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ANTIOXIDANT CAPACITY AND TOTAL PHENOLS OF WILD POINSETTIA (*Euphorbia heterophylla*) AS POTENTIAL TEA INFUSION PRODUCT

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ABSTRACT

The use of wild poinsettia (*Euphorbia heterophylla*) is known as a traditional medicinal ingredient. The potential of this plant is the latex which can be used as a protease enzyme and the leaves can be used as herbal tea or tea infusion. To provide the potential of dried wild poinsettia, the total phenols and antioxidant capacity were measured by the Folin-Ciocalteu method, and the antioxidant capacity was assessed by DPPH assays. Phytochemical screening of dried wild poinsettia leaves which were dried at different temperatures (50 ºC & 60 ºC) and drying times (2, 3, & 4 h) showed total phenols and antioxidant capacity which have potential health properties and benefits on human health, such as anti-inflammatory, antihypertensive, anticancer, and as antimicrobial agents. The antioxidant activity values varied from 40.07 – 56.50%, and the highest values (56.50 ± 2.35) were obtained at 50 ºC for 2 h of the drying process. Total phenols in those tea leave varied from 11.47 – 13.41 mg GAE/g. The highest phenol content (13.41 ± 0.30 mg GAE/g) was found in dried tea leaves which were dried at 60 ºC for 4 h of the drying process. According to the result, the significant inverse linear correlation ($p<0.05$) was confirmed between treatments of drying duration, while not in the temperature. Generally, these dried leaves have a high antioxidant capacity and total phenol content and may be an important food source as tea infused with antioxidant phenolic compounds to prevent oxidative stress diseases.

Key words: Bioactivity, *Euphorbia heterophylla*, tea infusion, tea leaves, wild plant

INTRODUCTION

The use of wild plants as raw materials for traditional herbs has been carried out since ancient times by the ancestors until now by modern society (Luczaj *et al*., 2012; Cruz-Garcia & Price, 2014). Ethnic communities consume wild plants as medicinal plants based on their local knowledge and wisdom, wild plant as traditional medicine is also used by primary health care providers in developing countries, and tea consumption is influenced by local culture and the diversity of the flora (Jin *et al*., 2016; Ismail *et al*., 2021). The potential of wild plants needs to be explored to prevent the knowledge of traditional processing of wild plants from becoming extinct and can be processed into functional food products that are beneficial to health (Menendez-Baceta *et al*., 2012; Sanchez-Bel *et al*., 2015; Gramza-Michałowska *et al*., 2016; Marrelli *et al*., 2020).

Wild poinsettia (scientific name is *Euphorbia heterophylla*) is one of the wild plants of the family Moraceae, widely distributed in tropical and subtropical regions (James & Friday, 2010). The characteristics are milky latex (Mahajan *et al*., 2014), and sticky sap; some are co-carcinogens, cause severe skin irritation, and are toxic to livestock and humans (Grongnet *et al*., 2013). One part of the wild poinsettia (*Euphorbia heterophylla*) plant that is easy to use is the leaf part. Traditionally this plant is used to treat various diseases of cardiovascular, respiratory (bronchitis and asthma), gastrointestinal tract (constipation) (Ismawati & Destryana, 2019), as well as antispasmodic and anti-inflammatory (Falodun *et al*., 2006; James & Friday, 2010). There is still a lack of scientific information about the bioactive compound of wild poinsettia leaf relating to antioxidant and anti-inflammation. This research is the first step to determining the potential of wild plants as functional food through the approach of processing wild plants into tea leaf products.

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Herbal tea is one of the beverage products from herbal plants that can help treat disease and as a body refreshing drink. Herbal teas can be made from the flowers, seeds, leaves, and roots of tree plants (Oh *et al*., 2013; Ravikumar, 2014; Chandrasekara & Shahidi, 2018; Poswal *et al*., 2019). The process of extracting the bioactive compound in the tea leaves in the water solvent is called infusion (Visht & Chaturvedi, 2012; Fotakis *et al*., 2016). In the process of making tea, the condition of the leaves, the withering process, the enzymatic oxidation process, the drying process, and the brewing process are thought to affect the quality of the final brewed leaf tea (Yamin *et al*., 2017; Sari *et al*., 2020). In addition, the effect of the drying process of leaf tea also affects the quality of the herbal tea produced (Jiménez-Zamora *et al*., 2016).

Temperature and time play an important role in the drying of tea leaves and the quality of their chemical content, color, and rehydration capacity (Xiangyang *et al*., 2010; Taufik *et al*., 2016; Mabai *et al*., 2018; Qu *et al*., 2019; Roslan *et al*., 2020). The effect of this drying process also affects the physical properties of tea leaves, the highest score of the sensory evaluation was obtained for green tea by microwave vacuum drying (Xiangyang *et al*., 2010). Drying processes that usually use are conventional hot-air drying processes such as sun, solar, and oven (40, 50, & 60ºC), using the sun drying method is too long can cause the quality of the tea to decrease, such as the texture of the tea being brittle, the aroma is not fresh, the color is too pale, and nutritional changes. On the other hand, if the drying time used is too fast, the water content in the tea leaves is still high enough that it cannot be stored for too long.

Phenolic content and antioxidant capacity are important information due to knowing the potent of the bioactive compound in the plants (Li *et al*., 2007; Fu *et al*., 2011; Ismail *et al*., 2021). Therefore, it was desirable to identify antioxidant activity and phenolic content from dried wild poinsettia leaves and to evaluate the relationship between these two parameters. This work aimed to study the effect of the drying process of wild poinsettia leaves (*Euphorbia heterophylla*) on the quantity of the antioxidant activities and phenolic content.

MATERIALS AND METHODS

Materials

The fresh leaves of wild poinsettia (wild plant) were plucked in the West Sumenep area in January–April 2021, immediately air drying for 6 hr before treatment (Sari *et al*., 2020). All chemicals used were analysis-grade, distilled water, 1,1-diphenyl-2-picrylhydrazil (DPPH) (Sigma-Aldrich), ethanol 96%, ethanol (Merck), methanol (Merck), Folin Ciocialteu (Sigma-Aldrich), gallic acid (Sigma-Aldrich).

Methods

The study was performed with a combination of drying temperature and time. The temperature variations used were 50 ºC and 60 ºC. The drying times used were 2, 3, and 4 h.

Antioxidant Activity

(DPPH Method, Büyükbalci & El, 2008). Prepare 50 ppm of DPPH stock solution. Stock solutions of DPPH were prepared by dissolving 5 mg of solid DPPH in 100 mL of methanol. Then, a comparison solution was prepared, namely, a control solution containing 2 mL of methanol PA and 1 mL of 50 ppm DPPH solution. For the test sample, prepare 2 mL of sample solution and 2 mL of DPPH solution each time. Then incubate for 30 min at 27 ºC until there is a color change from DPPH activity. All samples tripled.

Total phenol Content

(Folin-Ciocalteu Method). This analysis is defined according to (Li *et al*., 2007; Fu *et al*., 2011), 50 μL of tea infusion (water dilution factor) was added to 50 μL of 10% folin solution and 50 μL of bicarbonate solution (60 g/L) and then incubated at room temperature for 60 min. The absorbance of the solution was measured at 760 nm. Gallic acid is used as the standard, which is made by dissolving gallic acid in distilled water and is produced at a concentration of 3.125; 6.25; 12.5; 25; 50; 100 (g/mL). Distilled water was also used as a blank. Total phenols are expressed in mg of gallic acid equivalent (mg GAE)/L of infusion.

Data analysis

Study data were analyzed using SPSS for Windows version 21.0. The data were evaluated by descriptive quantitative and presented as mean and standard deviation. All comparisons were subjected to a one-way analysis of variance (ANOVA), and significant differences between treatments (temperature drying and drying duration) means were determined using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Water content

The drying method and its parameters affect the rehydration capacity of a product, not only by product composition, water temperature, and rehydration time. During the drying process, the amount of water gradually decreases, initiating irreversible cell damage and causing damage to cell structure and strength. The effect of different drying temperatures and drying duration on the water content of tea leaves is shown in Table 1. The water content of wild poinsettia tea leaves is shown in Table 1. Water content in those tea leaves varied from 5.47 – 7.28% (w/w).

Table 1. The water content of tea leaves of the wild poinsettia

Drying Temperature (ºC)	Drying Duration (h)	Water content (%)
50	2	7.28 ± 0.62[a]
	3	5.57 ± 0.16[b]
	4	5.60 ± 0.28[b]
60	2	6.06 ± 0.49[a]
	3	5.85 ± 0.17[b]
	4	5.47 ± 0.93[b]

Data are reported as mean ± standard deviation.

The lowest water content (5.47 ± 0.93%) was found in dried tea leaves which were dried at 60 ºC for 4 h of the drying process. According to the result, the significant inverse linear correlation ($p<0.05$) was confirmed between the amount of antioxidant activity of drying duration, while not the temperature. The decrease in water content during the drying process can affect the concentration of cell fluid that comes out and the enzymatic oxidation process becomes inactive. Water is needed in the enzymatic process, so a decrease in water content results in a decrease in enzyme activity.

Enzyme activity affects the steeping results produced in tea products. The high temperature of drying produces tea leaves with lower water content, can provide the polyphenolase enzymes in the ingredients be inactivated more quickly, and make the steeping color brighter (yellowish green). On the other hand, products dried at lower temperatures tend to produce darker colors (Taufik *et al*., 2016).

Antioxidant activity

It is important to identify new sources of organic and inexpensive antioxidants of natural origin. The process conditions, such as drying temperature and time were found the main factors to affect the nutritional values of dried green tea (Xiangyang *et al*., 2010). Some herbal teas and infusions traditionally used in the traditional treatment have been studied for their antidiabetic effects based on phenolic contents and antioxidant activities (Büyükbalci & El, 2008). The estimation of the crude yields of chemical constituents of the plant studied showed that the leaf of the plant (*E. heterophylla*) is rich to some extent in alkaloids, flavonoids, saponins, and tannins (James & Friday, 2010). The effect of different drying temperatures and drying duration on the antioxidant activity of tea leaves is shown in Table 2.

In this study, the antioxidants in tea leaves were analyzed based on the temperature and length of the drying process. The antioxidant capacity of the infusion was assessed using the DPPH assay, one of the most commonly used methods for measuring antioxidant capacity (Li *et al*., 2007). In this method, the total antioxidant potential of the samples was evaluated for the free radical scavenging capacity of the tea extracts using a 2,2-diphenyl-1picrylhydrazyl free radical assay (Büyükbalci & El, 2008).

Table 2 shows the antioxidant capacities of the 6 samples of tea leaves infusion. The antioxidant activity values varied from 40.07 – 56.50%. The best values (56.50 ± 2.35[a]) were obtained at a drying temperature of 50 ºC and a drying time of 2 h, while a drying time of hr at a drying temperature of 50 ºC was the lowest. According to the result, the significant inverse linear correlation ($p<0.05$) was confirmed between the amount of antioxidant activity of drying duration, while not the temperature. In this study, we studied and determined the drying temperature and time process commonly used for producing herbal teas. Using a low temperature of drying at 50 ºC for 2-3 h produced antioxidant activity of wild poinsettia tea leaves.

Table 2. Antioxidant activity of tea leaves of wild poinsettia (measured by DPPH assay, percentage %)

Drying Temperature (ºC)	Drying Duration (h)	Antioxidant activity
50	2	56.50 ± 2.35[a]
	3	48.74 ± 6.59[b]
	4	40.07 ± 2.20[b]
60	2	50.14[a] ± 3.00[a]
	3	50.32[a] ± 0.71[b]
	4	42.15[b] ± 4.43[b]

Data are reported as mean ± standard deviation.

Total Phenol

The wild plant used as medicinal plants is of great importance to the health of humans in general. Some chemical substances in medicinal plants produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of the indigenous medicinal plants are used as herbs, spices, food plants, and wild plants. The total phenolic contents of tea leaves were estimated using the Folin–Ciocalteu method, which is a simple and rapid method. This method relies on the transfer of electrons from phenolic compounds to the Folin–Ciocalteu reagent in an alkaline medium. The effect of different drying temperatures and drying duration on the total phenol content of tea leaves is shown in Table 3.

The content of phenolic compounds was calculated in milligrams of gallic acid equivalents per gram of tea leaves (mg GAE/g). The total amounts of phenolic compounds in the studied tea leaves are presented in Table 3. Total phenols in those tea leaves varied from 11.47 – 13.41 mg GAE/g. The highest phenol content (13.41 ± 0.30 mg GAE/g) was found in dried tea leaves which were dried at 60 ºC for 4 h of the drying process. The correlation between antioxidant activity and phenol content of 6 samples was found to be very weak. Thus, phenolic compounds were not the main contributors to the antioxidant capacity of these wild poinsettia tea leaves. The phytochemical components in aquatic extracts of *Euphorbia heterophylla* can produce a wide range of antioxidant compounds, including saponins, flavonoids, and terpenes (Falodun *et al*., 2006).

There are no claims for the phenol content and related antioxidant properties of wild poinsettia tea leaves or herbal plants, traditionally used in Indonesia. A special case is the wild plants that are brought back to the natural garden and directly planted for food and medicine (Luczaj *et al*., 2012). The use of different methods for brewing tea and assessing antioxidant capacity or controlled products makes comparisons more difficult. Good results for antioxidant activity and total phenolic content were also obtained for various edible wild plants as potential antioxidants for minimally processed beverages (Sanchez-Bel *et al*., 2015). The wild poinsettia has great potential in the food industry, not only because of its phytochemical properties but also because of its biological and medicinal benefits.

CONCLUSION

The temperature and duration of drying affect the water content and antioxidant activity. The lowest water content (5.47 ± 0.93%) was found in dried tea leaves which were dried at 60 ºC for 4 h of the drying process, while. The antioxidant activity values varied from 40.07 – 56.50%, and the highest values (56.50 ± 2.35[a]) were obtained at 50 ºC for 2 h of the drying process. Total phenols in those tea leaves varied from 11.47–13.41 mg GAE/g. The highest phenol content (13.41 ± 0.30 mg GAE/g) was found in dried tea leaves which were dried at 60 ºC for 4 h of the drying process. According to the result, the significant inverse linear correlation ($p<0.05$) was confirmed between treatments of drying duration, while not in the temperature. The results suggest that the dried leaves of poinsettia may show great promise and could be an economic food source as an infusion tea for the prevention of oxidative stress diseases.

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Table 3. Total phenol content of tea leaves of wild poinsettia (*Euphorbia heterophylla*)

Drying Temperature (ºC)	Drying Duration (h)	Total phenol mg GAE/g
50	2	11.47 ± 0.63
	3	12.85 ± 1.61
	4	11.61 ± 0.02
60	2	13.39 ± 0.78
	3	12.53 ± 0.22
	4	13.41 ± 0.30

Data are reported as mean ± standard deviation.

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