Journal of Tropical Biodiversity and Biotechnology

COUNTRY

Indonesia

Universities and research institutions in Indonesia

Media Ranking in Indonesia

SUBJECT AREA AND CATEGORY

Agricultural and Biological Sciences
- Agricultural and Biological Sciences (miscellaneous)
- Ecology, Evolution, Behavior and Systematics

Biochemistry, Genetics and Molecular Biology
- Biotechnology

Immunology and Microbiology
- Applied Microbiology and Biotechnology

PUBLISHER

Universitas Gadjah Mada, Faculty of Biology

H-INDEX

4

PUBLICATION TYPE

Journals

ISSN

25409573, 25409581

COVERAGE

2020-2022

INFORMATION

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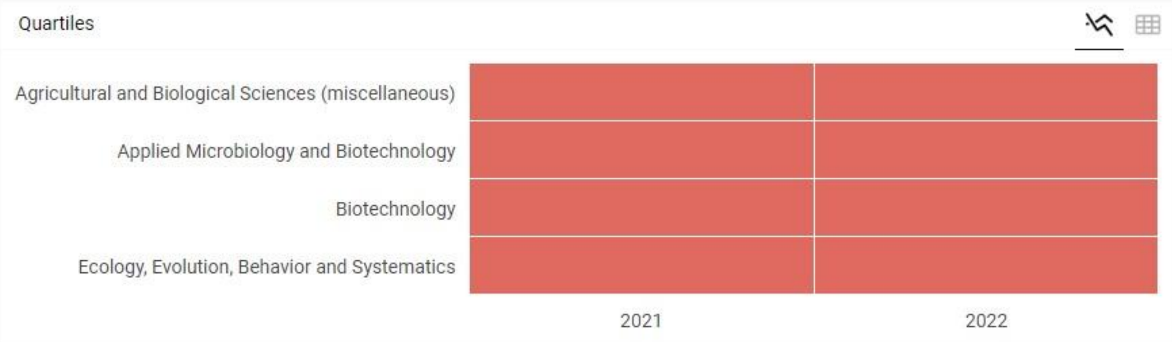
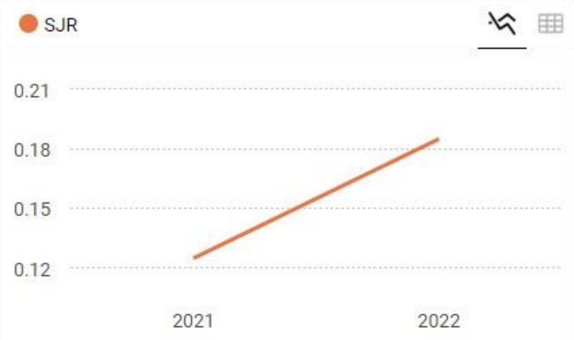
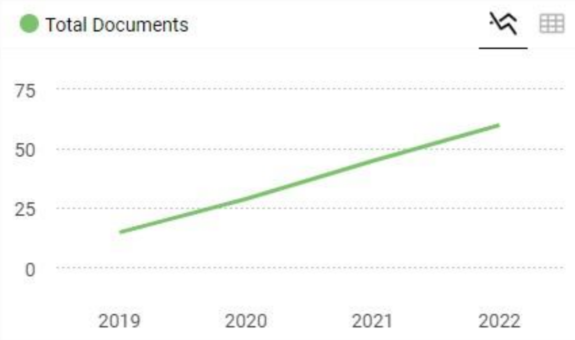
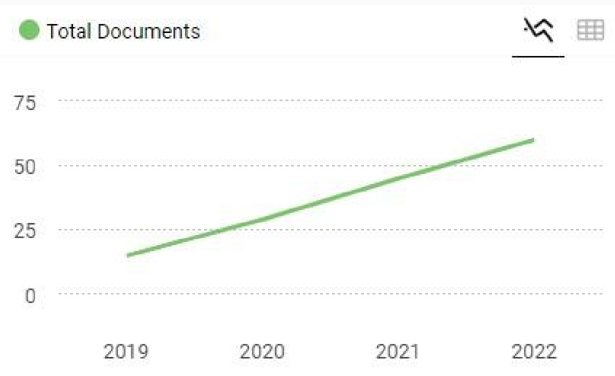
How to publish in this journal

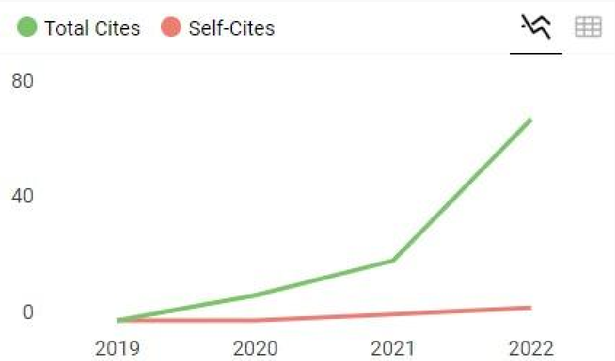
jtbb.biologi@ugm.ac.id

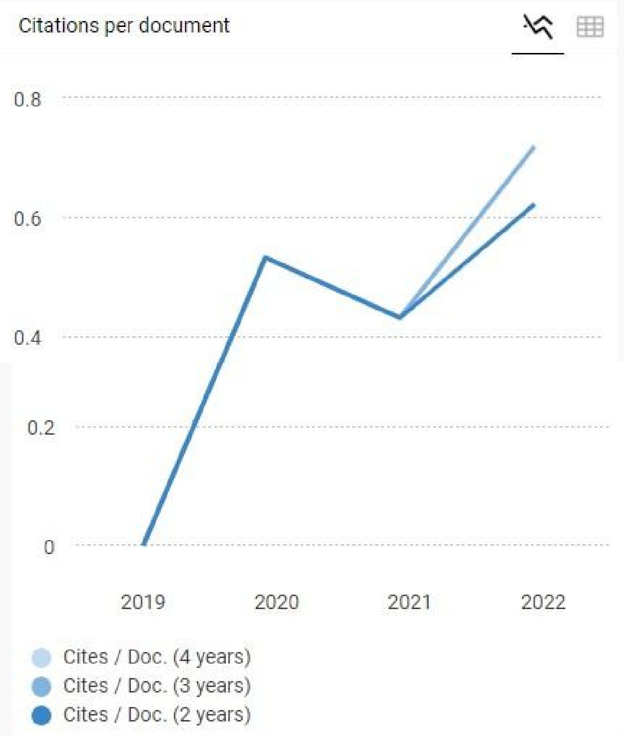


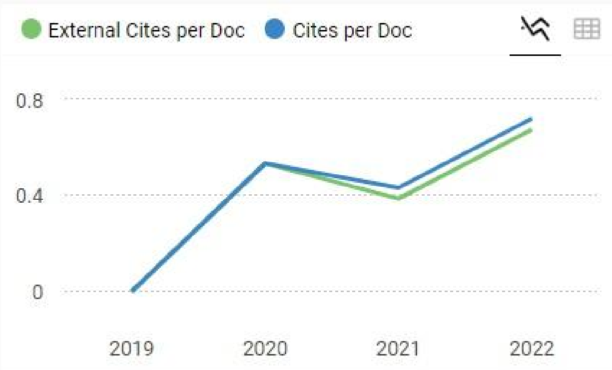


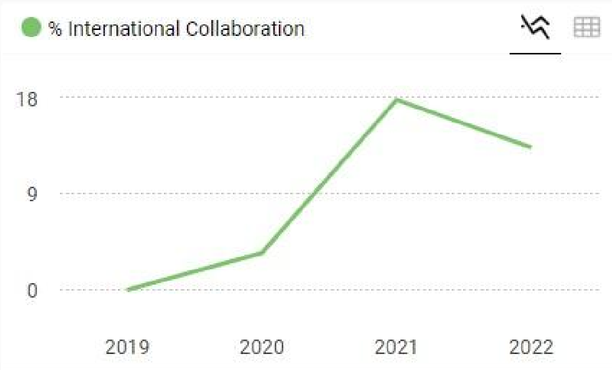


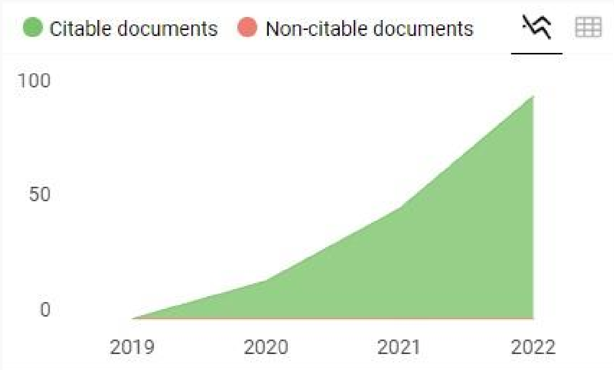


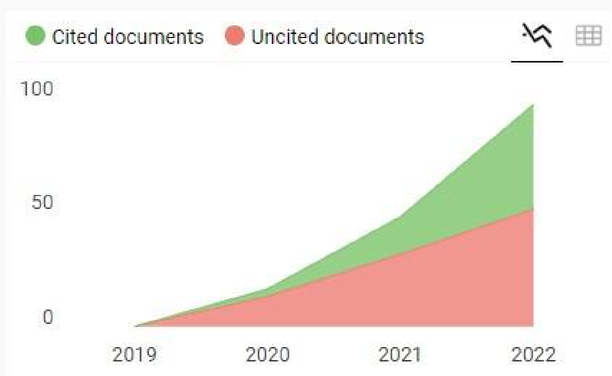


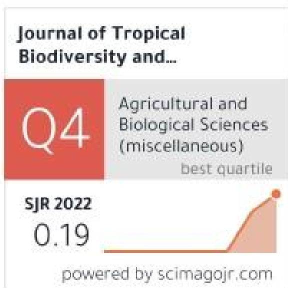












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Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 02 (2023): jtbb81339
DOI: 10.22146/jtbb.81339

Short Communications

Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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Keywords:
Antioxidant
Dolichos lablab
functional food
nutritional value
secondary metabolites
Submitted:
15 January 2023
Accepted:
29 March 2023
Published:
03 July 2023
Editor:
Miftahul Ilmi

ABSTRACT
To date, the data describing various nutritional and secondary metabolites content of Lablab beans is incomplete. Therefore, this study evaluated the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The results showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging activity. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity via the FRAP assay. Those who are keen in developing functional food from Lablab beans should consider this data as a reference.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only are they a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022a; Hermanto et al. 2022b). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their rich fibre and carbohydrate contents (Maass et al. 2010; Purwanti et al. 2019a). Moreover, Lablab beans also have superior

environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). Their innate nature may benefit the maintenance of food security, particularly in lands with low water supply. Thus, the cultivation of Lablab beans provides a promising means in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' colour, i.e., brown, black, and cream (figure 1). Although other variants may exist, these three are commonly found in several regions in Indonesia, such as East Java (Probolinggo and Madura Island) and West Nusa Tenggara (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional differences among the variants of Lablab beans. Lablab beans have numerous bioactivities including antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). These bioactivities make it a promising and excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

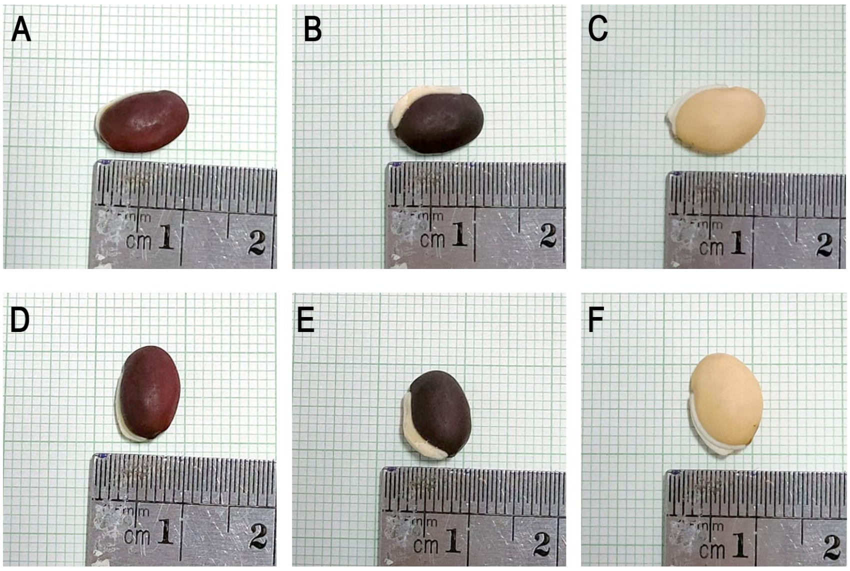

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A, D), black (B, E), and cream (C, F). The beans have been positioned to obtain the length (A-C) and the width (D-F).

The sample of the beans for this study was obtained from Sumenep, Madura Island, East Java, Indonesia during dry season in 2019. Details of the sample profiles and precise locations are as described in the previous study (Purwanti et al. 2019b). The beans were stored in 4°C until used. The beans were processed as per the extraction method previously mentioned in (Purwanti et al. 2022). Briefly, grounded beans were soaked in 96% ethanol with 3:1 ratio (volume in L and weight in kg) for 24 hours. The soaked beans powder then filtered to obtain the filtrate and homogenate. The filtrate then rotary evaporated to separate solvent and solute followed by freeze drying process to obtain Lablab beans' extract. The extract then processed to the subsequent analysis.

The crude fibre, total protein, and crude fat content were determined according to the previous protocol (Thiex 2009). Amylose and am-

ylopectin content were also measured colorimetrically using a previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories as per manufacturer's protocol.

To determine the secondary metabolites content, total phenol and total flavonoid was employed. Total flavonoid was performed as per a previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 μL of the dissolved extract was mixed with 10 μL of 5% $NaNO_2$, followed by the addition of 150 μL of water and 10 μL of 1 M CH_3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with gallic acid as the standard. The sample was diluted in water, and 100 μL of the sample was added to 1 mL of Folin Ciocalteu reagent and incubated for 5 minutes at room temperature with minimum light ambiance. 1 mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically at 725 nm. The total phenol was defined as percentage of Gallic Acid Equivalent (GAE) as per the build standard curve.

The antioxidant activity was measured by DPPH scavenging and Ferric Reducing Antioxidant Potential (FRAP) assays. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier work (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if the p-value is < 0.05. The data was then visualised as mean $\pm$ standard deviation.

All Lablab bean variants have good nutritional content according to the caloric, total fibre, protein, crude fat, amylose, and amylopectin content. The fibre content of black Lablab bean was the highest, with 8% fibre content, followed by the cream and brown variants (table 1). The considerable content of dietary fibre in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fibre consumption can improve physiological homeostasis, particularly in relations to lipid and glucose metabolism (Jahan et al. 2020). High fibre content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). With regards to total protein content, cream beans had the same protein content as brown beans, while black beans had the lowest content of total proteins (table 1). The high percentage of total protein content in Lablab beans would be valuable as a candidate for functional foods since plant-based protein have broad health benefits, such as antioxidant, antiviral, antidiabetic, and anticancer properties (Maphosa et al. 2017; Liu et al. 2020; Sipahli et al. 2021; Roy et al. 2022; Purwanti et al. 2022). Nevertheless, specific treatment, such as isoelectric preparation, was suggested to obtain a protein isolate with adequate quality and good functional properties (Subagio 2006).

Cream beans have the highest percentage of crude fat among all variants (table 1). The low-fat content of Lablab beans exhibit a great potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food

provides more health benefits with deleterious high-energy intake, particularly in areas with high level of famine cases (Delaš 2011; Robson 2013). For instance, West Nusa Tenggara province in Indonesia has the highest occurrence of hunger cases (Mone & Utami 2021). Interestingly, this region also founds a large distribution of Lablab beans (Jayanti et al. 2011). The utilization of Lablab beans to reduce the incidence of famine should be considered. Thus, the low-fat content of Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of its functional properties and energy source. The black Lablab bean has the highest amylose content, with 15% amylose content, followed by brown and cream (table 1). In contrast, black beans had the lowest amylopectin content compared to the other analysed variants (table 1). Similarly, black beans also had the lowest calorie per gram compared to other variants of Lablab beans (table 1). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose reflects better nutrient digestibility (Gao et al. 2020). A diet containing large portion of amylopectin positively associated with the postprandial insulin response resulted in more efficient nutrient uptake and glucose metabolism (Gao et al. 2020). Therefore, brown and cream beans may become potential candidates as functional food.

This study demonstrated that Lablab beans have been found to have comparable levels of total protein with *Vigna radiata* and *Pisum sativum*, and even higher levels than *Glycine max* and *Lens culinaris* (Singh et al. 2022). In addition, Lablab beans have a favourable nutritional profile with higher dietary fibre and lower fat content compared to *Phaseolus vulgaris*, *L. culinaris*, *P. sativum*, and Edamame (Dhingra et al. 2012; Mullins & Arjmandi 2021; Didinger & Thompson 2021). The amylose content in Lablab beans was higher than *Cicer arietinum* and *G. max* (Tayade et al. 2019). Moreover, the amylose and amylopectin content in Lablab beans also similar with *V. angularis*, a "red pearls" that has good nutrients and hypoglycemic activity (Zhang et al. 2022).

The total phenolic and flavonoid contents evaluation demonstrated that brown beans exhibited the greatest content of flavonoid ($p < 0.01$), while the cream beans had the most negligible flavonoid content (table 2). On the other hand, the phenolic content was highest in black beans compared to other Lablab variants ($p < 0.05$, table 2). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being the most abundant in brown beans. In other words, the other variants may comprise of other phenolic compounds like phenolic acids, tannins, and other phenolic compounds (Purwanti et al. 2022). The current result was also higher than several edible beans, such as *P. vulgaris*, *P. lunatus*, *V. radiata* and *C. arietinum* (Zhao et al. 2014). Nonethe-

Table 1. The comparison of primary metabolites and nutritional content among Lablab bean variants.

Variant	Fiber (%)	Protein (%)	Crude Fat (%)	Amylose (%)	Amylopectin (%)	Calorie (kcal/g)
Brown	7.02 ± 0.015^a	24.91 ± 0.06^a	0.36 ± 0.01^a	14.41 ± 0.095^a	87.18 ± 0.030^a	3.86 ± 0.005^a
Black	8.16 ± 0.040^b	23.43 ± 0.23^b	0.45 ± 0.03^b	15.46 ± 0.515^b	85.79 ± 0.015^b	3.83 ± 0.002^b
Cream	8.11 ± 0.005^c	24.82 ± 0.15^a	0.55 ± 0.04^c	13.52 ± 0.120^c	87.90 ± 0.025^c	3.85 ± 0.002^a

Note: The data was presented as mean $\pm$ standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

less, the total flavonoid contents of Lablab beans were lower compared to *P. sativum*, *C. arietinum*, *V. radiata*, *P. vulgaris*, *P. lunatus*, *L. culinaris*, *Vicia faba*, and *G. max* (Sharma & Giri 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with antioxidant activity through DPPH scavenging activity, where brown beans had the highest scavenging activity compared to the others (table 2). However, the ferric reducing activity was stronger in variant with higher phenolic contents (table 2). These results were supported by a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have an *ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Those structural differences influence the radical scavenging mechanism of flavonoids and other phenolic compounds in different antioxidant assay. Hydrogen Atom Transfer (HAT), Single-Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton-Loss Electron Transfer (SPLET) are taking place during the DPPH assay. Contrary, SPLET is the main mechanism during the electron transfer enthalpy in the FRAP reaction system (Chen et al. 2020). Ferulic Acid, Hydroxycinnamic Acid, Sinapinic Acid, Coumaric Acid, and Isovanillic Acid are identified phenolic acids in Lablab beans. Also, Rutin and Isoquercetin are flavonoids that also found in Lablab beans (Purwanti et al. 2022). Those compounds were identified in adequate abundance in Lablab beans and may perform as radical scavenger during this study. Nevertheless, future studies are required to compare the secondary metabolites among different variant of Lablab beans to comprehend the phytochemical content differences better. Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties.

This study shows that all Lablab bean variants have good amount of nutritional value, total phenol and flavonoid contents, and antioxidant activity. Lablab beans have adequate nutritional values surpassed other types of edible beans. Despite having lower flavonoid contents compared to commonly consumed beans, the phenolic compounds in these beans still exhibit superior performance. Finally, the cream variant shows slightly lower nutritional contents and bioactive compound compared to

Table 2. The total flavonoid, phenolic, and antioxidant capacity according to the DPPH scavenging capacity and FRAP analysis.

Variant	Total Flavonoid (%)	Total Phenol (%)	DPPH Scavenging (%)	FRAP (μM $FeSO_4$/mg)
Brown	26.25 ± 0.34^a	40.56 ± 0.69^a	78.87 ± 1.59^a	1154.58 ± 4.17^a
Black	12.99 ± 0.31^b	45.28 ± 2.56^b	40.01 ± 0.16^b	2398.05 ± 4.81^b
Cream	12.15 ± 0.06^c	40.32 ± 3.06^a	24.53 ± 0.16^c	1061.53 ± 2.41^c

Notes: The data was presented in mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

the other analysed Lablab beans. Still, some varieties possess a promising characteristic as a functional food candidate owing to their nutritional value.

AUTHORS CONTRIBUTION

EP designed the research and acquired the project funding, FE collected and analysed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

ACKNOWLEDGMENTS

The authors thank the Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 (Grant No. E.2.a/334/BAA-UMM/IV/2022) for funding this work.

CONFLICT OF INTEREST

There is no conflict of interest raised in this study.

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    - Initial review result
4. Article resubmitted to Journal of Tropical Biodiversity and Biotechnology (15-1-2023)
    - Submission acknowledgment
    - First short communication draft
5. First revision: Accepted with major revision (22-2-2023)
    - Article revision letter for authors
    - Review result
6. First revision submitted (6-3-2023)
    - Email response to editor
    - Author's response form to reviewer's comments
    - Revised article
7. Article accepted for publication (29-3-2023)
    - Decision letter to authors
8. Copyedited article sent to author (13-4-2023)
    - Notification letter
9. Edited article by author sent to editor (14-4-2023)
    - Edited article with track changes
    - Copyright transfer agreement
10. Draft layout sent to author (9-5-2023)
    - Notification letter
    - Draft layout
11. Draft layout revision sent to editor (10-5-2023)
    - Email response to editor
12. Article published (3-7-2023)
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- First short communication draft

Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development

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Abstract

To date, the data describing the nutritional and secondary metabolites comparison of Lablab beans is incomplete. Therefore, this study will evaluate the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The result showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity in FRAP assay. Developing functional food from Lablab beans should consider this data as a reference.

Keywords: Antioxidant, *Dolichos lablab*, functional food, nutritional value, secondary metabolites.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only as a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022b; Hermanto et al. 2022a). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their richness of fiber and carbohydrate contents (Purwanti et al. 2019a; Maass et al. 2010). Moreover, Lablab beans also have superior environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). This nature may benefit the maintenance of food security, particularly in lands with a low water supply. Thus, the cultivation of Lablab beans provides a promising result in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' color, i.e., brown, black, and cream (figure 1). Although other variants may exist, those three are commonly found in several areas in Indonesia, such as East Java (Probolinggo and Madura Island, and West Nusa Tenggara) (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional difference among the variants of Lablab beans. Lablab beans have a lot of bioactivities like antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). Those bioactivities make it promising as an excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A), black (B), and cream (C).

The sample of the beans for this study was obtained from Madura Island, East Java, Indonesia. The details of the sample profiles and precise locations as described in previous literature (Purwanti et al. 2019b). The beans extraction was processed as the previously mentioned method (Purwanti et al. 2022). The crude fiber, total protein, and crude fat were determined according to the previous protocol (Thiex 2009). Besides, the amylose and amylopectin were also measured colorimetrically using the previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) calculated total calories referring to the manufacturer's protocol.

To determine the secondary metabolite contents, total phenol and total flavonoid was employed. Total flavonoid was performed referring to the previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 μL of the dissolved extract was mixed with 10 μL of 5% $NaNO_2$, followed by the addition of 150 μL of water and ten μL of 1 M CH3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with Gallic acid determined as the standard for phenolic compound quantification. The sample was diluted in water, and 100 μL of the sample was added by 1 mL of Folin Ciocalteu reagent and incubated for five minutes at room temperature with minimum light ambiance. An mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically in 725 nm wavelength. The total phenol was defined as % of Gallic Acid Equivalent (GAE) referring to the build standard curve.

The antioxidant activity was measured by DPPH scavenging and a Ferric Reducing Antioxidant Potential (FRAP) assay. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier literature (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if $p < 0.05$. The data is then visualized as mean accompanied by standard deviation value.

All Lablab bean variants have good nutrition according to the total fiber, protein total, crude fat, amylose, amylopectin, and calories. The fiber content of black Lablab bean was the highest, with 8% fiber content, followed by cream and brown variants (figure 2A). The considerable content of dietary fiber in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fiber consumption could improve physiological homeostasis, particularly related to lipid and glucose metabolism (Jahan et al. 2020). The high fiber content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). Meanwhile, from the total protein point of view, the cream beans had the same protein content as brown beans, while the black beans showed the lowest content of total proteins (figure 2B). The high percentage of total protein would be valuable for Lablab beans as the candidate for functional foods since the plant-based protein have primary dietary sources for essential amino acid supply to perform more health benefits (Maphosa et al. 2017). However, the cream beans have the highest percentage of crude fat among all variants (figure 2C, $p < 0.05$ and < 0.01). The low-fat contents of the Lablab beans showed a high potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food provides more health benefits with deleterious high-energy intake (Delaš 2011; Robson 2013). Thus, the low-fat contents in Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of the functional properties and energy source. The black Lablab bean has the highest amylose percentage with 15% amylose content ($p < 0.05$ and < 0.01), followed by brown and cream (figure 2D). In contrast, black beans had the lowest amylopectin ($p < 0.01$) than other analyzed variants (figure 2E). Similarly, black beans also had the lowest calorie per gram ($p < 0.01$) compared to other variants of Lablab beans (figure 2F). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose has better nutrient digestibility (Gao et al. 2020). Therefore, brown and cream beans may become the potential candidate for functional food.

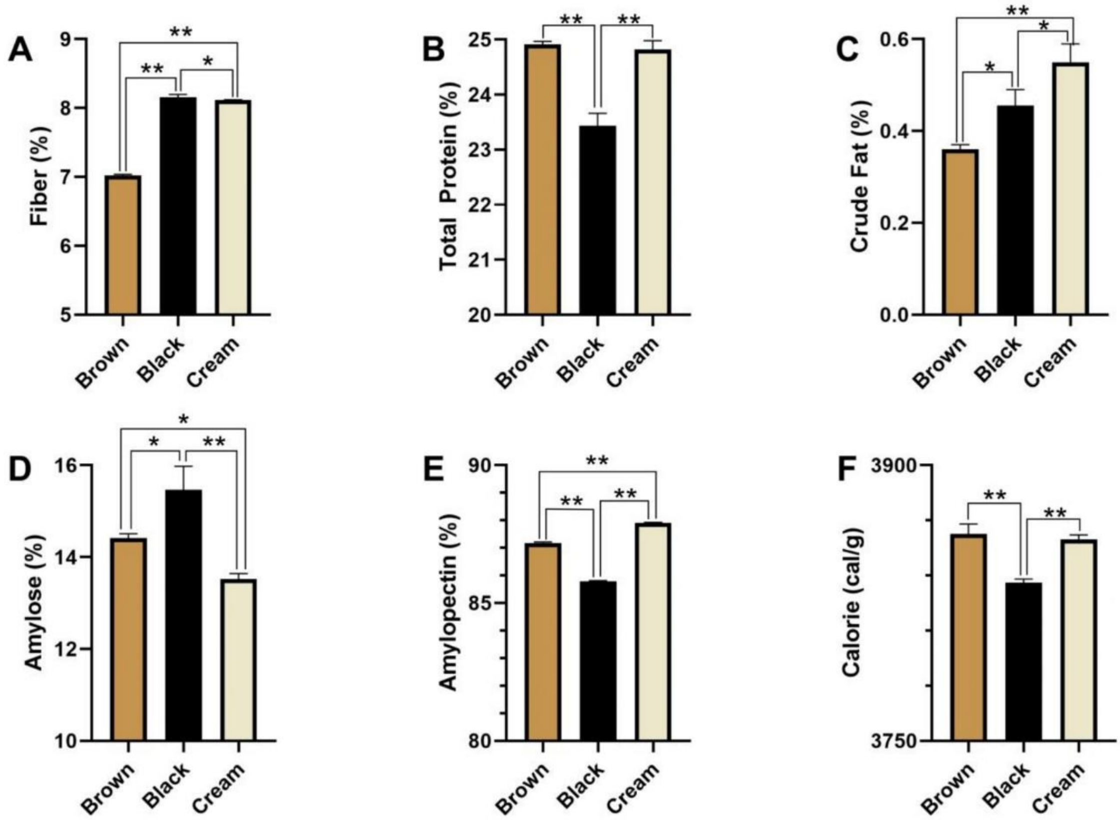

Figure 2. The comparison of primary metabolites and nutritional content among Lablab beans' variants. In consecutive order: crude fiber (A), total protein (B), crude fat (C), amylose (D), amylopectin (E), and total calories (F). The data was presented in mean with standard deviations (n = 3). The asterisk symbol determines the significant difference, with (*) being significant at 95% and (**) at 99% confidence intervals, respectively.

The secondary metabolites of Lablab beans were measured according to the total phenolic and flavonoid contents. Brown beans showed more great content of flavonoid ($p < 0.01$), while the cream beans comprised the most negligible flavonoid content (figure 3A). However, the phenolic compound was higher in black beans compared to other Lablab variants ($p < 0.05$). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being more abundant in brown beans. In other words, brown beans had the most secondary metabolites in phenolic and flavonoids. In contrast, the other variants may comprise other phenolic compounds like phenolic acids, tannins, and so on (Purwanti et al. 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with the antioxidant activity through DPPH scavenging activity, where the brown beans had the most excellent scavenging activity compared to the others (figure 3C). In contrast, ferric-reducing activity has similar results with the phenolic contents as the black beans outperformed the brown and cream beans variants (figure 3D). This result unsurprisingly occurred since there is a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have *an ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Electron Transfer Enthalpy (ETE) is the frequent mechanism during the radical scavenging of phenolic acids in the FRAP assay (Chen et al. 2020). Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties through different components of bioactive molecules in each bean. This study shows all Lablab bean variants shows good amount of nutritional value, secondary metabolites, and antioxidant activity. Although the cream variant shows slightly lower nutritional contents and bioactive compound compared to others analyzed Lablab beans, it is still a promising source of food mainly because the nutritional value.

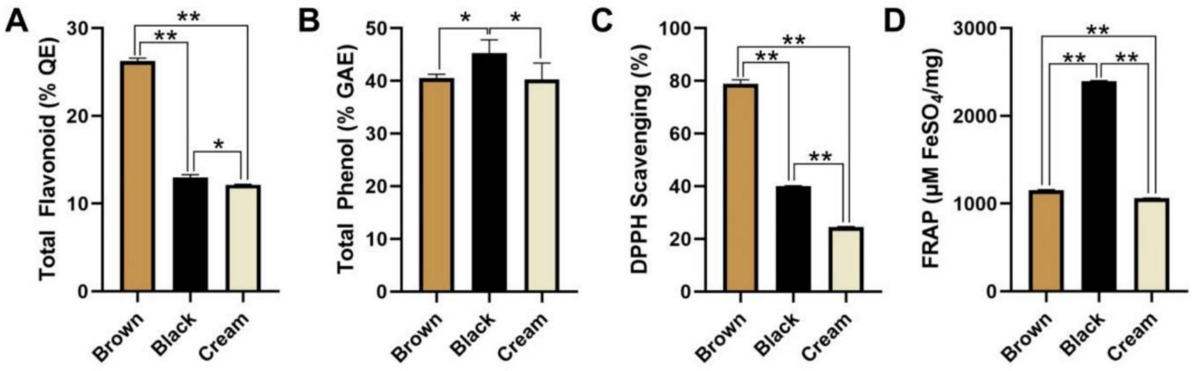

Figure 3. The secondary metabolites and antioxidant activity among Lablab beans' variants. Secondary metabolites were determined according to the total flavonoid (A) and total phenolic (B) contents, while the antioxidant activity was determined based on the DPPH scavenging (C) and FRAP analysis (D). The data was presented in mean with standard deviations (n = 3). The asterisk symbol determines the significant difference, with (*) being significant at 95% and (**) at 99% confidence intervals, respectively.

Author contribution

EP designed the research and acquired the project funding, FE collected and analyzed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

Acknowledgments

The authors thank to Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 for funding this work.

Conflict of Interest

There is no conflict of interest raised in this study.

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5. First revision: Accepted with major revision (22-2-2023)

- Article revision letter for authors

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From: Tanti Agustina <tantiagus5@gmail.com>
Sent: Wednesday, February 22, 2023 8:58 AM
To: Elly Purwanti <purwantielly@ymail.com>
Cc: Feri Eko Hermanto <feri.eko.hermanto@hotmail.com>; Wahyu Prihanta <wahyuprihanta@gmail.com>; Tutut Indria Permana <tutut_indria@umm.ac.id>; I Gusti Ngurah Agung Wiwekananda <wiwekananda@student.ub.ac.id>
Subject: [JTBB] Editor Decision

Dear Elly Purwanti,

Thank you for submitting your work, titled "Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development", to Journal of Tropical Biodiversity and Biotechnology. After reviewing your submission, we found that your manuscript has merit for publication.

However, we think that the manuscript needs a major revision to fulfill our publishing standard. We invite you to do the revision and return it to us to be reviewed again. Please activate the track changes mode in MS Word to track your revision. We enclosed the reviewer comments for you to learn.

Please send us answers to the reviewers' comments (attached) in a separate file.

We expect to receive your revision within four (4) weeks. If you fail to turn your revision in within the designated time, we may have to decline your submission without notice.

If you have any questions, please feel free to contact us.

Sincerely yours,
Tanti Agustina

- Review result

Review Form

Overall statement or **summary** of the article and its findings in your own words *

In general, this article is well written, and the study's findings can provide fresh information about phytochemical and antioxidant analyses of Lablabs bean growing in Indonesia. However, there are numerous errors that must be rectified before this article can be accepted and published.

Overall **strengths** of the article and what **impact** it might have in your field *

The strength of this article lies in a study of lablab beans that have been obtained in Indonesia

Specific comments on the **weaknesses** of the article and what could be done to improve it *

Title

Tittle- The species name must be written in italics.

Introduction

Images of sample must be scaled to reflect their true size.

Methodology

Please revised the method.

Explain in fully how the samples were collected (sampling time)?

Line 52, how much beans are extracted. Explain the specific procedure of the extraction process. detail

The extraction procedure is a critical step in this research. The author, however, fails to explain all the critical processes. The author just informs the reader to refer to past studies. Please describe the extraction procedure. What kind of solvent is employed? Authors should not expect readers to look up additional references to comprehend the experiments.

Please explain in detail the method for crude fibre, total protein, and crude fat analysis.

Result and discussion

Overall, the discussion is well written

Figure 3, the term secondary metabolite refers to phytochemical content. Please change it.

1.
2. Keputusan tidak consistent dengan kaedah. Rujuk Figure 2. Terdapat 6 analisis keputusan, tetapi berdasarkan kaedah tiada eksperimen bagi

6. First revision submitted (6-3-2023)

- Email response to editor

Re: [JTBB] Editor Decision

Yahoo/Inbox

Feri E. Hermanto <feri.eko.hermanto@hotmail.com>

Mon, Mar 6 at 5:11 PM

To: Tanti Agustina, Elly Purwanti

Cc: Wahyu Prihanta, Tutut Indria Permana, I Gusti Ngurah Agung Wiwekananda

Dear the Editor of JTBB,

On behalf of the corresponding author, we have submitted the revised version of our manuscript as well as the responses for the reviewer comments in your submission system. Please kindly check our submitted files whether those files are correct or not.

Thank your for considering our paper to be published and considered in your journal. We look forward to hear from you soon.

Sincerely,

Dr. Feri E. Hermanto, M.Si.

Department of Biology, Brawijaya University

Bioinformatics Research Center, Indonesia Institute of Bioinformatics

Malang, Indonesia

From: Tanti Agustina <tantiagus5@gmail.com>

Sent: Wednesday, February 22, 2023 8:58 AM

- Author's response form to reviewer's comments

Journal of
Tropical Biodiversity and Biotechnology

Author's Response Form to Reviewer's Comments

Manuscript Title: Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development

<table>
<tr><th>No.</th><th>Reviewer's Comments</th><th>Author's Response</th><th>Line</th></tr>
<tr><td colspan="4">Reviewer B
No evidence that these beans have been submitted to a herbarium for certification. PCR of DNA markers may also be used to confirm the identification of species.
<u>Response:</u> Thank you for your suggestion. However, due to funding limitations, we did not perform the molecular barcoding analysis. We continue the previous works related with this species, so we think that the taxonomic identity was confirmed and not confused with other related taxa.
Figure 2 and Figure 3 are best presented as a table as well to provide numerical values to their experiments.
<u>Response:</u> thank you. We have changed the figure into tables for better description.
The authors mentioned that based on their results, these beans are good candidates as functional food but fails to provide any in depth discussion on the benefits.
<u>Response:</u> We have added some discussions to strengthen our arguments.
The authors describe in detail regarding the chemistry behind DPPH and FRAP assay. They also state that brown and black beans exhibit solid antioxidant properties through different components of bioactive molecules in each bean but fails to mention what these bioactive molecules are.
<u>Response:</u> We have added the information about the biomolecules according to the previous work.
The discussion is superficial. No reference / comparisons to other legumes were made to justify their claims that these beans are superior functional food candidates.
<u>Response:</u> More comprehensive discussion has been made. The current results also compared to the previous results from different kind of beans to improve the arguments in the discussion section.
Also, there in no solid conclusion to their manuscript.
<u>Response:</u> We have modified the conclusion to improve the point of our study.</td></tr>
<tr><td>1.</td><td>too general. Kindly specify.</td><td>Several citations were added to specify the health benefits.</td><td>97</td></tr>
<tr><td>2.</td><td>Reconsider/elaborate on statement. In regions where food is scarce, functional food with greater fat content are better to overcome famine.</td><td>We have added the information of the data for hunger case in Indonesia to enrich our discussion at those points.</td><td>101-102</td></tr>
</table>

3.	Elaborate on statement	A sentence had been added to improve the clarity of the previous sentence.	113
4.	This is best presented as a table	The data have been restructured as table	117-118
5.	rephrase statement	The sentence has been erased and combined with the next sentence. We think that the next sentence is suitable enough to fulfill our discussion and not contra with the data.	129-130
6.	rephrase statement	The statement had been rephrased.	139-140
7.	Rephrase.	The statement had been amended.	141
8.	frequently occurs?	The sentence was revised to improve the clarity and compliance with the cited reference.	148-149
9.	Rephrase statement	The statement "…through different components of bioactive molecules in each bean" has been deleted.	150-153
10.	What are these bioactive molecules? How do you know they are different?	Actually, the result from total flavonoid and total phenolic compound have roughly described the difference of the bioactive compounds. Nevertheless, we do realize that those data are still insufficient to be discussed as in the previous statement. Thus, we think it would be better to delete that statement.	152-153
11.	..shows…shows.. Rephrase	First "shows" was replaced by "have"	153

12.	promising source of food?	Thank you for your critical evaluations. We did a revision with that sentence to improve the clarity.	156
13.	Grant number?	The grant number or contract number has been added to the funding statement.	172
Reviewer D Overall, the discussion is well written			
1.	**Title** Tittle- The species name must be written in italics.	The species name has been italicized.	
2.	**Introduction** Images of sample must be scaled to reflect their true size.	The scaled picture of each variant has been added to replace the old one.	
Methodology Please revised the method.			
3.	how much beans are extracted. Explain the specific procedure of the extraction process. detail	The extraction method was added.	52
4.	Explain in fully how the samples were collected (sampling time)?	We have added the information about the sampling of the beans.	
5.	The extraction procedure is a critical step in this research. The author, however, fails to explain all the critical processes. The author just informs the reader to refer to past studies. Please describe the extraction procedure. What kind of solvent is employed? Authors should not expect readers to look up additional references to comprehend the experiments.	The extraction method was added. Thank you for your critical evaluation.	

6.	Please explain in detail the method for crude fibre, total protein, and crude fat analysis.	Since no protocol modification from the cited reference, we think it's better to describe the method as it. Also, our paper was submitted as short communication paper, so the brief description of common method for crude fiber, total protein, and crude fat analysis would be less informative and make our paper exceed the maximum allowed words. Otherwise, we will revise it in the second round of revision if the method is urgently needed to be elaborated in the paragraph. Thank you for your suggestion.	
Result and discussion			
7.	The decision is not consistent with the rules. Refer to Figure 2. There are 6 decision analyses, but based on the method there is no experiment for.	Figure 2 describes about the content of fiber, protein, fat, amylose, amylopectin, and total calorie from each bean's variant. We have mentioned the method for each experiment in the figure 2 (now revised as a table following the suggestion from another reviewer) at the methodological section. We referred to a paper from Thiex (2009) to perform an analysis for crude fiber, protein, and fat content. Also, we referred to a protocol from McGrance et al. (1998) to determine the amylose and amylopectin content in our samples. Also, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories. We think that the method for figure 2's data is already covered in that paragraph. Thank you for your critical evaluations. If there is some	

		misunderstanding, please kindly elaborate your suggestion in the next round of reviewing step. We really acknowledge for your critical evaluations and constructive comments in our present works.	
8.	Figure 3, the term secondary metabolite refers to phytochemical content. Please change it.	We have modified that term in the revised version as table caption (as suggested by other reviewer). Thank you for your suggestion to enhance the clarity of our sentences.	

- Revised article

Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development

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Abstract

To date, the data describing the nutritional and secondary metabolites comparison of Lablab beans is incomplete. Therefore, this study will evaluate the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The result showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity in FRAP assay. Developing functional food from Lablab beans should consider this data as a reference.

Keywords: Antioxidant, *Dolichos lablab*, functional food, nutritional value, secondary metabolites.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only as a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022b; Hermanto et al. 2022a). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their richness of fiber and carbohydrate contents (Purwanti et al. 2019a; Maass et al. 2010). Moreover, Lablab beans also have superior environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). This nature may benefit the maintenance of food security, particularly in lands with a low water supply. Thus, the cultivation of Lablab beans provides a promising result in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' color, i.e., brown, black, and cream (figure 1). Although other variants may exist, those three are commonly found in several areas in Indonesia, such as East Java (Probolinggo and Madura Island, and West Nusa Tenggara) (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional difference among the variants of Lablab beans. Lablab beans have a lot of bioactivities like antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). Those bioactivities make it promising as an excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A), black (B), and cream (C).

The sample of the beans for this study was obtained from Madura Island, East Java, Indonesia. The details of the sample profiles and precise locations as described in previous literature (Purwanti et al. 2019b). The beans extraction was processed as the previously mentioned method (Purwanti et al. 2022). The crude fiber, total protein, and crude fat were determined according to the previous protocol (Thiex 2009). Besides, the amylose and amylopectin were also measured colorimetrically using the previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) calculated total calories referring to the manufacturer's protocol.

To determine the secondary metabolite contents, total phenol and total flavonoid was employed. Total flavonoid was performed referring to the previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 μL of the dissolved extract was mixed with 10 μL of 5% $NaNO_2$, followed by the addition of 150 μL of water and ten μL of 1 M CH3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with Gallic acid determined as the standard for phenolic compound quantification. The sample was diluted in water, and 100 μL of the sample was added by 1 mL of Folin Ciocalteu reagent and incubated for five minutes at room temperature with minimum light ambiance. An mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically in 725 nm wavelength. The total phenol was defined as % of Gallic Acid Equivalent (GAE) referring to the build standard curve.

The antioxidant activity was measured by DPPH scavenging and a Ferric Reducing Antioxidant Potential (FRAP) assay. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier literature (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if $p < 0.05$. The data is then visualized as mean accompanied by standard deviation value.

All Lablab bean variants have good nutrition according to the total fiber, protein total, crude fat, amylose, amylopectin, and calories. The fiber content of black Lablab bean was the highest, with 8% fiber content, followed by cream and brown variants (figure 2A). The considerable content of dietary fiber in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fiber consumption could improve physiological homeostasis, particularly related to lipid and glucose metabolism (Jahan et al. 2020). The high fiber content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). Meanwhile, from the total protein point of view, the cream beans had the same protein content as brown beans, while the black beans showed the lowest content of total proteins (figure 2B). The high percentage of total protein would be valuable for Lablab beans as the candidate for functional foods since the plant-based protein have primary dietary sources for essential amino acid supply to perform more health benefits (Maphosa et al. 2017). However, the cream beans have the highest percentage of crude fat among all variants (figure 2C, $p < 0.05$ and < 0.01). The low-fat contents of the Lablab beans showed a high potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food provides more health benefits with deleterious high-energy intake (Delaš 2011; Robson 2013). Thus, the low-fat contents in Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of the functional properties and energy source. The black Lablab bean has the highest amylose percentage with 15% amylose content ($p < 0.05$ and < 0.01), followed by brown and cream (figure 2D). In contrast, black beans had the lowest amylopectin ($p < 0.01$) than other analyzed variants (figure 2E). Similarly, black beans also had the lowest calorie per gram ($p < 0.01$) compared to other variants of Lablab beans (figure 2F). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose has better nutrient digestibility (Gao et al. 2020). Therefore, brown and cream beans may become the potential candidate for functional food.

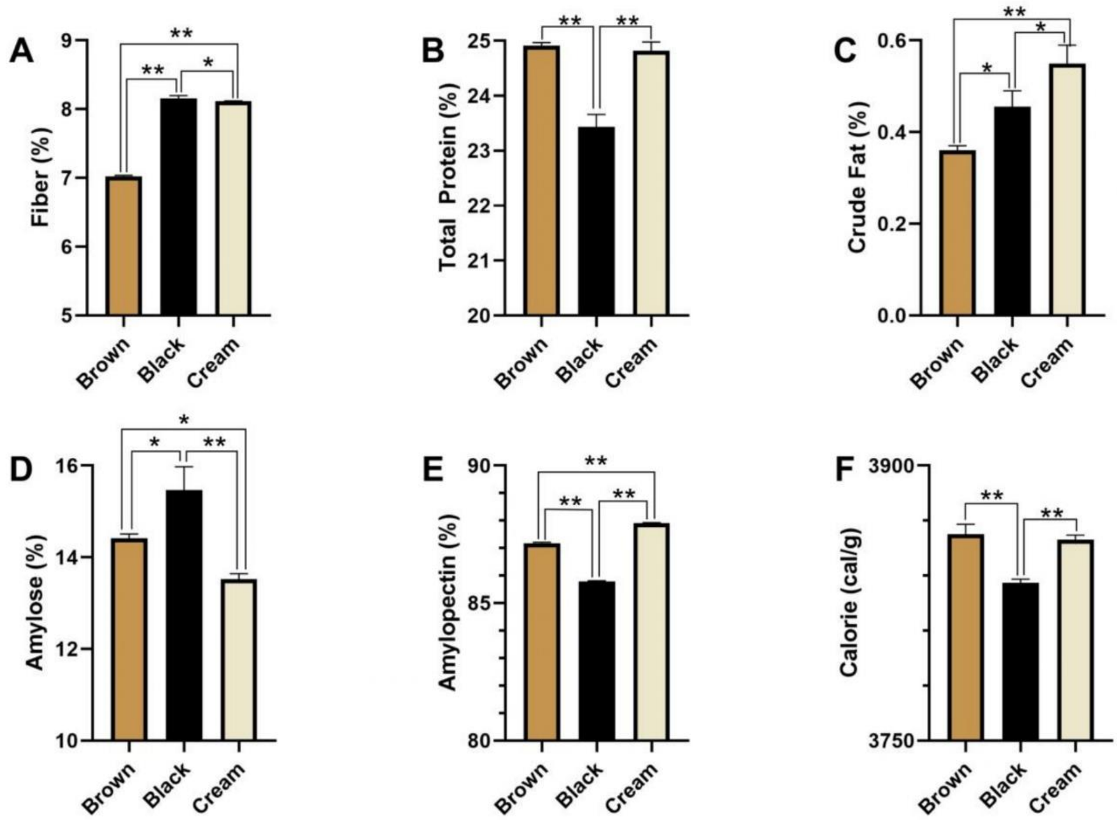

Figure 2. The comparison of primary metabolites and nutritional content among Lablab beans' variants. In consecutive order: crude fiber (A), total protein (B), crude fat (C), amylose (D), amylopectin (E), and total calories (F). The data was presented in mean with standard deviations (n = 3). The asterisk symbol determines the significant difference, with (*) being significant at 95% and (**) at 99% confidence intervals, respectively.

The secondary metabolites of Lablab beans were measured according to the total phenolic and flavonoid contents. Brown beans showed more great content of flavonoid ($p < 0.01$), while the cream beans comprised the most negligible flavonoid content (figure 3A). However, the phenolic compound was higher in black beans compared to other Lablab variants ($p < 0.05$). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being more abundant in brown beans. In other words, brown beans had the most secondary metabolites in phenolic and flavonoids. In contrast, the other variants may comprise other phenolic compounds like phenolic acids, tannins, and so on (Purwanti et al. 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with the antioxidant activity through DPPH scavenging activity, where the brown beans had the most excellent scavenging activity compared to the others (figure 3C). In contrast, ferric-reducing activity has similar results with the phenolic contents as the black beans outperformed the brown and cream beans variants (figure 3D). This result unsurprisingly occurred since there is a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have *an ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Electron Transfer Enthalpy (ETE) is the frequent mechanism during the radical scavenging of phenolic acids in the FRAP assay (Chen et al. 2020). Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties through different components of bioactive molecules in each bean. This study shows all Lablab bean variants shows good amount of nutritional value, secondary metabolites, and antioxidant activity. Although the cream variant shows slightly lower nutritional contents and bioactive compound compared to others analyzed Lablab beans, it is still a promising source of food mainly because the nutritional value.

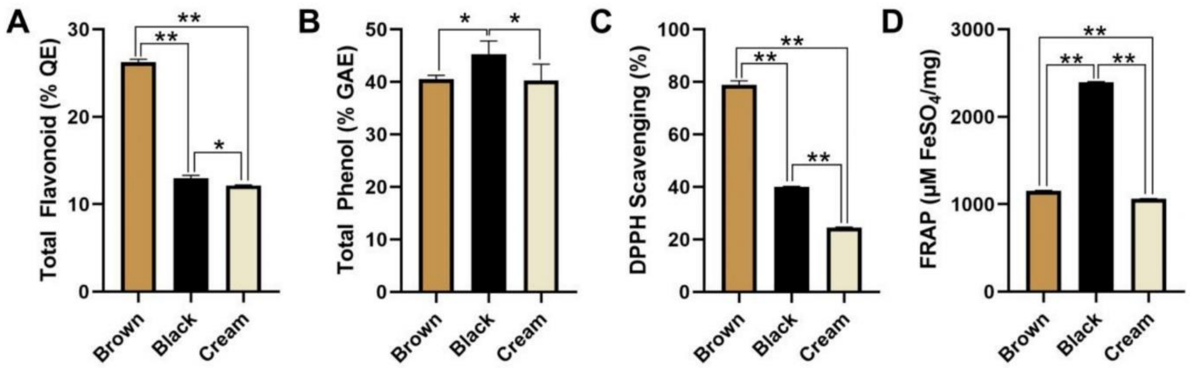

Figure 3. The secondary metabolites and antioxidant activity among Lablab beans' variants. Secondary metabolites were determined according to the total flavonoid (A) and total phenolic (B) contents, while the antioxidant activity was determined based on the DPPH scavenging (C) and FRAP analysis (D). The data was presented in mean with standard deviations (n = 3). The asterisk symbol determines the significant difference, with (*) being significant at 95% and (**) at 99% confidence intervals, respectively.

Author contribution

EP designed the research and acquired the project funding, FE collected and analyzed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

Acknowledgments

The authors thank to Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 for funding this work.

Conflict of Interest

There is no conflict of interest raised in this study.

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7. Article accepted for publication (29-3-2023)

- Decision letter to authors

[JTBB] Editor Decision 2

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Tanti Agustina <tantiagus5@gmail.com>

Wed, Mar 29 at 5:53 PM

To: Elly Purwanti

Cc: Feri Eko Hermanto, Wahyu Prihanta, Tutut Indria Permana, I Gusti Ngurah Agung Wiwekananda

Dear Dr. Dra. Elly Purwanti, M.P.,

Thank you for submitting your work, titled "Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development", to Journal of Tropical Biodiversity and Biotechnology. After reviewing your submission, we decided to accept your manuscript for publication.

Shortly after this, our copyeditor will contact you to review the final edited version of your manuscript. Please keep in mind that we will not publish the manuscript before you approve the final version.

If you have any questions, please feel free to contact us.

Sincerely yours,
Tanti Agustina
tantiagus5@gmail.com

Journal of Tropical Biodiversity and Biotechnology
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8. Copyedited article sent to author (13-4-2023)

- Notification letter

[JTBB] Copyediting Completed

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Salwa Shabria Wafi <wafisalwa@gmail.com>
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Thu, Apr 13 at 9:26 PM

Elly Purwanti:

We have now copyedited your submission "Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development" for Journal of Tropical Biodiversity and Biotechnology. To review the proposed changes and respond to Author Queries, please follow these steps:

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13. Edited article by author sent to editor (14-4-2023)

- Edited article with track changes

Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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Abstract

To date, the data describing [deleted text] various nutritional and secondary metabolites content of Lablab beans is incomplete. Therefore, this study [deleted text] **evaluated** the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The results showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging activity. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity via the FRAP assay. Those who are keen in developing functional food from Lablab beans should consider this data as a reference.

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Keywords: Antioxidant, *Dolichos lablab*, functional food, nutritional value, secondary metabolites.

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Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only are they a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022a; Hermanto et al. 2022b). Furthermore, the bioactive compounds in legumes also provide

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numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their rich **fibre** [deleted text] and carbohydrate contents (Purwanti et al. 2019a; Maass et al. 2010). Moreover, Lablab beans also have superior environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). Their innate nature may benefit the maintenance of food security, particularly in lands with low water supply. Thus, the cultivation of Lablab beans provides a promising means in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' colour, i.e., brown, black, and cream (figure 1). Although other variants may exist, these three are commonly found in several regions in Indonesia, such as East Java (Probolinggo and Madura Island, and West Nusa Tenggara) (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional differences among the variants of Lablab beans. Lablab beans have numerous bioactivities including antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). These bioactivities make it a promising and excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

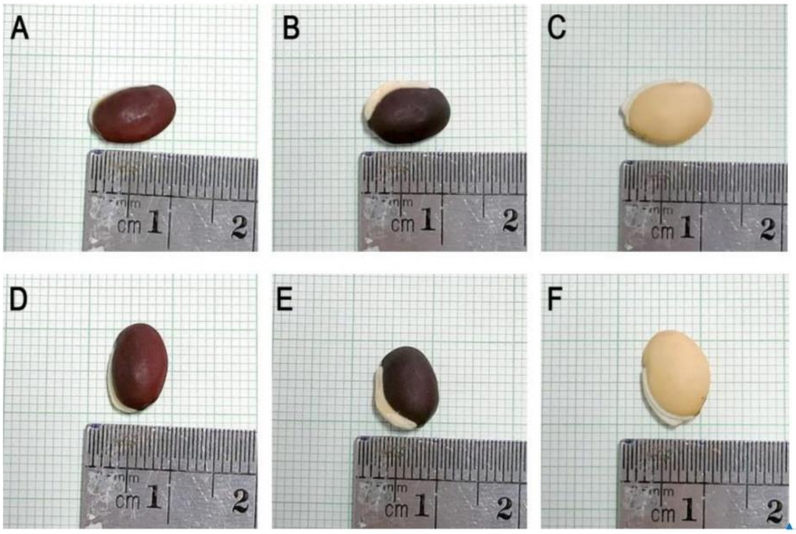

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A, D), black (B, E), and cream (C, F). The beans have been positioned to obtain the length (A-C) and the width (D-F).

The sample of the beans for this study was obtained from Sumenep, Madura Island, East Java, Indonesia during dry season in 2019. Details of the sample profiles and precise locations are as described in (Purwanti et al. 2019b). The beans were stored in 4°C until used. The beans were processed as per the extraction method previously mentioned in (Purwanti et al. 2022). Briefly, grounded beans were soaked in 96% ethanol with 3:1 ratio (volume in L and weight in kg) for 24 hours. The soaked beans powder then filtered to obtain the filtrate and homogenate. The filtrate then rotary evaporated to separate solvent and solute followed by freeze drying process to obtain Lablab beans' extract. The extract then processed to the subsequent analysis.

The crude **fibre** [deleted text], total protein, and crude fat content were determined according to the previous protocol (Thiex 2009). Amylose and amylopectin content were also measured colorimetrically using a previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories as per manufacturer's protocol.

To determine the secondary metabolites content, total phenol and total flavonoid was employed. Total flavonoid was performed as per a previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 µL of the dissolved extract was mixed with 10 µL of 5% $NaNO_2$, followed by the addition of 150 µL of water and 10 µL of 1 M CH_3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with gallic acid as the standard. The sample was diluted in water, and 100 µL of the sample was added to 1 mL of Folin Ciocalteu reagent and incubated for 5 minutes at room temperature with minimum light ambiance. 1 mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically at 725 nm. The total phenol was defined as [deleted text]~~%~~ percentage of Gallic Acid Equivalent (GAE) as per the build standard curve.

The antioxidant activity was measured by DPPH scavenging and Ferric Reducing Antioxidant Potential (FRAP) assays. The method for DPPH scavenging and FRAP reducing power was performed as described in (Irshad et al. 2012). All data were analysed by one-way

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ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if the p-value is < 0.05. The data was then **visualised** [deleted text] as mean ± standard deviation.

All Lablab bean variants have good nutritional content according to the caloric, total **fibre** [deleted text], protein, crude fat, amylose, and amylopectin content. The **fibre** [deleted text] content of black Lablab bean was the highest, with 8% **fibre** [deleted text] content, followed by the cream and brown variants (table 1). The considerable content of dietary **fibre** [deleted text] in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, **fibre** [deleted text] consumption can improve physiological homeostasis, particularly in relations to lipid and glucose metabolism (Jahan et al. 2020). High **fibre** [deleted text] content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). With regards to total protein content, cream beans had the same protein content as brown beans, while black beans had the lowest content of total proteins (table 1). The high percentage of total protein content in Lablab beans would be valuable as a candidate for functional foods since plant-based protein have broad health benefits, such as antioxidant, antiviral, antidiabetic, and anticancer properties (Maphosa et al. 2017; Sipahli et al. 2021; Liu et al. 2020; Roy et al. 2022; Purwanti et al. 2022). Nevertheless, specific treatment, such as isoelectric preparation, was suggested to obtain a protein isolate with adequate quality and good functional properties (Subagio 2006).

Cream beans have the highest percentage of crude fat among all variants (table 1). The low-fat content of Lablab beans exhibit a great potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food provides more health benefits with deleterious high-energy intake, particularly in areas with high level of famine cases (Delaš 2011; Robson 2013). For instance, West Nusa Tenggara province in Indonesia has the highest occurrence of hunger cases (Mone & Utami

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2021). Interestingly, this region also founds a large distribution of Lablab beans (Jayanti et al. 2011). The utilization of Lablab beans to reduce the incidence of famine should be considered. Thus, the low-fat content of Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of its functional properties and energy source. The black Lablab bean has the highest amylose content, with 15% amylose content, followed by brown and cream (table 1). In contrast, black beans had the lowest amylopectin content -compared to the other **analysed** [deleted text] variants (table 1). Similarly, black beans also had the lowest calorie per gram compared to other variants of Lablab beans (table 1). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose reflects better nutrient digestibility (Gao et al. 2020). A diet containing large portion of amylopectin positively associated with the postprandial insulin response resulted in more efficient nutrient uptake and glucose metabolism (Gao et al. 2020). Therefore, brown and cream beans may become potential candidates as functional food.

This study demonstrated that Lablab beans have been found to have comparable levels of total protein with *Vigna radiata* and *Pisum sativum*, and even higher levels than *Glycine max* and *Lens culinaris* (Singh et al. 2022). In addition, Lablab beans have a **favourable** nutritional profile with higher dietary **fibre** [deleted text] and lower fat content compared to *Phaseolus vulgaris*, *L. culinaris*, *P. sativum*, and Edamame (Mullins & Arjmandi 2021; Dhingra et al. 2012; Didinger & Thompson 2021). The amylose content in Lablab beans was higher than *Cicer arietinum* and *G. max* (Tayade et al. 2019). Moreover, the amylose and

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amylopectin content in Lablab beans also similar with *V. angularis*, a “red pearls” that has good nutrients and hypoglycemic activity (Zhang et al. 2022).

Table 1. The comparison of primary metabolites and nutritional content among Lablab bean variants.

Variant	Fiber (%)	Protein (%)	Crude Fat (%)	Amylose (%)	Amylopectin (%)	Calorie (kcal/g)
Brown	7.02 ± 0.015a	24.91 ± 0.06a	0.36 ± 0.01a	14.41 ± 0.095a	87.18 ± 0.030a	3.86 ± 0.005a
Black	8.16 ± 0.040b	23.43 ± 0.23b	0.45 ± 0.03b	15.46 ± 0.515b	85.79 ± 0.015b	3.83 ± 0.002b
Cream	8.11 ± 0.005c	24.82 ± 0.15a	0.55 ± 0.04c	13.52 ± 0.120c	87.90 ± 0.025c	3.85 ± 0.002a

Note: The data was presented as mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

The total phenolic and flavonoid contents evaluation demonstrated that brown beans exhibited the greatest content of flavonoid ($p < 0.01$), while the cream beans had the most negligible flavonoid content (table 2). **On** [deleted text] the other hand, the phenolic content was highest in black beans compared to other Lablab variants ($p < 0.05$, table 2). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being the most abundant in brown beans. In other words, the other variants may comprise of other phenolic compounds like phenolic acids, tannins, and other phenolic compounds (Purwanti et al. 2022). The current result **was** also higher than several edible beans, such as *P. vulgaris*, *P. lunatus, V. radiata* and *C. arietinum* (Zhao et al. 2014). Nonetheless, the total flavonoid

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contents of Lablab beans were lower compared to *P. sativum, C. arietinum, V. radiata, P. vulgaris, P. lunatus, L. culinaris, Vicia faba,* and *G. max* (Sharma & Giri 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with antioxidant activity through DPPH scavenging activity, where brown beans had the highest scavenging activity compared to the others (table 2). However, the ferric reducing activity was stronger in variant with higher phenolic contents (table 2). These results were supported by a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have *an ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Those structural differences influence the radical scavenging mechanism of flavonoids and other phenolic compounds in different antioxidant assay. Hydrogen Atom Transfer (HAT), Single-Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton-Loss Electron Transfer (SPLET) are taking place during the DPPH assay. Contrary, SPLET is the main mechanism during the electron transfer enthalpy in the FRAP reaction system (Chen et al. 2020). Ferulic Acid, Hydroxycinnamic Acid, Sinapinic Acid, Coumaric Acid, and Isovanillic Acid are identified phenolic acids in Lablab beans. Also, Rutin and Isoquercetin are flavonoids that also found in Lablab beans

(Purwanti et al. 2022). Those compounds were identified in adequate abundance in Lablab beans and may perform as radical scavenger during this study. Nevertheless, future studies are required to compare the secondary metabolites among different variant of Lablab beans to [deleted text] comprehend the phytochemical content differences **better**. Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties.

This study shows **that** all Lablab bean variants have good amount of nutritional value, total phenol and flavonoid contents, and antioxidant activity. Lablab beans have adequate nutritional values surpassed other types of edible beans. Despite having lower flavonoid contents compared to commonly consumed beans, the phenolic compounds in these beans still exhibit superior performance. Finally, the cream variant shows slightly lower nutritional contents and bioactive compound compared to the other **analysed** [deleted text] Lablab beans. Still, some varieties possess a promising characteristic as a functional food candidate owing to their nutritional value.

Table 2. The total flavonoid, phenolic, and antioxidant capacity according to the DPPH scavenging capacity and FRAP analysis.

Variant	**Total Flavonoid (%)**	**Total Phenol (%)**	**DPPH Scavenging (%)**	**FRAP (µM $FeSO_4$/mg)**
Brown	26.25 ± 0.34a	40.56 ± 0.69a	78.87 ± 1.59a	1154.58 ± 4.17a
Black	12.99 ± 0.31b	45.28 ± 2.56b	40.01 ± 0.16b	2398.05 ± 4.81b
Cream	12.15 ± 0.06c	40.32 ± 3.06a	24.53 ± 0.16c	1061.53 ± 2.41c

Notes: The data was presented in mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

Author contribution

EP designed the research and acquired the project funding, FE collected and **analysed** [deleted text] the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

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Acknowledgments

The authors thank the Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 (Grant No. E.2.a/334/BAA-UMM/IV/2022) for funding this work.

Conflict of Interest

There is no conflict of interest raised in this study.

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Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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Journal of Tropical Biodiversity and Biotechnology
http://jtbb.or.id
ISSN 2540-9573 (print)
ISSN 2540-9581 (online)

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- Draft layout

Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 02 (2023): jtbb81339
DOI: 10.22146/jtbb.81339

Short Communication

Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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Keywords:
Antioxidant
Dolichos lablab
functional food
nutritional value
secondary metabolites
Submitted:
15 January 2023
Accepted:
29 March 2023
Published:
xxxx
Editor:
Miftahul Ilmi

ABSTRACT
To date, the data describing various nutritional and secondary metabolites content of Lablab beans is incomplete. Therefore, this study evaluated the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The results showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging activity. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity via the FRAP assay. Those who are keen in developing functional food from Lablab beans should consider this data as a reference.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only are they a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022a; Hermanto et al. 2022b). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their rich fibre and carbohydrate contents (Maass et al. 2010; Purwanti et al. 2019a). Moreover, Lablab beans also have superior

environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). Their innate nature may benefit the maintenance of food security, particularly in lands with low water supply. Thus, the cultivation of Lablab beans provides a promising means in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' colour, i.e., brown, black, and cream (figure 1). Although other variants may exist, these three are commonly found in several regions in Indonesia, such as East Java (Probolinggo and Madura Island) and West Nusa Tenggara (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional differences among the variants of Lablab beans. Lablab beans have numerous bioactivities including antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). These bioactivities make it a promising and excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

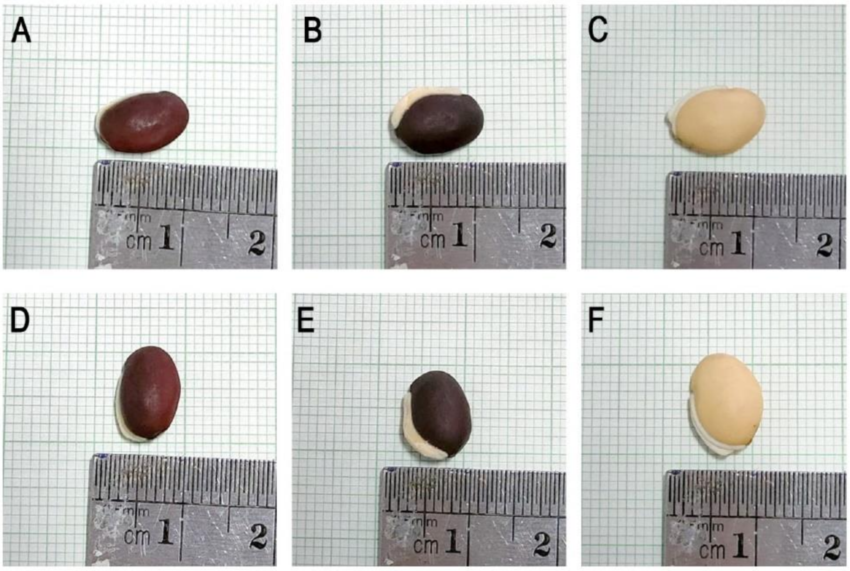

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A, D), black (B, E), and cream (C, F). The beans have been positioned to obtain the length (A-C) and the width (D-F).

The sample of the beans for this study was obtained from Sumenep, Madura Island, East Java, Indonesia during dry season in 2019. Details of the sample profiles and precise locations are as described in the previous study (Purwanti et al. 2019b). The beans were stored in 4°C until used. The beans were processed as per the extraction method previously mentioned in (Purwanti et al. 2022). Briefly, grounded beans were soaked in 96% ethanol with 3:1 ratio (volume in L and weight in kg) for 24 hours. The soaked beans powder then filtered to obtain the filtrate and homogenate. The filtrate then rotary evaporated to separate solvent and solute followed by freeze drying process to obtain Lablab beans' extract. The extract then processed to the subsequent analysis.

The crude fibre, total protein, and crude fat content were determined according to the previous protocol (Thiex 2009). Amylose and am-

ylopectin content were also measured colorimetrically using a previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories as per manufacturer's protocol.

To determine the secondary metabolites content, total phenol and total flavonoid was employed. Total flavonoid was performed as per a previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 μL of the dissolved extract was mixed with 10 μL of 5% $NaNO_2$, followed by the addition of 150 μL of water and 10 μL of 1 M CH_3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with gallic acid as the standard. The sample was diluted in water, and 100 μL of the sample was added to 1 mL of Folin Ciocalteu reagent and incubated for 5 minutes at room temperature with minimum light ambiance. 1 mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically at 725 nm. The total phenol was defined as percentage of Gallic Acid Equivalent (GAE) as per the build standard curve.

The antioxidant activity was measured by DPPH scavenging and Ferric Reducing Antioxidant Potential (FRAP) assays. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier work (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if the p-value is < 0.05. The data was then visualised as mean $\pm$ standard deviation.

All Lablab bean variants have good nutritional content according to the caloric, total fibre, protein, crude fat, amylose, and amylopectin content. The fibre content of black Lablab bean was the highest, with 8% fibre content, followed by the cream and brown variants (table 1). The considerable content of dietary fibre in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fibre consumption can improve physiological homeostasis, particularly in relations to lipid and glucose metabolism (Jahan et al. 2020). High fibre content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). With regards to total protein content, cream beans had the same protein content as brown beans, while black beans had the lowest content of total proteins (table 1). The high percentage of total protein content in Lablab beans would be valuable as a candidate for functional foods since plant-based protein have broad health benefits, such as antioxidant, antiviral, antidiabetic, and anticancer properties (Maphosa et al. 2017; Liu et al. 2020; Sipahli et al. 2021; Roy et al. 2022; Purwanti et al. 2022). Nevertheless, specific treatment, such as isoelectric preparation, was suggested to obtain a protein isolate with adequate quality and good functional properties (Subagio 2006).

Cream beans have the highest percentage of crude fat among all variants (table 1). The low-fat content of Lablab beans exhibit a great potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food

provides more health benefits with deleterious high-energy intake, particularly in areas with high level of famine cases (Delaš 2011; Robson 2013). For instance, West Nusa Tenggara province in Indonesia has the highest occurrence of hunger cases (Mone & Utami 2021). Interestingly, this region also founds a large distribution of Lablab beans (Jayanti et al. 2011). The utilization of Lablab beans to reduce the incidence of famine should be considered. Thus, the low-fat content of Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of its functional properties and energy source. The black Lablab bean has the highest amylose content, with 15% amylose content, followed by brown and cream (table 1). In contrast, black beans had the lowest amylopectin content compared to the other analysed variants (table 1). Similarly, black beans also had the lowest calorie per gram compared to other variants of Lablab beans (table 1). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose reflects better nutrient digestibility (Gao et al. 2020). A diet containing large portion of amylopectin positively associated with the postprandial insulin response resulted in more efficient nutrient uptake and glucose metabolism (Gao et al. 2020). Therefore, brown and cream beans may become potential candidates as functional food.

This study demonstrated that Lablab beans have been found to have comparable levels of total protein with *Vigna radiata* and *Pisum sativum*, and even higher levels than *Glycine max* and *Lens culinaris* (Singh et al. 2022). In addition, Lablab beans have a favourable nutritional profile with higher dietary fibre and lower fat content compared to *Phaseolus vulgaris*, *L. culinaris*, *P. sativum*, and Edamame (Dhingra et al. 2012; Mullins & Arjmandi 2021; Didinger & Thompson 2021). The amylose content in Lablab beans was higher than *Cicer arietinum* and *G. max* (Tayade et al. 2019). Moreover, the amylose and amylopectin content in Lablab beans also similar with *V. angularis*, a "red pearls" that has good nutrients and hypoglycemic activity (Zhang et al. 2022).

The total phenolic and flavonoid contents evaluation demonstrated that brown beans exhibited the greatest content of flavonoid ($p < 0.01$), while the cream beans had the most negligible flavonoid content (table 2). On the other hand, the phenolic content was highest in black beans compared to other Lablab variants ($p < 0.05$, table 2). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being the most abundant in brown beans. In other words, the other variants may comprise of other phenolic compounds like phenolic acids, tannins, and other phenolic compounds (Purwanti et al. 2022). The current result was also higher than several edible beans, such as *P. vulgaris*, *P. lunatus*, *V. radiata* and *C. arietinum* (Zhao et al. 2014). Nonethe-

Table 1. The comparison of primary metabolites and nutritional content among Lablab bean variants.

Variant	Fiber (%)	Protein (%)	Crude Fat (%)	Amylose (%)	Amylopectin (%)	Calorie (kcal/g)
Brown	7.02 ± 0.015^a	24.91 ± 0.06^a	0.36 ± 0.01^a	14.41 ± 0.095^a	87.18 ± 0.030^a	3.86 ± 0.005^a
Black	8.16 ± 0.040^b	23.43 ± 0.23^b	0.45 ± 0.03^b	15.46 ± 0.515^b	85.79 ± 0.015^b	3.83 ± 0.002^b
Cream	8.11 ± 0.005^c	24.82 ± 0.15^a	0.55 ± 0.04^c	13.52 ± 0.120^c	87.90 ± 0.025^c	3.85 ± 0.002^a

Note: The data was presented as mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

less, the total flavonoid contents of Lablab beans were lower compared to *P. sativum*, *C. arietinum*, *V. radiata*, *P. vulgaris*, *P. lunatus*, *L. culinaris*, *Vicia faba*, and *G. max* (Sharma & Giri 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with antioxidant activity through DPPH scavenging activity, where brown beans had the highest scavenging activity compared to the others (table 2). However, the ferric reducing activity was stronger in variant with higher phenolic contents (table 2). These results were supported by a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have an *ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Those structural differences influence the radical scavenging mechanism of flavonoids and other phenolic compounds in different antioxidant assay. Hydrogen Atom Transfer (HAT), Single-Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton-Loss Electron Transfer (SPLET) are taking place during the DPPH assay. Contrary, SPLET is the main mechanism during the electron transfer enthalpy in the FRAP reaction system (Chen et al. 2020). Ferulic Acid, Hydroxycinnamic Acid, Sinapinic Acid, Coumaric Acid, and Isovanillic Acid are identified phenolic acids in Lablab beans. Also, Rutin and Isoquercetin are flavonoids that also found in Lablab beans (Purwanti et al. 2022). Those compounds were identified in adequate abundance in Lablab beans and may perform as radical scavenger during this study. Nevertheless, future studies are required to compare the secondary metabolites among different variant of Lablab beans to comprehend the phytochemical content differences better. Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties.

This study shows that all Lablab bean variants have good amount of nutritional value, total phenol and flavonoid contents, and antioxidant activity. Lablab beans have adequate nutritional values surpassed other types of edible beans. Despite having lower flavonoid contents compared to commonly consumed beans, the phenolic compounds in these beans still exhibit superior performance. Finally, the cream variant shows slightly lower nutritional contents and bioactive compound compared to

Table 2. The total flavonoid, phenolic, and antioxidant capacity according to the DPPH scavenging capacity and FRAP analysis.

Variant	Total Flavonoid (%)	Total Phenol (%)	DPPH Scavenging (%)	FRAP (µM $FeSO_4$/mg)
Brown	26.25 ± 0.34[a]	40.56 ± 0.69[a]	78.87 ± 1.59[a]	1154.58 ± 4.17[a]
Black	12.99 ± 0.31[b]	45.28 ± 2.56[b]	40.01 ± 0.16[b]	2398.05 ± 4.81[b]
Cream	12.15 ± 0.06[c]	40.32 ± 3.06[a]	24.53 ± 0.16[c]	1061.53 ± 2.41[c]

Notes: The data was presented in mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

the other analysed Lablab beans. Still, some varieties possess a promising characteristic as a functional food candidate owing to their nutritional value.

AUTHORS CONTRIBUTION

EP designed the research and acquired the project funding, FE collected and analysed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

ACKNOWLEDGMENTS

The authors thank the Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 (Grant No. E.2.a/334/BAA-UMM/IV/2022) for funding this work.

CONFLICT OF INTEREST

There is no conflict of interest raised in this study.

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10. Draft layout revision sent to editor (10-5-2023)

- Email response to editor

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Elly Purwanti <purwantielly@ymail.com>
To: Salwa Shabria Wafi

Wed, May 10 at 10:43 AM

Dear the Editor of JTBB,

Thank you for the update. After thoroughly checked the draft, here are our revisions:

[page 2, 2nd paragraph, line 5] - REPLACE by (Probolinggo and Madura Island), and West Nusa Tenggara, from (Probolinggo and Madura Island, and West Nusa Tenggara)
[page 2, 3rd paragraph, line 3] - ADD by ... as described in the previous study (Purwanti et al. 2019b), from ... as described in (Purwanti et al. 2019b)
[page 3, 4th paragraph, line 4] - ADD by ... as described in the earlier work (Irshad et al. 2012), from ADD by ... as described in (Irshad et al. 2012)
[page 4, table 1] - REFORMAT - alphabetical notation in each row describing the value of the data should be superscripted
[page 5, 2nd paragraph, line 8] - REFORMAT - Flavonoids have an *ortho*-dihydroxyl ... , from Flavonoids have *an ortho*-dihydroxyl (from italic version of "an" into unitalicized)
[page 5, table 2] - REFORMAT - alphabetical notation in each row describing the value of the data should be superscripted

The part that need a revision as highlighted in yellow in the attached file. Please kindly inform us if there is something unclear.
Thank you.

Sincerely,
Dr. Elly Purwanti

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11. Article published (3-7-2023)

- Question for publication schedule

Question for Publication Schedule 3

Yahoo/Sent

Elly Purwanti <purwantielly@ymail.com>
To: Miftahul Ilmi, Salwa Wafi

Thu, Jun 8 at 4:53 PM

Dear the editors of JTBB,

Regarding the acceptance of our manuscript entitled "*Nutritional Contents and Bioactive Compounds among Several Variants of Dolichos lablab: Fundamental Facts for Functional Food Development*" in your journal, we would like to ask for the publication schedule of our manuscript. If possible, please kindly send us the link for the online first version if our manuscript not have been assigned into a certain volume.
Thank you in advance.

Sincerely,
Dr. Elly Purwanti

- Publication schedule confirmed

Salwa Wafi <wafisalwa@gmail.com>
To: Elly Purwanti
Cc: Miftahul Ilmi

Fri, Jun 9 at 9:43 AM

Dear Ms. Purwanti,

Thank you for your email. Your manuscript has been scheduled to be published on 3rd July 2023 in Volume 8 Issue 2. As it has not been published yet, there is no online version available. Please kindly wait until it is published, and we can give the link of the online version afterwards. I hope it answers your question. Thank you for your understanding.

Best regards,
Salwa Shabria Wafi
Admin of JTBB.

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ISSN 2540-9581 (online)

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- Final paper for publication

Journal of Tropical Biodiversity and Biotechnology
Volume 08, Issue 02 (2023): jtbb81339
DOI: 10.22146/jtbb.81339

Short Communications

Nutritional Contents and Bioactive Compounds among Several Variants of *Dolichos lablab*: Fundamental Facts for Functional Food Development

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Keywords:
Antioxidant
Dolichos lablab
functional food
nutritional value
secondary metabolites
Submitted:
15 January 2023
Accepted:
29 March 2023
Published:
03 July 2023
Editor:
Miftahul Ilmi

ABSTRACT
To date, the data describing various nutritional and secondary metabolites content of Lablab beans is incomplete. Therefore, this study evaluated the nutritional value, secondary metabolites, and antioxidant activity of three different variants of Lablab beans, i.e., brown, black, and cream beans. The results showed that the brown Lablab beans had outperformed other variants according to their nutritional value and flavonoid content with outstanding DPPH scavenging activity. However, the black beans also showed good bioactive contents through their total phenolic percentage with decent reducing activity via the FRAP assay. Those who are keen in developing functional food from Lablab beans should consider this data as a reference.

Legumes have provided nutritional value for years, contributing to the development of agriculture and food security (Considine et al. 2017). Not only are they a staple food in several regions of the globe, but legumes also provide valuable nutritional and health benefits (Polak et al. 2015; Piergiovanni 2021). The consumption of legumes has been reported to have protective roles against modern society's health problems, such as diabetes mellitus, hyperlipidemia, and cardiovascular diseases (Polak et al. 2015; Hermanto et al. 2022a; Hermanto et al. 2022b). Furthermore, the bioactive compounds in legumes also provide numerous biological activities to achieve physiological homeostasis (Çakir et al. 2019). Those facts are more than enough to describe the vital role of legumes in developing social health status. There are many species of legumes worldwide, but not all beans are known in the society. One of the underutilized legumes is Lablab beans (*Dolichos lablab*), also known as Koro Komak in Indonesia (Purwanti et al. 2019b). Natively grown in the African continent and Indian subcontinent, Lablab beans have become the primary source of energy due to their rich fibre and carbohydrate contents (Maass et al. 2010; Purwanti et al. 2019a). Moreover, Lablab beans also have superior

environmental adaptation due to their ability to grow in drought areas (Missanga et al. 2021). Their innate nature may benefit the maintenance of food security, particularly in lands with low water supply. Thus, the cultivation of Lablab beans provides a promising means in maintaining primary food stock in dry areas.

Three primary accessions or variants of Lablab beans have been identified in Indonesia (Purwanti et al. 2019b). Those accessions are commonly identified based on the beans' colour, i.e., brown, black, and cream (figure 1). Although other variants may exist, these three are commonly found in several regions in Indonesia, such as East Java (Probolinggo and Madura Island) and West Nusa Tenggara (Purwanti et al. 2019b). The previous study reported the bioactivity of bioactive compounds and nutritional values of Lablab beans (Purwanti et al. 2021; Purwanti et al. 2022). Nevertheless, no report addresses the nutritional differences among the variants of Lablab beans. Lablab beans have numerous bioactivities including antioxidant (Maheshu et al. 2013), antidiabetic (Purwanti et al. 2022), antivirus (Purwanti et al. 2021), antimicrobial (Bai-Ngew et al. 2021), and anti-inflammatory properties (An et al. 2020). These bioactivities make it a promising and excellent candidate for functional food development. The details on the nutritional comparison among Lablab beans will provide a fundamental guideline for determining suitable variants for functional food development, and it will be addressed by this study.

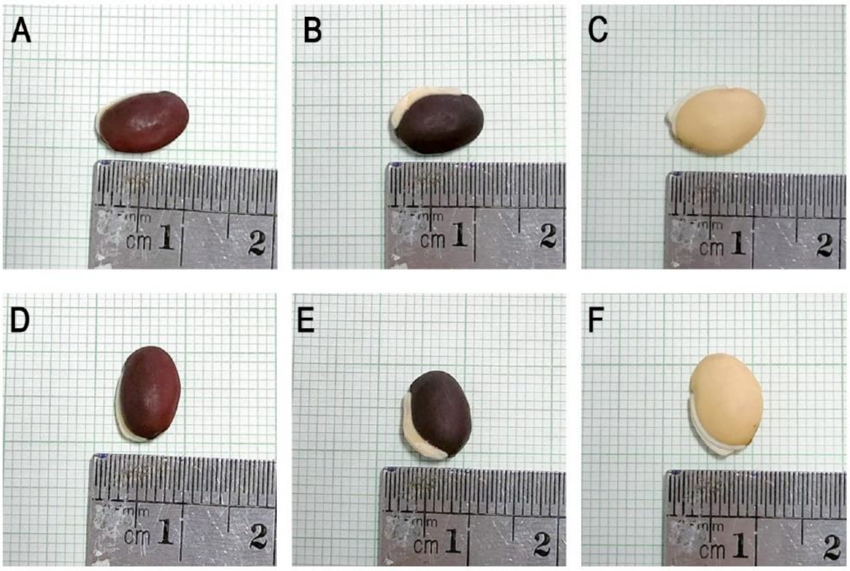

Figure 1. The most popular Lablab beans variant found in Indonesia are brown (A, D), black (B, E), and cream (C, F). The beans have been positioned to obtain the length (A-C) and the width (D-F).

The sample of the beans for this study was obtained from Sumenep, Madura Island, East Java, Indonesia during dry season in 2019. Details of the sample profiles and precise locations are as described in the previous study (Purwanti et al. 2019b). The beans were stored in 4°C until used. The beans were processed as per the extraction method previously mentioned in (Purwanti et al. 2022). Briefly, grounded beans were soaked in 96% ethanol with 3:1 ratio (volume in L and weight in kg) for 24 hours. The soaked beans powder then filtered to obtain the filtrate and homogenate. The filtrate then rotary evaporated to separate solvent and solute followed by freeze drying process to obtain Lablab beans' extract. The extract then processed to the subsequent analysis.

The crude fibre, total protein, and crude fat content were determined according to the previous protocol (Thiex 2009). Amylose and am-

ylopectin content were also measured colorimetrically using a previously described method (McGrance et al. 1998). On the other hand, IKA C2000 Calorimeter System (IKA Works, Germany) was employed to calculate the total calories as per manufacturer's protocol.

To determine the secondary metabolites content, total phenol and total flavonoid was employed. Total flavonoid was performed as per a previous protocol with minor modifications (Pratami et al. 2018). Quercetin was used as the standard flavonoid compound. The extract was dissolved in water, then 50 μL of the dissolved extract was mixed with 10 μL of 5% $NaNO_2$, followed by the addition of 150 μL of water and 10 μL of 1 M CH_3COONa, consecutively. The sample was then incubated at room temperature for 40 minutes. After incubation, the sample was quantified using a spectrophotometer at 415 nm wavelength. The total flavonoid concentration was described as percent (%) of Quercetin Equivalent (QE) according to the standard curve.

Total phenol was measured according to the previous study with minor modifications (Hyun et al. 2014), with gallic acid as the standard. The sample was diluted in water, and 100 μL of the sample was added to 1 mL of Folin Ciocalteu reagent and incubated for 5 minutes at room temperature with minimum light ambiance. 1 mL of 7.5% Na_2CO_3 was added to the mixture, followed by incubation for 90 minutes in the same condition mentioned beforehand. Upon incubation, the sample was then quantified spectrophotometrically at 725 nm. The total phenol was defined as percentage of Gallic Acid Equivalent (GAE) as per the build standard curve.

The antioxidant activity was measured by DPPH scavenging and Ferric Reducing Antioxidant Potential (FRAP) assays. The method for DPPH scavenging and FRAP reducing power was performed as described in the earlier work (Irshad et al. 2012). All data were analysed by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis. The data was determined as significantly different if the p-value is < 0.05. The data was then visualised as mean $\pm$ standard deviation.

All Lablab bean variants have good nutritional content according to the caloric, total fibre, protein, crude fat, amylose, and amylopectin content. The fibre content of black Lablab bean was the highest, with 8% fibre content, followed by the cream and brown variants (table 1). The considerable content of dietary fibre in Lablab beans displayed an immense potential to be developed as a functional food. As commonly known, fibre consumption can improve physiological homeostasis, particularly in relations to lipid and glucose metabolism (Jahan et al. 2020). High fibre content is also suitable for dietary intervention to prevent obesity (Dayib et al. 2020). With regards to total protein content, cream beans had the same protein content as brown beans, while black beans had the lowest content of total proteins (table 1). The high percentage of total protein content in Lablab beans would be valuable as a candidate for functional foods since plant-based protein have broad health benefits, such as antioxidant, antiviral, antidiabetic, and anticancer properties (Maphosa et al. 2017; Liu et al. 2020; Sipahli et al. 2021; Roy et al. 2022; Purwanti et al. 2022). Nevertheless, specific treatment, such as isoelectric preparation, was suggested to obtain a protein isolate with adequate quality and good functional properties (Subagio 2006).

Cream beans have the highest percentage of crude fat among all variants (table 1). The low-fat content of Lablab beans exhibit a great potential as functional food compared to other beans since most legumes contain around 1,5% crude fat total (Etiosa et al. 2017). Low-lipid food

provides more health benefits with deleterious high-energy intake, particularly in areas with high level of famine cases (Delaš 2011; Robson 2013). For instance, West Nusa Tenggara province in Indonesia has the highest occurrence of hunger cases (Mone & Utami 2021). Interestingly, this region also founds a large distribution of Lablab beans (Jayanti et al. 2011). The utilization of Lablab beans to reduce the incidence of famine should be considered. Thus, the low-fat content of Lablab beans displayed their potential as a functional food candidate.

This study also measured the amount of amylose and amylopectin as part of its functional properties and energy source. The black Lablab bean has the highest amylose content, with 15% amylose content, followed by brown and cream (table 1). In contrast, black beans had the lowest amylopectin content compared to the other analysed variants (table 1). Similarly, black beans also had the lowest calorie per gram compared to other variants of Lablab beans (table 1). A food source with high amylopectin induces a better glycemic response, especially during fasting (Singhania & Senray 2012). This starch also provides higher energy intake than low amylopectin sources (Singhania & Senray 2012). Moreover, the increasing ratio of amylopectin/amylose reflects better nutrient digestibility (Gao et al. 2020). A diet containing large portion of amylopectin positively associated with the postprandial insulin response resulted in more efficient nutrient uptake and glucose metabolism (Gao et al. 2020). Therefore, brown and cream beans may become potential candidates as functional food.

This study demonstrated that Lablab beans have been found to have comparable levels of total protein with *Vigna radiata* and *Pisum sativum*, and even higher levels than *Glycine max* and *Lens culinaris* (Singh et al. 2022). In addition, Lablab beans have a favourable nutritional profile with higher dietary fibre and lower fat content compared to *Phaseolus vulgaris*, *L. culinaris*, *P. sativum*, and Edamame (Dhingra et al. 2012; Mullins & Arjmandi 2021; Didinger & Thompson 2021). The amylose content in Lablab beans was higher than *Cicer arietinum* and *G. max* (Tayade et al. 2019). Moreover, the amylose and amylopectin content in Lablab beans also similar with *V. angularis*, a "red pearls" that has good nutrients and hypoglycemic activity (Zhang et al. 2022).

The total phenolic and flavonoid contents evaluation demonstrated that brown beans exhibited the greatest content of flavonoid ($p < 0.01$), while the cream beans had the most negligible flavonoid content (table 2). On the other hand, the phenolic content was highest in black beans compared to other Lablab variants ($p < 0.05$, table 2). This result showed that Lablab beans have many phenolic compounds, with the flavonoid group being the most abundant in brown beans. In other words, the other variants may comprise of other phenolic compounds like phenolic acids, tannins, and other phenolic compounds (Purwanti et al. 2022). The current result was also higher than several edible beans, such as *P. vulgaris*, *P. lunatus*, *V. radiata* and *C. arietinum* (Zhao et al. 2014). Nonethe-

Table 1. The comparison of primary metabolites and nutritional content among Lablab bean variants.

Variant	Fiber (%)	Protein (%)	Crude Fat (%)	Amylose (%)	Amylopectin (%)	Calorie (kcal/g)
Brown	7.02 ± 0.015^a	24.91 ± 0.06^a	0.36 ± 0.01^a	14.41 ± 0.095^a	87.18 ± 0.030^a	3.86 ± 0.005^a
Black	8.16 ± 0.040^b	23.43 ± 0.23^b	0.45 ± 0.03^b	15.46 ± 0.515^b	85.79 ± 0.015^b	3.83 ± 0.002^b
Cream	8.11 ± 0.005^c	24.82 ± 0.15^a	0.55 ± 0.04^c	13.52 ± 0.120^c	87.90 ± 0.025^c	3.85 ± 0.002^a

Note: The data was presented as mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

less, the total flavonoid contents of Lablab beans were lower compared to *P. sativum*, *C. arietinum*, *V. radiata*, *P. vulgaris*, *P. lunatus*, *L. culinaris*, *Vicia faba*, and *G. max* (Sharma & Giri 2022). Although flavonoids are the most abundant phenolic compounds with various biological activities (Kumar & Pandey 2013), other phenolic compounds, either simple phenols or polyphenols other than flavonoids, have also been reported to have bioactivities to improve physiological homeostasis, mainly through their antioxidant activity (Shahidi & Ambigaipalan 2015; Singh et al. 2017).

The high flavonoid content was positively correlated with antioxidant activity through DPPH scavenging activity, where brown beans had the highest scavenging activity compared to the others (table 2). However, the ferric reducing activity was stronger in variant with higher phenolic contents (table 2). These results were supported by a structure-activity relationship between radical scavenging from different phenolic compounds and the radical scavenging mechanism in DPPH and FRAP assay. Flavonoids have an *ortho*-dihydroxyl structure that plays a role in radical scavenging during DPPH assay by forming an intramolecular hydrogen bond and more stable *ortho*-hydroxyl phenoxyl radical during the oxidation process of radical scavenging (Zheng et al. 2010). Alternatively, other phenolic compounds, such as phenolic acids, have *ortho* or *para* position of the hydroxyl group in its benzene ring (Spiegel et al. 2020). Those structural differences influence the radical scavenging mechanism of flavonoids and other phenolic compounds in different antioxidant assay. Hydrogen Atom Transfer (HAT), Single-Electron Transfer followed by Proton Transfer (SET-PT), and Sequential Proton-Loss Electron Transfer (SPLET) are taking place during the DPPH assay. Contrary, SPLET is the main mechanism during the electron transfer enthalpy in the FRAP reaction system (Chen et al. 2020). Ferulic Acid, Hydroxycinnamic Acid, Sinapinic Acid, Coumaric Acid, and Isovanillic Acid are identified phenolic acids in Lablab beans. Also, Rutin and Isoquercetin are flavonoids that also found in Lablab beans (Purwanti et al. 2022). Those compounds were identified in adequate abundance in Lablab beans and may perform as radical scavenger during this study. Nevertheless, future studies are required to compare the secondary metabolites among different variant of Lablab beans to comprehend the phytochemical content differences better. Despite the different mechanisms and types of bioactive compounds in performing the antioxidant activity, it has been displayed that brown and black beans exhibit solid antioxidant properties.

This study shows that all Lablab bean variants have good amount of nutritional value, total phenol and flavonoid contents, and antioxidant activity. Lablab beans have adequate nutritional values surpassed other types of edible beans. Despite having lower flavonoid contents compared to commonly consumed beans, the phenolic compounds in these beans still exhibit superior performance. Finally, the cream variant shows slightly lower nutritional contents and bioactive compound compared to

Table 2. The total flavonoid, phenolic, and antioxidant capacity according to the DPPH scavenging capacity and FRAP analysis.

Variant	Total Flavonoid (%)	Total Phenol (%)	DPPH Scavenging (%)	FRAP (μM $FeSO_4$/mg)
Brown	26.25 ± 0.34^a	40.56 ± 0.69^a	78.87 ± 1.59^a	1154.58 ± 4.17^a
Black	12.99 ± 0.31^b	45.28 ± 2.56^b	40.01 ± 0.16^b	2398.05 ± 4.81^b
Cream	12.15 ± 0.06^c	40.32 ± 3.06^a	24.53 ± 0.16^c	1061.53 ± 2.41^c

Notes: The data was presented in mean ± standard deviations (n = 3). Different alphabetical notation indicates significant difference with $p < 0.05$ based on LSD test.

the other analysed Lablab beans. Still, some varieties possess a promising characteristic as a functional food candidate owing to their nutritional value.

AUTHORS CONTRIBUTION

EP designed the research and acquired the project funding, FE collected and analysed the data, WP and TIP wrote the manuscript, and IGNAW performed critical review and revision.

ACKNOWLEDGMENTS

The authors thank the Directorate of Research and Community Service (DPPM), University of Muhammadiyah Malang under PKID research grant scheme in 2022 (Grant No. E.2.a/334/BAA-UMM/IV/2022) for funding this work.

CONFLICT OF INTEREST

There is no conflict of interest raised in this study.

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