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# The Effect of Germination Time on the Level of Total Phenol and Antioxidant Activity of Ethanol Extract of Red Bean Sprouts (*Phaseolus vulgaris* L.)

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**ABSTRACT:** Red beans (*Phaseolus vulgaris* L.) is one type of legume that has high nutritional value and has various benefits, including as a source of minerals, vitamins, proteins, carbohydrates and antioxidants. Germination process can increase the activity of secondary metabolite compounds in red bean seeds so that it will increase the content of phenol and antioxidant. The total phenol content formed is greatly influenced by germination time. The purpose of this study was to determine the effect of germination time on the total phenol content and antioxidant activity of ethanol extract of red bean sprouts. To achieve this goal, a series of research stages were carried out, namely carrying out the germination process for several germination times followed by extraction of the formed sprouts by maceration technique in ethanol. Vacuum evaporation was run to obtain a thick extract. Furthermore, the extract was analyzed to determine the total phenol content using the UV-Vis spectrophotometry method at a wavelength of 660 nm and the antioxidant activity was analyzed using the DPPH method. The results showed that the sprouts produced in germination times of 0, 24, 48, 72, 96 and 120 hours contained total phenols of (1.09; 1.19; 2.02; 1.57; 5.82 and 4.95)%, respectively and the antioxidant activities were 187.04; 134.89; 94.54; 153.91; 52.79 and 57.91 mg/L, respectively. It was evident that the highest levels of total phenols and antioxidant activities were produced by the red bean sprouts obtained with a germination time of 96 hours.

**KEYWORDS:** Antioxidants, germination time, red beans, total phenol content

## INTRODUCTION

Bean seeds are a source of protein, minerals, vitamins and bioactive compounds [1], including phenolic compounds that play a role in various physiological and metabolic processes in humans [2]. Most phenolic compounds are concentrated in bean seeds [3, 4] which function as active and important compounds in determining the color, taste, and flavor of food. These compounds show the capacity to bind free radicals that interact with proteins. Red beans are one type of bean that has various benefits, including as a source of protein (20-30%), carbohydrates, minerals, vitamins (A, B, C, E), and antioxidants [5]. Red beans are known to contain secondary metabolite compounds flavonoids, tannins, steroids/triterpenoids, saponins which have the potential as antioxidants. In addition, red beans also contain other active compounds, including phenolic acids, organic acids, amino acids, and lipids [6]. Due to the content of various active compounds, red beans have potential activity in detoxification, anti-inflammatory, cholesterollowering, and diuretics [7, 8]. The germination process increases the activity of secondary metabolite compounds contained in the seeds. In the process of germination of red beans, the content of antioxidant polyphenols becomes higher compared to ungerminated seeds [9]. Red bean sprout polyphenol antioxidants are known to play a role in healing cardiovascular diseases, inhibiting and reducing the risk of certain cancers, and protecting against diabetes, osteoporosis, and neurodegenerative diseases [10]. Seed germination is determined by internal factors, namely genetic factors that affect the speed of germination, as well as external factors influenced by the conditions around the seeds, temperature, water, humidity, oxygen, and sunlight. Internal factors also determine the germination process such as food reserves and hormone content in seeds [11]. During the germination process, the protein content in the sprouts decreases. This happens due to the fact that the protein content is used during the germination process. The longer the germination period, the protein content will decrease because during the sprout growth process, nitrogen in protein is used to form new structures in line with the increasing age in the germination stage [12]. In the germination process, various biological changes occur, namely changes in complex compounds into simpler compounds that are ready to be utilized by

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the embryo for further growth. During the germination process, the carbohydrate content is converted into dextrin or smaller parts, namely in the form of maltose sugar, large proteins are broken down into amino acids. In addition, there are other factors that have a negative effect on the protein content of red bean sprouts, namely the length of germination. During the germination process, the protein content in the seeds will be broken down and utilized both as a source of energy and as materials for new tissue structures in the morphogenesis process in germination by phenol compounds. In the germination process, new tissues will be formed that form the plumule and radicle structures. For this morphogenesis process, a number of substrates are needed, both carbohydrates, proteins and fats, therefore the protein content in the seeds will decrease during the germination process, but phenol compounds will increase [13]. Germination of red beans has been shown to significantly increase total phenolic content due to both the activation of the phenylpropanoid pathway, which stimulates de novo synthesis of phenolic compounds, and the enzymatic release of bound phenolics from the seed matrix. These compounds play a vital role in protecting the developing seedling against oxidative stress and environmental factors. Typically, total phenolic content peaks during the early to mid-stages of germination before stabilizing or slightly declining as the seedling matures [14]. Therefore, the length of germination time is important to study to see the relationship between germination time and total phenol compounds contained in red bean sprouts.

## MATERIALS DAN METHODS

### *A. Research Materials and Tools*

The research materials used were red beans obtained from a traditional markets in Badung Regency of Bali-Indonesia. The chemicals used were of reagent grade included: ethanol 96%, distilled water, DPPH (2,2-Diphenyl-1-Picryl Hydrazil), methanol, gallic acid, Na-hypochlorite and Folin-Ciocalteu reagent.

The research tools used included a set of glass tools, micro pipette, pipette tip, ball filler, hot plate and magnetic stirrer, spatula, Whatman No.42 filter paper, rotary evaporator, oven, macerator, porcelain cup, vortex, oven, refrigerator, analytical balance, blender and Shimadzu 2600 UV-Vis spectrophotometer.

### *B. Research Procedures*

#### *1). Red bean germination*

A total of 2 kg of red beans were washed thoroughly with water to remove dirt, then soaked for 12 hours. After that, the beans were drained and washed. Six clean plastic trays that have been lined with wet cotton as a growing medium were prepared. Each tray was filled with 250 g of red beans then stored at room temperature for some variations of germination time, namely 0, 24, 48, 72, 96 and 120 hours. During the germination period, the beans were sprayed with water every 6 am, 12 pm and 6 pm. Then the sprouts were harvested according to their germination time.

#### *2). Red bean sprout extraction*

The harvested red bean sprout samples were cleaned of their skin, then macerated for 24 hours using 96% ethanol until they completely submerged and finnaly covered with aluminum foil and stirred every 1 hour for the first 6 hours. After 24 hours, the extract was filtered using filter paper, then the maceration results were evaporated using a rotary vacuum evaporator to obtain a thick sprout extract. All extracts obtained were used to determine total phenol content and antioxidant activity tests.

#### *3). Determination of total phenol content*

a. Preparation of 1000 ppm gallic acid stock solution
   Gallic acid stock solution by weighing 0.1 grams of gallic acid and then dissolving it using of distilled water to a volume of 100 mL.

b. Measurement of standard gallic acid solution
   A series of gallic solutions with concentrations of 10, 20, 40, 80 and 100 ppm as well as a blank were prepared then measured with UV-Vis spectrophotometer at a wavelength of 660 nm. Then, the absorbance and concentration were plotted to obtain a calibration curve.

c. Determination of total phenol content in ethanol extract of red bean sprouts
   The determination of total phenol content in ethanol extract of red bean sprouts was done by weighing 0.08 grams of thick sprout extract then in a beaker glass mixed with 70 mL of distilled water and added with 2 mL of Folin-Ciocalteu reagent. The mixture was the left for 2-3 minutes. Next, 20 mL of 10% sodium carbonate solution was added and then the mixture was removed to a

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100 mL volumetric flask and diluted with distilled water up to the mark. A blue color was allowed to develop for 1.5 hours, then the absorbance was read at a wavelength of 660. The same works were done for all treatments.

***4). Antioxidant activity test***

a. Preparation of 0.1 mM DPPH solution

A total of 0.039 g of DPPH powder was weighed and dissolved in 1000 mL of methanol. Then it was used for running the antioxidant activity tests to all extracts.

b. Testing the antioxidant activity of the extract using the DPPH method

A total of 0.1 grams of red bean sprout extract was added with 5 mL of methanol then homogenized and centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered, the filtrate was then diluted to a volume of 5 mL (this resulted in a solution of 20,000 ppm). Furthermore, this was used to prepared various concentrations, namely 20, 40, 60, 80, 100 mg/L. Each concentration was pippeted as much as 0.5 mL and placed in a test tube separately, then added with 3.5 mL of DPPH and incubated for 30 minutes. Then the absorbance of the solutions were read at a wavelength of 517 nm.

## RESULTS AND DISCUSSION

***1). Germination.***

Germination process triggers a series of biochemical reactions that transform a dormant seed into a young, actively growing organism. In this research, in the initial stage of the germination the beans were soaked with Na-hypochlorite for 30 minutes which aims to sterilize the media and samples from various contaminants. After 30 minutes of soaking, the skin of the red beans absorbed a little water so that it looked like swelling on the skin. By washing the beans three times with running water they were completely clean from impurities that can interfere the germination process. The imbibition process occurred when the beans were soaked in warm water at a temperature of 50°C for 5 hours at room temperature. The imbibition process is a diffusion process that occurs in plants, namely the entry of water into the intercellular space from low concentration to high concentration by the skin of red bean seeds. This can cause an increase in the water content of the seeds needed to trigger biochemical changes in the seeds so that the seeds can germinate. Figure 1 shows the growth of the sprouts during the germination.

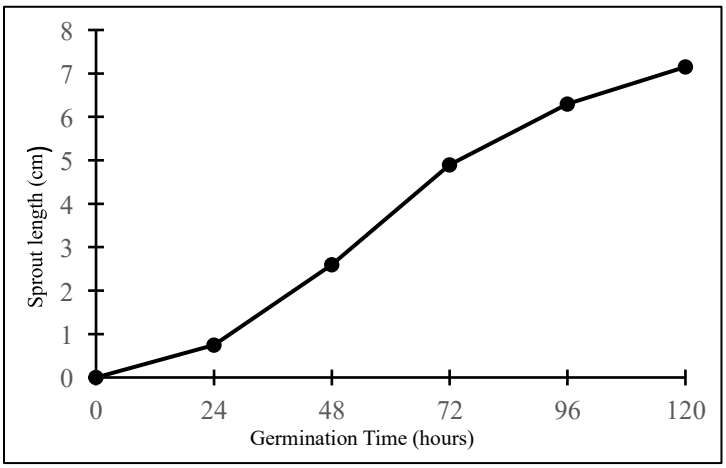


**Figure 1. The Growth of the Red Bean Sprouts during Germination**

Generally, the longer the germination, the longer the resulting sprouts. From the figure it can be seen that the red bean seeds had not changed the germination for 24 hours. On the second day or after 24 hours, the radicle appeared on several red bean seeds. At 48 hours of germination, the radicle which is a potential root candidate began to appear and several seeds experienced root elongation. The radicle grew during the germination period. Entering 72 hours of germination, the seed coat began to open and the seed flesh was visible. At 96 hours of germination, the root growth became longer and there was a release of the skin on several red bean seeds. When germination entered 120 hours, some red bean seeds rotten and some grew.

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***2). Red bean sprout extraction***

The extraction of the sprouts harvested from various germination times with ethanol resulted in thick extracts as showed in Figure 2. The figure shows that the longer the germination, the more thick extract is produced. This indicates that during the growth of the sprouts biochemical changes occur causing more ethanol-soluble compounds form, and finally they were extracted in a longer germination time. However, in germination time of 120 hours the sprouts have grown, therefore germination over 120 hours was not carried out.

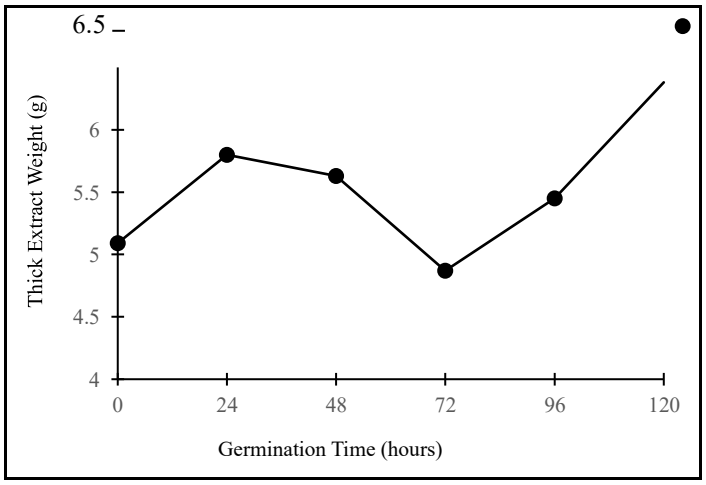


**Figure 2. The Yield of Ethanol Extract of the Red Bean Sprouts from Various Germination Times**

***3. Total Phenol Content***

The total phenol content in the ethanol extract of the red bean sprout can be seen in Figure 3. There was an increase in the total phenol content from 0 hours to 48 hours, namely from 1.09% to 2.02%. At 72 hours of germination, the total phenol content decreased to 1.57%. There was a significant increase in the total phenol content in germination for 96 hours, which was 5.82%, then decreased at 120 hours of germination. At a long germination period, the decrease in total phenol content generally occurs because phenolic compounds are used or broken down in the metabolism of growing seedlings. During germination, phenols are used as substrates for the synthesis of lignin, organic acids, or growth compounds. Oxidation by the enzymes polyphenol oxidase (PPO) and peroxidase (POD) converts phenols into quinones, which then react to form polymers or contribute to cell wall formation. This process reduces the measured free phenol content [15]. Moreover, during germination, bonds between phenols or other metabolites with proteins and polysaccharides can be degraded, making the compounds more freely soluble in ethanol [16].

***4). $IC_{50}$ Antioxidant activity test***

The determination of $IC_{50}$ antioxidant activity value in ethanol extract of red beans was carried out using the DPPH (2,2-diphenyl1-picryl hydrazyl) method. The principle of quantitative measurement of antioxidant activity is by measuring the capture of DPPH radicals by a compound that has antioxidant activity using UV-Vis spectrophotometry at a wavelength of 517 nm, so that the value of free radical scavenging activity was obtained expressed as the $IC_{50}$ value. The $IC_{50}$ value states the concentration of the test compound that can scavenge free radicals by 50%. The smaller the $IC_{50}$ value indicates the higher free radical scavenging activity. Figure 4 shows that the highest $IC_{50}$ antioxidant value of ethanol extract of red bean sprouts was obtained at a germination time of 96 hours with an $IC_{50}$ value of 52.79 mg/L while the lowest was at 0 hours of germination with a value of 187.04 mg/L.

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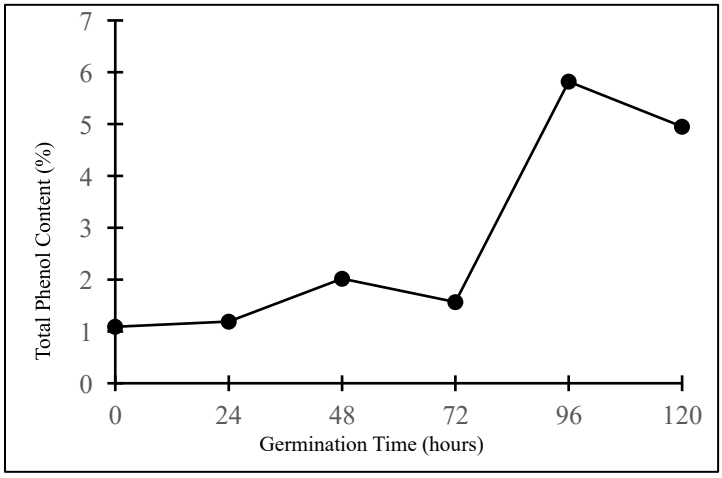


**Figure 3. Total Phenol Content in the Ethanol Extract of the Red Bean Sprouts with Various Germination Times**

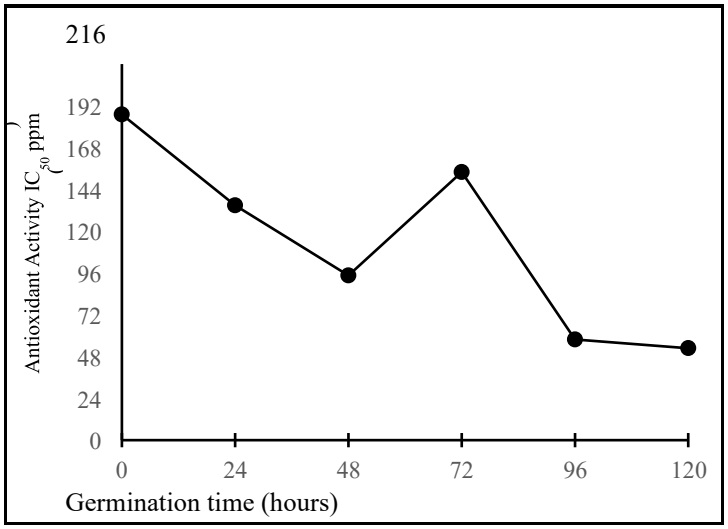


**Figure 4. Antioxidant Activity of Thick Ethanol Extract of Red Bean Sprouts with Various Germination Times**

The smaller the $IC_{50}$ value, the higher the antioxidant activity. The $IC_{50}$ value is the inhibition concentration, meaning the concentration of the sample solution required to inhibit 50% of DPPH free radicals, so it can be said that the smaller the $IC_{50}$ value, the stronger the antioxidant in counteracting free radicals or having strong antioxidant activity. The longer the germination time in red beans can increase the activity of enzymes that produce secondary metabolites such as phenols which have the ability as antioxidants. Germination triggers mild oxidative stress due to increased respiratory metabolism. Plants respond by producing enzymatic and non-enzymatic antioxidants to protect young cells. Once the seedlings are established, the need for oxidative protection decreases, leading to a decline in antioxidant production and hence decrease its activity [17].

## CONCLUSION

Based on the results of the research carried out, it can be concluded that the highest total phenol content (5.82%) was shown by the ethanol extract of red beans sprouts germinated for 96 hours. This extract showed the strongest antioxidant activity, namely $IC_{50}$ value of 52.79 ppm. Germination affects the total phenol content and ultimately affects its antioxidant activity.

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**REFERENCES**

1. Cominelli, E., Sparvoli, F., Lisciani, S., Forti, C., Camilli, E., Ferrari, M., Donne, C. L., Marconi, S., Vorster, B. J., Botha, A.M., Marais, D., Losa, A., Sala, T., Reboul, E., Ramos, K. A., Waswa, B., Ekesa, B., Aragão, F., and and Kunert, K. 2022. Antinutritional factors, nutritional improvement, and future food use of common beans: A perspective. Frontiers in Plant Science. DOI 10.3389/fpls.2022.992169. 01-08.
2. Magalhães, S. C. Q., Taveira, M. , Cabrita, A. R. J., Fonseca, A. J. M., Valentão, P., and Andrade, P. B. 2017. European marketable grain legume seeds: Further insight into phenolic compounds profiles. Food Chemistry 215. 177–184.
3. Diniyah, N., Badrul Alam, M., and Lee, S.H. 2020. Antioxidant potential of non-oil seed legumes of Indonesian's ethnobotanical extracts. Arabian Journal of Chemistry. 13 (5): 5208-5217.
4. Amarowicz, R., and Shahidi, F. 2017. Antioxidant activity of broad bean seed extract and its phenolic composition. Journal of Functional Foods. 38: 656- 662.
5. Chandrasiri, S. D., Liyanage, R., Vidanarachchi, J. K., Weththasinghe, P., and Jayawardana, B. C. 2016. Does processing have a considerable effect on the nutritional and functional properties of mung bean (Vigna radiata). Procedia Food Science. 6. 352–355.
6. Alcázar-Valle, M., Lugo-Cervantes, E., Mojica, L., Morales-Hernández,N, Reyes-Ramírez, N., Enríquez-Vara, J., N., and García-Morales, S. 2020. Bioactive Compounds, Antioxidant Activity, and Antinutritional Content of Legumes: A Comparison between Four Phaseolus Species. Molecules. 25(15), 3528.
7. Ganesan, K. and Xu, B. 2017. Polyphenol-Rich Dry Common Beans (*Phaseolus vulgaris* L.) and Their Health Benefits, Int J Mol Sci. 8(11):2331
8. Para, U., Chandra, B., and Rivai, H. 2020. Overview of Phytochemistry and Pharmacology of Chickpeas (*Phaseolus Vulgaris*). World Journal of Pharmacy and Pharmaceutical Sciences. 9(9):442-461.
9. Puyanda, I. R., Kuswanto, K. R., Margareta, L. A., and Anggraini, M. P. A. 2022. Antioxidant Activity of Sprouting Mungbean (Vigna radiata) Variety VIMA1. Journal of Applied Food Technology 9 (1) 11–15.
10. Rodríguez, L., Mendez, D., Montecino, H, Carrasco, B., Arevalo, B., Palomo, I., and Fuentes, E. 2022. Role of *Phaseolus vulgaris* L. in the Prevention of Cardiovascular Diseases—Cardioprotective Potential of Bioactive Compounds. Plants (Basel). Jan 11;11(2):186.
11. Sera. B. and Hnilicka, F. 2023. Genetic and Environmental Factors Affecting Seed Germination. Plants 12(24):4106
12. Bagarinao, N. C., King, J., Leong, S. Y., Agyei, D. and Sutton, K. 2024. Effect of Germination on Seed Protein Quality and Secondary Metabolites and Potential Modulation by Pulsed Electric Field Treatment. Foods. 13, 1598.
13. Abayechaw, D. and Wulchafo, K. 2020. The Concept and Process of Seed Germination – A Review. Int.J.Curr.Res.Aca.Rev.2020; 8(5): 100-112.
14. Xu, M., Jin, Z., Simsek, S., Hall, C., Rao, J., and Chen, B. 2019. Effect of germination on nutritional and physicochemical properties of whole grain highland barley. Food Chemistry, 297, 124991. https://doi.org/10.1016/j.foodchem.2019.124991
15. Wu, G., Johnson, S. K., Bornman, J. F., Bennett, S. J., and Fang, Z. (2016). Changes in whole grain polyphenols and antioxidant activity of six sorghum genotypes during germination. Food Chemistry, 194, 951–958.
16. Xu, J. G., Hu, Q. P., and Liu, Y. 2012. Changes in phytochemical content and antioxidant activities of sweet potato after germination. *Food Chemistry*, 135(4), 2716–2721.
17. Gan, R. Y., Wang, M. F., Lui, W. Y., Wu, K., and Corke, H. 2016. Dynamic changes in phytochemical composition and antioxidant capacity in mung bean sprouts. Food Chemistry, 194, 1102–1109.

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