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Antioxidant and anticancer activity test of Jewawut Extract (*Setaria italica* L.) Local Varieties of West Sulawesi

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Abstract. Jewawut (*Setaria italica* L.) is a local plant that is widely found and consumed by the people of West Sulawesi. This cereal plant has various benefits, one of which is as an anticancer. So, this study aims to determine the antioxidant and anticancer potential of methanol extract of Jewawut (*Setaria italica* L.). This research includes phytochemical test of secondary metabolites, antioxidant activity test with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and identification of anticancer potential based on toxicity test with Brine Shrimp Lethality Test (BSLT) method. The results showed that the methanol extract of Jewawut (*Setaria italica* L.) contained secondary metabolite compounds including alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. The extract has very weak antioxidant activity with an IC_{50} value of 4104.63 ppm, but the toxicity value (LC_{50}) is 41.65 µg/mL. That makes this species a promising source of anticancer agent.

Introduction

West Sulawesi is an area that has an abundance of diverse local foods, one of which is Jewawut (*Setaria italica L.*) or better known by the local name Tarreang. Jewawut (Tarreang) is widely grown and processed by local people as traditional food such as uleq-uleq, dodol, buras, baje, and jepa. Jewawut is also used in every traditional event of the local community such as sayyang pattu'du, weddings, makkuliwa, macceda, harvest celebrations, fishing parties and various other traditional events (Ramlah et al, 2020).

Apart from being used as a functional food ingredient, Jewawut also has various health benefits. According to Juhaeti et al (2019), West Sulawesi mandarin speciality jewawut has its own characteristics. This is because the amino acid content in West Sulawesi mandar jewawut is higher than jewawut from other regions. In addition, West Sulawesi mandar jewawut also has protein and fat content that far outperforms other cereals (Soeka and Sulistiani, 2017). Jewawut extract is known to contain secondary metabolites such as phenolics and flavonoids that function as antioxidants. (Normawati et al, 2020). Antioxidants are compounds that can counteract or neutralize free radical molecules by donating one of their electrons so as to inhibit oxidative reactions and prevent cell damage that can cause degenerative diseases such as cancer (Suwardi and Noer, 2020; Rudiana et al., 2018).

Cancer is one of the diseases that always increases every year. To reduce this risk, according to Clinton et al (2020), it is important to adopt a healthy diet by making fiber-rich cereals a major part of the daily diet. The results showed that there was a 17% reduction in the risk of cancer for every 90 grams of cereal consumed daily. Several studies have shown that cereal consumption can reduce the risk of cancer. This is because the fiber content in cereals can reduce the contact between carcinogens and the lining of the colon and rectum. In addition, bacteria from consumed cereals are able to ferment and produce short-chain fatty acids that have a protective effect against cancer (Song et al, 2015; Ben et al, 2014; Nindrea et al, 2018). Based on this, research using local food cereals of West Sulawesi is important to be carried out to see the content of secondary metabolite compounds contained in barley (*Setaria italica* L.) and test its activity as an antioxidant, and see the level of toxicity as an anticancer through the Brine Shrimp Lethality Test (BSLT) method.

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Experimental

Material and Methods

The materials used in this study were jewawut (*Setaria italica L.*) obtained from Balanipa District, West Sulawesi, methanol p.a, Mg powder, HCl (Merck), $FeCl_3$ 1%, distilled water, H_2SO_4 (Merck), wagner reagent, dragendorf reagent, mayer reagent, vitamin C, DPPH, *Artemia salina* Leach, DMSO and sea water.

Procedures

Sample Preparation and Extraction

Jewawut (*Setaria italica L.*) samples were washed thoroughly under running water and dried. After drying the sample was then mashed using a blender. Next, 500 grams of Jewawut sample powder was macerated with methanol for 3x24 hours. After that, it was stirred for 10 minutes and tightly closed. Then filtration was carried out using filter paper. Filtrate was then evaporated until the thick extract was obtained.

Phytochemical Test

Phytochemical identification was carried out to determine the presence of secondary metabolites of alkaloid, flavonoid, steroid, terpenoid, saponin and tannin groups with the following procedures:

Alkaloid. A total of 2 mL of extract was added to 5 mL of chloroform ($CHCl_3$), ammonia (NH_3) and sulfuric acid (H_2SO_4), then shaken gently and allowed to separate. Then the acid layer was taken and put into three test tubes. Then each tube is added a few drops of Mayer, Wagner and Dragendorff reagents. Positive results for the presence of alkaloids if a white precipitate is formed with Mayer's reagent, a brown precipitate with Wagner's reagent and orange with Dragendorff's reagent.

Flavonoid. A total of 2 mL of extract was put into a test tube and then added magnesium powder (Mg) and 5 drops of concentrated hydrochloric acid (HCl). If an orange or red colour is formed then the solution is positive for flavonoid compounds.

Steroid and Terpenoid. A total of 2 mL of extract was put into a test tube, then added a few drops of anhydrous acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4). If the solution forms a red colour, it is positive for terpenoids and if a bluish green colour is formed, it is positive for steroids.

Saponin. The extract was put into a test tube and added 1 mL of hot distilled water, cooled and shaken vigorously for 1 minute until excessive foam formed. Then a few drops of hydrochloric acid (HCl) are added, if the foam does not disappear for 5 minutes, the sample is positive for saponins.

Tannin. A total of 2 mL of extract was put into a test tube then added 5 drops of FeCl 1%. If a green or blue-black colour is formed in the solution, it is positive for tannin compounds.

Antioxidant Activity Test

Antioxidant testing of jewawut extract (*Setaria italica L.*) was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A total of 1 gram of sample was dissolved in 1000 mL of methanol (1000 ppm). Then a concentration series of 10 ppm, 100 ppm, 1000 ppm was made. Then as much as 3.5 mL of each sample concentration series solution was put in a test tube and 0.5 mL of DPPH was added. The solution mixture was homogenised and incubated in a dark room for 20 minutes. Furthermore, measurement of antioxidant activity was carried out using a UV-VIS spectrophotometer at a wavelength of 517 nm. Percent inhibition is calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(AA\text{-}BB)}{AA} \text{ x } 100\% \quad (1)$$

Explanation:
AA: Blank Absorption
AB: Sample absorbance.

Toxicity Test by BSLT Method

The extract of jewawut (*Setaria italica L.*) was tested for toxicity against A. salina shrimp larvae using the Brine Shrimp Lethality Test (Tahar et al, 2023).

Preparation of A. salina Leach Shrimp Larvae. 15 mg of A. salina Leach shrimp eggs were placed in a container containing seawater and kept under a 40-60watt incandescent lamp. The lamp was switched on for 48 hours until the A. salina eggs hatched into larvae.

Sample Preparation. Preparation of sample solution was carried out by making an initial concentration of 2000 µg/mL as a mother solution by dissolving 20 mg of sample in 200 µL of DMSO, then diluted with 9800 µL of seawater until the total volume became 10,000 µL. Dilutions and measurements were done in triplicate in vial tubes with concentration series of 1000, 100, 10, 1, and 0.1 µg/mL.

Toxicity Test. A total of 10 hatched *A. salina* larvae were put into vial tubes at each concentration and incubated for 24 hours. After 24 hours, the number of dead and alive *A. salina* in the test tube was counted to obtain the LC_{50} value (Tahar et al, 2023).

Result and Discussion

Sample Preparation and Extraction

The samples used in this study were barley (Setaria italica L.) obtained from Balanipa District, Polewali Mandar, West Sulawesi. Samples taken were put in ziplock plastic and brought to the Integrated Laboratory of West Sulawesi University. Then the samples were washed with running water to remove impurities that could interfere with the extraction process. Furthermore, the samples were dried with the aim of reducing water content and preventing enzymatic reactions/microbial activity and preventing the growth of fungi so that they can be stored longer and are not easily damaged so that their chemical composition does not change (Hendrisno et al., 2023; Handayani et al., 2014). The dried samples were then mashed using a blender. The sample powder that has been obtained is then weighed as much as 500 g, then extracted by maceration method which is done by soaking the sample in a closed container using methanol for 3×24 hours and every 1×24 hours filtering is done. The filtrate obtained was then evaporated using an evaporator to obtain a concentrated extract which was then used for phytochemical tests, antioxidant activity tests and toxicity tests.

Phytochemical Test

Phytochemical test aims to determine the class of secondary metabolite compounds contained in methanol extract of jewawut (*Setaria italica L.*) by looking at the colour changes that occur.

Table 1. Phytochemical Test of Methanol Extract of Jewawut (*Setaria italica L.*).

Compound Group	Observation Result	Description
Alkaloid		
- Mayer Reagent	white precipitate	+
- Wagner Reagent	brown precipitate	+
- Dragendorf Reagent	yellow/orange precipitate	+
Flavonoid	Orange/red	+
Terpenoid	red	+
Tannin	blackish blue	+
Saponin	foam	+

Based on the results of phytochemical tests (Table 1), it is known that there are groups of alkaloid compounds, flavonoids, steroids, terpenoids, tannins, and saponins in jewawut extract (*Setaria italica L.*). These results are in accordance with research conducted by Aini et al (2021) that the results of phytochemical tests on jewawut flour used as a base for making cookies are alkaloid, flavonoid, phenolic, and tannin compound groups.

Antioxidant Activity Test

Antioxidants are chemical compounds used to counteract free radicals caused by an imbalance of oxidative processes in the body. This is what causes various diseases such as cancer, kidney disease and diabetes mellitus (Khaira, 2010). Antioxidant compounds can be found from natural and synthetic materials. However, the use of synthetic drugs has side effects. So, the search for natural antioxidant compounds is a better alternative.

Antioxidant activity testing of methanol extract of jewawut (*Setaria italica L.*) was conducted using DPPH method. The working principle of the DPPH method is the provision of electrons (H+) antioxidant compounds in the sample on DPPH compounds(2,2-diphenyl-1-picrylhydrazyl). Thus, converting DPPH free radicals into non-radical compounds (Figure 1), which is indicated by a colour change from purple to pale yellow. The DPPH method was chosen because the test requires only a few samples, simple, easy, and fast in the process (Hanani et al, 2005; Handayani et al, 2014). In addition, the DPPH method is also a method with a sensitive test level with a very significant correlation (Maesaroh et al, 2018).

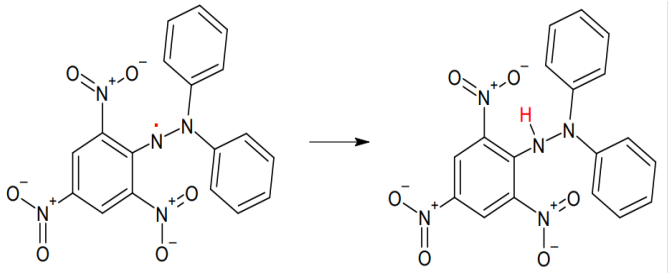

Figure 1. Reaction of DPPH radical with antioxidant compounds.

The parameter used to see antioxidant activity is Inhibitor Concentration (IC_{50}). The IC_{50} value is defined as the amount of antioxidant concentration of the test compound required to reduce free radicals by 50%. The smaller the IC_{50} value, the higher the activity in suppressing free radicals, indicating that the sample has stronger antioxidant activity. If the IC_{50} value is below 50 ppm, the antioxidant activity is very strong, the IC_{50} value is between 50-100 ppm indicates strong antioxidant activity, the IC_{50}

value is between 100-150 ppm means moderate antioxidant activity, the IC_{50} value is between 150-200 indicates weak antioxidant activity, and if the IC_{50} value is above 200 ppm means very weak antioxidant activity (Rahman et al, 2014). The results of antioxidant activity IC_{50} obtained from methanol extract of jewawut (*Setaria italica L.*) amounted to 4104.63 ppm. This result indicates that the sample has very weak antioxidant activity (Table 2). However, this value does not mean that the sample extract has no antioxidant ability. Antioxidant testing of samples can be influenced by several things such as, contamination of materials, incubation time and sample preparation process. In addition, it is suspected that the content of flavonoid compounds that act as antioxidants is small in the sample and the flavonoid compounds in the extract are not pure. Therefore, the process of fractionation and purification of compounds needs to be done next.

Table 2. Antioxidant Test Results of Methanol Extract of Jewawut (*Setaria italica L.*).

Sample	IC_{50} Value (ppm)			Average IC_{50} Value (ppm)
	Simplo	Duplo	Triplo	
Methanol Extract of Jewawut (*Setaria italica L.*)	4073,86	4093,57	4146,75	4104,63

Toxicity Test by BSLT Method

To find out whether a compound in a plant has potential as an anticancer agent, it is necessary to conduct a test as the initial stage of research, namely through toxicity testing with the Brine Shrimp Lethality Test (BSLT) method. The advantages of this method are easy, fast, simple, and cheap. The parameter used in this test is based on the LC50 value (Rosyadi et al., 2021). LC50 is the concentration of extract or sample that can cause death by 50% of the test animals (Utami and Ardiyanti, 2019; Afriani et al, 2016).

The mechanism of mortality of A. salina larvae is closely related to the function of the compound flavonoids, alkaloids, tannins, saponins, terpenoids compounds contained in the Jewawut extract. The mechanism of larval death is thought to be related to the function of alkaloid compound that can inhibit larval feeding. The way the compound works is by acting as a poison, therefore if this compound enters the larval body, the digestive system will be disrupted. In addition, the compound also inhibits taste receptors in the larval mouth area. This causes the larvae to fail to get a taste stimulus so that they are unable to recognise their food, causing them to die of starvation (Nguyen & Widodo, 1999; Cahyadi, 2009). How work of secondary metabolite compounds Saponins are by binding oxygen in water, this is because saponins contain glycosides in plants that resemble soap which can bind oxygen that is dissolved in water so that oxygen levels in the water decrease and can kill larvae. While the function of flavonoid compounds is to reduce the activity of digestive enzymes and food absorption. In addition, it also acts as a stomach poisoning or stomach poison so that A. Salina larvae become starved and die (Yunita et al, 2009). The impact of this metabolic damage is also rapid and can be observed within 24 hours (Mokosuli, 2021). The use of A. salina in the BSLT method is done because A. salina has the same response or stress response as humans in the form of behavioural and physiological responses to environmental stress (Nuralifa et al, 2021). So that this BSLT method has a positive correlation with anticancer potential.

According to Hamidi et al (2014) the toxicity category with the LC50 value parameter, namely if the LC50 value is below 100 (µg/mL) is very toxic, LC50 100-500 (µg/mL) is toxic, 500-1000 (µg/mL) is weakly toxic, and if it is above 1000 (µg/mL) is very weak. The toxicity value (LC50) obtained in this study is 41.65 (µg/mL) which means the toxicity is very strong (Table 3). Secondary metabolite compounds contained in methanol extract of Jewawut (*Setaria italica L.*) are toxic to A. salina Leach larvae due to the content of alkaloid compounds, terpenoids, steroids, saponins, flavonoids and tannins. The regression curve between log [sample] the and probit value to be shown in Figure 2.

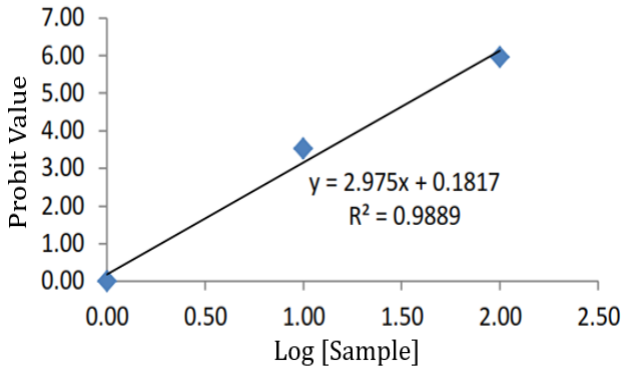

Figure 2. Regression curve between log [sample] and probit value.

Table 3. Toxicity Test Results of Jewawut Methanol Extract with BSLT Method.

Sample	Concentration (µg/mL)	Log Concentration	% Mortality	Probit	LC_{50}
Methanol Extract of Jewawut (*Setaria italica L.*)	1	0	0	0,00	41,65
	10	1	7	3,52	
	100	2	83	5,95	

Conclusion

Based on the results of the research conducted, it can be concluded that the methanol extract of Jewawut (*Setaria italica L.*) contains secondary metabolite compounds such as alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. In addition, the extract also has very weak antioxidant activity with an IC_{50} value of 4104.63 ppm and has potential as an anticancer with a toxicity value (LC50) of 41.65 µg/mL which means the toxicity is very strong. So it is important to do further research at the fractionation stage and compare the results of antioxidant activity and toxicity obtained.

Conflict of Interest

The authors declare that there is no conflict of interest.

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