



# Phytochemical Characterization and Sensory Evaluation of Vinegar from *Melastoma malabathricum* L. Flowers with Variations in Starter Concentration and Fermentation Time

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**Abstract:** *Melastoma malabathricum* L. or Senggani is a shrub that is easily found in open meadows. Acehese people often use senggani as traditional medicine. The purpose of this study was to determine the phytochemical content and sensory evaluation of natural vinegar from Senggani flowers. The research design used a completely randomized design (CRD) which consisted of 5 days and 10 days of *Saccharomyces* administration. Both groups were added with *Acetobacter aceti* with concentrations of 5%, 10%, and 15% respectively in each sample. The result showed that phytochemical screening Senggani vinegar contains tannins, flavonoids, saponins, dragendrof, polyphenols, and terpenoids. The results of the organoleptic test show that the average ranking of panelists' preference for taste from the combination of the Senggani flower vinegar fermentation treatment ranges from 5.8-6.04, meaning that the taste of vinegar is slightly sour, while the panelists' preference ranking on acceptability ranges from 4.4-5, which means acceptability of panelists is neutral. The Senggani flower vinegar product with added *Saccharomyces* for 10 days and the fermentation of *Acetobacter aceti* with concentrations of 15% is the best formula preferred by the panelists. Senggani flower vinegar has the potential to be used as a health drink.

**Keywords:** Organoleptic; Phytochemical; Sensory; Senggani flower vinegar.

## Introduction

Indonesia has various types of plants that are used as daily food ingredients, as well as traditional medicines. One of the traditional medicines that are often used is herbal medicine. Herbs are an important source of medicine, and various examples have shown the potential to treat human diseases, especially complex and chronic diseases such as T2DM (Weng, 2019).

Recently, several medicinal plants have been reported to be useful for diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic treatment. The antihyperglycemic activity of plants is mainly due to their ability to restore pancreatic tissue function by causing increased insulin output or inhibiting intestinal glucose absorption or

facilitating metabolites in insulin-dependent processes (Balamurugan, 2014).

*Melastoma* is a type of wild plant that can be used as herbal medicine. The fruit, flowers and leaves of this plant are used for medicine and natural food coloring. *Melastoma malabathricum* Linn (MM) is a small shrub from the family Melastomaceae commonly found in tropical and temperate Southeast Asian countries, locally known by Malays as Sensaat, Indians as Phutki. *M. malabathricum* consists of three different varieties, having dark purple-magenta petals found in India, other dark purple-magenta petals, pink-magenta petals and other rare varieties that have white petals. Generally, different parts of *M. malabathricum* are used in traditional medicine for the treatment of dysentery, diarrhea, hemorrhoids, vaginal discharge, wounds and sores especially in India, Malay and Indonesia. Another

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infection used during confinement and also used to prevent smallpox scarring and hemorrhoids (Kumar, 2013).

*M. malabathricum* flower is known to contain quercetin and kaempferol, quercetin and kaempferol are natural polyphenols that have several antioxidant properties that can eliminate ROS through free radicals, metal ion chelation, inhibition of pro-oxidant enzymes, antioxidant activation, and enzyme detoxification (Isnaini, 2019).

The biological properties of several *Melastoma* species have been investigated. In particular, its anti-inflammatory, hemostatic, anticoagulant, cytotoxic, antibacterial, antioxidant, hepatoprotective, gastroprotective, and hypoglycemic activities have been studied. Tannins, flavonoids, and organic acids are recognized as the main active constituents in *Melastoma* spp (Zheng, 2021).

*M. malabathricum* is also considered a potential source of anthocyanins and these compounds often accumulate at high concentrations in flowers; so, in the future, it can be used as a source for the pharmaceutical industry and health supplements. To date, more than 30 phytochemicals have been identified in the flower extract of *M. malabathricum*; most of these constituents are common flavonoids, anthocyanins, and alkaloids (Zheng, 2019).

The use of vinegar as a functional drink is very popular today. Vinegar is produced from ethanol fermentation by acetic acid bacteria. Intake of vinegar is considered a food that raises blood glucose in humans. This review aims to discuss studies investigating the impact of vinegar intake on the glycemic profile in humans and putative mechanistic cellular pathways in human and animal models (Santos, 2019).

Vinegar is produced from raw materials containing starch or sugar by sequential fermentation of ethanol and acetic acid. The production of vinegar usually involves a first fermentation in which the simple sugars in the raw material are converted to alcohol by yeast. The resulting alcohol is further oxidized to acetic acid by AAB during the last fermentation (Budak, 2014).

Vinegar can also be used to treat joint pain, minimize the effects of bee stings, increase calcium absorption, eliminate toxins in the food you eat, eliminate stinging on reddened skin, lower cholesterol, prevent swelling in the body, protect the skin from the effects of the sun's heat, smoothen the passage of blood that is congested in the stomach (menstrual problems), smoothes the release of mother's milk, treats bile problems (clears bile from fat that forms stones), removes toxins, and removes dirty blood and improves blood flow.

Senggani vinegar products were carried out with various treatments and the length of fermentation has

not been reported. This research also carried out organoleptic tests to assess the quality of vinegar products. Sometimes organoleptic assessment can give very accurate assessment results. In some respects, a sensory assessment exceeds the accuracy of even the most sensitive instruments.

This study aims to determine the phytochemical characterization and sensory evaluation of natural vinegar from *M. malabathricum* L. flowers with variations in starter concentration and fermentation duration.

## Method

This research belongs to the type of experimental research with qualitative and quantitative approaches, using a completely randomized design (CRD), following the method of Rachmawati et al., (2020) with a modification of the addition of starter concentration (addition of *Acetobacter aceti* inoculum with a concentration of 5%, 10%, and 15%) and fermentation time (5 days and 10 days) and 2 storage treatments for 14 and 28 days.

In this study using 6 treatments, which consist of:

P1 : 5 days *Saccharomyces* +5% concentration of *Acetobacter aceti* + 28 days of storage.

P2 : 5 days *Saccharomyces* +10% concentration of *Acetobacter aceti* + 28 days of storage.

P3 : 5 days *Saccharomyces* +15% concentration of *Acetobacter aceti* + 28 days of storage.

P4 : 10 days *Saccharomyces* +5% concentration of *Acetobacter aceti* + 28 days of storage.

P5 :10 days *Saccharomyces* +10% concentration of *Acetobacter aceti* + 28 days of storage.

P6 : 10 days *Saccharomyces* +15% concentration of *Acetobacter aceti* + 28 days of storage.

The acetic acid product was analyzed using the phytochemical test method, to determine the presence or absence of alkaloids, flavonoids, tannins, saponins, quinones, triterpenoids, and steroids (Rumagit, 2015) in fruit and flower vinegars and flowers of *Melastoma malabathricum*. Alcohol content and sugar content were measured using UV-Vis spectrophotometry method. While the pH value is known by using a universal pH indicator. The dose used in the phytochemical test of fruit vinegar and senggani flowers was 2 mL in each test (Rosidah, 2016). Then, the quantitative approach of this research includes data collection and data analysis using statistical methods.

### *Senggani Flower Vinegar Manufacturing Procedure*

Weighed 250gram flower, sAfter that, the senggani flowers are cleaned or washed with water. Then added 750 ml of water (aquadest) and 50 grams of sugar.

The mixture of leathers is then filtered to get the filtrate or juice of the fruit and senggani flowers. The filtrate was pasteurized at 65°C for 15 minutes at the Chemistry Education Laboratory, Faculty of Teacher Training and Education, Syiah Kuala University. Then pasteurized fruit and flower juices were added with *Saccharomyces cerevisiae* as much as 1% of the total sample weight. Then each sample was fermented at room temperature for 5 and 10 days (each fruit, flower and mixture filtrate was divided by 2) under anaerobic conditions (without air). After the 5th and 10th days, the sample was added with *Acetobacter aceti* inoculum with 3 concentrations, namely 5%, 10% and 15%. Then the filtrate was fermented again into 2 storage treatments, namely 14 and 28 days under aerobic conditions (with air).

*Vinegar Organoleptic Test Procedure Senggani Flower*

Organoleptic testing can be classified in several ways, namely the differentiation test, the acceptance test, the scale test and the descriptive test. This study uses acceptance test as the method used, acceptance test is used to assess new products, by predicting consumer (market) acceptance.

The chemical and organoleptic properties of vinegar are a function of the starting material and the fermentation method. Acetic acid, the volatile organic acid that identifies the product as vinegar, is responsible for the sour taste and pungent odor of vinegar. Vinegar is also a dietary source of polyphenols, compounds synthesized by plants to defend against oxidative stress. Ingestion of polyphenols in humans increases antioxidant protection in vivo and reduces cancer risk (Johnston, 2006).

The results obtained with this test are very subjective, so no extreme panelists are used for certain products. In the development of the need for market acceptance testing is important, in this context to test whether a product is accepted or not accepted by the market (society). This method is used to measure the subjective attitude of consumers towards products based on organoleptic properties. The results obtained are acceptance (like or dislike), aroma (typical acetic acid or not), taste (very sour or not) and also color assessment of the Senggani flower vinegar product (Table 1). There are 20 untrained panelists and 5 trained panelists.

**Table 1.** Assessment criteria on the hedonic test scale

Numerical Scale	Color	Smell	Flavor	Hedonic Scale Reception
1	Clear	Very atypical of acetic acid	not very sour	Very dislike
2	Yellowish brown	Very atypical of acetic acid	Not too sour	Very dislike
3	Brownish Yellow	Not typical of acetic acid	Not sour	hate
4	Pale Brownish Purple	Slightly unlike acetic acid	a little not sour	Kinda don't like it
5	Medium Brownish Purple	Neutral	Neutral	Neutral
6	brownish purple	Slightly characteristic acetic acid	slightly sour	kinda like
7	pale purple	Typical acetic acid	Wry	Like
8	Medium Purple	Very characteristic of acetic acid	Very Acid	Really like
9	Purple	Very many characteristics of acetic acid	Very sour	Really like

*The procedure of Senggani Flower Vinegar Phytochemical*

The method in carrying out the phytochemical test follows the method of Rosidah et. al., (2016) (with the following modifications:

a. Identification of Tannins

A total of 50 mg of sample was added to 5 mL of distilled water then boiled for 5 minutes and then filtered. Then 3 drops of the filtrate were added with 3 drops of 1% FeCl<sub>3</sub>. The presence of tannins is indicated by the formation of a dark blue or greenish-black color.

b. Identification of Alkaloids

A total of 50 mg of the sample was added with 10 mL of chloroform and 3 drops of ammonia. The chloroform fraction was separated and acidified with 10 drops of 2M H<sub>2</sub>SO<sub>4</sub>. The acid fraction formed was taken and divided into 3 parts, then each part was added with 1 drop of Dragendorff reagent, 1 drop of Meyer's reagent,

and 1 drop of Wagner's reagent. The presence of alkaloids was indicated by the formation of a red precipitate on Dragendorff's reagent, a white precipitate on Meyer's reagent and a brown precipitate on Wagner's reagent.

c. Identification of Flavonoids

A total of 50 mg of sample was added to 10 mL of distilled water and then heated for 5 minutes. Then filtered and the filtrate was taken. The filtrate obtained was added with Mg powder, 1 mL concentrated HCl, and 1 mL amyl alcohol and then shaken. The presence of flavonoid compounds is indicated by the appearance of red, yellow, or orange colors on the amyl alcohol layer.

Flavonoids are naturally occurring polyphenol metabolites distributed throughout the plant kingdom, and are found in large amounts in fruits, vegetables,

whole grains, nuts, seeds, teas, and traditional medicinal herbs (Han, 2016).

d. Identification of Saponins

A total of 50 mg of the sample was added with 5 mL of distilled water and then boiled for 5 minutes. Then the solution was filtered and the filtrate was shaken vigorously. The presence of saponins was indicated by the appearance of a stable foam for 10 minutes after shaking.

Saponins are natural sugar conjugated compounds that have many biological activities such as medicinal properties, antimicrobial activity, antiviral activity, etc. Saponin production is part of the normal growth and development process in many plants and plant extracts (Sharma, 2021). The structural classification of saponins is mainly based on their sapogenin framework, which can be divided into two main groups—triterpenoid saponins and steroidal saponins (Juang, 2020).

e. Identification of Triterpenoids and Steroids

A total of 50 mg of the sample was added with 2 mL of 30% ethanol then heated for 5 minutes and filtered. The filtrate formed was evaporated and then 1 mL of ether was added. Furthermore, Liberman Buchard's reagent was added which contained 3 drops of acetic anhydride and 1 drop of H<sub>2</sub>SO<sub>4</sub>. The presence of triterpenoids is indicated by the formation of a red or purple color, while the presence of steroids is indicated by the formation of a green color.

Data analysis

Organoleptic test data were analyzed by non-parametric statistical methods using Friedman's test. Taste and aroma were analyzed manually using analysis of variance and if the variance had a significant or very significant effect (P<0.05), then proceed with the Least Significant Difference Test (BNT) (Yitnosumarto, 1993). Meanwhile, to determine the best treatment in the study, it was calculated using the value of the effectiveness index (Lindriati, 2021).

Result and Discussion

Senggani Flower Vinegar Phytochemical Test

Phytochemical testing is carried out to identify the content of active compounds contained in plants. In this study, the test was carried out by taking a small sample of the vinegar to be tested, then adding reagents or solutions according to the compounds to be identified. Based on the results of phytochemical screening on *Melastoma* flower vinegar, it shows that *Melastoma malabathricum* flower vinegar contains bioactive

compounds, namely flavonoids, saponins, dragendraf, tannins, polyphenols, and terpenoids (Table 2).

Table 2. Phytochemical test of senggani flower vinegar in various treatments

Phytochemical Test	P1	P2	P3	P4	P5	P6
Flavonoids	+	+	+	+	+	+
Dragendrof Wagner	+	+	+	+	+	+
Mayer	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Quinone	-	-	-	-	-	-
Polyphenol	+	+	+	+	+	+
Steroids	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+

Flavonoids are one of a group of antioxidants found in plants such as vegetables, fruits, and cereals that have been widely published. Flavonoids function as antioxidants by donating hydrogen atoms or through their ability to chelate metals, in the form of glucosides (containing glucose side chains) or in free forms called aglycones (Redha, 2010). In previous studies, it was explained that the levels of bound flavonoids in corn, wheat, oats and rice were relatively higher than other plants.

In the results of the flavonoid test, the sample showed positive results, which changed color to reddish purple. Flavonoids are polar compounds because they have a number of hydroxyl groups. Therefore, flavonoids are generally soluble in polar solvents such as ethanol. Ethanol functions as a flavonoid liberator from its salt form. The addition of concentrated hydrochloric acid serves to pronotate flavonoids to form flavonoid salts. After the addition of propanol and allowed to stand for 15-30 minutes, positive results were indicated by a change in the color of the solution to reddish purple. Reddish purple color indicates the presence of flavonoids as a result of reduction by concentrated hydrochloric acid and magnesium. (Harborne, 1996).

Wagner and Mayer tests showed negative results because these compounds were more sensitive when tested. The steroid test showed negative results, as evidenced by the fact that there was no color change to red-green-blue when the sample was added with Lieberman Burchard. The compound analysis was based on the ability of these compounds to form colors with concentrated H<sub>2</sub>SO<sub>4</sub> in an anhydrous acetic acid solvent (Rumagit, 2015).

Dragendorff's test is one of the phytochemical screening methods of the division of the alkaloid test

which serves to determine whether there are potassium alkaloid compounds in a plant. The dregendroff test showed positive results, indicated by a change in the color of the solution to brown-orange. The results of the dregendroff test were obtained by adding a solution of chloroform and 2N HCl or 2N H<sub>2</sub>SO<sub>4</sub> to the sample and then allowed it to stand, and giving 2 drops of Dragendorff.

Saponins are one of the chemical compounds of steroidal glycosides or triterpenes found in most plants and have an important role in health because they have broad biological activities. (Yanuartono, 2017). The saponin test showed positive results, indicated by the presence of bubbles in the solution. The saponin test was carried out by shaking the sample vertically, if there were already bubbles of approximately 1 cm and then 1 drop of 0.1 N HCl was added, then if there were permanent bubbles, it indicated the presence of saponins in the sample solution being tested. In the tannin test, positive results were obtained which were indicated by the presence of a cloudy white solution in the sample when gelatin was added. In the quinone test, negative results were obtained because the solution did not change color to red when 1% NAOH was added (Al-Rimawi, 2017).

Phytochemical tests are the first step in an effort to determine the content of active compounds contained in local medicinal plants so that they can be used appropriately and more widely (Kartina, 2019). Based on the results of phytochemical screening, it contains tannins, flavonoids and saponins that function as antioxidants. Trina (2014), explained that antioxidant compounds have potential as antidiabetics that can prevent the oxidation of glucose in the blood. It is known that tannic acid compounds (tannins) have antioxidant activity and have an antidiabetic effect. According to Parawansah (2015), the mechanism of action of flavonoid compounds is to increase glycogenesis so that there is no accumulation of glucose in the blood.

Vinegar is a liquid fermented from ingredients that contain starch and sugar. Vinegar made from fruits contains many functional components such as organic acids, vitamins, minerals, amino acids and phenolic compounds (Hamidatun, 2014). Vinegar produced by ingredients fed a normal (standard) diet. containing starch and sugar through two stages of alcoholic and acetous fermentation containing at least 4%. The combination of acetic acid, flavonoid active compounds and antioxidants contained in flower vinegar is thought to prevent oxidative reactions caused by free radicals (Zubaidah, 2015).

Vinegar has been widely researched and contains many benefits such as antioxidants, lowering cholesterol, blood sugar and preventing cardiovascular disease (Yulianti, 2019). In the melastoma plant, there

are many parts that can be used as drugs to lower blood sugar levels, as research has been done previously, because the melastoma plant contains many benefits for lowering cholesterol, one of which is anthocyanins. Anthocyanin is one of the flavonoid components from plants that can function as antioxidants that can prevent various types of damage caused by oxidative stress.

Phytochemical testing is carried out to identify the content of active compounds contained in plants that may be used as alternative medicines for some symptoms of the disease. In this study, the test was carried out by taking a small sample of the macerated extract, then adding reagents according to the compounds to be identified. There were several characteristics tested in the *Melastoma malabathricum* flower vinegar, namely flavonoids, dragendroff, wagner, maer, saponins, tannins, quinones, polyphenols, steroids, and terpenoids.

### *Senggani Flower Vinegar Sensory Evaluation*

Based on the results of the sensory test, it can be seen that the Senggani flower vinegar product was favored by the panelists. The results of the organoleptic test show that the average ranking of panelists' preference for taste from the combination of the Senggani flower vinegar fermentation treatment ranges from 5.8 to 6.04. The higher the average preference ranking of the panelists, the higher the level of preference of the panelists for the taste. The highest taste of Senggani flower vinegar in the treatment 5 days of *Saccharomyces* + 10% concentration of *Acetobacter aceti* + 28 days of storage (P2). The panelists' preference for color from the combination of Senggani flower vinegar fermentation treatment was around 3, all panelists liked the color of senggani fruit vinegar, which was brownish yellow. The panelists' preference for the acceptability of the combination of Senggani flower vinegar fermentation treatment ranged from 4.4 - 5. The highest public acceptance of senggani flower vinegar was obtained in the treatment 5 days of *Saccharomyces* + 10% concentration of *Acetobacter aceti* + 28 days of storage (P2), which is with an average value of 5. meaning that the panelists' acceptance is neutral to senggani flower vinegar. The taste of the senggani flower vinegar fermentation treatment of senggani flower vinegar ranged on average from 4-6 and the highest results were in the taste of the treatment 5 days of *Saccharomyces* + 10% concentration of *Acetobacter aceti* + 28 days of storage (P2), which is with an average rating of 6, meaning that the taste of vinegar is slightly sour after being fermented for a long time (Table 3). According to Trinugroho, (2012), it takes a relatively long time to produce the optimal amount of vinegar, so the process of increasing the level of vinegar takes place slowly on the effect of the taste of vinegar as for the

aroma, the average rating is 9 because the smell of vinegar is very distinctive with the aroma of acetic acid.

**Table 3.** Sensory test results of Senggani Flower vinegar

Treatment	Flavor	Color	Smell	Receptivity
P1	5.8 ±1.95	3 ±0	9 ±0	4.92 ±1.82
P2	6.04±1.67	3 ±0	9 ±0	5 ±1.80
P3	5.88±1.58	3 ±0	9 ±0	4.96 ±1.85
P43	4.88±2.00	3 ±0	9 ±0	4.4 ±1.93
P5	4.88±1.87	3 ±0	9 ±0	4.72 ±2.07
P6	5.16±1.95	9 ±0	9 ±0	4.64 ±1.8

Vinegar is a liquid that tastes sour and is made through alcoholic fermentation and acetate fermentation which is obtained from sugar-rich ingredients such as grapes, apples, malt, etc. Flavor or taste is a stimulus caused by the material eaten, which is felt by the sense of taste or smell, as well as other stimuli by the mouth. The taste parameter plays a role in determining the level of acceptance of a food ingredient by consumers. The lowest average panelist's preference is on 10 days of *Saccharomyces* + 5% concentration of *Acetobacter aceti* + 28 days of storage (P4) and 10 days of *Saccharomyces* + 10% concentration of *Acetobacter aceti* + 28 days of storage (P5), this is because the taste of the senggani flower vinegar has become slightly sour, bitter as well as bad.

Color becomes very important because of the visual factor in making decisions before buying and consuming food (Capule, 2016). The color obtained in the Senggani flower vinegar is strongly influenced by the time of sampling for the manufacture of vinegar. Because the longer the distance between the time of flower sampling and the time of manufacture, it will affect the color to be a little darker, due to the wilting factor of the flowers.

Smell has a key role in delicacy in food (Boesveldt, 2017) and quickly gives an outcome judgment of like or dislike (Anwara, 2019). The results of the organoleptic test on aroma showed that the panelists on average chose a value of 9, which is very typical of acetic acid. This is because during the process of making vinegar using acetic acid and also fermenting it the aroma of the acetic acid comes out into a distinctive aroma in vinegar.

Organoleptic is a test of food ingredients based on preferences and willingness to use a product. Panelists' sensory test results can be used to minimize risk in making decisions needed to identify the nature of food ingredients and can describe the product (Buckle et al, 1999). The organoleptic test is a test carried out with the human senses on food ingredients. This test is carried out by taking into account 4 parameters, namely odor, taste, acceptability, and color. The level of suitability of vinegar was observed with the level of preference of the panelists at the time of the assessment. The

questionnaire given uses a hedonic scale and an organoleptic test table according to SNI 2729:2013 issued by the National Standardization Agency (BSN).

The conditions that must exist in the organoleptic test are the presence of samples (samples), the presence of panelists, and honest response statements. There are six stages in this sensory assessment, namely first receiving the material, recognizing the material, clarifying the properties of the material, recalling the material that has been observed, and re-elaborating the sensory properties of the product (Rifky, 2013).

The acceptability test is an organoleptic test that involves panelists on the experiment or experiment being carried out. The organoleptic test was carried out by 5 trained panelists, namely the lecturer of the Family Welfare Education Study Program who would test the vinegar product under study. Panelists gave their assessment of flower vinegar that had been stored for 28 days. The assessment includes several factors, namely taste, aroma, color, and public acceptance of flower vinegar. Taste is one of the factors that affect the acceptance of a food product that has been produced. Taste can be determined by taste and oral stimulation. The texture and consistency of an ingredient will affect the taste caused by the material (Safrida, 2022). Taste arises due to chemical stimuli received by the sense of taste. If consumers do not like the taste of a food product, consumers will not accept the product.

#### *Best Treatment Selection*

Determination of the best treatment for various combinations of Senggani flower vinegar fermentation treatment was carried out using the effective index method (Susrini, 2005). The best treatment test results were obtained from the combination of P2 fermentation treatment with a product value of 5 with the average characteristic value of the panelist preference for taste 6, colors 3, and aroma 9.

## **Conclusion**

The Senggani flower vinegar product with added *Saccharomyces* for 10 days and the fermentation of *Acetobacter aceti* with concentrations of 15% is the best formula preferred by the panelists. Senggani flower vinegar has the potential to be used as a health drink.

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