Ciência Rural, Santa Maria, v.53:7, e20210913, 2023
http://doi.org/10.1590/0103-8478cr20210913
ISSNe 1678-4596
FOOD TECHNOLOGY

Total phenolic and flavonoid contents, antioxidant, and α-glucosidase inhibitory activities of several big chili (*Capsicum annuum* L.) genotypes

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ABSTRACT: Big chili has the largest fruit size compared to other types of chili. In addition to having a spicy taste, big chilies contain other beneficial biochemicals such as phenolics, flavonoids, antioxidants, and α-glucosidase inhibitors that have the potential to be developed for functional tropical plants. Information on the content is still not widely found. Therefore, this study identified biochemical compounds (TPC, TFC, DPPH, FRAP, AGI) in several genotypes of big chili peppers. From October to November 2021, we conducted a study using the microplate reader method. We repeated the experiment three times. The research design used was a completely randomized design with a single factor genotype. A total of 22 genotypes of big chili were used in this study. The big chili genotype F6074136-2-3-2-3 had the highest content of α-glucosidase inhibitor and antioxidant FRAP compared to other genotypes 67.62% and 71.20 mol TE/g extract. The genotypes CK 12 and F6074077-1-1-3-1 indicated the highest phenolic and flavonoid contents. The correlation between biochemical parameters showed that TPC-AGI and TPC-DPPH were significantly negatively correlated. The conclusion is that the information in this study can be the basis for the development of functional tropical plants in future research.
Key words: antioxidant, big chili, phenolic, flavonoid, inhibitor α-glucosidase activities.

Conteúdo fenólico total e flavonóide, atividades antioxidantes e inibitórias da α-glucosidase de vários genótipos de pimentão (*Capsicum annuum* L.)

RESUMO: O pimentão grande tem o maior tamanho de fruta em comparação com outros tipos de pimentão. Além de ter um sabor picante, os pimentões grandes contêm outros bioquímicos benéficos, como fenólicos, flavonóides, antioxidantes e inibidores de α-glicosidase, que têm potencial para serem desenvolvidos para plantas tropicais funcionais. Informações sobre o conteúdo ainda não são amplamente encontradas. Portanto, este estudo teve como objetivo identificar compostos bioquímicos (TPC, TFC, DPPH, FRAP, AGI) em diversos genótipos de pimentão. O estudo foi conduzido de outubro a novembro de 2021 usando o método do leitor de microplaca. Os dados obtidos foram repetidos três vezes. O desenho de pesquisa usado foi um desenho inteiramente casualizado com um genótipo de fator único. Um total de 22 genótipos de pimentão grande foi usado neste estudo. O genótipo big chili F6074136-2-3-2-3 apresentou o maior teor de inibidor de α-glucosidase e antioxidante FRAP em comparação com outros genótipos 67,62% e 71,20 mol TE / g de extrato. O maior teor de fenólicos e flavonóides neste estudo foi indicado pelos genótipos CK 12 e F6074077-1-1-3-1. A correlação entre os parâmetros bioquímicos mostrou que TPC-AGI e TPC-DPPH foram significativamente correlacionados negativamente. Conclui-se que as informações deste estudo podem servir de base para o desenvolvimento de plantas tropicais funcionais em pesquisas futuras.
Palavras-chave: antioxidante, big chili, fenólico, flavonóides, inibidor da atividade da α-glucosidase.

INTRODUCTION

Plants have complex biochemical compounds resulting from the process of photosynthesis. These biochemical compounds can be utilized for the development of the herbal pharmaceutical industry. Biochemical compounds commonly reported in plants are antioxidants (WEN et al., 2020). Antioxidants are compounds that inhibit oxidation reactions. The way antioxidants work varies, one of which is the binding of free radicals formed in the human body (SUEISHI & NII, 2019). The formation of free radical compounds causes a chain reaction that can damage cells or tissues, causing degenerative diseases, cancer, and diabetic (JEONG et al., 2021).

Received 12.28.2021 Approved 08.03.2022 Returned by the author 09.23.22
CR-2021-0913.R1
Editors: Leandro Souza da Silva Juliane Welke

Humans naturally produce antioxidant compounds in the body (LAMMI et al., 2019). The human body requires sufficient antioxidants to help prevent and overcome the free radical compounds contained in it. However, due to the influence of various factors that cause a lot of free radical compounds to form in the body, it is necessary to take additional antioxidants from outside the human body.

Generally, antioxidants in plants are reported in horticultural groups (BURRI et al., 2017; HEGEDŰS et al., 2019). Plants produce excess antioxidants when experiencing growth disorders as a self-protection mechanism (GIORDANO et al., 2021). One of the horticultural crops consumed by humans is chili. Chili is a plant that generally gives a spicy taste to food consumed by humans (SAHID et al., 2020). Behind its spiciness, chili has many secondary metabolites compounds that can potentially be developed including phenolic compounds (MORENO et al., 2018; ANJOS et al., 2022), flavonoids (VAZQUEZ-FLORES et al., 2020), antioxidants (ALAM et al., 2018), and even *α-glucosidase* inhibitory activities (NANOK & SANSENYA, 2019). *α-glucosidase* inhibitors are enzymes that function to inhibit the absorption of sugar in the blood (STEFANO et al., 2018). Recent research has shown that *α-glucosidase* inhibitors may be helpful as COVID-19 antivirals (SPENCER & ETHAN, 2020).

Chili is one type of horticultural plant that has many consumers and has high economic value (HERATH et al., 2021). The majority of the world's population uses chili as the basic ingredient for spicy food. In addition, the pharmaceutical industry uses chili peppers for making chili patches (FATTORI et al., 2016; SALEH et al., 2018). However, based on previous research, it turns out that chili has the potential to be developed as an herbal medicine for diabetic because it has antioxidant compounds and *α-glucosidase* inhibitors. Information on inhibitor *α-glucosidase* content in chili is still rare.

The distribution of chili plants in the world is grouped by species. The most widely circulated species in Asia, especially Indonesia, is *Capsicum annuum* (KARIM et al., 2021). This is partly due to the climatic factors of the tropics, which are very optimum for *Capsicum annuum*. Based on the size of the fruit, chilies are divided into three namely cayenne pepper, large chili, and curly chili (ISTIQLAL et al., 2018). Big chili has the advantage of large fruit size so that it affects the productivity produced. Therefore, it is expected that the content of antioxidants and *α-glucosidase* inhibitors produced is more than other types of chili. Seeing this potential, we identified the content of phenolic, flavonoid, antioxidant and inhibitor content in large chili species *Capsicum annuum*.

MATERIALS AND METHODS

The genetic material used in this study is shown in table 1. The genetic material consisted of 5 genotypes of pure-line chili and 17 genotypes of chili that are commonly cultivated in Indonesia. All species used comes from *Capsicum annuum,* which is also a type of sweet pepper that is commonly cultivated in the world. Chili planting was carried out at the Alam Sinarsari D80 Greenhouse by fertilizing using AB Mix dissolved in water. The chilies used for sample extraction in this study are perfectly ripe chilies.

Sample Extraction

Fresh chilies were dried in an oven at 80^0C for 24 hours. The dried fruit is crushed into powder using a blender until smooth. Extraction using a ratio of 1:20 between chili powder and ethanol 80%. This study used 3 grams of chili powder dissolved in 60 mL of 80% ethanol by maceration method for 2x24 hours in a water bath shaker. The extract that had been shaken for 2x24 hours was then filtered using filter paper and transferred to a glass bottle. Then, repeated maceration and re-filtering was carried out until the final volume of the extract was 60 mL. The filtered extract was then stored at 15^0C for 24 hours before use.

Measurement of total phenolic content (TPC), total flavonoid content (TFC), antioxidant DPPH, antioxidant FRAP, and inhibitor α-glucosidase Activity (AGI)

Measurement of biochemical parameters carried out in this study using ELISA Reader. The different wavelengths used in this study are: 750 nm (TPC), 415 nm (TFC), 517 nm (DPPH), 595 nm (FRAP), and 410 nm (AGI). All methods used in this measurement refer to SAHID et al. (2021). The standard curves used in this study are Trolox (DPPH and FRAP), Quercetin (TFC), and Gallic Acid (TPC). Calculation of the percentage of AGI inhibition using the method of WIBISONO et al., (2019).

Table 1 - List of genotypes used in this study.

Number	Genotype
1	CK 12
2	CK 11
3	CK 3
4	CK 2
5	ANIES 1-5-1
6	ARISA
7	SELOKA 4-10-2-1-3
8	SELOKA 3-10-2-2
9	F6074
10	F7 IMPERIAL 10-2-4
11	F6074077-1-4-2-1
12	F6074077-1-1-3-1
13	F6136074-1-4-3-1
14	F6074136-2-3-2-3
15	F6074035-2-1-2-4
16	C 143
17	C 141
18	C 37
19	C 18
20	C 5
21	CK 5
22	C4VIA

Data analysis

This study used SAS 9.0 and R 4.0.5 applications for further testing in this article. Further tests performed included: ANOVA, 5% DMRT test, Pearson correlation analysis, and HCA analysis. HCA analysis determined the relationship between the genotypes used and the R 4.0.5 packages PheatMap Library.

RESULTS AND DISCUSSION

Table 2 shows the average biochemical content of several genotypes of large chili n in table 2. The total phenolic content was measured in the range 8.11 (C 5) - 17.99 (CK 12). The CK 12 genotype has a total phenolic content that is significantly different from all genotypes except the CK 11 genotype. Phenolic compounds are secondary metabolites in plants that are reported in plant cell walls (MNICH et al., 2020). This compound serves to prevent the decay of plant tissue (ŽEBELJAN et al., 2021). This can indicate that C5 chilies will easily spoil compared to CK 12 chilies.

Antioxidants in the CK 12 genotype as measured using the FRAP method showed the highest yield of 71.67. CK12 was a bell pepper (paprika) that isy used as a base for pizza making around the world. Antioxidant FRAP is an antioxidant content that is measured to see the ability of the extract to convert Fe^{3+} compounds into Fe^{2+} (SVEČNJAK et al., 2020). This means that the CK 12 genotype contains antioxidants that function to reduce iron. The total phenolic content of the C 5 genotype was inversely proportional to the antioxidant value of DPPH produced, which was 13.73 where this value was the highest value compared to other genotypes. This shows that this genotype contains antioxidants that are able to ward off free radicals better than other genotypes. In accordance with research (ADEGBOLA et al., 2020) that DPPH antioxidants are measured using the principle of free radical scavenging.

Table 2 - Total phenolic content (TPC), total flavonoid content (TFC), antioxidant DPPH, and antioxidant FRAP in chili genotypes.

Genotype	TPC	TFC	DPPH	FRAP
CK 12	17.99^{a}	1.49^{abc}	7.64^{gh}	71.67^{a}
CK 11	15.41^{ab}	1.55^{ab}	6.71^{hi}	61.71^{ab}
CK 3	12.00^{c-g}	1.06^{b-g}	10.67^{cd}	26.06^{d}
CK 2	13.03^{b-f}	1.42^{a-d}	10.35^{cde}	55.00^{ab}
ANIES 1-5-1	10.09^{e-h}	1.25^{a-e}	11.74^{abc}	59.40^{ab}
ARISA	11.40^{c-h}	0.97^{b-g}	8.74^{d-h}	52.45^{b}
SELOKA 4-10-2-1-3	11.04^{d-h}	1.08^{b-g}	9.91^{c-f}	46.90^{bc}
SELOKA 3-10-2-2	10.01^{e-h}	1.00^{b-g}	8.93^{d-g}	58.94^{ab}
F6074	9.89^{e-h}	0.91^{c-g}	6.71^{hi}	17.73^{d}
F7 IMPERIAL 10-2-4	9.14^{gh}	0.61^{fg}	7.35^{gh}	16.34^{d}
F6074077-1-4-2-1	11.48^{c-h}	1.12^{a-g}	8.59^{d-h}	30.46^{cd}
F6074077-1-1-3-1	10.77^{d-h}	1.68^{a}	7.81^{fgh}	43.89^{bc}
F6136074-1-4-3-1	10.05^{eh}	1.10^{a-g}	8.95^{d-g}	58.47^{ab}
F6074136-2-3-2-3	10.49^{d-h}	1.34^{a-e}	11.62^{bc}	71.20^{a}
F6074035-2-1-2-4	14.65^{bc}	0.76^{efg}	8.47^{e-h}	51.76^{b}
C 143	9.97^{e-h}	0.76^{fg}	10.28^{cde}	52.22^{b}
C 141	13.82^{bcd}	0.63^{fg}	5.12^{ij}	22.36^{d}
C 37	13.35^{b-e}	1.25^{a-e}	4.57^{j}	31.62^{cd}
C 18	9.62^{fgh}	0.85^{d-g}	8.68^{d-h}	22.82^{d}
C 5	8.11^{h}	0.55^{g}	13.73^{a}	23.29^{d}
CK 5	9.58^{fgh}	1.19^{a-f}	13.07^{ab}	25.14^{d}
C4VIA	9.65^{fgh}	0.91^{c-g}	12.77^{ab}	20.51^{d}

Means followed by equal letters (vertical) do not differ statistically from each other by the duncan multiple range test at 5%.

The measurement results of *α-glucosidase* inhibitors (Figure 1) showed that the pure line genotype F6074136-2-3-2-3 was the highest (67.62%) compared to other genotypes but was not significantly different from the C 5 genotype (65.53%). The content of *α-glucosidase* measured in all genotypes was in the range of 9.55-67.62%. The pure line F6074136-2-3-2-3 has relatively high biochemical compounds. C 5 genotype was also observed to have the highest DPPH antioxidant compared to all observed genotypes. This is consistent with the results of the study (ZAYAPOR et al., 2021), which showed that the genotype with the highest antioxidant content of DPPH also showed the highest content of *α-glucosidase* inhibitor. To explain the relationship between the observed variables for biochemical content, a Pearson correlation analysis was performed (Figure 2).

The relationship between genotypes and between biochemical content can be seen by using HCA analysis. The results of the HCA analysis are shown in figure 3. The genotypes observed in this article were divided into 5 major groups. Groups 1 and 2 each consisted of 3 genotypes (CK 12, CK 11, CK 2) and (F6074077-1-1-3-1; C 37; F6074077-1-4-2-1). Group 3 only consisted of 2 genotypes, namely F6074035-2-1-2-4 and C 141. While groups 4 and 5 had the highest number of genotypes of 7 genotypes in each group, namely (F7 Imperial 10-2-4, F6074, C 18, CK 3, CK 5, C4VIA, C 5) in groups 4 and C 143, SELOKA 3-10- 2-2, F6136074-1-4-3-1, ARISA, SELOKA 4-10-2-1-3, ANIES 1-5-1, F6074136-2-3-2-3 in group 5. HCA analysis also can show the highest value based on the color listed (GARCIA et al., 2019). The lighter the color shown on the genotype, the genotype has the highest biochemical content compared to other genotypes and other biochemical contents. CK 12 in the HCA analysis had the highest TPC content compared to other genotypes, and also compared to other biochemical contents.

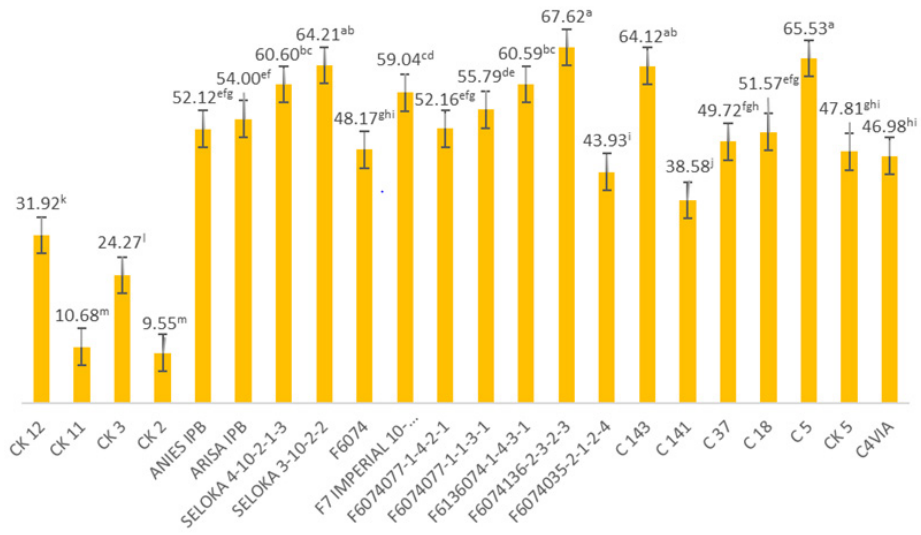

Figure 1 - Inhibitor α-glucosidase activity (AGI) in big chili genotypes. Means followed by equal letters (vertical) do not differ statistically from each other by the Duncan multiple range test at 5%.

CONCLUSION

Twelve genotypes contain *α-glucosidase* inhibitors above 50%. The content of *α-glucosidase* inhibitor has a positive correlation with antioxidant DPPH, but has a significant negative correlation with TPC and TFC. The results of the HCA analysis in this article are able to provide information on the relationship between the genotype and the observed biochemical content. The genotypes were divided into 5 major groups. Functional chili plants development are directed

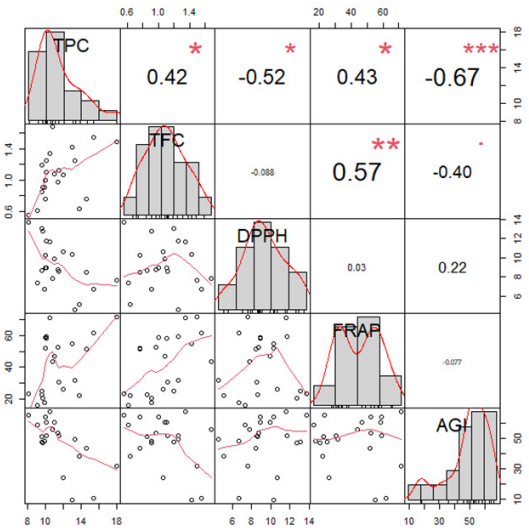

Figure 2 - Pearson correlation of biochemical content in chili genotypes. *: significantly (< 0.05), **: significantly (< 0.01), ***: very significantly (< 0.001).

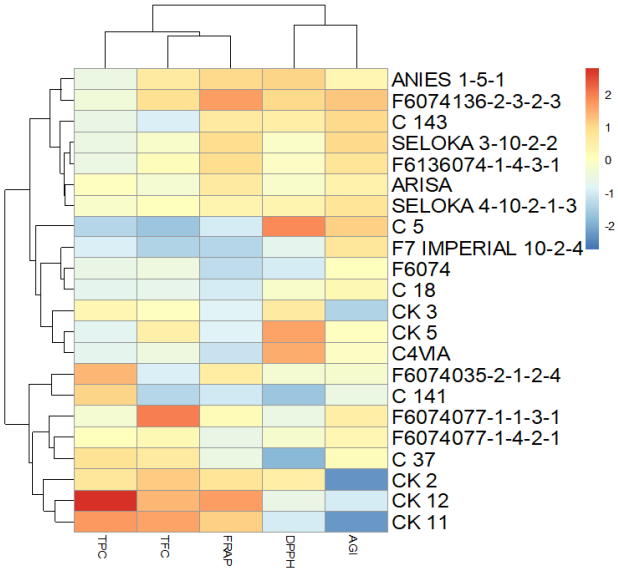

Figure 3 - HCA Analysis of biochemical content in chili genotypes. Color shows intensity based on legend.

to alternative plants for diabetic treatment. In addition, plant breeders can use the information in this article as a basis for selection of elders in both conventional and biotechnology plant breeding activities.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support by the Ministry of Education, Culture, Research and Technology of Republic Indonesia through the Doctoral Dissertation Research scheme.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

MS, AM, and WN conceived and designed experiments. ZDS performed the experiment and carried out the lab analyses. ZDS and WN performed statistical analyses of data and prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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