Effect of drying and composition ratio of herbal tea prepared from *Clitoria ternatea* L. and *Ocimum sanctum* L. on its antioxidant capacity

Supriatno\textsuperscript{1,*}, H Rahmatan\textsuperscript{1}, Lelifajri\textsuperscript{2}, and S K Andesa\textsuperscript{1}

\textsuperscript{1}Department of Biology Education, Faculty of Teacher Training and Education, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
\textsuperscript{2}Department of Chemistry, Mathematics and Basic Science Faculty, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

*supriatno@unsyiah.ac.id

\textbf{Abstract.} Lemon Basil (*Ocimum sanctum* L.) and Butterfly Pea (*Clitoria ternatea* L.) have been prepared as an herbal tea source and evaluated their secondary metabolites content and antioxidant values. The purpose of this study was to determine the effect of drying time and composition ratio of herbal tea on their antioxidant value. The experimental design of Factorial Completely Randomized Design with nine treatment combination and three replications was applied. The results were analysed by ANOVA and Tukey’s HSD test. The results showed that the treatment affected the antioxidant value with the F-value > F-table (hypothesis accepted) at the test level 0.01 with a value of 94.26 > 3.71. The best treatment is P2K3 by showing an antioxidant value of 38.61 ppm. A total of 37.01 ppm concentration of combined herbal tea samples as a whole can reduce 50% of the radical effects of DPPH compounds. The secondary metabolite compounds contained in combination herbal teas are alkaloids, flavonoids, terpenoids, and saponins.

1. Introduction

Tea is an aromatic beverage containing tannins and polyphenols, an infusion made from the results of brewing dried leaves, shoots, or petioles \cite{1}. Nowadays, tea is not only produced from tea leaves (*Camelia sinensis*), but can also be made from plants that are not derived from the tea plant, which is called herbal tea. Herbal teas can be generated from flowers, seeds, leaves or roots of various plants, such as *Clitoria ternatea* L. and *Ocimum sanctum* L. \cite{2}. Lemon basil leaves (*Ocimum sanctum* L.) and butterfly pea flowers (*Clitoria ternatea* L.) have good potential as an antioxidant source due to their secondary metabolite contents. Some studies have reported that flavonoid ursolic acid contained in basil leaves consists of apigenin, polyphenols, anthocyanins, luteolin, eugenol and thymol or sesquiterpenic alcohol \cite{3}. Meanwhile, Kamkaen and Wilkinso \cite{4} have reported that water extracts of *Clitoria ternatea* have stronger antioxidant activity which was tested with DPPH, compared with ethanol extracts \cite{4}. However the application of *Ocimum sanctum* L and *Clitoria ternatea* L. as herbal medicines has not been intensively reported.

Preparation process of herbal tea could affect their antioxidant capacity. Preparation process such as drying process with high temperatures and a long drying time can reduce antioxidant activity in the...
dried material. Sari [5] has reported that drying avocado leaves at 50°C with 120 minutes drying time produced the best avocado leaf tea with antioxidant activity of 85.11% [5]. Evaluation of tea preparation process with variations on drying time of 90, 120, and 150 minutes has been conducted by Adri and Wikanastri [6]. They reported that the highest antioxidant activity was found at a temperature of 50 °C with a drying time of 150 minutes [6].

In this research, we evaluate the effect of drying and composition ratio of herbal tea prepared from Clitoria ternatea L. and Ocimum sanctum L. on its antioxidant capacity.

2. Materials and Methods
The research was conducted at the Laboratory of Food and Agriculture Product Analysis Faculty of Agriculture, Laboratory of Department of Biology Education, and Laboratory of Department of Chemistry Education. The research was conducted during October until December 2019.

2.1. Preparation of Herbal Tea
*Ocimum sanctum* L. leaves and *Clitoria ternatea* L. flowers that are not deformed are picked and then washed and wiped dry in the wind for 48 hours. The sample was then dried using an oven with different drying times (60 minutes, 90 minutes and 120 minutes). Samples that have been roasted and then blended and packed into a tea bag.

2.2. Antioxidant Test with DPPH method (1,1-diphenyl-2-picril-hidrazil)
DPPH is dissolved and homogenized (4 mg) with 30 ml of distilled water and 70 ml of methanol. Homogeneous solution then stored in a dark place. This DPPH solution is intended as a blank. Then, each treatment sample of tea was brewed and taken as much as 0.2 ml and added 3.8 ml of DPPH solution, then homogenized. Then put into a measuring flask that has been coated with aluminum foil and stored in a dark place for 30 minutes. The samples were measured it’s percent inhibition using a UV-Vis spectrophotometer at a 517 nm wavelength. Free radical uptake (% inhibition) is measured using the formula [8]:

\[
% \text{ of Inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100\% \quad (1)
\]

Based on the damping percentage value at each concentration, then made a regression curve, so that the equation \( y = bx + a \) is obtained where the extract concentration (ppm) as abscissa (x axis) and the percentage of damping as ordinate (y axis). Then do the calculation of the value of IC\(_{50}\) (inhibitory concentration) that is the concentration of the sample which has an inhibition of DPPH absorbance of 50%. Based on the linear regression equation IC\(_{50}\) values will be obtained where the lower IC\(_{50}\) values indicate higher antioxidant activity [9]. Then determine the IC\(_{50}\) value of each sample based on the Table [7].

| Table 1. Antioxidant Properties Based on IC Value |  |
|---|---|
| IC\(_{50}\) Value | Characteristic of Antioxidant |
| 50 ppm< | Very Strong |
| 50 ppm-100 ppm | Strong |
| 100 ppm – 150 ppm | Mediate |
| 150 ppm – 200 ppm | Weak |

As a comparison, quercetin compounds are used as standardization of antioxidant activity with the same composition as DPPH blanks.
2.3. Secondary Metabolite Compound Test
Secondary metabolite compounds tested include alkaloid, flavonoid, tannin, saponin, and triterpenoid tests, respectively [10].

2.3.1. Alkaloid Test. A total of 50 mg extract was dissolved with 5 mL chloroform and a few drops of NH₄OH and filtered. The filtrate was added with 1 mL of concentrated H₂O then the fractions were tested on a drip plate. Meyer's reagent contains mercury chloride and potassium iodide with a positive white precipitate. Dragendorf reagents contain potassium iodide and bismuth subnitrate in glacial acetic acid with positive red precipitate results. Wagner reagents consist of iodine and potassium iodide with positive results of chocolate deposits.

2.3.2. Flavonoid Test. A total of 50 mg of extract was added with 5 mL of methanol and heated at a temperature of 50°C. The filtrate was separated into two parts. Added concentrated H₂SO₄ and a red discoloration showed the presence of flavonoids.

2.3.3. Tannin Test. A total of 50 mg extract was dissolved with distilled water and then heated for 5 minutes and filtered. The filtrate added 1% FeCl₃ and the presence of blackish green indicates tannin. Tannins have a hydroxyl group that can react with a 10% iron (III) chloride solution.

2.3.4. Saponin Test. The filtrate obtained from the tannin test was cooled, and shaken strongly. The positive saponin test results are characterized by the formation of stable froth for about 10 minutes.

2.3.5. Triterpenoid Test. A total of 50 mg of extract was added with 10 mL of ethanol, heated, and filtered. The residue is added to 1 mL diethyl ether. The ether layer was added with Lieberman Buchard reagent containing 3 drops of anhydrous acetic acid and 1 drop of concentrated H₂SO₄. The positive results of the triterpenoid test are marked by the formation of red or purple.

3. Results and Discussion

3.1. Standard Curve of Quercetin
The linear regression equation is obtained \( Y = 2.3412x + 31,763 \) (Figure 1) with an \( r \) value of 0.9873. A positive \( r \) value indicates a positive correlation between quercetin concentration variables and absorbance values. Furthermore, the equation is used to determine the concentration of antioxidants in ppm units (parts per million) by substituting the absorbance value of the sample with a value of \( Y \).

![Figure 1. Standard of Quercetin](image-url)
3.2. Antioxidant Activity of Herbal Tea Combinations

The results of statistical analysis using ANOVA and carried out further tests with Honestly Significant Difference Test (HSD)/ Tukeys test. The results of the analysis of variance showed that the effect of tea drying time and concentration on antioxidant ability to reduce free radical activity with $F$-count > $F$-table at $\alpha$ test level of 0.01 with $\alpha$ value 94.26 > 3.71.

Analysis of antioxidant activity showed that the antioxidant activity in P2K3 treatment produced the greatest concentration of antioxidants with a value of 38.61 ppm compared to other treatments. The P2K3 treatment is dried for 90 minutes in combination (1: 2). When antioxidant levels are high, the antioxidant activity is large [11]. Differences in the treatment of raw material preparations affect antioxidant activity [12].

Table 2. Data on Antioxidant Levels of Herbal Tea Combination.

| Factor         | Replication | Averages (ppm) |
|----------------|-------------|----------------|
| Drying Time (P)| Tea Combination (K) | I | II | III |
| P1 (60 minute) | K1 (1:1)     | 20,0 | 20,0 | 17,9 | 19,34 |
|                | K2 (2:1)     | 20,0 | 17,9 | 17,9 | 18,62 |
|                | K3 (1:2)     | 17,9 | 15,7 | 17,9 | 17,20 |
| P2 (90 minute) | K1 (1:1)     | 35,0 | 32,9 | 35,0 | 34,33 |
|                | K2 (2:1)     | 28,6 | 26,4 | 24,3 | 26,48 |
|                | K3 (1:2)     | 39,3 | 39,3 | 37,1 | 38,61 |
| P3 (120 minute)| K1 (1:1)     | 20,0 | 17,9 | 20,0 | 19,34 |
|                | K2 (2:1)     | 24,3 | 22,1 | 22,1 | 22,90 |
|                | K3 (1:2)     | 30,7 | 30,7 | 28,6 | 30,05 |

Duration and drying techniques affect the antioxidant activity of herbal tea. The longer the drying process and the hotter the drying, the antioxidant activity of herbal tea decreases. This is because strong antioxidants will be damaged by heat and cooking [13].

The DPPH method is a chemical reaction in which antioxidant compounds will react with DPPH through a hydrogen atom donation mechanism and cause a change in color from purple to yellow [14].

The factors that influence the speed of drying of a food material are the area of the surface and the heating temperature the higher the temperature used the faster the material becomes dry. With the reduction of water in food, the content of compounds such as protein, carbohydrates, fats and minerals will increase in concentration, but vitamins and dyes are reduced [15].

3.3. Inhibit Concentration ($IC_{50}$)

$IC_{50}$ value is defined as the magnitude of the concentration of the test compound that can reduce free radicals by 50%. The smaller the $IC_{50}$ value, the higher the free radical mitigation activity [16]. Based on antioxidant classification on (Table 1) shows that the sample has antioxidant activity in the range < 50 ppm, which indicate a very strong activity.
Furthermore, based on the regression equation \((Y = 0.9625x + 14.291)\) with showed the relationship between the concentration of antioxidant samples and percent inhibitors (Figure 2), the IC\(_{50}\) value of the sample was found at 37.01 ppm.

![Figure 2. The relationship between antioxidant concentration and percent inhibitors](image)

3.4. Secondary Metabolites Compounds
The flavonoid test showed positive results with dark red color changes. Flavonoid compounds, simple phenolics and tannins are antioxidant compounds that contain phenol structures and have several hydroxy functional groups that are abundant in plants. Non-phenolic compounds that can have antioxidant activity include alkaloids, essential oils, and saponins. This is consistent with previous research which states the presence of alkaloid compounds and saponins in the *Graptophyllum pictum* Griff and *Gynura procumbens* Merr plants. \(^{[17]}\) and the presence of essential oil content in *Ocimum basilicum* \(^{[18]}\). For saponin test positive results were indicated by the presence of foam bubbles as high as 1 cm. This is due to the presence of hydrophilic and hydrophobic groups in which the formation of froth is due to hydrophilic groups that bind to water while hydrophobic bind to air \(^{[19]}\).

| Table 3. Screening of Secondary Metabolite Compound |
|-----------------------------------------------|
| Tests                  | Reagents       | Result                  |
|------------------------|----------------|-------------------------|
| **Alkaloid**           | Burchard       | (+) Green colored       |
|                        | Dragendrof     | (+) Red sediment        |
|                        | Meyer          | (-) No color changes    |
| **Flavonoid**          | Propanol + HCl| (+) Dark red colored    |
| **Terpenoid**          | Libermann-burchhard | (+) Purplish red    |
| **Tannin**             | Glatin         | (-) No color changes    |
| **Saponin**            | HCL 2 N        | (+) Foam for 1 cm       |

Information: 
(+): Contains secondary metabolites
(-): Does not contain secondary metabolite compounds
For alkaloid testing with Burchard and Dragendorf reagents showed positive test results evidenced by a change in green color in the Burchard test and the presence of Burchard test red sediment in the Dragendorf test, while in the Meyer test showed negative results with no change in color. This is assumed because the methanol extract of basil leaves has slightly alkaloids where nitrogen is not used to form covalent coordinate bonds with K+ which is a metal ion, so orange deposits are formed[19].

For the terpenoid test, it showed positive results with a change in color to purplish red. For tannin test negative results in the absence of discoloration. The content of flavonoids and alkaloids contained in combination herbal teas shows that, combined herbal teas have antioxidant potential.

4. Conclusion

Based on the present study, it can be concluded that the drying process and composition of herbal tea affected its antioxidant activity with the best treatment was observed at P2K3 composition with an antioxidant value of 38.61 ppm. A total of 37.01 ppm sample concentration can reduce 50% of DPPH free radicals. The metabolite compounds contained in the sample are alkaloids, flavonoids, terpenoids, and saponins.

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