Phytochemical test fraction N-hexane allelopathy goat weed extracts (Ageratum conyzoides L.) on the growth of thorn spinach (Amarantus spinosus L.)

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Abstract. Plants release allelopathy compounds that can inhibit the growth of other plants so that it has the potential as a vegetable herbicide. One of the allelopathy characters that can be used in determining the criteria as a basis for plant biocides is allelopathy that specifically inhibits the growth of target and non-target plants. The source of allelopathy used in this study was goat weed (A. conyzoides) using N-hexane on the growth of thorn spinach (A. spinosus). The research has been carried out at Weed Laboratory, Agrotechnology Study Program, Faculty of Agriculture, Syiah Kuala University. Further analysis and test activities were carried out at the Laboratory of Agriculture and Food Analysis Laboratory of the Faculty of Agriculture, Syiah Kuala University. The first factor was the type of sub-fraction of weed extract consisting of 4 levels: control, sub-fraction A, sub-fraction B and sub-fraction C, and the second factor is the extract of goat weed consisting of 3 levels: 5%, 10% and 15%. The results showed that the use of various types of A. conyzoides extracts with various levels significantly affected the stem diameter; shoot dry weight and root dry weight of A. spinous on 7 DAT (days after treatment) up to 28 DAT. Sub-fraction types A and B can inhibit the growth of A. spinous.

1. Introduction

The term of weed has a broad meaning. Weed is a plant that grows in the unexpected places, competing cultivated plants and has a negative value and not yet known its usefulness, potential and the right of its presence in agricultural business [1]. Moreover, another negative caused by weed is that it can be a temporary host for plant diseases or parasites so that it can inhibit plant growth and reduce crop yields [2].

Efforts that can be done to reduce the loss of crop yields caused by rivalry is to take action to control the weeds preventively, technically, biologically, chemically, and mechanically. Among these methods, chemical control that uses synthetic herbicides is mostly done by farmers [3]. However, excessive use of synthetic herbicides has negative impact to the environment, leads to herbicide resistant weeds and the residues are poison to plants [4]. Therefore, alternative ways to control weeds are needed. It should be environmentally friendly and leaving no residue in agricultural products. Such control can be done by looking for the potential of secondary metabolites (allelochemicals) derived from other plants that can be used as plant-based herbicides [5].

Allelopathy is a phenomenon in the form of interactions among one another living thing through chemical compounds. The effect of allelopathy on plants can be both harmful and
beneficial [6]. The ability to inhibit the growth of other plants is due to the presence of a certain chemical compound found in a plant species [7]. Chemicals or organic substances that are allelopathic can be divided into two groups based on their effects on its plant or others plants, namely autotoxin that can kill or inhibit the growth of similar plants and antitoxic that can kill or inhibit the growth of other different types of plants [8]. Allelopathic compounds can affect plant activities including: nutrient absorption, cell division, growth inhibitors, photosynthesis, respiration, and protein synthesis and enzyme activity [9].

Organic herbicides can be produced from various types of plants that produce allelopathy. One of the plants that produce allelopathy is goat weed. This is in line with the results of previous studies that have been done for ten species of plants originating from goat weed allelopathy (A. conyzoides), siam weed (Chromolaena odorata), reeds (Imperata cylindrica), nut grass (Cyperus rotundus), tropical almond (Terminalia catappa), acacia (Chromolaena odorata), pine (Imperata cylindrica), teak (Tectona grandis), physic nut (Jatropha curcas), and paitan grass (Paspalum conjugatum). Goat weed is able to control thorn spinach weed at the concentration of 20% which has the potential as an organic herbicide that can inhibit the growth of thorn spinach weed in 7, 14 and 21 days after the utilization [10]. Goat weed is the plant that is mostly found on agricultural land. It can be used as a safe and environmentally friendly plant-based pesticide, also reducing the use of excessive chemical pesticides, production costs and bad impacts on farmers' health and environment. Goat weed leaf extract contains active compounds such as saponin, flavonoid, tannin, essential oil and polyphenol [11].

2. Materials and Methods

This research was conducted at the Laboratories and Experimental Garden of Agriculture Faculty of Syiah Kuala University from September 2017 - May 2018. The materials used in this study were goat weed leaves obtained from Indrapuri, Aceh Besar District and thorn spinach seeds (A. spinosus) obtained from Delima, Pidie District.

2.1. Research Materials and experimental design

A completely randomized factorial design with 2 factors and 3 replications was employed in this experiment. Goat weed extracts from sub-fraction A, B and C; and concentration of A. conyzoides extract of 5, 10 and 15% were the combination of treatments to be studied. Analysis of variance was used to study the effect of each treatments and its interaction. Duncan New Multiple Range Test was used as the post-hoc test for the significant effect.

2.2. Phytochemical Test

Phytochemical test becomes important for guidelines in the isolation of secondary metabolites. There are many ways for researchers to do phytochemical test, but in general the most frequently used method is phytochemical analysis with staining. The stain appearance of each group is different. Alkaloid can be identified using Mayer, Wagner, and Dragendorf reagents. Steroids and terpenoids usually are identified using Liberman-Bourchard reagents and flavonoids with FeCl3 reagents.

2.2.1. Alkaloids test

A 10 g samples goat weed leaf previously moistened with concentrated ammonia for 2 hours were macerated in 5 ml of dichloromethane, and then crushed and grounded. The slurry was added with 5 ml of 5% hydrochloric acid and shaken vigorously. It will be let stand until the hydrochloric acid and dichloromethane separated. The layer of hydrochloric acid is taken and divided into three test tubes and each tube is tested to determine the presence of alkaloids. Addition of Meyer, Dragendorf and Wagner reagent will lead to white, reddish brown, and yellowish precipitation, respectively.
2.2.2. Terpenoids, steroids and saponins test
A sample of 10 g of goat weed leaf were crushed and extracted with n-hexane and filtered. The filtrate was evaporated using rotary evaporator. Identification using the Liebermann-Burchard reagent resulted green or blue color for steroids and purple or red color for terpenoids.

Saponin tests was done by adding water into the extract, followed by shaking. Saponins did not show sensitivity to the Liebermann-Burchard reagent. It needs to be hydrolyzed with hydrochloric acid to break the glycoside bond. The broken glycoside bonds will be identified with the Liebermann-Burchard reagent. Positive result is indicated by the presence of purple or red for triterpene saponins and green or blue for steroid saponins.

2.2.3. Flavonoid phytochemical test
The extract from each sample was added with n-hexane and shaken vigorously. After that, the residue is extracted with 10 ml ethanol 80%. Addition of 0.5 mg magnesium and hydrochloric acid 0.5 M resulted pink or purple as indication of the presence of flavonoids.

2.3. Research Implementation

2.3.1. Sample preparation
Sample preparation was carried out by collecting A. conyzoides leaves taken from Indrapuri, Aceh Besar. The leaves should have optimum growth, not too young and not too old. They looked physically fresh green with perfect shape. The leaves were dried for about 10 days in room temperature without direct exposure of sunlight. The dried leaves were mashed using a wooden mortar and put into a container.

2.3.2. Extraction
The dry goat weed leaves were moistened using 25% ammonia for about one hour. The sample was then macerated using n-hexane for 72 hours. After filtration, the maceration was continued for the second times in n-hexane for another 72 hours. The extract from the first and second filtration was collected and evaporated using rotary evaporator.

2.3.3. Isolation
Fractionation of compounds in the extract of goat weed leaves was conducted using a column chromatography with a height of 73 cm and a diameter of 5 cm. The column was filled with silica gel 60 (0.2-0.5 mm) for about 125 grams. Sand was placed on the bottom and the top of silica gel to avoid the spread of the gel while pouring the sample or eluent. Fraction that was separated by the chromatography was concentrated using rotary evaporator.

2.3.4. Planting media preparation
Top soil was dried for 3 days, separated from fragments of plants and root. The soil was sieved and mixed with compost in a ratio of 1:1.

2.3.5. Thorn spinach seed planting
Thorn spinach seed was collected from Delima Sub-District, Pidie. The seeds were soaked for 24 hours before planting in a seeding trail. Five seeds were grown in a pot. One week after planting, a plant with best growth performance was selected while the others were removed. The plants were watered twice a day.

2.3.6. Plant Extract Application
Plant extract was sprayed to the experimental units 21 days after planting (DAP). Before the application of goat weed extract, calibration was first implemented by using water to determine the solvent need of goat weed extract that would be needed to spray weeds on each repetition with
the same concentration and time of application in the afternoon. The application is done by spraying the liquid extract using hand sprayer throughout the whole parts of thorn spinach seeds.

3. Results and Discussion

3.1. Stem Diameter (cm)

The following table 1 shows the average stem diameter of *A. spinous* due to bioherbicide with several types of n-hexane sub-fractions at 7, 14, 21, and 28 DAT. The results showed that the use of *A. conyzoides* bioherbicide with several n-hexane sub-fractions on the diameter of *A. spinousus* in sub-fraction type factors and concentration factors at 7, 21, and 28 DAT show a real effect but not on the both interactions.

| Treatment          | Observation Time |
|--------------------|------------------|
|                    | 7 DAT            | 14 DAT | 21 DAT | 28 DAT |
| Control            | 4.67             | 5.67   | 6.63   | 7.4    |
| Sub-fraction types |                  |        |        |        |
| Sub-fraction A     | 3.30             | 2.71   | 2.13   | 1.31   |
| Sub-fraction B     | 3.91             | 3.16   | 2.59   | 1.21   |
| Sub-fraction C     | 4.52             | 4.36   | 4.36   | 2.63   |
| Concentration (%)  | 5                | 4.33   | 3.69   | 3.13   | 2.52   |
|                    | 10               | 3.94   | 3.37   | 3.13   | 1.40   |
|                    | 15               | 3.46   | 3.17   | 2.81   | 1.23   |

Numbers followed by the same letters in the same column are not significantly different at the 5% level according to the DMRT test.

The effect of sub-fraction A and B had an impact on the suppression of *A. spinous* stem diameter from 7 to 28 DAT with a range of 1.21 and 3.30 cm, which were significantly different from sub-fraction C. This is because *A. conyzoides* L. contains secondary metabolites such as flavonoids, alkaloids, terpenoid, chromene, chromon, benzofuran, coumarin, essential oils, sterols and tannins [12]. Phenol compounds in the leaves of *A. conyzoides* L. can inhibit the growth of weeds. According to [13], mitotic abnormalities caused by phenol compounds are caused because the phenol leads to damage of the spindle threads during metaphase. Hindrance to cell division by allelochemical compounds extracts of *A. conyzoides* L. leaves can also be through the disruption of the activity of plant hormones such as cytokinins that play a role in stimulating cell division.

Stem diameter is one of the plant morphologies representing vegetative growth. Stems in plants have complex physiology; therefore [14] stated that stems are part of the body of the plant as an important organ in addition to roots and leaves. Nutrients obtained from planting media such as soil, absorbed by roots and distributed by stems through xylem and phloem tissue.

This study used *A. conyzoides* L. extracts with 3 sub-fractions (sub-fraction A, sub-fraction B, sub-fraction C) and 3 levels of concentration (5, 10 and 15%). The extract of *A. conyzoides* L contains allelopathic compounds. According to [15] allelopathic compound has become a poison that inhibits seed germination and vigor in corn and soybeans. Hence, this is related to the decrease of stem diameter of *A. spinous*, where the treatment of sub-fraction A with concentration of 15% indicated the best performance in this study.
3.2. Shoot Dry Weight (g)

The following Table 2 is the average results of observations of *A. spinosus* shoot dry weight due to the application of bioherbicides with several types of n-hexane sub-fractions at 7, 14, 21, and 28 DAT. The results showed that the use of *A. conyzoides* bioherbicides with several n-hexane sub-fractions on the shoot dry weight of the *A. spinosus*, had a significant effect on sub-fraction type factors at 7, 14, 21 and 28 DAT, but did not significantly affect the concentration and interaction between both of them.

| Treatment          | Observation Time |
|--------------------|------------------|
|                    | 7 DAT  | 14 DAT  | 21 DAT  | 28 DAT  |
| Control            | 4.37   | 5.10    | 5.77    | 6.40    |
| Sub-fraction types |        |         |         |         |
| Sub-fraction A     | 1.74 a    | 1.33 a    | 1.08 a    | 0.80 a    |
| Sub-fraction B     | 2.19 b    | 1.59 a    | 1.26 a    | 0.90 a    |
| Sub-fraction C     | 2.81 c    | 3.27 b    | 2.67 b    | 2.79 b    |

Concentration (%)

| Concentration (%) | 5 | 10 | 15 |
|-------------------|---|----|----|
| 5                 | 2.31 | 2.20 | 1.69 | 1.64 |
| 10                | 2.29 | 1.98 | 1.72 | 1.42 |
| 15                | 2.14 | 2.01 | 1.62 | 1.43 |

Numbers followed by the same letters in the same column are not significantly different at the 5% level according to the DMRT test.

The effect of giving sub-fraction A and B of goat weed extract has been observed at 7 to 28 DAT. There was a decrease of shoot dry weight. This is because the sub-fractions A and B contain components of caryophyllene and benzopyran. Those compounds have the potential as allelopathy which can inhibit plant growth, according to the study.

The results of the study reported that the giving of goat weed extract at the concentration of 20% was able to suppress the growth of weed tillers. N-hexane extract contains alkaloids and terpenoids. The compounds isolated by the two solvents have bioherbicide effects in inhibiting the growth of thorn spinach [16]. The difference in the level of polarity of the solvent determines the chemical structure of the extracted compounds. According to research [17] on phytochemical test of Massoi wood, ethyl acetate extract is known to contain essential oil compounds, flavonoids, tannins, steroids, triterpenoids and coumarin.

From the results of [18], it is known that the shoot wet weight of *Chromolea odorata L.* was lower than control. Application *A. conyzoides* extract up to 20% can reduce shoot wet weight up to 0.12 g, while in the control treatment or 0% shoot wet weight reaches 0.34 g. This is in line with the results of the following study, that *A. spinosus* dry weight also decreased. It can be concluded that the use of *A. conyzoides* extract tends to be effective in suppressing shoot dry weight of weeds.

3.3. Root Dry Weight (g)

The following table is the average observations of the root dry weight of *A. spinosus* due to the giving of bioherbicides with several types of n-hexane sub-fractions at 7, 14, 21 and 28 DAT. The results showed that the type of sub-fraction affected the root dry weight of *A. spinosus* at 7, 14, 21 and 28 DAT, but did not significantly affect the concentration and interaction factors between both of them. In the observation of 21 DAT, the n-hexane extract of sub-fraction B was not significantly different from sub-fraction A, but it was significantly different from the treatment of sub-fraction C. This is due to the increase of concentration of *A. conyzoides* extract which is able to reduce the root dry weight of *A. Spinous*. As root is the most important part on plant body as
a nutrient transporter from the soil, this function can be disrupted if the application of *A. conyzoides* extract contains phenolic compounds with the right concentration. The same thing occurred in the study of [18] that the root dry weight of *Chromolena odorata* L. decreased to 0.08 g at the concentration of 20% of *A. Conyzoides* L. extract.

Table 3. Mean Effect of Bioherbicides *A. conyzoides* with Several Types of n-hexane Sub-fractions on Root Dry Weights of *A. spinosus* at 7, 14, 21 and 28 DAT.

| Treatment                      | Time          | Observation |
|--------------------------------|---------------|-------------|
| Control                        | 7 DAT         | 2.47        |
|                                | 14 DAT        | 2.7         |
|                                | 21 DAT        | 3.07        |
|                                | 28 DAT        | 3.37        |
| Sub-fraction types             |               |             |
| Sub-fraction A                 | 7 DAT         | 1.09 a      |
|                                | 14 DAT        | 0.87 a      |
|                                | 21 DAT        | 0.34 a      |
|                                | 28 DAT        | 0.11 a      |
| Sub-fraction B                 | 7 DAT         | 1.04 a      |
|                                | 14 DAT        | 0.93 a      |
|                                | 21 DAT        | 0.75 b      |
|                                | 28 DAT        | 0.33 a      |
| Sub-fraction C                 | 7 DAT         | 2.28 b      |
|                                | 14 DAT        | 2.34 b      |
|                                | 21 DAT        | 1.65 c      |
|                                | 28 DAT        | 1.01 b      |
| Concentration (%)             |               |             |
| 5                              | 1.63 a        |
|                                | 1.44 a        |
|                                | 1.10 a        |
|                                | 0.61 a        |
| 10                             | 1.48 a        |
|                                | 1.40 a        |
|                                | 0.87 a        |
|                                | 0.45 a        |
| 15                             | 1.30 a        |
|                                | 1.30 a        |
|                                | 0.78 a        |
|                                | 0.39 a        |

Note: Numbers followed by the same letters in the same column are not significantly different at the 5% level according to the DMRT test.

Inhibition of the nut grass growth lead to decrease of dry weight on thorn spinach. This is happened because the allelopathy contained in goat weed leaf extract can cause a decrease in cell membrane permeability and disrupt the ability to absorb water and dissolved nutrients. A structural damage of cell membranes occurs due to the presence of allelochemical compounds such as phenolic compounds [19].

3.4. Phytochemical Test

The samples of goat weed leaf extract of n-hexane sub-fraction A, B and C have been tested for phytochemical test to determine the group of active chemical compounds contained in the samples. n-Hexane fraction of sub-fraction A, B and C of *A. conyzoides* leaf extract indicated that *A. conyzoides* leaf contained alkaloids and terpenoids compounds. Test using Mayer, Dragenderoff and Wagner reagents resulted color change of the reagent mixtures. In addition to the positive alkaloid test, the n-hexane fraction (sub-fractions A, B and C) of goat weed extract was also positive containing terpenoids which is marked by the formation of red precipitate [20]. Moreover flavonoid, steroids, saponins and tannins compounds were not presence in the n-hexane fraction of goat weed leaves.

Alkaloid test on *A. conyzoides* leaf powder added with Mayer reagent produces white precipitate; it is caused by the Mayer reagent bounded to alkaloids through the bonds between N alkaloid atoms with Hg Mayer reagent, resulting in non-polar mercury complex compounds settling in white. Goat weed leaf powder added with Wagner reagent produces brown color because the K + metal ion formed coordinate covalent bond with the N atom in the alkaloid to form precipitated alkaloid. Dragendorff reagent added to goat weed leaf powder produces orange color because the Dragendorff reagent contained bismuth nitrate and mercury chloride [21].

Secondary metabolite compounds from the terpenoid group can role as plant growth inhibitors [22]. Compounds from the terpenoid group can reduce membrane permeability [23]. Compounds which belong to the terpenoid group are neophytadiene, 3,7,11,15-2,6,10,14,18,22-tetracosahexane that are suspected to cause microtubule depolymerization so that the microtubule
spindle is not formed and there is no chromosome retraction towards the pole at the telophase stage.

**Table 4.** Compounds of phytochemical test results of n-hexane fraction (A, B and C sub-fractions) *A. conyzoides* leaf extract.

| No. | Compounds | Sub-Fraction |
|-----|-----------|--------------|
|     |           | A | B | C |
| 1   | Alkaloid  | + | + | + |
|     | -Meyer    | + | + | + |
|     | -Dragendorff | + | + | + |
|     | -Wagner   | + | + | + |
| 2   | Flavonoid | - | - | - |
| 3   | Terpenoid | + | + | + |
| 4   | Steroid   | - | - | - |
| 5   | Saponin   | - | - | - |
| 6   | Tanin     | - | - | - |

4. Conclusion
The Result that the application bioherbicide of *A. conyzoides* with several types of subfraction n-hexane can inhibit the growth of weed *A. spinous* on the parameters of stem diameter, root dry weight and shoot dry weight on observation 7 to 28 DAT. Through phytochemical test results of n-hexane fraction of sub-fraction A, B and C of *A. conyzoides* leaf extract showed that goat weed leaf contains many alkaloids and terpenoids compounds. *A. conyzoides* contain secondary metabolite compounds such as flavonoids, alkaloids, terpene, chromen, chromon, benzofuran, coumarin, essential oils, sterols, and tannins.

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