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

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ABSTRACT
Curcuma is one of the abundant natural resources used as a medicinal plant in Indonesia. It contains desmetocurcumin and bis-desmetocurcumin compounds which have effects antidiuretic, anti-inflammatory, antioxidant, antihypertensive, antivirus, 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-), DPPH solution, positive control group (K+), vitamin C solution, EET 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 temperature 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.

INTRODUCTION
Antioxidants are used to stop damage to cell membranes, DNA mutations, and lipid peroxidation due to Reactive Oxygen Species (ROS).\textsuperscript{1,2} Free radicals are formed from the use of teeth whitening (bleaching) materials, which generally uses solutions such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and carbamide. These materials cause sensitive teeth, micro-leakage, and changes in structure.\textsuperscript{1,4} The interaction of these chemicals increases the release of hydrogen from H\textsubscript{2}O\textsubscript{2} and creates unstable free radicals, such as Perhydroxyl, hydroxyl, superoxide, and Perhydroxyl anions, when it diffuses into the teeth, thereby, causing oxidation.\textsuperscript{3,4} 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.\textsuperscript{7} It is divided into two groups, namely natural and synthetic antioxidants.\textsuperscript{8} Natural antioxidants are obtained from nutritious plants, also known as herbs, with the ability to capture free radicals.\textsuperscript{8} Curcuma (Curcuma xanthorrhiza) is a medicinal plant that grows in many tropical regions such as Indonesia,\textsuperscript{9,11} and empirically used to cure various diseases such as liver damage, hypertension, diabetes, anti-cholesterol, anti-inflammation, anemia, antioxidants, cancer prevention, and antimicrobial.\textsuperscript{9,13} 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-picrylhydrazyl), superoxide anion, ferric reducing antioxidant power (FRAP) and metal bonding activity.\textsuperscript{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.\textsuperscript{17} The effectiveness of a medicinal plant extract is proven and acquired 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 temperature to 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 ethanolic extract.

MATERIALS AND METHODS
Plant collection
The research material used is simplicia 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 between 7-8 months.

The production of Curcuma ethanol extract (EET)
Simplicia is analyzed in the production of Curcuma rhizome ethanol extract(Curcuma xanthorrhiza 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.\textsuperscript{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, divortexed, 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.\textsuperscript{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 one-way ANOVA test with a significant level of 95% (\(p<0.05\)).

**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>
<thead>
<tr>
<th>Groups</th>
<th>DPPH absorbance ((X \pm SD))</th>
<th>T0</th>
<th>p-value</th>
<th>T3</th>
<th>p-value</th>
<th>T7</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-</td>
<td></td>
<td>0.610</td>
<td></td>
<td>0.591</td>
<td></td>
<td>0.599</td>
<td></td>
</tr>
<tr>
<td>K+ (Vitamin C)</td>
<td></td>
<td>0.142</td>
<td>(p&lt;0.05)</td>
<td>0.145</td>
<td>(p&lt;0.05)</td>
<td>0.144</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td>EET37</td>
<td></td>
<td>0.160</td>
<td></td>
<td>0.253</td>
<td></td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td>EET4</td>
<td></td>
<td>0.158</td>
<td></td>
<td>0.224</td>
<td></td>
<td>0.412</td>
<td></td>
</tr>
</tbody>
</table>

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 an antioxidant.

**DISCUSSION**

The use of antioxidants has presently increased in the field of dentistry. 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.

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. 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 \( A^- \) and \( AH \), to obtain the following reaction

\[
A^- + AH \rightarrow ZH + A^-
\]

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.

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.

Ethanol is used to extract these substances due to its solubility. 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.\textsuperscript{29} Treatment using traditional plants needs to fulfill the same requirements before being used for a long time.\textsuperscript{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 4°C. The increase in DPPH absorbance values of Curcuma extract increases at 4°C, compared at when stored at room temperature (37°C). 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.\textsuperscript{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 4°C 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**