The effect of brewing time herbal tea from mother-in-law’s tongue leaves (*Sansevieria trifasciata* Prain) on total phenol, total flavonoid, and antioxidant activity

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**Abstract.** Indonesia is a tropical country known for its biodiversity, one of which is the mother-in-law’s tongue (*Sansevieria trifasciata* Prain). Mother-in-law’s tongue leaves extract contains bioactive compounds, such as triterpenoids, steroids, and flavonoids and traditionally used especially as antidiabetics. The phenolic and flavonoid compounds in mother-in-law’s tongue are potential antioxidants, and usually processed into herbal tea. This research aimed to determine the effect of duration brewing time herbal tea from mother-in-law’s tongue leaves (*Sansevieria trifasciata* Prain) on total phenol, total flavonoid, and antioxidant activity. This study using Completely Randomized Designed (CRD) with 5 treatments (different brewing time that were 1, 3, 6, 9, 12 minutes) and 3 replications. The result showed that brewing time had a significant effect on total phenol, total flavonoid, and antioxidant activity. The optimum of brewing time herbal tea from mother-in-law’s tongue leaves is 6 minutes.

**Keyword:** mother-in-law’s tongue leaves, brewing time, total phenol, total flavonoid, antioxidant activity

**INTRODUCTION**

Indonesia is a tropical country known for its biodiversity, one of which is the mother-in-law’s tongue (*Sansevieria*). Sansevieria is known as the Snake Plant, but Indonesians call it the Lidah Mertua [1]. Sansevieria has the potential to be a pollution-absorbing plant (anti-pollutant), fiber producer, cosmetic ingredients, and medicinal herb (materia medica). As a medicinal herb, people process sansevieria by boiling it to cure diabetes, toothache, antidote, ambient, and prevent influenza [2].

Mother-in-law’s tongue leaves extract contains bioactive compounds, such as triterpenoids, steroids, and flavonoids [3]. Total flavonoid mother-in-law’s tongue leaves ethanol extract was 13.934 mgQE/g [4]. Flavonoids are one of the phenolic compounds that have many health benefits, namely as antioxidants, anti-inflammatory, and antidiabetics[5]. Flavonoids as antioxidants can ward off free radicals so as to prevent cell damage to the body's immune system. The antioxidant activity (IC\(_{50}\)) of mother-in-law’s tongue leaves ethanol extract was 9.44 ppm [6]. Ethanol extract of mother-in-law’s tongue leaves at a dose of 0.083 g/kg body weight (bw) had the effect of reducing blood sugar levels [7]. Based on this description, mother-in-law’s leaves have the potential to be a product that can be beneficial for health. Tea products can be used in the development of innovations.

Tea is a refreshing drink with a characteristic aroma and taste, containing tannins and polyphenols, made by brewing leaves, leaf tops or petioles dried from the *Camellia sinensis* plant. During this time tea was known to come from tea leaves (*Camellia sinensis*). However, along with the increasing demand of society and the development of science, a lot of teas were developed made from other than tea leaves, which are referred to as tisane. Tisane or herbal tea is a common terms for tea made from herbal ingredients instead of tea plants [8]. Herbal teas are made from flowers, seeds, leaves, or roots of various plants. Several studies on herbal tea from leaves and flowers have been conducted, including kenanga flowers, soursop leaves, telang flowers, lotu flowers, avocado leaves tabah bamboo, and others [9, 10, 11, 12, 13, 14, 15].

In general, tea is consumed by brewing it using hot water. Research shows that the use of hot water with a long brewing time can increase the polyphenol content of green tea and black tea [16, 17] as well as increasing the antioxidant activity of white tea [18]. However, research on the effect of brewing time on the content of phenol compounds, flavonoids, and antioxidant activities of mother-in-law’s tongue leaves herbal tea has never been conducted. Based on this, this study
aims to study the effect of brewing time on the content of phenol compounds, flavonoids, and antioxidant activity of mother-in-law’s tongue leaves herbal tea.

METHODOLOGY

Materials and Instruments

The raw material used in this study was mother-in-law’s tongue leaves (S. trifasciata Prain.) obtained from Medan City. The chemicals used were quercetin (Sigma Aldrich), DPPH (Sigma Aldrich), reagent Folin-Ciocalteu 10% (Merck), gallic acid (Merck), Na₂CO₃ 7.5% (Merck), CH₃COONa 1M (Merck), AlCl₃ 10% (Merck), ethanol p.a and 70% (Merck), ethanol p.a and 96% (Merck) and aquaades. The tools used in this study were analytical balances (Sartorius), UV-Vis spectrophotometers (Orion Aquamate 8000), cabinet dryers (Modification), micro pipettes (Eppendrorf), blenders (Philips HR-2056), glass tools, baskets, knife, 40 mesh sieves, tea strainers, clip plastics (Polypropylene), glass cups, aluminum foil (Klin Pak), glass drip pipettes, test tubes, glass thermometers, and vortex.

Research Design

In this study, it used a Complete Randomized Design (CRD) with 5 treatments and 3 replications. The data obtained were statistically analyzed with the F test and if they differed significantly continued with Duncan's New Multiple Range Test (DNMRT) test at a real level of 5%. The study treatment was brewing time of mother-in-law’s tongue leaves herbal tea, with the following details:

A = brewing time 1 minute
B = brewing time 3 minutes
C = brewing time 6 minutes
D = brewing time 9 minutes
E = brewing time 12 minutes

The Production of Mother-in-law’s Tongue Leaves Herbal Tea

The production of mother-in-law’s tongue leaves herbal tea was modified from the research of Lagawa [19]. Leaves were disorted, cleaned and cut to a size of 1-2 cm. Leaves were stored for 24 hours at room temperature. The leaves were then dried in the oven at 60 °C for 26 hours and a reversal was carried out once every 12 hours. The dried leaves were ground in a blender to prepare a simplicia powder with a size of 40 mesh.

Brewing of Mother-in-law’s Tongue Leaves Herbal Tea

The mother-in-law’s tongue leaves herbal tea was weighed 2.5 g and brewed with boiling water by 200 ml [20]. The length of tea brewing time corresponds to the predetermined time (1 minute, 3 minutes, 6 minutes, 9 minutes, and 12 minutes). The mother-in-law’s tongue leaves herbal tea was filtered using a tea cloth strainer and stopped until the last drop of tea steeping water.

Total Phenol Analysis

Total phenol analysis using Folin Ciocalteu reagents and gallic acid as standard. The water steeping mother-in-law’s tongue leaves herbal tea with concentration 500 µg/ml was transferred 0.5 ml into the test tube, added 1.25 ml of 10% Folin Ciocalteu reagent and in the vortex for 1 minute. The solution was allowed to stand for 5 minutes. After that, 1 ml of sodium carbonate (Na₂CO₃) of 7.5% was added. The mixture was incubated for 30 minutes. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 746 nm. The standards concentrations of gallic acid used were 5, 10, 15, and 20 g/ml [21].

\[
\text{Total Phenol} = \frac{\text{concentration} \times \text{sample vol} \times \text{DF}}{\text{sample weight (g)}}
\]

DF= dilution factor

Total Flavonoid Analysis

Total flavonoid analysis using the colorimetric method using AlCl₃ and quercetin as standard. The water steeping mother-in-law’s tongue leaves herbal tea was transferred 2 ml into a test tube, added 0.1 ml of 10% AlCl₃, 0.1 ml of sodium acetate (CH₃COONa) 1M and 2.8 ml of aqueous, incubated for 40 minutes. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 426 nm. The standard concentrations of quercetin used were 10, 20, 30, and 40 µg/ml [21].

\[
\text{Total Flavonoid} = \frac{\text{concentration} \times \text{sample vol} \times \text{DF}}{\text{sample weight (g)}}
\]

DF: dilution factor

Determination of Antioxidant Activity (IC₅₀)

Determination of antioxidant activity using the DPPH method [21]. The water steeping mother-in-law’s tongue leaves herbal tea was diluted to 1000 ppm. Variations in solution concentrations of 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm were made by transferring 62.5 µl, 125 µl, 250 µl, 500 µl from the mother liquor, each added with DPPH solution of 50 ppm as 1 ml and the volume was adjusted to 5 ml with ethanol p.a and container covered with aluminium foil. Then it was homogenized with vortex and incubated for 30 minutes at 37 °C. It absorption was measured at a wavelength of 516 nm.

Control/blank solution was made by transferring 1 ml of 50 ppm DPPH solution and adjusted its volume to 5 ml with ethanol p.a and container covered with aluminium foil. Then it was homogenized with vortex and incubated for 30 minutes at 37 °C. It absorption was measured at a wavelength of 516 nm.
The effect of brewing time herbal tea (tisane) from mother-in-law’s tongue leaves (Sansevieria trifasciata Prain) …

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%Inhibition = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{sample}}} \times 100

A: Absorbance

**Determination of IC\textsubscript{50} Value**

Four concentration series solutions for each sample (12.5 ppm, 25 ppm, 50 ppm, and 100 ppm) whose absorbance was measured were then plotted for concentration values (X axis) and percent inhibition (Y axis) to obtain a calibration curve [21]. The IC\textsubscript{50} calculation was obtained from the linear regression equation \( y = ax + b \)

where, \( y \): % inhibition
\( a \): slope/gradient
\( x \): concentration IC\textsubscript{50}
\( b \): intercept

IC\textsubscript{50} concentration was calculated by entering the price \( y = 50 \), so that the value of \( x \) can be calculated as IC\textsubscript{50}.

**RESULTS AND DISCUSSION**

**Total Phenol**

The results of the analysis of variance showed that the brewing time had a significantly different effect at the 5% level on the total phenol of the mother-in-law’s tongue leaves herbal tea steeping water. The effect of brewing time on the total phenol of the mother-in-law’s tongue leaves herbal tea can be seen in Figure 1.

The total phenol water steeping of mother-in-law’s tongue leaves herbal tea produced ranges from 5.29-14.09 mg GAE/g. The highest total phenol content in the water steeping of mother-in-law’s tongue leaves herbal tea was obtained in treatment C (6 minutes of brewing time) which was 14.09 mg GAE/g, while the lowest total phenol was obtained in treatment A (1 minute of brewing time) 5.29 mg GAE/g.

![Figure 1](image1.png)

**Figure 1.** The effect of brewing time on total phenol of mother-in-law’s tongue leaves herbal tea.

![Figure 2](image2.png)

**Figure 2.** The effect of brewing time on total flavonoid of mother-in-law’s tongue leaves herbal tea.

![Table](table1.png)

The same letter shows no significant difference at the 5% level

A: brewing time 1 minute
B: brewing time 3 minutes
C: brewing time 6 minutes
D: brewing time 9 minutes
E: brewing time 12 minutes
Water is used in the preparation of mother-in-law’s tongue leaves herbal tea. As water is a polar solvent, therefore, during the brewing process, secondary metabolites that have the same polarity as water will be dissolved [20]. Based on previous research, the mother-in-law’s tongue leaves contain polar secondary metabolites, such as phenolics, flavonoids, alkaloids, steroids, and saponins [6, 21].

Total phenols have increased to 6 minutes of brewing. This is consistent with previous research, which discovered that the phenolic content increased by 0.5-8 minutes of brewing [16, 17, 22]. A decrease in total phenol after 6 minutes of brewing may occur due to the use of a high brewing temperature, which is 100°C. Prolonged tea brewing at high temperatures can cause total phenols to degrade [23, 24].

**Total Flavonoid**

The results of the analysis of variance showed that the brewing time had a significantly different effect at the 5% level on the total flavonoid of the mother-in-law’s tongue leaves herbal tea steeping water. The effect of brewing time on the total flavonoid of the mother-in-law’s tongue leaves herbal tea can be seen in Figure 2. The total flavonoid water of the mother-in-law’s tongue leaves herbal tea produced ranges from 1.63-2.22 mg GAE/g. The highest total flavonoid content in the water steeping of mother-in-law’s tongue leaves herbal tea was obtained in treatment C (6 minutes of brewing time) which was 2.22 mg GAE/g, while the lowest total flavonoids were obtained in treatment A (1 minute of duration time) 1.63 mg GAE/g. Increase in total flavonoids occurred at the duration of brewing minutes 1, 3, and 6, while the decrease in total flavonoids occurred at minutes 9 and 12. The decrease in total flavonoids is in line with the decrease in total phenols.

The extraction time will give the optimal time for each material and solvent to make contact, but extraction with too long a time can also make the solvent become quickly saturated and unable to extract optimally so that it will lower flavonoid levels [25]. The optimum time obtained will vary for each material used. An optimum black tea brewing time of 6 minutes [26], while an optimum time of green tea brewing was 15 minutes [27].

According to the literature, the total flavonoids of the ethanol extract of the mother-in-law’s tongue leaves were 13,934 mg QE/g [4], whereas this study produced a total flavonoid of 2.22 mg QE/g. This demonstrates that the total flavonoids produced are lower than previously reported. This difference in total flavonoids can be influenced by differences in the solvents used. The 96% ethanol solvent is able to extract more flavonoid compounds from the mother-in-law’s tongue leaves compared to water solvents. This is supported by the research of Maulana et al., which showed that a 96% ethanol extract of Surian leaves produces higher total flavonoids than a water extract of Surian leaves [28].

**Antioxidant Activity (IC₅₀)**

The results of the analysis of variance showed that the brewing time had a significantly different effect at the 5% level on the antioxidant activity (IC₅₀) of the mother-in-law’s tongue leaves herbal tea steeping water. Calibration curve of the % inhibition DPPH in the steeping water of mother-in-law’s tongue leaves herbal tea can be seen in the Figure 3. The effect of brewing time on the antioxidant activity (IC₅₀) of the mother-in-law’s tongue leaves herbal tea can be seen in Table 1.

Based on Figure 3, the higher the concentration of the solution, the higher the % inhibition value of DPPH. This shows that there is antioxidant activity, where the steeping water of mother-in-law’s tongue leaves herbal tea provides 1 hydrogen atom to the DPPH radical, which converts it into DPPH-H [29].

**Table 1. The effect of brewing time on antioxidant activity (IC₅₀) of mother-in-law’s tongue leaves herbal tea**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Regression Equations</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (brewing time 1 minute)</td>
<td>Y = 0.0379x + 2.2987</td>
<td>1263.80 a</td>
</tr>
<tr>
<td>B (brewing time 3 minutes)</td>
<td>Y = 0.0546x + 1.620</td>
<td>888.40 bc</td>
</tr>
<tr>
<td>C (brewing time 6 minutes)</td>
<td>Y = 0.1x + 0.627</td>
<td>503.40 d</td>
</tr>
<tr>
<td>D (brewing time 9 minutes)</td>
<td>Y = 0.0651x + 2.2343</td>
<td>743.00 c</td>
</tr>
<tr>
<td>E (brewing time 12 minutes)</td>
<td>Y = 0.0501x + 2.385</td>
<td>955.60 b</td>
</tr>
</tbody>
</table>

Note: The same letter shows no significant difference at the 5% level.
The IC50 values ranging from 503.40 – 1263.80 μg/ml. The highest antioxidant activity was obtained by the C treatment (6 minutes of brewing time) which was 503.40 μg/ml (the smallest IC50 value), while the lowest antioxidant activity was obtained by treatment A (1 minute brewing time), which was 1263.80 μg/ml (the largest IC50 value). The antioxidant activity of the mother-in-law’s tongue leaves herbal tea increases as the brewing gets longer, but there is a decrease in antioxidant activity after reaching the optimum condition, which is for 6 minutes. Brewing time is one of the driving factors for hydrolyzing the bonds of the compounds contained in the material. However, prolonged brewing can also reduce its content in steeping water, because antioxidants are also susceptible and easily degraded [30].

The smallest IC50 value obtained was 503.40 μg/ml. IC50 value of <50 ppm has very strong antioxidant properties [31]. This shows that the mother-in-law’s tongue leaves herbal tea has weak antioxidant properties. One of the factors affecting the antioxidant activity is the content of phytochemicals contained in the ingredients. Phenolic compounds that are known to play a very important role in antioxidant activity, the greater the content of phenol group compounds, the greater the antioxidant activity [32]. This is in accordance with the research conducted, namely the highest total content of phenols and flavonoids in the mother-in-law’s tongue leaves herbal tea is obtained at a time of 6 minutes of brewing, as well as its antioxidant activity. According to the literature, the antioxidant activity (IC50) of mother-in-law’s tongue leaves was 9.44 ppm [6], while in this study, 503.4 ppm was obtained. This is due to the difference in the drying process of the mother-in-law’s tongue leaves. Sarjani et al. used sunlight to dry the mother-in-law’s tongue leaves, while this study used an oven at a temperature of 60 °C for 26 hours, which allowed contact between the sample and heat to last a long time, causing damage to active compounds that act as antioxidants. This is supported by Rusnayanti's research, where the high temperature and length of drying time of cocoa leaf green tea can reduce the total phenol content [33].

CONCLUSION

Based on the research that has been carried out, it can be concluded that the time of brewing mother-in-law’s tongue leaves herbal tea causes a significant effect on the parameters of total phenols, total flavonoids, and antioxidant activity. The optimum time of brewing mother-in-law’s tongue leaves herbal tea is 6 minutes. The results obtained that herbal tea contains total phenols 14.085 mgGAEE/g, 2.22 mgQE/g total flavonoids, and IC50 of antioxidant activity is 503.4 ppm.

REFERENCES


