosterols (Table 4).

Based on Liebermann-Burchard Reaction test, UV-Vis and IR spectral analyses, melting point determination and chromatographic mobility, the isolate could be β-Sitosterol, a phytosterol (Table 4). The Liebermann-Burchard reaction is a common test for the presence of phytosterols. The formation of complex pentaenyl carbonium ion is the one responsible for the characteristic blue-green product indicative of a positive test for phytosterols (Burke et al, 1974). The UV spectrum of the isolate showed $\lambda_{\text{max}}$ of 253 nm, which is close to standard β-Sitosterol ($\lambda_{\text{max}} = 251$ nm). The obtained experimental melting point range of the isolate was 134°C - 138°C. The reported melting point value of β-sitosterol is 138°C, respectively (Rajput, 2012).

Table 4. Determination of the possible identity of the major isolate of dichloromethane extract of *T. Paniculatum*.
Potential Allelopathic Activity Of Dichloromethane Extract From The Leaves Of Talinum paniculatum (Jacq.) Gaertn Against Cyperus iria

Methods used | Results | Literature | Possible Identity
---|---|---|---
Liebermann-Burchard Reaction | Blue green complex was formed | Positive test is the formation of blue green complex (Phytosterols) | β-Sitosterol
UV spectrum & maximum wavelength of absorption | $\lambda_{max} = 253$ nm | $\lambda_{max} = 251$ nm (β-Sitosterol, chloroform as solvent)
Melting Point Determination | 134–138 °C | 138 °C (β-Sitosterol)
IR Spectrum | Wavenumber: O-H stretch, medium: 3346; Aliphatic C-H stretching: 2921; C = C stretch, weak: 1641; C-H bending: 1466; C-O stretch, medium: 1052 | β-Sitosterol | 3331; 2959; 1654; 1464; 1062

Bioherbicidal Assay of Dichloromethane Extract from Talinum paniculatum

Lettuce Seed Germination Inhibition Assay

Among the four organic extracts, the dichloromethane extract had the highest seed germination inhibiting activity (Figure 5). As reported by Khanh et al. (2007), one of the phytotoxins responsible for the allelopathic activity of some plants includes terpenoids which are soluble in dichloromethane. Terpenoids are also one of the three most known classes of allelochemicals found in sunflower (Vyvyan, 2002). A phytochemical screening on chloroform extract of Talinum triangulare carried out by Swarna and Ravindhran (2013) also revealed the presence of saponins and steroids. These compounds may also be present on the obtained dichloromethane extract.

As the concentration is increased, the % inhibition of the lettuce seeds also increased (Figure 6). Using this plot, the IC50 of the most active extract was determined to be at 477 ppm. (Fig. 6).

Whole Plant Dose-response Assay of Dichloromethane Leaf Extract of Talinum paniculatum

Results showed that at increasing concentration of the dichloromethane extract, the growth of the Cyperus iria was inhibited (Figure 7). Figure 8 shows the actual growth of sedge applied with increasing concentrations of the extract after seven days. Statistical analysis showed that at 95% confidence interval, the height of the weeds applied with the extract was significantly different with that of the control (Fig. 7).
This study shows that the dichloromethane extract of the leaves of *Talinum paniculatum* can be used as a potential source of allelochemicals for the development of bioherbicides against *Cyperus iria*, one of the world’s worst weeds of rice.

**Acknowledgement**

We would like to thank the UPLB-OVCRE for funding this research.

**Bibliography**


Other Plants. Institute of Food and Agricultural Sciences, University of Florida, 1-3.


