Comparison of Antioxidant Activity and Phytochemical Screening of Fresh and Dried *Moringa Oleifera L.* Tea

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ABSTRACT

The world is being hit by a new variant of Covid-19, namely Omicron. The Omicron variant is easily dispersed from the previous variant. To prevent transmission of this variant, people need to increase their body's resistance by consuming foods and drinks rich in antioxidants. Moringa leaf tea can be an alternative drink rich in antioxidants to increase endurance and prevent the speed of transmission of the Omicron variant of Covid-19. This study analyzes the presence or absence of secondary metabolites and antioxidant activity in fresh and dry moringa tea. Secondary metabolite compounds were tested using phytochemical screening, which included testing for alkaloids, flavonoids, phenolics, saponins, tannins, steroids, terpenoids, and glycosides, while for antioxidant activity, the DPPH method was used. Based on the test results, the two types of tea did not have antioxidant activity because the IC50 value was more than 250 ppm, namely 868.72 ppm for fresh moringa tea and 2851.67 ppm for dry moringa tea. However, when compared between fresh and dry moringa tea, both have an IC50 value of 1:3 ratio, where the IC50 value of fresh moringa tea is three times lower than that of dried moringa tea. The contribution of this study is to provide the phytochemical screening of the two types of tea that were positive for several secondary metabolites, namely flavonoids, alkaloids, and saponins.

1. Introduction

Today, most diseases are caused by the food and drink consumed daily. Food and beverages are consumed as long as there is a feeling of satiety and the elimination of thirst. Most people do not care about the nutritional value or the side effects of consuming various food and drinks. Even without realizing it, food and beverages that enter the body are not necessarily beneficial for health. Instead, they become a source of free radicals that trigger various types of degenerative diseases and chronic diseases in the body [1]. Free radicals are molecules that contain free electrons in their outermost trajectory, so they are reactive, damaged tissues, and accelerate the process of the pathology of living things [2].

Apart from being a source of free radicals, various types of food and drink have the potential to be anti-free radicals. Anti-free radicals are electron-donor compound molecules because there are free electrons in the outermost orbital so that they can bind and stabilize the free electrons possessed by free radicals [3]. Sources of anti-free radicals come from various plant parts (stems, fruits, seeds, leaves, roots). The presence of anti-free radicals is due to the content of secondary metabolite compounds [4]. Secondary metabolite compounds are compounds used for plant self-protection

against environmental conditions. Secondary metabolites contain many special compounds, such as alkaloids, flavonoids, phenolics, saponins, tannins, steroids, terpenoids, and glycosides [5].

Secondary metabolites are also found in tea. Tea is a type of beverage that people of all ages favor. Tea is suitable for drunk in hot, warm, and cold conditions because it gives a fresh taste and is easy to consume. Tea is categorized into various types and comes from multiple leaves, fruits, flowers, and roots brewed with hot water [6]. In Indonesia, there are multiple plants whose leaves can be used as a tea, including Moringa leaves. Moringa is a nutrient-rich plant that can live in tropical and subtropical regions. The nutrients contained in Moringa leaves inhibit the increase of free radicals due to the high antioxidant activity of Moringa leaves [7].

The antioxidant content in Moringa leaves can increase endurance, especially in the post-Coronavirus Disease-2019 (Covid-19) pandemic. The world is being hit by a new variant of Covid-19, namely Omicron. Omicron has different characteristics because it experienced a type shift from SARS-CoV-2 to variant B.1.1.529. The Omicron variant is easily dispersed from the previous variant [8]. To prevent the transmission of this variant, people need to increase their immune system, one of which is by consuming antioxidant-rich foods and beverages. Moringa leaf tea can be an alternative to antioxidant-rich drinks to increase endurance and prevent the speed of transmission of the Omicron variant of Covid-19. This study analyzes the presence or absence of secondary metabolites and antioxidant activity in fresh and dry moringa tea, while the contribution of this study is to provide the phytochemical screening of the two types of tea that were positive for several secondary metabolites, namely flavonoids, alkaloids, and saponins.

2. Research Methodology

This research was conducted in July-September 2022 at the Chemistry Laboratory, Widya Mandira Catholic University Kupang. As for this study, there were several stages, starting with the making of moringa leaf tea, then phytochemical screening, and continued testing of the antioxidant activity of both types of tea, namely fresh and dried Moringa leaf tea.

2.1. Materials

The tools used in this study consisted of an analytical balance, knife or scissors, UV-Vis spectrophotometer, oven, vortex, rotary vacuum evaporator, flask, water bath, and glassware, which is common in the laboratory. The materials used were Moringa leaves originating from Ende district East Nusa Tenggara Province, absolute ethanol, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Mg metal powder, 2 N HCl, 1% $FeCl_3$ solution, concentrated HCl, concentrated H_2SO_4, concentrated anhydrous acetic acid ($C_4H_6O_3$), distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.2. Making Moringa Leaf Tea

Moringa leaves are washed and drained, then aerated to dry. Each Moringa leaf is weighed as much as 200 mg and then chopped or sliced. Fresh moringa leaves that have been chopped taken as much as 100 mg and brewed with hot water at a temperature of 100ºC as much as 100 ml for 20 minutes, then closed. The remaining 100 mg of Moringa leaves were dried using an oven at a temperature of 50ºC with a drying time of 2 hours. After drying Moringa leaves, proceed with the same treatment as in fresh ones.

2.3. Phytochemical Screening

2.3.1. Flavonoid Test

The sample was added with a spatula of Mg metal powder and four drops of concentrated HCl. The presence of flavonoids will be indicated by a change in the color of the filtrate to orange-red [9].

2.3.2. Tannin Test

The sample was added 1-2 drops of 1% $FeCl_3$ reagent. The presence of tannins will be indicated by a change in the color of the filtrate to green or blue-black [10].

2.3.3. Saponin Test

The sample was added with hot water and shaken for 10 seconds. After that observed the changes that occurred. Then one drop of 2 N HCl was added, and the changes were observed again. Positive results when stable foam appears for 10 minutes [11].

2.3.4. Alkaloid Test

The sample was added with a few drops of 2 N HCl and distilled water. After that, it was heated in a water bath for 2 minutes, then cooled. The filtrate used for the alkaloid test, namely, every three drops of the filtrate, was added with two drops of Mayer reagent, Dragendorf reagent, and Wagner reagent, and the changes that occurred from the three were observed. Alkaloids are positive if a precipitate or turbidity exists in at least two of the three experiments above. The characteristic of a positive reaction for alkaloids is the formation of a brownish-yellow color with Wagner's reagent, a yellow precipitate is formed with Meyer's reagent, and a white precipitate is formed with Dragendorff's reagent [12], [13].

2.3.5. Steroid and Terpenoid Test

The sample is added 2-3 drops of concentrated anhydrous acetic acid ($C_4H_6O_3$), then stirred slowly until dry, then 1-2 drops of concentrated H_2SO_4 are added, and the staining is observed. Ring staining of red, brownish red, or purple-red indicates triterpenoids, whereas ring staining is bluish-green for steroids [14], [15].

2.3.6. Phenolic Test

The sample was added with ten drops of 1% $FeCl_3$. If a green, red, purple, blue, or black color is formed, it indicates the presence of phenol [16].

2.3.7. Glycoside Test

The sample was dissolved in absolute ethanol, evaporated over a water bath, dissolved the remainder in 5 mL of concentrated anhydrous acetic acid ($C_4H_6O_3$), and added ten drops of concentrated H_2SO_4. If a blue or green color is formed, it indicates the presence of glycosides [17].

2.4. Antioxidant Activity Test

The free radical activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) was analyzed based on a linear regression equation followed by the determination of the median value of Inhibitory Concentration (IC50). The test solution was prepared by inserting 0.1 mL of liquid extract of fresh Moringa tea and dry Moringa tea, which had been prepared at a concentration of 1000, 2000, and 3000 g/mL, respectively, into a dark-colored vial, then added 2 mL of DPPH solution (60 g/mL in methanol) and 2 mL of methanol. The mixture was then vortexed and incubated at room temperature for 30 minutes in the dark. The absorbance of this solution was then measured at a max of 515 nm. The IC50 value is then determined. The absorbance measurement data were analyzed for the percentage of antioxidant activity using equation 1 [18]:

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \quad (1)$$

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening is carried out to see the presence of secondary metabolites in a plant by the test method using dyes. During the reaction process, there may be changes in tea water in color changes, precipitates, ring formation, and foam formation [19]. The results of tea screening from two types of Moringa leaf samples, namely fresh and dried, can be seen in Table 1.

Based on the results of phytochemical screening in Table 1, it can be seen that tea water derived from fresh and dried Moringa tea leaves both contain secondary metabolites, namely flavonoids, saponins, and alkaloids. That can be seen in the change in color to orange in the flavonoid test, the formation of stable foam in the saponin test, the shape of a yellow precipitate in the alkaloid test using Mayer's reagent and brownish yellow precipitate using Wagner's reagent. Any changes in the tested samples indicated that the positive samples contained secondary metabolites [20]. Previous

studies revealed that fresh tea contains flavonoids, tannins, and triterpenoids, while dried Moringa tea also contains flavonoids, alkaloids, tannins, and saponins [21], [22].

Table 1. Phytochemical Screening of Fresh and Dried Moringa (*Moringa oleifera* L.) Tea

Phytochemical Compounds	Moringa Tea Screening Results		Information
	Fresh Leaves	***Dried Leaves***	
Flavonoids	+	+	The color changes to orange.
Terpenoids	-	-	Does not form a red, brownish red, or purple-red ring
Tannins	-	-	Does not change color to green or blue-black
Phenolics	-	-	Does not change color to green, red, purple, blue, or solid black
Saponins	+	+	Forms stable foam
Glycoside	-	-	Does not change color to blue or green
Alkaloids Mayer	+	+	Forms a yellow precipitate
Wagner	+	+	Forms a yellow-brown precipitate
Steroids	-	-	It does not form a bluish-green ring.

Moringa significantly contributes to treating diseases because it has pharmacological activity. The secondary metabolites contained in Moringa are alkaloids, flavonoids, carotenoids, tannins, anthraquinones, anthocyanins, proanthocyanidins, saponins, steroids, triterpenoids, coumarins, phenols, quinines [23]. Saponins, flavonoids, and alkaloids are metabolites with anti-cancer and anti-inflammatory activities. Alkaloid compounds in high concentrations have cytotoxic activity for cancer cells. Alkaloids have components of piperine, magnoflorine, pseudoephedrine, indirubin, esculetin, peiminine, and triptantin. These components have anti-ulcer, anti-carcinogenic, anti-asthmatic, anti-amoebic properties, rhinitis medicine, cold medicine, lowering blood pressure, anti-oxidation, anti-diabetic, anti-fungal, anti-viral, antibacterial, intestinal disease medication, tuberculosis (TB) drugs, and *Escherichia coli* infection drugs [24].

Saponin compounds inhibit the proliferation of cancer cells [25]. In addition, saponins can also treat hypertension and heart disease [26], as well as chemotherapeutic agents for malignant tumors in the brain (anti-glioblastoma) [27]. Other compounds are flavonoids. Flavonoids can reduce fat accumulation, which is beneficial for heart health or anti-atherosclerotic activity [28]. The components in flavonoid compounds such as quercetin, liquiritigenin, naringenin, diosmetin, baicalein, puerarin, formononetin, chamaejasmine, chrysin, and sulfurethine. These components have antioxidant, anti-tumor, and anti-inflammatory activity but have antibacterial, liver protection, anti-allergic, anti-oxidation, nerve protection, reduced insulin resistance, pain reliever (analgesic), vasodilating, sugar lowering drugs, blood and immunosuppressive drugs [29].

3.2. Fresh and Dried Moringa Tea Antioxidant

Moringa (*Moringa oleifera L.*) is a plant with mostly beneficial parts. The leaves can be used as food in the rainy and dry seasons and as a source of protein [30]. In addition, Moringa leaves also contain nutrients magnesium, iron, phosphorus, manganese, calcium, sodium, zinc, potassium, and sodium. Moringa seeds and flowers provide vitamin C, protein, calcium, and potassium. Moringa root can be an essential ingredient in making medicines as a paste [31]. Moringa is a green vegetable throughout Asia and Africa [32]. Moringa leaves are rich in nutrients and compounds that play a role in the treatment and improvement of human and animal health. One of the benefits of Moringa is as an antioxidant. Natural antioxidants are increasingly in demand because of their role in treating disease. Several studies have shown that antioxidant and anti-inflammatory peptides protect against reactive oxygen species (ROS), which contribute to reducing oxidative stress [33], [34], [35].

To determine the percentage of inhibition, samples of fresh and dried Moringa leaf tea were measured for absorbance using a UV-Vis spectrophotometer. The absorbance obtained from these measurements is to see the ability of the sample antioxidant compounds to capture free radicals at each concentration of the two types of Moringa tea. The test result data can be seen in Table 2.

Table 2. Test Result Data for Test Samples (Fresh and Dried Moringa Leaf Tea)

Test Sample	Concentration (ppm)	Sample Absorbance	% Inhibition
Fresh	125	1.305	11.10
	250	1.196	18.53
	500	1.044	28.88
	1250	0.432	70.57
Dry	500	0.881	20.63
	1250	0.796	28.29
	2500	0.583	47.48
	5000	0.26	76.58

From the data in Table 2, each test sample is regressed to obtain a linear regression equation y = ax + b, with concentration variations as x values and % inhibition as y values. The graph of the relationship between concentration and % inhibition in each test sample and vitamin C can be seen in Fig. 1., 2., and 3.

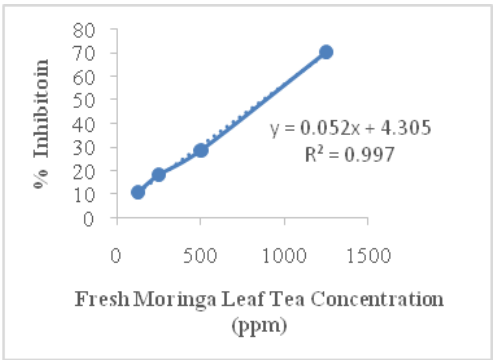

Fig. 1. Graph of the relationship of % inhibition with fresh Moringa Leaf tea concentration

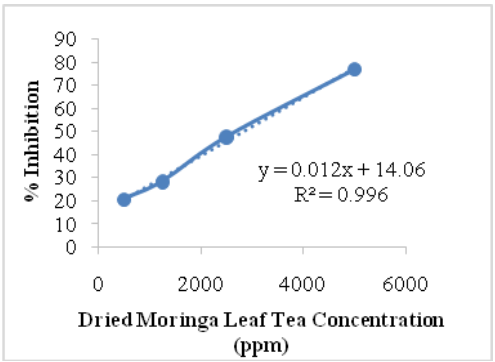

Fig. 2. Graph of the relationship of % inhibition with dried Moringa Leaf tea concentration

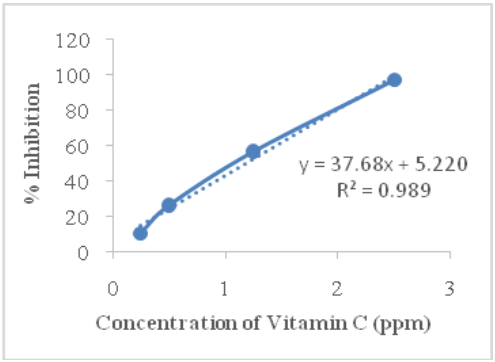

Fig. 3. Graph of the relationship of % inhibition with vitamin C concentration

Fig. 1., 2., and 3. show that the correlation coefficient (R2) of both test samples and vitamin C is above 0.75 for fresh Moringa tea 0.997, dry 0.996, and vitamin C 0.989. The three values are categorized as having a robust correlation, where the percentage of inhibition is influenced by the concentration of the sample [36]. Based on Fig. 1., 2., and 3., it can be seen that there was an increase in the percentage of inhibition at each increase in concentration. In other words, the higher the sample concentration, the higher the rate of inhibition [37].

To obtain the effective concentration of the extract in reducing DPPH free radicals (IC50), the linear regression equation obtained from Fig. 1., 2., and 3. above was used [38]. The comparison of the IC50 values of fresh, dried, and vitamin C Moringa tea is presented in Table 3.

Table 3. IC50 Value of Fresh, Dried Moringa Tea and Vitamin C

Sample	Line Equations	y Value	x Value
Fresh Moringa Tea	y = 0.052x + 4.305	50	868.72
Dry Moringa Tea	y = 0.012x + 14.06	50	2851.67
Vitamin C	y = 37.68x + 5.220	50	1.19

From Table 3 above, the IC50 value of fresh Moringa tea samples is 868.72 ppm, while the IC50 of dried Moringa tea samples is 2851.67 ppm. A substance has powerful antioxidant properties if it has an IC50 value of <10 ppm, strong if the value is between 10-50 ppm, moderate if the value is 50-100 ppm, weak if the value is between 100-250 ppm, and inactive if the value is >250 ppm [39], [40]. Previous research also explained that the smaller the IC50 value, the stronger the antioxidant activity of a compound or extract [41], [42], [43]. Based on the IC50 value, the results showed that the action of antioxidant compounds in fresh and dried Moringa tea was inactive because the value was more than 250 ppm. Several factors, including the solvent, the number of tea leaf samples, and the surface area of the tea leaves, cause the inactivation of antioxidant compounds in fresh and dry moringa tea. These factors are also supported by previous studies, which also stated that the low antioxidant activity measured was influenced by the solvent used. This study uses water as a solvent. The resulting tea water is then used to measure antioxidant activity. Water is a polar solvent, so not all of the antioxidant compounds in Moringa leaves are dissolved in tea water [44], [45]. In addition, the amount of moringa used was only a small amount, the surface area of the tea leaves was small, and the size of the chopped moringa leaves was not the same, causing no antioxidant activity. Moringa tea leaves are better made in powder form so that the surface area is large, the tea's compounds are also attracted, and the antioxidants can be measured properly [46], [47].

Although both of these moringa teas do not have antioxidant activity when compared to fresh and dry moringa tea, both of them have a ratio of IC50 value of 1:3, where the IC50 value of fresh moringa tea is three times lower than that of dry moringa tea. The antioxidant activity of moringa tea is also affected by the drying temperature of the tea leaves. Drying can reduce the water content in the process of making tea leaves. The lower air content can increase the levels of several components of secondary metabolites, which function as antioxidant compounds in Moringa tea leave [48]. In addition to temperature, climatic conditions or seasons, soil type, and location of growing moringa also affect. Previous research stated variations in antioxidant activity in moringa harvested at different locations and seasons [49], [50]. The low antioxidant activity in moringa tea does not change the benefits of moringa as a traditional medicine. However, one of the drawbacks of moringa tea is its unpleasant taste. Therefore, to eliminate the sour taste, it is usually added with honey or sweetener with other flavors according to taste to remove the bad taste [51].

The results of the antioxidant activity obtained from fresh and dry moringa tea differ from vitamin C, used as a standard in antioxidant tests. Based on Table 3, vitamin C has vigorous antioxidant activity because it has an IC50 of less than 50 ppm, namely 1.19 ppm. Previous research stated that the antioxidant activity of moringa tea has a high IC50 value ranging from 832.8 ppm to 4477 ppm. The range of IC50 values indicated that the tea samples had low antioxidant activity but had good antioxidant activity compared to ascorbic acid. This shows that the components that have antioxidant activity in Moringa leaves are polar compounds [52], [53], [54].

4. Conclusion

Based on the results of research and discussion, it can be concluded that both types of tea do not have antioxidant activity because the IC50 value is more than 250 ppm, namely 868.72 ppm for fresh Moringa tea and 2851.67 ppm for dry Moringa tea. However, when compared between fresh and dried Moringa tea, both have an IC50 value ratio of 1:3, where the IC50 value for fresh Moringa tea is three times smaller than that of dried Moringa tea. In addition, the results of the phytochemical screening of both types of tea were positive for containing several secondary metabolites, namely flavonoids, alkaloids, and saponins. Given the limitations in the study, it is hoped that for future research to conduct similar studies but using different drying techniques, the test samples will be made in the same size. Also, the surface area will be enlarged to make it easier for the components of the secondary metabolite to be tested to be attracted all into the solvent. Also, the immersion time of tea is made a relatively long duration to be able to prove the antioxidant activity of Moringa leaf tea (*Moringa oleifera* L.).

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