POTENCY OF ANTIOXIDANT COMPOUNDS OF INDIAN GOOSEBERRY (Phyllanthus emblica) LEAVES AS HERBAL TEA

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ABSTRACT

Indian gooseberry leaves (Phyllanthus emblica) have been used as traditional medicine to treat a variety of diseases. Methanolic extract of the leaves contains high of antioxidant components, such as flavonoids, alkaloids, coumarins, dihydrochalcones, flavones, gallic tannins, glycosides, phenols and triterpenoids. Therefore, the leaves extract have antioxidant potency as herbal tea. The purpose of this study was to determine the effect of fermentation and drying methods during Indian gooseberry leaves tea processing on antioxidant content, total phenol content, water content and ash content of the tea. The study was designed with consisted of 2 factors. The first factor was fermentation time (0, 60, 90 and 120 minutes) and the second factor was drying methods (indirect sun-drying and roasting method). The experiment was carried out in triplicate. Water content of fresh kimalaka leaves is 49.6%. Based on analysis of Duncan Multiple Range Test (DMRT) showed that the highest water content of the herbal tea was provided by indirect sun-dried method (9.125%) and the lowest water content was given by roasted dried method (3.417%). Ash content of the tea powder from roasted method without fermentation obtained the higher value (4.42%) which was significantly different with others treatment. Based on analysis by FTIR spectroscopy method, FTIR spectra of the brewed tea indicated the presence of several functional groups of antioxidant components. The spectra showed that there were C-O, C=O and O-H groups which were thought to be functional groups of phenol compounds, flavonoids and tannins. The highest antioxidant activity of the brewed tea was provided by indirect sun-dried method (79.05%) and the lowest antioxidant activity of the tea was given by roasted dried method (68.99%). The highest total phenol of the tea was provided by roasted method (71.52 mg gallic acid/50 ml), while the lowest total fenol of the tea was given by indirect sun-dried method (50.28 mg gallic acid/50 mL). The results of the qualitative tannin analysis showed that in the brew of Indian gooseberry leaves tea was positively containing tannin compounds

Keywords: antioxidant, flavonoid, herbal tea, Indian gooseberry, phenol, Phyllanthus emblica.

INTRODUCTION

Indonesia is one of tropical countries with many plants. One of potential plants that grow well in Indonesia is Indian gooseberry (Phyllanthus emblica). In Indonesia this plant is known as kimalaka (Uji, 2006). Indian gooseberry plants generally grow in regions with tropical and subtropical climates such as India, China, Indonesia, Semenanjuang Malaysia, Thailand (Charoenteeraboon et al., 2010) Pakistan, Uzbekistan and Sri Lanka (Khan, 2009).

Indian gooseberry plant (Phyllanthus emblica) usually used as traditional medicine. Moreover, Indian gooseberry leaves can be combined with other ingredients such as honey and milk to treat diarrhea by mixing one tablespoon of Indian gooseberry leaves paste and honey or
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milk (Dasaroju and Gottumukkala, 2014). Based on the study, Indian gooseberry leaves has an important component that presence of antineutrophil and antiplatelet activity in vitro. The leaves extract has also pharmacological compounds such as anti-virus (HIV, AIDS, Herpes Virus, CMV), antimutagenic, anti-allergic and anti-bacterial (Sukanya *et al*., 2013).

Narasimhudu and Raju (2012) stated that Indian gooseberry leaves which were extracted by methanol contain alkaloids, coumarin, dihydrochalcones, flavonoids, flavones, gallon tannins, glycosides, phenols and triterpenoids. Besides that, the Indian gooseberry leaves were extracted by methanol and ethyl acetate contain chemical components such as alkaloids, carbohydrates, flavonoids, saponins, tannins and terpenoids (Sukanya *et al*., 2013).

The processing of Indian gooseberry leaves into herbal tea was expected to be able to maintain the nutritional content, the shelf life of this leaves and to facilitate their application. Herbal tea is a beverage product that produced from tea and herbal plants and provides benefits for health, such as a disease treatment and a body refreshing drink (Hambali *et al*., 2005). Furthermore, herbal tea has different functions and benefits for health. The benefits are obtained because of the content of phenolic and flavonoids which have function as antioxidants in counteracting free radicals (Komes *et al*., 2010).

There are several factors that influence the tea quality, including the duration of fermentation time and the drying method. Fermentation process is a process of oxidation polyphenol compounds by polyphenol oxidase enzymes and produce theaflavin and thearubigin substances. This substance will determine the nature of strength, color, quality, and briskness of the tea water (Setyamidjaja, 2000).

Another factor influenced the tea quality is the drying method to reduce the water content of tea leaves up to 3-4% (Ajisaka, 2012). The lowest temperature causes the drying
process runs slowly, so the material is easily moldy. Meanwhile, high temperature impacts on the outer part of the leaves which dry faster than inside. Therefore, the drying temperature should be carried out at 60°C (Agromedia, 2008). Therefore, this research would examine the effect of fermentation time and drying method of Indian gooseberry leaves on its chemical properties, and would evaluate the antioxidant potency of the tea.

**MATERIALS AND METHODS**

Plant materials of Indian gooseberry were obtained fresh from Blang Bintang, Aceh Besar, Indonesia. Then the chemicals used for analysis were 1,1-diphenyl-2-picrylhydrazly free radical (DPPH), galat acid, metanol, natrium carbonate, Follin-Ciocalteau phenol reagent, aquades, follin buffer, NaCl, gelatin and FeCl₃.

**Preparation of Raw Materials**

Indian gooseberry leaves are picked and separated from foreign objects and stalks. Then washed and drained. The leaves withering on dry conditions until 18 hours at of 27-28°C. Furthermore, the leaves and bone was reduced by measuring the way with a knife. After that Indian gooseberry leaves are begin to fermentation process with a determined time.

**Fermentation Process and Dry Method (Modification Rahmawati and Suhartatik, 2015)**

The fermentation process of leaves Indian gooseberry was carried out with different time. The treatments fermentation was 0 minutes, 60 minutes, 90 minutes and 120 minutes. After the fermentation process done in accordance with predetermined time, the leaves would be dried use indirect sun method and roaster method. Then reduced the size of sample up to 16 mesh.

**Water Content Analysis (AOAC, 2007)**

Petri dishes were dried in an oven at 105°C for one hour. The petri was placed in desiccatar then weighed. About 5 g sample put in the petri, then dried in oven at 105°C for 5 hours or until the weight was constant. Inserted the petri in to desiccator and weighed again. The percentage of water content can be calculated as follows:

\[
Water \, content \, (\%) = \frac{A - B}{A} \times 100\%
\]

Description: \( A \) = the weight of sample before drying  
\( B \) = sample weight after drying
Ash Content Analysis (AOAC, 2007)

The dried plate was drying in oven for 1 hour at temperature 105°C then put in to desiccator for 15 minutes and weighed. About 2 gram sample was inserted in to the ignition cup and sprinkled over the flame until it was no longer smoky. The sampel of put in to a furnace with a temperature of 600°C for 5 hours, then weighed until a constant weight was obtained ash content could be calculated by the following formula:

\[
\text{Ash content (\%) = } \frac{\text{weight of sample and cup after furnace} - \text{weight of empty cup}}{\text{initial sample weight}} \times 100\%
\]

FTIR Analysis

Kimalaka tea powder (1 gram) was brewed in 100 ml of water and was allowed to stand for 6 minutes. It was then filtered and read the wavelength spectrum using the fourier infrared spectrum.

Indian Gooseberry Tea Brew Processing (SNI, 1995)

The sample was weighed as much as 1 g, put in a beaker glass measuring 50 ml. Simmer pure water until it boils, then pour it into a cup that contains the experimental sample, closed, leave for 6 minutes and strain the tea solution from the pulp.

Antioxidant Activity Determination Using DPPH Free Radical Scavenging Method

The DPPH free radical scavenging activity of each sample was measured used Spectrophotometer (UV-Vis 1800, Shimadzu) according to the method of Sompong et al., 2011. Briefly, 2 ml of DPPH (Merk) solution (4.73 mg DPPH in 100 ml of methanol) was dissolved in a reaction tube containing 1 ml of brewed tea. The mixture was further shaken and incubated for 30 minutes in dark space and room temperature. The absorbance of the solution was measured at 517 nm. The degree of decoloration of the solution indicated the scavenging efficiency of the added substance. The free radical scavenging activity was calculated as a percentage of DPPH decoloration used the following equation:

\[
\text{DPPH (\%) = } \frac{(\text{absorbance515 nm control} - \text{absorbance515 nm sample})}{\text{absorbance515 nm control}} \times 100\%
\]

Total Polyphenol Contents Determination Folin-Ciocalteau Method (Indarwati, 2015)

About 9 ml the Folin-Ciocalteau (Merk) reagent was diluted with water 90 ml and homogenized. Added 6 gr Na₂CO₃ mixed with 100 ml of aquades and homogenized. The preparation a solution of galic acid was carried out by dissolving 0.010 g of galic in 100 ml of
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aquades and homogenized. Standard curve creation was made by varying the gallic to six samples. The first flask was filled with 10 ml aquades, the second, mixed 2 ml gallic + 8 ml aquades, third, mixed 4 ml gallic + 6 ml aquades, fourth, 6 ml gallic + 4 ml aquades, fifth with 8 ml gallic + 2 ml aquades and sixth with 10 ml gallic, then vortex. Each gallic sample was tested as a treatment on the material sample. The test of the material sample was carried out by taked 0.2 ml of tea brewed solution then diluted in 5.8 ml of aquades, then about 0.2 ml of the solution and mixed with 1.8 ml of follin solution and 1.8 ml of Na$_2$CO$_3$ and vortex. Entered in a cuvette and read its absorbance at 760 nm wavelength. The reading results were compared with the standard curve used gallic acid (Brand).

**Qualitative Tanin Analysis (Mukhriani et al., 2014)**

About 1 ml brewed Indian gooseberry tea was mixed distilled water then was heated. After that gelatin was added. The formation of white precipitates indicates tannins. Other analysis with addition FeCl$_3$ solution in 1 ml of water from Indian gooseberry tea, the formation of bluish black color indicates the presence of tannin compounds.

**RESULTS AND DISCUSSION**

**Water Content**

Water content affects the quality of dry tea and shelf life (Herawati and Nurawan, 2006). Fresh Indian gooseberry leaves contain 49.6% of water content. After dried process the water content declined about 2.47-9.87%. According to Indonesia National Standar (SNI 01-3836-2003), the water content of tea products was maximum 8%. Thus the water content of the tea powder roasted method has qualified the SNI, meanwhile indirect method neither.

The result of analysis of variant (ANOVA) showed that dried method significantly influence (P<0.01) on the water content of the tea powder. Based on Duncan Multiple Range Test (DMRT), the water content can be seen in Figure 2. Figure 2 show that indirect sun dried have the higher water content value than roast method dried. In this case, the roasting method (60°C) has higher temperature than indirect sun drying (33°C), thus more water is evaporated from the tea leaves. Winangsih *et al.*, (2013) showed water content of dried oven treatment (50°C) was lower than direct sun and dried wind. According Sinurat (2014) dried proses was influence by several factors such as, dried temperature, initial water content and have relations with dry conditions.
Figure 2. Effect of drying method on the water content of Indian gooseberry tea powder (the value followed by the same letter indicates an insignificant difference).

**Ash Content**

Ash content is a parameter to indicate the value of the inorganic substances (minerals) in a material or product. The inorganic components in product are very varies in type and amount (Roni, 2008). The result of ANOVA shows that interaction of drying method and fermentation time is significantly influence (P<0.05) the as content of the tea powder (Figure 3).

Figure 3 stated the ash content of tea powder was range from 3.28-4.42%. According to Indonesia National Standar (SNI) (2013), the ash content of tea products was maximum 8%. Thus, the ash content of the tea powder has in this research has made the SNI. Analysis of Duncan Multiple Range Test (DMRT) showed ash content of the tea powder from roasting method without fermentation obtained the higher value (4.42%) which was significantly
different with others treatment. Ash content in ingredient was affected by water content. The higher water content in ingredients, would make the dry matter content such as fat and protein would increases the percentage of ash content.

**FTIR Analysis**

FTIR analysis was to identify functional groups of chemical compounds in brewed indian gooseberry tea which indirect sun without fermentation treatment. The wavelength range used in this analysis was from 800-4000 cm\(^{-1}\). The infrared absorption spectrum of the that can be seen in Figure 4. The result of FTIR analysis showed there are several absorption in brewed tea of indian gooseberry which different wavelength frequencies. Estimation of wavelength can be seen of Table 1.

![Infrared specturm in Indian gooseberry brewed tea.](image)

Sari (2018) identified at wavelengths of 1576 cm\(^{-1}\) and 1633 cm\(^{-1}\) in Aceh cucumber extract as absorption of flavonoids. Sukandana (2010) states that a common feature of flavonoid compounds is the presence of carbonyl groups (C=O). Suspected that the vibrations at the wavelength of 1636 cm\(^{-1}\) contained in the FTIR spectrum of indian gooseberry brew tea as a vibration of flavonoid compounds. Allegedly the spectrum in brew indian gooseberry was stretching the C-H functional group which is a functional group of tannin compounds. Tanin is
one of the polyphenol compounds which has a large molecular weight consisting of hydroxyl groups and carbonyl groups (Hovart, 1981). The results of FTIR analysis based on detected wavelengths indicate that there are functional groups O-H, C-OH, C=O and C-H which identify Indian gooseberry brew tea contains flavonoid compounds which are organic compounds that have antioxidant properties to counteract free radicals.

<table>
<thead>
<tr>
<th>No</th>
<th>Brew Tea Wavelength cm(^{-1})</th>
<th>Potential of functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1219 1000-1230</td>
<td>O-H (alcohol dan phenol)</td>
</tr>
<tr>
<td>2</td>
<td>1356 1308-1370</td>
<td>C-OH (phenol)</td>
</tr>
<tr>
<td>3</td>
<td>1635 1540-1870</td>
<td>C=O (flavonoid)</td>
</tr>
<tr>
<td>4</td>
<td>3282 3000-3500</td>
<td>C-H (tannin)</td>
</tr>
<tr>
<td>5</td>
<td>3327 3000-3500</td>
<td>O-H (alkuna)</td>
</tr>
</tbody>
</table>


**Total Polyphenol**

Phenolic compounds one of the secondary metabolites and included the largest group in plants. These metabolite compounds were included as aromatic alcohol because their hydroxyl groups attached to the benzene ring. In general, phenolic compounds have the potential as bactericidal, antiseptic, antioxidant (Penggelly, 2006). The result of analysis of variant (ANOVA) stated that dried method significantly influence (P<0.01) to total phenol content. The result of Duncan Multiple Range Test (DMRT) showed in Figure 5.

![Figure 5. Effect of drying method on the total phenol of Indian gooseberry in brew tea (the value followed by the same letter indicates an unsignificant difference).](image-url)
The highest total phenol average value obtained from the treatment roaster dried method as 71.08 mg gallic acid/50 mL which was significantly different from indirect sun method as 50.31 mg gallic acid/50 mL. The results of antioxidant activity analysis and total phenol showed non-linear results. Tea samples that have the highest antioxidant activity have a low total phenol. According to Nasution and Tjiptadi (1975), tea contains other components besides polyphenols such as organic matter, carbohydrates, pigments, enzymes that affects the measurement of antioxidant capacity. Thus, the antioxidant capacity of a product is not always linear with the total phenol produced, and vitamins. This vitamin component can act as an antioxidant.

**Antioxidant Activity Using DPPH Free Radical Scavenging Method**

Antioxidants were substances needed to neutralize free radicals and prevent damage by completing the lack of radicals possessed by electrons (Yuliarti, 2008). Antioxidants contained in the brew of Indian gooseberry tea ranges from 49.07 to 83.17%. The result of analysis of variant (ANOVA) showed that dried method significantly influence (P<0.05) to antioxidant activity. Figure 6 showed that the highest antioxidant value was obtained from indirect sun treatment which was significantly different from the value of antioxidant activity with roasted drying. This is thought to be due to roasting dried used higher temperatures so that antioxidant compounds that sensitive to heat were reduced.

Duncan Multiple Range Test (DMRT) showed antioxidant activity compound in brew tea indirect sun method (79.05%) was significantly different from roasted method (68.99%). In
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In this case, because different temperature both dried method. Widyanto and Nelisyta (2008) stated that an inappropriate dried process can reduce the antioxidant content. Antioxidants contained in brew Indian gooseberry leaves tea with all indirect sun treatments were higher than other herbal teas. Comparison of antioxidant Indian gooseberry tea with other teas could be seen in Table 2.

### Table 2. Activities of antioxidants of different types of tea leaves.

<table>
<thead>
<tr>
<th>Herbal Tea</th>
<th>Antioxidants Activities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacar Air Leaf (<em>Impatiens balsamina</em> L.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.43</td>
</tr>
<tr>
<td>Soursop Leaf&lt;sup&gt;b&lt;/sup&gt; (<em>Annona muricata</em> L.)</td>
<td>76.06</td>
</tr>
<tr>
<td>Lotus Flower&lt;sup&gt;c&lt;/sup&gt; (<em>Nelumbo nucifera</em>)</td>
<td>32.19</td>
</tr>
</tbody>
</table>


**Tanin Content**

The results of the qualitative tannin analysis showed that in the brew of Indian gooseberry leaves tea was positively containing tannin compounds. This was seen in brew tea which had a bluish black discoloration after added FeCl₃ solution, and also had a slightly cloudy color change after adding gelatin. So it was concluded that the brew of Indian gooseberry tea contained tannin compounds.

**CONCLUSIONS**

The water content of Indian gooseberry tea powder which was dried by indirect sun had a higher value (9.125%) than roasted method (3.417%). The ash content of tea powder from all treatment was qualified to SNI (3.28-4.42%). FTIR analysis indicates that there are functional groups O-H, C=OH, C=O and C-H which identify Indian gooseberry brew tea contains flavonoid compounds. Indirect sun-dried treatment showed a higher antioxidant content compared to the roasted dried. Where as the highest phenol amount was obtained in the treatment of roasted methods than indirect sun dried. The results of the qualitative analysis of tannins showed that the brew tea contained tannins.

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