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Article

Antioxidant Activity of Red Dragon Fruit Teabag (*Hylocereus polyrhizus*) Peels with the Addition of Ginger (*Zingiber officinale* var. amarum) and Cinnamon (*Cinnamomum zeylanicum*, Bl)

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Abstract. Free radicals have very unstable and reactive molecules. Excessive free radicals can trigger oxidative stress and cause various diseases. The peel of red dragon fruit contains chemical compounds that shows potential as an antioxidants. This study aims to optimize the benefits of red dragon fruit peels as tea bags because its convenient and simple to use. Design of this study was a randomized block design (RBD) which consisted of two factors, the drying temperature of the red dragon fruit skin (T) and the teabag formulation (F). Ginger and cinnamon are added as flavoring ingredients to the formula. The result showed that the drying temperature of the peel red dragon fruit affects the antioxidant activity of the teabag, where T1 has the highest antioxidant activity. The formulation of teabags also affects the antioxidant activity of the teabag, where F1 has the highest antioxidant activity. T1F1 had the highest antioxidant activity, and the interaction between the two (T and F) had a significant effect on antioxidant activity ($p < 0.05$).

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1. Introduction

Free radicals have unpaired electrons so they are unstable and very reactive. The free radicals become more stable when they gain electrons from the metabolism of proteins, carbohydrates, lipids, and deoxyribonucleic acid (DNA) [1-3]. Endogenous free radicals originate within the body, whereas exogenous radicals originate outside of the body. Sources of free radicals that come from outside the body for example air pollution, ultraviolet radiation, alcohol, cigarettes, heavy metals, pesticides and when excessive can trigger oxidative stress [4-6]. Continuous and unstoppable exposure to free radicals can cause disease such as diabetes mellitus, neurodegenerative disorders, gastrointestinal disorders, cardiovascular disorders, respiratory disorders, cataracts, rheumatoid arthritis, cancer, an immunity deficiency, inflammation, and aging [7-11].

Antioxidants are compounds that work by neutralizing free radicals and stopping the reaction [3, 9]. Several studies have reported that the use of antioxidants can repair cell damage due to oxidative stress. It also prevent several diseases such as cancer, neurological disorders, and cardiovascular disease [5, 12]. There are two types of antioxidants: natural antioxidants and synthetic antioxidants but the use of synthetic antioxidants has been restricted because the side effect issue [13, 14]. Natural antioxidants exist that come from inside the body (endogenous) and from outside the body (exogenous). Exogenous natural antioxidants can be obtained from natural ingredients containing phenolic compounds, flavonoids, vitamin E, vitamin C, and carotenoids [3, 14].

Dragon fruit is one of fruit that rich of antioxidants [15-17]. The most frequently used part of the dragon is the fruit. The peel of the dragon fruit is part of the dragon fruit that has been wasted because it has not been used optimally [17-19]. The peel of the red dragon fruit contains pectin and several chemical compounds such as betacyanin, flavonoids, and phenols which also show antioxidant activity. The pectin also can be used for the development of functional foods. [20-22].

In order to optimize the use of dragon fruit peels is formulate it as tea bags so that they have economic value and reduce the impact of waste on the environment. Teabags are one of the preparations that are easy to use and affordable. The tea processing process includes withering and drying, where the process affects the quality of the tea. Teabags are made with a combination of ginger and cinnamon to reduse the "unpleasant flavor" of the dragon fruit peel and enhance the taste of the tea. This study aims to determine the optimal conditions in the process of making teabags contains red dragon peel to produce the highest antioxidant activity. Antioxidant testing used the DPPH (2,2-diphenyl-2-picryl hydrazyl) method. Knowing the optimal conditions is expected to increase antioxidant activity and make a positive contribution to health.

2. Experimental Section

2.1. Materials and Instrumentations

This study used analytical scales (Ohaus), blenders, ovens, sieves, UV-Vis spectrophotometers (Shimadzu), and glassware. The materials used in this study were red dragon fruit peel, emprit ginger, cinnamon, aquadest, methanol, and DPPH.

2.2. Research Design

This is an experimental study that used a randomized block design (RBD), which consisted of two factors. The first factor is the drying temperature of the peel of the red dragon fruit (T) and the second factor is the formulation of the teabag (F), where in this study, emprit ginger and cinnamon are added as ingredients used to cover the "langu taste" of the red dragon fruit peel. The red dragon fruit peel drying temperature factor (T) is made in three temperature variations, 40°C (T1), 50°C (T2) and 60°C (T3), while the formulation where the dragon fruit peel teabag preparation will be added emprit ginger and cinnamon in three variations concentration. F1 (ginger 2.5% and cinnamon 7.5%), F2 (ginger 5% and cinnamon 5%), and F3 (ginger 7.5% and cinnamon 2.5%) so there are 9 treatment combinations as shown in table 1.

Table 1. Variety of treatment combinations

Drying Temperature	Formulation		
	F1	F2	F3
T1	T1F1	T1F2	T1F1
T2	T2F1	T2F2	T2F2
T3	T3F1	T3F1	T3F3

Description: T1 = 40 °C, T2 = 50 °C, T3=60 °C, F1 = ginger 2.5% and cinnamon 7.5%, F2 = (ginger 5% and cinnamon 5%), F3 = (ginger 7.5% and cinnamon 2.5%). The teabag each formulas is 2 g.

2.3. Plant Determination

Plant determination is carried out at the Politeknik Negeri Jember.

2.4. Sample Preparation

Samples of red dragon fruit (*Hylocereus polyrhizus*), emprit ginger (*Zingiber officinale* var. amarum), and cinnamon (*Cinnamomum zeylanicum*, BI) were obtained from the Muncar, Banyuwangi, East Java. The peel of the red dragon fruit which has been separated from the flesh then washed using water and then drained. The purpose of this sorting is to separate the dirt and parts that are not needed. Dragon fruit peel is cut into small pieces (± 0.5 cm) and then dried in the oven at 40°C, 50°C and 60°C for 24 hours. Emprit ginger and cinnamon are treated in the same way. Dried of emprit ginger and cinnamon is carried out at a temperature of 60°C for 6 hours. The dried simplisia is then mashed and sieved through a 30 mesh.

2.5. Teabag Formulation

Teabags are made in several formulations according to Table 1. The formulation variations in this study were nine formulas. The total weight of the powder made into the teabag is 2 grams, then put into a tea bag.

2.6. Antioxidant Activity Test

Antioxidant activity was tested using the DPPH method according to previous studies with minor modifications [23].

a. DPPH preparation
   A total of 10 mg of DPPH crystals were dissolved in 10 mL of methanol (1000 ppm). The DPPH solution is then diluted to a concentration of 80 ppm.
b. Determination of the maximum wavelength of DPPH
   The DPPH solution was pipetted 5 mL at a time, and absorption was measured at 490-534 nm. The maximum of DPPH wavelength is 516 nm.
c. Sample preparation
   Each formulation of the teabag is added to 150 mL of boiling water for 5 minutes. After that, the teabag is removed.
d. Antioxidant Activity Test
   Each sample that has been made is then tested for antioxidant activity using the DPPH method by adding 50.0 µL of the sample to an 80 ppm DPPH solution of 80.0 µL. After that, the samples were covered with aluminum foil and incubated for 30 minutes in a dark place, avoiding sunlight, at room temperature. The absorbance of each samples is then read at the maximum wavelength.

2.7. Data analysis

Calculation of the value of % inhibition using the formula below:

$$\% \text{ inhibition} = \frac{(\text{Abs blanko} - \text{Abs sample})}{\text{Abs blanko}} \times 100\%$$

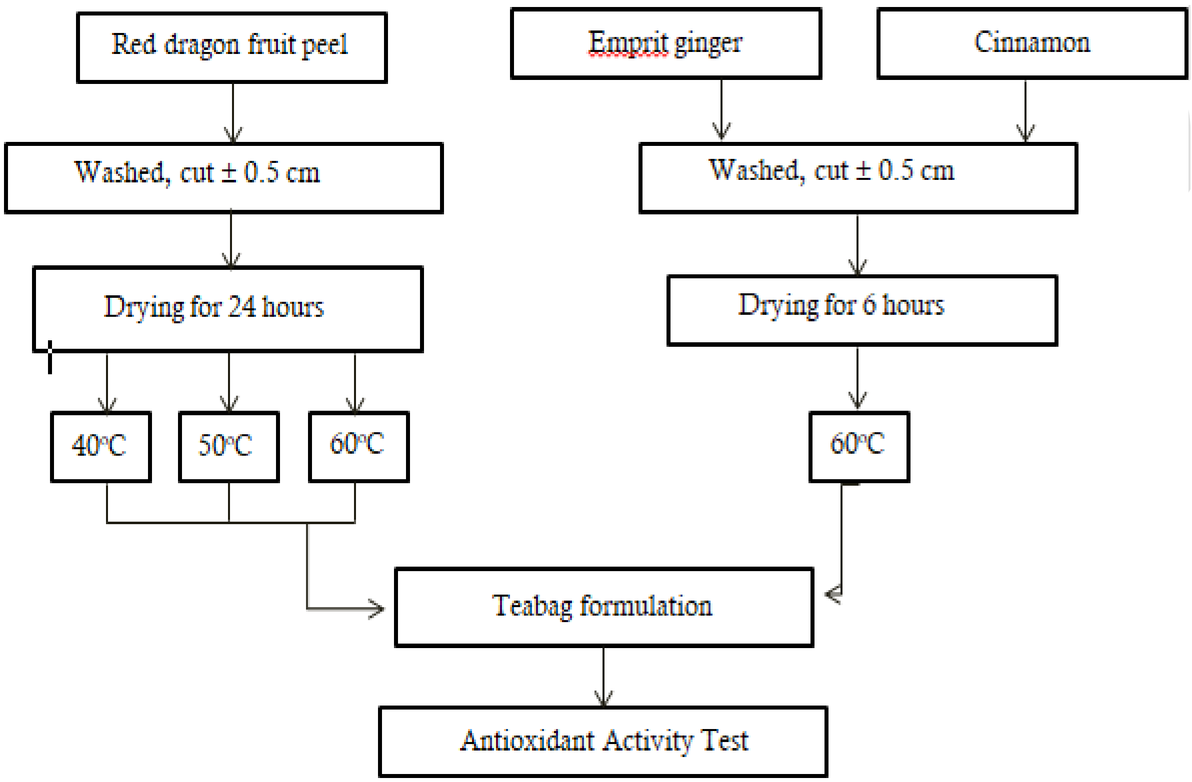

Figure 1. Research design

3. Results and Discussion

This research aims to optimize the utilization of red dragon fruit peel waste by making it into teabag. Teabags are a convenient, cost-effective, and simple of dosage form. In the teabag formula made from dragon fruit peel, additional flavors and aromas are needed to cover the "langu taste" of dragon fruit. Herbs and spices such as ginger and cinnamon are additional flavors that can be added to teabag formula[24]. Emprit ginger contains 1.5–3.3% essential oil and also contains phenols, namely oleoresin. This substance, in addition to providing the characteristic spicy taste of ginger, also acts as an antioxidant that can prevent the oxidation process so that it can also function as an antioxidant [25, 26]. Cinnamon contains eugenol, cinnamaldehyde, safrole resin, tannins, calcium oxalate, tanning agents, and several other components that are often used as aroma and flavor ingredients in food and beverage products [27-30].

The initial stage of this research is the process of determining the three plants used as samples. Determination of plants aims to ensure the correctness of plants used for research [31]. Plant determination is carried out at the Politeknik Negeri Jember. The results of the determination of plants used in this study are contained in the Plant Identification Certificate with Number 132/

PL17.8/PG/2022 for red dragon fruit, Number 133/PL17.8/PG/2022 for cinnamon, and 134/ PL17.8/PG/2022 for emprit ginger.

3.1. Antioxidant Activity

Antioxidant activity testing in this study used DPPH to determine the inhibitory ability of plant samples against DPPH free radicals which were measured for absorption using a UV-Vis spectrophotometer at maximum wavelength. The maximum wavelength obtained from the optimization results is 516 nm. The DPPH method has several advantages, including its simplicity, ease of use, speed, sensitivity, low sample requirement, and high stability [32-34]. The results of measuring the antioxidant activity of red dragon fruit peel water tea preparations in nine formulation variations showed in Table 2.

Table 2. Antioxidant activity of teabag preparations in each formulation

No	Formulation	% Inhibition
1.	T1F1	84.795 ± 1.358
2.	T1F2	77.662 ± 0.581
3.	T1F3	61.830 ± 1.477
4.	T2F1	76.235 ± 2.367
5.	T2F2	62.909 ± 2.625
6.	T2F3	49.548 ± 1.358
7.	T3F1	62.944 ± 0.478
8.	T3F2	44.398 ± 4.177
9.	T3F3	21.816 ± 7.376

To see the effect of each factor and the interaction between factors on antioxidant activity, statistical analysis was carried out using ANOVA. If there is a significant difference ($p<0.05$), the test is continued with the LSD test. The test results showed that both the drying temperature treatment of the red dragon fruit peel (T) and the teabag formulation (F), as well as the temperature interaction with the teabag formulation (T x F), had a significant effect ($p < 0.05$) on the antioxidant activity of teabags. The effect of temperature on antioxidant activity can be seen in figure 2.

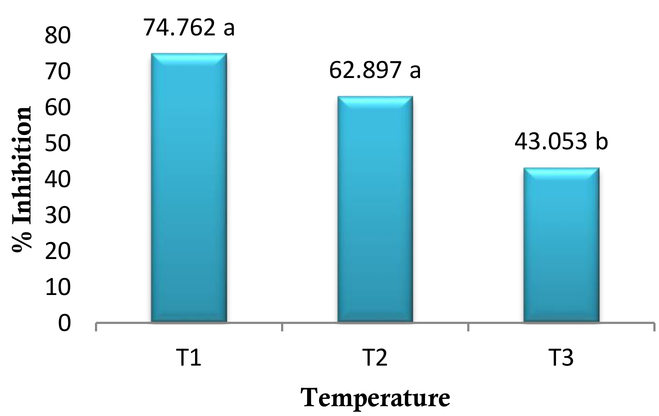

Figure 2. Effect of drying temperature on the antioxidant activity of teabags (values marked with different letters indicate a noticeable difference ($p< 0.05$)). Description: T1 = 40 °C, T2 = 50 °C, T3=60 °C.

The drying of the peel of the red dragon fruit at the lowest temperature (40^0C) has the greatest antioxidant activity compared to the temperatures of 50^0C and 60^0C (Figure 2). This is possible because of the difference in betacyanin levels. Betacyanin is a flavonoid compound that produces red to purple pigments in red dragon fruit. Betacyanin belongs to the group of flavonoid compounds that have antioxidant properties. The high and low antioxidant activities are effected by the content of compounds in the preparation, one of which is betacyanin. The stability of betacyanin is affected by several things, including drying temperature, pH, storage temperature, light, and oxidizer [35-37]. In addition to the drying temperature of the peel of the red dragon fruit (T), the formulation (F) also has a significant effect on antioxidant activity. The effect of the formulation on antioxidant activity can be seen in Figure 3.

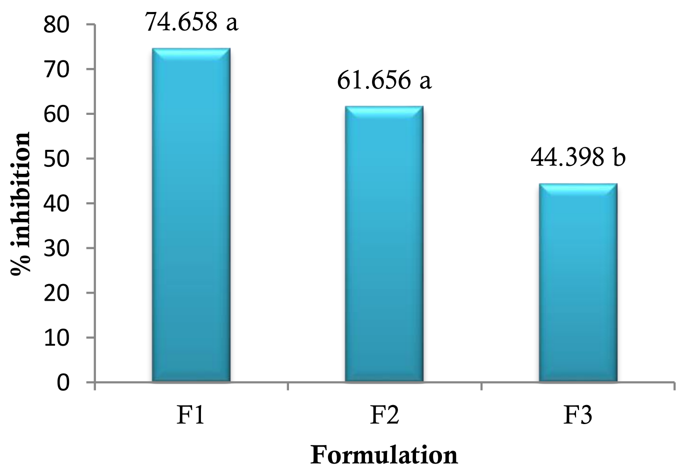

Figure 3. Effect of the formulation on the antioxidant activity of teabags (Values marked with different letters indicate a noticeable difference ($p< 0.05$)). Description: F1 = ginger 2.5% and cinnamon 7.5%, F2 = (ginger 5% and cinnamon 5%), F3 = (ginger 7.5% and cinnamon 2.5%).

From Figure 3 above, it can be seen that F1 has the highest antioxidant activity. F3 has the lowest antioxidant activity and differs significantly from F1 and F2. The antioxidant activity of cinnamon in teabags is increasing. Some studies reported that cinnamon has antioxidant activity [38-41]. It is also used to add aroma and flavor to teabags. This is possible because cinnamon contains several compounds that also have antioxidant activity, such as polyphenols, flavonoids, phenolic essential oil compounds, cinnamic acid, protocatechuic acid, quercetin, catechin, epicatechin, 3,4-dihydroxybenzaldehyde, and procyanidin B2 [38, 39, 42]. The interaction between the drying temperature of the red dragon fruit peel (T) and the formulation (F) also affects the antioxidant activity of the red dragon fruit peel. The effect of the interaction between the drying temperature of the red dragon fruit peel (T) and the formulation (F) showed in figure 3.

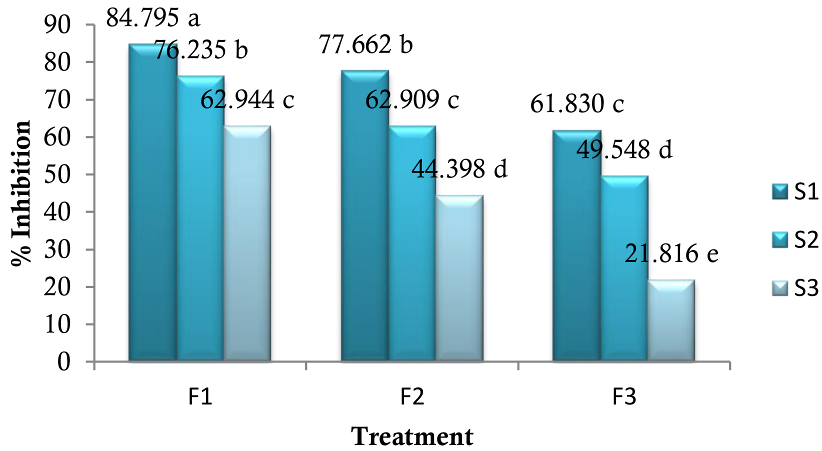

Figure 4. Effect of the interaction of the drying temperature of the peel of the red dragon fruit (T) with the formulation (F) on the antioxidant activity of teabags (values marked with different letters indicate a significant difference ($p < 0.05$))

From Figure 4, it can be seen that the T1F1 teabag has the highest antioxidant activity and has significant differences with other groups. The lower the drying temperature of the red dragon fruit peel and the greater the amount of cinnamon, the greater antioxidant activity of the dragon fruit peel teabag. Some compounds such as flavonoids are usually more stable at low temperatures. This is due to the fact that the lower the drying temperature of the dragon fruit peel effect on the higher antioxidant activity [43].

4. Conclusion

The drying temperature of the red dragon fruit peel affects the antioxidant activity of the teabag where the lowest dragon fruit peel drying temperature T1 has the highest antioxidant activity. The formulation also affects antioxidant activity, where F1 has the highest antioxidant activity. The interaction between the drying temperature of the red dragon fruit peel and the formulation (T x F) also affects the antioxidant activity of the teabag. The T1F1 formulation has the highest antioxidant activity compared to other formulas.

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