ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 09, Issue 12, pp.8257-8262, December, 2022

RESEARCH ARTICLE

A STUDY ON THE ANTI-OBESITY EFFICACY OF WHEAT GERM HYDROTHERMAL EXTRACT

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ARTICLE INFO

Article History:
Received 09th September, 2022
Received in revised form
28th October, 2022
Accepted 24th November, 2022
Published online 30th December, 2022

Key words:

Antiobesity, α-Glucosidase, Hydrothermal Extract, Lipase, Wheat Germ Extracts, Staphylococcus, Aureus.

ABSTRACT

In this study, to confirm the antiobesity efficacy of wheat germ hot-water extract, hot-water extraction with 20%, 50%, and 80% ethanol content was used to analyze antioxidant efficacy, *Staphylococcus aureus* antibacterial activity, lipase inhibitory activity, and α-glucosidase activity inhibitory activity. As a result of the antioxidant test of wheat germ extracts, the IC_{50} values of ascorbic acid used as a control was 2.16, whereas the values for 20%, 50%, and 80% ethanol of wheat germ extracts were 3.13, 2.91, and 2.64, respectively. The values were lowers in all samples than that in ascorbic acid, but antioxidants showed concentration-dependent effects. The result of the antibacterial analysis of wheat germ extracts showed that the antimicrobial activity increased as the ethanol content increased, and specifically, the 80% ethanol extract showed a high bacterial reduction rate of 75.7%. In the lipase activity inhibition analysis of wheat germ extracts, the lipase inhibitory activity of orlistat (positive control) was 81.9% ± 1.17%, whereas those of 20%, 50%, and 80% ethanol extract were 76.0% ± 7.42%, 85.5% ± 5.01%, and 95.5% ± 3.26%, respectively. All extracts showed lipase inhibitory activity. Specifically, 80% ethanol extract showed higher lipase inhibitory activity than orlistat (positive control). In the α-glucosidase activity inhibitory activity analysis of wheat germ extracts, the inhibitory activity of acarbose (positive control) was 87.5% ± 3.21%; 20% ethanol extract, 71.3% ± 3.40%; 50% ethanol extract, 88.2% ± 4.00%; and 80% ethanol extract, 90.2% ± 90.2 ± 4.00%. All extracts showed α-glucosidase inhibitory activity at 2.12%. Specifically, the 80% ethanol extract showed a higher α-glucosidase inhibitory activity than acarbose (positive control). This is caused by the effect of complex extracts such as 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid.

INTRODUCTION

In modern society, the incidence of metabolic syndrome and obesity due to physical inactivity and high-calorie eating habits is rapidly increasing (1). According to the World Health Organization, the world's obese population is doubling every 5 years, warning that obesity is a chronic disease requiring treatment and is one of the world's serious health problems (2). Obesity is a state in which surplus energy is excessively stored in body fat tissues when energy intake is greater than the energy used by the body because of excessive food intake and lack of exercise. Excessive accumulation of energy and fatty acids caused by factors such as shortage, stress, and endocrine disorders have been reported as causes of obesity (3). Excessive fat accumulation because of obesity is reported to promote insulin resistance, diabetes, metabolic syndrome, cardiovascular disease, hyperglycemia, and blood pressure by inducing modifications in the endocrine function of adipose tissues (4).

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Obesity is caused by the accumulation of intracellular triglycerides (TG) in adipocytes owing to the differentiation and adipogenesis of preadipocytes. Therefore, controlling the adipogenic mechanism is an effective treatment for suppressing obesity. Orlistat, the most used obesity treatment, is known to help in antiobesity by blocking the absorption of approximately 30% of ingested fat by inhibiting the activity of lipase, a lipolytic enzyme secreted from the pancreas (5-8). However, side effects such as insomnia, increased blood pressure, vomiting, abdominal distension, constipation, diarrhea have been reported after long-term use; thus, a therapeutic agent with proven safety and efficacy is an urgent need. Recently, many studies on the development of antiobesity materials using natural substances have focused on exploring factors with potential use as a natural lipase activity inhibitor (9-12). Carbohydrates are first decomposed by amylase, decomposed into oligosaccharides and disaccharides, and arrive in the small intestine, where they are decomposed into monosaccharides by enzymes of each substrate and absorbed into the body. Briefly, α-glucosidase decomposes carbohydrates contained in the diet into glucose by acting as a catalyst in the final stage of digestion (13, 14, 15).

Therefore, if α-glucosidase activity is inhibited, the breakdown of disaccharides into monosaccharides is inhibited, thereby delaying the absorption of sugar and mitigating postprandial blood sugar increase, reducing the absorption of excess carbohydrates into the body, and preventing obesity (16, 17). Thus, interest in antiobesity and natural products or foods having the effect of controlling sugar absorption and digestion and inhibiting fatty acid absorption is increasing (18, 19). Wheat germ is a by-product of milling wheat. Only pure wheat germ can be separated; thus, it is easy to supply and demand. It is mainly used for feed or discarded because the oil component of wheat germ causes rancidity and its bitter taste affects flavor. However, it has high value as a food additive because it accounts for 2%–3% of wheat grains and is rich in nutrients such as alpha-tocopherol (vitamin E), vitamin B complex, dietary fiber, protein, and minerals (20, 21). Studies have reported that wheat germ has antioxidant (22), antibacterial (23, 24), anti-inflammatory (25), immunity-enhancing (26), and anticancer effects (27). A study also reported that wheat germ is valuable health functional food (28). However, studies on the antiobesity efficacy of wheat germ are still lacking. Previous studies have reported that wheat germ extracts contain large amounts of linoleic acid, which is a major body fat reduction and anti-diabetic component (29, 30). This study aimed to confirm the antiobesity efficacy of wheat germ extracts, which was obtained during the wheat milling process, as a functional food material by hot-water extraction; thus, antioxidant, antibacterial, and antiobesity efficacy experiments were conducted on wheat germ extracts.

MATERIALS AND METHODS

Materials: The wheat germ extracts used in this experiment were provided by Daehang Flour Co., Ltd. in November 2021.

Preparation of wheat germ extracts: For the hot-water extraction of wheat germ, 30 g of wheat germ powder was used, the solvent was 300 mL of water and ethanol, and the ethanol contents were 20%, 50%, and 80%. The sample was extracted using a high-pressure hot-water extractor (KSP-240L, KYUNGSEO E&P, Incheon, Korea) at 80°C and a pressure of 0.06 MPa for 3 h. The extracted solution was filtered under reduced pressure (DOA-P704-AC, GAST Manufacturing Inc., USA) with a 5–8 μm filter (20 HM Hyundai Micro, Korea) and concentrated under reduced pressure (EYELA N-1300, Shanghai EYELA Co., China) and kept refrigerated at 4°C. In addition, the extract yield (%) of the extracted sample was calculated by formula (1) using the mass of the extracted sample after filtration under reduced pressure containing ethanol and the weight of the concentration after ethanol had completely evaporated.

$$\text{Extract yield (\%)} = \frac{Mass\ after\ concentration}{Mass\ after\ decompression} \times 100 \qquad (1)$$

Gas chromatography–mass spectrometry (GC-MS) analysis of wheat germ extracts: The active components of the hot-water extract of the wheat germ were analyzed using GC-MS. The column (Agilent 19091S-433UI: 1456957H, Agilent technology, USA) used for active ingredient analysis was HP-5ms (30 m × 250 μm × 0.25 μm). The carrier gas was helium, and the pressure and flow rate were set to 7.0699 psi and 1 mL/min, respectively. The temperature range of the column was set to –60°C–325°C, and the oven temperature was 40°C–300°C for 45 min (31).

2,2-diphenyl-1-picryhydrazyl (DPPH)-scavenging activity assay for wheat germ extracts: The antioxidant effect of wheat germ extracts was confirmed by measuring the DPPH-scavenging ability. The antioxidant efficacy of wheat germ extracts was analyzed using DPPH standard sample (Alfa Aesar, MA, USA) (free radical) and 95% powder. Moreover, 3 g of all extracts of 20%, 50%, and 80% ethanol content were diluted in 100 mL of methanol and then diluted again in methanol at concentrations of 5%, 10%, 15%, 20%, and 30%. A 1.5 mM DPPH solution was dissolved in methanol, and the diluted wheat germ extracts and ethanol were added at a ratio of 6 (600 μL):3 (300 μL):1 (100 μL) and reacted for 15 min in a shaded dark room. After the reaction, absorbance was measured at 517 nm with a UV/Vis spectrophotometer (KLAB, Deajeon, Korea). As for the control ascorbic acid, 0.1 mg/mL was diluted with the same methanol ratio as the sample, and the experiment was conducted under the same conditions. The inhibition rate for the DPPH-radical–scavenging activity was calculated using formula (2), and the IC_{50} value was converted into the inhibition rate value.

$$\text{Inhibition (\%)} = \frac{Abs_{(control)} - Abs_{(sample)}}{Abs_{(control)}} \text{x } 100 \qquad (2)$$

($Abs_{sample} = Abs_{test} - Abs_{color}$)

Analysis of the antibacterial efficacy of wheat germ extracts: *Staphylococcus aureus* is an aerobic or facultative anaerobic Gram-positive bacterium that forms colonies on human skin or mucous membranes and causes very common infections in humans because of its high carrier rate. *S. aureus* is a gram-positive cocci that produces many pathogenic factors, such as enterotoxin, coagulase, leukocidin, and staphylococcal protein A. It not only causes skin infectious diseases such as pneumonia but also occurs in many organs such as enteritis, abscess, gastroenteritis, endocarditis, toxin syndrome, and sepsis and recently acts as an etiological factor causing endogenous atopic dermatitis (32, 33). For the antibacterial activity test, the ASTM E2149-20 standard test method was applied. *S. aureus* (ATCC 6538) obtained from the National Institutes of Health was test sterilized for 60 min in an autoclave (121°C ± 2°C) for the strain used to measure antibacterial activity. Phosphate buffer (pH 7.2, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) was set to 50 mL, and the weight of the sample was set to 1.0 mL for analysis.

Analysis of lipase activity inhibition of wheat germ extracts: Lipase, a lipolytic enzyme secreted from the pancreas, is an enzyme that hydrolyzes TG into glycerol and one fatty acid. Lipase activity inhibition is a very useful test method for predicting the antiobesity activity of a sample (34). In this study, the ability of wheat germ extracts to inhibit lipase activity was measured: 0.1 mL of the sample solution in 0.85 mL of buffer (pH 7.0) with 1 M HCl was added to a mixture of 100 mM Tris and 5 mM CaCl2, 0.9 mL of 1 mM EDTA and 0.9 mL of 1 mM MOPS, and 0.009 g of lipase (L3126 -25G, Sigma Aldrich, St. Louis, MO, USA) was added and centrifuged at 13,500 rpm for 5 min, only the supernatant was taken, and 0.06 mL of the enzyme buffer solution was added and reacted at 37°C for 15 min with the substrate solution 10 mM 4-Nitrophenyl. After adding 0.02 mL of butyrate (N9876-1G, Sigma Aldrich) and reacting at 37°C for 15 min, absorbance was measured at 400 nm. In the positive control group, the experiment was conducted under the same conditions using orlistat.

The lipase activity inhibition rate was calculated by substituting the control group without the addition of the sample solution and the experimental group with the addition of the sample solution into formula (3).

$$\text{Inhibition (\%)} = \left(1 - \frac{Sample\ \text{O.D}}{Sample\ \text{O.D}}\right) \text{x } 100 \qquad (3)$$

Analysis of the α-glucosidase activity inhibition of wheat germ extracts: Carbohydrate-degrading enzyme α-glucosidase inhibition was analyzed using the following method: 0.05 mL of enzyme solution in which 0.0012 g of α-glucosidase (G5003-100UN, Sigma Aldrich) was dissolved in 2 mL of 0.2 M potassium phosphate buffer (PH 6.8) was taken, and 0.05 mL of the sample and 0.05 mL of the buffer solution were added. After reacting at 37°C for 15 min, 0.1 mL of the substrate p-Nitrophenyl-α-Dglucopyranoside (Source BCCH4822, Sigma Aldrich) was diluted to a concentration of 0.5 mM and mixed in buffer, and again at 37°C, after reacting for 15 min, 0.04 g of NaOH was added to 10 mL of the buffer solution, and 0.05 mL was added to terminate the reaction at a concentration of 0.1 M. Then, absorbance was measured at 405 nm. Acarbose (A8980-1G, Sigma Aldrich), a positive control, was diluted in DMSO at a concentration of 0.05 M, and the experiment was conducted in the same manner. The inhibition rate of α-glucosidase was calculated using formula (3) for the control group without the addition of the sample solution and the experimental group with the addition of the sample solution.

RESULTS AND DISCUSSION

The values were marked as A for 20%, B for 50%, and C for 80% according to the ethanol content.

GC-MS analysis of wheat germ extracts: As shown in Tables 1–3, more active ingredients were detected as the ethanol content increased. Specifically, cyclotrisiloxane, an antioxidant, antibacterial, and active ingredient, was detected in extract A. In extract B, active ingredients such as hexadecanoic acid, conjugated linoleic acid, and 9, 12-octadecadienoic acid were detected. In extract C, in addition to hexadecanoic acid, conjugated linoleic acid, and 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid were additionally confirmed active ingredients (31).

Antioxidant efficacy analysis result of wheat germ extracts: The results of measuring the DPPH-radical–scavenging activity of wheat germ extracts are shown in Figure 1. The IC_{50} value of ascorbic acid used as the control was 2.16, and the IC_{50} value of extracts A, B, and C were 3.13, 2.91, and 2.64, respectively. As shown in Table 1, the IC_{50} value was lower than that of ascorbic acid in all samples, but extract C showed the highest antioxidant effect. This is expected because extract C contains antioxidant active ingredients such as 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, compared with extracts A and B.

Antibacterial efficacy analysis result of wheat germ extracts: The results of the antibacterial analysis of wheat germ extracts are shown in Table 2, and the bacterial reduction rate of each extract is shown in Figure 2. The results of the experiment revealed that the antimicrobial activity increased as the ethanol content increased; specifically, a high bacterial reduction rate of 75.7% was found in the 80% ethanol extract after 60 min. This is expected because extract C contains active antioxidant ingredients such as 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid compared with extracts A and B.

Lipase activity inhibition assay result of wheat germ extracts: The lipase activity inhibitory ability of wheat germ extracts is shown in Figure 3. All extracts showed lipase inhibitory activity: orlistat, 81.9% ± 1.17% (positive control); extract A, 76.0% ± 7.42%; extract B, 85.5% ± 5.01%; and extract C, 95.5% ± 3.26%. Specifically, extract C showed much higher lipase inhibitory activity than orlistat (positive control). This is expected because extract C contains 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, which are active ingredients for reducing body fat, compared with extracts A and B.

Table. 1 GC-MS chromatogram profile of *wheat germ* in 20% ethanol

Retention time (RT)	Name of the compound	Quality(%)	Area peak (%)
39.054	Cyclotrisiloxane	47	17.0

Table 2. GC-MS chromatogram profile of *wheat germ* in 50% ethanol

Retention time(RT)	Name of the compound	Quality(%)	Area peak (%)
25.267	Hexadecanoic acid	99	7.84
28.058	9,12-Octadecadienoic acid	99	53.3
28.139	Conjugated linoleic acid	95	38.8
39.054	Cyclotrisiloxane	47	18.0

Table 3. GC-MS chromatogram profile of *wheat germ* in 80% ethanol

Retention time (RT)	Name of the compound	Quality(%)	Area peak (%)
25.251	Hexadecanoic acid	99	8.33
28.206	9,12-Octadecadienoic acid	99	57.7
28.266	Conjugated linoleic acid	99	27.5
28.536	Linoleic acid ethyl ester	99	3.34
28.629	Linoelaidic acid	97	2.71
39.054	Cyclotrisiloxane	47	19.0

Table. 1 Half maximal inhibitory concentration (IC_{50})

No.	IC_{50}
Ascorbic acid	2.16
A	3.13
B	2.91
C	2.64

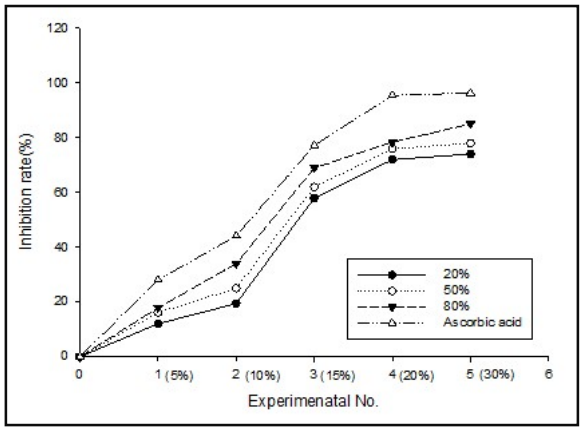

Figure. 1 Antioxidant ability according to extraction conditions

Table. 2 Results of the antimicrobial analysis of *wheat germ* extracts against *Staphylococcus aureus*

	A	B	C
Initial number of bacteria	2.8×10^6	2.8×10^6	2.8×10^6
After 60 min	1.6×10^6	1.2×10^6	6.8×10^5
Bacterial reduction rate	42.9	57.1	75.7

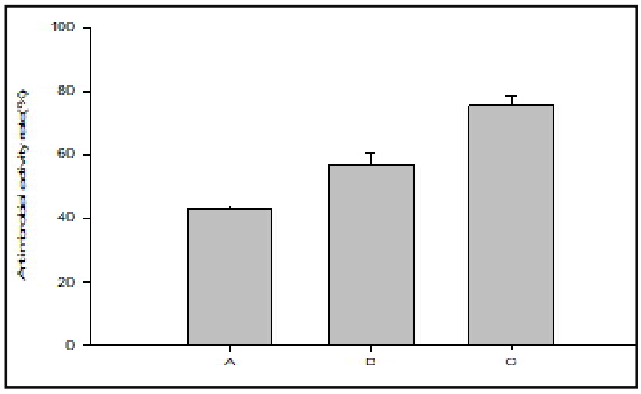

Figure. 2 Comparison of the antibacterial activity of *wheat germ* extracts against *Staphylococcus aureus*.

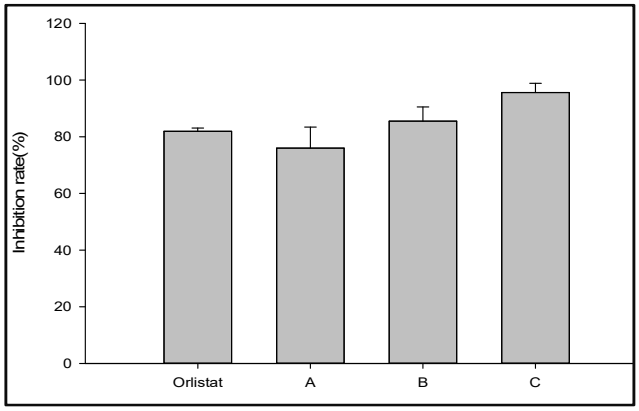

Figure. 3 Effect of *wheat germ* extracts on lipase inhibitory activity

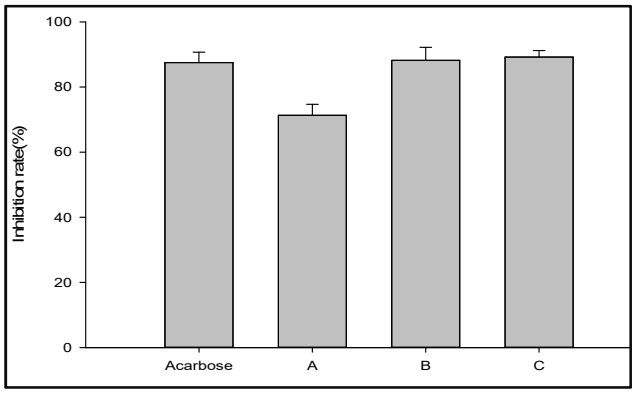

Figure 4. Effect of *wheat germ* extracts on α-glucosidase inhibitory activity

Assay result of the α-glucosidase activity inhibitory ability of wheat germ extracts: A shown in Figure 4, acarbose (positive control) had α-glucosidase activity inhibitory ability of 87.5% ± 3.21%; extract A, 71.3% ± 3.40%; extract B, 88.2% ± 4.00%; and extract C, 90.2% ± 2.12%. All extracts showed α-glucosidase inhibitory activity. Specifically, extract C exhibited higher α-glucosidase inhibitory activity than acarbose (positive control). This is expected because extract C contains 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, which are active ingredients for reducing body fat, compared with extracts A and B.

Conclusion

The results of the experimental analysis of the hot-water extract according to the ethanol content of wheat germ are as follows:

- Extract A contains the antioxidant, antibacterial, and active ingredient cyclotrisiloxane. Extract B contains active ingredients such as hexadecanoic acid, conjugated linoleic acid, and 9,12-octadecadienoic acid. In addition to hexadecanoic acid, extract C contained conjugated linoleic acid, 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid.
- As a result of measuring the DPPH-radical–scavenging ability of wheat germ extracts, the IC_{50} value of ascorbic acid used as a control group was 2.16, whereas the IC_{50} values of extract A, B, and C were 3.13, 2.91, and 2.64, respectively. The DPPH-radical–scavenging ability was lower in all samples than in ascorbic acid, but antioxidants showed concentration-dependent effects. This is expected because extract C contains antioxidant active ingredients such as 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, compared with extracts A and B.
- The A result of the antibacterial analysis of wheat germ extracts revealed that the antibacterial activity increased as the ethanol content increased; specifically, 80% ethanol extract showed a high bacterial reduction rate of 75.7% after 60 min. This is expected because extract C contains antioxidant active ingredients such as 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, compared with extracts A and B.
- In the analysis of the lipase activity inhibitory activity of wheat germ extracts, all extracts showed lipase inhibitory activity. Extract C showed much higher lipase inhibitory activity than orlistat (positive control). This is expected

because extract C contains 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, which are active ingredients for reducing body fat, compared with extracts A and B.

- In the analysis of the α-glucosidase activity inhibitory activity of wheat germ extracts, all extracts showed α-glucosidase inhibitory activity. Extract C exhibited higher α-glucosidase inhibitory activity than acarbose (positive control). This is expected because extract C contains 9,12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid, which are active ingredients for reducing body fat, compared with extracts A and B.

In this study, the wheat germ hot-water extract was confirmed to have antibacterial activity against *S. aureus*, α-glucosidase activity inhibitory effect, and lipase activity inhibitory effect. Therefore, the wheat germ hot-water extract may be applicable as an antiobesity food ingredient.

Acknowledgements

This work supported by National Research Foundation of Korea (NRF-2020R1F1A1074571).

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