ISOLATION AND BIOACTIVE ASSAY OF γ-ORYZANOL FROM RICE BRAN (*ORYZA SATIVA*)

Nguyen Thanh Chung[1], Luu Duc Huy[2], Ho Van Khanh[2], Hoang Quoc Viet[2], Nguyen Phuong Thao[2], Do Bich Hoa[2], Duong Ngoc Tu[2*]

[1]Institute of Biological and Food technology – Hanoi Open University

[2]Institute of Chemistry – Vietnam Academy of Science and Technology

ARTICLE INFO

Received: 01/3/2023

Revised: 05/3/2024

Published: 06/3/2024

KEYWORDS

Rice bran oil

γ-oryzanol

24- methylenecycloartanyl ferulate

α-glucosidase inhibitor

Antioxidant

ABSTRACT

Vietnam is currently the second-largest exporter of rice in the world. Rice bran is a by-product of the milling process. It makes up 14% of the paddy rice seed weight. In the past, rice bran was only used as feed for livestock. This gives very low economic value. Recently, a little amount of rice bran is used in cosmetics and food by using whole bran or bran oil. For improving the value and application of rice bran, we study the composition of rice bran of Thai Binh rice variety. Rice bran oil was extracted by using ethyl acetate extract. From that, a γ-oryzanol compound, 24-methylenecycloartanyl ferulate (**CG55**), was successfully isolated. Its structure was elucidated by mass spectrometry (MS) and nuclear magnetic resonance (NMR). Compound CG55 showed strong inhibition of enzyme α-glucosidase with IC_{50} at 5.03 µg/mL, weak antioxidant, and nontoxicity.

CHIẾT TÁCH VÀ XÁC ĐỊNH HOẠT TÍNH SINH HỌC CỦA γ-ORYZANOL TỪ CÁM GẠO (*ORYZA SATIVA*)

Nguyễn Thành Chung[1], Lưu Đức Huy[2], Hồ Văn Khánh[2], Hoàng Quốc Việt[2], Nguyễn Phương Thảo[2], Đỗ Bích Hòa[2,] Dương Ngọc Tú[2*]

[1]Viện Công nghệ Sinh học và Công nghệ Thực phẩm - Trường Đại học Mở Hà Nội

[2]Viện Hóa học - Viện Hàn lâm Khoa học và Công nghệ Việt Nam

THÔNG TIN BÀI BÁO

Ngày nhận bài: 01/3/2023

Ngày hoàn thiện: 05/3/2024

Ngày đăng: 06/3/2024

TỪ KHÓA

Dầu cám gạo

γ-oryzanol

24- methylenecycloartanyl ferulate

Ức chế α-glucosidase

Chống oxy hóa

TÓM TẮT

Việt Nam hiện nay là nước xuất khẩu gạo lớn thứ hai trên thế giới. Cám gạo là sản phẩm phụ của quá trình xay xát gạo. Nó chiếm khoảng 14% trọng lượng hạt thóc. Trước đây, cám gạo chỉ được dùng làm thức ăn cho gia súc. Điều này mang lại giá trị kinh tế rất thấp. Gần đây, một lượng nhỏ cám gạo được sử dụng trong mỹ phẩm và thực phẩm bằng cách sử dụng nguyên cám hoặc giàu cám. Để nâng cao giá trị và ứng dụng của cám gạo, chúng tôi tiến hành nghiên cứu thành phần cám gạo của giống lúa Thái Bình. Dầu cám gạo được chiết suất bằng cách sử dụng ethyl acetate. Từ đó, một hợp chất γ-oryzanol là 24-methylenecycloartanyl ferulate (**CG55**) đã được tinh sạch thành công. Cấu trúc của nó đã được xác định bằng phương pháp khối phổ (MS) và cộng hưởng từ hạt nhân (NMR). Hợp chất CG55 thể hiện khả năng ức chế mạnh enzyme α-glucosidase với IC_{50} là 5,03 µg/mL, hoạt tính chống oxy hóa yếu và không độc.

DOI: https://doi.org/10.34238/tnu-jst.7466

---

[*] Corresponding author. *Email: duongngoctu@yahoo.com*

1. Introduction

Vietnam is a long-standing wet rice agricultural country, so rice has a great value in the country's economy. Rice is the main food for more than half the world’s population and as one of the earth’s three major grain crops. It contains more than 100 bioactive compounds including phytic acid, isovitexin, phytosterols, γ-oryzanol, octacosanol, γ-aminobutyric acid (GABA), squalene, tocopherol, and tocotrienol derivatives [1], [2]. During the production and processing of rice, a large by-product is rice bran. Most of the rice bran is used only as animal feed, but its potential has not been fully exploited. In rice bran, there are many valuable nutrients such as proteins, lipids, vegetable oils, glucosides, and vitamins. Among of those, γ-oryzanol shows good antioxidant activity, is good for health widely used in cosmetics, and has high economic value [3]-[5]. Currently, γ-oryzanol has been applied a lot in the production of pharmaceuticals and cosmetics, but all are from imported sources. The γ-oryzanol is a mixture of phytosterols esterified with phenol, ferulic acid obtained from rice bran or rice bran oil [6], [7]. γ-Oryzanol was first isolated from rice bran oil by Kaneko and Tsuchiya in 1954 and has been used in Japan to treat anxiety, menopausal symptoms, stomach ulcers, and hyperlipidemia [8], [9]. The major components of γ-oryzanol include cycloartenyl ferulate (Oryzanol A), 24 methylenecycloartanyl ferulate (Oryzanol C), campesteryl ferulate, Δ^7-stigmastenyl ferulate, stigmasteryl ferulate, Δ^7-campestenyl ferulate, Δ^7-sitostenyl ferulate, sitosteryl ferulate, compestanyl ferulate, and sitostanyl ferulate [10].

In this study, we decided to isolate γ-oryzanol from BC15 Thai Binh rice bran. Some bioactive properties of γ-oryzanol were also tested. The aim of this study is to improve the value and application of rice bran in Vietnam.

2. Materials and methods

2.1. Materials

Rice bran was collected from BC15 Thai Binh rice variety. After milling to a fine-soft standard, rice bran was sieved through a 180 µm (80 mesh) filter, and dried at 60°C for about 45 minutes.

2.2. Isolation methods

The rice bran (1,5 kg) was extracted three times with n-hexane, ethyl acetate, and ethanol, respectively. Then, the total extracts of each solvent were filtered and dried by a vacuum evaporator to obtain n-hexane extracted crude (35.95g), ethyl acetate extracted crude (131g), and ethanol extracted crude (27.80g). Ethyl acetate extracted crude (45g) was separated on a silica gel column, using a gradient n-hexane/ethyl acetate solvent system to obtain 100 fractions (F1-F100). The F55 fraction was crystallized and purified by recrystallization in n-hexane to obtain compound 1 (CG55; 120 mg) as a white, slightly yellow crystal.

2.3. Column chromatography, LC-MS, and NMR

Column chromatography (CC) was applied to purified γ-oryzanol using silica gel as an absorbent (Merck, Germany) with a particle size of 40-63 µm.

Compounds obtained from rice bran were measured on a high-resolution mass spectrometry system LC-MS QTOF, X500R at the Institute of Chemistry.

Purified compound (CG55) was dried and dissolved in $CDCl_3$, then, measured ^1H NMR spectra on a Bruker AV 600 spectrometer with TMS (tetramethylsilane) as the internal standard at the Institute of Chemistry.

2.4. α-glucosidase inhibitory activity assay

The α-glucosidaseinhibitory activity was performed on a 96-well elisa plate. Each well contains: 20 µL α-glucosidase (0.5 U/mL), 130 µL phosphate buffer 100 mM (pH 6.8) and 50 µL sample diluted in different concentrations in 10 mM phosphate buffer (pH 6.8) to achieve the concentrations of 500, 100, 20.4 µg/mL, respectively. Mix well and incubate at 37°C for 15 mins. Then, 50 µL of p-nitrophenyl-α-D-glucopyranoside (pNPG) (5 mM) was added to each well and further incubated at 37°C for 60 mins. The reaction was stopped by adding 80 µL of 0.2 M Na_2CO_3 and measuring the OD at 405 nm using an ELISA Plate Reader meter (Biotek). The IC_{50} value (50% inhibitory concentration) will be determined using TableCurve2Dv4 computer software.

2.5. Antioxidant activity assay

The reaction consisted of 10 µL of reagent prepared in deionized water and 190 µL of 1 mM DPPH solution in Methanol (MeOH) on a 96-well plate. The antioxidant activity of CG55 was determined at concentrations of 2.8, 32, 128, 512 µg/mL. The antioxidant activity of quercetin was determined at concentrations of 0.5, 2, 8, 32 µg/mL. The reaction mix was incubated for 30 mins at 37°C. The free radical scavenging capacity of DPPH was determined by measuring at 517 nm. The experiment was repeated 3 times.

The % free radical trap of DPPH of the sample was calculated according to the following formula:

SC% = (OD of control – OD of sample)/ OD of control (%)

EC_{50} is calculated according to the SC value correlating with different concentrations of the reagent.

2.6. Cytotoxic activity assay

Carcinoma cell line KB was grown in DMEM (Dulbeccos Modified Eagle Medium) supplemented with 7-10% FBS (Fetal Bovine Serum), 1% antibiotics at standard conditions (5% CO2, 95% relative humidity, 37°C). Cells grown in log phase will be used for toxicity testing.

Add to each well 10 µl of substrate and 190 µl of cell solution at 3×10^4 cells/ml. The substrate concentrations in the wells were 128, 32, 8, 2, and 0.5 µg/ml. The reference substrate used was Ellipticine with concentrations of 12.8, 3.2, 0.8, and 0.2 µg/ml. The plate was incubated at standard conditions. After 72 hours, each well was added 10 µl of MTT (5 mg/ml) and incubated for 4 hours. After removing the medium, formaran crystals were dissolved with 100 µl of 100% DMSO. The experimental results were determined by the OD at 540 nm on a Biotek spectrophotometer. The experiment was repeated 3 times.

3. Results and discussion

3.1. Structure of CG55

After extracting the oil from the rice bran by using ethyl acetate. The oil crude was separated on a silicagel column. **CG55** was achieved in fraction 55 of purification process. **CG55** was analyzed by LC-MS and ^1H NMR. The results showed in Figures 1 and 2. **CG55** molecular formula $C_{41}H_{59}O_4^-$ was established by the LC-MS spectrometry at m/z 615.4415 [M - H^+] (calcd., 615.4413). Figure 2 and Table 1 shows ^1H NMR spectral data of CG55. ^1H NMR of CG55 data show that CG55 contains 60 protons at δ_H 7.60 (1 H, H-3', dd, 15.9, 1.6 Hz), δ_H 7.07 (1 H, H-9', ddd, 8.1, 6.1, 1.9 Hz), δ_H 7.04 (1 H, H-5', dd, 5.2, 1.9 Hz), δ_H 6.91 (1 H, H-8', dd, 8.2, 1.8 Hz), δ_H 6.29 (1 H, H-2', dd, 15.9, 13.8 Hz), δ_H 5.86 (1 H, H-OH, s), δ_H 4.71 (1 H, H-3, d, 11.4 Hz), δ_H 3.93 (3 H, H-10', d, 8.4 Hz), δ_H 2.24 (1 H, H-25', pd, 6.8, 1.1 Hz), δ_H 2.13 (1 H, H-23, ddd, 15.5, 11.3, 4.7 Hz), δ_H 2.01 (1 H, H-11, ddd, 13.8, 6.7, 3.1 Hz), δ_H 1.93 (1 H, H-16, m), δ_H 1.89 (1 H, H-23, m), δ_H 1.84 (1H, H-2, m), δ_H 1.69 (2 H, H-1, H-2, H- m), δ_H 1.64 (1 H, H-12, m), δ_H 1.63 (1 H, H-17, m), δ_H 1.60 (1 H, H-6, m), δ_H 1.58 (1 H, H-22, m), δ_H 1.53 (1 H, H-8, m), δ_H 1.45 (1H, H-5, m), δ_H 1.41 (1 H, H-20, dt, 6.2, 3.2 Hz), δ_H 1.34 (1 H, H-7, m), δ_H 1.30 (3 H, H-1, H-15, H-16, m), δ_H 1.16 (1 H, H-11, H-11, m), δ_H 1.13 (1 H, H-22, m), δ_H 1.10 (1 H, H-7, m), δ_H

1.04 (3 H, H-26, d, 5.3 Hz), δ_H 1.03 (1 H, H-27, d, 3.3 Hz), δ_H 0.98 (6 H, H-18, H-30, s), δ_H 0.92 (3 H, H-31, s), δ_H 0.91 (3 H, H-29), s), δ_H 0.90 (3 H, H-21, s), δ_H 0.82 (1 H, H-6, d, 7.2 Hz), δ_H 0.61 (1 H, H-19, d, 4.1 Hz), δ_H 0.37 (1 H, H-19, d, 4.1 Hz). When comparing these data with ^{1}H NMR data of 24-methylenecycloartanyl ferulate by Ito et al. [11], it is showed that CG55 is the 24-methylenecycloartanyl ferulate (Figure 3).

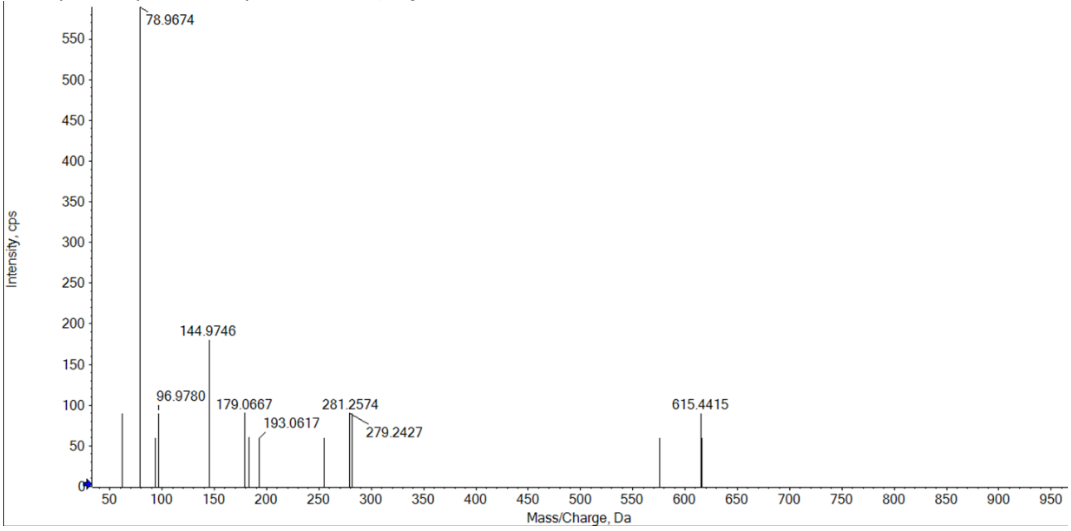

Figure 1. *Mass spectra of **CG55***

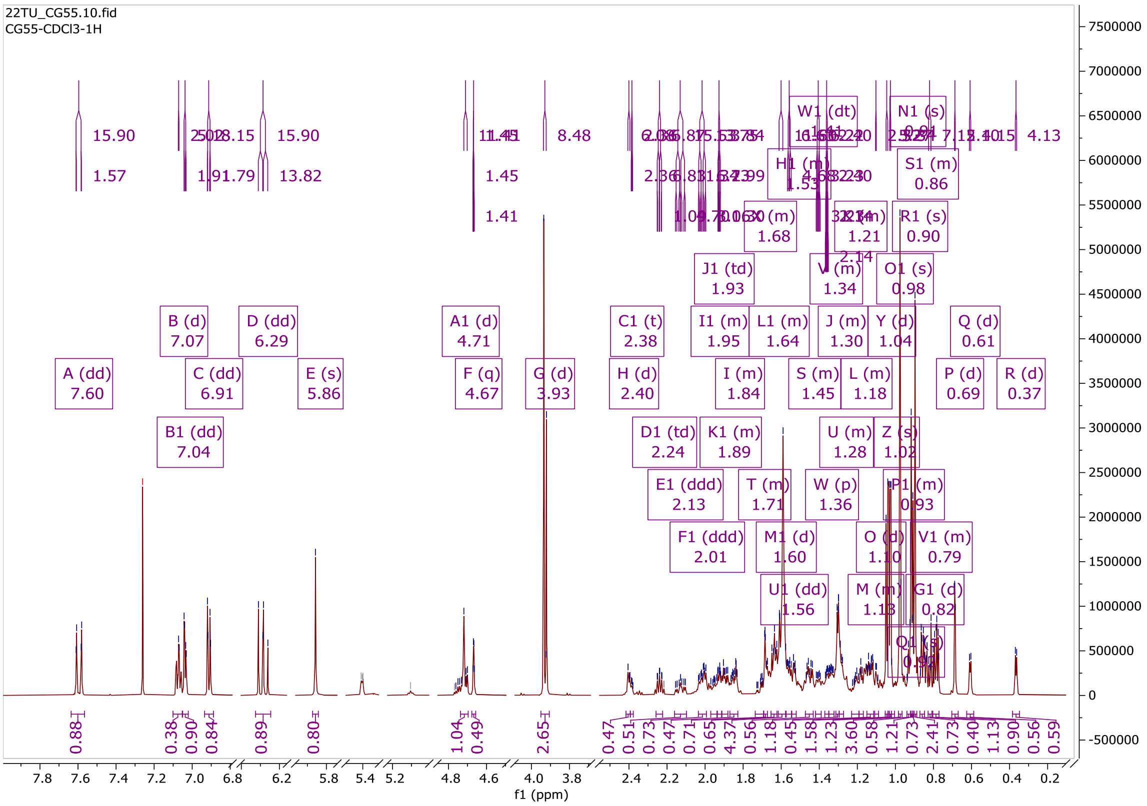

Figure 2. *^{1}H NMR spectra of CG55*

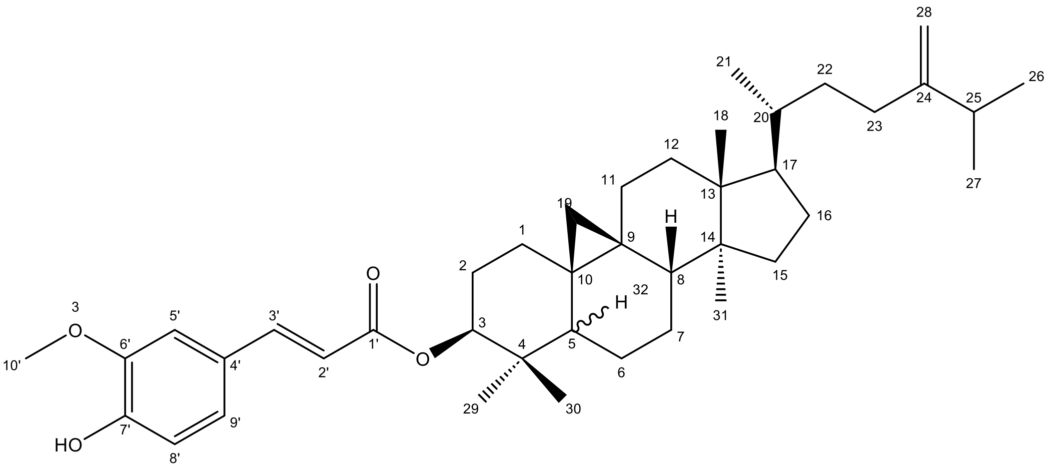

Figure 3. *Structure of 24 methylencycloartanyl ferulate (**CG55**)*

Table 1. *Comparison of ^{1}H NMR data of CG55 and 24-Methylenecycloartanyl ferulate*

24-Methylenecycloartanyl ferulate			**CG55** (^{1}H NMR (600 MHz, CDCl$_3$))	
Carbon position C	**δH (ppm)**	**Type, Hz, No. of H**	**δH (ppm)**	**Type, Hz, No. of H**
1	1.68	m, 1H	1.69	m, 1H
	1.29	m, 1H	1.30	m, 1H
2	1.84	m, 1H	1.84	m, 1H
	1.68	m, 1H	1.69	m, 1H
3	4.72	dd (10.8, 4.8), 1H	4.71	d (11.4), 1H
5	1.45	dd (16.2, 4.2), 1H	1.45	m, 1H
6	1.60	m, 1H	1.60	m, 1H
	0.82	dq (12.6, 2.4), 1H	0.82	d (7.2), 1H
7	1.34	m, 1H	1.34	m, 1H
	1.10	m, 1H	1.10	m, 1H
8	1.53	dd (12.8, 4.8), 1H	1.53	m, 1H
11	2.01	dt (15.0, 8.4), 1H	2.01	ddd (13.8, 6.7, 3.1), 1H
	1.15	m, 1H	1.16	m, 1H
12	1.63	t (7.8), 2H	1.64	m, 1H
15	1.30	m, 2H	1.30	m, 1H
16	1.93	m, 1H	1.93	m, 1H
	1.30	m, 1H	1.30	m, 1H
17	1.62	m, 1H	1.63	m, 1H
18	0.98	s, 3H	0.98	s, 3H
19	0.60	d (3.9), 1H	0.61	d (4.1), 1H
	0.37	d (3.9), 1H	0.37	d (4.1), 1H
20	1.40	m, 1H	1.41	dt (6.2, 3.2), 1H
21	0.90	d (6.0), 3H	0.90	s, 3H
22	1.58	m, 1H	1.58	m, 1H
	1.14	m, 1H	1.13	m, 1H
23	2.13	ddd (14.4, 11.7, 4.5), 1H	2.13	ddd (15.5, 11.3, 4.7), 1H
	1.89	m, 1H	1.89	m, 1H
25	2.24	sep (7.2), 1H	2.24	pd (6.8, 1.1), 1H
26	1.04	d (7.2), 3H	1.04	d (5.3), 3H
27	1.03	d (7.2), 3H	1.03	d (3.3), 1H
28	4.72	br s, 1H	4.71	d (11.4), 1H
	4.67	1H	4.67	q (1.4), 1H
29	0.90	s, 3H	0.91	s, 3H
30	0.98	s, 3H	0.98	s, 3H
31	0.92	s, 3H	0.92	s, 3H

24-Methylenecycloartanyl ferulate			CG55 (^{1}H NMR (600 MHz, $CDCl_3$)	
Carbon position C	**δH (ppm)**	**Type, Hz, No. of H**	**δH (ppm)**	**Type, Hz, No. of H**
2’	6.30	d (15.9), 1H	6.29	dd (15.9, 13.8), 1H
3’	7.60	d (15.9), 1H	7.60	dd (15.9, 1.6), 1H
5’	7.05	d (1.8), 1H	7.04	dd (5.2, 1.9), 1H
8’	6.92	d (8.4), 1H	6.91	dd (8.2, 1.8), 1H
9’	7.08	dd (8.4, 1.8), 1H	7.07	ddd (8.1, 6.1, 1.9), 1H
10’	3.94	s, 3H	3.93	d (8.4), 3H
OH	5.84	s, 1H	5.86	s, 1H

3.2. α-glucosidase inhibitory activity of CG55

Table 2. *α-glucosidase inhibitory activity of CG55 and Acarbose*

Concentration µg/mL	% Inhibitor	% Inhibitor
	CG55	**Acarbose**
500	95.75	76.97
100	92.69	48.15
20	91.42	18.90
4	30.67	9.66
IC$_{50}$	**5.03± 0.39**	**133.32± 5.45**

After that, α-glucosidase inhibition activity of **CG55** was performed. The IC$_{50}$ of α-glucosidase inhibition of **CG55** in Table 2, it was showed that CG55 showed a very good α-glucosidase inhibitory activity with IC$_{50}$ of 5.03 µg/mL. It is 26,5-fold higher than that of the Acarbose with IC$_{50}$ of 133.32. µg/mL. It is shown that the α-glucosidase inhibition activity of **CG55** is strong.

3.3. Antioxidant activity of CG55

Table 3. *Antioxidant activity of CG55*

No.	Sample	Concentration (µg/ml)	% free radical scavenging	EC$_{50}$ (µg/ml)
1	**CG55**	512	100	**149.74±5.06**
		128	47	
		32	6	
		8	0	
		2	0	
Control	Quercetin	32	100	**9.97±0.25**
		8	45,5	
		2	0	
		0,5	0	

Also, CG55 was tested of antioxidant activity. From the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical inhibitory activity of CG55 compared with Quercetin in Table 3, it indicated that the EC$_{50}$ value of CG55 is 15 times higher than that of Quercetin. So, CG55 has weak antioxidant activity than Quercetin.

3.4. Cytotoxic activity of CG55

Table 4. *Cytotoxic activity of CG55 and Ellipticine*

No.	Sample	Concentration (µg/ml)	% Inhibition
1	CG55	128	51
		32	33
		8	26
		2	19
		IC$_{50}$	**122.67±2.58**
2	Ellipticine	**IC$_{50}$**	**0.22±0.01**

From the activity of **CG55** and Ellipticine in Table 4, it is showed that the inhibition of CG55 with KB was decreased from 51 to 19% when the CG55 concentration from 128 to 2 µg/mL. And CG55 has a 557,59-folded greater inhibitory activity than Ellipticine (Table 4). This means that CG55 is not cytotoxic.

4. Conclusion

Compound CG55 was isolated from rice ban and elucidated the structure as 24-Methylenecycloartanyl ferulate.

The biological activity test of CG55 indicated that it showed high α-glucosidase inhibitory activity, weak antioxidant and non-cytotoxic.

Acknowledgment

This study is funded by the Institute of Chemistry - Vietnam Academy of Science and Technology (Project number: VHH.2022.05).

REFERENCES

[1] P. Chanapiwat and K. Kaeoket, "Breed of boar influences the optimal concentration of gamma-oryzanol needed for semen cryopreservation," *Reprod Domest Anim*, vol. 50, no. 2, pp. 221-226, 2015.

[2] H. W. Kim, J. B. Kim, P. Shanmugavelan, S. N. Kim, Y. S. Cho, H. R. Kim, J. T. Lee, W. T. Jeon, and D. J. Lee, "Evaluation of γ-oryzanol content and composition from the grains of pigmented rice-germplasms by LC-DAD-ESI/MS," *BMC Res Notes*, vol. 6, p. 149, 2013.

[3] F. V. Francisqueti-Ferron, J. L. Garcia, A. J. T. Ferron, E. T. Nakandakare-Maia, C. S. Gregolin, J. P. D. C. Silva, K. C. Dos Santos, A. T .C. Lo, J. S. Siqueira, L. de Mattei, B. H. de Paula, F. Sarzi, C. C. V. A. Silva, F. Moreto, M. R. Costa, A. L. A. Ferreira, I. O. Minatel, and C. R. Corrêa, "Gamma-oryzanol as a potential modulator of oxidative stress and inflammation via PPAR-y in adipose tissue: a hypothetical therapeutic for cytokine storm in COVID-19?" *Mol Cell Endocrinol.*, vol. 520, 2021, Art. no. 111095.

[4] A. Najafi, H. Daghigh-Kia, M. Mehdipour, H. Mohammadi, and H. Hamishehkar, "Comparing the effect of rooster semen extender supplemented with gamma-oryzanol and its nano form on post-thaw sperm quality and fertility," *Poult Sci.*, vol. 101, no. 3, 2022, Art. no. 101637.

[5] S. Chalermpong, C. Chaiyavat, C. Sunee, S. Maitree, and S. Busaban, "Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran," *Journal of Medicinal Plants Research*, vol. 6, no. 6, pp. 1070-1077, 2012.

[6] L. Mattei, F. V. Francisqueti-Ferron, J. L. Garcia, A. J. T. Ferron, C. C. V. A. Silva, C. S. Gregolin, E. T. Nakandakare-Maia, J. D. C. P. Silva, F. Moreto, I. O. Minatel, and C. R. Corrêa, "Antioxidant and anti-inflammatory properties of gamma- oryzanol attenuates insulin resistance by increasing GLUT- 4 expression in skeletal muscle of obese animals," *Mol Cell Endocrinol*, vol. 537, 2021, Art. no. 111423.

[7]T. H. Pham, V. H. Nguyen, T. T. T. Nguyen, T. N. D. Dinh, A. D. Cao, V. S. Tran, and T. T. Nguyen, "Efficiency of additional release of Trichogramma chilonis in Tay Ninh province," *Journal of Vietnam Agricultural Science and Technology*, vol. 4, no. 101, pp.100-105, 2019.

[8] K. B. Wheeler and K. A. Garleb, "Gamma oryzanol-plant sterol supplementation: metabolic, endocrine, and physiologic effects," *Int J Sport Nutr.*, vol. 1, no. 2, pp. 170-177, 1991.

[9] S. H. Huang and L. T. Ng, "Quantification of tocopherols, tocotrienols, and γ-oryzanol contents and their distribution in some commercial rice varieties in Taiwan," *J Agric Food Chem.*, vol. 59, no. 20, pp. 11150-11159, 2011.

[10] N. B. Tuncel and N. Yılmaz, "Gamma-oryzanol content, phenolic acid profiles and antioxidant activity of rice milling fractions," *Eur Food Res Technol.*, vol. 233, 2011, Art. no. 577.

[11] J. I to, K. Sawada, Y. Ogura, *et al.*, "Definitive evidence of the presence of 24-methylenecycloartanyl ferulate and 24-methylenecycloartanyl caffeate in barley," *Sci Rep.*, vol. 9, 2019, Art. no. 12572.