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An Investigation of the Allelopathic Properties of Various Weed Species in the Eastern Denpasar Region of Bali

A. A. S. P. R. Andriani[1,6], N. L. P. P. Setianingsih[2,6], N. Nazir[3,6], W. S. Dewi[4,6], E. R. Indrayatie[5,6], and P.K. Kalimutu[6]*

[1]*Agriculture Department, Faculty of Agriculture, Warmadewa University, Denpasar, Indonesia*
[2] *Food Science and Technology, Faculty of Agriculture, Warmadewa University, Denpasar, Indonesia*
[3]*Faculty of Agricultural Technology, Andalas University. Indonesia*
[4]*Soil Science Department, Faculty of Agriculture, Sebelas Maret State University, Surakarta, Indonesia*
[5]*Faculty of Forestry, Universitas Lambung Mangkurat. Indonesia.*
[6]*SAFE Innovation Center Indonesia*

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CORRESPONDING AUTHOR

*E-mail: putriameell@gmail.com

A B S T R A C T

Weeds are plants that live wild on cultivated land, which can reduce the productivity of cultivated plants. Phytochemical content in weeds has various benefits besides food, such as vegetable pesticides, pharmaceuticals, industry, and cosmetics. For this reason, this research aims to analyze the content of secondary metabolite compounds and measure the levels of allelopathic phytochemical compounds in several weed plants. Various substances have been identified in the form of allelopathy, namely flavonoids, tannins, phenolic acids, ferulic acid, coumarins, terpenoids, steroids, cyanohydrins, quinones, cinnamic acid, and their derivatives. Compounds identified as allelochemical compounds are flavonoids, tannins, phenolic acids, ferulic acid, coumarins, steroids, terpenoids, cinnamic acids, and their derivatives. Exploratory methods to identify plant types, experimental methods with phytochemical screening tests to characterize types of secondary metabolite compounds, and allelopathic toxicity tests Based on the results and discussion that have been presented, it can be concluded that the weeds *Echinochloa crus-galli, Panikum repen, Euphorbia hirta, Ageratum conyzoides,* and *Imperata cindrica* contain flavonoid, tannin, and phenol compounds. The weeds with the highest scores in the phytochemical test are *Echinochloa crusgalli* and *Ageratum conyzoides.*

1. INTRODUCTION

1.1. Research Background

Biotic and abiotic factors influence plant growth on agricultural land. Biotic factors such as pests, diseases, and weeds, while abiotic factors such as temperature, humidity, wind, rain, and sunlight, the presence of weeds is one of the biotic factors that can cause a decrease in sugarcane production. Weeds are wild plants that cause competition with other plants. The presence of weeds needs to be identified to find effective ways to control them [1].

Weeds are plants that live wild on cultivated land, which can reduce the productivity of cultivated plants [2]. Besides, weeds are plants that disturb or harm productive crops planted by humans, so farmers try to control them. Weeds can cause losses slowly as long as they interact with plants [3]. According to Ref. [4], the presence of weeds in cultivated plants will reduce crop yields. Losses caused by weeds are due to competition with cultivated plants in terms of nutrients, water, sunlight, and growing space. In addition, weeds can emit allelopathic compounds and can become hosts for pests and pathogens in cultivated plants.

The presence of weeds that dominate can reduce plant growth and yields. But apart from that, wild plants that live around cultivated plants can be used as biological resources. The diversity of weed types is influenced by planting patterns, plant density levels, soil fertility, and land processing methods [5]. Each plant has its chemical content and properties, especially in medical use for various human diseases [6].

Siam weed is efficacious as an antidiarrheal, astringent, antispasmodic (reduces spasms in the gastrointestinal tract), antihypertensive, anti-inflammatory (reduces inflammation), diuretic tonic (urinating agent), antipyretic (fever reducer) and heart tonic [7], used for skin diseases and treatment wound

healing [8], human burns, soft tissue wounds, ulcerated wounds, postnatal wounds and also for the treatment of leech bites, digestive disorders, and skin infections [9], antiprotozoal (antibiotic), antitrypanosome, antibacterial, antifungal and hepatotropic (liver inflammation relief) [10], amenorrhea, catarrh, diabetes, fever, pertussis and rheumatism, and as an anthelmintic [11], in agriculture it can be used as organic fertilizer, biopesticide and herbicide and in the medical field traditionally It can be used as a cough medicine and to stop bleeding [12], a mouthwash for treating sore throats, a malaria medicine, an antimicrobial, a headache, and a diuretic [13].

Weeds contain secondary metabolite chemical compounds such as Cynodon dactylon, Cyperus rotundus, Imperata cylindrica, Paspalum conjugatum, Ageratum conyzoides, and Euphorbia hirta L. Wild plants have potential if their chemical content is further investigated. Phytochemical content in weeds has various benefits besides food, such as vegetable pesticides, pharmaceuticals, industry, and cosmetics. For this reason, this research aims to analyze the content of secondary metabolite compounds and measure the levels of allelopathic phytochemical compounds in several weed plants.

1.2. Literature Review

1.2.1. Allelopathy

Various substances have been identified in the form of allelopathy, namely flavonoids, tannins, phenolic acids, ferulic acid, coumarins, terpenoids, steroids, cyanohydrins, quinones, cinnamic acid, and their derivatives.

Allelopathy is a phenomenon characterized by reciprocal biochemical interactions that encompass both inhibitory and stimulating effects across many plant species, including microbes. Allelopathy refers to the adverse impact exerted by a plant, including microbes, on neighbouring plants. This influence can occur either directly or indirectly, as a result of the release of hazardous chemical compounds into the surrounding environment[14]. Allelopathic compounds can affect nutrient absorption, cell division, growth inhibition, photosynthesis, respiration, protein synthesis, and enzyme activity. Allelopathy can cause conditions that can inhibit the growth of other plants [15].

Allelochemical compounds are secondary metabolites in plants. These compounds can be found in all plant tissues, including leaves, stems, roots, rhizomes, flowers, fruit, and seeds. Allelochemicals are compounds found in a plant that has the property of suppressing other plants around it. These allelochemical compounds can be released into the environment through evaporation, root exudates, and washing. Compounds identified as allelochemical compounds are flavonoids, tannins, phenolic acids, ferulic acids, coumarins, steroids, terpenoids, cinnamic acids, and their derivatives [16].

1.2.2. Weed Echinochloa crus-galli

Echinochloa crus-galli is a type of annual weed. E. crusgalli belongs to the class Poales, family Poaceae. The E. crus-galli weed is very competitive with lowland rice plants due to its high seed production, fast growth, and the C4 photosynthesis pathway [17]. This weed can cause harm if not controlled. E. crus-galli, reducing lowland rice production by around 30% [18] and reducing the weight of paddy-filled grain by 46.20% [19].

1.2.3. Weed Panicum Repens

Panicum repens L., known locally as lempuyangan grass, is an herbaceous plant in the Poaceae family. Its morphological characteristics include narrow leaves and creeping stolons, which can grow to a height of 0.2–0.8 meters. This grass grows easily and spreads even on land that is poor in nutrients, so it is called perennial grass [20]. This plant has the potential to be used as animal feed, a shelter for organisms in the water to lay their eggs, and a natural pesticide [21].

1.2.4. Weed Euphorbia Hirta

Patikan kebo (Euphorbia hirta L.) is a plant that has antibacterial activity against Staphylococcus epidermidis bacteria, which is the bacteria that causes acne. Flavonoids and tannins are compounds that are thought to act as active anti-acne compounds.

Euphorbia hirta L. has many properties for treating several diseases, such as dysentery, improving urination, bitter melon abscess and chronic bronchitis, breast abscess, abdominal typhus and eczema, kidney inflammation, sore throat, and asthma [22]. Apart from that, patikan kebo has anti-inflammatory properties and relieves itching (antipruritic) [23]. Patikan kebo (Euphorbia hirta L.) has been proven to inhibit the growth of Staphylococcus epidermidis bacteria with a minimum inhibitory concentration of 10 mg/mL [24].

1.2.5. Weed Ageratum Conyzoides

The active compounds contained in Ageratum conyzoides weed are saponins, alkaloids, tannins, flavonoids, and polyphenols [25]. According to Edwin et al. (2018) [26], abandon contains monoterpenoids, diterpenoids, sesquiterpenoids, and other compounds such as curcumin, flavonoids, benzofurans, alkaloids, terpenoids, chromenes (Conyzorigum) and sterols, which make these leaves have a repellent effect, are anti-food, larvicide, ovicidal, and toxic to various types of pests. All the active compounds in this plant can hinder the ability to eat, so pests refuse to eat, interfere with the development of eggs into pupae, and make it difficult for female pests to reproduce. One pest that is now widespread is Spodoptera frugiperda. According to Ramadan et al. (2018), [27] abandon extract has the potential to be an insecticide that can kill Plutella xylostella L. pests at a concentration of 5%, which has been proven to result in 46.67% of Plutella xylostella L. pest deaths in 12 hours.100% of pests occur within 72 hours after application [28].

1.2.6. Weed Imperata Cilindrica

Imperata cylindrica (L.) Beauv. is a plant known as a weed [29]. According to Kusuma and Zaky (2005) [30], weeds are defined simply as plants that are undesirable and harmful. These plants are detrimental because they can compete for growing space, nutrients, and air with other cultivated plants. However, weeds also have a positive side because they can be used as functional foods. The rhizomes of weeds are often used as a helpful food source because they contain natural antioxidants. One of the available food products based on weed rhizomes is in the form of herbal tea [31].

Thatch rhizomes contain many chemical compounds, one of which is phenolic. Phenolic compounds and flavonoids in thatch rhizomes can be used as a source of antioxidants [32]. Flavonoids can act as antioxidants because they contain

hydroxyl groups bound to the carbon of the aromatic ring, so they can capture free radicals by donating electrons (reductants), producing more stable products and inhibiting free radical chain reactions [33].

1.3. Research Objective

This research aims to determine the secondary metabolite content in several types of weeds, the process of allelopathy in various kinds of weeds, and the allelopathic potential of grass, nut, and broadleaf weeds.

2. MATERIALS AND METHODS

2.1. Preparation sampel

This research was conducted at the Laboratory of the Faculty of Agriculture, Warmadewa University. The research method used is a mixed method of exploration and experimentation. Exploratory methods to identify plant types, experimental methods with phytochemical screening tests to characterise types of secondary metabolite compounds, and allelopathic toxicity tests the tools used in this research were test tubes, tube racks, blenders, rotary evaporators, dropper pipettes, volume pipettes, beakers, stationery and observation tables, rulers, digital cameras, label paper, key books on plant determination (Flora book), scissors, knives, scales, and plastic samples. The materials used in this research were distilled water and clean water. The samples in this study were Cynodon dactylon, Cyperus rotundus, Imperata cylindrica, Paspalum conjugatum, Ageratum conyzoides, Euphorbia hirta L., and green bean seeds.

2.2. Phytochemical Test

The experimental method was carried out at the characterization stage of secondary metabolite compounds, namely by qualitative testing of secondary metabolite compounds with the following stages:

First, sample preparation. Fresh leaves, stems, and roots of weed plants are cut into small pieces and then washed with water and distilled water. Then, it dried indoors until the water content disappeared. After drying, the samples were ground using a blender.

Second, Maceration. The ground sample was then extracted using a 96% ethanol solvent using the maceration method until a filtrate was obtained.

Third, Evaporation. The filtrate obtained from the maceration results is then evaporated.

Fifth, phytochemical screening.

Alkaloid Test: The evaporation process results are added to Mayer's reagent, Wagner's reagent, and Dragendorff's reagent. The formation of a white precipitate or cloudy solution when Mayer's reagent is added indicates the presence of alkaloid compounds. The formation of a brown precipitate when Wagner/Dragendorff reagent is added indicates the presence of alkaloid compounds. Phenolic Test: The evaporation process results are added to 10% NaOH. If it produces a red color, then it contains phenolics. Steroid and triterpenoid test The results of the evaporation process are added to the Liebermann-Bauer reagent. The formation of a green or blue color means it contains steroid compounds. The appearance of a purple or red color means it contains triterpenoid compounds.

Saponin Test: The results of the evaporation process are added to distilled water and then shaken. The formation of stable foam means it contains saponin.

Flavonoid Test: The evaporation process results are added to HCl-Mg metal. The formation of a red, orange, or purple color means it is positive for flavonoids.

In the Tannin Test, $FeCl_3$ is added to the evaporation process results. The formation of a dark blue or blackish-green color indicates the presence of tannin compounds.

This research uses descriptive data analysis techniques, namely research that aims to create information (descriptions) about situations or events. The data analysis technique for this research was determined by the names of the types of plants and compounds found. Determining the type of plant is done by comparing photos or pictures and the characteristics of the plant with references in various plant books. The determination of the type of compound is carried out using a phytochemical screening test in the laboratory.

3. RESULT AND DISCUSSION

3.1. Allelopathic Phytochemical Content of Weeds

The allelopathic phytochemical content test carried out on Echinochloa crus-galli weed extract showed that Echinochloa crus-galli weed extract contained flavonoid compounds, tannins, and phenols. Based on the results of quantitative phytochemical tests obtained on Echinochloa crus-galli weed samples, three repetitions were carried out to test the content of flavonoid compounds, tannins, and phenols (mg/100g). The average content of flavonoid compounds was 2869.9 mg/100 g; the average tannin compound content was 10884.35 mg/100 g; and the average phenolic compound content was 35011.017 mg/100 g (Table 1).

Ref. [34] reported that Echinochloa crus-galli contains 59 types of allelopathy, including phenols, terpenoids, steroids, lactose, and others. Ref. [35] revealed that triterpenoid compounds and steroids can slow down the metabolic activity of the weed Echinochloa crus-galli, causing the cell division system in the leaves to be disrupted, which will then have an impact on the weed's leaf area.

The allelopathic phytochemical content test carried out on Panikum repen weed extract showed that Panikum repen weed extract contained flavonoid compounds, tannins, and phenols. Based on the results of quantitative phytochemical tests obtained on Panikum repen weed samples, three repetitions were carried out to test the content of flavonoids, tannins, and phenols (mg/100g). The average content of flavonoid compounds was 2561.9367 (mg/100g); the average tannin compound content was 6891.1533 (mg/100g); and the average phenolic compound content was 4684.34 (mg/100g).

Panicum repen can grow in warm to hot climates, growing in several types of soil, from sandy, clay soil to waterlogged soil with high soil moisture. Panicum repens grows well in wet organic soil but can also grow at high altitudes in drought conditions [36].

The allelopathic phytochemical content test carried out on Euphorbia hirta weed extract showed that it contained flavonoid compounds, tannins, and phenols. Based on the results of

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quantitative phytochemical tests obtained on Euphorbia hirta weed samples, three repetitions were carried out to test the content of flavonoid compounds, tannins, and phenols (mg/100g). The average content of flavonoid compounds was 1513.1133 (mg/100g); the average tannin content was 2654.18 (mg/100g); and the average phenolic compound content was 2147.37 (mg/100g).

Patikan kebo has the scientific name Euphorbia hirta, which grows wild in tropical areas in Indonesia. All parts of the plant can be used as medicine [23]. The existence of this plant still receives little attention from the public, even though it is a plant. This plant has many properties for treating several diseases, such as dysentery, improving urination, bitter melon abscess and chronic bronchitis, breast abscess, abdominal typhus and eczema, kidney inflammation, sore throat, and asthma [22]. Apart from that, patikan kebo has anti-inflammatory properties and relieves itching (antipruritic) [23]. Patikan kebo (Euphorbia hirta L.) has been proven to inhibit the growth of Staphylococcus epidermidis bacteria with a minimum inhibitory concentration of 10 mg/mL [24].

Table 1. Allelopathic Phytochemical Content of Weeds

No.	Sample	Test	Test results		
			Flavonoid Test (mg/100g)	Tannin Test (mg/100g)	Phenol Test (mg/100g)
1.	Echinochloa crus-galli	1	3082.48	10438.27	8343.11
		2	2763.61	11664.99	8577.68
		3	2763.61	10549.79	88112.26
		Average	**2869.9**	**10884.35**	**35011.017**
2	Panikum Repen	1	2477.48	6699.16	4563.53
		2	2618.24	6965.00	4780.99
		3	2590.09	7009.30	4708.50
		Average	**2561.9367**	**6891.1533**	**4684.34**
3	Euphorbia Hirta	1	1940.52	27,57.80	2212.04
		2	1188.02	2338.44	2001.52
		3	1410.80	2969.92	2228.55
		Average	**1513.1133**	**2654.18**	**2147.37**
4	Ageratum Conyzoides	1	2754.24	4634.62	3649.91
		2	3036.08	5125.95	3641.72
		3	3658.23	5615.40	4157.09
		Average	**3149.5167**	**5125.3233**	**3816.24**
5	Imperata Cilindrica	1	1772.39	4851.97	3557.21
		2	1772.39	4839.74	3548.64
		3	1784.05	4839.74	3548.64
		Average	**1776.2767**	**4843.8167**	**3551.4967**

The allelopathic phytochemical content test carried out on Ageratum conyzoides weed extract showed that Ageratum conyzoides weed extract contained flavonoid compounds, tannins, and phenols. Based on the results of quantitative phytochemical tests obtained on Ageratum conyzoides weed samples, three repetitions were carried out to test the content of flavonoids, tannins, and phenols (mg/100g). The average content of flavonoid compounds was 3149.5167 (mg/100g); the average tannin compound content was 5125.3233 (mg/100g); and the average phenolic compound content was 3816.24 (mg/100g).

According to Ref,) [37], Ageratum conyzoides is one of the plants that can be used as a vegetable insecticide because the leaves of this plant contain toxic allelopathic compounds that can be used as a control agent for pests and diseases that disturb the host plant. The leaves of this plant can act as a repellent against pests because it has a distinctive aroma, and the leaves contain antifeedant substances that contain essential oils that cause a decrease in the appetite of the target pest.

According to Ref. [38], there are several ways to get insecticide into the pest's body, which causes the pest to become poisoned and eventually die. First, it has the property of being a contact poison where all the active compounds contained in an extract enter through the pest's body wall. Second, as a stomach poison, all the active compounds contained in an extract enter the pest's body through the mouth and enter the digestive system of the pest. Thirdly, it acts as a fumigant, meaning that all the active compounds in an extract enter the pest's body through the respiratory system.

The allelopathic phytochemical content test on the Imperata Cilindrica weed extract showed that the extract contained flavonoid compounds, tannins, and phenols. Based on the results of quantitative phytochemical tests obtained on Imperata cylindrical weed samples, three repetitions were carried out to test the content of flavonoids, tannins, and phenols (mg/100g). The average content of flavonoid compounds was 1776.2767 (mg/100g); the average tannin compound content was 4843.8167 (mg/100g); and the average phenolic compound content was 3551.4967 (mg/100g).

Reeds are often found in open fields and cultivated land. Reeds contain allelopaths that can affect plant growth [39]. Phenolic compounds, which are allelopathic to sedge, can function as contact bioherbicides [40]. To determine the potential of reeds as a bioherbicide, we must know the weeds that are suppressed by the bioherbicide.

The phytochemical test is a qualitative test to determine the types of secondary metabolite compounds contained in the crude

extract of a plant. The principle is a color-testing reaction with several active compound detection reagents [41].

Based on the results of flavonoid tests on five types of weeds, namely Echinochloa crus-galli, Panikum repen, Euphorbia hirta, Ageratum conyzoides, and Imperata cirlindrica, Based on the data in Figure1, the comparison between the five weeds shows that the weeds that have the highest to lowest levels are Ageratum conyzoides > Echinochloa crus-galli > Panikum repen > Imperata citrica > Euphorbia hirta.

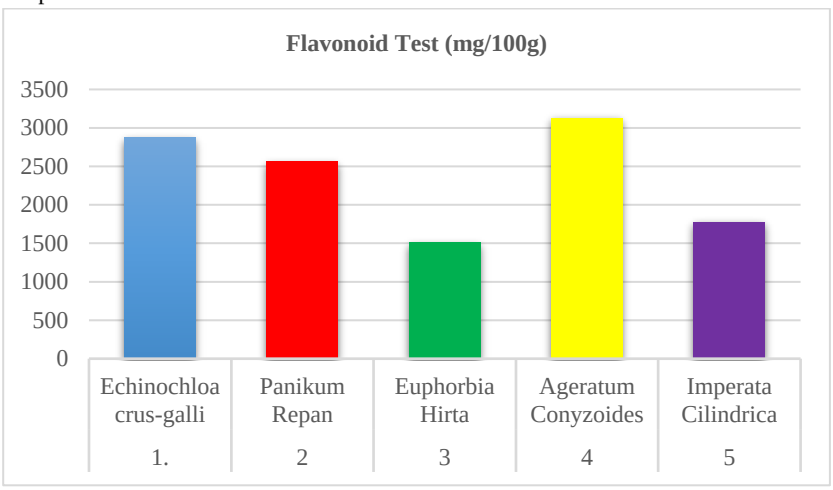

Figure 1. Weed allelopathic flavonoid test

Flavonoids are compounds consisting of C6–C3 – C6. Flavonoids are generally found in plants as glycosides. The sugar group is compounded on one or more phenolic hydroxyl rings (Figure 2). Flavonoids are found in all parts of plants, including fruit, pollen, and roots [42].

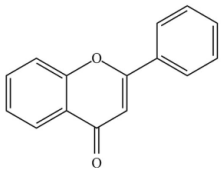

Figure 2. Flavonoid structure

Based on the results of flavonoid tests on five types of weeds, namely Echinochloa crus-galli, Panikum repen, Euphorbia hirta, Ageratum conyzoides, and Imperata cirlindrica, Based on the data in graph 2, the comparison between the five weeds shows that the weeds that have the highest to lowest levels are Echinochloa crus-galli > Panikum repen > Ageratum conyzoides > Imperata cirlindrica > Euphorbia hirta.

Based on the results of flavonoid tests on five types of weeds, namely Echinochloa crus-galli, Panikum repen, Euphorbia hirta, Ageratum conyzoides, and Imperata cirlindrica, Based on the data in Figure 3, the comparison between the five weeds shows that the weeds that have the highest to lowest levels are Echinochloa crus-galli > Panikum repen > Ageratum conyzoides > Imperata cirlindrica > Euphorbia hirta

Tannins are a class of phenolic compounds found in leaves and unripe fruit (Figure 4) They are a class of active plant compounds that belong to the flavonoid group, have an astringent taste, and have the ability to tan the skin. Chemically, tannins are divided into two groups, namely condensed tannins, or catechin tannins, and hydrolyzed tannins, or gallic tannins.

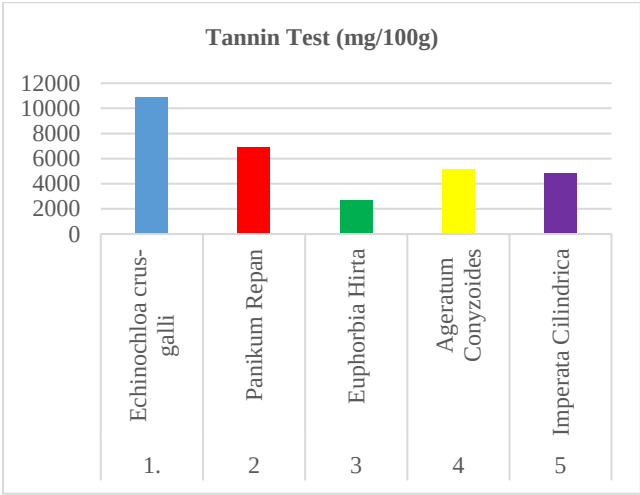

Figure 3. Weed allelopathic tannin test

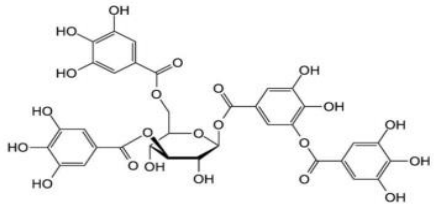

Figure 4. Tannin structure

Ref. [43] stated that phenol compounds can cause a decrease in permeability in cell membranes, thus inhibiting the transport and diffusion processes resulting from the breakdown of food reserves through the cell membrane, causing inhibition of the cell growth process.

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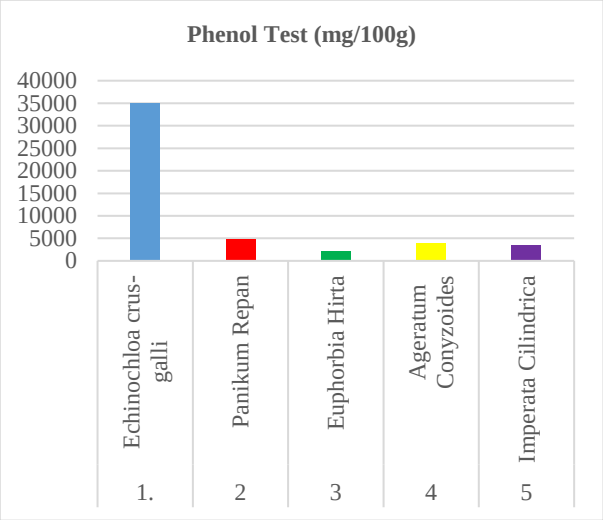

Figure 5. Weed allelopathic Phenol test

To dissolve phenolic substances in plants, you must use an ethanol solution. According to Ref [44], the solubility of substances in solvents depends on polar and non-polar bonds. Polar compounds only dissolve in polar solvents. Polar compounds that can be attracted by the 70% ethanol solvent in the reed rhizomes include tannins, phenols, and flavonoids [45]. Tannins, phenols, and flavonoids are polar compounds and can be extracted using polar solvents [46].

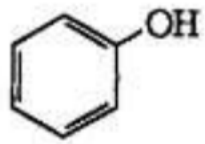

Figure 6. Phenol structure

4. CONCLUSION

Based on the results and discussion that have been presented, it can be concluded that the weeds Echinochloa crus-galli, Panikum repen, Euphorbia hirta, Ageratum conyzoides, and Imperata cindrica contain flavonoid, tannin, and phenol compounds. The weeds with the highest scores in the phytochemical test are Echinochloa crus-galli and Ageratum conyzoides.

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