

Nutrition profile, antioxidant, and inhibitor alpha-glucosidase effect of hanjeli (*Coix lacryma-jobi* L. var. *ma-yuen*) seed extract

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Abstract

Hanjeli, a functional food plant still rarely known to the general public, with the Latin name *Coix lacryma-jobi* L with the variant *ma-yuen*. Hanjeli has fruit seeds that can be used as a nutritious food source and have biological activity. This study aimed to record the nutritional content of hanjeli seeds and evaluate the antioxidant activity and alpha-glucosidase inhibitor activity as antidiabetic. Hanjeli seeds were evaluated for their nutritional profile by analyzing the proximate, mineral, phytoconstituent, and total phenolic and flavonoid contents of hanjeli seed extract. The extract was tested for antioxidant activity with DPPH radical reduction, ABTS and iron reduction (FRAP) parameters, and antidiabetic evaluation with alpha-glucosidase inhibition parameters. The results of the nutritional content analysis show that the macronutrients and micronutrients (minerals Na, K, Ca, Mg, and Fe) were by Indonesia Standard National. Hanjeli seed extract contains phytoconstituents in phenolics, flavonoids, saponins, alkaloids, and terpenoids. Total phenolic and flavonoid levels were 98.17 ± 0.053 mg EGA/g extract and $15.36 \pm 0,068$ mg EQ/g extract, respectively. Hanjeli seed extract has antioxidant potential in reducing DPPH and ABTS radicals with an IC_{50} of 662.69 ± 2.77 and 164.053 ± 0.156 μ g/mL, respectively, and an iron-reducing power (FRAP assay) of 70.21 ± 0.93 μ M/g extract. Hanjeli seed extract showed strong potential in inhibiting alpha-glucosidase with an IC_{50} value of 84.47 μ g/mL. The nutritional content and biological activity of hanjeli seeds showed promising potential to be developed as functional food products for the public.

1. Introduction

The availability of food sources is a problem in several developing countries, including Indonesia. This case is based on the increasing need for food raw material sources, so the price of raw materials has increased, which causes problems related to nutritional imbalance (Rozaki, 2021). Nutritional problems such as malnutrition, stunting, and wasting are dietary problems, especially on the Southeast Asian continent. Based on data from the World Health Organization (2018), the Southeast Asian continent has 91% of cases of stunting and wasting, 73% of overweight children, and 82% of children with low birth weight (Estecha Querol *et al.*,

2021). Therefore, several researchers are interested in developing functional foods with good nutritional adequacy and a dual effect in preventing diseases such as diabetes mellitus. As far as possible, raw materials used as functional food sources do not cause other effects so sustainable use can be well accepted. Therefore, to overcome the problem of unbalanced nutrition, several developments are needed that come from natural ingredients that can function as functional foods that have good nutritional content to fulfill balanced nutrition and have beneficial biological effects such as preventing and even treating diabetes (Konstantinidi and Koutelidakis, 2019).

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Hanjeli or *Coix lacryma-jobi* L. is a cereal plant from the Poaceae family that originates from tropical and subtropical regions in Asia and has been used as food since prehistoric times (Juhaeti, 2015; Kang *et al.*, 2018). Besides food, hanjeli has also been used as a tea and traditional medicine. Hanjeli is widespread on various islands in Indonesia, such as Sumatra, Kalimantan, Java, Sulawesi, Nusa Tenggara, Papua, and the Maluku Islands (Fauzi *et al.*, 2021). Therefore, hanjeli has various local names such as hajeli, barley, jali, japen or jelim, jole, kasa lore, and jejeane. Meanwhile, hanjeli in other countries is known as Job's tears (Australia and England), hatomugi (Japan), adlay (Philippines), damu daud (Arabic), and mayuen (China) (Wicaksono *et al.*, 2022).

Two variants of hanjeli are commonly found in Indonesia, namely the -ma-yuen and stenocarpa variants (Wicaksono *et al.*, 2022). The ma-yuen variant of Hanjeli is a variant that has a soft seed shell so that the seeds are mostly used for consumption for the seed part (Fauzi *et al.*, 2021; Wicaksono *et al.*, 2022). The ma-yuen variant of hanjeli seeds has also been used for generations as a medicine for various gastrointestinal disorders and anti-inflammatory and respiratory diseases. The ma-yuen variant is a type of hanjeli that is widely cultivated by the people of Bandung Regency, especially in Rancaekek, Punclut, Tanjungjaya, Cicalengka, and Cipongkor (Fauzi *et al.*, 2021). Meanwhile, the stenocarpa variant of hanjeli, or what can be called the stone type, is a plant that grows wild in dry lands and is planted in yards as decoration and is more often used as a craft or ornament such as prayer beads or hanging curtains. This plant is still conventionally cultivated as a rare plant by farmers. It is sporadically found in Sumedang, Garut, Ciamis, Indramayu, Sukabumi, and Bandung (West Java Province in Indonesia) (Fauzi *et al.*, 2021).

The ma-yuen variety of hanjeli has high levels of amylopectin, a polysaccharide compound that provides easily digestible energy. Hanjeli ma-yuen is reported to have a lower carbohydrate content than rice, corn, millet, and sorghum, namely 76.4%, and a higher fat, protein, and vitamin B1 content (Bajaj *et al.*, 2018; Weng *et al.*, 2022). Based on research conducted by Saragih (2018), the increase in blood glucose levels in subjects who consumed white rice was 39.83 mg/dL, while subjects who consumed ma-yuen hanjeli seeds experienced a rise in blood glucose of only 13.42 mg/dL. These results indicate that hanjeli ma-yuen seed cereal produces lower blood glucose release. In addition, hanjeli ma-yuen seeds are gluten-free, so they have the potential to be used as food for diabetes patients (Comino *et al.*, 2013). Additional information also states that hanjeli ma-yuen

seeds contain polyphenolic and flavonoid compounds (Choi *et al.*, 2017), where these compounds are compounds that have the potential to act as antioxidants and inhibit alpha-glucosidase. Based on research by Wang *et al.* (2016), the ethanol fraction of hanjeli ma-yuen seeds contains several flavonoid compounds, one of which is rutin and quercetin (Al-Ishaq *et al.*, 2019). Flavonoid compounds can be alpha-glucosidase inhibitors that work in a reversible competitive manner because these compounds have a chemical structure similar to natural glucosidase substrates (Xiao, 2022). This study explored the benefits of hanjeli seeds as a functional food rich in nutrients and antioxidants. It can be used as a substitute for rice for people with diabetes mellitus.

2. Materials and methods

2.1 Materials

Ethanol p.a. (Merck, Germany); methanol p.a. (Merck, Germany); alpha-glucosidase enzyme from *Saccharomyces cerevisiae* (Sigma Aldrich, United States); p-Nitrophenyl- α -D-Glucopyranoside (pNPG) (Sigma Aldrich, United States); bovine serum albumin (Sigma Aldrich, United States); acarbose (Sigma Aldrich, United States); sodium carbonate (Merck, Germany); potassium dihydrogen phosphate (Merck, Germany); sodium hydroxide (Merck, Germany); purified water (Brataco); DMSO (Merck, Germany); sulfuric acid (Merck, Germany); aluminum chloride (Merck, Germany); iron (III) chloride (Merck, Germany); anhydrous acetic acid P (Merck, Germany); ethanol 96%; hydrochloric acid (Sigma Aldrich); zinc powder; magnesium powder; ethyl acetate (Merck, Germany); gelatin (Merck, Germany); ferric chloride; sodium sulfate (Merck, Germany); benzene (Merck, Germany).

2.2 Sample preparation

Seeds from hanjeli plants were collected from Padjadjaran University, West Java (6°55'17.8"S 107° 46'24.1" E). Wet sorting was carried out by separating impurities or other foreign materials that had to be removed. Washing was done to clean and remove dirt that sticks to the plants. Dry sorting was then done to ensure no dirt or other unwanted contaminants were present. Hanjeli seed powder was made by grinding dry seeds using a grinder. The aim of grinding simplicia into powder was to increase the efficiency of the extraction process.

2.3 Macronutrient analysis

2.3.1 Protein content analysis

The hanjeli seed powder (1 g) was put into a 100 mL

Kjedhal flask followed by the addition of 2 g of the selene and 25 mL of concentrated H₂SO₄. The mixture was heated over an electric heater until it boiled and the solution turned greenish. The solution was then cooled and transferred into a 100 mL volumetric flask. The test sample was diluted with distilled water to the limit mark. Then, 10 mL of solution was taken and put into a distiller, and 10 mL of 40% NaOH was added along with a few drops of phenolphthalein indicator. Next, it was distilled for approximately 10 mins. As a reservoir, 10 mL of 2% boric acid solution mixed with phenolphthalein indicator was used. Next, the mixture was titrated with 0.01 N HCl solution (2.3.2 1992). The total protein content was determined using Equation 1.

$$\text{Protein total} = \frac{\text{Volume (titration)} \times 0.014 \times \text{conversion factor}(6.25)}{\text{Sample weight}} \times 100\% \quad (1)$$

2.3.2 Carbohydrates total analysis

Determination of carbohydrate levels was carried out following the procedure by the Indonesia Nasional Standard (SNI 01-2891-1992). A volume of 50 mL of sample was taken into an Erlenmeyer flask and added with 200 mL of 3% HCl. The mixture was heated at reflux with a hotplate temperature of 100°C for 1.5 hrs, then cooled and added with 0.5 mL phenolphthalein indicator and 30% NaOH until the color of the solution changed to pink. Then, 3% acetic acid solution was added until the solution's color became clear. The mixture was put into a 500 mL flask, 10 mL was taken, and 25 ml of Luff Schoorl was added. The mixture was boiled under reflux for 12 mins at a hotplate temperature of 200°C, then cooled, and 15 mL of 20% KI and 25 ml of 25% H₂SO₄ were added. Next, titration was carried out with 0.1N Sodium Thiosulfate until a yellow color was formed, and 0.5 mL of 1% starch indicator was added. The titration was continued until the end point of titration (TAT) was milky white (Standar Nasional Indonesia, 1992).

2.3.3 Fat content analysis

Determination of total fat in hanjeli seeds was carried out using the Indonesian National Standard method SNI 01-2891-1992. Hanjeli seed powder was extracted using the Soxhlet method. The extraction flask on a Soxhlet device was dried in an oven at 105°C for 1 hr and weighed empty. A set of Soxhlet tools was prepared and installed first, then the sample was placed in the Soxhlet flask, and n-hexane solvent was added in a ratio of 1:10. Next, the bath was turned on, and extraction continued for 5 hrs, then continued with the solvent evaporation process for 1 hr. The extracted fat flask was placed in an oven at 105°C and weighed until constant (Standar Nasional Indonesia, 1992). Total fat content was calculated using the equation 2:

$$\text{Fat content} = \frac{\text{weight of Soxhlet flask and fat} - \text{weight of empty flask}}{\text{Sample weight}} \times 100\% \quad (2)$$

2.3.4 Crude fiber analysis

Fiber content was analyzed based on Standar Nasional Indonesia (SNI) 01-2891-1992. The residue from Soxhlet extraction from the fat content analysis was dried, weighed 4 g of the sample, and put into an Erlenmeyer flask. The sample was added with 50 mL of 1.25% H₂SO₄ and then boiled for 30 mins using an upright cooler. Approximately 50 mL of 3.25% NaOH was added and boiled for 30 mins. The filtrate was filtered hot using a Buchner funnel and Whatman filter paper (Note: the filter paper was dried and weighed). The residue on the filter paper was washed successively with 1.25% H₂SO₄, hot water, and 96% ethanol. Lift the filter paper and its contents, then weigh, then dry in the oven (105°C), then cool and weigh until the weight remains constant (Standar Nasional Indonesia, 1992). Crude fiber content was calculated using the equation 3:

$$\text{Crude fiber} = \frac{\text{Residue weight on filter paper}}{\text{Sample weight}} \times 100\% \quad (3)$$

2.3.5 Water content analysis

Determination of water content was carried out using the thermogravimetric method SNI 01-2354-2006. Place the empty crucible in the oven for at least 2 hrs. The crucible was moved into a desiccator for 30 mins until it reached room temperature, and then the empty weight was weighed. The crushed sample (2 g) was placed into the crucible. The crucible containing the sample was placed in the oven at 105°C for 16-24 hrs. The crucible was moved into a desiccator for 30 mins and weighed until the weight was constant (SNI, 2006). Water content was calculated using the equation 4:

$$\text{Water content} = \frac{\text{Wet sample weight} - \text{dry sample weight}}{\text{Wet sample weight} - \text{weight of empty crucible}} \times 100\% \quad (4)$$

2.3.6 Ash content analysis

Determination of ash content was carried out using the thermogravimetric method SNI-01-2354.2-2006. Place the empty crucible in the oven for at least 2 hrs. The empty crucible was transferred to a desiccator for around 30 mins until it reached room temperature, and then the empty weight was weighed. The sample was put into a crucible and then oven at 100°C for 24 hrs. The sample was transferred to the furnace by gradually increasing the temperature until it reached 550°C and maintained for 8 hr until white ash was obtained. The furnace temperature is reduced to around 40°C. Then, the crucible was removed and placed in a desiccator for 30 mins. Weigh immediately after cooling (SNI, 2006). Ash content was calculated using the equation 5:

$$\text{Ash content} = \frac{\text{Weight of ashes and crucibles} - \text{crucibles empty}}{\text{Sample weight}} \times 100\% \quad (5)$$

2.3.7 Micronutrients analysis

Analysis of micronutrient content (Na, K, Ca, Mg, and Fe) by following the procedure SNI 01-2891-1992. Approximately 20 g of hanjeli seed powder was put into an Erlenmeyer glass and added with 5 mL of concentrated HNO₃ and 1 mL of concentrated H₂SO₄. The sample was left for 24 hrs, and then 2-3 drops of HCl and HNO₃ solution (2:1) were added. Next, the solution was heated until brownish at a low temperature and reheated at 150°C to 200°C until the solution became yellow and clear. After cooling, the sample was added with concentrated HNO₃ and distilled water to 100 mL while stirring. The filtrate was filtered and then analyzed for mineral content using an Atomic Absorption Spectrophotometer (AAS) (SNI, 1992).

2.4 Extraction

Extraction of hanjeli seed powder was carried out using the maceration method. The extraction process was carried out separately with the same solvent and hanjeli dry powder ratio. The solvent used was 70% ethanol, with a dry sample and solvent ratio of 1:10. The weight of the dry sample used for each extraction was 100 g. Maceration extraction was carried out in three cycles, where one cycle was carried out for 24 hrs with occasional stirring. The extraction results from each method are filtered using Whatman paper to separate the filtrate from the residue. The filtrate was collected in an Erlenmeyer glass, and the remaining solvent was removed by evaporation using a rotary vacuum evaporator and a water bath to obtain an extract with a constant weight. Extract yield can be calculated using Equation 6 (Nur et al., 2022).

$$\% \text{Yield} = \frac{\text{Extract weight}}{\text{Hanjeli seed weight}} \times 100\% \quad (6)$$

2.5 Phytochemical screening

2.5.1 Alkaloid test

A total of 50 mg of thick extract was put into a test tube and dissolved in 1 mL of 2N HCl and 9 mL of water. Next, the test tube was placed in a beaker filled with hot water and heated for two mins. After the solution was cooled and filtered, the filtrate was collected, and the filtrate was used as a test solution. A total of three test tubes with 1 mL of the filtrate were prepared and added with two drops of Bouchardatt's reagent. If a black or brown precipitate forms, the test solution is positive for containing alkaloids. In the second test tube, two drops of Mayer's reagent were added to 1 mL of the filtrate, and the formation of a yellow or white lumpy precipitate, which was soluble in methanol, showed that the sample was positive for alkaloids. In the third reaction tube, 1 mL of the filtrate was added with two drops of Dragendorff Reagent where

the sample showed positive for alkaloids if an orange-brown precipitate was formed (Harborne, 1998).

2.5.2 Flavonoid test

A total of 50 mg of thick extract was dissolved in 3 mL of 96% ethanol, then 100 mg of Zn powder and 2 mL of 2N HCl were added. The mixture was left for one min, then ten drops of concentrated HCl were added and left for five mins. Positive results are indicated in intensive red. Apart from that, the formation of a purple-red to orange-red color indicates positive flavonoids, while the orange-yellow color indicates positive flavonoids such as flavone, aurone, and chalcone (Harborne, 1998).

2.5.3 Phenolic test

A total of 50 mg of extract was dissolved in 15 mL of hot distilled water, then stirred and cooled. After that, filtration was carried out, and 1 mL was taken. Then, two drops of 5% FeCl₃ solution were added, and the color change to blue or violet-green was observed, which indicated that the sample was positive for containing phenolics (Harborne, 1998).

2.5.4 Terpenoid test

A total of 100 mg of thick extract was dissolved in 3 mL of ethyl acetate, and then Lieberman-Bouchard reagent was added, which is a mixture of anhydrous acetic acid and concentrated sulfuric acid in a ratio of 2:1. The formation of a red-green or violet-blue color indicates positive results contained terpenoid (Iqbal et al., 2015).

2.5.5 Tanin test

A total of 50 mg of extract was dissolved in 15 mL of hot distilled water and cooled. The filtrate was filtered, and 1 mL each was taken into two test tubes to be treated differently as follows: In the first reaction tube, 3 mL of 10% gelatin solution was added and then observed for the formation of a white precipitate. In the second test tube, 3 mL of NaCl-gelatin solution was added and a white precipitate was observed, which showed it was positive for containing tannin (Iqbal et al., 2015).

2.5.6 Saponin test

The saponin identification process was done by dissolving 50 mg of the extract in 10 mL of hot distilled water in a test tube. Shake until foam was formed as high as 1 to 10 cm and does not disappear after adding one drop of 2N HCl, indicating the presence of saponin (Harborne, 1998).

2.5.7 Anthraquinones test

A total of 20 mg of thick extract was dissolved in 5 mL of 2N sulfuric acid, heated, and cooled. Next, 10 mL of concentrated benzene was added to the solution, shaken, and left to stand. The benzene layer formed was separated and then filtered. The benzene layer was shaken with 1-2 mL of 2N NaOH and allowed to stand. Samples positive for containing anthraquinone were indicated by the formation of a colorless benzene layer and an intense red water layer (Iqbal *et al.*, 2015).

2.5.8 Glycoside test

A total of 300 mg of extract was diluted with 3 mL of 10% HCl solution, then heated to boiling, cooled, and filtered. The filtrate obtained was washed using 10 mL of petroleum benzene and repeated thrice. The washed filtrate was collected and added with anhydrous sodium sulfate. The results were then filtered, and the filtrate obtained was added with 2 mL of methanol. The mixture was added with five drops of Molisch reagent. Next, 2 mL of concentrated sulfuric acid was added, and the color formed was observed. If a purple ring is at the liquid boundary, the sample is positive for glycosides (Iqbal *et al.*, 2015).

2.6 Phenolic and flavonoid total content

In determining the total phenolic content in the ethanol extract of *Hanjeli* using the procedure described by Nur *et al.* (2023a) with slight modifications. The gallic acid solution as standard with various concentrations (4, 5, 6, 7, 8, dan 9 µg/mL) and hanjeli seed extract solution (800 µg/mL) were taken in 1 mL each, and 5 mL of Folin-Ciocalteu reagent was added. Next, the mixture was incubated at room temperature for 8 mins. Then, 4 mL of NaOH was added to the test solution and incubated at room temperature for 1 hr. The absorbance of standard solutions and samples was measured with the UV/Vis Spectrophotometer instrument (T80+, PG Instrument Ltd, UK) at 635 nm. The blank solution was prepared without adding the test solution. The total phenolic content was expressed as mg Gallic Acid Equivalent per gram of extract.

Determination of total flavonoid levels in Hanjeli seed ethanol extract was carried out colorimetrically using the procedure from Nur *et al.* (2019). In this study, Quercetin was used as a standard to create a standard curve with a series of levels of 4, 5, 6, 7, 8, and 9 µg/mL. 10 mg of hanjeli seed extract was dissolved in 10 mL of 70% ethanol in a volumetric flask. Approximately 0.5 mL of each quercetin concentration series and sample solution was taken and added with 1.5 mL of ethanol. Next, each mixture was added with 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M sodium acetate,

and the volume was increased with distilled water to 5 mL. Each test mixture was incubated at room temperature for 30 mins. Absorbance measurements of standard solutions and samples were carried out using a UV/Vis Spectrophotometer instrument (T80+, PG Instrument Ltd, UK) at 430 nm. A blank solution was prepared without adding the test solution. Total flavonoid content was expressed as mg Quercetin Equivalents (QE) per gram of extract. Total phenolic and flavonoid levels were calculated using the following formula (Nur *et al.*, 2019):

$$\text{Total phenolic or flavonoid content} = \frac{C \times F_p \times V}{\text{Sample Weight}} \times 100\% \quad (7)$$

2.7 Antioxidant evaluation

2.7.1 Anti-radical DPPH assay

DPPH radical inhibitory activity was carried out according to the procedure of Nur *et al.* (2023a). Hanjeli seed extract was weighed in 50 mg and dissolved in 70% ethanol to 50 mL (1000 µg/mL). Volumes of 0.75, 1.50, 2.25, 3.00, and 3.75 mL of hanjeli extract solution were taken and reacted with 1 mL of 0.4 mM DPPH solution. The volume of the mixture was increased with pro-analysis ethanol to 5 mL in a volumetric flask. The final concentrations of the sample solution were 150, 300, 450, 600, and 750 µg/mL. The mixture was incubated at room temperature and in a dark place for 30 mins. The absorbance of each sample solution was measured at 516 nm with a UV-Vis spectrophotometer (T80+, PG Instrument Ltd, UK).

2.7.2 Anti-radical ABTS assay

Determination of antioxidant activity in reducing ABTS radicals was carried out according to the method of Nur, Aswad, Yulianty *et al.* (2023) and Nur *et al.* (2023b). A mixture of 1:1 ABTS (7 mM in 10 mL distilled water) and potassium persulfate (2.45 mM in 10 mL distilled water) was incubated in the dark for 12-16 hrs to form an ABTS radical solution. The solution mixture was then diluted with ethanol to 50 mL. ABTS working solution is prepared by mixing it with ethanol (1:10). Approximately 1 mL of ABTS working solution was diluted to 5 mL with ethanol and incubated for 30 mins as a blank solution. Absorbance was measured with a UV-visible spectrophotometer (745 nm; Shimadzu UV-1900). Varying concentrations of each Hanjeli extract were reacted with 1 mL of ABTS working solution, and the volume was made up to 5 mL with ethanol. Absorbance measurements at 745 nm were carried out after the mixture was incubated for 30 mins in the dark at room temperature.

The antioxidant strength of each sample solution in the DPPH and ABTS assay was expressed by the value of 50% inhibitor concentration (IC₅₀). The inhibition

percentage and IC₅₀ value of each sample were calculated as follows in Equation 8:

$$\text{Inhibition (\%)} = \frac{\text{Abs.blank} - \text{Abs.sample}}{\text{Abs.blank}} \times 100\% \quad (8)$$

2.7.3 Iron reduction power by ferric reducing antioxidant power assay

The antioxidant activity of hanjeli seed extract in reducing iron was carried out using the Ferric Reducing Antioxidant Power (FRAP) testing method (Abbas *et al.*, 2022). FRAP reagent was made by reacting acetate buffer (pH 3.6), Tris Pyridyl Triazine (TPTZ, 1 mM), and FeCl₃ (0.02 M) in a ratio of 10:1:1. Each extract solution (0.1% w/v) was mixed with 2 mL of FRAP reagent, and distilled water was added to make up the volume (5 mL). Incubation at room temperature for 30 mins was followed by an absorbance measurement at 595 nm (Shimadzu UV-1900). The FRAP value for each extract was calculated using quercetin as the standard curve. The quercetin equivalent value per gram of extract ($\mu\text{MFSV/g}$ extract) was used to estimate its antioxidant capability (Nur *et al.*, 2019).

2.8 Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibitory activity of hanjeli seed extract was carried out by following the established procedure of Nur, Wierson, Sami *et al.* (2021). Hanjeli seed extract (10 mg) was dissolved with 1 mL of phosphate buffer (pH 6.8) in a microtube (10,000 $\mu\text{g/mL}$). The extract solution was diluted with buffer to 1000 $\mu\text{g/mL}$ by taking 100 μL and diluted with phosphate buffer to 1 mL. A series of extract solution concentrations of 200, 300, 400, 500, and 600 $\mu\text{g/mL}$ were made, and 30 μL of each concentration series was taken and put into the well, then 36 μL of phosphate buffer solution and 17 μL of pNPG substrate (5 mM) were added. The solution was incubated for 5 mins at 37°C. Then, 17 μL of alpha-glucosidase solution (0.12 Unit/mL) was added and set for 15 mins at 37°C. After incubation, 100 μL of 200 mM sodium carbonate was added to the test solution to stop the reaction between the enzyme and the substrate. In this study, the final concentrations of the sample extract were 30, 45, 60, 75, and 90 $\mu\text{g/mL}$. The absorbance of the test solution was measured at a wavelength of 405 nm using a microplate reader.

2.9 Data analysis

Data from determining total phenolic and flavonoid levels and their activity as anti-alpha-glucosidase were produced as mean \pm SD using Microsoft Excel 19 software.

3. Results and discussion

The distribution of hanjeli plants worldwide, especially in Asia, has become abundant and is used as a staple food ingredient. In Indonesia, especially in Sumedang Village, West Java Province, it is cultivated as a food ingredient whose nutritional value is no less than that of the main food, rice (Mayasti *et al.*, 2021). Although several previous studies have explained many facts about the nutritional content of hanjeli seeds, in this study, it is deemed necessary to provide additional information regarding the important nutritional content of hanjeli seeds cultivated in Sumedang Village, West Java. This study also provides an overview of the benefits of hanjeli seeds, which can be used to prevent and treat diabetes mellitus.

The nutritional content of hanjeli seeds is shown in Table 1. The nutritional contents of Hanjeli seeds were evaluated in terms of macronutrients and micronutrients. Macronutrient analysis includes water content, ash content, carbohydrate content, protein, total fat, and crude fiber. Meanwhile, micronutrients were evaluated for mineral levels in the form of sodium (Na), potassium (K), calcium (Ca), magnesium (mg), and iron (Fe). Macronutrient evaluation showed that hanjeli seeds have a high carbohydrate content of 71.01% and protein of 21.37%.

Table 1. Nutritional component in hanjeli seeds.

	Parameters	Results
Macronutrients (%)	Water content	5.62 \pm 0.02
	Ash content	0.90 \pm 0.00
	Carbohydrate	71.01 \pm 0.79
	Protein	21.37 \pm 0.26
	Fat content	0.11 \pm 0.01
Micronutrients (mg/Kg)	Crude fiber	0.99 \pm 0.00
	Na	16.18 \pm 0.91
	K	863.81 \pm 10.23
	Ca	22.18 \pm 0.42
	Mg	20.72 \pm 0.08
	Fe	15.89 \pm 0.11

Meanwhile, the total fat content was quite low at 0.11%. Some research reports stated that the protein content of hanjeli seeds ranges from 20 to 31%. The results showed that the hanjeli seeds cultivated in Sumedang village had almost the same nutritional characteristics as the hanjeli seeds that had been reported previously (Mayasti *et al.*, 2021; Weng *et al.*, 2022; Wei *et al.*, 2023). The results of the macronutrient evaluation carried out in this study are slightly different from the research carried out by Mayasti *et al.* (2021), which showed that the protein and fat levels in hanjeli seeds are 13.00 \pm 0.12% and 2.44 \pm 0.14%, respectively. The protein content of hanjeli seeds in this study showed better

results than in previous research (Mayasti *et al.*, 2021), while the total carbohydrate content obtained showed similar results. Hanjeli seeds have also been reported to contain 18 types of amino acids, building blocks of complete protein (Liu *et al.*, 2015). The macronutrient content in the form of carbohydrates and protein in hanjeli seeds is relatively high, which is very supportive in fulfilling the nutrition needed in the body so that it can be used as a functional food source to fulfill nutritional imbalances but still be low in calories. This differs from other food sources, such as rice, which is reported to contain high calories (Bellissimo and Akhavan, 2015).

The protein and amino acid content is more significant than other food sources. Apart from that, reports regarding the carbohydrate content in the form of coixan in hanjeli seeds have many benefits. The type of polysaccharide coixan in hanjeli seeds is reported to be able to reduce blood sugar levels. Also, it has other pharmacological effects, including improving the immune system, antioxidants, and protecting pancreatic beta cells (Weng *et al.*, 2022). The macronutrient content is beneficial and supports the development of hanjeli seeds as a functional food product.

This research also reported that hanjeli seeds contain high amounts of micronutrients in the form of minerals needed in the body to help increase metabolism. The mineral content in the form of Na, K, Ca, Mg, and Fe respectively amounted to 161.18, 863.8, 22.18, 20.72, and 15.89 mg/kg BW, showing relatively high results and by the requirements mineral needs in the body every day. However, there is no complete information regarding the mineral content contained in hanjeli seeds that was reported in other studies. The minerals sodium and potassium have a major role in metabolic processes in the body. Sodium and potassium help in maintaining osmotic balance in the body. The mineral calcium helps increase bone resilience and strength, so it is suitable for all groups, including children, teenagers, and the elderly. At the same time, the minerals magnesium and iron play an important role in modulating and regulating the immune system in the body. The mineral content in hanjeli seeds has great prospects as a staple or functional food source (Weyh *et al.*, 2022).

Apart from the nutritional content of hanjeli seeds. This study also reported the content of secondary metabolites, which may play a role in providing disease treatment effects. Other chemical contents in the hanjeli seed extract were evaluated colorimetrically using specific reagents to determine the class of compounds contained in the hanjeli seed extract (Nur, Angelina, Aswad *et al.*, 2021; Nur *et al.*, 2022). The compounds identified include flavonoids, phenolics, saponins,

alkaloids, terpenoids, tannins, glycosides, and anthraquinones. The results of the phytochemical screening of hanjeli seed extract can be seen in Table 2. Table 2 shows that hanjeli seed extract is generally positive for containing phenolic compounds, flavonoids, alkaloids, terpenoids, saponins, and glycosides.

Table 2. Screening phytochemicals of hanjeli seeds extract.

Class of compounds	Identification result
Phenol	+
Flavonoid	+
Alkaloid	+
Terpenoid	+
Tanin	+
Saponin	+
Glycoside	+
Anthraquinones	-

(+) indicate positive in the extract, (-) indicate absence in the extract

Meanwhile, negative tannin and anthraquinone compounds were found in the hanjeli seed extract. The large number of groups of compounds that were positively identified indicates that there are secondary metabolites in hanjeli seed extract that may have a role in treatment. Phenolic and flavonoid compounds have been widely reported to have antioxidant effects and inhibit the activity of the alpha-glucosidase enzyme, which can cause an increase in blood glucose levels (Wang *et al.*, 2016; Al-Ishaq *et al.*, 2019; Xiao, 2022). The total phenolic and flavonoid levels in hanjeli seed extract can be seen in Table 3. The phenolic and flavonoid levels in hanjeli seed extract were determined based on the standard curve equation of gallic acid in total phenolics (Figure 1a) and quercetin in total flavonoids (Figure 1b). The results showed that the hanjeli seed extract contained a complete phenolic content of 98.17 ± 0.053 mgEGA/g extract. Meanwhile, the results obtained were 15.36 ± 0.068 mgEQ/g extract of flavonoid content. The high total phenolic and flavonoid content in hanjeli seed extract has the potential to provide biological effects such as antioxidants and alpha-glucosidase inhibitors.

Table 3. Phenolic and flavonoid content of Hanjeli seeds extract.

Content	Triplicate (n = 3)	Average (mean \pm SD)
Phenolic total (mgEGA/g extract)	96.44	98.17 \pm 0.053
	98.21	
	99.85	
Flavonoid total (mgEQ/g extract)	15.44	15.36 \pm 0.068
	15.32	
	15.34	

The nutritional content and secondary metabolite compounds from hanjeli seed extract play an essential role in biological activities, including as antioxidants and

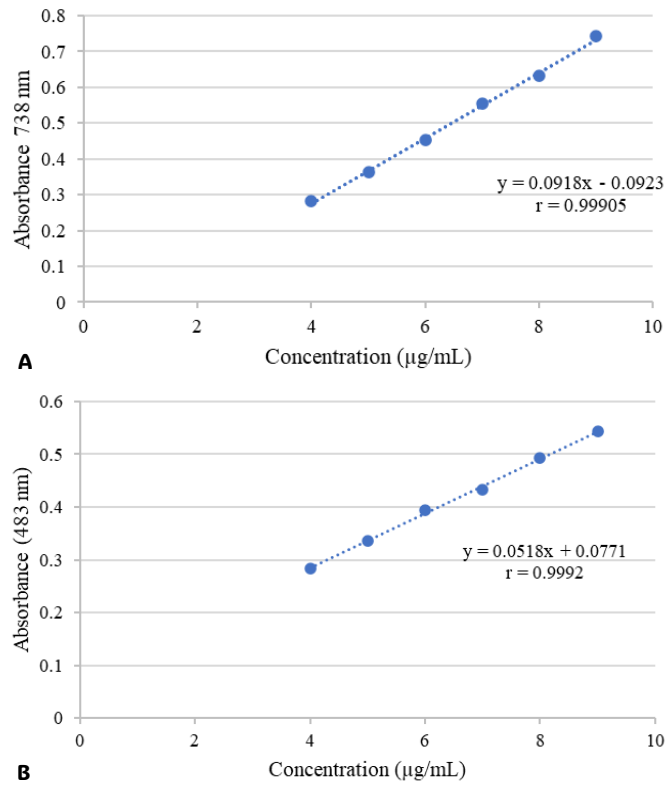


Figure 1. Standard curve calibration of gallic acid (A) and quercetin (B) with serial concentrations of 4, 5, 6, 7, 8, and 9 µg/mL.

alpha-glucosidase inhibitors. The high levels of total phenolics and flavonoids allow it to have antioxidant and alpha-glucosidase inhibitory effects. This study evaluated antioxidant activity using three test parameters: DPPH radical reduction, ABTS, and iron reduction, the FRAP test. The phenolic and flavonoid content plays a role in inhibiting DPPH and ABTS radicals through electron donor and hydrogen donor mechanisms. Giving electrons or hydrogen to radicals causes the radicals to become stable and will not harm the environment. Meanwhile, the FRAP test illustrates the ability of hanjeli seed extract to reduce iron through a redox reaction mechanism. These three mechanisms represent compounds with antioxidant properties that can inhibit the action of radicals in the body (Aisyah *et al.*, 2022; Nur *et al.*, 2023b). Based on the results of testing the antioxidant activity of hanjeli seed extract is shown in Table 4. Hanjeli seed extract has moderate activity in reducing DPPH radicals and intense action in reducing ABTS radicals with IC_{50} values of 662.69 ± 2.77 and 164.053 ± 0.156 µg/mL, respectively. Meanwhile, the antioxidant activity in reducing iron was 70.21 ± 0.93 uMEFeSO₄/g extract. The mechanism of action of the phenolic compounds or flavonoids in the extract greatly influences the existence of different IC_{50} values in reducing DPPH radicals with ABTS. Compounds with low polarity have a strong potential to reduce DPPH radicals compared to compounds with high polarity. This is because the DPPH system tends to work on low-

polarity compounds. Meanwhile, the ABTS method can be suppressed by phenolic or flavonoid compounds with low or high levels of polarity so that these compounds enable them to work synergistically in inhibiting ABTS radicals. However, the mechanism of inhibition has the same effect (Ghica *et al.*, 2023; Hussien and Endalew, 2023).

Table 4. Antioxidant activity of hanjeli seeds extract.

Antioxidant assay	Hanjeli seeds extract (n = 3)	Average (mean±SD)
DPPH assay (IC_{50} , µg/mL)	665.72	662.69±2.77
	660.29	
	662.06	
ABTS assay (IC_{50} , µg/mL)	163.91	164.053±0.156
	168.22	
	164.03	
FRAP assay (uMEFeSO ₄ /g extract)	69.42	70.21±0.93
	69.96	
	71.23	

In this study, the biological activity of anti-diabetes mellitus of hanjeli seed extract was also carried out enzymatically against alpha-glucosidase. The alpha-glucosidase inhibitory activity of hanjeli seed extract can be seen in Table 5. The alpha-glucosidase inhibitory activity of hanjeli seed extract was carried out at 30 to 90 µg/mL. The inhibitory activity by the extract appeared to increase according to increasing extract concentration. The higher the concentration, the greater the percent inhibition of alpha-glucosidase activity. At a high concentration of 90 µg/mL, the results showed that hanjeli seed extract could inhibit alpha-glucosidase activity by >50%. From the test results for each concentration of hanjeli seed extract, an IC_{50} value of 84.47 µg/mL was obtained, which means that the extract solution concentration of 84.57 µg/mL can inhibit 50% of alpha-glucosidase activity (Table 5). This inhibition by hanjeli seed extract was considered to have a strong activity (Nur, Wierson, Sami *et al.*, 2021). The positive control test for acarbose also showed strong inhibitory activity of 53.77 µg/mL (Table 6). However, the inhibitory activity of acarbose is still considered better than the extract because it still has a lower IC_{50} value than the IC_{50} of the hanjeli seed extract. Nevertheless, the hanjeli seed extract has potent activity in inhibiting the action of alpha-glucosidase. Activities in this strong category support the development of hanjeli seeds in treating diabetes mellitus.

Phenolic or flavonoid compounds are known to have a role as antihyperglycemic agents by inhibiting the activity of the alpha-glucosidase enzyme, especially phenolic compounds or flavonoid glycosides. Based on research by Wang *et al.* (2016), the ethanol fraction of

Table 5. Alpha-glucosidase inhibition activity of hanjeli seeds extract.

Concentration of extract ($\mu\text{g/mL}$)	Absorbance	Average (Absorbance)	Inhibition (%)	IC ₅₀ ($\mu\text{g/mL}$)
30	0.596	0.5993	13.47 \pm 0.76	84.47
	0.608			
	0.594			
45	0.535	0.5316	23.24 \pm 0.49	
	0.526			
	0.534			
60	0.463	0.4606	33.49 \pm 0.40	
	0.463			
	0.456			
75	0.385	0.3840	44.56 \pm 0.56	
	0.378			
	0.389			
90	0.317	0.3243	53.17 \pm 0.67	
	0.326			
	0.330			

Table 6. Alpha-glucosidase inhibition activity of acarbose as positive control.

Concentration of acarbose ($\mu\text{g/mL}$)	Absorbance	Average (Absorbance)	Inhibition (%)	IC ₅₀ ($\mu\text{g/mL}$)
15	0.518	0.5157	25.55 \pm 1.27	53.77
	0.502			
	0.527			
30	0.462	0.4560	34.16 \pm 0.66	
	0.457			
	0.449			
45	0.373	0.3813	44.94 \pm 0.73	
	0.384			
	0.387			
60	0.319	0.3213	53.61 \pm 1.16	
	0.311			
	0.334			
75	0.253	0.2487	64.1 \pm 0.51	
	0.243			
	0.250			

hanjeli ma-yuen seeds contains several flavonoid glycoside compounds, one of which is rutin and quercetin. In previous research, routine quercetin and kaempferol inhibited the alpha-glucosidase enzyme (Zhang *et al.*, 2016). This compound has a hydroxyl group (OH) in the C-3' and C-4' positions on ring B. Based on in silico structure-activity relationship studies, there is a hydroxylation process or the introduction of an OH group in this position on the active site of the alpha-glucosidase enzyme. Hydrogen bonds prevent the substrate from interacting with the enzyme, decreasing the alpha-glucosidase enzyme's work (Sakulkeo *et al.*, 2022). In addition, the glycosylation process or substitution of the sugar group to the C-3 hydroxy group on flavonoids will form a new glycoside complex with a structure similar to the natural alpha-glucosidase substrate, namely pNPG (Hossain *et al.*, 2020) inhibits

the action of the alpha-glucosidase enzyme in a reversible competitive manner.

The research results on hanjeli seeds that have been carried out have provided information regarding the nutritional content and chemical compounds, which are pretty significant, making it possible for them to be used as a source of essential nutrition, which is no less important than other basic ingredients.

4. Conclusion

Research has been conducted on the nutritional content and bioactivity of antioxidants and alpha-glucosidase inhibitors from hanjeli seeds. This research provides data that hanjeli seeds have complete nutrition, both macronutrients and micronutrients, which are

needed to fulfill nutritional sources in the body. Also, chemical contents such as high levels of phenolics, flavonoids, and other compounds that support the bioactivity of hanjeli seed extract as an antioxidant and alpha-glucosidase inhibitor have been identified. Therefore, hanjeli seeds have the prospect of being developed as a primary functional food source and a source of antioxidants and can maintain blood sugar levels to minimize the risk of diabetes mellitus.

Conflict of interest

The authors declare no conflict of interest.

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