

# Microwave-Assisted Extraction and Identification of $\gamma$ -Oryzanol from Rice Bran (*Oryza sativa* L. cv Ciliwung)

Maryono<sup>1,\*</sup>, Netti Herawati<sup>1</sup>, Meuthia Aulia Farhani Gaffar<sup>1</sup>, Sartini<sup>2</sup>, Aliyah<sup>2</sup>, Elly Wahyudin<sup>2,\*</sup>

Maryono<sup>1,\*</sup>, Netti Herawati<sup>1</sup>,  
Meuthia Aulia Farhani Gaffar<sup>1</sup>,  
Sartini<sup>2</sup>, Aliyah<sup>2</sup>, Elly Wahyudin<sup>2</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences, Makassar State University, Makassar, South Sulawesi, INDONESIA.  
<sup>2</sup>Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, INDONESIA.

## Correspondence

### Maryono

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Makassar State University, Makassar, South Sulawesi, INDONESIA.

Phone no. +6282290862121

E-mail: bintangyono1@gmail.com

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## ABSTRACT

Rice Bran (*Oryza sativa* L. cv Ciliwung) is a by-product of the rice milling process, consisting of an outer layer of rice grains with the whole seed institute. Rice bran oil is a result of rice bran extraction. Rice bran oil contains antioxidant compounds, one of which is oryzanol, so this study aims to determine the levels of or-oryzanol in rice bran. The stages of the research included sample preparation, extraction using Microwave-Assisted Extraction (MAE), followed by Vacuum Liquid Column Chromatography (VLCC) was extracted using the MAE method produced 30 mL of black-brown rice bran oil extract. A total of 8 grams of rice bran extract was obtained at VLCC using eluent (n-hexane: ethyl acetate) with ratios 9:1, 7:3, and 1:1, respectively. Then the five fractions obtained were characterized using HPLC, and GC/MS identified the mass of the  $\gamma$ -oryzanol compound.

**Key words:**  $\gamma$ - Oryzanol, Microwave Assisted Extraction, Rice Bran (*Oryza sativa* L. cv Ciliwung), Secondary Metabolite.

## INTRODUCTION

Rice production in Indonesia has considerable productivity. Every increase in rice production increases. According to the Central Statistics Agency revealed rice production in 2018 was estimated at 80 million tons of Dry Grain (GKG), or an increase of 15 million tons compared to 2011. The rice production conditions that continue to increase like this can trigger a good chance for Indonesia<sup>1</sup>.

On the other hand, the large rice production also results in greater residual yields from grinding rice to rice. The results of this milling produce side products such as groats, broken rice, husks, and bran (rice bran). Bran is a byproduct of the rice milling process, consisting of an outer layer of rice grains with a number of seed institutions.

Rice bran oil or known as rice bran oil is the result of rice bran extraction. Bran oil can be consumed and contains vitamins, antioxidants, and nutrients that the human body needs. Bran oil also contains natural antioxidants tocopherol, tocotrienol, and oryzanol, which are beneficial against free radicals in the body, especially cancer cells, and help reduce cholesterol in blood and liver cholesterol, and inhibit menopause. Therefore, bran oil can be used as a food supplement to improve the quality of human health<sup>2</sup>.

Gamma oryzanol ( $\gamma$ -oryzanol) is a mixture of phytosterols ferulates in rice bran oil. Xu and Godber (1999) found that 24-methylene cycloartanyl ferulate, cycloartanyl ferulate, campesteryl ferulate, beta-sitosterol ferulate, and campesteryl ferulate which had been identified as the main component and were found to have antioxidant activity ten times greater than tocopherol and tocotrienol, which are components of the ferrite<sup>3</sup>. The main of vitamin E. reported that or-oryzanol is not only beneficial in the treatment

of menopausal symptoms, it is also beneficial in reducing cholesterol in the blood, liver and beneficial against free radicals.

In 2009, Siti Zullaikah isolated oryzanol using the 2-stage crystallization method. In this method, Siti Zullaikah isolated oryzanol at a low temperature in the first crystallization stage to reach  $-60^{\circ}\text{C}$  and the yields were relatively high. The content of Oryzanol in Liquid Phase 1 (LP1) produced was 13.68%, with a recovery of 63.34%. While the temperature of  $-22^{\circ}\text{C}$  in the first crystallization stage also obtained oryzanol with a concentration in Liquid Phase 1 (LP1) that is 12.59% with 47.17% recovery, and at the end of the second crystallization phase obtained oryzanol crystals with a purity of 93-95% and recovery of 59%. However, with the results given, the drawback of this method is that the temperature carried out in the first crystallization process is too low, so it is not easy to apply to simple refrigerators<sup>4</sup>.

Therefore, the research conducted by the author at this time is in the form of the development of a method that Siti Zullaikah has carried out by using the soxhlet extraction method<sup>4</sup>. Extraction of  $\gamma$ -oryzanol from rice bran (*Oryza sativa* L. cv Ciliwung) using the Microwave-Assisted Extraction (MAE) method has not been reported. The reason researchers in this study use the MAE method is because it has advantages, one of which is the extract results obtained more than a simple extraction method<sup>5</sup>. Other than that, the time required to do one extraction takes 15-30 minutes. This study aims to apply the MAE method and identify the target compounds of  $\gamma$ -oryzanol from rice bran.

## MATERIALS AND METHODS

### Materials

The sample of Rice bran (*Oryza sativa* L. cv Ciliwung) was achieved by a rice mill located in Barru District, South Sulawesi, Indonesia. The chemical materials

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were used in this study include n-hexane (>99%), ethyl acetate (>99%), and secondary metabolite test reagent were purchased from PT. SmartLab Indonesia, Indonesia. Silica gel (60H), methanol grade HPLC, aqua pro injection grade HPLC, dichloromethane grade HPLC, and acetonitrile grade HPLC were purchased from Sigma-Aldrich (via PT. Elo Karsa, Indonesia). The equipment was utilized, such as rotary evaporator (Buchi), glass column (2.5 x 25 cm), Hight Performance Liquid Chromatography (HPLC) (Shimadzu), and Gas Chromatography/Mass Spectrometer (GC/MS), and the modified domestic microwave (Modena).

### Microwave-assisted extraction process and secondary metabolite screening

The extraction process was performed using the Microwave-Assisted Extraction (MAE) method according to previous studies<sup>6,7</sup>. Prepare a sample of rice bran to be extracted. Filter rice bran to dissolve impurities and other particles using a 100mesh screen. 50-gram rice bran measuring 100 mesh mixed with n-hexane as much as 200 mL and as much as 30 ml ethyl acetate and extracted using Microwave-Assisted Extraction (MAE) for 15 minutes. The extraction results are a mixture of solvent and rice bran pulp and then filtered using a Buchner funnel to separate the solvent and rice bran pulp. In the centrifuge for 10 minutes at 180 rpm, evaporate at 50 °C with 50 rpm to remove the remaining solvent. Furthermore, a preliminary test (group test) was carried out on n-hexane extract obtained with various reagents, including reagents, FeCl<sub>3</sub> 1% (flavonoids), Dragendorff, and Wagner reagents (alkaloids).

### Semipurification of extracted $\gamma$ -oryzanol compound

Semipurification  $\gamma$  -Oryzanol Using Vacuum Liquid Column Chromatography (VLCC). The glass column (2.5 x 25 cm) packed with 55 g silica (grade 60) removes triglycerides and other lipids. Extracted crude oil is dissolved in 100 mL solvent (hexane/ethyl acetate = 9: 1). Then 100 mL of solvent (hexane/ethyl acetate = 7:3) has flowed through the column, and the eluant is collected. The column was then washed with 100 mL hexane/ethyl acetate (1:1), and the purified oryzanol was obtained after the solvent was evaporated.

### Characterization High-Performance Liquid Chromatography (HPLC) and Gas Chromatography/Mass Spectrometer (GC/MS)

The HPLC system was used to record chromatograms of fraction results obtained so that a profile of each fraction appears. Five fractions were characterized using HPLC. The eluent used consisted of methanol, aqua pro injection, dichloromethane, acetonitrile (40: 56: 2: 2%), and the flow rate was 1 mL/min. The injection volume used was 20  $\mu$ L, and the wavelength used was 370 nm. Peak with fraction two selected continued to identify levels of  $\gamma$ -oryzanol compounds using Gas Chromatography/Mass Spectrometer (GC/MS).

## RESULTS

The application of microwave-assisted extraction (MAE) aims to obtain selectively extract compounds from various raw materials. The microwave radiation was applied to heat solvents quickly and efficiently to reduce the extraction time<sup>8</sup>. Microwave power used to extract rice bran crude oil is 380 watts, while the volume of solvent used is 230 ml. The oil extract obtained is yellowish-brown. The yield obtained was 2.404%.

A phytochemical test is a qualitative test to determine the active compound contained in the sample. The secondary metabolite compounds tested were alkaloids and flavonoids. The following results of phytochemical testing of bran extract reagents can be seen in Tables 1 and 2.

**Table 1: Secondary metabolite testing of rice bran extract.**

Secondary Metabolites	Reagent	Observation	result
Alkaloids	Wegner	Brownish / Red Brick Deposition	+
	Dragendorff	Brownish / Red Brick Deposition	+
Flavanoids	FeCl <sub>3</sub>	Yellowish Green	+

**Table 2: Fraction of rice bran extract using Vacuum Liquid Chromatography.**

No. Fraction	Eluent Comparison Hexane-Ethyl	Weight (g)	Information
Fr-1	(9:1)	-	-
Fr-2	(9:1)	4,37	Orange
Fr-3	(7:3)	1,54	Brownish yellow
Fr-4	(7:3)	0,35	Dark green
Fr-5	(1:1)	0,16	Tosca
Fr-6	(1:1)	0,12	Clear Green

The thick extract obtained subsequently at Vacuum Liquid Column Chromatography (VLCC) uses grade 60 silica gel adsorbent as the stationary phase and eluent, which was continuously enhanced in ethyl acetate, and n-hexane as the mobile phase. The results of vacuum liquid chromatography obtained six fractions can be seen in Figure 1. The solvent is then evaporated to dryness and weighed and characterized using HPLC.

The HPLC is a widely accepted separation technique for analyzing certain purification compounds in a sample. This test aims to see the profile of the  $\gamma$ -Oryzanol compound in the sample using HPLC instrument. The mobile phase used is a mixture of methanol, API, dichloromethane, acetonitrile with a ratio of 40: 56: 2: 2. In contrast, the stationary phase used is column C18 which is non-polar and eluting for 45 minutes.

Chemical content analysis of the GC-MS method was carried out to identify levels of compounds contained in the extract of rice bran oil (*Oryza sativa* cv Ciliwung). The stationary phase used is SPB-5 with a column length of 30 meters and an internal diameter of 0.25 mm. While the mobile phase used is Helium with a flow speed of one mL/min. In MS instruments used the electron impact ionizing method with 70 Ev energy. The results of GC-MS testing of n-hexane rice bran extract are expressed in the chromatogram Figure 2. The results of GC-MS testing of n-hexane rice bran extract are expressed in the chromatogram Figure 3.

## DISCUSSION AND CONCLUSION

In MAE, extraction occurs due to changes in cell structure caused by electromagnetic waves, heat transfer, and mass out of parts in irradiated media. In contrast, in conventional extraction, the mass transfer occurs from the inside to the outside even though heat transfer occurs from the outside to the parts in solids<sup>7</sup>. The bran samples were extracted by the MAE method.

As much as 1 kg of bran samples using 4 liters of n-hexane and added with ethyl acetate 600 mL then stirred evenly, n-hexane was chosen because according to research conducted by Nasir *et al.* (2009), n-hexane is a solvent that is widely used and provides a yield of good rice bran oil<sup>9</sup>. The n-hexane extract of rice bran that has been obtained is then evaporated using a rotary evaporator. This is done to remove n-hexane, which is still mixed, which is then obtained by crude rice bran oil of 30 ml (24.0462 grams).

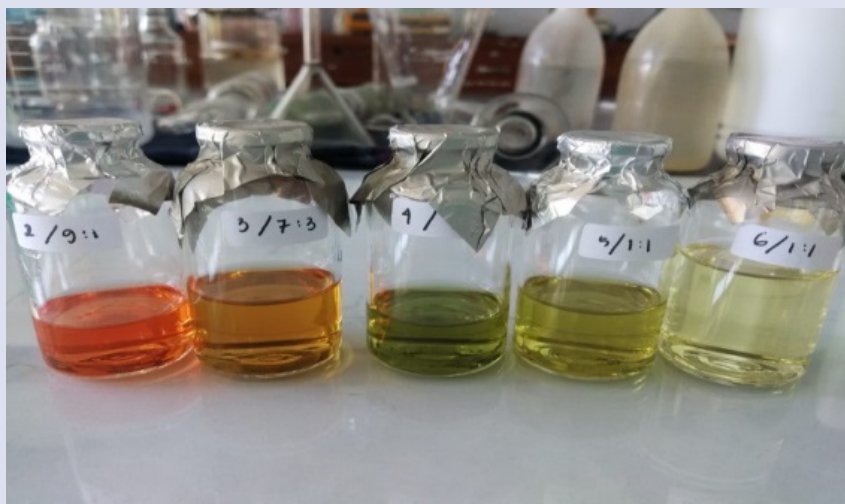


Figure 1: The fraction from rice bran extract.

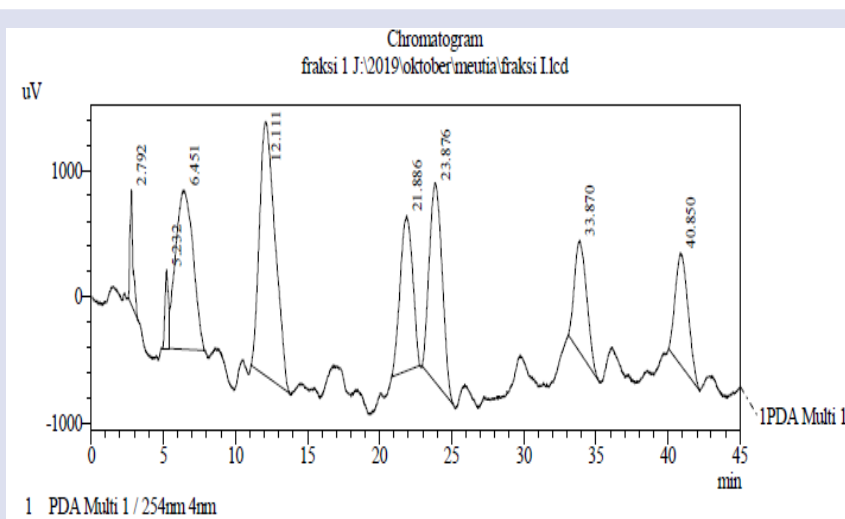


Figure 2: Chromatogram  $\gamma$ -oryzanol fraction 2 (9:1).

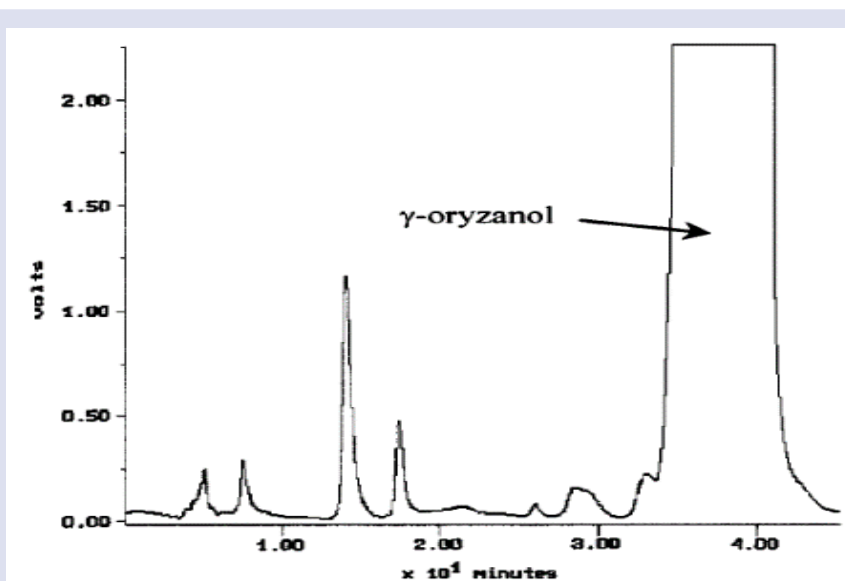


Figure 3: The semi-purified  $\gamma$ -oryzanol chromatogram in normal preparative HPLC phase (column, silica; mobile phase, 4% ethyl acetate in hexane; flow rate, 21.6 mL/min; UV detector, 330 nm).

The calculation shows that the percentage of crude rice bran oil obtained from pressing results obtained 2.404%. The temperature can cause the lack of percent yield produced at the time of extraction, i.e., the optimum temperature that should be used in rice bran extraction is below 100° C. If the temperature used is very high will make the components contained in the bran to be damaged and affect the yield bran oil produced<sup>1</sup>.

Compared with the results of research conducted by (Nasir 2009) on "Extraction of rice bran into crude oil with n-hexane and ethanol solvents" with an extraction time of 1 hour, 2 hours, and 3 hours gives a reasonably high percent yield this has an effect on the quality of the oil produced<sup>9</sup>. In principle, the extraction process of bran must be carried out as soon as possible because the longer the extraction process takes place, the % of oil increases and the higher the content of Free Fatty Acid (FFA) in bran oil. The size of the % FFA can affect the quality of oil, where the higher the % FFA content in oil, the oil will be more difficult to refine and affect the quality of the oil produced<sup>9</sup>. Based on Yulianti (2014), research using cold-pressed extraction yields a yield of 17.055%<sup>10</sup>. Cold pressure or expression are all physical processes in which the essential oil glands on the skin of the fruit are destroyed or damaged to release essential oils. Making essential oils by cold pressure is by pressing without heating, carried out on materials in the form of seeds, fruit, or fruit skin produced from plants, including types of plants that have oil from plant types that will be damaged if made by distillation<sup>11</sup>. So that the most optimum extraction process to produce good yield and quality of rice bran oil is to use cold-pressed extraction.

Based on the observation of phytochemical tests, it is known that n-hexane extract contains alkaloids and flavonoid compounds. Alkaloid compounds tested using Wegner and Dragondroff reagents were characterized by forming a brick-red precipitate. The flavonoid compounds tested using FeCl<sub>3</sub> were marked by the formation of a yellowish-green color. This shows that there is a possibility that the  $\gamma$ -oryzanol compound is present in n-hexane rice bran extract where the  $\gamma$ -oryzanol compound belongs to the flavonoid compound<sup>12</sup>.

The viscous extract obtained later at VLCC uses grade 60 silica gel adsorbent as the stationary phase and eluent, which continues to be improved in the form of ethyl acetate and n-hexane as the mobile phase. The results of vacuum liquid chromatography obtained five fractions. The solvent is then evaporated to dryness and weighed and characterized using HPLC.

This test aims to look at the profile of the O-Oryzanol compound in the sample using the HPLC instrument to be compared with the HPLC chromatogram results of the  $\gamma$ -oryzanol compound that have been examined<sup>3</sup>. The basic principle of HPLC is based on differences in the distribution of components - components in the sample between two phases, the mobile phase and the stationary phase. The mobile phase used is a mixture of methanol, API, dichloromethane, acetonitrile with a ratio of 40:56:2:2. In contrast, the stationary phase used is column C18 which is non-polar and eluting for 45 minutes. Therefore, this test uses the reverse phase HPLC method because the mobile phase used is polar while the stationary phase is non-polar.

Based on the results of the HPLC characterization of the five fractions, the results of the chromatogram in fraction 2 (9:1) have similarities to the results of the chromatogram conducted by Gunaratne et al. (2013)<sup>13</sup>. Where Ret. The time for the  $\gamma$ -oryzanol compound was at 40.850 minutes, with an area of 54810. So that fraction two was continued to the process of determining the levels using GC/MS instruments.

Chemical content analysis of the GC-MS method was carried out to identify levels of compounds contained in rice bran extract. The stationary phase used is SPB-5 with a column length of 30 meters and an internal diameter of 0.25 mm. While the mobile phase used is

Helium with a flow speed of 1 mL/min. In MS instruments used the electron impact ionizing method with 70 Ev energy.

The skinative analysis test of  $\gamma$ -oryzanol compounds using the GC/MS method shows that the positive rice bran contains  $\gamma$ -oryzanol compounds. The quantitative tests using this method show that at 69 m/z molecular weight, clearly visible peak  $\gamma$ -oryzanol compounds have a retention time of 33.050 with an area of 5035 with a concentration of 244.30327 ppm.

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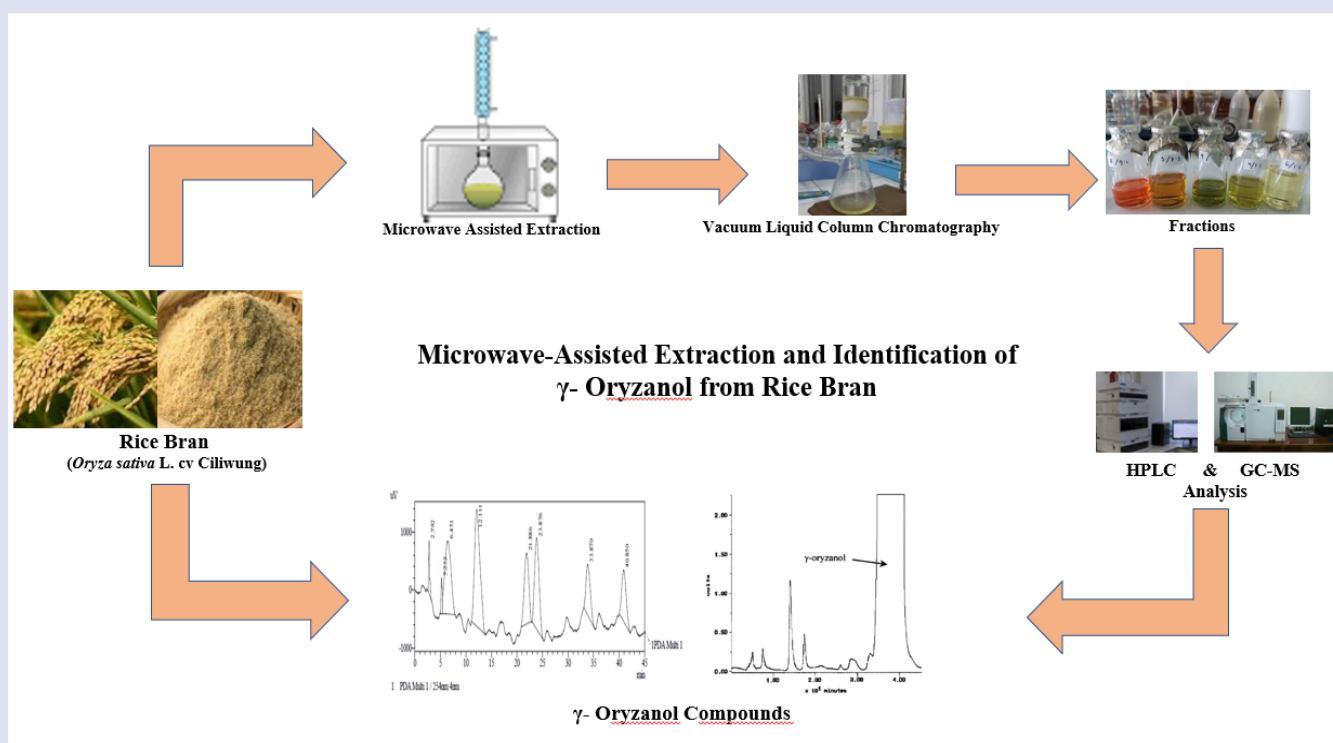
## CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

## REFERENCES

- Hapsari RP, Fikri AY, Zullaikah S, Rachimoellah HM. Isolasi dan Karakterisasi Oryzanol dari Minyak Dedak Padi. Jurnal Teknik Pomits. 2013;1(1): 1-7
- Cho JY, Lee HJ, Kim GA, Kim GD, Lee YS, Shin SC, Park KH, Moon JH. Quantitative analyses of individual  $\gamma$ -oryzanol (steryl ferulates) in conventional and organic brown rice (*Oryza sativa* L.). Journal of Cereal Science. 2012; 55: 337-343.
- Xu H, Godber JS. Antioxidant Activity of Tocopherols, Tocotrienols, and  $\gamma$ -Oryzanol Components from Rice Bran Against Cholesterol Oxidation Accelerated by 2,2'-azobis (2-methylpropionamide) dihydrochloride. Journal Agricultural Food Chem.2000; 49: 2077-2081.
- Zullaikah S, Melwita E, Ju YH. Isolation of oryzanol from crude rice bran oil. Bioresource Technology 2009; 100: 299-302.
- Ahmad I, Yanuar A, Mulia K, Mun'im A. Ionic liquid-based microwave-assisted extraction: Fast and green extraction method of secondary metabolites on medicinal plant. Pharmacognosy Review. 2018; 12(23): 20-26.
- Ahmad I, Yanuar A, Mulia K, Mun'im A. Application of ionic liquid based microwave-assisted extraction of the secondary metabolite from *Peperomia pellucida* (L) Kunth. Pharmacognosy Journal. 2017; 9(2): 227-234.
- Chen Y, Xie MY, Gong XF. Microwave-Assisted Extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. Journal of Food Engineering. 2007; 80: 170-172.
- Aladedunye F, Przybylski R, Rudzinska M, Pawlik DK.  $\Gamma$ -Oryzanol of North American Wild Rice (*Zizania palustris*). Journal of the American Oil Chemists' Society. 2013; 90(8): 1101-1109.
- Nasir S, Fitriyanti, Kamila H. Ekstraksi dedak padi menjadi minyak mentah dedak padi (crude rice bran oil) dengan pelarut n-heksana dan etanol. Jurnal Teknik Kimia 2009; 2(16): 1-6.
- Yulianti D, Susilo B, Yulianingsih R. Pengaruh lama ekstraksi dan konsentrasi pelarut etanol terhadap sifat fisika-kimia ekstrak daun stevia (*Stevia rebaudiana* Bertoni M.) dengan metode microwave-assisted extraction. Jurnal Bioproses Komoditas Tropis. 2014; 2(1): 7-10.
- Ghatak SB, Shital S, Panchal. Antidiabetic activity of oryzanol and its relationship with the antioxidant property. International Journal of Diabetes in Developing Countries. 2012; 32: 185-192.
- Goffman FD, Shannon P, Christine B. Genetic diversity for lipid content and fatty acid profile in rice bran. JAOCS. 2003; 80(5): 485-490.
- Gunaratne A, Wu K, Li D, Bentota A, Corke H, Cai YZ. Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. Food Chemistry. 2013; 138(2-3): 1153-1161.

## GRAPHICAL ABSTRACT



## SUMMARY

- Rice bran oil contains antioxidant compounds, one of which is  $\gamma$ -oryzanol.
- A total of 8 grams of rice bran extract was obtained at KKCW using eluent (n-hexane: ethyl acetate) with ratios 9:1, 7:3, and 1:1, respectively.
- Five fractions obtained were characterized using HPLC, and GC/MS identified the mass of the  $\gamma$ -oryzanol compound.

## ABOUT AUTHORS



**Maryono**, a doctoral candidate at Postgraduate Program, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia. As a lecturer and researcher at the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar, South Sulawesi, Indonesia. Research interest in Natural Products Chemistry.



**Dr. Netti Herawati**, a lecturer and researchers at Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar, South Sulawesi, Indonesia. She has experience in the area of Natural products and Organic Chemistry.



**Meuthia Aulia Farhani Gaffar**, an undergraduate student at Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar, South Sulawesi, Indonesia.



**Dr. Sartini**, a Professor of Pharmaceutical Microbiology and Biotechnology at Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia. She has experience in the area of Pharmaceutical Microbiology and Biotechnology, mainly in infection diseases and pharmaceutical Biotechnology products.



**Dr. Aliyah**, Associate Professor of Pharmaceutical Technology at Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia. She has experience in the area of Pharmaceutical Calculation, Drug Delivery System, and Nano-herbal development.



**Dr. Elly Wahyudin**, a Professor of Pharmacodynamic and Pharmacokinetic at Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia. She has experiences in the area pharmacology of natural product research, mainly Pharmacodynamic and Pharmacokinetic profile of phytocompounds from natural products.

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