Sys Rev Pharm 2020; 11(4): 332 – 336
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

The Difference of Temperature and Storage Time on the Antioxidant Activity of Curcuma Ethanol Extract (*Curcuma zanthorrhiza*) using the DPPH

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Article History: *Submitted: 23.01.2020* *Revised: 20.03.2020* *Accepted: 09.04.2020*

ABSTRACT
Curcuma is one of the abundant natural resources used as a medicinal plant in Indonesia. It contains desmetoxicurcumin and bis-desmetoxicurcumin compounds which have effects antidiuretic, anti-inflammatory, antioxidant, antihypertensive, antihepatotoxic, antibacterial and antifungal. Degenerative diseases tend to occur due to the ability of the antioxidant compounds to react with the free radical in the body. This study aims to determine the effect of temperature and storage time of Curcuma ethanol extract (EET) as antioxidant. Curcuma was made from the ethanol extract preparations. The antioxidant activity test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) by measuring the absorbance with a UV-Visible (UV-Vis) spectrophotometer at a wavelength of 517 nm. The study comprises of four research groups, namely the negative control group (K-) using DPPH solvent, positive control group (K+) using vitamin C solution, EET37 group in the form of EET with 100% concentration stored at 37°C and the EET4 group in the form of EET with 100% concentration stored in a refrigerator at 4°C. All groups were tested for DPPH on days 0, 3, and 7. There were antioxidant activity in the group treated with vitamin C and EET compared to the control group, which showed a decrease in the absorbance value of DPPH ($p<0.00$). The difference of EET storage at room and refrigerator temperatures in significantly affects the antioxidant activity at ($p>0.05$). The prolonged storage time for EET to 3 and 7 days showed an increase in DPPH absorbance value compared to the 0th day ($p<0.05$). The differences in temperature and storage time influences the antioxidant activity of Curcuma ethanol extract.

Keywords: Curcuma ethanol extract, Antioxidant, DPPH, Temperature, Storage time.

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DOI: 10.31838/srp.2020.4.48

INTRODUCTION
Antioxidants are used to stop damage to cell membranes, DNA mutations, and lipid peroxidation due to Reactive Oxygen Species (ROS).[1,2] Free radicals are formed from the use of teeth whitening (bleaching) materials, which generally uses solutions such as hydrogen peroxide (H2O2) and carbamide. These materials cause sensitive teeth, micro-leakage, and changes in structure.[3,4]

The interaction of these chemicals increases the release of hydrogen from H_2O_2 and creates unstable free radicals, such as Perhydroxyl, hydroxyl, superoxide, and Perhydroxyl anions, when it diffuses into the teeth, thereby, causing oxidation.[5, 6]In addition, anti-oxidative drugs are needed to reduce the side effects associated with the use of teeth whitening ingredients such as discomfort and toothache.

Antioxidants have the ability to provide electrons, bind, and end free radical chain reactions.[7] It is divided into two groups, namely natural and synthetic antioxidants.[8] Natural antioxidants are obtained from nutritious plants, also known as herbs, with the ability to capture free radicals.[9]

Curcuma (Curcuma xanthorrhiza) is a medicinal plant that grows in many tropical regions such as Indonesia,[10,11] and empirically used to cure various diseases such as liver damage, hypertension, diabetes, anti-cholesterol, anti-inflammation, anemia, antioxidants, cancer prevention, and antimicrobial.[12-14] The results obtained from the isolation and identification of EET contained several active compounds such as curcumin, xanthorrhizol, and essential oils. Its antioxidant potential has been tested through several methods such as the DPPH (1,1-diphenyl-2-picrihydazi), superoxide anion, ferric reducing antioxidant power (FRAP) and metal bonding activity.[15,16] DPPH is a stable radical compound and is a simple method used to determine the antioxidant potential of a plant extract. The materials used to determine the temperature effects of curcumin were dissolved with the absorbance of DPPH measured using spectrophotometry at a wavelength of 517 nm.[17]

The effectiveness of a medicinal plant extract is proven and acquires maximum results when repeatedly conducted. Furthermore, due to the common usage of Curcuma as an antioxidant compound by the public, it needs to be stored in optimum temperatureto prevent its properties from changing over a long period. Therefore, this research aims to determine the effect of temperature and storage time on the antioxidant potential of Curcuma ethanol extract.

MATERIALS AND METHODS
Plant collection
The research material used is simplisia Curcuma from the Technical Implementation Unit (UPT) of the East Java Provincial Health Office located in Batu City. The Materia Medica Batu office is located in Pesanggrahan, which borders the Ngaglik Village in the Batu City area. Based on its geographical location Materia Medica is located at an altitude and temperature of ± 875 D.P.L and ± 20-25 C, respectively. The plant is harvested at 11-12 months because its yields better quality compared to when it is harvested between7-8 months.

The production of Curcuma ethanol extract (EET)
Simplicia is analyzed in the production of Curcuma rhizome ethanol extract(Curcuma xanthorriza Roxb.) using 250 g macerated with 1000 mL of 70% ethanol solution for 3 days.

The solution is further filtered till a liquid is obtained which is evaporated to obtain a thick extract.[18]

Sample preparation

This study used two variables, namely temperature and storage time, comprises of the negative/ blank control group (K-), vitamin C group (K+), Curcuma extract group stored at 37°C (EET37), and Curcuma stored at 4°C (EET4). A blank solution was made by weighing 4 mg of DPPH dissolved with 100 mL ethanol. The comparison solution was prepared by weighing 200 mg of vitamin C dissolved with 200 mL of distilled water. In addition, the sample solution was prepared by dissolving 10 µL of Curcuma extract in ethanol and homogenized till a volume of 1 mL is obtained. Subsequently, each solution is stored for 3 to 7 days.

The antioxidant activity using DPPH method analysis

This activity was carried out by adding 1 mL of DPPH solution into 2 mL of distilled water, divortex, and incubated at 37°C in a dark room. This was followed by measuring the absorbance of the blank solution with a UV-VIS spectrophotometer at a wavelength of 517 nm. The absorbance measurements in the Curcuma and vitamin C groups were carried out by pipette 2 mL of the sample solution, then adding 1 mL of DPPH, divortexed, and incubated at 37°C in a dark room. The DPPH absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 517 nm.[17]

Statistical analysis

The DPPH absorbance measurements were tabulated, presented in mean and standard deviations, with statistical tests performed using *Statistical Package for the Social Sciences* (SPSS) software version 21. The differences between study groups used the oneway ANOVA test with a significant level of 95% (α=0,05).

RESULTS

The antioxidant activity of Curcuma ethanol extract through the DPPH method

The antioxidant activity was analyzed from EET through the DPPH using the UV-VIS spectrophotometry with a wavelength of 517 nm. This showed a significant difference between the DPPH absorbance in the control group towards the vitamin C, EET37, and EET4 on day 0 ($p<0.05$). There was no significant difference between the DPPH absorbance of the vitamin C, EET37, and EET4 groups ($p>0.05$), as shown in table 1.

Table 1: Difference of DPPH absorbance in Curcuma ethanol extract

Groups	DPPH absorbance ($\bar{X}$±SD)					
	T0	p-value	T3	p-value	T7	p-value
K-	0.610	p<0.05	0.591	p<0.05	0.599	p<0.05
K+ (Vitamin C)	0.142		0.145		0.144	
EET37	0.160		0.253		0.451	
EET4	0.158		0.224		0.412	

Significance of DPPH absorbance in the negative control group (K-), vitamin C (K+), concentration 100% of ethanol extract stored in room temperature at 37°C (EET37) and concentration 100% of ethanol extract stored in a refrigerator at 4°C (EET4) measured on day 0 (T0), day 3 (T3) and day 7 (T7).

The decrease in antioxidant activity of EET affected by temperature and storage time

The results of DPPH absorbance measurements of EET stored at room temperature of 37°C was higher than at the refrigerator temperature of 4°C on the 3rd day. The increase in DPPH absorbance continued to occur close to the absorbance value of the K- group till the 7th day, with significant differences ($p<0.05$). A significant difference of $p<0.05$ was obtained when DPPH absorbance was compared between the vitamin C group towards the EET37 and EET4 groups. The DPPH absorbance of EET stored for a long time led to a decrease in antioxidant efficacy, as shown in figure 1.

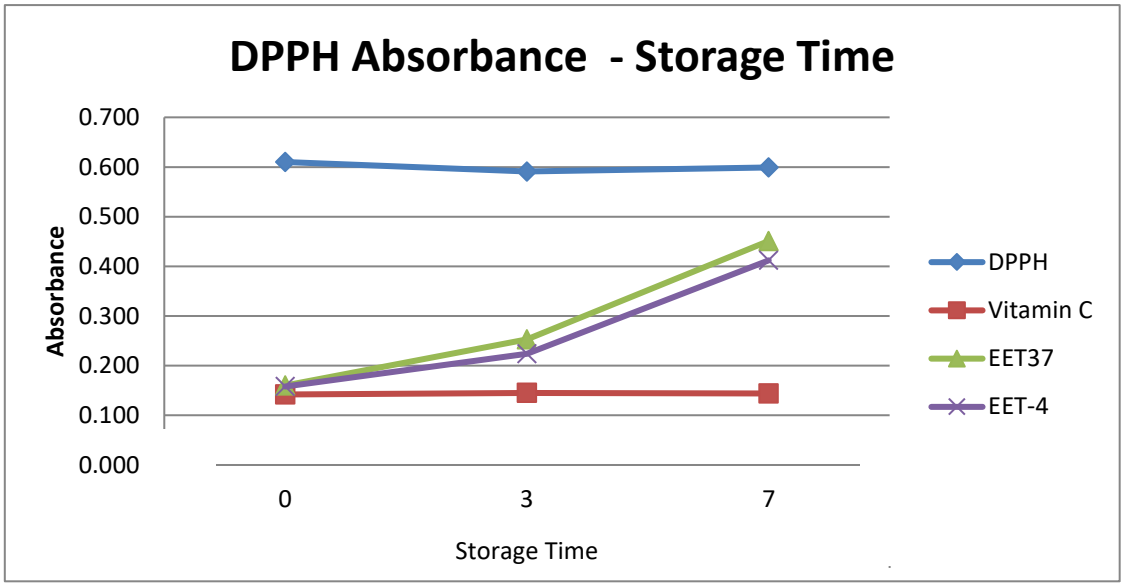

Figure 1: DPPH absorbance value with UV-VIS spectrophotometry at a wavelength of 517 nm.This shows that the lower the absorbance value, the higher its activity as antioxidant.

DISCUSSION

The use of antioxidants has presently increased in the field of dentistry.[19-21] Generally, they are in the form of oral health topical such as mouthwashes, gels, pastes, and lozenges. Antioxidants are used to reduce free radicals, oxygen-reactive species, inflammation in gum and periodontal tissue diseases.[22-24]

The dissolution of DPPH powder in ethanol produces a purple color. Furthermore, when mixed with compounds with antioxidants potential, it produces hydrogen atoms and turns yellow.[25] The provision of Curcuma stored at room (EET37) and refrigerator (EET4) temperatures reduces DPPH free radicals, therefore the solution turns yellow, as shown in figure 2. The DPPH radical reaction and donor molecule are denoted with $A \bullet$ and AH, to obtain the following reaction

$$A\bullet + AH = ZH + A\bullet$$

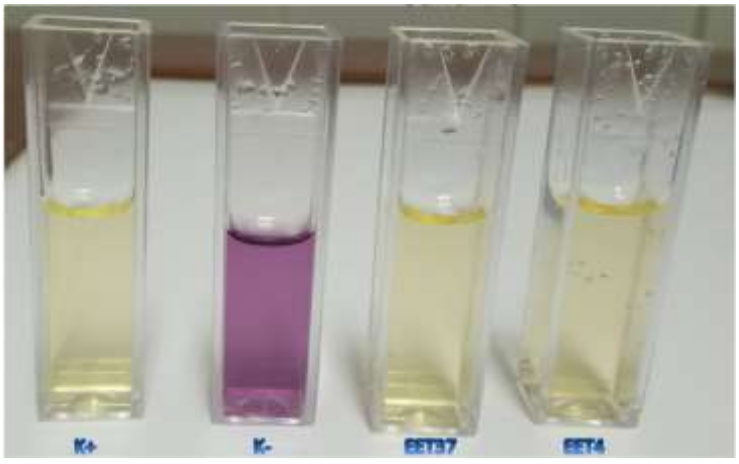

Figure 2: Antioxidant testing via the DPPH method. The ability of hydrogen atom donors causes purple to yellow discoloration obtained in the groups of vitamin C (K+), Curcuma Ethanol Extract stored at 37 ^{o}C (EET37), Curcuma Ethanol Extract was stored at 4 ^{o}C and purple blanks (K-).

One of the benefits of using the DPPH is its slow reactivity, therefore, it is possible to react with weak and strong antioxidant compounds. In addition, polar and nonpolar organic solvents are used to examine hydrophilic and lipophilic antioxidants.[26]

The main ingredients of Curcuma rhizome are proteins, carbohydrates, and essential oils consisting of camphor, glucoside, turmerol, and curcumin. Curcuminoids are the derivative compounds of curcumin, which comprises of desmetoxicurcumin and bis-desmetoxicurcumin materials.[18, 27] Ethanol is used to extract these substances due to its solubility.[28]

Furthermore, the separation of active compounds from plants using solvents is known as extracts. The solvent is

removed by evaporating the concentrated extracts with the therapeutic technique used for the pharmacological and non-pharmacological healing process. Generally, drugs are repeatedly used to treat diseases, therefore it needs to fulfill the requirements in accordance with time without changing the physical properties and usefulness.[29] Treatment using traditional plants needs to fulfill the same requirements before being used for a long time.[30]

An extract consists of many active compounds with effects capable of strengthening or weakening, the activity. This is indicated by an increase in the DPPH absorbance value of Curcuma extract in accordance with the length of storage time, despite being stored at 4ºC. The increase in DPPH absorbance values of Curcuma extract increases at 4ºC, compared at when stored at room temperature (37ºC). However, the antioxidant ability of vitamin C does not change even when stored over a prolonged time, thereby resulting in a constant absorbance value.

The high water content in plant extracts is one of the causes of damages, therefore the efficacy of a medicinal plant is easily reduced due to the development and production of toxic microorganisms. The production of none sterile extracts do not fulfill the method of making a standardized medicine, due to the decrease in the efficacy of medicinal plants.[31]

CONCLUSION

In conclusion, the storage of Curcuma ethanol extract decreased the antioxidant activity of the DPPH test compared to vitamin C, although it was stored at 4ºC on the 7th day. The community should make a fresh herbal medicine extract to more efficacy and no stored more than 7 days at refrigerator.

REFERENCES

1. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11–26. doi:10.1007/s12291-014-0446-0
2. Ighodaro OM, Akinloye OA.First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018;54(4): 287-93.
3. ALF Briso, APB Lima, RS Gonçalves, MO Gallinari, and PH dos Santos. Transenamel and Transdentinal Penetration of Hydrogen Peroxide Applied to Cracked or Microabrasioned Enamel. Oper Dent. 2014;39(2):166-73.
4. B Cvikl, A Lussi, A Moritz, and S Flury (2016) Enamel Surface Changes After Exposure to Bleaching Gels Containing Carbamide Peroxide or Hydrogen Peroxide. Operative Dentistry: January/February 2016, Vol. 41, No. 1, pp. E39-E47.
5. Kohji K, Yasuhisa T. Effects of the Hydroxyl Radical and Hydrogen Peroxide on Tooth Bleaching. Journal of endodontics. 2004;30:45-50.
6. Lima Neto CF, Silva CB, Silva MAB, Vitti RP, Silva Zanta CLP, Tonholo J. Tooth whitening affects bond strength of adhesive systems in enamel. Matéria (Rio de Janeiro). 2018; 23(4):e12227.
7. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev. 2012;70: 257-65.
8. Akbarirad H, Gohari AA, Kazemeini SM, Mousavi KA. An overview on some of important sources of natural antioxidants. Int Food Res J. 2016;23(3):928-33.
9. Toshiya M, Junko I, Akiko J, Nobuji N. Antioxidative curcuminoids from rhizomes of Curcuma xanthorrhiza. Phytochem. 1992;31(10):3645-7.
10. Elfahmi Y, Herman W, Oliver K. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J Herb Med. 2014;4(2):57-73.
11. Sholikhah EN. Indonesian medicinal plants as sources of secondary metabolites for pharmaceutical industry. J Med Sci. 2016;48(4):226-39.
12. Reyes-Gordillo K, Segovia J, Shibayama M, Vergara P, Moreno MG, Muriel P. Curcumin protects against acute liver damage in the rat by inhibiting NF-kappaB, proinflammatory cytokines production and oxidative stress. Biochim Biophys Acta. 2007;1770(6):989–96.
13. Khan N, Afaq F, Mukhtar H. Cancer chemoprevention through dietary antioxidants: progress and promise. Antioxid Redox Signal. 2008;10(3):475–510.
14. Oon SF, Nallappan M, Tee TT, Shohaimi S, Kassim NK, Sa'ariwijay a MS, et al.Xanthorrhizol: a review of its pharmacological activities and anticancer properties. Cancer Cell Int. 2015;21:15.
15. Devaraj S, Ismail S, Ramanathan S, Yam MF: Investigation of antioxidant and hepatoprotective activity of standardized Curcuma xanthorrhiza rhizome in carbon tetrachloride-induced hepatic damaged rats. Scientif World J. 2014:353128:Epub 2014.
16. Dosoky NS, Setzer WN. Chemical Composition and Biological Activities of Essential Oils of Curcuma Species. Nutr. 2018;10:1196.
17. Rosidi A. The difference of Curcumin and Antioxidant activity in Curcuma xanthorrhiza at different regions. J Adv Pharm Edu Res. 2020;10(1):14-8.
18. Jitoe A, Masuda T, Tengah IGP, Suprapta DN, Gara LW, Nakatani N. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. J Agric Food Chem. 1992;40:1337-40.
19. Aksakalli S. Antioxidants in dentistry: Review of literature. Dent. 2013;4:181.
20. Shivanna V, Gupta S. Antioxidants; stressbusters in dentistry-a review. J Dent Practice Res. 2013;1(2):9-19.
21. Patel S, Hans MK, Chander S, Ahluwalia AS. Antioxidants in endodontics: A strategic review. J Clin Diagn Res. 2015;9(5):ZE12–ZE15.
22. Bansal N, Gupta ND. Role of dietary antioxidants in periodontitis: A preventive approach. J Dent Med Sci. 2014;13(9):81-84.
23. Villa-Correa YA, Isaza-Guzmán DM, Tobón-Arroyave SI. Influence of periodontal clinical status on salivary levels of glutathione reductase. J Periodont. 2016;87(6):716-24.

24. Bains VK, Bains R. The antioxidant master glutathione and periodontal health. Dent Res J (Isfahan). 2015;12(5):389-405.
25. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011;48(4):412-22.
26. Elnour AAM, Mirghani MES, Musa KH, Kabbashi NA, Alam MZ. Challenges of Extraction Techniques of Natural Antioxidants and Their Potential Applications Opportunities as Anti-Cancer Agents. Health Sci J. 2018;12(5):596.
27. Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Crit Rev Food Sci Nutr. 2017;57(13):2889-95.
28. Pertamawati. Opportunities to elevate leves of curcumin in Curcuma xanthorriza plants. J Jamu Indonesia. 2018;3(1):10-17.
29. Albert V, Lanz M, Imanidis G, Hersberger KE, Arnet I. Stability of medicines after repackaging into multicompartment compliance aids: eight criteria for detection of visual alteration. Drugs Ther Perspect. 2017;33(10):487-96.
30. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177.
31. Kosalec I, Cvek J, Tomić S. Contaminants of medicinal herbs and herbal products. Arh Hig Rada Toksikol. 2009;60:485-501.