

# Brewing Time Affects the Total Phenol Content and Antioxidant Activity of Combined Herbal Teas

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**Abstract.** Various natural products have been studied to have positive benefits in increasing endurance/immunity, including meniran leaves, moringa leaves, pegagan leaves and green tea leaves. We combine various natural ingredients with these properties in the form of herbal tea formulations. Next, we conduct an analysis process to measure the total phenol content and antioxidant activity of this herbal tea formula with variations in brewing duration. The purpose of this study was to prove the effect of brewing duration on the total phenol content and antioxidant activity of herbal tea formulas. The study was conducted in the chemistry laboratory of the State Polytechnic of Malang. The results of this study indicate that the brewing duration has a positive effect on the total phenol content and antioxidant activity of herbal tea. Furthermore, research needs to be conducted to determine the maximum duration that is still effective for the herbal tea brewing process as well as the effect of the brewing water temperature. The results of this study will be important information for the public to get optimal benefits from herbal tea with the correct processing and serving techniques.

**Keywords:** herbal tea, brewing duration, total phenol, antioxidant activity

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## Introduction

The Covid-19 pandemic, which became a worldwide pandemic a few years ago, has taught us all a very valuable lesson. During the pandemic, changes in awareness and healthy living behavior have shown positive developments. We should be able to maintain these positive changes as a healthy lifestyle in our daily lives. At the individual level, what is also very important in this situation is efforts to increase endurance/immunity (Nilashi et al., 2020). Various natural products have been studied to have positive benefits in increasing endurance/immunity, including meniran leaves (Atmadja & Yunianto, 2019), moringa leaves (Hasriani et al., 2020), pegagan leaves (Khusnawati et al., 2016) and green tea leaves (Yusni et al., 2015).

Meniran (*Phyllanthus niruri*) is a wild plant originating from Tropical Asia which is spread throughout Asia, including Indonesia. Empirically, meniran herb functions as an antioxidant, antibacterial, antihepatotoxic,

antipyretic, antitussive, anti-inflammatory, antiviral, diuretic, expectorant, hypoglycemic, and as an immunostimulant (Atmadja & Yunianto, 2019). Moringa leaves contain various micronutrients including: thiamine, riboflavin, niacin, beta carotene, calcium, iron, phosphorus, magnesium, zinc, vitamin C, which can be an alternative to replace multiple micronutrient supplements in improving the nutritional status of pregnant women. This plant is rich in protein, amino acids, minerals, vitamins, antioxidants and anti-inflammatories (Hasriani et al., 2020). *Centella asiatica* (L) Urban or known as pegagan is also a type of plant that has many benefits, including diuretics, mouth ulcer medicine, fever reducers, appetite enhancers and others. The benefits of pegagan as an immunomodulator are reported to be related to its ability to increase lymphocyte proliferation (Khusnawati et al., 2016)

In line with the circular letter of the Minister of Health number HK.02.02/IV.2243/2020 concerning the Utilization of Traditional Medicine for Health Maintenance, Disease Prevention and Health Care, we formulated herbal-based tea preparations to increase body resistance/immunity. This herbal formulation for immunity was made by researchers in the form of tea preparations with the consideration that drinking tea is a local habit/wisdom that is very common in everyday life for almost everyone, in terms of presentation it is very easy and practical because it only needs water to brew, and is also easy to distribute because it does not require special storage techniques. With this consideration, it is hoped that the presence of herbal tea preparations for immunity will be more easily accepted than other forms of preparations such as pills or capsules that give the impression of taking medicine. This herbal-based tea will be an alternative solution for the community and has the potential to be accepted by the wider community.

Flavonoids are phenolic components found in various natural materials that act as good reservoirs for hydroxyl and superoxide radicals by protecting membrane lipids from damaging oxidation reactions. The efficacy of herbal tea for health is largely determined by the content of active ingredients that can be dissolved. Based on literature, there are several factors that affect the solubility of active ingredients, including temperature and brewing time. In this study, we conducted an experiment to measure the total phenol content and antioxidant activity of herbal tea combinations with variations in brewing duration to obtain information on the total phenol content and optimal brewing time of this herbal tea preparation, we conducted a test on the total phenol content of herbal tea with variations in brewing time.

## Methods

In this study we measured the total phenol content of herbal tea formulas with variations in brewing time (10, 20 and 30 minutes), the analysis was carried out in the chemistry laboratory of the State Polytechnic of Malang.

### Herbal tea formula

1. Each tea powder is weighed  $\pm 1$  gram
2. Then brewed with water at a temperature of  $T = \pm 85-90$  °C,
3. The brewing time variable is carried out for 10, 20, 30 minutes each
4. Then filtered
5. The remaining solids and liquids are separated using a centrifuge

### TPC (Total Phenolic Content) testing

- a. A number of samples are taken/pipetted
- b. 1.25 ml of distilled water is added
- c. 1.25 ml of Folin-Ciocalteu is added, left for 5 minutes

d. Added 1.0 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution, then incubated at a temperature of T = 45 °C, and time t = 15 minutes

#### Antioxidant testing using IC-50 parameters

- a. A number of samples were taken/pipetted
- b. Added 4 ml of methanol
- c. Added 1 ml of DPPH solution, then incubated in a dark room at room temperature for t = 30 minutes

8. Furthermore, points (6) and (7) carried out absorbance measurements at the maximum wavelength for TPC 765 nm and Antioxidant IC-50 517 nm.

### Result and Discussion

The following is a presentation of the research data on herbal tea that we have conducted:

#### 1. Analysis of total phenol content

Flavonoids are phenolic components found in various natural materials that act as good reservoirs for hydroxyl and superoxide radicals by protecting membrane lipids from damaging oxidation reactions. In this test we measured the total phenol content of the herbal tea with variations in brewing time (10, 20 and 30 minutes), the analysis was carried out in the chemistry laboratory of the State Polytechnic of Malang.

The results of the analysis of the total phenol content of the samples are as follows:

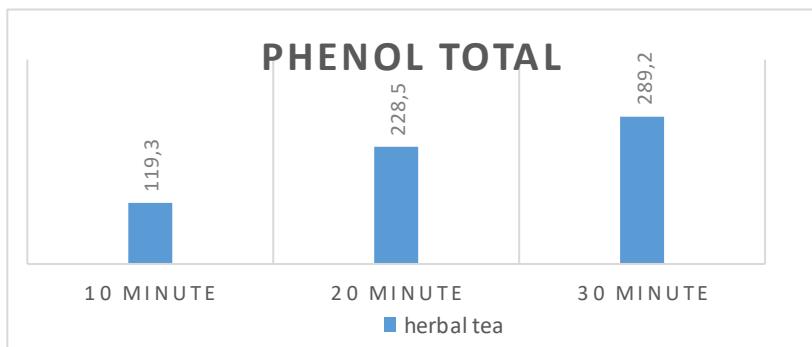


Figure 1. Total phenol analysis profile of herbal tea with various brewing durations (10, 20, and 30 minutes) in µg/g units.

In Figure 1 above, it can be seen that the optimal brewing temperature is 30 minutes, as evidenced by the total phenol content measured with 10-minute brewing of 119.3 µg/g, then with 20-minute brewing the total phenol content was 228.5 µg/g, and with the longest brewing time, namely 30 minutes, the total phenol content was 289.2 µg/g.

#### 2. Analysis of antioxidant activity

Free radicals are a form of reactive compounds, which are generally known as compounds that have unpaired electrons in their outer shell. The presence of free radicals in the human body can cause various diseases. Free radicals can be counteracted or suppressed by administering antioxidants or by consuming antioxidants. Antioxidants are compounds that provide electrons (electron donors) or reductants.

Antioxidants are also compounds that can inhibit oxidation reactions by binding free radicals and highly reactive molecules, as a result cell damage will be inhibited.

In this test we measured the antioxidant activity of the immunity tea formula. Antioxidant analysis using the DPPH method, was carried out in the chemistry laboratory of the Malang State Polytechnic. The results of the antioxidant activity analysis are as follows (the results are the results of measuring antioxidant activity in 1 gram of sample):

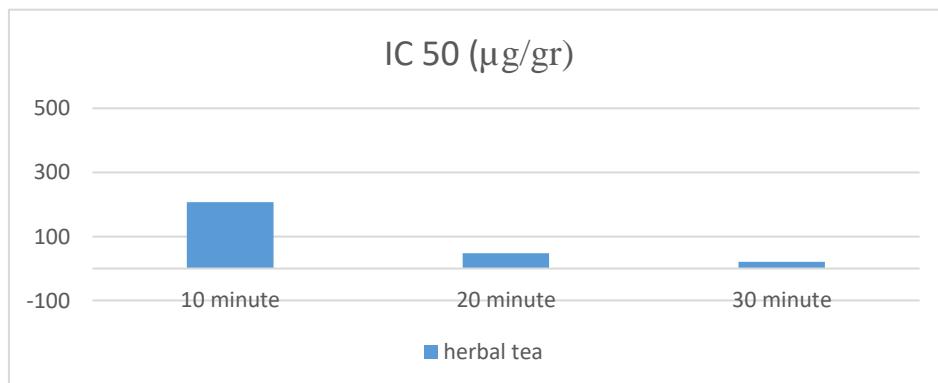


Figure 2. Antioxidant Activity Profile of herbal tea with varying brewing duration (10, 20, and 30 minutes). Note: the lower the IC50 value indicates the higher the antioxidant activity.

In Figure 2 above, it can be seen that the optimal brewing duration is 30 minutes, where with a brewing duration of 30 minutes, the IC50 of herbal tea is at the lowest position ( $21.39\mu\text{g/g}$ ) and much lower than the IC50 of tea with 20 minutes and 10 minutes of brewing.

Similar results were obtained by Fillianti et al. (2023) who studied the Effect of Brewing on the Total Phenol Content of Coriander Seed and Soursop Leaf Herbal Tea (Fillianti, 2023). The effect of temperature and brewing time on increasing antioxidant activity was also reported in the publication of Fauzan et al. (2022) who studied the Effect of Brewing Time and Temperature on the Antioxidant Activity of Pedada Fruit Peel Extract. In this study, it was concluded that antioxidant activity increased with increasing temperature and brewing time. This can be explained by the longer brewing time that can increase polyphenol levels because there is longer contact between tea and water as a solvent (Maharani et al., 2021). The total phenolic content of a sample is directly proportional to antioxidant activity. The higher the total phenolic content of a sample, the greater the antioxidant activity. This is in line with the statement of Kao et al. (2007) who proved that the phenol and flavonoid content in blackberries is directly proportional to antioxidant activity. This increase can occur because there are more hydroxyl groups of phenolic compounds that can act as electron donors. Antioxidant compounds will act as free radical stabilizers by complementing their electron deficiencies, thereby inhibiting the chain effects of the formation of free radicals (Anwar and Triyasmmono, 2016).

## Conclusion

From this study it can be concluded that the duration of brewing has a positive effect on the total phenol content and antioxidant activity of herbal tea. The longer the brewing, the higher the phenol content and the stronger the antioxidant activity.

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