# Comparison of Antioxidant Activity between Turmeric- Tamarind Infused Water and Traditional Herbal using DPPH Method

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Abstract: Recently, functional foods and beverages have been many advances, some of which are traditional herbal and infused water. The traditional herbal is a product of traditional Indonesian ingredients that have been developed in the community and used from generation to generation. Meanwhile, the infused water is water in which there are pieces of fruits, vegetables, or spices so that the water has a distinctive aroma, flavour, and colour according to the ingredients infused in the water. The efficacy of using turmeric-tamarind infused water and traditional herbal still largely based on empirical data. Several studies related to the antioxidant activity of turmeric- tamarind infused water and traditional herbal are still limited. This study aimed to determine the antioxidant activity of turmeric tamarind in infused water and traditional herbal products using the DPPH method. The percentage of inhibition and IC50 were measured. The results showed that turmeric-tamarind traditional herbal was known to be a strong antioxidant (IC50 value of 94.21 ppm), and turmeric-tamarind infused water was included in the moderate antioxidant group (IC50 value 142,08 ppm). Based on the statistical test, it can be concluded that there was a significant difference in IC50 value between turmeric-tamarind traditional herb and turmeric-tamarind infused water.

## 1 INTRODUCTION

The presence of a new trend related to functional drinks shows the high public awareness regarding body health. Several types of functional drinks that are well known in Indonesia are infused water and traditional herb. Traditional herb is a product of traditional Indonesian ingredients that have been developed in the community and used from generation to generation (A'yunin, 2019). Traditional herbs are generally referred to by the name of *jamu*. The names of these *jamu* are the main components in the herbal ingredients, such as *jamu kunir asem*, then the components are turmeric and tamarind. The observations found that the ingredients used to make the herbs came from nature without synthetic chemicals, so that the herbs can be consumed regularly and there are relatively no side effects (Sumarni, 2019).

Different from traditional herb, infused water is water in which there are pieces of fruits, vegetables or spices so that the water has a distinctive aroma, flavor and color according to the ingredients infused in the water (Muaris, 2014).

Infused water is different from juice, because it does not use added sugar or other additives so that infused water is more natural for consumption (Harifah, 2017). Infused water can be a recommendation for those who don't like to consume water or mineral water because of the taste and aroma of the fruit (Muzaifa, 2019). In addition, the infused water works as a toxin neutralizer in the body and is very suitable for daily consumption to replace drinking water. Consuming infused water can help maintain health (Aprilia, 2014).

The fruit used in making functional drinks, both in herbal medicine and in infused water, mainly contains vitamin C which is good for maintaining the immune system and has antioxidant activity that is able to ward off free radicals in the body. (Harifah, 2017). Antioxidants are compounds that give electrons to unpaired free radicals. Antioxidants are believed to play a very important role in body defense and protect cells from damage caused by free radicals. Antioxidants have been investigated for the prevention of diseases such as cancer, coronary heart disease, and even altitude sickness (Yadav et al. 2016). Antioxidant activity in beverages can be influenced by several factors including: Variations of



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fruits, stepping time, temperature, and particle size (Castiglioni, 2015).

Tamarind and turmeric are examples of plants that are known to have high antioxidant activity and can be used in various types of functional drinks. The antioxidant activity of dry turmeric rhizomes is known to have high antioxidant activity with an IC50 value of 46.7686 g/m (Pratiwi and Wardaniati, 2019). Meanwhile, the antioxidant activity in tamarind fruits extracts expressed in percentage ranged between 29.27±0.06% and 40.11±0.03% (Mbunde, 2018)

Antioxidant activity can be measured by various methods. One of the methods is the radical scavenger DPPH (1,1-diphenyl-2-picrylhydrazyl). DPPH method is the most widely reported method for screening antioxidant activity because this method is simplest, fast, sensitive, accurate. Antioxidant activity is expressed in terms of $IC_{50}$ (inhibitory concentration). The concentration of antioxidant compounds necessary to inhibit free radicals by 50% is called the $IC_{50}$ value (Chow et al, 2003).

The efficacy of using herbal tamarind infused water and drink is still largely based on empirical data. Several studies related to the antioxidant activity of tamarind and turmeric infused water and traditional herbs are still limited, therefore this study aimed to determine the proportion of tamarind turmeric $IC_{50}$ as antioxidant in infused water and traditional herb products, using the DPPH method.



# 2 MATERIALS AND METHOD

## 2.1 Materials

Turmeric from species *Curcuma longa* L. and tamarind from species *Tamarindus indica* L., determination of turmeric is evaluated at the Laboratory of Materia Medica Batu, Malang, and tamarind at the Laboratory of Agrotechnology University of Darussalam Gontor. The chemicals used in this study include DPPH (1,1-diphenyl-2-pikrihydrazil), ascorbic acid, methanol pro analysis, aquadest, aluminum foil, and drinking water.

## 2.2 Preparation of Infused Water

Tamarind turmeric herbal traditional was made by modification of Haitami (2017). Tamarind turmeric infused water was made by cleaning the skin of the turmeric rhizome, then washing it with running water. Turmeric was weighed 25 g. While the tamarind fruit, separated from the seeds, then the flesh is weighed as much as 12.5 g. The two samples were put into a glass bottle filled with 500ml of drinking quality water. Infused water was allowed to stand for 12 hours at room temperature. After 12 hours the turmeric acid-infused water was concentrated using rotary evaporator.

## 2.3 Preparation of Traditional Herbal

Tamarind turmeric herbal traditional was made by modification of A'yunin (2019). Skin of the turmeric rhizome was cleaning, then washing it with running water. The rhizome was grated and weighed as much as 25 g, while the tamarind fruit was separated from the seeds and weighed as much as 12.5 g. After that, grated turmeric rhizome and tamarind meat that has been weighed and then boiled with 500 ml of boiling water for 10 minutes. After 10 minutes, tamarind-turmeric herbal solution was filtered through filter paper and allowed to cool. After letting it cool down, the tamarind turmeric herbal traditional was concentrated by using rotary evaporator.

## 2.4 Preparation of Solution Test

### 2.4.1 Preparation of DPPH Stock Solution

DPPH was weighed 4 mg and dissolved in 100mL methanol p.a. until concentration 40 ppm in a 100 ml volumetric flask. The solution must be made fresh and covered with aluminium foil.

### 2.4.2 Preparation of Ascorbic Acid Standard Solution

Preparation of a standard ascorbic acid comparison solution by dissolving 100 mg of ascorbic acid powder into distilled water in a 100 ml volumetric flask to obtain a solution with a concentration series of 1000 ppm. Then pipette 1, 2, 3, 4, 5, 6, 7, 8, 9 ml to obtain a solution with a concentration series of 100, 200, 300, 400, 500, 600, 700, 800, and 900 ppm.

### 2.4.3 Preparation of Tamarind- Turmeric Solution Test

A total of 100 mg of the filtrate of the tamarind turmeric herbal medicine was taken, then added methanol to 100 ml, so that the concentration of the test solution of the tamarind turmeric test solution was 1000 ppm. Then pipette 1, 2, 3, 4, 5, 6, 7, 8, 9 ml to obtain a solution with a concentration series of 100, 200, 300, 400, 500, 600, 700, 800, and 900 ppm. Optimization of antioxidant activity test methods.

### 2.5 Determination of the Operating Time (OT)

A total of 1 ml of 400 ppm DPPH stock solution was put into a 5 ml volumetric flask, then 3 ml of 400 ppm sample was added. The solution is measured at the maximum wavelength for ± 5-60 minutes. Furthermore, the same treatment was carried out for a solution of ascorbic acid.

### 2.6 Determination of the Maximum Absorption Wavelength (λ Maximum)

A total of 1 ml of 40 ppm DPPH stock solution was added to 3 ml of the test solution in a 5 ml volumetric flask. The solution was homogenized, allowed to stand for OT, then scanned the maximum with a visible spectrophotometer at a wavelength of 400-600 nm

### 2.7 Antioxidant Activity Test

In a 10 ml volumetric flask, 2 ml of each stock solution of DPPH was added, then 2 ml of the test solution was added. The solution was added with methanol, vortexed, and allowed to stand for OT. The absorbance of the solution was measured at maximum by visible spectrophotometry. The work was replicated 3 times. Antioxidant activity is calculated using the formula:

$$\%IC_{50} = \frac{A - B}{A}$$

Note : A : Absorbance of DPPH solution
B : Absorbance of Sample solution

The data obtained from this study is by comparing the absorbance value, which is the result of antioxidant levels by calculating IC50 by using a linear regression line equation between each concentration of infused water and *jamu* turmeric acid (x axis) with % IC (y axis). Then, statistical tests were carried out using an unpaired T test for determined the significance of the difference in IC50 values of infused water and *jamu* turmeric acid. The IC50 data of turmeric acid in the infused water and herbal medicine preparations were then compared with the IC50 of ascorbic acid. The use of ascorbic acid as a comparison because ascorbic acid is one of the most commonly used antioxidant compounds. In addition, ascorbic acid belongs to the group very strong antioxidant because ascorbic acid is one of the antioxidant compounds that are pure without a mixture of other compounds (Wibawa, 2020).

## 3 RESULTS AND DISCUSSION

### 3.1 Preparation of Turmeric-Tamarind Infused Water and Traditional Herb

The preparation of turmeric-tamarind traditional herb, the part of the turmeric that was taken as the test material in this study was the rhizome part. This section was chosen because it was more yellow in colour than the other parts, so it was believed to contain more antioxidant compounds. The process of washing the turmeric rhizomes should not be too long because the water could carry the colour of the rhizomes, which affected the antioxidant compounds contained in the rhizomes. After washing, the turmeric was grated and weighed, while the tamarind part was extracted from the flesh. The tamarind was separated from the seeds and then weighed. After weighing the grated turmeric and tamarind pulp, the mixture was boiled in boiling water for 10 minutes. The herbs were cooled and then filtered.

In addition to being in the form of traditional herbs, tamarind turmeric was made with infused water. The difference between these two preparations in the manufacturing process. The turmeric rhizome which had been peeled and washed was weighed. The same as turmeric tamarind, had been separated from the seeds and then weighed. The weighed turmeric rhizome and tamarind flesh were placed in a glass bottle filled with 500 ml of drinking water. The infused water was stored for 12 hours at room temperature.

In this study, the samples to be analysed for spectrophotometry were made in the form of filtering. The method of using the filtrate was chosen to facilitate the reading of absorbance by spectrophotometry. Infused water and traditional herb filtrate were prepared using a water bath, and the filtrate performance was shown in the following table:

Table 1: The result of concentrating tamarind turmeric in infused water and traditional herb preparations.

| Sample weight | Total | | Yield |
|---|---|---|---|
| | Preparation | Filtrate | |
| Turmeric-tamarind 37,5 g | Infused water 183 g | 20,2 g | 11,03 % |
| Turmeric-tamarind 37,5 g | Traditional herb 194 g | 55,8 g | 28,76 % |

The infused water filtrate was browner in colour, while the herbal filtrate was more yellowish. This was because, in the process of make tamarind-turmeric

traditional herb, the turmeric used was completely crushed, while the turmeric-tamarind infused water was only cut into small pieces and soaked for several hours. Yield is the relationship between the number of metabolites obtained after the extraction process and the weight of the sample used. Based on percent yield, traditional herb (28,76 %) are known to perform better than infused water (11,03 %). Yield data have something to do with the active compound in a sample, so if the yield is higher, the number of active compounds contained in the sample will also increase (Dewatisari, 2017).

## 3.2 Determination of Maximum Wavelength (λ Maximum)

The purpose of determined the maximum wavelength in this study is to determine the wavelength at which the DPPH solution has the maximum absorption. The experimental conditions and the tools used were not always the same, so the maximum wavelength generated in each study might be different. If the measurement was carried out at the maximum wavelength, a small change in the concentration of the solution to be analyzed could lead to a large difference in the absorption results. This would increase the sensitivity of the method (Yulia, 2015).

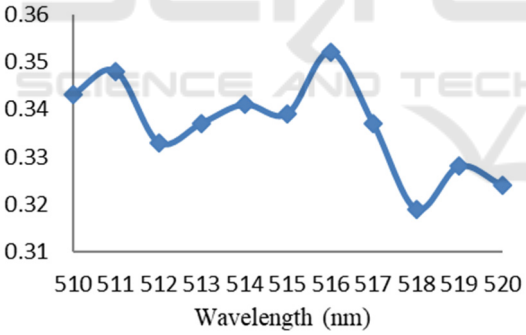


Figure 1: Result of the maximum λ determination curve.

The determination of the wavelength of maximum absorption had been measured the wavelength of a series of concentrations of DPPH, namely 40 ppm. The maximum wavelength measurement was carried out at a wavelength of 510 nm to 520 nm. This wavelength range was chosen to see if there was a change in wavelength compared to the theoretical maximum wavelength, which was 517 nm. From the determination of the wavelength carried out, the maximum wavelength at 516 nm was obtained. This wavelength shift is called a hypsochromic shift, which is a shift in absorbance to a shorter wavelength region due to the substitution or solvent effect (Dachriyanus, 2004). Another possibility is a factor in the difference in equipment and experimental conditions.

## 3.3 Determination of Operating Time (OT)

The operating time was the time that the absorption reading was taken with a visible spectrophotometer, where the test solution had perfectly reduced the DPPH radical to obtain a stable absorbance value. The operating time determination was performed by measuring the relationship between absorbance and measurement time.

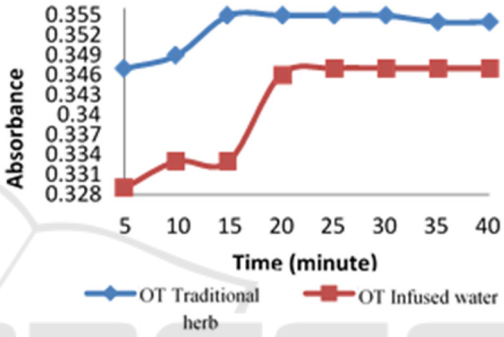


Figure 2: The operating time of turmeric-tamarind traditional herb and infused water. Blue line is for OT traditional herbar and Red line is OT infused water.

In this study, the determination of OT for sample of turmeric-tamarind traditional herb and OT turmeric-tamarind infused water. The OT in this study was measured from minute 5 to minute 40 at a maximum wavelength of 516 nm. The OT measurement results between the tamarind and turmeric-infused water samples showed a time difference, with a stable absorbance result of 0.355 at 15 minutes for turmeric OT traditional herb and a stable absorbance result was 0.347 at 25 minutes for OT turmeric-tamarind infused water. From the results of the investigation, the most stable OT was selected to minimize absorption variations and have a high degree of reproducibility in the new measurement.

## 3.4 Results of the Determination of Antioxidant Activity by the DPPH Method

Measurement of the antioxidant activity of the traditional herbs of tamarind and turmeric and the water infused with tamarind was carried out at various concentrations. This was done to determined

the effect of concentration on antioxidant activity. The higher concentration of sample solution, the more hydrogen atoms were donated, so the antioxidant activity was higher. DPPH was a stable dark purple free radical that had a strong absorbance at a wavelength of 517 nm (Molyneux, 2004). The dark purple color of the DPPH solution was formed because DPPH radicals could resonated to form long chromophore groups.

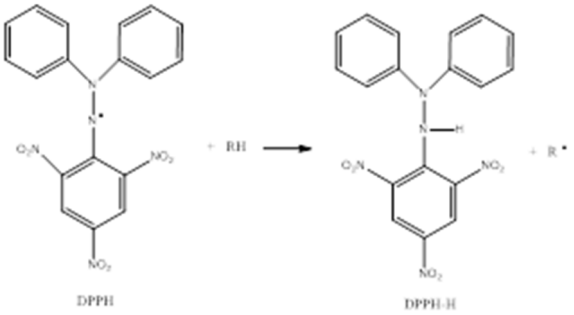


Figure 3: The reaction of the radical DPPH with antioxidants.

In this study, the DPPH solution was a solution that acted as a control solution that had a higher absorbance than the sample solution. This was because the control solution only contained DPPH and did not contain compounds that had antioxidant activity. The turmeric-tamarind traditional herb and infused water filtrate samples were diluted with concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm. Every 2 ml of sample solution and 2 ml of 40 ppm DPPH solution were added which were homogenized and allowed to stand for OT for each preparation, then the absorbance was measured used UV-Vis spectrophotometry. After obtained the absorbance value, % inhibition calculated using the formula:

$$\%IC_{50} = \frac{A - B}{A}$$

After obtained the % inhibition, a linear regression equation was performed to calculate the antioxidant activity. Antioxidant activity was determined based on the curve of the linear regression equation between concentration and % IC. Therefore, three repetitions were carried out to obtain a linear regression equation. Linearity was expressed as the correlation coefficient (r). The antioxidant activity was expressed by $IC_{50}$, which was the concentration of antioxidants necessary to capture 50% of the DPPH radicals. The lower the $IC_{50}$ value, the less concentration was needed to scavenge free radicals.

According to the linear regression equation between each concentration of tamarind-turmeric traditional herb, water infused with tamarind turmeric, and ascorbic acid with % IC, the $IC_{50}$ value could be seen in the following curve:

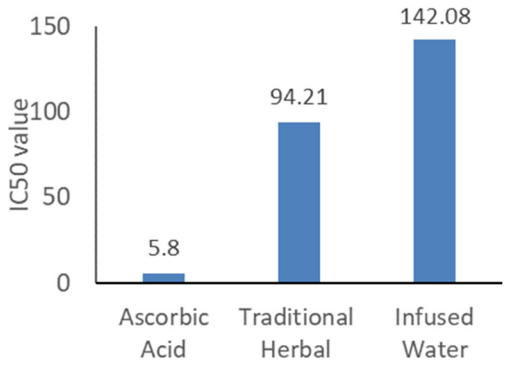


Figure 4: $IC_{50}$ value of ascorbic acid, turmeric-tamarind infused water, and traditional herb.

From the diagram above, it was known that turmeric-tamarind traditional herb had an $IC_{50}$ value of 94.21 ppm, and turmeric-tamarind infused water of 142.08 ppm. The smaller IC50 value, show the greater the antioxidant activity. Both samples had different groups of antioxidant activity according to the $IC_{50}$ classification table below:

Table 2: Antioxidant power with DPPH reactive compounds following Haerani, (2019).

| Classification of $IC_{50}$ | |
|---|---|
| $IC_{50} \leq 50$ ppm | Very strong |
| $IC_{50}$ between 50-100 ppm | Strong |
| $IC_{50}$ between 101-150 ppm | Moderate |
| $IC_{50}$ more than 151 ppm | Weak |

From the table above, turmeric-tamarind traditional herb was known to be a strong antioxidant, and turmeric-tamarind infused water was included in the moderate antioxidant group. This was probably due to the difference in particle size between the two preparations. Turmeric-tamarind traditional herb had a smaller particle size because the ingredients used were subjected to a refining process used a blender. As for the infused water preparation, the ingredients were only sliced with a knife. This was in line with one of the extraction principles, that was the particle size important to ensure that the extraction of the compound could take place as much as possible. The particle size was related to the area of the surface that would be in contact with the solvent. (Sapri et al., 2004). The main process in the transport of compounds in infused water and traditional herb is the diffusion process. Diffusion is the process of moving a substance or particle from a highly

concentrated part to a low concentration part. Apart from the particle size factor, another factor that affects is the surface area of an area. The wider the surface of a diffusion area, the wider the parts that can be crossed.

In this study, the standard solution used was ascorbic acid which had an $IC_{50}$ value of 5.8 ppm. Ascorbic acid was used as the standard reference solution because ascorbic acid is one of the most common antioxidant compounds. Also, ascorbic acid is included in the very strong antioxidant group because ascorbic acid is one of the antioxidant compounds that is pure without any other compounds.

To determine the significant difference between the $IC_{50}$ value of the turmeric-tamarind traditional herb and the turmeric-tamarind infused water, a statistical test was performed, namely the independent T-test. The independent T-test requires that the data be normally distributed. Therefore, the first step to take was a normality test used the Shapiro-Wilk test. This test had a sample requirement of ≤ 50. The next step was to perform an analysis to see the significance of the $IC_{50}$ value between turmeric-tamarind infused water used an independent T-test. The alternative hypothesis used was the $IC_{50}$ value of turmeric-tamarind traditional herb a was different from turmeric-tamarind infused water. The null hypothesis ($H_0$) was that the $IC_{50}$ value of turmeric-tamarind was not different from turmeric-tamarind infused water. The results obtained with a significance value of 0.000 between turmeric-tamarind infused water. When compared to the specified significance value, namely 0.05, H0 was rejected because it was 0.000 < 0.05. Therefore, it could be concluded that there was a significant difference in $IC_{50}$ value between turmeric-tamarind traditional herb and turmeric-tamarind infused water.

# 4 CONCLUSIONS

Turmeric-tamarind infused water ($IC_{50}$ 142.08 ppm) had lower antioxidant activity than turmeric-tamarind traditional herb ($IC_{50}$ 94.21 ppm). Based on statistical tested, there were differences in the $IC_{50}$ value of turmeric-tamarind in traditional herb and infused water, influenced by differences in dosage forms and the manufacturing process of these preparations.

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