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Scientific article

Total phenolics, flavonoids, antioxidant activity, and α-glucosidase inhibitory activity of ornamental pepper and several other lines

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Abstract

Ornamental chili is a type of chili plant that favors the value of beauty but still has a spicy taste. It generally has a purple fruit color because they contain anthocyanins. The biochemical content in ornamental chili is believed to have benefits for human health. This study aims to identify several biochemical compounds in several genotypes of ornamental chili and pure lines. The study was conducted using the microplate method which was repeated three times. The research design used was a completely randomized design with a single factor genotype. Sixteen genotypes of chili were used, consisting of 4 genotypes of ornamental chilies and 12 genotypes of pure lines. The results showed that the ornamental chili genotype Nazla IPB contained 83.27% α-glucosidase inhibitory activities. The pure lines genotype F10 145174-9-7-1-5-3-1-2-5 contained the second-largest α-glucosidase inhibitory activities (80.67%) and the highest antioxidant DPPH (15.40 µmol TE g^{-1} extract). The highest antioxidant of the FRAP method was shown in Adelina IPB's ornamental chili. The correlation between biochemical parameters showed that DPPH-AGI was significantly positively correlated and FRAP-AGI was negatively correlated. It is concluded that the information in this study can be the basis for the development of functional ornamental plants in future ornamental chili breeding research.
Keywords: DPPH, FRAP, inhibitor α-glucosidase activities, ornamental chili.

Resumo

Fenólicos totais, flavonóides, atividade antioxidante e atividade inibitória de α-glicosidase de pimenta ornamental e outras linhagens

A pimenta ornamental é um tipo de planta de pimenta favorecida pela beleza, mas ainda tem um sabor picante. Geralmente, tem uma cor roxa devido à presença de antocianinas. Acredita-se que o conteúdo bioquímico da pimenta ornamental tenha benefícios para a saúde humana. Este estudo visou a identificar compostos bioquímicos em diversos genótipos de pimentas ornamentais e linhagens puras. O estudo foi realizado usando o método de microplaca que foi repetido três vezes. O delineamento utilizado foi inteiramente casualizado com genótipo de fator único. Foram utilizados 16 genótipos de pimenta, sendo 4 genótipos de pimentas ornamentais e 12 genótipos de linhagens puras. Os resultados mostraram que o genótipo de pimenta ornamental Nazla IPB continha 83,27% de atividades inibitórias de α-glicosidase. As linhagens puras genótipo F10 145174-9-7-1-5-3-1-2-5 continham a segunda maior atividade inibitória de α-glicosidase (80,67%) e o maior antioxidante DPPH (15,40 µmol TE/g de extrato). O maior antioxidante do método FRAP foi demonstrado no pimentão ornamental de Adelina IPB. A correlação entre os parâmetros bioquímicos mostrou que o DPPH-AGI foi correlacionado significativa e positivamente e o FRAP-AGI foi correlacionado negativamente. Conclui-se que as informações deste estudo podem ser a base para o desenvolvimento de plantas ornamentais funcionais em futuras pesquisas de melhoramento de pimenta ornamental.
Palavras-chave: DPPH, FRAP, inibidor de atividades de α-glicosidase, pimenta ornamental.

Introduction

Chili is a type of horticultural plant that is widely cultivated throughout the world and almost everyone consumes chili. Continents of Europe and America generally cultivate chili plants of the species *Capsicum sinensis*, *Capsicum pubescens* and *Capsicum baccatum*. While on the Asian and African continents, there are many famous chili cultivation species *Capsicum annuum* and *Capsicum frutescens*. Various types of chili in the same species found many variations including cayenne pepper, large chili, curly chili and ornamental chili. Ornamental

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chili is one type of chili that has a unique morphology so that it can be used as an ornamental plant (Rêgo and Rêgo, 2018). The ornamental chili that is widely spread and famous is the chili plant which has a purple fruit color. The purple color of chili is caused by the chemical compounds contained in it, especially anthocyanins (Tang et al., 2020).

The primary metabolite commonly found in chili plants is capsaicin. Capsaicin plays an important role in controlling the spiciness of chilies (Lu et al., 2020). Fresh chilies contain capsaicin as much as 0.1%-1.0%. Capsaicin can be found in fruit skins, fruit flesh, and chili seeds. The capsaicin content found was also influenced by chili species (Sahid et al., 2020). Besides containing capsaicin, chili also has other secondary metabolite compounds including: antioxidants, phenolics, flavonoids, and α-glucosidase inhibitory activities so that they can potentially be developed into functional food plants that have medicinal properties.

Antioxidants function to overcome free radical damage in the body and play a role in preventing various diseases due to oxidative stress (Ramírez et al., 2019). Antioxidant content is also influenced by environmental stress factors both biotically (Ramzan et al., 2021) and abiotically (Kopta et al., 2020). However, in this study we focused on the optimal conditions for chili growth in greenhouse. This was because we want to focus on obtaining the potential biochemical content of chili peppers at optimal growth-controlled conditions. Phenolic compounds are secondary metabolites that are hydrosoluble and very sensitive to enzyme oxidation (Puangbanglang et al., 2019). Meanwhile, flavonoids are also secondary metabolites found in chili peppers and can function as dyes in plant tissues scattered in plant body parts (Mathesius, 2018). The color of chilies containing flavonoids is generally purple, purplish red, or dark blue (Palma et al., 2020). In this study, red chilies that have been fully ripened are used as initial biochemical profiling.

The α-glucosidase inhibitors play a role in absorbing sugar in the blood (Smith et al., 2021). In addition, information on biochemical content is useful in the development of plant varieties (Lemos et al., 2019). Several other plants that contain α-glucosidase inhibitors have been investigated including flower plants (Zhang et al., 2020), fruits (Islam et al., 2021), vegetables (Assefa et al., 2020; Papoutsis et al., 2021), onions (Ahmed et al., 2020), seaweed (Liu et al., 2018), and winged bean (Calvindi et al., 2020). Cultivation of ornamental vegetables in urban areas as urban farming is currently a new trend in the era of the covid pandemic. Ornamental vegetable plants grown in urban areas can be directed as ornamental vegetable plants that have useful biochemical compounds (Nascimento et al., 2019). Information on biochemical content, especially α-glucosidase inhibitors in chili genotypes, has not been widely published. Therefore, this study aims to identify the biochemical content of several genotypes of ornamental chilies and pure lines that can potentially be developed for functional ornamental plants.

Materials and Methods

Genetic materials and field technical

The genotypes of chili used in this study are shown in Table 1. The genotypes used consisted of 4 genotypes of ornamental chilies that are well known in Indonesia and 12 genotypes of pure lines from crosses in 2017.

The fruit and plant appearance of the four ornamental chilies used are shown in Figure 1.

Table 1. List of genotypes used in this study

Number	Genotype
1	NAZLA IPB
2	SSH C 2
3	ADELINA IPB
4	AYESHA IPB
5	VIOLA IPB
6	F10 145291-10-7-1-1-2-1-3-6
7	F10 145293-19-8-3-113-1-1-1-1
8	F10 145174-9-7-1-5-3-1-2-5
9	F8 145291-14-9-3-12-1-1
10	F11 160291-3-12-5-511-1-2-2-4
11	F11 145291-115-15-8-1-1-2-5-1 (H)-6
12	F11 160291-9-4-3-2-1-1-1-1-1
13	F11 145291-115-15-8-1-1-2-5-3 (K)-3
14	F11 160291-14-10-10-4-9-1-1-1-1
15	F12 160291-3-12-3-4-5-1-1-5-1-1
16	F12 160291-3-12-5-51-1-1-2-1-1-2

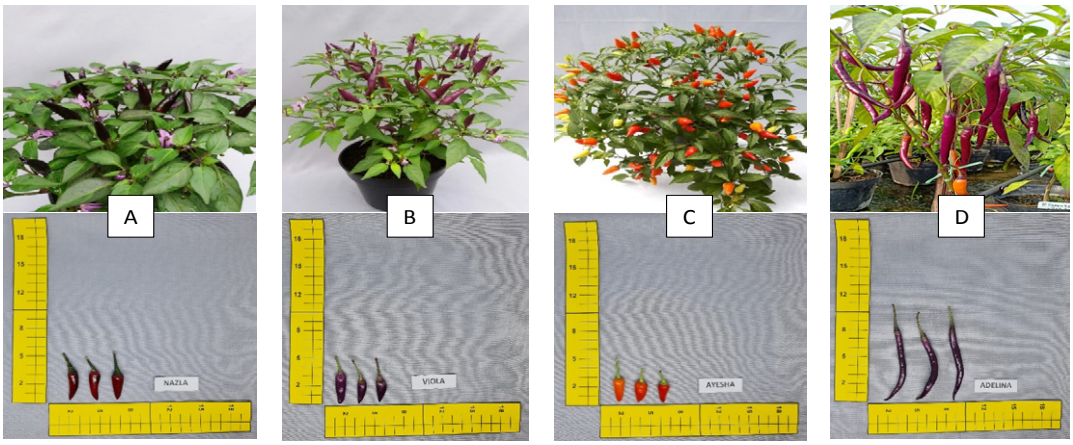

Figure 1 (A) Nazla IPB; (B) Viola IPB; (C) Ayesha IPB; (D) Adelina IPB

Planting ornamental chili was carried out in the D80 Alam Sinar Sari greenhouse. The treatment includes fertilizing with AB Mix dissolved in water. In addition to fertilization, pesticides are sprayed every three weeks. The ripe chilies were then harvested as sample extraction material for biochemical analysis.

Sample Extraction

The chilies that have been harvested are then dried using an oven at 50 ˚C for 72 hours. After drying, the grinding process is carried out until it becomes smooth. Chilli powder that has been finely weighed 3 grams is added 80% ethanol as much as 60 mL into a sterilized glass jam bottle. The bottle is then wrapped in aluminum foil and shaken for 48 hours. After the sample was shaken, it was filtered and transferred to a glass bottle to a volume of 50 mL. The extract solution was stored in a refrigerator at 15 ^{0}C for 24 hours before further analysis.

Biochemical parameters measurements

All biochemical parameters were measured using a microplate using an ELISA Reader. Measurements were repeated three times. The results that emerge from the ELISA Reader are sample absorbances which are then converted using Microsoft Excel. The wavelength used in each parameter is different. Measurement of Total Phenolic Content (TPC) using a wavelength of 750 nm with 500 ppm Gallic Acid as standard. The final unit used for TPC parameters is mg GAE g^{-1} extract. Total Flavonoid Content (TFC) was measured at a wavelength of 415 nm and the standard Quercetin was used in this study. The final unit for TFC parameters was mg QE g^{-1} extract. The measurement of antioxidants using the DPPH and FRAP methods uses the same standard, namely Trolox and the final unit used is also µmol TE (Trolox Equivalent)/g extract. The difference is shown in the wavelength used, namely 517 nm for DPPH and 595 nm for FRAP. Measurement of α-glucosidase inhibitory activities using a wavelength of 410 nm (Loodu and Rupasinghe, 2019; Wibisono et al., 2019; Nur et al., 2020; Gaber et al., 2021; Jo et al., 2021).

Data analysis

This research was conducted using a completely randomized design with three repetitions for each genotype. Each replication used ripe chilies from twenty chili plants for biochemical analysis. Biochemical analysis used microplate reader ELISA method (Sahid et al., 2021). ANOVA, DMRT 5% test, and Pearson correlation analysis were performed using the SAS 9.0. PCA analysis was performed to display dendrograms and quadrants using the PB-STAT 3.1. HCA analysis uses the R 4.0.5 program with the Performance Analytics packages (Peterson et al., 2014).

Results and Discussion

The results of ANOVA are shown in Table 2. The mean genotype values for all observed biochemical parameters showed significant differences which could be the basis for the DMRT test. Further testing could be carried out, if the ANOVA results were significantly different (Rohini et al., 2017). The replications in Table 2 showed no significant difference, indicating that the results of the biochemical parameter test were stable in the tested genotypes. The value of the coefficient of variation produced in this study is in the range of 3.20%-18.99%. The small value of the coefficient of variation (20% <) indicates that the validity of the conclusions generated is also getting better (Döring and Reckling, 2018).

Table 2. ANOVA of the biochemical parameters of chili genotypes.

Sources	df	Mean Square				
		TPC	TFC	DPPH	FRAP	AGI
Repetition	2	3.22	0.08	0.14	94.08	12.21
Genotype	15	7.11*	0.52**	7.58**	1276.39**	125.91**
Error	30	5.14	0.05	1.02	31.11	5.24
Coefficient of Variance (%)		18.99	17.52	7.77	10.04	3.20

Notes: **= very significantly at level α 1%; *= significantly at level α 5%; TPC= Total Phenolic Content, TFC= Total Flavonoid Content, DPPH= Antioxidant DPPH Method, FRAP= Antioxidant FRAP Method, AGI= α-glucosidase inhibitory activity.

Nazla IPB is an ornamental chili genotype that has the highest value of α-glucosidase inhibitory activity (83.27%) compared to other genotypes. The high value of α-glucosidase inhibitory activity is thought to be caused by the purple color contained in this chili fruit. The purple color in plants was caused by the content of anthocyanins and total flavonoids (Guevara et al., 2019). Anthocyanin is one of the compounds that play a role in α-glucosidase inhibition activities (Papoutsis et al., 2021). In addition to Nazla IPB, the genotypes of ornamental chilies Adelina IPB and Viola IPB which have a purple color on the fruit also showed high α-glucosidase inhibitory activity values of 78.68% and 79.68%, respectively. The value of α-glucosidase inhibitory activity produced in this study was in the range of 62.83%-83.27%. The value of α-glucosidase inhibitory activity produced in this study was above 50%, which means that the chili genotype used in this study has the potential to be developed for functional ornamental plant varieties to help overcome diabetes control. This is in line with the statement that α-glucosidase inhibitory activity of more than 50% can potentially be used to help prevent diabetes (Hossain et al., 2020).

In addition to α-glucosidase inhibitory activity, Nazla IPB also showed the highest total phenolic content (15.49 mg GAE g^{-1} extract) and total flavonoid content (2.21 mg QE g^{-1} extract) results compared to other genotypes. The antioxidant content measured using the DPPH method (13.40 µmol TE g^{-1} extract) showed higher results compared to those measured using the FRAP method (23.29 µmol TE g^{-1} extract). This shows that the antioxidant content of the Nazla IPB genotype has a greater ability to ward off free radicals than to reduce Fe compounds. The basic principle of the DPPH method is the ability of the extract to ward off free radicals, while the FRAP method has the principle of the ability of the extract to convert Fe^{3+} into Fe^{2+} (Sethi et al., 2020). The highest antioxidant of the FRAP method was shown in the Adelina IPB genotype, which was 87.87 µmol TE g^{-1} extract. The highest antioxidant of the DPPH method was shown by the pure lines genotype F10 145174-9-7-1-5-3-1-2-5. In addition to the highest antioxidant content of the DPPH method, the measured α-glucosidase inhibitory activity also showed the second high yield after Nazla IPB.

The results of other studies indicate that antioxidant activity is influenced by environmental stress. In high saline soils, it can increase the activity of antioxidant enzymes in sweet pepper plants (Abdelaal et al., 2020). Antioxidant activity increases in stressed plant conditions because it is a form of plant self-defense against a stressed environment (Mahmood et al., 2021). Therefore, in this study an optimal chili growth environment was used for initial screening of antioxidant content of several genotypes of chili plants grown in a controlled environment in a greenhouse.

The results of the Pearson correlation analysis are in line with the results of the mean value shown in Table 3. Pearson correlation analysis results are shown in Table 4.

Table 3. Means value of 16 genotypes based on biochemical parameters in chili.

Genotype	TPC	TFC	DPPH	FRAP	AGI
NAZLA IPB	15.49^{a}	2.21^{a}	13.40^{b-e}	23.29^{f}	83.27^{a}
SSH C 2	14.26^{ab}	1.74^{b}	13.34^{b-f}	75.60^{b}	74.09^{c}
ADELINA IPB	13.15^{ab}	1.72^{b}	14.55^{abc}	87.87^{a}	78.68^{b}
AYESHA IPB	10.33^{b}	0.74^{g}	12.79^{c-f}	11.94^{g}	69.83^{def}
VIOLA IPB	10.33^{b}	0.91^{efg}	11.89^{ef}	31.62^{f}	79.68^{ab}
F10 145291-10-7-1-1-2-1-3-6	10.17^{b}	0.93^{efg}	14.32^{a-d}	43.19^{e}	72.13^{cd}
F10 145293-19-8-3-113-1-1-1-1	12.83^{ab}	1.38^{bcd}	14.93^{ab}	70.97^{b}	64.42^{ghi}
F10 145174-9-7-1-5-3-1-2-5	10.85^{b}	1.49^{bc}	15.40^{a}	48.52^{de}	80.67^{ab}
F8 145291-14-9-3-12-1-1	10.81^{b}	0.80^{g}	11.84^{ef}	48.29^{de}	67.69^{efg}
F11 160291-3-12-5-511-1-2-2-4	11.96^{ab}	1.25^{c-f}	11.43^{f}	73.75^{b}	67.40^{efg}
F11 145291-115-15-8-1-1-2-5-1 (H)-6	11.60^{ab}	0.87^{fg}	9.43^{g}	56.85^{cd}	62.83^{j}
F11 160291-9-4-3-2-1-1-1-1-1	12.63^{ab}	1.00^{d-g}	14.32^{a-d}	68.43^{b}	74.60^{c}
F11 145291-115-15-8-1-1-2-5-3 (K)-3	12.00^{ab}	1.57^{bc}	12.58^{def}	52.92^{de}	67.16^{e-h}
F11 160291-14-10-10-4-9-1-1-1-1	10.33^{b}	1.51^{bc}	14.30^{a-d}	65.88^{bc}	63.09^{hi}
F12 160291-3-12-3-4-5-1-1-5-1-1	12.95^{ab}	1.32^{b-e}	12.14^{ef}	73.29^{b}	71.32^{cde}
F12 160291-3-12-5-51-1-1-2-1-1-2	11.36^{ab}	1.00^{d-g}	11.64^{ef}	56.16^{cd}	66.74^{f-i}

Notes: Numbers followed by the same letter in the same column were not significantly different according to DMRT 5% level; TPC= Total Phenolic Content (mg GAE/g extract); TFC= Total Flavonoid Content (mg QE/g extract); DPPH= Antioxidant DPPH Method (µmol TE/g extract); FRAP= Antioxidant FRAP Method (µmol TE/g extract); AGI= α-glucosidase inhibitory activity (%).

Table 4. Pearson correlation analysis of biochemical parameters and capsaicin for 16 genotypes of chili.

	TPC	TFC	DPPH	FRAP	AGI
TPC	1.00				
TFC	0.227	1.00			
DPPH	0.035	0.369**	1.00		
FRAP	0.191	0.212	0.137	1.00	
AGI	0.245	0.363*	0.298*	-0.256	1.00

Notes: **= very significantly at level α 1%; *= significantly at level α 5%; TPC= Total Phenolic Content, TFC= Total Flavonoid Content, DPPH= Antioxidant DPPH Method, FRAP= Antioxidant FRAP Method, AGI= α-glucosidase inhibitory activity.

The correlation between AGI and FRAP shows a negative result, which means that when the resulting AGI value is high, the resulting FRAP value is negative or low. It is shown in the Nazla IPB genotype which has the highest AGI value but the lowest FRAP value. A significant positive correlation was shown between DPPH-TFC, DPPH-AGI, and TFC-AGI. Significant positive correlation means that the observed parameters show mutually supportive results (Feng et al., 2019). When the measured parameter is high, then the parameter that has a significant positive correlation also shows high results.

PCA analysis was carried out to determine the distribution of the observed genotype coordinates. The results showed that Nazla IPB and F10 145174-9-7-1-5-3-1-2-5 clustered in the same quadrant (Figure 2).

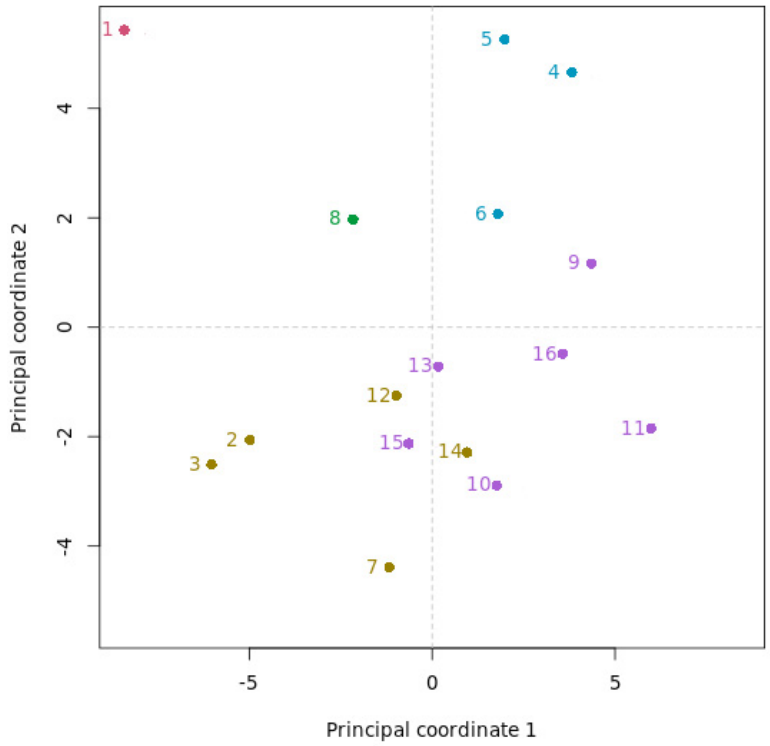

Figure 2. PCA analysis of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant DPPH, Antioxidant FRAP and α-glucosidase inhibitory activity (AGI) on chili genotypes.

This is due to the trend that occurs in the biochemical parameters observed by the two genotypes. The third and fourth quadrants each grouped into five genotypes. While the second quadrant grouped four genotypes (Ayesha IPB, Viola IPB, F10 145291-10-7-1-1-2-1-3-6, F8 145291-14-9-3-12-1-1). The relationship between genotypes was shown by the results of the HCA analysis (Figure 3) and the dendrogram (Figure 4).

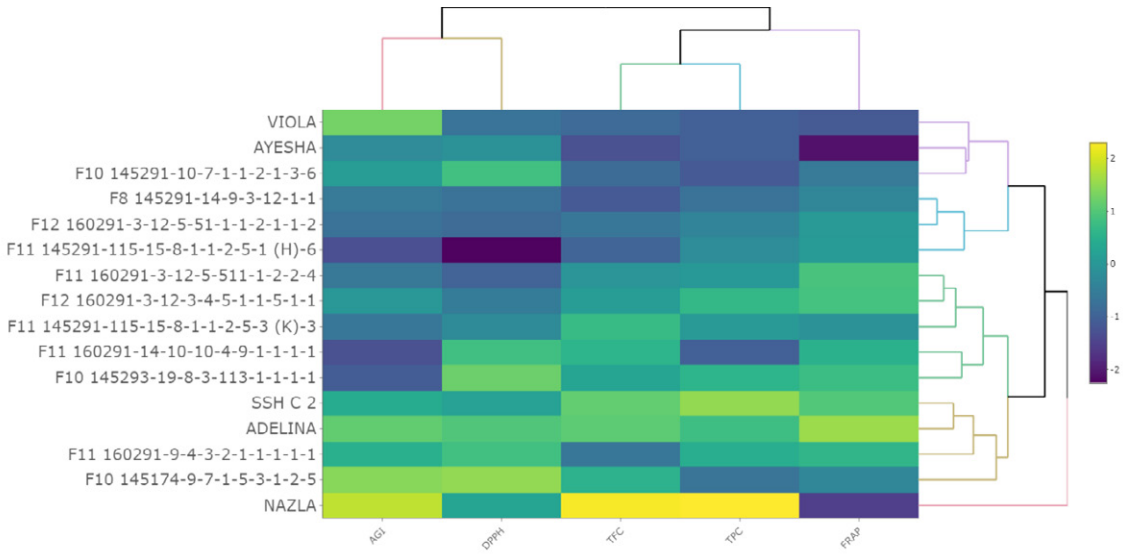

Figure 3. HCA analysis of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant DPPH, Antioxidant FRAP and α-glucosidase inhibitory activity (AGI) on chili genotypes.

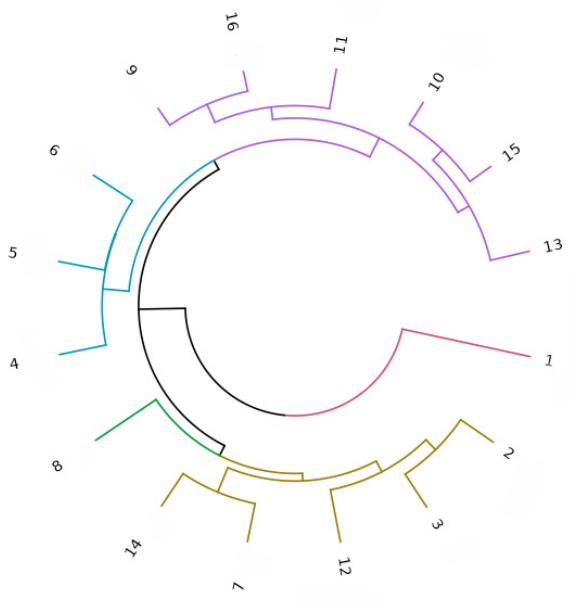

Figure 4. Dendrogram of 16 chili genotypes.

The dendrogram shown is part of the results of PCA analysis on the PB-STAT online application. Both analyzes showed the same kinship results in the tested genotypes and were divided into five major clusters. The difference seen in the two analysis results is that HCA analysis is able to show the relationship between each tested biochemical parameter, while PCA analysis is able to group based on quadrants. Ayesha IPB, Viola IPB, and F10 145291-10-7-1-1-2-1-3-6 showed a close relationship in one cluster according to the quadrant of the results of PCA analysis.

Conclusions

Concluded based on the results of the ornamental chili genotype Nazla IPB has the highest AGI content of 83.27% and has the potential to be further developed to overcome diabetic prevention herbally. Adelina IPB's ornamental chili genotype has the ability to reduce Fe^{3+} to Fe^{2+} as indicated by the highest FRAP antioxidant results compared to all observed genotypes (87.87). The results of PCA and HCA analysis showed that the genotypes based on biochemical parameters were divided into five clusters. while the Pearson correlation resulted in a positive correlation between DPPH antioxidants and AGI in ornamental chili pepper.

Author contribution

ZDS: performed experiments, obtained results, took photographs, compiled the data and drafted the manuscript; **MS, AM, WN**: helped in designing the experiment, supervised the laboratory work, and edited the manuscript, helped in statistical analysis of the data and in the laboratory work.

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