Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 10, No. 2, April 2025

Research Paper

Phytochemical Profile, Antioxidant Activity and Anticancer Activity of Gamma-Irradiated Black Rice Bran *(Oryza sativa L.)* Ethanolic Extract: In-Vitro and In-Silico Study

Rizal Maarif Rukmana[1*], Rantika Silfarohana[2], Aditya Dwi Permana Putra[1], Devi Safrina[1], Dian Susanti[1], Nur Rahmawati Wijaya[1], M Bakti Samsu Adi[1], Muhammad Evy Prastiyanto[3], Yanuar Ashari Cahyaningrum[4], Rina Nurmaulawati[4], Weka Sidha Bhagawan[5]

[1]*Research Center for Pharmaceutical Ingredients and Traditional Medicine, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia*
[2]*Research Center for Vaccine and Drug, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia*
[3]*Department of Medical Laboratory Technology, Faculty of Health and Nursing Science, Universitas Muhammadiyah Semarang, Semarang, 50273, Indonesia*
[4]*The College of Health Sciences Bhakti Husada Mulia, Madiun, 63139, Indonesia*
[5]*Department of Pharmacy, Faculty of Health and Sciences, Universitas PGRI Madiun, Madiun, 63118, Indonesia*
***Corresponding author**: riza007@brin.go.id

Abstract
Black rice is a food crop with black pigments, which helps reduce the risk of various diseases and improve health. One way to improve food quality and avoid food contaminants is by irradiating. This study evaluated the phytochemical profile, antioxidant activity, and anticancer activity of irradiated black rice bran extract (IBRBE) in vitro and in silico. Black rice was irradiated with a Gamma cell 220 type irradiator at a 7.5 kGy/hour dose rate. Extraction of irradiated black rice bran was carried out using the maceration method. Phenolic and flavonoid components were quantified using the Folin–Ciocalteau and $AlCl_3$ methods, respectively. Phytochemical compounds were identified by liquid chromatography-high-resolution mass spectrometry (LC−HRMS). The antioxidant activity of IBRBE was carried out against 2,2-diphenyl-1picrylhydazyl (DPPH). The cytotoxic activity of IBRBE against WiDr cells (colorectal cancer) and Vero (nonhuman cell lines) used the MT test method. Prediction of the inhibitory mechanism of compounds in the extract against target proteins EGFR and GPX7 was carried out in silico. Total phenolics and flavonoids were 2.57 ± 0.28 mg GAE/g and 19.12 ± 0.18 μg QE/ml, respectively. Twenty-four types of active compounds were obtained in IBRBE. The results of antioxidant activity obtained an IC_{50} value of 1198.45 ± 92.86 μg/ml. IC_{50} in WiDr cells and Vero cells were obtained at 36.08 ± 11.71 μg/ml and 570.58 ± 130.25 μg/ml, respectively. In silico results, the compound $_4$−Dodecylbenzene sulfonic acid has the highest binding affinity to the EGFR protein, with a value of -5.9. Meanwhile, the Monoolein compound has the highest binding affinity to the GPX7 protein, with a value of -5.4.

Keywords
Anticancer, Antioxidant, Black Rice Bran, In Vitro, In Silico

Received: 19 December 2024, Accepted: 23 March 2025
https://doi.org/10.26554/sti.2025.10.2.628-643

1. INTRODUCTION

Indonesia boasts the world's highest biodiversity, including various fauna, microbes, and plants (Indrayati et al., 2023). Black rice, a recently popular food crop, is particularly noteworthy for its unique seed color and high nutritional value (Nashrurrokhman et al., 2019). Black rice *(Oryza sativa L.)* is rich in a variety of primary metabolites such as carbohydrates, proteins, lipids, and amino acids, making it an excellent nutritional source (Zarei et al., 2018). It contains minerals and fiber and consists mainly of carbohydrates (80%), proteins (7–8%), and fats (3%) (Wisetkomolmat et al., 2022). For certain amino acids, black rice bran types can vary widely whereas some cultivars and metabolic tests have reported as many as 122 amino acids (Zarei et al., 2018). These metabolites mainly serve to provide energy (carbohydrates), assist growth and repair (proteins and amino acids), and help general health by means of beneficial lipids such as tocopherols and tocotrienols, which have antioxidant characteristics (Cherif and Messaouda, 2014). Black rice also contains bioactive compounds accumulated in the aleurone layer, known as rice bran (Yuliana et al., 2020). These compounds include phenolics, flavonoids, alkaloids, triterpenoids, saponins, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol, phytosterols, and polyphenols (Rukmana et al., 2017a). These compounds offer various health benefits, such as antioxidant, anticancer, anti-mutagenic, anti-inflammatory, anti-bacterial, anti-diabetic, anti-cholesterol,

anti-allergenic, and anti-carcinogenic properties (Rukmana et al., 2017b). As a functional food crop, black rice must maintain its quality to ensure the availability of raw materials free from contaminants (Talib et al., 2022).

Gamma-ray radiation is a technique for maintaining food quality and preserving raw materials. Studies show that administering 10 kGy of gamma ray radiation reduces microbial growth but does not affect plant bioactive compounds (Winarno et al., 2019). Radiation of *Andrographis paniculata* simplicia at 7.5 kGy does not reduce its anticancer activity. Methanol extract of *Portulaca oleracea* subjected to 9 kGy gamma ray radiation increases antioxidant and antimicrobial activity. This research also explains the content of phytochemical compounds analyzed by HPLC, revealing 24 active compounds in *Portulaca oleracea* (Santos et al., 2018; Sallam and Anwar, 2017).

Black rice bran contains various phytochemical compounds with health benefits, including antioxidants and anticancer properties. Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol, and phytic acid are the main antioxidants (Talib et al., 2022). Anthocyanin is the dominant compound, while cyanidin and peonidin are potential food additives (Nuriliani et al., 2023). Phenolic and flavonoid groups in black rice bran have been shown to have anticancer activity, inhibiting cancer cells in various organs (Pratiwi and Purwestri, 2017).

An in-silico approach complements in vitro studies of red rice bran by predicting and analyzing intermolecular interactions to evaluate black rice bran extract's bioactivity, reducing time and cost (Mushebenge et al., 2023). This approach optimizes drug candidates, predicts pharmacokinetics and toxicology, reduces animal model dependence, and supports precision medicine (Mohammed et al., 2023; Xu et al., 2024). Molecular docking will determine the inhibitory mechanism of compounds in irradiated black rice bran against EGFR and GPX7 proteins. The Epidermal Growth Factor Receptor (EGFR) and glutathione peroxidase 7 (GPX7) proteins present in humans are expressed in various human tumors. EGFR proteins are abnormally expressed and detected in tumors of the lung, head and neck, colon, pancreas, breast, ovary, bladder and kidney, and gliomas. In addition, excessive abnormal expression of EGFR can lead to cancer prognosis (Yatebe and Yatebe, 2009). Meanwhile, abnormal expression of GPX7 causes the emergence and development of various tumors such as esophageal adenocarcinoma, breast cancer, HCC, and others (Liu et al., 2019). In silico research has attracted many researchers, such as the in silico analysis of phenolic compounds from *Ceriops decandra* Griff. which was conducted in Puspitasari et al. (2023). Since 2009, in silico *Oryza sativa* has been the focus of study Hatorangan et al. (2009). Research has been conducted on many rice variations and protein structures. However, there has been no investigation into the irradiation of black rice (Faustino et al., 2025; Shamshad et al., 2023). Thus, this study investigated gamma-irradiated black rice bran's phytochemical profile, antioxidant activity, and anticancer activity in vitro and in silico.

2. EXPERIMENTAL SECTION

2.1 Materials

Black rice is obtained from black rice farmers in Bogor, West Java, Indonesia. The colon cancer cells used were the WiDR cell line model (ATCC@CCL-218^{TM}), and the normal cells used were Vero cells (ATCC@CCL-81^{TM}) obtained from the Laboratory of Traditional Medicine Raw Materials, National Research and Innovation Agency (BRIN) Tawangmangu, Central Java, Indonesia. Filter paper, methanol (PA), ethanol (96%), distilled water, chloroform, and $AlCl_3$ were obtained from Merck, Darmstadt, Germany. Quercetin hydrate reagent, gallic acid reagent, sodium carbonate (Na_2CO_3), Folin-Ciocalteu Reagent (FCR), 2,2-diphenyl-1picrylhydazyl (DPPH) were purchased from Sigma Aldrich Singapore. Cell growth media and cytotoxicity tests include Roswell Park Memorial Institute 1640 (RPMI 1640), Dulbecco's Modified Eagle Medium (DMEM) media powder, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), Sodium Bicarbonate, Fetal Bovine Serum (FBS) 10%, Penicillin-Streptomycin, Trypsin EDTA 0.25%, Phosphate Buffer Saline (PBS), MTT reagent (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), So dium Dodecyl Sulfate (SDS) 10%, HCl 0.01 N, Dimethyl sulfoxide (DMSO) purchased from Sigma Aldrich Singapore.

2.2 Methods

2.2.1 Black Rice Radiation

Black rice with broken skin weighed 2.5 kg and was then put in plastic and taped. The broken black rice was irradiated with gamma rays using a Gamma cell 220-type irradiator with a 7.5 kGy/hour dose rate. After irradiation, the black rice with broken skin was stored for 14 days at 20°C (Winarno et al., 2019).

2.2.2 Black Rice Extraction

Black rice with broken skin is sanded to obtain the aleurone layer of the seed (rice bran). Black rice bran is dried in the oven at 35-40°C until the weight is stable, then sieved using a 40 mesh sieve (Maharani et al., 2024). The water content value was calculated using a moisture analyzer for black rice bran resulting from a 40-mesh sieve. 100 g of rice bran was mixed into 96% ethanol solvent and macerated for 3×24 hours while stirring occasionally. The filtrate is collected and filtered using filter paper. The filtered filtrate is then evaporated in an oven at 35-40°C, and the weight is calculated (Rukmana et al., 2023).

2.2.3 Total Phenol Analysis

Total phenol measurement was carried out using a standard solution of gallic acid. The standard curve was prepared by making a series of gallic acid concentrations of 4 ppm, 8 ppm, 12 ppm, 16 ppm, and 20 ppm. A total of 20 μL of standard solution and 100 μL of Folin-Ciocalteu Reagent (FCR) were mixed homogeneously in a 96-well plate and incubated at room temperature for 5 minutes. An 80 μL of sodium carbonate

© 2025 The Authors.

Table 1. Results of the Extraction of Irradiated Black Rice Bran

Sample	Yield	Organoleptic characteristics of the extract		
		shape	color	odor
Irradiated black rice bran	17.41 %	paste	Blackish purple typical	odorless

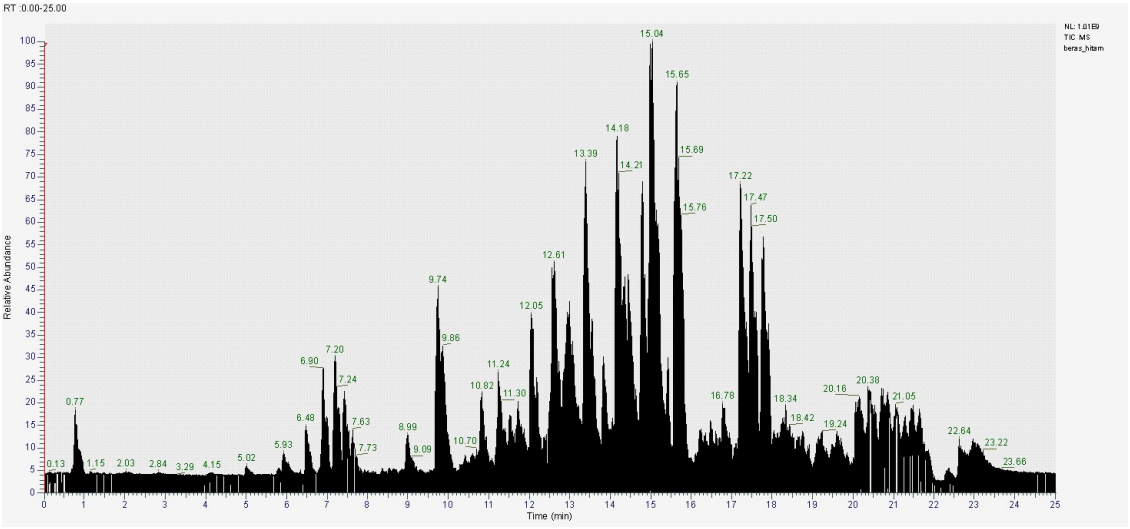


Figure 1. Total Ion Chromatogram (TIC) of the Irradiated Black Rice Bran Extract

solution was added to the mixture and incubated for 120 minutes at room temperature. Then, the absorbance measurement was carried out at 720 nm, and the absorbance results obtained were made into a linear regression equation for the standard curve. IBRBE in 96% methanol was taken at 20 μL, and 100 μL of FCR was mixed homogeneously in a 96-well plate. Then, incubation was carried out at room temperature for 5 minutes. 80 μL of sodium carbonate solution was added to the mixture and incubated for 120 minutes at room temperature. The extract was measured for absorbance at a wavelength of 720 nm, and the quantitative value of phenol was calculated using the linear regression equation of the gallic acid standard curve (Widodo et al., 2019; Vardhani et al., 2024).

2.2.4 Identification of Compounds with Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)

The extracts' phytochemical composition was evaluated utilizing the non-targeted mode of the Q-Exactive Quadrupole Orbitrap^{TM} Mass Spectrometer instrument (ThermoScientific®). The extract was solubilized in 50 μg/mL MS-grade methanol and subsequently filtered using a 0.22 μm filter. The device was configured in the following manner (Windarsih et al., 2022). The mobile phases consisted of (A) MS-grade water with 0.1% formic acid and (B) MS-grade methanol with 0.1% formic acid; the injection volume was 5 μL, and the flow rate was 0.3 mL/min. The gradient protocol was structured as follows: 0 to 16 minutes (B 5% to 90%), 16 to 20 minutes (B 90%), and 20.1 to 25 minutes (B 5%). The ion source utilized was the 3.62 kV ESI positive mode. Subsequently, the unprocessed chromatograms were examined utilizing Compound Discoverer 3.2 software (ThermoScientific®) (Sholikhah et al., 2024), referencing both local and internet databases, including MzCloud and Predicted Compositions.

2.2.5 Antioxidant Activity of IBRBE

The DPPH (2,2-diphenyl-1picrylhydazyl) method carried out the antioxidant activity test. A stock DPPH solution was prepared by weighing 10 mg DPPH and dissolving it in 1 ml methanol. The DPPH solution was then made into a concentration series of 2000 ppm, 1600 ppm, 1200 ppm, 800 ppm, 400 ppm, and 0 ppm. This concentration series was used as a DPPH control, and the absorbance value was measured at a wavelength of 517 nm. IBRBE was made into a stock of 10,000 ppm by weighing 100 mg of the extract and dissolving it with 10 ml of methanol. Next, concentration variations were made at 2000 ppm, 1600 ppm, 1200 ppm, 800 ppm, 400 ppm, and 0 ppm. Each sample concentration was taken as 100 μl and added to the DPPH solution according to the concentration with a volume of 200 μl. The extract was then calculated for its absorbance value at a wavelength of 517 nm ((Riyana et al., 2022). The antioxidant activity was estimated by calculating the percent inhibition value of the extract and creating a linear regression equation so that the IC_{50} value could be obtained (Rao et al., 2019).

© 2025 The Authors.

Table 2. Results of Compound Identification from IBRBE by LC-HRMS

Name	Formula	Retention Time (min)	Sum of Abundance (%)
Ethyl palmitoleate	$C_{18}H_{34}O_2$	15.64	9.94
Monoolein	$C_{21}H_{40}O_4$	29.75	7.88
Stearic acid	$C_{18}H_{36}O_2$	17.49	7.81
1-Linoleoyl glycerol	$C_{21}H_{38}O_4$	28.51	7.37
Ethyl oleate	$C_{20}H_{38}O_2$	34.28	7.13
α-Eleostearic acid	$C_{18}H_{30}O_2$	31.80	6.64
α-Linolenic acid	$C_{18}H_{30}O_2$	29.12	3.08
Dihexyl adipate	$C_{18}H_{34}O_4$	11.25	1.86
Erucamide	$C_{22}H_{43}NO$	33.77	1.37
1-Stearoylglycerol	$C_{21}H_{42}O_4$	31.01	1.28
Ethyl myristate	$C_{16}H_{32}O_2$	49.33	1.26
2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	9.85	1.09
Oleamide	$C_{18}H_{35}NO$	10.43	0.91
1,2,3,4-Tetramethyl-1,3-cyclopentadiene	C_9H_{14}	15.04	0.84
Linolenic acid ethyl ester	$C_{20}H_{34}O_2$	31.51	0.80
Choline	$C_5H_{13}NO$	0.77	0.79
Linoleoyl ethanolamide	$C_{20}H_{37}NO_2$	13.54	0.43
Citral	$C_{10}H_{16}O$	30.69	0.32
Jasmone	$C_{11}H_{16}O$	15.04	0.31
Hexadecanamide	$C_{16}H_{33}NO$	14.41	0.31
Oleoyl ethanolamide	$C_{20}H_{39}NO_2$	16.30	0.29
Palmitoleic acid	$C_{16}H_{30}O_2$	14.64	0.24
Palmitoyl ethanolamide	$C_{18}H_{37}NO_2$	16.93	0.22
4-Dodecylbenzene sulfonic acid	$C_{18}H_{30}O_3S$	8.99	0.21

2.2.6 Cytotoxic Activity using the MTT Assay Method

WiDr cells were removed from the cryogenic tube and cultured in RPMI media. Meanwhile, Vero cells were cultured in DMEM media. Cells transferred to the respective media are stored in a 5% CO_2 incubator at 37°C (Seran et al., 2020). Cells reaching 80-90% confluency are ready to be harvested and used for treatment (Djamal et al., 2020). WiDr cells and Vero cells were counted and placed in a 96-well microplate (each well 100 μl) with 104 cells/mL cell density. The stock of radiation black rice bran extract was prepared by weighing 10 mg of extract, dissolving it in 50 μl DMSO, and adding a complete medium to a volume of 10 ml. The extract stock was then made into a concentration series of 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml, 15.625 μg/ml and used for treatment. Cells that have been treated are incubated in a CO_2 incubator at 37°C for 24 hours. The treatment medium was discarded after 24 hours and replaced with MTT solution (sterile stock solution 5 mg/ml) added to the cell medium at a final concentration of up to 100 μg/ml. This solution was incubated for 4-6 hours at 37°C in a 5% CO_2 incubator. The MTT reaction was stopped with stopper reagent (10% SDS in 0.01 N HCl) and then incubated overnight at room temperature. Test results were measured at 595 nm (microplate ELISA reader) (Prastiyanto et al., 2020). The percentage of cell viability can be calculated from the absorbance value using equations 1 (Widiyastuti et al., 2018, 2019).

$$\text{Cell Viability}(\%) = \frac{\text{Sample absorbance} - \text{Media control absorbance}}{\text{Cell control absorbance} - \text{Media control absorbance}} \times 100\% \quad (1)$$

The obtained IC_{50} values of T47D cells and IC_{50} of Vero cells were then calculated for their selectivity index. The selectivity index value can be calculated using the equations 2 (Yusuf et al., 2022).

$$\text{Selectivity index} = \frac{IC_{50} \text{ of Vero cell}}{IC_{50} \text{ of cancer cell}} \quad (2)$$

© 2025 The Authors.

Table 3. Results of Antioxidant Activity of Irradiated Black Rice Bran Extract

Sample	IC_{50}			Average of IC_{50} (µg/ml)
	1	2	3	
Irradiated Black Rice Bran Extract	1258.61	1245.24	1091.50	1198.45±92.86*

2.2.7 In Silico Evaluation of Potential Compounds from Black Rice Bran Extract Targeting EGFR and GPX7

A bioavailability prediction was employed to assess and visualize the pharmacokinetic characteristics of compounds derived from irradiated black rice bran. This analysis utilized the SwissADME Predictor (http://www.swissadme.ch/), incorporating lipophilicity, size, polarity, solubility, saturation, and flexibility. Subsequently, the SwissADME Predictor also provided physicochemical data for all constituents. The collected data encompassed molecular weight (g/mol), number of rotatable bonds, H-bond acceptors, H-bond donors, topological polarity, MLOGP, and Lipinski violations ((Gonzales et al., 2023).

Toxicity analysis is indispensable for identifying and developing novel drugs, particularly from naturally active compounds. This analysis predicted the toxicity effects of the phytochemical utilizing the pkCSM pharmacokinetics (https://biosig.\lab.uq.edu.au/pkcsm/prediction). Both bioavailability radar and toxicity analysis were conducted by inputting the SMILES code for each compound and predicting its properties based on numerous parameters within the application. Parameters in toxicity analysis included AMES Toxicity, maximum tolerated dose (human) (log mg/kg/day), hERG 2 inhibitor, hepatotoxicity, and skin sensation (Cacace et al., 2021; Izatunnafis et al., 2023).

Twenty-four compounds derived from irradiated black rice bran were subsequently subjected to molecular docking simulations, targeting EGFR and GPX7 as their respective receptors. All three-dimensional structures of the compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/), with those not available in the database being generated using Avogadro 1.2.0. Concurrently, the receptors were obtained from PDB (https://www.rcsb.org/), with EGFR (PDB ID: 1M17) at a resolution of 2.60 Åand GPX7 (PDB ID: 2P31) at a resolution of 2.00 Å. The molecular docking process was performed using Chimera UCSF integrated with Autodock Vina. This analysis encompasses ligand and receptor preparation, including solvent removal, hydrogen addition, and charge assignment, followed by docking. LigPlot facilitated the two-dimensional visualization of residue interactions, while the three-dimensional visualizations were generated using PyMol 3.1.3 (Gonzales et al., 2023).

2.2.8 Analysis of data

Data from LC-HRMS analysis were determined using XCalibur 4.4 software with Predicted Compositions and mzCloud Search MS/MS library. The antioxidant test result data were normalized using the Shapiro-Wilk test. The cytotoxic test result data were analyzed statistically using one-way ANOVA with a significance level of 95%; if there was a significant difference, then continued using the post hoc Tukey HSD test. Data analysis was performed using IBM statistic 30 (Gondokesumo et al., 2025).

3. RESULTS AND DISCUSSION

3.1 Yields of Extraction Sample

The results of the extraction of IBRBE, including the yield weight and the organoleptic characteristics of the extract, can be seen in Table 1. Extraction is an important step to obtain phytochemical compounds in a plant. One of the characteristics that can be observed when carrying out extraction is the organoleptic properties of the extract and the extract yield. High extract yield is one indicator of the success of the extraction technique. The results of previous studies have shown that a high percentage of extract yield can increase the diversity of chemical compounds from the extract (Prastiyanto et al., 2020).

3.2 Total Phenol and Flavonoid

The total phenols in the IBRBE were measured to be 2.57 ± 0.28 mg GAE/g. Polyphenols can scavenge free radicals, exhibit anti-inflammatory properties, and reduce oxidative stress. Consequently, they can play a significant role in the human diet by avoiding neurological disorders, cancer, and other diseases. In the realm of plants, they have a crucial role in fostering resistance against diseases, promoting growth, enhancing pigmentation, and facilitating reproduction. Polyphenols provide a protective effect against bacterial and viral infections (Milella et al., 2023).

The total flavonoids from irradiated black rice bran extract were calculated using quercetin as a standard. The results of the calculation of total flavonoids from IBRBE obtained a value of 19.12 ± 0.18 μg QE/ml. Flavonoids are secondary metabolites of the polyphenol group widely found in various plants. They have various pharmacological activities, including those against immunological disorders, cardiovascular disease, antioxidants, and cancer. Flavonoids can directly eliminate reactive oxygen species (ROS) and bind to metal ions because they can stabilize free radicals through the presence of phenolic hydroxyl groups. Flavonoids' indirect antioxidant effects are associated with the activation of antioxidant enzymes, inhibition of pro-oxidant enzymes, and promotion of the synthesis of antioxidant enzymes and phase II detoxifying enzymes. Both antioxidants and pro-oxidants have a role in flavonoids' anticancer impact

© 2025 The Authors.

(Kopustinskiene et al., 2020). As an anticancer agent, flavonoids can inhibit cancer cell proliferation, cell cycle arrest, metastasis, apoptosis induction, and autophagy (Mir et al., 2024).

3.3 LC-HRMS Profiling Analysis

Compounds from analysis with LC-HRMS were determined using XCalibur 4.4 software with Predicted Compositions and mzCloud Search MS/MS library. The results of the analysis of black rice bran extract irradiated with LC-HRMS can be seen in Table 2. A total of 24 active components have been identified in the IBRBE. This demonstrates that the black rice bran extract exposed to radiation retains various chemical components. LC-HRMS analysis is an untargeted compound analysis that detects all compounds in the sample. Figure 1 displays the irradiated black rice bran extract's total ion chromatogram (TIC).

The LC-HRMS method enables the identification of a wide range of compounds that exhibit a variety of different biological activities. According to this research, twelve chemical substances have a concentration greater than one percent. These 12 compounds are the prevailing chemicals in IBRBE. From a pharmacological perspective, these chemicals are crucial in promoting and maintaining good health. The alpha-linolenic acid compound is one of the potential fatty acids that can be used as an anticancer. The alpha-linolenic acid compound can reduce cell viability, inhibit metastasis, induce apoptosis, and arrest the cell cycle in breast cancer cells (Huang et al., 2022). Another compound with the potential as an anticancer and antioxidant is alpha-eleostearic acid. Previous research showed that the compound α-eleostearic acid has anti-breast cancer activity through several mechanisms, including apoptosis induction, cell cycle arrest, decreased cell viability (cytotoxicity), and reduced cell proliferation. In addition, administration of α-eleostearic acid can increase the expression of various proteins related to apoptosis induction, such as PPARy, p21, Bax, p53, and caspase-3 proteins (Zhang et al., 2012).

3.4 Antioxidant and Anticancer Activity of IBRBE

IBRBE was tested for antioxidant activity using the DPPH method. The results of the antioxidant activity of IBRBE can be seen in Table 3. Based on the Shapiro-Wilk test, the significance value is 0.138 with a p-value>0,05. It shows the data distributed normally. This study showed that IBRBE's antioxidant activity was very weak. The IC_{50} value reached 1198.45 ± 92.86 μg/ml. The IC_{50} value category is classified as highly potent if the IC_{50} value is less than 10 μg/ml, strong if it is between 10 and 50 μg/ml, moderate if it ranges from 50 to 100 μg/ml, weak if it is between 100 and 250 μg/ml, and not active if it exceeds 250 μg/ml. Several factors can affect antioxidant activity, including plant varieties and geographical conditions (Reviana et al., 2021). Previous studies showed that the black rice variety 'Cempo Ireng' irradiated has very strong antioxidant activity and is more potent than the unirradiated. The black rice variety 'Cempo Ireng' is grown in Yogyakarta, Indonesia (Suryanti et al., 2020).

IBRBE was tested for cytotoxic activity against WiDr and Vero cells. The IBRBE showed higher cytotoxic activity on WiDr colon cancer cells and lower cytotoxic activity on normal Vero cells. The results of the cytotoxic test of irradiated black rice bran extract can be seen in Table 4. The results of the one-way ANOVA test stated that the IC_{50} of each treatment of the test cells had a P value <0.001 or less than 0.05, so each treatment was stated to be significantly different. The further test (post hoc test) showed that the IBRBE treatment of Vero cells had a P value <0.05, indicating that this treatment was significantly different from other treatments. Meanwhile, the administration of IBRBE to WiDr cells was not significantly different from the administration of doxorubicin because the P value was>0.05. The National Cancer Institute (NCI) of the United States classifies the cytotoxicity of a compound based on its IC50 value. A compound is considered to have high cytotoxic activity if its IC_{50} is less than 20 μg/mL, moderate cytotoxic activity if its IC_{50} ranges between 21-200 μg/mL, weak cytotoxic activity if its IC_{50} ranges between 201-500 μg/mL, and no cytotoxic activity if its IC_{50} is greater than 500 μg/mL (Damasuri et al., 2020).

The IC_{50} value of WiDr cells and Vero cells can be used to calculate the selectivity index value of IBRBE. The calculated selectivity index for the IBRBE is 15.81. Therefore, IBRBE is specifically employed and exhibits cytotoxicity towards WiDr cells while deemed harmless for normal cells (Vero cells). The results of this study indicate that IBRBE has cytotoxic activity against WiDr cells in the moderate category, with an IC_{50} value of 36.08 ± 11.71 μg/ml. This indicates that IBRBE has potential as an anticancer. Previous research results showed that ethanolic extracts of black rice bran varieties 'Cempo Ireng,' 'Woja Laka,' and 'Toraja' have cytotoxic activity against HepG2 cells with IC_{50} values of 1494.47 ± 87.81, 857.23 ± 99.19, 1896.55 ± 83.80 μg/ml, respectively. In addition, ethanolic extracts of black rice bran varieties 'Cempo Ireng,' 'Woja Laka', and 'Toraja' have cytotoxic activity against Raji cells with IC_{50} values of 1874.14 ± 169.56, 1295.20 ± 37.00, 1232.07 ± 165.51 µg/ml, respectively (Rukmana et al., 2017b). Prior studies have demonstrated that exposing *Curcuma zanthorrhiza* extract to gamma rays at a dosage of 10 kGy reduces the antiproliferative effects of F3 on lymphoma HUT78 cancer cells, A549 lung cancer cells, HeLa cervical cancer cells, and THP1 mouse leukemia cancer cells by 32%, 48%, 42%, and 31% respectively (Winarno et al., 2019).

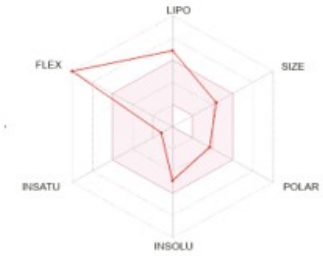
3.5 ADME, Physicochemical Properties, and Toxicity Prediction

Multiple chemicals discovered in irradiated black rice bran were evaluated using ADME to ascertain their potential as therapeutic candidates based on physicochemical features. This prediction was conducted with a bioavailability radar that illustrates their resemblance to medication compounds. A molecule is considered optimum if many measured parameters fall within the ideal range (Bakchi et al., 2022). This polygonal radar illustrates a chemical's probable oral bioavailability.

The analyzed physicochemical property parameters in-

© 2025 The Authors.

Ethyl palmitoleate



Monoolein

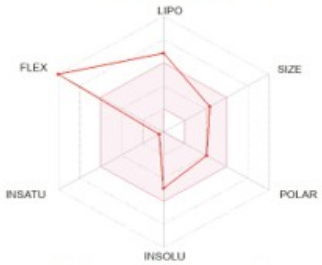
Stearic acid

1-Linoleoyl glycerol

Ethyl oleate

Dihexyl adipate

Erucamide



1-Stearoylglycerol

Ethyl myristate

2-Amino-1,3,4-octadecanetriol

Oleamide

1,2,3,4-Tetramethyl-1,3-cyclopentadiene

Linolenic acid ethyl ester

Choline

Linoleoyl ethanolamide

© 2025 The Authors.

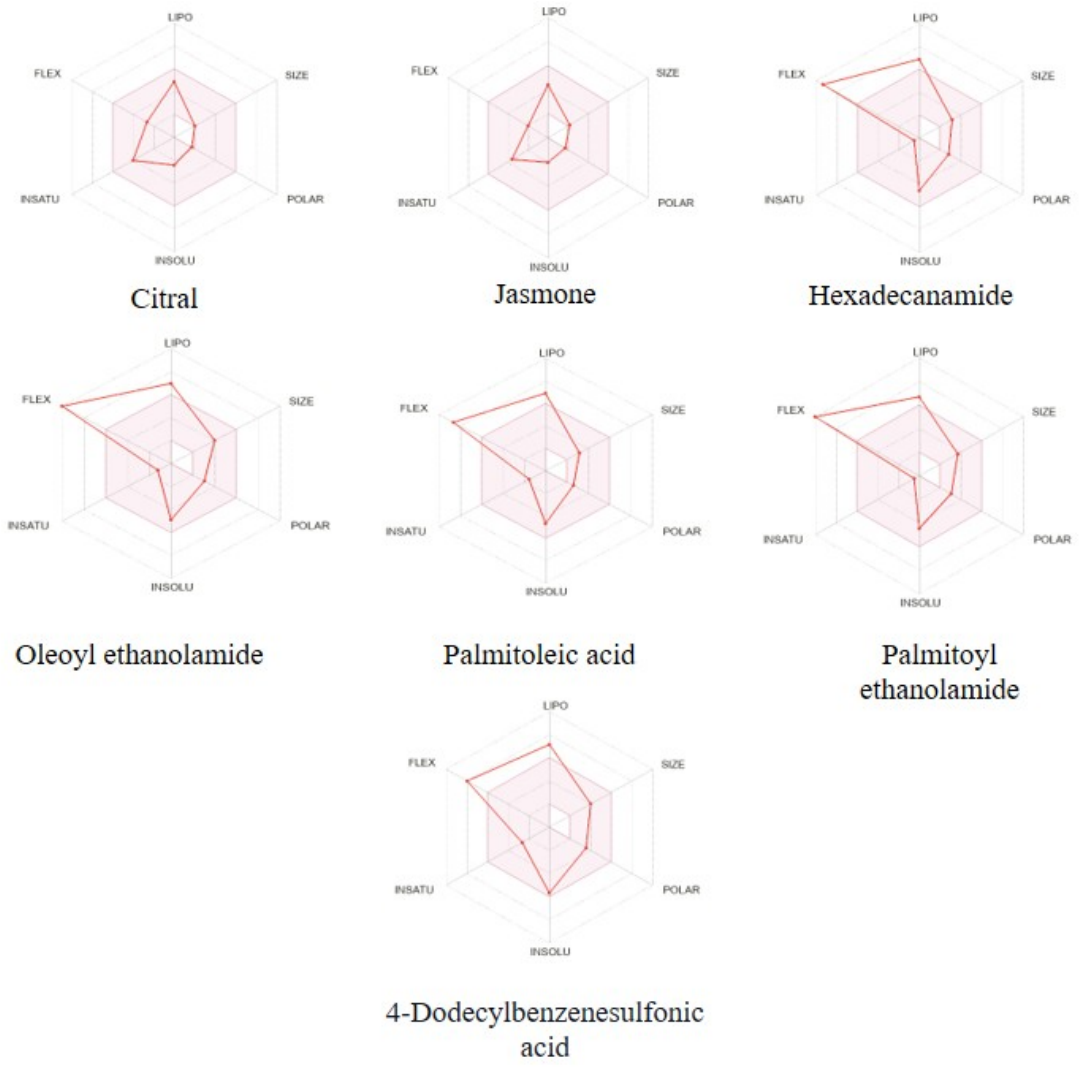


Figure 2. Identified Compounds from Irradiated Black Rice Bran Extract Were Assessed for Drug-Likeness Using the SwissADME Bioavailability Radar

cluded FLEX, which reflects the flexibility of a molecule characterized by the number of rotatable bonds (≤ 9 bonds); SIZE, which pertains to the molecular weight (150-500 g/mol); POLAR, indicating the polarity of the molecule as observed from the topological surface area (20-130 Å^2); INSOLU, which denotes the solubility of the molecule in water based on the LogS value (≤6); and INSATU, representing the saturation of the carbon fraction in sp^3 hybridization, specifically ≤0.25 (Daina et al., 2017). The findings from the radar bioavailability analysis of compounds in irradiated black rice bran indicate that only a limited number of compounds satisfy the criteria for molecules with potential oral bioavailability. These include 1,2,3,4-Tetramethyl-1,3-cyclopentadiene, choline, citral, and jasmone (Figure 2).

In many of studies, the prospective drug candidates discerned through molecular docking simulations are those compounds that demonstrate superior docking characteristics, taking into account their binding affinity. It is crucial to evaluate both the pharmacokinetic characteristics and the physicochemical attributes in the endeavor of identifying alternative pharmaceuticals. The physicochemical parameters anticipated to substantially influence a molecule's efficacy, safety, or metabolism were assessed for all the designed compounds in accordance with Lipinski's rule of five (Olasupo et al., 2021).

Lipinski's rule of five (Ro5) serves as a methodical approach, both computational and experimental, to assess compounds' pharmacological efficacy alongside their membrane permeability and solubility in water and lipids during the drug discovery process (Lipinski et al., 2012). The comprehensive assessment of all compounds obtained from the irradiated black rice bran is meticulously presented in Table 5. The findings indicated that seven compounds contravene Lipinski's rule of five, including

© 2025 The Authors.

Table 4. Results of the Cytotoxic Activity Test of Irradiated Black Rice Bran Extract

Sample	Cell line type	IC_{50} 1	IC_{50} 2	IC_{50} 3	Average of IC_{50} (µg/ml)
Irradiated black rice bran extract	WiDr	48.48	25.19	34.59	36.08 ± 11.71^{a}
Doxorubicin	WiDr	1.50	0.67	2.61	1.59 ± 0.96^{a}
Irradiated black rice bran extract	Vero	438.50	574.30	698.94	570.58 ± 130.25^{b}

^{a,b} shows that there are significant differences between treatments.

Table 5. Predicted Physicochemical Properties of the Chemicals from Irradiated Black Rice Bran Extract

Compounds	Molecular Weight (g/mol)	No of Rotatable Bonds	No of H-bond Acceptors	No of H-bond Donors	Topological Polar Surface Area (Å)	MLOGP	Lipinski Violations
Ethyl palmitoleate	282.46	15	2	0	26.30	4.57	1
Monoolein	356.54	19	4	2	66.76	3.52	0
Stearic acid	284.48	18	2	1	37.30	4.67	1
1-Linoleoyl glycerol	354.52	18	4	2	-	-	-
Ethyl oleate	310.51	17	2	0	26.30	5.03	1
α-Eleostearic acid	-	-	-	-	-	-	-
α-Linolenic acid	-	-	-	-	-	-	-
Dibutyl adipate	314.46	17	4	0	52.60	3.65	0
Eucarvone	357.58	19	1	0	77.76	5.06	1
1-Stearoylglycerol	358.56	18	4	2	-	2.81	0
Ethyl myristate	256.42	14	2	0	26.30	4.19	1
2-Amino-1,3,4-octadecanetriol	-	-	-	-	-	-	-
Oleamide	281.45	15	1	1	-	4.16	1
1,2,3,4-Tetramethyl-1,3-cyclopentadiene	122.21	0	0	0	2.96	-	0
Linolenic acid ethyl ester	-	-	-	-	-	-	-
Choline	104.87	3	1	2	20.03	-3.46	0
Linoleoyl ethanolamide	323.51	17	2	2	49.33	2.49	0
Citral	-	-	-	-	-	-	-
Jasmine	164.24	3	1	0	17.07	2.39	0
Hexadecanamide	-	-	-	-	-	-	-
Oleoyl ethanolamide	325.53	18	2	2	49.33	3.74	0
Palmitoleic acid	254.41	13	2	1	37.30	4.94	0
Palmitoyl ethanolamide	317.53	17	2	2	49.33	3.39	0
4-Dodecylbenzenesulfonic acid	326.49	12	3	1	62.75	4.49	1

ethyl palmitoleate, stearic acid, ethyl oleate, erucamide, ethyl myristate, oleamide, and 4-dodecylbenzene sulfonic acid. A compound that violates the Ro5 can diminish oral bioavailability (Roskoski, 2023).

The results of the toxicity prediction analysis of irradiated black rice bran compounds can be seen in Table 6. Several parameters used in the toxicity analysis include AMES toxicity, maximal tolerated dose, hERG 2 inhibitor, hepatotoxicity, and skin sensitization. The AMES toxicity parameter indicates that the compound can cause mutations in the DNA structure. At the same time, the maximal tolerated dose is the maximum dose of a compound that the human body can still accept. The hERG 2 inhibitor parameter indicates that the compound has the potential to become a channel blocker in the heart, thus disrupting the heart's rhythm. In contrast, hepatotoxicity indicates that the compound can damage the liver. The skin sensitization parameter indicates that the compound can cause skin allergies (Rahman et al., 2023). The results of the toxicity

© 2025 The Authors.

Table 6. In Silico Toxicity Profiles from Irradiated Black Rice Bran Extract

Compounds	AMES Toxicity	Max Tolerated Dose (Human) (log mg/kg/day)	hERG 2 Inhibitor	Hepatotoxicity	Skin Sensitisation
Ethyl palmitoleate	No	0.143	No	No	Yes
Monoolein	No	0.141	No	No	Yes
Stearic acid	No	-0.791	No	No	Yes
1-Linoleoyl glycerol	No	0.09	No	No	Yes
Ethyl oleate	No	0.07	No	No	Yes
α-Eleostearic acid	-	-	-	-	-
α-Linolenic acid	-	-	-	-	-
Dibutyl adipate	No	0.7	No	No	Yes
Eucarvone	No	-0.469	No	No	Yes
1-Stearoylglycerol	No	-0.263	No	No	Yes
Ethyl myristate	No	0.261	No	No	Yes
2-Amino-1,3,4-octadecanetriol	No	0.279	No	No	Yes
Oleamide	No	-0.265	No	No	Yes
1,2,3,4-Tetramethyl-1,3-cyclopentadiene	No	-0.705	No	No	Yes
Linolenic acid ethyl ester	No	-0.051	No	No	Yes
Choline	No	0.952	No	No	Yes
Linoleoyl ethanolamide	No	-0.365	No	No	Yes
Citral	No	5.43	No	No	Yes
Jasmine	No	0.885	No	No	Yes
Hexadecanamide	No	-0.074	No	No	Yes
Oleoyl ethanolamide	No	-2.099	No	No	Yes
Palmitoleic acid	No	-0.713	No	No	Yes
Palmitoyl ethanolamide	No	-0.087	No	No	Yes
4-Dodecylbenzenesulfonic acid	No	0.35	No	No	Yes

prediction analysis show that most of the active compounds from irradiated black rice bran are non-toxic (safe). However, three compounds, namely erucamide, oleamide, and hexadecanamide, are estimated to be toxic because they can specifically cause an inhibitory effect on the hERG 2 receptor.

3.6 Molecular Docking

Based on the analysis of amino acid residue interaction (Table 7), it is known that the ligand 4-Dodecylbenzenesulfonic acid has binding affinity with EGFR protein with the highest value of -5.9, followed by ligands α-Eleostearic acid, linolenic acid ethyl ester, linoleoyl ethanolamide, and jasmone with binding affinity value of -5.7. The interaction of 4-Dodecylbenzenesulfonic acid with EGFR protein is found at amino acid residues Thr766 (A) and Thr830(A). Linolenic acid ethyl ester ligand interacts with EGFR protein at amino acid Thr830(A), and Linoleoyl ethanolamide interacts at amino acids Ala719(A), Thr 766(A) and Thr830(A). α-Eleostearic acid ligand interacts at amino acids Gln767(A) and Met769(A). Meanwhile, the Jasmone ligand did not find any interaction with amino acids in the EGFR protein. Compounds that are identified as having high target protein binding affinity are thought to have anticancer activity (Ghazi et al., 2021). Amino acids Thr830 and Th766 are thought to have a role in the process of enzyme phosphorylation and play an important role in promoting the metabolic reprogramming of cancer cells (Taddei et al., 2020). Thyroxine, in general, has the role of increasing the length of time of tumor cell inhibitory signals based on measurements of tyrosine receptor phosphorylation Ala719 acts as one of the key components in peptide and protein synthesis (Cruz et al., 2024). Met769 is one of the forms of Methionine that plays a role in initiating amino acids in eukaryotic protein synthesis. Methionine has a major metabolic function in changing its form to S-adenosylmethionine, which is the main biological methylation agent through the processes of transmethylation, remethylation, and transsulfuration (Brosnan et al., 2007). Gln767 is a form of Glutamine that is generally able to act as a fence for the microenvironment of tumors and cancer cells (Jin et al., 2023). The presence of these amino acid residues is thought to synergistically play a role in inhibiting cancer cell

© 2025 The Authors.

Table 7. Binding Affinity and Interaction of Compounds from Irradiated Black Rice Bran Extract with Protein Target By Molecular Docking Analysis

Ligand/Compound	EGFR			GPX7		
	Binding Affinity (Kcal/mol)	Hydrogen Bond Acid Amino Residue	Length (A)	Binding Affinity (Kcal/mol)	Hydrogen Bond Acid Amino Residue	Length (A)
Ethyl palmitoleate	-5.1	-	-	-4.5	Arg34(A)	3.01
Monoolein	-5	Ala719(A)	2.98	-5.4	Arg34(A)	3.24
		Leu764(A)	3.04		Arg34(A)	4.25
		Thr766(A)	3.2		Arg105(A)	2.92
		Thr766(A)	2.88		Arg105(A)	2.9
		Thr766(A)	2.76		Arg105(A)	2.83
					Met114(A)	3.01
					Met114(A)	2.92
Stearic acid	-5.3	Met769(A)	3.14	-4.4	Asp73(A)	2.8
		Gln767(A)	2.94		Arg169(A)	2.93
1-Linoleoyl glycerol	-5.6	Glu738(A)	2.82	-4.7	Gln71(A)	3.04
		Thr830(A)	2.7		Gln71(A)	3.02
		Asp831(A)	3.27		Gly75(A)	3.13
		Asp831(A)	2.95		Phe79(A)	3.16
					Phe79(A)	3.01
					Phe79(A)	2.9
					Asn80(A)	2.91
Ethyl oleate	-5.3	Thr830(A)	3.12	-4	Arg65(A)	3.03
α-Eleostearic acid	-5.7	Gln767(A)	2.87	-4.9	Asp73(A)	2.95
		Met769(A)	3.03		Arg169(A)	2.99
α-Linolenic acid	-5.4	Met769(A)	3.04	-4.8	Gly75(A)	2.99
					Gly75(A)	2.95
					Phe79(A)	3.22
Dihexyl adipate	-5.3	Lys721(A)	3.13	-4.5	Gln62(A)	3.09
		Thr830(A)	2.87		Arg65(A)	2.98
Erucamide	-5.3	Glu738(A)	2.97	-3.8	Val120(A)	2.82
		Asp831(A)	3.2		Gly122(A)	3.13
					Phe129(A)	3.21
1-Stearoylglycerol	-5.2	Thr766(A)	3.18	-5	His63(A)	3.06
		Thr766(A)	3		Pro161(A)	3.02
		Thr766(A)	2.78		Pro161(A)	2.89
		Thr830(A)	3.21		Val163(A)	2.82
		Thr830(A)	2.94			
Ethyl myristate	-5.2	-	-	-4.3	His63(A)	3.13

© 2025 The Authors.

2-Amino-1,3,4-octadecanetriol	-5.5	Thr766(A)	3.25	-4.5	Gln69(A)	3.1
		Thr830(A)	3.17		Gln69(A)	3.01
		Thr830(A)	2.8		Tyr108(A)	3.33
		Asp831(A)	3.23			
		Asp831(A)	3.06			
Oleamide	-5.5	Thr766(A)	3.14	-4.5	Tyr108(A)	3.24
		Thr830(A)	3.32			
1,2,3,4-Tetramethyl-1,3-cyclopentadiene	-4.8	-	-	-4.5	-	-
Linolenic acid ethyl ester	-5.7	Thr830(A)	2.99	-4.1	Gly75(A)	2.99
					Gly75(A)	2.95
					Phe79(A)	3.22
Choline	-3.4	Arg105(A)	2.9	-3.1	Arg105(A)	2.9
		Met114(A)	3.01		Met114(A)	3.01
		Met114(A)	2.8		Met114(A)	2.8
Linoleoyl ethanolamide	-5.7	Ala719(A)	2.99	-5	Gln71(A)	3.06
		Thr766(A)	2.97		Gly75(A)	3.15
		Thr766(A)	2.91		Phe79(A)	3.34
		Thr830(A)	2.85		Asn80(A)	2.79
Citral	-4.9	Thr830(A)	3.11	-4.5	-	-
Jasmone	-5.7	-	-	-4.8	Arg105(A)	3.08
					Arg105(A)	3.06
Hexadecanamide	-5.2	Thr766(A)	3.13	-4.1	Arg105(A)	3.13
		Thr830(A)	3.24		Arg105(A)	2.87
					Ser111(A)	2.9
Oleoyl ethanolamide	-5.5	Ala719(A)	3.02	-4	Gly58(A)	2.7
		Thr766(A)	3.17		Gln62(A)	2.87
		Thr830(A)	2.76		Asp61(A)	3.34
Palmitoleic acid	-5.3	Ala719(A)	2.81	-4.7	-	-
		Thr766(A)	3.26			
Palmitoyl ethanolamide	-5.3	Glu738(A)	3.15	-4.4	Phe79(A)	3.24
		Asp831(A)	3.12		Phe79(A)	3.05
					Asn80(A)	3.15
					Asn80(A)	3.03
4-Dodecylbenzenesulfonic acid	-5.9	Thr766(A)	3.21	-5.1	Asp27(A)	2.92
		Thr830(A)	3.15		Lys29(A)	3.07
					Lys29(A)	2.83

© 2025 The Authors.

growth (Sguizzato et al., 2022).

The interaction of amino acid residues shows that the Mono olein ligand has a binding affinity with the GPX7 protein with the highest value of -5.4, followed by the ligands 4-dodecylbenz ene sulfonic acid (-5.1), 1-stearoylglycerol and linoleoyl ethanol amide (-5), and α-eleostearic acid with a binding affinity value of -4.9 (Table 7). Monoolein functions as an antioxidant primarily through its ability to form stable nanostructures in aqueous environments, which can encapsulate and deliver other antioxidant compounds effectively. Monoolein self-assembles in water to create various nanostructures, including bicontinuous cubic phases and cubosomes. These structures are biocompatible and can encapsulate hydrophilic and lipophilic molecules, enhancing the solubility and stability of antioxidants like ascorbyl palmitate (AP) and alpha-tocopherol (AT) when incorporated into monoolein aqueous dispersions (MADs) (Falchi et al., 2015). GPX7 (Glutathione Peroxidase 7) and 4-dodecylbenzene sulfonic Acid (DBSA) involve the enzyme's role in oxidative stress responses and the potential impact of surfactants like DBSA on cellular mechanisms. DBSA is an anionic surfactant known for its ability to interact with biological membranes and potentially induce oxidative stress. Surfactants like DBSA can disrupt cellular homeostasis and influence the activity of antioxidant systems, including those involving GPX enzymes (Pei et al., 2023; Zhang et al., 2021).

The extract of *Oryza sativa L.* reveals binding interactions that assist in identifying the amino acid residues that stabilize protein-ligand complexes and or those that may be involved in antioxidant or free radical scavenging activity (Cruz et al., 2024). Among other issues, the methionine residue is a direct antioxidant due to its sulfur-containing side chain structure. Some amino acid residues containing sulfur that contain aromatic ring systems and the imidazole ring systems (histidine) are very effective against free radicals and cellular protection. The amino acid residues responsible for binding, their antioxidant and free radical scavenging properties, and their hydrogen bonding emphasize their structural stability and ligand interaction specificity, which helps design ligands or inhibitors for oxidative stress pathway proteins (Nwachukwu and Aluko, 2019).

4. CONCLUSIONS

IBRBE has low antioxidant activity and moderate cytotoxic activity against WiDr cells but no cytotoxic activity against normal Vero cells. IBRBE contains total phenolics and total flavonoids of 2.57 ± 0.28 mg GAE/g and 19.12 ± 0.18 μg QE/ml, respectively, and 24 active phytochemical compounds were identified. The compound 4-Dodecylbenzenesulfonic acid has the highest binding affinity to the EGFR protein with a value of -5.9, followed by other compounds, namely α-eleostearic acid, linolenic acid ethyl ester, linoleoyl ethanolamide, and jasmone with a binding affinity value of -5.7. The compound monoolein has the highest binding affinity to the GPX7 protein with a value of -5.4, followed by the compounds 4-dodecylbenzene sulfonic acid (-5.1), 1-stearoylglycerol and linoleoyl ethanolamide (-5), and α-eleostearic acid with a binding affinity value of -4.9. This research shows a positive correlation between in vitro and in silico study results. Compounds in IBRBE extract show more significant potential as anti-cancer agents than as antioxidants, both in vitro and in silico.

5. ACKNOWLEDGMENT

The authors acknowledge the facilities, scientific and technical support from Advanced Characterization Laboratories Yogyakarta, National Research and Innovation Agency through E-Layanan Sains BRIN. Addition, the research is supported by the facilities, scientific and technical support from Laboratory Standardization of Traditional Medicine Raw Materials, National Research and Innovation Agency through E- Layanan Sains-BRIN.

REFERENCES

Bakchi, B., A. D. Krishna, E. Sreecharan, V. B. J. Ganesh, M. Niharika, S. Maharshi, and A. B. Shaik (2022). An Overview on Applications of SwissADME Web Tool in the Design and Development of Anticancer, Antitubercular and Antimicrobial Agents: A Medicinal Chemist's Perspective. *Journal of Molecular Structure*, **1259**; 132712

Brosnan, J. T., M. E. Brosnan, R. F. P. Bertolo, and J. A. Brunton (2007). Methionine: A Metabolically Unique Amino Acid. *Livestock Science*, **112**(1–2); 2–7

Cacace, F., M. Menci, M. Papi, and V. Piemonte (2021). In-Silico Prediction of Oral Drug Bioavailability: A Multi-Boluses Approach. *Medical Engineering & Physics*, **98**; 140–150

Cherif, A. O. and M. B. Messaouda (2014). Floating Drug Delivery Systems for Eradication of *Helicobacter pylori* in Treatment of Peptic Ulcer Disease. In *Trends in Helicobacter pylori Infection*, volume 9. InTech, pages 1–13

Cruz, H., A. Llanes, and P. L. Fernández (2024). Computational Prediction of Amino Acid Preferences of Potentially Multispecific Peptide-Binding Domains Involved in Protein-Protein Interactions. *Journal of Visualized Experiments*, **17**(203); 786–798

Daina, A., O. Michielin, and V. Zoete (2017). SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports*, **7**(1); 42717

Damasuri, A. R., E. N. Sholikhah, and Mustofa (2020). Cytotoxicity of ((E)-1-(4-Aminophenyl)-3-Phenylprop-2-En-1-One)) on HeLa Cell Line. *Indonesian Journal of Pharmacology and Therapy*, **1**(2); 54–59

Djamal, J. M., J. M. P, and R. M. Rukmana (2020). Cytotoxic and Antiangiogenesis Activity of Papasan Cucumber Tuber (*Coccinia grandis* L. Voight) against bFGF Protein-Induced HeLa Cancer Cells. *Riset Informasi Kesehatan*, **9**(1); 51

Falchi, A. M., A. Rosa, A. Atzeri, A. Incani, S. Lampis, V. Meli, and S. Murgia (2015). Effects of Monoolein-Based Cubo-

© 2025 The Authors.

some Formulations on Lipid Droplets and Mitochondria of HeLa Cells. *Toxicology Research*, **4**(4); 1025–1036

Faustino, M., S. Strobbe, R. Sanchez-Muñoz, D. Cao, R. C. Mishra, T. Lourenço, and D. V. D. Straeten (2025). In Silico, In Vitro, and In Vivo Characterization of Thiamin-Binding Proteins from Plant Seeds. *Biochemical Journal*, **482**(04); 181–195

Ghazi, F., Y. A. Mahdi, and M. Dawood (2021). Theoretical Drug Design, Molecular Docking and ADME Study of New 1,3,4-Oxadiazole Derivatives: Promising Anticancer Agents Against Both Breast and Lung Cancers. *Egyptian Journal of Chemistry*, **64**(11); 0–0

Gondokesumo, M. E., A. Novilla, S. Prahastuti, H. S. W. Kusuma, W. Widowati, F. H. Zahiroh, and W. Surakusumah (2025). Anticancer Effectivity of Nanocrystals Derived from Mangosteen (*Garcinia mangostana*) Peel Extract on Leukemia HL-60 Cells. *Science and Technology Indonesia*, **10**(1); 228–237

Gonzales, A. L., S. K.-H. Huang, U. T. A. Sevilla, C.-Y. Hsieh, and P. Tsai (2023). In Silico Analysis of Anti-Inflammatory and Antioxidant Properties of Bioactive Compounds from *Crescentia cujete* L. *Molecules*, **28**(8); 3547

Hatorangan, M. R., E. Sentausa, and G. Y. Wijaya (2009). In Silico Identification of Cis-Regulatory Elements of Phosphate Transporter Genes in Rice (*Oryza sativa* L.). *Journal of Crop Science and Biotechnology*, **12**(1); 25–30

Huang, W., X. Guo, C. Wang, A. Alzhan, Z. Liu, X. Ma, and Q. Shu (2022). α-Linolenic Acid Induces Apoptosis, Inhibits the Invasion and Metastasis, and Arrests Cell Cycle in Human Breast Cancer Cells by Inhibiting Fatty Acid Synthase. *Journal of Functional Foods*, **92**; 105041

Indrayati, A., Nurfitriyawatie, and R. M. Rukmana (2023). Exploring the Anti-Photoaging Potential of *Bacillus cereus* Superoxide Dismutase in New Zealand Rabbit Skin. *HAYATI Journal of Biosciences*, **31**(2); 357–366

Izatunnafis, I., Y. B. Murti, and B. S. A. Sudarmanto (2023). In Silico Pharmacokinetic and Toxicity Prediction of Compounds from *Andrographis paniculata* (Burm.f.) Nees. *Journal of Food and Pharmaceutical Sciences*, **11**(2); 830–838

Jin, J., J.-K. Byun, Y.-K. Choi, and K.-G. Park (2023). Targeting Glutamine Metabolism as a Therapeutic Strategy for Cancer. *Experimental & Molecular Medicine*, **55**(4); 706–715

Kopustinskiene, D. M., V. Jakstas, A. Savickas, and J. Bernatoniene (2020). Flavonoids as Anticancer Agents. *Nutrients*, **12**(2); 457

Lipinski, C. A., F. Lombardo, B. W. Dominy, and P. J. Feeney (2012). Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews*, **64**(SUPPL.); 4–17

Liu, L.-D., Y.-N. Zhang, and L.-F. Wang (2019). GPX7 Promotes the Growth of Human Papillary Thyroid Carcinoma via Enhancement of Cell Proliferation and Inhibition of Cell Apoptosis. *Translational Cancer Research*, **8**(7); 2570–2580

Maharani, A. A., N. Puspawati, and R. M. Rukmana (2024). The Phytochemical Composition of Medicinal Plants in Indonesia and Their Potential as Antibacterial Agents Against *Salmonella typhi* ATCC 13311. *IOP Conference Series: Earth and Environmental Science*, **1312**(1); 012035

Milella, R. A., M. D. Rosso, M. Gasparro, I. Gigante, G. Debiase, L. R. Forleo, and R. Flamini (2023). Correlation Between Antioxidant and Anticancer Activity and Phenolic Profile of New Apulian Table Grape Genotypes (*V. vinifera* L.). *Frontiers in Plant Science*, **13**(January); 1–14

Mir, S. A., A. Dar, L. Hamid, N. Nisar, J. A. Malik, T. Ali, and G. N. Bader (2024). Flavonoids as Promising Molecules in the Cancer Therapy: An Insight. *Current Research in Pharmacology and Drug Discovery*, **6**(November 2023); 100167

Mohammed, A. E., S. S. Alghamdi, A. Shami, R. S. Suliman, K. Aabed, M. O. Alotaibi, and I. Rahman (2023). In Silico Prediction of *Malvaviscus arboreus* Metabolites and Green Synthesis of Silver Nanoparticles – Opportunities for Safer Anti-Bacterial and Anti-Cancer Precision Medicine. *International Journal of Nanomedicine*, **18**(April); 2141–2162

Mushebenge, A. G. A., S. C. Ugbaja, N. A. Mbatha, R. B. Khan, and H. M. Kumalo (2023). Assessing the Potential Contribution of In Silico Studies in Discovering Drug Candidates that Interact with Various SARS-CoV-2 Receptors. *International Journal of Molecular Sciences*, **24**(21); 15518

Nashrurrokhman, M., P. R. Sayekti, A. Safitri, Y. A. Purwestri, and R. Pratiwi (2019). Macronutrient and Mineral Contents of Five Local Black Rice *(Oryza sativa)* Cultivars in Indonesia. *Biodiversitas Journal of Biological Diversity*, **20**(12); 3647–3653

Nuriliani, A., F. C. Conara, G. Oktavya, L. T. N. Hidayah, and Y. A. Purwestri (2023). Ethanolic Extract of Black Rice 'Sembada Hitam' Bran Did not Show Cytotoxic Effect on HeLa Cell (Cervical Cancer Cell Line). *Bioma: Berkala Ilmiah Biologi*, **25**(1); 49–59

Nwachukwu, I. D. and R. E. Aluko (2019). Structural and Functional Properties of Food Protein-Derived Antioxidant Peptides. *Journal of Food Biochemistry*, **43**(1); e12761

Olasupo, S. B., A. Uzairu, G. A. Shallangwa, and S. Uba (2021). Unveiling Novel Inhibitors of Dopamine Transporter via In Silico Drug Design, Molecular Docking, and Bioavailability Predictions as Potential Antischizophrenic Agents. *Future Journal of Pharmaceutical Sciences*, **7**(1); 63

Pei, J., X. Pan, G. Wei, and Y. Hua (2023). Research Progress of Glutathione Peroxidase Family (GPX) in Redoxidation. *Frontiers in Pharmacology*, **14**(March); 1–14

Prastiyanto, M. E., R. M. Rukmana, D. K. Saraswati, S. Darmawati, E. T. W. Maharani, and Y. Tursinawati (2020). Anticancer Potential of Methanolic Extracts from Pleurotus Species on Raji Cells and Antibacterial Activity Against Methicillin-Resistant *Staphylococcus aureus*. *Biodiversitas Journal of Biological Diversity*, **21**(12); 5644–5649

Pratiwi, R. and Y. A. Purwestri (2017). Black Rice as a Functional Food in Indonesia. *Functional Foods in Health and Disease*, **7**(3); 182

Puspitasari, Y. E., M. A. Alfikri, R. Sitanggang, J. E. Tambunan,

© 2025 The Authors.

and H. Hardoko (2023). In Silico Analysis of Phenolic Compounds from *Ceriops decandra* Griff. Leaves and Molecular Interaction as Anti Diabetes. *Science and Technology Indonesia*, **8**(4); 542–553

Rahman, A. T., R. Rafia, A. Jethro, P. Santoso, V. D. Kharisma, A. A. A. Murtadlo, and D. A. P. Sari (2023). In Silico Study of the Potential of Endemic Sumatra Wild Turmeric Rhizomes (*Curcuma Sumatrana*: Zingiberaceae) As Anti-Cancer. *Pharmacognosy Journal*, **14**(6); 806–812

Rao, S., K. Chinkwo, A. Santhakumar, S. Johnson, and C. Blanchard (2019). Apoptosis Induction Pathway in Human Colorectal Cancer Cell Line SW480 Exposed to Cereal Phenolic Extracts. *Molecules*, **24**(13); 2465

Reviana, R., A. N. Usman, I. Raya, Aliyah, A. Dirpan, A. Arsyad, and F. Fendi (2021). Analysis of Antioxidant Activity on Cocktail Honey Products as Female Pre-Conception Supplements. *Gaceta Sanitaria*, **35**(2014); S202–S205

Riyana, A., F. Fathurrahman, and E. Roebiakto (2022). Javanese Ginseng Root Extract (*Talinum paniculatum* (Jacq) Gaertn) to Increase Superoxide Dismutase Activities in White Male Sprague Dawley Rats by Using a Forced Swimming Test Model. *Tropical Health and Medical Research*, **5**(1); 54–60

Roskoski, R. (2023). Rule of Five Violations Among the FDA-Approved Small Molecule Protein Kinase Inhibitors. *Pharmacological Research*, **191**(April); 106774

Rukmana, R. M., A. A. D. Sawal, and D. A. A. Wibawa (2023). Combination of Antibacterial Activity of Ethanol Extract of Meniran Leaves and Kenikir Leaves Against *Shigella dysenteriae*. In *Proceedings of the 1st International Conference for Health Research – BRIN (ICHR 2022)*, volume 2. Atlantis Press International BV, pages 7–20

Rukmana, R. M., N. P. Soesilo, Rumiyati, and R. Pratiwi (2017a). Chemopreventive Activities of 'Woja Laka' Black Rice Bran Fractions on Liver Carcinoma HepG2 Cells. *Biomedical and Pharmacology Journal*, **10**(4); 1677–1684

Rukmana, R. M., N. P. Soesilo, R. Rumiyati, and R. Pratiwi (2017b). The Effect of Ethanolic Extract of Black and White Rice Bran (*Oryza sativa* L.) on Cancer Cells. *Indonesian Journal of Biotechnology*, **21**(1); 63

Sallam, E. M. and M. M. Anwar (2017). Antioxidant Activity of Some Extracts from Gamma Irradiated Purslane (*Portulaca oleracea*) Plant. *International Journal of Agriculture and Biology*, **19**(1); 48–52

Santos, G. H. F. D., A. Amaral, and E. B. da Silva (2018). Antibacterial Activity of Irradiated Extracts of *Anacardium occidentale* L. on Multiresistant Strains of *Staphylococcus aureus*. *Applied Radiation and Isotopes*, **140**; 327–332

Seran, A. A., J. M. Peranginangin, and R. M. Rukmana (2020). Cytotoxic Activity and Antiangiogenesis of Extract Fraction of *Coccinia grandis* (L.) Voigt) Base Towards T47D Breast Cancer Cells and Chorio Allantoic Membrane (CAM) of Chicken Embryo Induced by bFGF. *JURNAL ILMIAH SAINS*, **20**(1); 37

Sguizzato, M., M. Drechsler, A. Baldisserotto, R. Cortesi, and E. Esposito (2022). Antioxidant-containing Monoolein Aqueous Dispersions: A Preliminary Study. *Drug Delivery and Translational Research*, **12**(8); 1873–1880

Shamshad, A., M. Rashid, and Q. uz Zaman (2023). In-silico Analysis of Heat Shock Transcription Factor (OsHSF) Gene Family in Rice (*Oryza sativa* L.). *BMC Plant Biology*, **23**(1); 395

Sholikhah, E. N., T. Wiyono, and W. R. Pratiwi (2024). Metabolic Profiling, Antioxidant, and Anti-lipase Activity from Combined Leaves Extracts of *Tamarindus indica* and *Murraya paniculata*: A Simplex Lattice Design Approach. *Science and Technology Indonesia*, **9**(4); 828–839

Suryanti, V., Riyatun, S. Suharyana, S. Sutarno, and O. A. Saputra (2020). Antioxidant Activity and Compound Constituents of Gamma-irradiated Black Rice (*Oryza sativa* L.) var. Cempo Ireng Indigenous of Indonesia. *Biodiversitas Journal of Biological Diversity*, **21**(9); 4205–4212

Taddei, M. L., E. Pardella, E. Pranzini, G. Raugei, and P. Paoli (2020). Role of Tyrosine Phosphorylation in Modulating Cancer Cell Metabolism. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, **1874**(2); 188442

Talib, W. H., A. I. Mahmod, D. Awajan, R. A. Hamed, and I. H. Al-Yasari (2022). Immunomodulatory, Anticancer, and Antimicrobial Effects of Rice Bran Grown in Iraq: An In Vitro and In Vivo Study. *Pharmaceuticals*, **15**(12); 1502

Vardhani, A., N. B. Anggraini, M. Jufri, E. Purwaningsih, and H. S. Perdanastuti (2024). Total Phenolic Content in Black Rice (*Oryza sativa* L. Indica) Bran Ethanolic Extract from Two Different Regions in Java, Indonesia. *Jurnal Jamu Indonesia*, **9**(1); 3–7

Widiyastuti, Y., R. Pratiwi, S. Riyanto, and S. Wahyuono (2018). Cytotoxic Activity and Apoptosis Induction of Avocado *Persea americana* Mill. Seed Extract on MCF-7 Cancer Cell Line. *Indonesian Journal of Biotechnology*, **23**(2); 61

Widiyastuti, Y., I. Y. M. Sholikhah, and S. Haryanti (2019). Cytotoxic Activities of Ethanolic and Dichloromethane Extract of Leaves, Stems, and Flowers of Jarong [*Stachytarpheta jamaicensis* (L.) Vahl.] on HeLa and T47D Cancer Cell Line. In *AIP Conference Proceedings*, volume 2202. page 020101

Widodo, H., S. Sismindari, W. Asmara, and A. Rohman (2019). Antioxidant Activity, Total Phenolic and Flavonoid Contents of Selected Medicinal Plants Used for Liver Diseases and Its Classification With Chemometrics. *Journal of Applied Pharmaceutical Science*, **9**(6); 99–105

Winarno, E. K., H. Winarno, and S. Susanto (2019). Antiproliferative Activity of Extracts and Fractions From Irradiated *Curcuma zanthorrhiza rhizomes* Against Mouse Leukemia and Human Cancer Cell Lines. *Atom Indonesia*, **45**(3); 159

Windarsih, A., Suratno, H. D. Warmiko, A. W. Indrianingsih, A. Rohman, and Y. I. Ulumuddin (2022). Untargeted Metabolomics and Proteomics Approach Using Liquid Chromatography-Orbitrap High Resolution Mass Spectrometry to Detect Pork Adulteration in *Pangasius hypopthalmus* Meat. *Food Chemistry*, **386**; 132856

Wisetkomolmat, J., C. Arjin, A. Satsook, M. Seel-audom,

© 2025 The Authors.

W. Ruksiriwanich, C. P. u Thai, and K. Sringarm (2022). Comparative Analysis of Nutritional Components and Phytochemical Attributes of Selected Thai Rice Bran. *Frontiers in Nutrition*, **9**; 1–12

Xu, X., J. E. Riviere, S. Raza, N. I. M. Gedara, R. A. Ramachandran, L. A. Tell, and M. Jaberi-Douraki (2024). In-Silico Approaches to Assessing Multiple High-Level Drug-Drug and Drug-Disease Adverse Drug Effects. *Expert Opinion on Drug Metabolism & Toxicology*, **20**(7); 579–592

Yatebe, T. M. and Y. Yatebe (2009). Epidermal Growth Factor Receptor in Relation to Tumor Development: EGFR Gene and Cancer. In *EGFR Gene and Cancer*. pages 301–308

Yuliana, N. D., M. Z. Tuarita, A. Khatib, F. Laila, and S. Sukarno (2020). GC–MS Metabolomics Revealed Protocatechuic Acid as a Cytotoxic and Apoptosis-Inducing Compound From Black Rice Brans. *Food Science and Biotechnology*, **29**(6); 825–835

Yusuf, H., M. Fahriani, C. Murzalina, and R. D. Mawaddah (2022). Inhibitory Effects on HepG2 Cell Proliferation and Induction of Cell Cycle Arrest by *Chromolaena odorata* Leaf Extract and Fractions. *Pharmacia*, **69**(2); 377–384

Zarei, I., E. Luna, J. E. Leach, A. McClung, S. Vilchez, O. Koita, and E. P. Ryan (2018). Comparative Rice Bran Metabolomics Across Diverse Cultivars and Functional Rice Gene–Bran Metabolite Relationships. *Metabolites*, **8**(4); 63

Zhang, J., Y. Luo, X. Chen, G. Wu, and L. Yuan (2021). Does the Anionic Surfactant Isopropylamine Dodecylbenzene Sulfonate Induce Hepatic Oxidative Stress and Impairment in *Carassius auratus* Gibelio? *Bulletin of Environmental Contamination and Toxicology*, **107**(3); 487–493

Zhang, T., Y. Gao, Y. Mao, Q. Zhang, C. Lin, P. Lin, and X. Wang (2012). Growth Inhibition and Apoptotic Effect of Alpha-Eleostearic Acid on Human Breast Cancer Cells. *Journal of Natural Medicines*, **66**(1); 77–84

© 2025 The Authors.