

# Determination of Phytochemicals and Antioxidants Activity in Herbs Spices of Kitchen

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## Abstract

Maintaining immunity is important because it can prevent infection of various viruses. One way to maintain immunity is by consuming herbal plants. The properties and benefits of several herbal plants that are often used as kitchen spices have been explained scientifically in the book *Herbal Spices for Kitchen*. In Alor Regency, East Nusa Tenggara Province, Indonesia, turmeric, which is rich in curcumin, is usually used as a wound medicine by rural communities. Likewise, ginger, which has a spicy taste, is often used by rural communities to warm cold babies by occasionally placing it on the baby's armpit. Meanwhile, lemongrass is usually used to add flavor to dishes at parties. Based on consideration of the local community's experience in using these three plants and combined with scientific studies, it was deemed necessary to analyze the content of phenolic compounds, saponins, steroids, terpenoids, and alkaloids, as well as antioxidant activity in a mixture of turmeric, ginger, and lemongrass with the ratio of 50% turmeric, 33.33% ginger, 16.67% lemongrass. The distribution of phenolic compounds, saponins, steroids, terpenoids, and alkaloids, even in the mixture of turmeric, ginger, and lemongrass plants is very strong antioxidant activity as indicated by the  $IC_{50}$  value of the mixture of 4.817  $\mu\text{g}/\text{ml}$ . The composition of the mixture of turmeric, ginger, and lemongrass can contribute to maintaining and even strengthening the body's immune system.

## 1. Introduction

Maintaining immunity is important because it can prevent infection with various viruses [1]. One way to maintain immunity is by consuming herbal plants [2]. The definition of herbs in the glossary of the book *Herbal Spices for Kitchen* [2] is that in fact, herbs are plants whose plant parts, whether in the form of seeds, fruit, flowers, leaves, stems, wood, or roots, have been scientifically identified and known to have compounds that are beneficial for preventing to cure disease. According to [3], kitchen spices are family medicinal plants that are always available around people's homes as living pharmacies. Family medicinal plants, in simple terms, are used by traditional communities to fulfill various life needs, especially food and medicine [4].

Traditional medicine is the total of knowledge, skills, and practices based on theories, beliefs, and experiences from different cultural customs used in healthcare, which have been used for generations from one generation to the next [5]. Traditional medicine begins with the experience of illness felt by oneself or others, thus encouraging the development of reasoning about the causes of illness from visible signs or felt symptoms, which then triggers the formation of concepts about how to prevent and treat the illness or disease [4]. Herbs have actually been used traditionally since ancient times, and have continued to be maintained until now and are often called herbal medicine.

Some herbal plants that can be studied for their benefits are turmeric, ginger, lemongrass, etc. They are formulated in herbal forms such as Kitchen Spices Herbs [2], Ready-to-Fast Herbs [6], Herbs for Health [7], Herbal Medicine for Body Endurance [8], How to Make Traditional Herbal Medicine for Body Endurance According to National Agency of Drug and Food Control [9], Utilization of Family Medicinal Plants [3], Utilization of Medicinal Plants (Biopharmaca) as Leading Community Products [10], and others.

The properties and benefits of several herbal plants that are often used as kitchen spices have been explained scientifically in the book *Herbal Spices for Kitchen* [2]. Especially turmeric, ginger, and lemongrass, which are also available in Alor Regency, East Nusa Tenggara Province, Indonesia. Turmeric, which is rich in curcumin, is usually used as a wound medicine by rural communities. Likewise, ginger, which has a spicy taste, is often used by rural communities to warm cold babies by occasionally placing it on the baby's armpit. Meanwhile, lemongrass is usually used to add flavor to dishes at parties. Some scientific data about these three plants can be described below.

### 1.1 Turmeric

Turmeric from the Zingiberaceae family with the species name *Curcuma longa* L. [11], has high levels of curcumin of 891,120.7 ppm [12] which has activity of anti-inflammatory, antitumor, antibacterial, etc. [13]. Turmeric also contains terpenoid and phenolic compounds [14]. The benefits of turmeric for health include healing joint pain, preventing cancer [15], reducing creatinine, and others [16]. Research results [17] show the antioxidant activity of turmeric with an  $IC_{50}$  value of 2.72  $\mu\text{g}/\text{ml}$  sample.

### 1.2 Ginger

Ginger has a spicy taste caused by a ketone compound called Zingeron. Ginger belongs to the Zingiberaceae family which contains terpenoid and phenolic compounds [18]; [19]. Ginger has antimicrobial, anti-inflammatory, antioxidant, antitumor, and other activities [10]; [20]. Ginger also has a protective effect on the digestive tract and eliminates symptoms of nausea [18], cures gout, and others [16]. Research results [21] show the antioxidant activity of ginger with an  $IC_{50}$  value of 0.654  $\mu\text{g}/\text{ml}$  sample. However, in their research, [22] added 3 grams of ginger to rabbit feed, which was considered excessive and could cause liver damage. Meanwhile, [23] stated that 6 grams of ginger can cause stomach irritation. In their conclusion, [24] advises using the correct dose of lemongrass and ginger to avoid side effects from these two ingredients.

### 1.3 Lemongrass

Lemongrass from the Poaceae family or grasses is a *Cymbopogon citratus* species that is quite abundant in Indonesia. Lemongrass contains some secondary metabolite compounds with the main component being citronellal ( $C_{10}H_{16}O$ ) with a concentration of 32-45% which is antioxidant [25]; [26]. The benefits of lemongrass are anti-inflammatory, anti-cancer, antioxidant, improving blood circulation, relieving coughs, easing phlegm, etc. [27]; [28]; [29]; [30]; [31]. In fact [32] obtained antioxidant activity in dried lemongrass leaves with an  $IC_{50}$  value of 17.15  $\mu\text{g}/\text{ml}$  sample. The benefits of lemongrass as a urine laxative, sweat laxative, lowering blood pressure, and several other benefits [28]; [33] can have a negative impact, especially on certain health conditions if not used regularly. So in their conclusion, [24] advises using the correct dose of lemongrass and ginger to avoid side effects from these two ingredients.

So far, including in Alor Regency, East Nusa Tenggara Province, Indonesia, no research has analyzed the characteristics of the combination of turmeric, ginger, and lemongrass herbal plants as herbal medicine. Even though they are consumed in mixed form, these herbal plants are always analyzed separately [3]; [16] so the exact combination of herbal mixtures that are right for consumption is not yet known. Considering the experience of the people in Alor Regency, East Nusa Tenggara Province, Indonesia, in utilizing these three plants and combining them with scientific studies, it is deemed important to analyze the mixture of ingredients from these three herbal plants. The desired combinations

of the three mixed herbal plants are 50% turmeric powder, 33.33% ginger powder, and 16.67% lemongrass powder to obtain certainty of their efficacy/benefits for survival.

Phytochemical analysis is a testing method used to qualitatively identify the content of secondary metabolite compounds in plants by observing the color changes that occur after adding reagents [34]; [35]. Secondary metabolites themselves are metabolic compounds that are not essential but have a very vital role in plants, namely functioning to defend themselves from environmental conditions [34]. These secondary metabolites consist of a group of terpenoid compounds (antioxidant, antimicrobial, etc.), alkaloids containing nitrogen (pharmacological properties), and phenolics (antioxidant, anti-inflammatory, anticancer, etc.) [34].

Apart from phytochemical tests [36], it is also important to know the antioxidant activity of these kitchen spice plants. This is to confirm the ability of these plants to ward off free radicals. Antioxidants are one of the properties of a group of secondary metabolite compounds that aim to ward off free radicals and prevent cell damage in the body. According to [37], antioxidants can prevent or delay several types of cell damage due to the oxidation process by oxidants.

Oxidants themselves are free radicals that are produced naturally in the body and the environment [38]; [39]. Free radicals in the environment usually come from cigarette smoke, air pollution, environmental polluting chemicals, and others [40]. The free radical used as a model in antioxidant activity test research is 2,2-diphenyl-1-picrylhydrazyl (DPPH) [40] to determine the  $IC_{50}$  value of the sample [41]. A compound is said to be a very strong antioxidant if the  $IC_{50}$  value is less than 50 ppm, strong if the  $IC_{50}$  value is between 50-100 ppm, medium if the  $IC_{50}$  value is between 100-150 ppm, and weak if the  $IC_{50}$  value is between 150-200 ppm [42]. In other words, the smaller the  $IC_{50}$  value in a sample, the stronger the antioxidant in warding off free radicals, or it can be said that the sample has stronger antioxidant activity. Inhibitory Concentration fifty ( $IC_{50}$ ) is the concentration of a test sample made using a certain method to measure the ability of antioxidants to inhibit the activity of DPPH free radicals by up to 50%. The  $IC_{50}$  value is calculated based on the percentage of inhibition of each sample solution concentration based on the formula:

$$\% \text{ inhibition} = \frac{\text{control absorbance} - \text{test sample absorbance}}{\text{control absorbance}} \times 100 \dots\dots\dots (1)$$

The % inhibition values at various concentrations are then entered into the regression equation with sample concentration as the x-axis and % inhibition as the y-axis. The  $IC_{50}$  value is obtained from calculations when the % inhibition is 50% from the equation  $y = a + bx$  [42].

This research aims to obtain an even distribution of secondary metabolite compounds and strong antioxidant properties from a combination mixture of turmeric, ginger, and lemongrass originating from Alor Regency, East Nusa Tenggara Province, Indonesia which can be used in maintain immunity and as a strong inhibitor of free radicals in the body, including supporting the regeneration of cells, nerves, and other organs of the body that are weak due to climate, work, or old age.

## 2. Material

The materials used in this research were ammonia ( $NH_3$ ), Iron (III) chloride ( $FeCl_3$ ), concentrated sulfuric acid ( $H_2SO_4$ ), chloroform ( $CHCl_3$ ), distilled water, anhydrous acetic acid ( $C_4H_6O_3$ ), Meyer's reagent,  $HgCl_2$ , KI, 2,2-diphenyl-1-picrylhydrazyl (DPPH), solvent (DMSO, ethanol, methanol), and test samples in the form of turmeric, ginger and lemongrass rhizomes taken from Alor Regency, East Nusa Tenggara Province, Indonesia. The types of equipment used during the research were a blender, analytical balance, 60 mesh sieve, baking sheet, drying cabinet, thermometer, micropipette, tip, oven, autoclave, incubator, refrigerator, pH meter, vortex, UV-Vis spectrophotometer, burette and stand, measuring flask, cuvettes, dark vials, and aluminum cups. Prepare samples of kitchen spice concoctions namely turmeric rhizome, ginger rhizome, and lemongrass stem which have been cleaned, sliced thinly, and dried using sunlight. Next, the samples were ground separately using a blender to produce powder and sieved using a sieve with mesh size No. 60 (0.25mm). The powder for each sample was then mixed with a concentration ratio of 3 parts turmeric powder (50%): 2 parts ginger powder (13.33%): and 1 part lemongrass powder (16.67%) to obtain herbal medicine powder from kitchen spice plants.

### 3. Method

#### 3.1 Phytochemical Test

The phytochemical testing procedure was carried out based on the procedure carried out by [34] with several modifications for this research.

A total of 2 grams of powdered samples of kitchen spices were put into a test tube and then macerated with ethanol in a water bath for 15 minutes. Next, the sample is filtered hot into a test tube and left until all the ethanol evaporates and dries. After that, the residue and filtrate are separated.

The filtrate was added with chloroform and distilled water in a ratio of 1:1, 5 mL each. Shake until completely even, then transfer to a test tube, and leave for a moment until a 2-layer solution is formed, they are a layer of chloroform and a layer of water. Next, it is separated between the chloroform layer and the water layer. The water layer is used to test phenolic, flavonoid, and saponin content while the chloroform layer is used to test steroids and terpenoids [43].

##### 3.1.1 Phenolic Test

A layer of water was put into the drop plate, after which 10 drops of 1% FeCl<sub>3</sub> were added gradually. If blue, purple, blackish green and dark black colors are formed, this indicates the presence of phenolic compounds.

##### 3.1.2 Saponin Test

The water layer was pipetted into a test tube and then shaken vigorously. If the foam is formed after a few minutes, this indicates the presence of saponin.

##### 3.1.3 Test for Steroids and Terpenoids

A layer of chloroform is put into a Pasteur pipette which contains charcoal or norite. The filtrate that comes out of the pipette is put into the 3 holes of the drop plate and left to dry. Next, concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is added to the first hole, a drop of anhydrous acetic acid and a drop of sulfuric acid are added to the second hole, and the third hole is used as a blank. If a purple-blue color is formed, it indicates the presence of steroids, whereas if a red color forms, it indicates the presence of terpenoids.

##### 3.1.4 Alkaloid Test

A total of 2-4 grams of powdered samples of kitchen spices were put into a container, then 10 mL of 0.05 N chloroform-ammonia was added and stirred gently. After that, the solution was filtered using a small funnel in which cotton was placed as a filter and the filtered results were put into a test tube. The filtrate was added with 10 drops of 2 N sulfuric acid and shaken gently. After that, left it until an acid layer and a chloroform layer were formed. Next, a drop of Meyer's reagent to the acid layer 1.358 g HgCl<sub>2</sub> + 60 mL distilled water and 5 grams KI + 10 mL distilled water mixed and added distilled water until it reached the 100 mL limit) to obtain the results. (Reaction with Mayer's reagent will form a white precipitate, with Dragendorff's reagent a red-orange precipitate will form, and with Wagner's reagent a brown precipitate will form).

#### 3.2 Antioxidant Activity Test

The antioxidant test procedure was carried out based on the procedure carried out by [44] and [42] with several modifications for this research.

##### 3.2.1 Preparation of DPPH Solution

Weighed 10 mg of DPPH solid using an analytical balance, dissolved it with 20 ml of ethanol in a dark vial, and homogenized it. The solution was then put into a 25 ml measuring flask to the mark and incubated in the refrigerator for 30 minutes. The DPPH control solution is ready to use. The DPPH control solution was tested on a UV-Vis spectrophotometer with a wavelength of 516 nm as a control absorbance in this test.

##### 3.2.2 Sample Preparation and Antioxidant Testing

A total of 2 mg of the test sample was made into a solution with concentrations of 1.25 ppm, 2.5 ppm, 5 ppm, and 10 ppm. 1 ml of each test solution was taken and 2 ml of DPPH and 1 ml of ethanol were added

each and then incubated for 30 minutes at room temperature. After that, samples of each concentration were transferred to a cuvette and measured using a UV-Vis spectrophotometer at a wavelength of 516 nm to determine the absorbance value. Next, antioxidant activity was determined using the % inhibition formula in equation (1). The % Inhibition value at each concentration is then entered into the regression equation with sample concentration as the x-axis and % inhibition as the y-axis. The  $IC_{50}$  value is obtained from calculations when the % inhibition is 50% from the equation  $y = ax \pm b$

#### 4. Results and Discussion

Phytochemical tests on herbal kitchen spice samples have been conducted to identify the content of bioactive compounds that play a role in various health benefits. A sample of the herbal spice, consisting of a combination of turmeric, ginger, and lemongrass, showed the presence of various important phytochemical components, each of which contributed to its therapeutic potential. The results of phytochemical tests on herbal samples of kitchen spice are shown in Table 4.1.

**Table 4.1** *The results of phytochemical tests on herbal samples of kitchen spices*

No	Compounds	Analysis Results
1	Phenolic	(++)
2	Saponins	(+)
3	Steroids	(+)
4	Terpenoids	(++)
5	Alkaloids	(+)

The test results in Table 4.1 show that the herbal kitchen spice samples contain several important phytochemical components with the indicated intensity.

- Phenolic (++)  
The herbal spice samples contain high amounts of phenolic compounds, as indicated by the test results with an intensity of (++) . Phenolic compounds are known for their strong antioxidant properties, which play an important role in protecting the body from oxidative damage caused by free radicals. The significant presence of phenolics in herbal spice samples indicates strong potential as antioxidant agents.
- Saponin (+)  
Phytochemical tests also showed the presence of saponin with (+) intensity. Saponin is known for its antimicrobial, anti-inflammatory properties, and its ability to boost immunity. The presence of saponin in herbal kitchen spice samples indicates that this drink can help fight infections and reduce inflammation.
- Steroids (+)  
Steroids, which have anti-inflammatory effects and are often associated with regulating the immune system, were also detected with (+) intensity in the herbal kitchen spice samples. This indicates that this herbal medicine has the potential to help reduce inflammation and support immune system health.
- Terpenoid (++)  
The terpenoid content in the herbal kitchen spice samples was detected with high intensity (++) . Terpenoids have various benefits, including as antioxidants, anti-cancer, and anti-microbial. The high terpenoid content adds to the therapeutic value of the herbal kitchen spice samples, making them potential in preventing various degenerative diseases.
- Alkaloids (+)  
Phytochemical tests also revealed the presence of alkaloids with (+) intensity. Alkaloids are known to have analgesic (pain relief) and antioxidant activities. Their presence in herbal kitchen spice samples indicates that these herbs can help in reducing pain as well as fighting damage caused by free radicals.

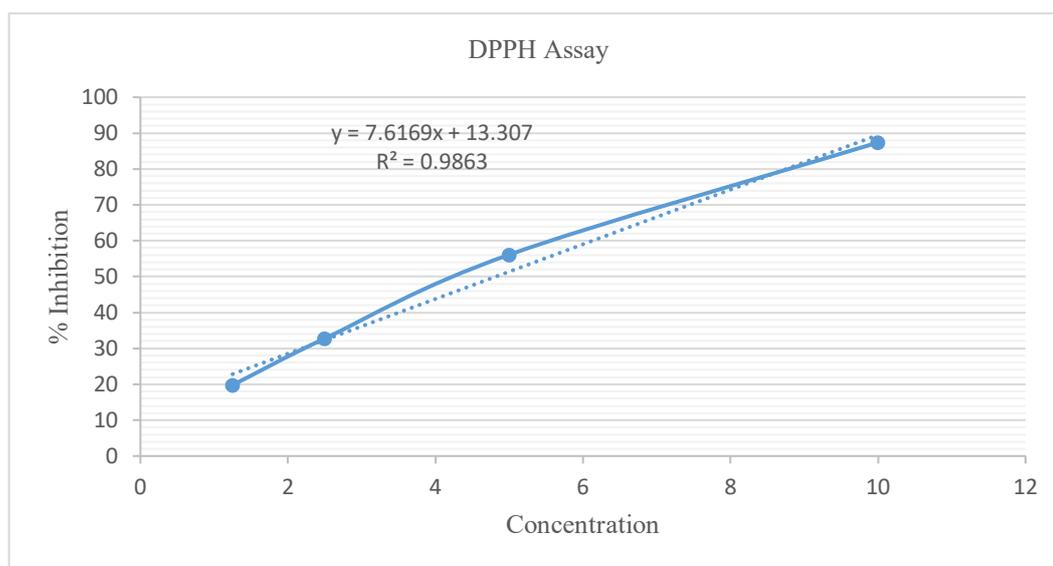
The results of phytochemical tests showed that the herbal kitchen spice samples were rich in various bioactive compounds, including phenolics, saponins, steroids, terpenoids, and alkaloids. The combination of these compounds makes herbal kitchen spices have great potential as herbal drinks that can provide various health benefits, especially in protecting the body from oxidative stress, inflammation, and other disease risks.

This can also be proven through the inhibition test ( $IC_{50}$ ) against free radicals, showing that its antioxidant activity is very strong. Analysis data can be shown in Table 4.2 the following.

**Table 4.2 Inhibition test results ( $IC_{50}$ )**

Concentration (ppm)	Control absorbance	Sample absorbance	% inhibition
1.25	0.809	0.649	19.7775
2.50	0.809	0.544	32.7565
5.00	0.809	0.355	56.1187
10.00	0.809	0.102	87.3918

The inhibition value of each sample concentration is obtained from equation (1). From the data in Table 4.2 shows that the higher the concentration, the higher the inhibition value, but the absorbance of the sample is smaller. From this data, a graph of the relationship between concentration as the x-axis and inhibition as the y-axis was created, as shown in Figure 4.1 the following.



**Figure 4.1** Graph of the relationship between concentration and inhibition of kitchen spices herbs

Based on Figure 4.1 the linear regression equation obtained is  $y = 7.6169x + 13.307$  with a correlation coefficient of  $R^2 = 0.9863$ . The  $R^2$  value describes the linearity of concentration to the percentage of inhibition. An  $R^2$  value that is close to 1 indicates that as the concentration of the extract increases, the antioxidant activity also increases. By entering the y value, namely 50% inhibitory power in a sample, an  $IC_{50}$  value of 4.817  $\mu\text{g/ml}$  is obtained. The  $IC_{50}$  value shows that the combination of these three plants provides antioxidant activity in the very strong category, so it can be used to maintain the body's resistance to various diseases.

With these results, it can be said that the herbal medicine sample with a concentration ratio of 50% turmeric powder, 33.33% ginger powder, and 16.67% lemongrass powder can be used as a medicinal ingredient. herbs, including to maintain the body's resistance to various diseases.

## 5. Conclusion

The combination of a mixture of 50% turmeric powder, 33.33% ginger powder, and 16.67% lemongrass powder originating from Alor Regency, East Nusa Tenggara Province, Indonesia, shows that the herbal spice sample is rich in various bioactive compounds, including phenolics, saponins, steroids, terpenoids, and alkaloids. In addition, the  $IC_{50}$  value of 4.817  $\mu\text{g/ml}$  indicates that the combination of the mixture is the best source of antioxidants. Thus, it can be concluded that the composition of the mixture of turmeric, ginger, and lemongrass in this sample is a balanced combination of mixtures that can contribute to maintaining, protecting, and even strengthening the immune system, including supporting the regeneration of cells, nerves, and other organs that are weak due to climate, work, or old age.

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## Conflict of Interest

The authors declare that there is no conflict of interest regarding the paper's publication.

## Author Contribution

The authors confirm their contribution to the paper as follows: **study conception and design:** Author 1, Author 2; **data collection:** Author 3, Author 4; **analysis and interpretation of results:** Author 1, Author 2, Author 3, Author 4; **draft manuscript preparation:** Author 1; All authors reviewed the results and approved the final version of the manuscript.

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