http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.380.386

Research Article
Phenolic, Flavonoid and Antioxidant Capacities Evaluation of *Celosia cristata* Resulted from Induced Mutation Using Ethyl Methane Sulphonate

[1]Yoshua Shandy Yudha, [1]Syarifahlis Aisyah, [1]Dewi Sukma and [2,3]Waras Nurcholis

[1]Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia
[2]Department of Biochemistry, IPB University, Bogor, Indonesia
[3]Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia

Abstract

Background and Objective: *Celosia cristata* L. is an ornamental plant that has the potency to be developed as a medicinal plant. The mutation breeding of *C. cristata* targets the increase of biochemical compounds that are potential as antioxidants. This study aimed to evaluate the phenolic and flavonoid contents and antioxidant activity of the putative mutants of *C. cristata* in the M1 generation. **Materials and Methods:** The samples used in this study were flowers and seeds. Induced mutation, using ethyl methane sulphonate (EMS) was used to obtain M1 generation of putative mutants and twelve putative mutants were selected for polyphenol contents analysis composed of total phenolic (TPC), total flavonoid (TFC) and antioxidant activities analysis using two approaches, i.e., 2,2-diphenyl picrylhydrazyl (DPPH) method and ferric reducing antioxidant power (FRAP). **Results:** This study showed that total phenolics were varied from 11.73-18.06 mg GAE g^{-1} DW and total flavonoids were varied from 2.34-3.11 mg QE g^{-1} DW. Meanwhile, the antioxidant activity gain using the DPPH method ranged from 16.43-19.02 µmol TE g^{-1} DW and the FRAP method ranged from 40.72-59.61 µmol TE g^{-1} DW. The clustering analysis results formed three clusters with two clusters consisting of potential mutants with higher biochemical content and antioxidant capacities. It was found that total phenolic and flavonoids highly correlated with the antioxidant FRAP. **Conclusion:** Induced mutation using EMS can increase the diversity of biochemical characters and antioxidant activity of *C. cristata* and provide potential genetic material with higher chemical content for further development.

Key words: Polyphenols, mutation breeding, putative mutants, genetic material, *C. cristata*

Citation: Yudha, Y.S., S. Aisyah, D. Sukma and W. Nurcholis, 2022. Phenolic, flavonoid and antioxidant capacities evaluation of *Celosia cristata* resulted from induced mutation using ethyl methane sulphonate. Pak. J. Biol. Sci., 25: 380-386.

Corresponding Author: Waras Nurcholis, Department of Biochemistry, IPB University, Bogor, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Celosia cristata L. is an ornamental plant included in the *Amaranthaceae* family. *Celosia cristata* has been grown widely in Asia, Africa and America. In Indonesia, *C. cristata*, is also called 'Jengger Ayam', is cultivated as an ornament and medicinal plant. The aesthetic value of this plant comes from the floral shape and enchanting colours of its flower, therefore, this planet is widely planted as a potted and landscape ornamental plants[1]. Apart from their aesthetic value, these plants also provide the potential chemical compounds beneficial for health and industry[2]. *Celosia cristata* contains flavonoids, phenols, saponins, tannins, alkaloids and betalain[3]. Further analysis shows that kaempferol, hydroquinone, p-hydroxybenzoic acid and several fatty acids such as octanoic acids, lauric acids and palmitic acids are found in *C. cristata*[4]. Furthermore, various chemical properties in *C. cristata* are helpful to overcome several diseases, such as dysentery, diarrhoea, hemoptysis, hypertension and some diseases of the urinary tract[5].

Among the many secondary metabolites produced by *C. cristata*, flavonoids and phenolics are important compounds actively involved in overcoming environmental stress in plants and valuable to maintain human body health[6–8]. Plants' flavonoids and phenolics protect cells against abiotic stresses, including heat and drought stress[9,10] and abiotic stresses[11-13]. The presence of flavonoids and phenolics, which act as antioxidants, suppresses free radicals that harm humans[14]. Therefore, the crop improvement of *C. cristata* is focused on increasing the diversity of chemical content in polyphenol compounds.

Mutation breeding plays an essential role in increasing morphology and metabolites variability in plants. Mutation can be induced using physical and chemical agents. Ethyl methane sulphonate (EMS), an alkylating agent, is a chemical mutagen frequently used in mutation breeding programs and produces many potential mutants. Several studies reported the improvement of secondary metabolite compounds in plants due to the application of EMS. Ghani *et al.*[15] also reported an increase in phenolic compounds and enzymes that play a role in antioxidant activity in *Gerbera* plantlets induced by EMS. Research results from Gurdon *et al.*[16] showed that the application of EMS to lettuce resulted in potential mutants obtained in M2 segregated populations with high flavonoid contents. The increase in protein and amino acid contents of methionine in *Vigna mungo* due to EMS was also reported by Rindita *et al.*[17].

Mutation breeding to increase the diversity of biochemical characters of *C. cristata* has been conducted by Aisyah *et al.*[18] and Muhallilin *et al.*[3] using gamma-ray irradiation. These studies reported that new compounds were identified in putative mutants that were not present in control plants. In this study, chemical mutation induction using EMS was carried out to increase the biochemical diversity of *C. cristata*. Furthermore, evaluation and quantification of the polyphenols content and antioxidant activity were carried out to ascertain the effect of EMS in improving the polyphenol contents and antioxidant capacity of *C. cristata*.

MATERIALS AND METHODS

Study area: This study was conducted from January to February, 2021.

Plant material and extraction: Twelve genotypes of *C. cristata* in the M1 generation were selected for polyphenol content and antioxidant activities analysis. The twelve genotypes consisted of two control plants in Fig. 1a and b and ten putative mutants in Fig. 2a-j. The putative mutants of *C. cristata* were obtained by immersing *C. cristata* seeds into EMS solutions (treatment levels ranging from 0.5-4% (v/v)). Plants grown due to EMS treatment were considered to be putative mutants. The samples used in this work were flowers, consisting of sterile flowers and fertile flowers and seeds. Extraction of *C. cristata* samples was carried out in consonance with Khumaida *et al.*[19] with slight modifications. After the samples were harvested from the experimental field of IPB Pasir Sarongge, West Java, samples were dried in an oven for one day at a temperature of 50°C. Soon afterwards, the sample was blended until it became a powder. A total of three grams of the sample was dissolved using 80% ethanol then the solution was homogenized for two days using a shaker. The filtrate was filtered and the extraction process was carried out repeatedly. Eventually, two filtrates were obtained to be combined to obtain a final volume of 60 mL and stored at -20°C.

Fig. 1: Two control samples of *C. cristata* in M1 generation, (a) C3 and (b) C11

Fig. 2(a-j): Ten putative mutant samples of *C. cristata* in M1 Generation, (a) C1, (b) C2, (c) C4, (d) C5, (e) C6, (f) C7, (g) C8, (h) C9, (i) C10 and (j) C12

Total phenolic and flavonoid content: The total phenolic content was analyzed based on the Folin-Ciocalteu method by Sidhiq *et al.*[20] with slight modifications. A total of 20 µL of the sample was added with 100 µL of Folin-Ciocalteu reagent. It was incubated for 5 min, then 80 µL of Na_2CO_3 was added to a 96-well microplate and incubated for 30 min. Samples were analyzed using a microplate reader (Epoch BioTek, USA) with a wavelength of 750 nm. The test sample was composed of three replications and expressed as mg gallic acid equivalent per gram dry weight extract (mg GAE g^{-1} DW). The concentration of gallic acid is made with a concentration range of 10-500 ppm.

Analysis of flavonoid content using aluminium chloride solution method used[21]. A total of 10 µL of the sample was added with 60 µL of methanol, 10 µL of $AlCl_3$ (10%), 120 µL of distilled water was put into a 96-well microplate and incubated for 30 min. Absorbance samples were measured using a microplate reader (Epoch BioTek, USA) with a wavelength of 415 nm. The test sample was composed of three replications and expressed as mg quercetin equivalent per gram dry weight extract (mg QE g^{-1} DW). Quercetin is made with a concentration range of 5-100 ppm.

Antioxidant analysis using DPPH dan FRAP methods: The antioxidant activity of DPPH (1, 1-diphenyl-2-picrylhydrazyl) was carried out in pursuance of Calvindi *et al.*[21] with minor modifications. A total of 100 L of DPPH (125 µM) was added with 100 µL of the sample injected into a 96-well microplate. The test sample solution was incubated in the dark for 30 min before being analyzed using a microplate reader (Epoch BioTek, USA). The absorbance was measured at a wavelength of 517 nm. The test sample solution was prepared in three replications, with the DPPH radical scavenging activity measured by Trolox equivalents (TE mol TE g^{-1} dry weight). Trolox was composed with a concentration ranging from 2.5-100 µM.

Antioxidant activity using the FRAP method refers to the method of Pandey and Rizvi[22] with minor modifications. The FRAP reagent preparation consisted of 10 mM TPTZ (within 40 mM HCl), 20 mM $FeCl_3$ (in distilled water) and acetate buffer pH 3.6. The FRAP reagent was prepared with a ratio of TPTZ, $FeCl_3$ and acetate buffer of 1:1:10, after which the reagent was incubated for 30 min at 37°C. A total of 10 µL of sample and 300 µL of FRAP reagent were put into a 96-well microplate and incubated for 30 min in a dark room at 37°C

for 30 min. The test samples were analyzed using a microplate reader (Epoch BioTek, USA) with three replications and absorbance was measured at a wavelength of 593 nm. Measurement of antioxidant activity was determined by Trolox equivalent (TE mol TE g^{-1} dry weight). Trolox was prepared in a concentration ranging from 5-700 µM.

Data analysis: Analysis of variance (ANOVA) was performed using R software applying the ExpDes package. Heatmap analysis used the heatmaply package and correlation analysis used the Performance Analytics package available in the R software.

RESULTS AND DISCUSSION

Polyphenol contents and antioxidant capacities: The means of polyphenol contents and antioxidant properties of twelve putative mutants of *C. cristata* are summarized in Table 1. The analysis of variance on the polyphenol contents and antioxidant activities were statistically significant ($p<0.05$). The mean total phenolics content ranged from 11.73-18.06 mg GAE g^{-1} DW. Five putative mutant genotypes showed significant differences with the control (C3 and C11), namely C1, C2, C4, C6 and C9, with the highest mean phenolic content found in the genotype C9. The means flavonoids content ranged from 2.34-3.11 mg QE g^{-1} DW. Four genotypes of putative mutants were significantly different from control plants, i.e., C2, C4, C7 and C8. Genotype C8 showed the highest mean in total flavonoids content. Significantly, TPC and TFC in this study were higher than Sukweenadhi *et al.*[23], which evaluated TPC and TFC on seven Indonesian medicinal plants. However, this result was lower than the results of research that have been done by Musdalipah *et al.*[24] evaluated TPC and TFC on Meister Chinensis at 30.72 (mg GAE g^{-1} DW) and 8.02 (mg QE g^{-1} DW), respectively. Internal factors such as the complexity of the biosynthesis of polyphenolic compounds are known to affect the number of flavonoids and phenolics in plants, while external factors such as temperature, light, environmental stress and free radicals can alter the number of polyphenols content[6,8,19,23-25].

The means of antioxidant activity of DPPH analysis was varied from 16.43-19.02 µmol TE g^{-1} DW. The C10 was the highest average DPPH, while genotype C1 was recorded with the lowest average DPPH. Five mutants were statistically significantly different from the control, including genotypes C6, C7, C8, C9, C10. Meanwhile, the antioxidant activity of FRAP was varied from 40.72-59.61 µmol TE g^{-1} DW. Six putative mutants were significantly different from the control, i.e., C2, C4, C6, C7, C8, C9. Genotype C6 was the putative mutant with the highest antioxidant capacity based on the FRAP method, while genotype C11 showed the lowest content. In this study, there was an increase in the polyphenols contents of several putative mutants of *C. cristata* due to the application of EMS. Four mutant genotypes, including C9, C8, C10 and C6, have the highest average value for each biochemical character. The EMS also enhanced polyphenol contents and antioxidant activities observed in mutants with means of polyphenol content and antioxidant activity above and below control plants. Thus, EMS mutagenesis can provide genetic material with increased polyphenols content that is beneficial to health. However, it is necessary to conduct further evaluations regarding the polyphenol contents and antioxidant activities of *C. cristata* in the M2 generation to assess genetic diversity and to obtain potential mutants with higher polyphenols content. The availability of natural compounds belonging to polyphenols is beneficially related to resisting oxidative stress caused by reactive oxygen species (ROS). In this case, the presence of free radicals can damage the entity of lipids, proteins and DNA[26,27].

Table 1: Means value of polyphenols content and antioxidant capacities

Genotypes	EMS induced (%)	TPC (mg GAE g^{-1} DW)	TFC (mg QE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)	FRAP (µmol TE g^{-1} DW)
C1	2.0	15.21^{a}	2.70^{b}	16.43^{b}	45.56^{b}
C2	2.0	16.73^{a}	2.95^{a}	16.84^{b}	54.39^{a}
C3	0.0	13.25^{b}	2.54^{b}	17.23^{b}	45.00^{b}
C4	0.8	16.73^{a}	2.85^{a}	17.40^{b}	57.39^{a}
C5	0.7	11.73^{b}	2.44^{b}	17.07^{b}	40.33^{b}
C6	0.5	15.87^{a}	2.65^{b}	18.31^{a}	59.61^{a}
C7	0.8	14.59^{b}	2.95^{a}	17.98^{a}	54.11^{a}
C8	2.0	16.92^{a}	3.11^{a}	18.09^{a}	58.11^{a}
C9	0.7	18.06^{a}	2.65^{b}	18.28^{a}	55.56^{a}
C10	0.8	12.59^{b}	2.54^{b}	19.02^{a}	41.89^{b}
C11	0.0	13.83^{b}	2.34^{b}	17.11^{b}	40.72^{b}
C12	0.5	14.35^{b}	2.49^{b}	18.51^{b}	44.00^{b}

Means with the same letter are not significantly different based on the Scott-Knott test at the 5% level

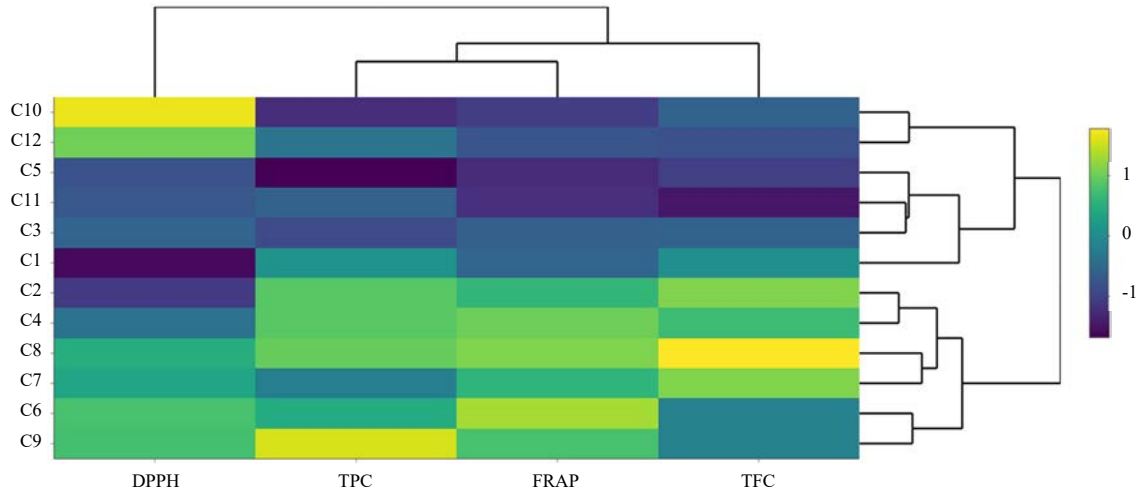

Fig. 3: Clustering analysis of twelve genotypes of *C. cristata*

Heatmap of clustering analysis of twelve putative mutants of *C. cristata*, the default color gradient estimates the lowest value in the heat map to dark blue, the highest value to a bright yellow and mid-range values to light blue

The presence of phenolics and flavonoids is closely related to antioxidant capacities in plants[28]. Assessment of antioxidant activity using the DPPH method to evaluate the scavenging activity of free radicals[19]. The FRAP method evaluates the activity of reducing the power of free radicals[21]. In this study, the mean value of antioxidant activity in the FRAP test was higher than that in the DPPH test, indicating that the ability of antioxidant activities in the *C. cristata* sample was greater to reduce the power of the free radical. The existence of polyphenolic compounds in the defence system against free radicals can occur by either electron donor or ROS binding mechanisms[11,29].

Clustering analysis: The clustering analysis results using a heatmap of twelve putative mutants were divided into three groups according to their polyphenol contents and antioxidant activity, as shown in Fig. 3. The first group comprises two putative mutants, C10 and C12 characterized by the lower total flavonoid, total phenolic and FRAP antioxidant activity but has the highest DPPH antioxidant activity. The second group is composed of four putative mutants, namely C5, C11, C3 and C1 characterized by the lowest total flavonoids, total phenolic, antioxidant activity of DPPH and antioxidant FRAP. The third group consisted of six genotypes, namely C2, C4, C8, C7, C6 and C9, with the highest of total flavonoids, total phenolic, the antioxidant activity of FRAP and antioxidant DPPH. The first and third groups consist of potential mutants with more improved biochemical compounds and antioxidant capacity than the second group. These potential mutants are expected to help the breeding program of *C. cristata* in the improvement of the biochemical compound. The clustering method is very helpful in exploring individuals with better genetic potential in a set of plant breeding programs. In addition, the clustering method is important to understand the size of the diversity of the evaluated genotypes[30]. Nurcholis *et al.*[28] grouped 20 accessions of *Curcuma aeruginosa* into three groups based on the similarity of total phenolic, total flavonoid and antioxidant activity of DPPH. Clustering analysis is also important in classifying accessions based on the type of important compounds. The research results by Li *et al.*[8] conducted a clustering analysis of forty-two *Hemerocallis* accessions forming four clusters based on ten types of flavonoids identified using HPLC. The cluster analysis by Jorkesh *et al.*[31] on fifty-two accessions of *Froriepia supinates* obtained twenty-eight potential accessions with more desirable morphology and chemical contents than other accessions.

Correlation between phenolics, flavonoids and antioxidant capacities of *C. cristata*: The results of the correlation analysis between polyphenol contents and antioxidant activities are presented in Fig. 4. Determining the correlation value is helpful to figure out the relationship between several characters, particularly in a series of plant breeding programs in the interest of selection. Total phenolic and total flavonoid showed a positive correlation of 0.64. Furthermore, total phenolic strongly correlates with antioxidant FRAP which is 0.84, while the correlation value between total flavonoid and antioxidant FRAP is 0.77. In this study, the correlation value between total phenolic and FRAP was higher than the correlation between flavonoids and FRAP despite both phenolic and flavonoids having a positive correlation with

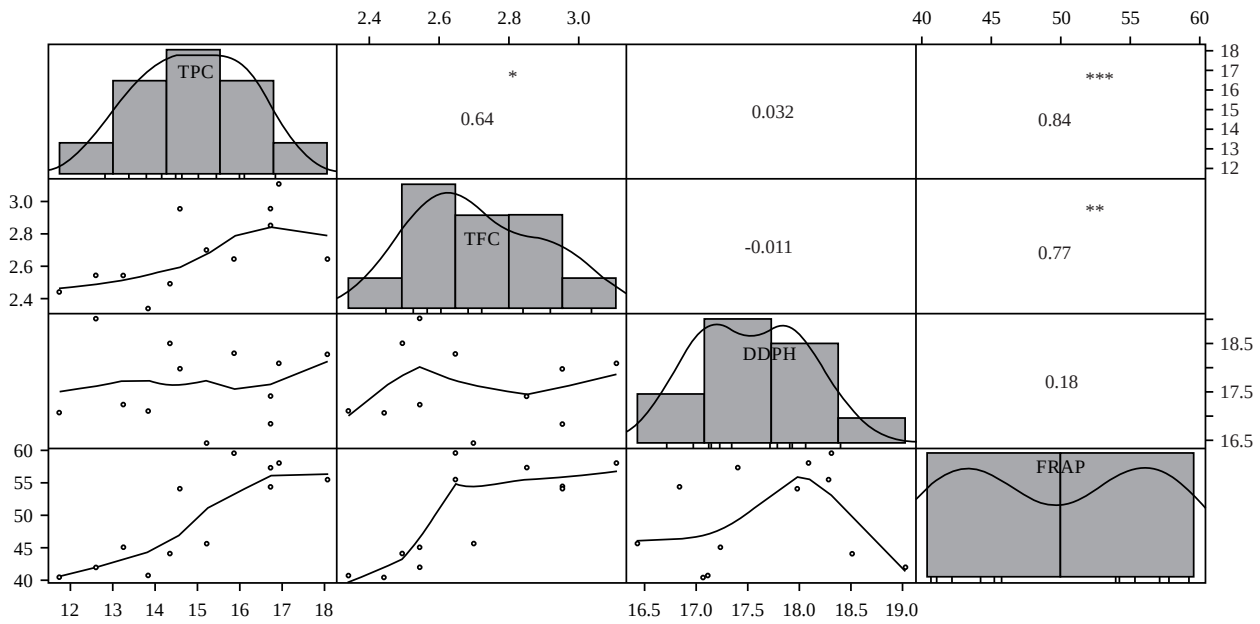

Fig. 4: Correlation between phenolics, flavonoids and antioxidant capacities of *C. cristata*

Correlation for total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant properties (DPPH: 22-diphenyl picrylhydrazyl, FRAP: Ferric reducing antioxidant power) in putative mutants of *C. cristata* and ***, **, *Significant level with p-values of 0.001, 0.01, 0.05 and 0.1, respectively

FRAP. However, both total phenolic and total flavonoid did not correlate with DPPH. The results of this study correspond with the report of Calvindi *et al.*[21] and Nurcholis *et al.*[28] stated that total phenolic and total flavonoid were not correlated with DPPH. However, in contrast to the research results from Zheng *et al.*[6] and Suleria *et al.*[7] stated that the total phenolic was strongly correlated with the antioxidant capacity of DPPH.

CONCLUSION

In conclusion, induced mutation using EMS can affect the polyphenols content and the antioxidant capacity of *C. cristata*. Clustering analysis of twelve putative mutants showed that two groups of putative mutants could be further evaluated based on their potential polyphenol contents and antioxidant properties.

SIGNIFICANCE STATEMENT

This study discovered the potential mutants of *C. cristata* with more improved polyphenol contents and antioxidant capacities compared to wild-type plants that can be beneficial for the plant breeding program of *C. cristata* as a medicinal plant. This study will help the researchers to uncover the critical areas of mutation breeding in the improvement of biochemical contents that many researchers were not able to explore.

REFERENCES

1. Islam, S., M.S. Shajib and T. Ahmed, 2016. Antinociceptive effect of methanol extract of *Celosia cristata* Linn. in mice. BMC Complementry Altern. Med., Vol. 16. 10.1186/s12906-016-1393-5.
2. Sultan, F.I., 2018. Chromatographic separation and identification of many fatty acids and phenolic compounds from flowers of *Celosia cristata* L. and its inhibitory effect on some pathogenic bacteria. Aust. J. Basic Appl. Sci., 12: 25-31.
3. Muhallilin, I., S.I. Aisyah and D. Sukma, 2019. The diversity of morphological characteristics and chemical content of *Celosia cristata* plantlets due to gamma ray irradiation. Biodiversitas, 20: 862-866.
4. Kim, Y.S., J.W. Hwang, S.H. Sung, Y.J. Jeon and J.H. Jeong *et al.*, 2015. Antioxidant activity and protective effect of extract of *Celosia cristata* L. flower on tert-butyl hydroperoxide-induced oxidative hepatotoxicity. Food Chem., 168: 572-579.
5. Sayeed, R., M. Thakur and A. Gani, 2020. *Celosia cristata* Linn. flowers as a new source of nutraceuticals- A study on nutritional composition, chemical characterization and *in-vitro* antioxidant capacity. Heliyon, 10.1016/j.heliyon.2020.e05792.
6. Zheng, J., X. Yu, M. Maninder and B. Xu, 2018. Total phenolics and antioxidants profiles of commonly consumed edible flowers in China. Int. J. Food Prop., 21: 1524-1540.
7. Suleria, H.A.R., C.J. Barrow and F.R. Dunshea, 2020. Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. Foods, Vol. 9. 10.3390/foods9091206.

8. Li, S., H. Cui, J. Wang, F. Hou, X. Xiong, X. Kang and G. Xing, 2021. Qualitative and quantitative analysis on flavonoid distribution in different floral parts of 42 *Hemerocallis* accessions. Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.670506.
9. Yu, D., T. Huang, B. Tian and J. Zhan, 2020. Advances in biosynthesis and biological functions of proanthocyanidins in horticultural plants. Foods, Vol. 9. 10.3390/foods9121774.
10. Singh, A.K., S. Dhanapal and B.S. Yadav, 2020. The dynamic responses of plant physiology and metabolism during environmental stress progression. Mol. Biol. Rep., 47: 1459-1470.
11. Chepel, V., V. Lisun and L. Skrypnik, 2020. Changes in the content of some groups of phenolic compounds and biological activity of extracts of various parts of heather (*Calluna vulgaris* (L.) hull) at different growth stages. Plants, Vol. 9. 10.3390/plants9080926.
12. Aoki, T., M. Kawaguchi, H. Imaizumi-Anraku, S. Akao, S.I. Ayabe and T. Akashi, 2021. Mutants of *Lotus japonicus* deficient in flavonoid biosynthesis. J. Plant Res., 134: 341-352.
13. Šamec, D., E. Karalija, I. Šola, V.V. Bok and B. Salopek-Sondi, 2021. The role of polyphenols in abiotic stress response: The influence of molecular structure. Plants, Vol. 10 10.3390/plants10010118.
14. Nurhasnawati, H., R. Sundu, S. Sapri, R. Supriningrum, H. Kuspradini and E.T. Arung, 2019. Antioxidant activity, total phenolic and flavonoid content of several indigenous species of ferns in East Kalimantan, Indonesia. Biodiversitas, 20: 576-580.
15. Ghani, M., S. Kumar and M. Thakur, 2016. Physiological and biochemical responses of gerbera (*Gerbera jamesonii* hook.) to physical and chemical mutagenesis. J. Hort. Sci. Biotechnol., 89: 301-306.
16. Gurdon, C., A. Poulev, I. Armas, S. Satorov, M. Tsai and I. Raskin, 2019. Genetic and phytochemical characterization of lettuce flavonoid biosynthesis mutants. Sci. Rep., Vol. 9. 10.1038/s41598-019-39287-y.
17. Rindita, R., V. Anggia, E. Rahmaesa, R.K. Devi and L.F. Alawiyah, 2020. Exploration, phenolic content determination, and antioxidant activity of dominant pteridophytes in Gunung Malang village, mount Halimun Salak National Park, Indonesia. Biodiversitas, 21: 3676-3682.
18. Aisyah, S.I., I. Muhallilin, D. Sukma and W. Nurcholis, 2019. The morphological and phytochemical studies on the effect of acute and recurrent irradiation in *Celosia cristata* seeds. Biodiversitas, 20: 3766-3771.
19. Khumaida, N., M. Syukur, M. Bintang and W. Nurcholis, 2019. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of Curcuma aeruginosa accessions growing in West Java, Indonesia. Biodiversitas, 20: 656-663.
20. Sidhiq, D.F., Y. Widiyastuti, D. Subositi, B. Pujiasmanto and A. Yunus, 2020. Morphological diversity, total phenolic and flavonoid content of *Echinacea purpurea* cultivated in Karangpandan, Central Java, Indonesia. Biodiversitas, 21: 1265-1271.
21. Calvindi, J., M. Syukur and W. Nurcholis, 2020. Investigation of biochemical characters and antioxidant properties of different winged bean (*Psophocarpus tetragonolobus*) genotypes grown in Indonesia. Biodiversitas, 21: 2420-2424.
22. Pandey, K.B. and S.I. Rizvi, 2012. Ferric reducing and radical scavenging activities of selected important polyphenols present in foods. Int. J. Food Prop., 15: 702-708.
23. Sukweenadhi, J., O. Yunita, F. Setiawan, Kartini, M.T. Siagian, N.P. Danduru and C. Avanti, 2020. Antioxidant activity screening of seven Indonesian herbal extract. Biodiversitas, 21: 2062-2067.
24. Musdalipah, M., S.A. Tee, K. Karmilah, S. Sahidin, A. Fristiohady and A.W.M. Yodha, 2021. Total phenolic and flavonoid content, antioxidant, and toxicity test with BSLT of *Meistera chinensis* fruit fraction from Southeast Sulawesi. Borneo J. Pharm., 4: 6-15.
25. Walvekar, S. and P. Kaimal, 2014. Comparative study of effect of physical and chemical mutagens on phytochemical content and antioxidant activity of *Aegle marmelos* Corr. Int. J. Adv. Res., 2: 590-595.
26. Newsholme, P., V.F. Cruzat, K.N. Keane, R. Carlessi and P.I.H. de Bittencourt, 2016. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem. J., 473: 4527-4550.
27. Juan, C.A., J.M.P. de la Lastra, F.J. Plou and E. Pérez-Lebeña, 2021. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22094642.
28. Nurcholis, W., N. Khumaida, M. Syukur and M. Bintang, 2016. Variability of total phenolic and flavonoid content and antioxidant activity among 20 *Curcuma aeruginosa* Roxb. accessions of Indonesia. Asian J. Biochem., 11: 142-148.
29. Lama-Muñoz, A., M. del Mar Contreras, F. Espínola, M. Moya, I. Romero and E. Castro, 2020. Content of phenolic compounds and mannitol in olive leaves extracts from six Spanish cultivars: Extraction with the Soxhlet method and pressurized liquids. Food Chem., Vol., 320. 10.1016/j.foodchem.2020.126626.
30. Nafees, M., M.J. Jaskani, S. Ahmad, M. Shahid, Z. Malik and M. Jamil, 2017. Biochemical diversity in wild and cultivated pomegranate (*Punica granatum* L.) in Pakistan. J. Hort. Sci. Biotechnol., 92: 199-205.
31. Jorkesh, A., Y. Hamidoghli, J. Olfati, H. Samizadeh, D. Bakhshi and J. Palá-Paúl, 2020. Morphological and biochemical variability of Froriepia. Int. J. Veg. Sci., 26: 262-274.