Trop J Nat Prod Res, November 2021; 5(11):1958-1961 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant Activity and Sun Protection Factor of Black Glutinous Rice (*Oryza sativa* var. glutinosa)

Anggun H. Kusumawati[1], Farhamzah Farhamzah[1], Maulana Y. Alkandahri[1]*, Asman Sadino[2], Lilis S. Agustina[1], Sukma D. Apriana[1]

[1]*Faculty of Pharmacy, Buana Perjuangan Karawang University, Karawang, West Java, Indonesia*
[2]*Department of Pharmacy, Faculty of Mathematics and Natural Science, Garut University, Garut, West Java, Indonesia*

ARTICLE INFO

Article history:
Received 13 June 2021
Revised 26 October 2021
Accepted 10 November 2021
Published online 05 December 2021

ABSTRACT

Indonesia is geographically located on the equator and is presently faced with several complex problems, among which are air pollution and ultraviolet radiation. On average, Indonesians lose approximately 1.2-5 years of their life expectancy due to air pollution and high exposure to ultraviolet radiation, which tends to increase risk factors for skin cancer (melanoma), skin aging, sunburn, damage to collagen, inflammation, and skin elasticity. However, some plants, such as black glutinous rice (*Oryza sativa* var. glutinosa) are believed to have hereditary protection against ultraviolet radiation and contain antioxidant compounds. Therefore, this study tries to find out the antioxidant activity and sun protection factor of *O. sativa* extract applying DPPH (1,1-diphenyl-2-picrylhydrazyl) and UV-Vis spectrophotometry. The result showed that the *O. sativa* extract showed weak antioxidant activity with an IC_{50} value of 318.883 µg/mL, while the SPF (Sun protection factor) test was at the ultra-protection level against UV-B with a value of 36.65 ± 0.377 at a concentration of 10,000 µg/mL. Therefore, *O. sativa* extract has antioxidant activity and protection against ultraviolet rays and has the potential to be considered as a new sunscreen agent.

Keywords: *Oryza sativa*, Antioxidant, Sun protection factor, Air pollution, Ultraviolet.

Introduction

According to the World Health Organization (WHO), Indonesians lose 1.2-5 years of their life expectancy due to air pollution, with a significant percentage contributed by the energy sector such as transportation, power generation activities, industry, and housing.[1,2] Furthermore, due to the country's geographic location on the equator, it is highly exposed to ultraviolet radiation.[3] The effects of exposure to ultraviolet radiation and air pollution increases risk factors for skin cancer (melanoma), extrinsic aging, elasticity, sunburn, damage to collagen, and inflammation, such as contact and atopic dermatitis psoriasis, and acne.[4,5] Therefore, it is necessary to protect the body from air pollution and ultraviolet radiation using antioxidants and sunscreen products.[6-8] The sun protection factor (SPF) of sunscreen is described as the amount of UV energy needed to create a minimum erythema dose (MED) on protected skin separated by the UV energy demanded to provide a MED on unprotected skin. MED was established as the shortest time interval or lowest dosage of UV irradiation that produces the least apparent erythema on exposed skin.[9] The bigger the SPF number, the greater the protection given toward UV rays. However, several studies reported that the application of sunscreen to protect the skin from UV is not completely harmless.[10] Therefore, it is essential to examine the production of sunscreen chemicals from natural components. Several studies have demonstrated that natural chemicals can be exploited as possible sources of sunscreen due to their UV absorption and antioxidant activities.[11]Furthermore, natural extracts as antioxidants and protection from ultraviolet radiation are widely used because they are flexible, economical, easy to obtain, used as cosmetics, and have the least side effects on the body.[12,13] Indonesia has the second-largest biodiversity globally with approximately 28,000 plant species, of which 2,500 are medicinal.[14] Black glutinous rice (*Oryza sativa var. glutinosa*) (Figure 1) is a plant that has hereditary protection against ultraviolet radiation and contains antioxidant compounds. Therefore, this study was carried out to determine the antioxidant activity of *O. sativa* extract and to measure its value of Sun Protection Factor (SPF) using *in vitro* testing.

*Corresponding author. E mail: alkandahri@gmail.com
Tel: +6282167757738

Citation: Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD. Antioxidant Activity and Sun Protection Factor of Black Glutinous Rice (*Oryza sativa* var. glutinosa). Trop J Nat Prod Res. 2021; 5(11):1958-1961. doi.org/10.26538/tjnpr/v5i11.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Materials

Methanol solvent, ether, sulfuric acid, Mg powder, ferric chloride, hydrogen chloride, ethanol, amyl alcohol, chloroform, potassium hydroxide, gelatin, ascorbic acid, vanillin, and DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma Chemical Co. (St. Louis, MO, USA)). All reagents are of analytical grade.

Plant determination and extraction

In November 2019, fresh rice of *O. sativa* weighing 10 kg, were taken from Bungur Gede Village, Subang Regency, West Java, Indonesia, and transported to Central Laboratory, Buana Perjuangan Karawang University, for cleaning, air drying, grinding, and extraction. The plant was identified as *O. sativa* by Dr. Iriawati from the School of Biological Science and Technology, Bandung Institute of Technology (No. 6721/11.CO2.2/PI./2019).

Preparation of extract

The *O. sativa* powder weighing 500 g was macerated thoroughly in 96% ethanol (2 L) until 72 hours. The liquid extract was obtained and concentrated using a rotary evaporator (Eyela OSB-2100) at a temperature of 50°C to produce a concentrate of 5.05%, with the fixed weights obtained by dividing simplicia weights multiplied by 100%.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Phytochemical screening
The *O. sativa* extract was screened qualitatively to analyse the presence of secondary metabolites, such as alkaloids, terpenoids, flavonoids, phenolic compounds, saponins, tannins, and quinones.[15]

DPPH free radical scavenging activity
Determination of antioxidant activity was carried out using DPPH.[16] A stock solution of 50 µg/mL DPPH was prepared by dissolving 5 mg of DPPH in 100 mL methanol. The sample solution was obtained by dissolving 250 mg of *O. sativa* extract with 25 mL of methanol, then the solution was diluted to 100, 250, 750, 2,500, and 7,500 µg/mL. After that, 2 mL of each solution was mixed with 2 mL of DPPH stock solution until homogeneous and incubated at 30°C for 30 minutes. Antioxidant activity was read using UV-Vis spectrophotometry (Thermo Fisher Scientific, USA) at a wavelength of 515.50 nm and repeated four times. The blank sample was 1 mL of DPPH solution in 10 mL of methanol measured at the same time and wavelength (Ab). Ascorbic acid was used as a comparison with various concentrations of 1, 2, 4 and 8 µg/mL. The percentage of DPPH radical scavenging activity was calculated by the following equation:

$$Inhibition\ rate\ (\%) = \frac{Ab - As}{Ab}\ x\ 100$$

Ab and As denote the absorbents of blank and sample. The percentage of inhibition was plotted against the concentration and calculated from the IC_{50} graph.

Sun protection factor (SPF) assay
SPF is described as the increase in exposure time needed to induce erythema, and it is used to measure screening activity against the sun.[17] A total of 250 mg of *O. sativa* extract with 25 mL ethanol was prepared and diluted to 1,000, 5,000, and 10,000 µg/mL. After that, the solution was filtered and precipitated at 3,000 rpm for 10 minutes using a centrifuge (Gemmy Industrial Corp., Taiwan) and filtered in a Whatman no.1 paper.[18,19] The SPF value was determined using a UV-Vis spectrophotometer at 290, 295, 300, 305, 310, 315, and 320 nm wavelengths. It was also compared four times using an ethanol solution as a blank. The SPF value was estimated using the Mansur equation.[20]

$$SPF\ spectrophotometric = CF\ \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times abs\ (\lambda)$$

Where:
CF = 10 (correction factor).
EE = Erythemaous radiation effect spectrum.
I = Sunlight intensity spectrum.
Abs = Absorbance value of sunscreen products.

Table 1: SPF effectiveness category

SPF value	Protection level
2-4	Weak protection
4-8	Medium protection
8-15	Maximum protection
15-24	Very maximum protection
25-39	Ultra-protection
40-50+	Power protection

Statistical analysis
Data were investigated using SPSS version 22. One-way analysis of variance (ANOVA) was used for statistical analysis. The results were reported as the mean±standard deviation (SD) of the four replicates with p-value less than 0.05 considered significant. Meanwhile, the IC_{50} value at 50% inhibition was obtained from the regression plot using the AAT Bioquest application.

Results and Discussion

Phytochemical screening
Phytochemical screening of *O. sativa* extract showed the existence of chemical elements such as alkaloids, polyphenols, flavonoids, quinones, sesquiterpenoids, monoterpenoids, triterpenoids, and steroids. Table 2 shows a summary of the phytochemical screening of *O. sativa* extracts.

Antioxidant activity
Antioxidant activity was quantitatively determined by the DPPH method expressed as IC_{50}, which is the concentration needed to inhibit 50% of DPPH free radicals.[16] The antioxidant activity of *O. sativa* extract was carried out at different concentrations. It was observed that the higher the concentration of the extract, the higher the percentage value of inhibition (Table 3). The results proved that the antioxidant power of *O. sativa* extract is in the weak antioxidant range, in contrast to the ascorbic acid, which has a very strong antioxidant power.

Table 2: Classification of antioxidants based on IC_{50} values.[21]

IC_{50} value (µg/mL)	Antioxidant activity
< 50	Very strong
50-100	Strong
101-250	Medium
250-500	Weak
> 500	Not active

Table 3: Phytochemical screening of *O. sativa* extract

No.	Phytochemical screening	Result
1	Alkaloids	+
2	Flavonoids	+
3	Polyphenols	+
4	Tannins	-
5	Quinones	+
6	Saponins	-
7	Monoterpenoids and Sesquiterpenoids	+
8	Triterpenoids and Steroids	+

(+) = Present, (-) = Absence

Figure 1: Black glutinous rice (*Oryza sativa* var. glutinosa).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Sun protection factor (SPF)

The SPF value is a measure of protection from UV-B radiation, where the higher the SPF value, the higher the protection against UV-B radiation.[22] The simple mathematical equation developed by Mansur in 1986 is used to determine its value,[23] as well as the efficacy and effectiveness of sunscreen.[22] The results of determining the SPF value of *O. sativa* extract is shown in Table 5. The effectiveness of protection against UV-B of *O. sativa* extract at each concentration showed a very good result. The SPF value of *O. sativa* extract is in accordance with the Indonesian National Standard,[24] which outlines that a sunscreen preparation needs to have a minimum protection factor of 4. Most Indonesians have skin types IV and V, therefore the recommended SPF used is 5-15.[25,26] The high SPF value in the *O. sativa* extract is due to the flavonoid compounds contained in it which have protective activity from ultraviolet radiation. This process occurs because flavonoid compounds can activate antioxidant enzymes,[27,28,29] catalyze metal chelates, transfer-free electrons,[30,31] reduce alpha-tocopherol radical,[32] inhibit oxidase,[33] and acts as an immunomodulator.[34]

Table 4: Antioxidant activity of *O. sativa* extract and ascorbic acid

No	Sample	Concentration (g/mL)	% Inhibition	IC_{50} (µg/mL)
1	*Oryza sativa*	100	4.017 ± 0.048	318.883
		250	28.730 ± 0.016	
		750	71.640 ± 0.005	
		2,500	77.912 ± 0.019	
		7,500	82.647 ± 0.006	
2	Ascorbic acid	1	14.719 ± 0.013	3.688
		2	23.869 ± 0.002	
		4	53.612 ± 0.019	
		8	73.842 ± 0.014	

Table 5: Category of SPF value effectiveness of *O. sativa* extract

No	Concentration (µg/mL)	SPF value	Effectiveness	Level of protection against UV-B (%)
1	1,000	11.93 ± 0.051	Maximum protection	91.62
2	5,000	23.61 ± 0.194	Very maximum protection	95.77
3	10,000	36.65 ± 0.377	Ultra-protection	97.27

Conclusion

This research revealed that *O. sativa* extract has weak antioxidant activity compared to ascorbic acid. It also has an ultra-protection level against UV-B at a concentration of 10,000 µg/mL. All SPF values in this test are in accordance with the Indonesian National Standard (SNI). Therefore, *O. sativa* can be used as a new sunscreen agent for protection against ultraviolet radiation. However, further research still needs to be carried out to determine the action mechanism of the active compounds contained in *O. sativa*, which protect against ultraviolet radiation.

Conflicts of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Rosyidah M. Air pollution and respiratory health. Integrasi. 2016; 1(2):1-5.
2. Abidin J and Hasibuan FA. The effect of the impact of air pollution on health to increase the general public's understanding of the dangers of air pollution. Proceedings of the National Seminar on Physics, Riau University IV (SNFUR-4) Pekanbaru. 2019: 3002-1- 3002-7.
3. Seran YYT, Pasangka B, Sutaji HI. Characteristics of exposure to ultraviolet a (UV-A) and visible light radiation in the city of Kupang. J Biotrop Sci. 2018; 15(3):49-56.
4. Zegarska B, Pietkun K, Zegarski W, Bolibok P, Wiśniewski M, Roszek K, Czarnecka J, Nowacki M. Air pollution, UV irradiation and skin carcinogenesis: what we know, where we stand and what is likely to happen in the future? Postepy Dermatol Alergol. 2017; 34(1):6-14.
5. Puri P, Nandar SK, Kathuria S, Ramesh V. Effects of air pollution on the skin: A review. Indian J Dermatol Venereol Leprol. 2017; 83(4):415-423.
6. Ngoc LTN, Tran VV, Moon JY, Chae M, Park D, Lee YC. Recent Trends of Sunscreen Cosmetic: An Update Review. Cosmet. 2019; 6(4):1-14.
7. Bernstein EF, Sarkas HW, Boland P, Bouche D. Beyond sun protection factor: An approach to environmental protection with novel mineral coatings in a vehicle containing a blend of skincare ingredients. J Cosmet Dermatol. 2020; 19(2):407-415.
8. Poljšak B, Jamnik P, Raspor P, Pesti M. Oxidation-antioxidation-reduction processes in the cell: Impacts of environmental pollution. Encycloped Environ Health. 2019; 2(1):300-306.
9. Bilbao J and de Migue A. Erythemal solar irradiance, UVER, and UV index from ground-based data in central Spain. Appl Sci. 2020; 10(18):1-14.
10. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Charati JY. Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. Iranian J Pharm Res. 2014; 13(3):1041-1047.
11. Paul SP. Ensuring the safety of sunscreens, and their efficacy in preventing skin cancers: challenges and controversies for clinicians, formulators, and regulators. Front Med. 2019; 6(195):1-7.
12. Nunes AR, Vieira ÍGP, Queiroz DB, Leal ALAB, Maia Morais S, Muniz DF, Calixto-Junior JT, Coutinho HDM. Use of flavonoids and cinnamates, the main photoprotectors with natural origin. Adv Pharmacol Sci. 2018; 1-9.
13. Dunaway S, Odin R, Zhou L, Ji L, Zhang Y, Kadekaro AL. Natural antioxidants: Multiple mechanisms to protect skin from solar radiation. Front Pharmacol. 2018; 9(392):1-14.
14. Alkandahri MY, Maulana YE, Subarnas A, Kwarteng A, Berbudi A. Antimalarial activity of extract and fractions of *Cayratia trifolia* (L.) Domin. Int J Pharm Res. 2020; 12(1):1435-1441.

15. Alkandahri MY, Berbudi A, Utami NV, Subarnas A. Antimalarial activity of extract and fractions of *Castanopsis costata* (Blume) A.DC. Avicenna J Phytomed. 2019; 9(5):474-481.
16. Alkandahri MY, Nisriadi L, Salim E. Secondary metabolites and antioxidant activity of methanol extract of *Castanopsis costata* leaves. Pharmacol Clin Pharm Res. 2016; 1(3):98-102.
17. Geoffrey K, Mwangi AN, Maru SM. Sunscreen products: Rationale for use, formulation development and regulatory considerations. Saudi Pharm J. 2019; 27(7):1009-1018.
18. Widyawati E, Ayuningtyas ND, Pitarisa AP. Determination of SPF value of extract and losio of solar treatment of Kersen leaf ethanol extract (*Muntingia calabura* L.) using UV-Vis spectrophotometry method. Indonesian Pharm Res J. 2019;1(3):189-202.
19. Ermawati DE, Yugatama A, Wulandari W. Physical properties, sun protecting factor, and *in vivo* test of ZnO-dispersed into nanoemulgel. J Pharm Sci Clin Res. 2020; 5(1):49-60.
20. Mansur JS, Breder MNR, Mansur MCA, Azulay RD. Determination of sun protection factor for spectrophotometry. Anais Brasileiros de Dermatologia. 1986; 61:121-124.
21. Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. Comparison of antioxidant activities of Isoflavones from kadzu root (*Puerari lobata* ohwi). J Food Sci. 2003; 68(6):2117-2122.
22. Donglikar MM and Deore SL. Sunscreens: A review. Pharmacogn J. 2016; 8(3):171-179.
23. Dutra EA, da Costa e Oliveira DAG., Kedor-Hackmann ERM, Santoro MIRM. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Braz J Pharm Sci. 2004; 40(3):381-385.
24. SNI. Sunscreen. In:National Standardization Board; 1996. 1-3 p.
25. Apriliyani YP. The relationship between the use of sunscreen and the severity of melasma (MASI score) in women in Grogol-Sukoharjo District. Thesis. Muhammadiyah Surakarta University; 2017. 3 p.
26. Baumanm L. Cosmetic dermatology principles and practice. (2nd ed.). The Mc Graw-Hill Book Companies Inc; 2009. 245-254 p.
27. Elliott AJ, Scheiber SA, Thomas C, Pardini RS. Inhibition of glutathione reductase by flavonoids. Biochem Pharmacol. 1992; 44:1603-1608.
28. Alkandahri MY, Shafirany MZ, Rusdin A, Agustina LS, Pangaribuan F, Fitrianti F, Farhamzah, Kusumawati AH, Sugiharta S, Arfania M, Mardiana LA. *Amomum compactum*: A review of pharmacological studies. Plant Cell Biotechnol Mol Biol. 2021; 22(33&34):61-69.
29. Shafirany MZ, Indawati I, Sulastri L, Sadino A, Kusumawati AH, Alkandahri MY. Antioxidant activity of red and purple rosella flower petals extract (*Hibiscus sabdariffa* L.). J Pharm Res Int. 2021; 33(46B):186-192.
30. Ferrali M, Signorini C, Caciotti B, Sugherini L, Ciccoli L, Giachetti D, Comporti M. Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating activity. FEBS Lett. 1997; 416(2):123-129.
31. Alkandahri MY, Patala R, Pratiwi MI, Agustina LS, Farhamzah, Kusumawati AH, Hidayah H, Amal S, Frianto D. Pharmacological studies of *Durio zibethinus*: A medicinal plant review. Ann Rom Soc Cell Biol. 2021; 25(4):640-646.
32. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J Nutr Sci Vitaminol. 2001; 47(5):357-362.
33. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, Pieters L, Vlietnck AJ, Vanden Berghe D. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod. 1998; 61(1):71-76.
34. Alkandahri MY, Subarnas A, Berbudi A. Review: Immunomodulator activity of Sambiloto plants (*Andrographis paniculata* Nees). Farmaka. 2018; 16(3):16-21.