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The Characterization of Active Compounds in Indonesian Local Rice that is Resistant to Environmental Stress

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Abstract: The purpose of this study was to characterize the active compounds in Indonesian local rice that are resistant to environmental stress in order to identify the nature of resistance to environmental stress, one of the indicators of which is the presence of flavonoid compounds. This step is one of the efforts that will lead to the acquisition of gene donors in the assembly of varieties resistant to biotic stress (pests and diseases) and abiotic stress (drought, high salinity, low temperature), which are very necessary in rice breeding programs. The method used is to plant 8 local rice varieties that are resistant to environmental stress in buckets in greenhouses. Next, analyze the content of active compounds by extracting the leaves and stems of rice plants, then macerate with methanol for 48 hours and test the content of active compounds. Then the total flavonoid content test was carried out using a quercetin standard and measured using a spectrophotometer. Whatever the results of this study, all samples showed active compounds containing flavonoids, alkaloids, tannins and saponins which were indicated by indicators of color change when tested with certain reagents. The results of the Flavonoid content analysis showed that the pendok variety had the highest flavonoid content, namely 3.9253 ± 0.06, and the Sigupai variety had the lowest flavonoid content, with a value of 1.8073 ± 0.07.

Keywords: Active compound; Favonoid; Local rice

Introduction

Local rice is one of the potential germplasm sources for genes that control important traits in rice plants. This is because local rice has evolved over the years, influenced by both natural and artificial selection and migration (Siwi, 1998). The genetic resources of local rice are an asset in rice breeding that must be continuously studied to support the Ministry of Agriculture's program in achieving the Sustainable Development Goals (SDGs) of Food Security and Sustainable Agriculture, as well as the acceleration of self-sufficiency in staple foods and the World Food Barn by 2045.

Local rice has certain advantages as it has been cultivated for generations and has adapted well to various specific climate and land conditions (Hayward, 1993; Sitaresmi, 2013). Some of the benefits of cultivating local rice varieties are that they are easy to obtain and maintain (Nafisah, 2007; Gunawan, 2016), stable, and the quality of the rice is favored by consumers (Iskandar, 2018), as well as resistant to biotic and abiotic environmental stress (Hairmansis, 2015). However, local rice also has some drawbacks, such as long harvest times (Hayward, 1993) and low productivity (Nurnayetty, 2013).

The low productivity of local rice has caused many farmers to abandon these varieties and switch to planting superior varieties with shorter harvest times and higher yields. However, these superior varieties are more sensitive to pest attacks and biotic environmental stress (Supanggat, 2017; Haeria, 2013). This sensitivity to biotic environmental stress in superior rice varieties has made farmers increasingly dependent on synthetic pesticides, which have negative environmental impacts,

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including on humans as rice consumers, livestock as consumers of agricultural waste, and other organisms interacting with rice plants.

A great deal of research and inventory has been conducted on local rice varieties resistant to biotic and abiotic environmental stress. The results of these inventories must undergo systematic review to optimize the use of germplasm for breeding purposes. One way to explore these genetic resources is by identifying the morphological characteristics of local rice, as these morphological traits reflect the plant's adaptation to environmental stress.

The aim of this study is to characterize the active compounds in local Indonesian rice that are resistant to environmental stress, in order to identify the traits related to this resistance. One of the indicators is the production of active compounds in the rice plant. This effort is part of a broader attempt to obtain gene donors for breeding varieties resistant to both biotic (pests and diseases) and abiotic stress (drought, high salinity, low temperatures), which are crucial in rice breeding programs.

Method

The planting of local rice samples was carried out in buckets with soil media and water saturation. The number of samples planted was 18 plants of each variety. The varieties used were 8 local rice varieties resistant to environmental stress, namely Sigupai, Pendok, SOJ 01, Hitam Melik, Pari Wangi, Genjah Arum, Planggini, and Sri Kuning.

The samples, consisting of the leaves and stems of the rice plants, were air-dried and then weighed until a constant weight was achieved. The dried leaves and stems were blended until fine and then sieved to obtain a crude drug (simplisia). This simplisia was then used for testing active compounds.

One gram of the sample powder was soaked in 90 mL of methanol for 3x24 hours, with occasional stirring. The extract solution was then filtered. The extract filtrate was concentrated using a rotary evaporator for about 15 minutes. The concentrated extract obtained was used for qualitative testing of active compounds.

The flavonoid test was conducted by dissolving 1 mL of the sample in 1 mL of 70% ethanol, followed by the addition of 0.1 g of Mg powder and 10 drops of concentrated HCl, and then shaken vigorously. A positive flavonoid test is indicated by the formation of a red, yellow, or orange color (Mainawati et al., 2017).

The alkaloid test was performed by adding 1.5 mL of 2N HCl to 10 mL of the sample solution, heating for 5 minutes, and then filtering. The filtrate was then added with 5 drops of Dragendorff's reagent. A positive alkaloid test is indicated by the formation of an orange/orange precipitate (Mainawati et al., 2017).

The saponin test was carried out by adding 1 mL of the sample to 1 mL of distilled water and shaking for 15 minutes. A positive saponin test is indicated by the presence of stable foam for 5 minutes (Mainawati et al., 2017; Felipe, 2006). The tannin test was performed by diluting 1 mL of the sample with 2 mL of distilled water, followed by the addition of 3 drops of FeCl3 solution. A positive tannin test is indicated by a color change in the solution to bluish-black or greenish-black (Mainawati et al., 2017).

The determination of the maximum wavelength of quercetin was done by running the quercetin solution within a wavelength range of 400-450 nm. The results of this run would indicate the maximum wavelength of the quercetin standard. (Aminah et al., 2017). This maximum wavelength was used to measure the flavonoid absorption of the samples.

The compound used as the standard for determining flavonoid content was quercetin (Haeria, 2018; Ningsih, 2016), as quercetin is a flavonol-type flavonoid that has a keto group at the C-4 atom and hydroxyl groups at the C-3 and C-5 atoms (Aminah et al., 2017). Ten mg of the quercetin standard was weighed and dissolved in 10 mL of methanol to obtain a concentration of 1000 ppm. One mL of the stock solution was pipetted and added to 10 mL of methanol to obtain a concentration of 100 ppm. From the 100 ppm quercetin standard solution, several concentrations were made: 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. For each concentration of the quercetin standard solution, 0.5 mL was pipetted and then added to 1.5 mL of methanol, 0.1 mL of 10% AlCl3 solution, and 0.1 mL of 1M potassium acetate, followed by 2.8 mL of distilled water. The sample was incubated for 30 minutes at room temperature. The absorbance was measured using the UV-Vis spectrophotometric method at the maximum wavelength obtained (Aminah et al., 2017).

Ten mg of simplisia was weighed and dissolved in 10 mL of methanol to obtain a concentration of 1000 ppm. From this solution, 0.5 mL was pipetted, followed by the addition of 1.5 mL of methanol, 0.1 mL of 10% AlCl3 solution, and 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water was added. The sample was incubated for 30 minutes at room temperature. The absorbance was measured using the UV-Vis spectrophotometric method at the maximum wavelength. The samples were prepared in three replications for each analysis, and the average absorbance value was obtained (Aminah et al., 2017). The absorbance values obtained from each extract solution concentration were entered into the regression equation of the quercetin standard solution, resulting in the total flavonoid content, expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

Results and Discussion

Based on the results of the qualitative tests, the rice plant extract was found to contain flavonoids in all samples. The test results showed a color change from green in the leaves and stems to orange/yellow after being treated with Mg-HCl reagent. The yellow or orange color formed indicates that the rice leaves and stems contain flavonoids. This is supported by the research of Mainawati et al. (2017) and Yulianti (2017), who stated that plant extracts contain active flavonoids if, after being treated with Mg-HCl reagent, a red, yellow, or orange color is formed. Robinson's research (1995) showed that a flavonoid test would turn red or orange after adding HCl to the sample extract. HCl in the flavonoid test is used to hydrolyze flavonoids into aglycones, specifically hydrolysis of O-glycosides. Glycosides are replaced by H+ from the acid due to its electrophilic nature. Deglycosylation with Mg and HCl results in a red or orange complex in flavonols, flavones, flavanones, and xanthones. The red color produced indicates the presence of flavonoids due to reduction with concentrated hydrochloric acid and magnesium, forming flavium salts (Achmad, 1986).

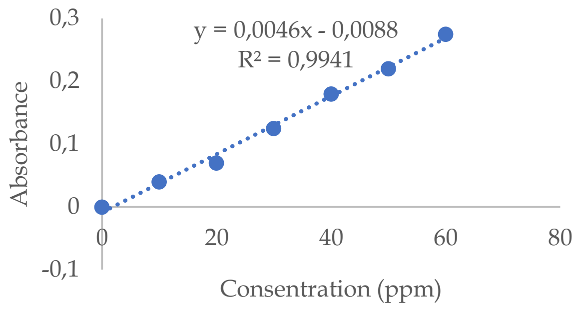
Tabel 1. Qualitative test of phytochemical compounds in several rice varieties

	Flavonoids	Alkaloids	Tannins	Saponins
Possitive Indicators	Yellow, red, orange	Red precipitate	Green, blue	Foam
Sigupai	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)
Hitam melik	Orange (+)	Red precipitate (+)	Light Green (+)	Foam (+)
Planggini	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)
SOJ 01	Yellow (+)	Red precipitate (+)	Dark Green (+)	Foam (+)
Pari wangi	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)
Pendok	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)
Genjah arum	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)
Sri kuning	Yellow (+)	Red precipitate (+)	Green (+)	Foam (+)

The qualitative alkaloid test on the rice plant extract showed that all rice plants contained alkaloids. This was indicated by the formation of a brick-red precipitate after treatment with Dragendorff's reagent. This is supported by the research of Ningsih et al. (2016), which showed that plant extracts contain positive alkaloids if, after being treated with Dragendorff's reagent, a brick-red (brown) to orange precipitate is formed. The principle of using Dragendorff's reagent is that the precipitation reaction occurs due to ligand substitution (Sangi et al., 2012). The nitrogen atom in alkaloids has a lone pair of electrons that can replace the iodine ion in Dragendorff's reagent. The brick-red (brown to orange) precipitate is formed because nitrogen forms a covalent bond with the K+ metal ion. This precipitate is potassium alkaloid (Marliana et al., 2005).

The qualitative analysis of tannins in rice plant samples showed that all tested rice varieties contained active tannin compounds. This was indicated by a color change in the sample extract from green to blue when FeCl3 reagent was added (Sangi, 2012). Tannins are compounds commonly found in vascular plants, containing phenol groups, having astringent properties, and the ability to darken skin due to their cross-linking ability with proteins (Taiz, 2022). When reacting with protein, they form stable, water-insoluble copolymers. Chemically, tannins are grouped into two categories: condensed tannins and hydrolyzable tannins. Condensed or flavolan tannins are thought to form through the condensation of simple catechins into dimer compounds and then higher oligomers (2012). Hydrolyzable tannins contain ester bonds that can be hydrolyzed when boiled in dilute hydrochloric acid (Harborne, 1987).

The saponin analysis showed that all samples contained saponins (Setyorini, 2016). This was indicated by the formation of foam when the green sample extract, which previously had no foam, was shaken after a drop of distilled water was added. Saponins are triterpenoid and sterol glycosides found in more than 90 plant genera. Glycosides are complexes between a reduced sugar (glycon) and a non-sugar (aglycon). Many saponins consist of up to five sugar units, and the common component is glucuronic acid. The presence of saponins in plants is indicated by foam formation during plant extraction or extract concentration (Harborne, 1987; Tan, 2018). Saponins have the ability to hemolyze blood cells, reduce cholesterol levels, and prevent the narrowing of heart blood vessels (arteriosclerosis). Saponins can penetrate the cell walls of some organisms and may be toxic (Illing et al., 2017).



Picture 1. Quercetin Standard Curve

The quercetin standard curve was created to determine the relative flavonoid concentration. Quercetin is a flavonoid derivative (Sa'adah, 2017 and Rahayu, 2003), making it suitable as a standard for measuring quercetin levels in various samples.

Table 2. Total Flavonoid content

Variety Name	Total Flavonoid content
Sigupai	1,8073±0,07
Hitam melik	3,5440±0,07
Planggini	2,7917±0,04
SOJ 01	2,9003±0,05
Pari wangi	3,1637±0,04
Pendok	3,9253±0,06
Genjah arum	2,8317±0,06
Sri kuning	3,3243±0,02

The highest total flavonoid content was found in the Pendok rice plant sample, with a value of 3.9253 ± 0.06, while the lowest was in the Sigupai variety, with a value of 1.8073 ± 0.07. The concentration of flavonoids correlates with the plant's resistance to pest attacks. In this case, active compounds, including flavonoids, are produced by the plant when it is attacked by pests.

Conclusion

From the research conducted, it was found that all the rice plants contained active compounds such as flavonoids, tannins, saponins, and alkaloids. The total flavonoid analysis revealed that the highest total flavonoid content was in the Pendok variety, with a value of 3.9253 ± 0.06, and the lowest was in the Sigupai variety, with a value of 1.8073 ± 0.07.

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Author Contribution
The authors contributed as follows: "Research idea, Tri Ratnasari and Wahyu Indra Duwi Fanata.; methodology, Tri Ratnasari.; software, Ahmad Ilham Tanzil.; validation, Tri Ratnasari, Wahyu Indra Duwi Fanata, Ummi Sholikhah, and Ahmad Ilham Tanzil.; formatting, Tri Ratnasari.; data analysis, Tri Ratnasari.; writing, Tri Ratnasari.; review, Ummi Sholikhah.; supervision, Wahyu Indra Duwi Fanata.; project administration, Ahmad Ilham Tanzil.; funding acquisition, LP2M Universitas Jember."

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