

Toxicity and Antioxidant Activity of Teabags of Mangrove Lenggadai (*Bruguiera parviflora*) and Stevia (*Stevia rbaudiana* Bertoni) Leaves

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ABSTRACT

Introduction: Mangrove plants are known to have a lot of potential as medicine. One of the most commonly found mangroves is the lenggadai mangrove (*Bruguiera parviflora*). Utilization of herbal drinks is made to prevent the onset of various diseases. The mixed teabag of mangrove lenggadai leaves and stevia leaves (*Stevia rbaudiana bertoni*) is named Mangrove Lenggadai Stevia (MLS) teabag. **Aims:** the purpose of this study is to see which MLS teabag formulation has good antioxidant activity and toxicity activity. **Methods:** The research conducted was experimental in nature. The data collected are quantitative and qualitative data which are taken from the results of sample collection, physical characteristics of MLS teabag quality, phytochemical screening of simplisia, antioxidant activity test using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) method, toxicity test using Brine Shrimp Lethality Test (BSLT) method on *Artemia salina* Leach shrimp larvae, stability and hedonic level. **Result:** MLS teabags have characteristics according to the quality requirements of SNI 4324:2014. The results of determining the antioxidant activity of MLS teabags obtained an IC_{50} value of 13.38 $\mu\text{g/mL}$. The results of the toxicity test of MLS teabags obtained an LC_{50} value of 8.74 $\mu\text{g/mL}$. **Conclusion:** MLS tea bags have physical quality characteristics that meet the requirements of SNI 4324: 2014 and have the potential as antioxidants and toxicity activity against *Artemia salina* Leach.

KEYWORDS: Antioxidant, lenggadai, mangrove, stevia, toxicity.

INTRODUCTION

Mangrove forests have been utilized by coastal communities for generations as a source of food and traditional medicines. However, knowledge related to the utilization of mangrove forests has not been scientifically proven. (Gazali et al., 2020). Mangrove plants are known to have a lot of potential as medicine. Mangrove plants themselves are known to contain secondary metabolite compounds that are

used as anticancer, antibacterial, antimalarial, antiviral and antioxidant. (Rahmah et al., 2021). One of the most commonly found mangroves is the lenggadai mangrove (*Bruguiera parviflora*), a member of the Rhizophoraceae family that grows scattered in mangrove forests. (Thuy & Tung, 2022).

The leaves of lenggadai mangrove contain more simple polyphenols around

10.27% than other mangrove species. All mangrove species contained catechin compounds ranging from 0.81-1.83%, indicating their potential as green tea ingredients (Septiana & Harlis, 2018). According to research conducted by Thuy & Tung (2022) *Bruguiera parviflora* showed antioxidant activity with an IC₅₀ value of 14.4 µg/mL. These findings contribute to the knowledge of the phytochemical properties of *Bruguiera parviflora* leaves and suggest that this plant is a natural source of antioxidants. According to research conducted by Egra et al., (2023) Toxicity testing with the Brine Shrimp Lethality Test (BSLT) method obtained very strong category results, namely ethyl acetate and methanol leaf extracts, *Bruguiera parviflora* stem bark extracts with LC₅₀ values of each plant less than 250 µg/mL.

Herbal concoctions derived from plants are proven to treat diseases (Puspita et al., 2018). Utilization of herbal drinks is made to prevent the onset of various degenerative diseases and to increase endurance (Syafurudin & Hamidah, 2009). Indonesian people have long utilized plants into traditional medicinal herbs as an effort to prevent disease, maintain and care for health. In addition to being used as a traditional medicinal herb, one of the innovative plant utilization products is

antioxidant herbal tea (Triandini et al., 2022).

Natural antioxidants come from secondary metabolites in plants, namely flavonoids (Supriatna et al., 2019). Flavonoids are one of the natural compounds found in plants and food (Arifin & Ibrahim, 2018). Flavonoid, alkaloid and triterpenoid compounds are also said to be anticancer by inhibiting the division mechanism and activating the cancer cell apoptosis pathway (Gusungi et al., 2020).

Based on this description, the researcher is interested in conducting research with the title “toxicity test of mangrove lenggadai leaf (*Bruguiera parviflora*) and stevia (*Stevia rbaudiana* Bertoni) tea bags and antioxidant activity test”. Herbal teabag of lenggadai mangrove leaves has a distinctive smell and taste of herbal tea, therefore, efforts need to be made to cover this taste by adding stevia leaves as a natural sweetener. The mixed teabag of lenggadai mangrove leaves and stevia leaves was named MLS teabag. the purpose of this study is to determine MLS tea bags that have antioxidant activity, toxicity activity and characteristics according to the requirements of SNI 4324: 2014.

Table 1. Teabag formula

Materials	Quantity (g)				
	F1	F2	F3	F4	F5
Mangrove lenggadai	0	0.5	1	1.5	2
Stevia	2	1.5	1	0.5	0

MATERIAL AND METHODS

The tools used in this study are microplate reader 96 (Epoch BioTek) and micro pipette (Eppendorf), oven (B-One), water bath (B-One), azeotrope, desiccator (Duran), furnace (Muffle Furnace), ultrasonic cleaner (Granbo), *Artemia salina* Leach egg hatching vessel.

The materials used in this study were lenggadai mangrove leaves (*Bruguiera parviflora*), Stevia leaves (*Stevia rbaudiana bertonii*) in Meranti Islands Regency, Riau Province, Sodium Hydroxide (NaOH) 2N (Emsure), iodine-free salt (Refina), *Artemia salina* Leach eggs (Supreme Plus), methanol p. a (Smart lab), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Smart lab).

The teabag formula of lenggadai mangrove leaves and Stevia leaves which can be referred to as MLS teabag, is made with various mixtures in Table 1.

Characterization of MLS Teabags

Determination of MLS tea bag moisture content

Heat the crucible and lid in an oven (105±2°C) for ±1 hour and cool in a desiccator. Put 5 g of sample into the

crucible, cover and weigh (W1). Heat the crucible containing the sample in an open state by placing the crucible lid next to the crucible in the oven at (105±2°C) for three hours. Close the crucible while it is still in the oven, transfer it immediately to a desiccator and cool it for 20 minutes to 30 minutes then weigh it. Warm up again for one hour and weigh until it reaches a fixed weight (W2). Do triplo work and calculate the water content in the sample (Badan Standarisasi Nasional, 2014).

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W} \times 100\%$$

Description:

W are sample weight (g)

W1 are weight of crust and sample before drying (g), W2 are weight of crust and sample after drying (g) Determination of ash

Determination of total ash

Heat the crucible in a furnace at a temperature of (525±25°C) for approximately one hour and cool in a desiccator for 30 minutes then weigh with an analytical balance (W0). Carefully weigh a 5 g sample, place it in the crucible and weigh it (W1). Place the crucible containing the sample in a furnace at a temperature of (525±25°C) until white ash is formed. Transfer immediately to a desiccator and cool for 30 minutes then weigh (W2). Do triplo work and calculate the total ash content in the sample (Badan Standarisasi Nasional, 2014)

$$\text{Total ash content (\%)} = \frac{W_2 - W_1}{W} \times 100\%$$

Description:

W are sample weight (g)

W1 are weight of empty krus (g), W2 are weight of krus and sample after drying (g)

Determination of Water Soluble ash Content in MLS Tea Bag

The sample used is the ash from the determination of total ash content (W). Add 20 mL of distilled water to the crucible containing the total ash, heat until almost boiling and filter with ash-free filter paper. Rinse the crucible and filter paper and its contents with hot water. Transfer the filter paper and its contents to the original crucible, evaporate carefully over a water bath. Fry in an electric furnace at (525±25°C) until carbon-free. Transfer immediately to a desiccator and cool for 30 minutes then weigh (W2). Do triplo work and calculate the water soluble ash content (Badan Standarisasi Nasional, 2014).

Water soluble ash content (%) :

$$\frac{W_1 - W_2}{W} \times \frac{100}{100 - KA} \times 100\%$$

Description:

W are sample weight in the determination of total ash (g)

W1 are total ash weight (g), W2 are weight of water insoluble ash (g), KA are moisture content (%)

Determination of Acid Insoluble ash Content in MLS Teabag Acid

The test sample is water insoluble ash. Add 25 mL of 10% HCl to the cup

containing the sample, cover the cup to avoid splashing and boil the solution carefully for ten minutes on a water bath. Cool and filter the solution using ash-free filter paper, Rinse using hot water until the wash water is free of acid. Place the filter paper and contents back into the crucible, evaporate carefully over a boiling water bath, then heat in a furnace at (525±25°C) until the particles are carbon-free. Immediately remove and cool the crucible in a desiccator for 30 minutes and weigh (W1). Do triplo work and calculate the acid insoluble ash content (Badan Standarisasi Nasional, 2014).

Acid insoluble ash content (%) :

$$\frac{W_1 - W_2}{W} \times 100\% \times 100\%$$

Description:

W are sample weight in determining total ash content (g)

W1 are weight of crust and acid insoluble ash (g), W2 are weight of empty crucible (g).

Antioxidant Activity

Antioxidant activity test with DPPH method used sample brew formulas F1, F2, F3, F4 and F5, each taken 50 µL was inserted into plate A, plate B, plate C, plate D, plate E. Blank DPPH methanol 50 µL was inserted into plate F. Aquadest 50 µL was put into plate G. Plate A to plate G except plate F was put 80 µg/mL DPPH as much as 80 µL. The test solution was allowed to stand for 30 minutes at room temperature

and dark conditions by wrapping the 96 well microplate using aluminum foil. After 30 minutes, the absorbance of the test solution was measured at a wavelength of 517 nm with a microplate reader and then processing or analyzing the test data to calculate the % inhibition value and IC₅₀ of the test sample (Lonteng et al., 2020), with the formula :

%Inhibition:

$$\frac{\text{DPPH absorbance} - \text{DPPH with extract}}{\text{DPPH absorbance}} \times 100\%$$

$$y = ax \pm b$$

Description:

y: % inhibition

a: slope

b: intercept

x: test concentration

Toxicity Testing with BSLT Method

The test vial was calibrated as much as 10 mL, put 5 mL of water of various formulas F1, F2, F3, F4 And F5 of mangrove leaf tea bags (*Bruguiera parviflora*) and Stevia leaves (*Stevia rbaudiana bertonii*) into the vial (each made in 3 vials). Furthermore, each vial is added with seawater up to 10 mL, for the control, 10 mL of seawater is put into the vial, then 10 *Artemia Salina* Leach larvae are put in each vial (Fatimah & Santoso, 2020).

Stability Level of MLS Teabags

The stability test was carried out by placing teabags for each formula in a container and stored for 2 weeks with open

and closed packaging (Hutabarat et al., 2022). Testing the stability of the preparation and ensuring that there is no change in color, odor, taste and shape. The test is carried out by storing the preparation in two different places, namely at room temperature (15-30 °C) and cool temperature (8-15 °C). (Puspita et al., 2018)

MLS Teabags Hedonics

The data analysis used in this study was analysis using the statistical method of the SPSS (Statistical Product and Service Solution) 20 program. In this study, it was determined that the selection of the tea formula most preferred by respondents using organoleptic tests. Organoleptic testing carried out is the Hedonic Test which is carried out on 25 untrained panelists who are taken randomly. The panelists were given steeped tea bags of mangrove lenggadai leaves (*Bruguiera parviflora*) and Stevia leaves (*Stevia rbaudiana bertonii*) according to the mixture. Each panelist will taste the five tea formulas and be given a questionnaire and then the panelists are asked to give their opinions about the mangrove leaf and

Table 1. Water content of MLS teabag

Formula	Water content (%)	Requirement (SNI 4324:2014)
F1	5.9 + 0.577	≤10%
F2	5.3 + 0.057	
F3	3.7 + 0.208	
F4	2.4 + 0.288	
F5	1.5 + 0.305	

Table 2. Total ash content of MLS teabags

Formula	Water content (%)	Requirement (SNI 4324:2014)
F1	6.7 ± 0.115	4-8%
F2	6.7 ± 0.251	
F3	6.4 ± 0.208	
F4	5.4 ± 0.288	
F5	5.8 ± 0.650	

Table 3. Water soluble ash content of MLS teabag

Formula	Water content (%)	Requirement (SNI 4324:2014)
F1	4.2 ± 0.251	>45%
F2	5.9 ± 0.251	
F3	4.9 ± 0.513	
F4	4.5 ± 0.230	
F5	5.2 ± 0.854	

Stevia leaf teabags by filling out the questionnaire provided with a value scale of 1-5, where scale 1 = very dislike, scale 2 = dislike, scale 3 = neutral, scale 4 = like and scale 5 very like (Soekarto, 2012).

RESULTS AND DISCUSSION

Characteristics of MLS Teabags

The results of water of MLS formula 1 clear yellowish green color, typical tea smell and typical tea sweetness, formulas 2, 3 and 4 clear yellowish color, typical tea smell and typical tea sweetness, while the results of water of lenggadai tea formula 5 clear yellowish color, typical tea smell and typical tea taste.

The results of the water content test of the teabags obtained are F1 5.9%, F2 5.3%, F3 3.7%, F4 2.4% and F5 1.5% which all formulas meet the requirements based on SNI 4324: 2014 for water content below 10%.

Table 4. Acid insoluble ash content of MLS teabag

Formula	Water content (%)	Requirement (SNI 4324:2014)
F1	0.7 ± 0.057	≤1%
F2	0.8 ± 0.000	
F3	0.7 ± 0.000	
F4	0.6 ± 0.000	
F5	0.4 ± 0.057	

The results in this study indicate that the determination of total ash content in MLS tea bags aims to determine the nature of the tea bags that have been made, the results of the total ash content of the tea bags obtained are F1 6.7%, F2 6.7%, F3 6.4%, F4 5.4% and F5 5.8% which all formulas meet the requirements based on SNI 4324: 2014 for total ash content of 4-8%.

The results in this study indicate that the determination of water-soluble ash content in MLS teabags aims to determine the nature of the teabags that have been made, the results of the water-soluble ash content of the teabags obtained are F1 4.2%, F2 5.9%, F3 4.9%, F4 4.5% and F5 5.2% which all formulas do not meet the requirements based on SNI 4324: 2014 for water-soluble ash content of at least 45%.

The results in this study indicate that the determination of acid insoluble ash content in MLS tea bags aims to determine the nature of the tea bags that have been made, the results of the acid insoluble ash content of the tea bags obtained are F1 0.7%, F2 0.8%, F3 0.7%, F4 0.6% and F5 0.4% which all formulas meet the requirements based on SNI 4324: 2014 for acid insoluble ash content

Table 5. Antioxidant MLS tea bag

Formulas	Concentration (µg/mL)	Ln Concentration	Average $\pm$ SD	Absorbance	% Inhibition	IC ₅₀ (µg/ml)
F1	0	0	0.229 $\pm$ 0.001	0.178	40.994	13.38
F2	5000	8.517	0.143 $\pm$ 0.001	0.091	69.724	
F3	10000	9.210	0.136 $\pm$ 0.001	0.084	72.044	
F4	15000	9.616	0.126 $\pm$ 0.001	0.074	75.359	
F5	20000	9.903	0.113 $\pm$ 0.002	0.062	79.558	

of maximum 1%.

The results of this study indicate that the determination of the extract content in water in MLS tea bags aims to determine how the properties of the tea bags that have been made, the results of the extract content in water from the tea bags obtained are F1 39.6%, F2 40.5%, F3 43.2%, F4 45.5% and F5 46.3% which all formulas meet the requirements based on SNI 4324: 2014 for extract content in water of at least 32%.

Antioxidant Activity Testing with DPPH

An increase in concentration leads to a decrease in absorbance value and an increase in percent inhibition. This indicates that the antioxidant components as H atom donors in the test solution have DPPH free radical reduction activity. The higher the concentration of the test solution, the less residual free radicals will be measured in the test solution because the test solution contains more components that can neutralize or reduce DPPH free radicals (Moektiwardoyo et al., 2019).

The antioxidant activity of the sample was determined by the magnitude of DPPH inhibition through % inhibition. % inhibition on free radical scavenging is the ability of an antioxidant to inhibit free radicals in relation to the concentration of the sample tested. The greater the percentage of sample inhibition, the higher the antioxidant activity, in other words, the more DPPH free radicals that are successfully suppressed. The higher the antioxidant activity value of a sample, the smaller the IC₅₀ value. Antioxidant activity is better if the IC₅₀ value is smaller because only a low concentration is needed to inhibit free radical activity by 50%. Specifically, it can be said that a sample has very strong antioxidant activity if it has an IC₅₀ value of <50 µg/mL, strong if it has an IC₅₀ value of 50-100 µg/mL, moderate if it has an IC₅₀ value of 101-250 µg/mL, weak if it has an IC₅₀ value of 251-500 µg/mL, while if it has IC₅₀> 500 µg/mL it can be categorized as inactive as an antioxidant (Muchtadi, 2013).

In the antioxidant activity test, the absorbance of the solution was measured

with a microplate reader. The test solution was first allowed to stand for 30 minutes so that the reaction between the compounds in the extract with DPPH free radicals takes place perfectly, causing a change in DPPH color from purple to yellow. Then the absorbance was measured at a wavelength of 517 nm, because at that wavelength the DPPH solution can absorb UV light maximally. The theoretical wavelength for DPPH measurement ranges from 515-520 nm (Tamunu et al., 2022).

The results of antioxidant activity testing of MLS leaf teabags can be seen in Table 5.

Furthermore, for testing the antioxidant activity of teabag water MLS. The purpose of making a formula is to see which formula is more effective against antioxidant activity. Then the measurement of the sample is then obtained absorbance of each formula on the sample. Then plotted the percent inhibition and concentration data so that the regression equation is obtained. In Table 5, the results of the linear regression equation for steeped tea water of mangrove leaves lenggadai and stevia are $y = 3.6117x + 40.631$ with a correlation coefficient value of $R^2 = 0.9825$. From the linear regression equation, the IC_{50} value of lenggadai mangrove leaf and stevia steeped tea water can be determined, which is 13.38 $\mu\text{g/mL}$, which can be said that the lenggadai mangrove leaf and stevia

steeped tea water used has very strong antioxidant activity because $IC_{50} < 50 \mu\text{g/mL}$. With the following calculation :

$$y = ax + b$$

$$50 = 3,6117x + 40,631$$

$$50 - 40,631 = 3,6117x$$

$$IC_{50} = 13,38 \mu\text{g/mL}$$

Antioxidant activity in tea is influenced by phenol compounds contained in the raw materials for making tea. Phenol compounds in each material include flavonoids, polyphenols, anthocyanins, tannins and essential oils (Arumsari et al., 2019). Mangrove plants themselves are known to contain flavonoids, polyphenols, tannins, phenolic compounds, chlorophyll, carotenoids, terpenoids and alkaloids (Rahmah et al., 2021). Research conducted by Bui et al (2022) found that *Bruguiera* genus plants contain secondary metabolite compounds of alkaloids, flavonoids, terpenoids. According to research conducted by Egra et al (2023) *Bruguiera parviflora* has antioxidant activity based on its activity. In addition, based on phytochemical tests conducted, *Bruguiera* mangrove plants contain secondary metabolite compounds such as tannins, flavonoids and saponins. According to research conducted by Hesturini et al (2023) the results of stevia extract contain alkaloids, flavonoids, tannins. Natural antioxidants come from secondary

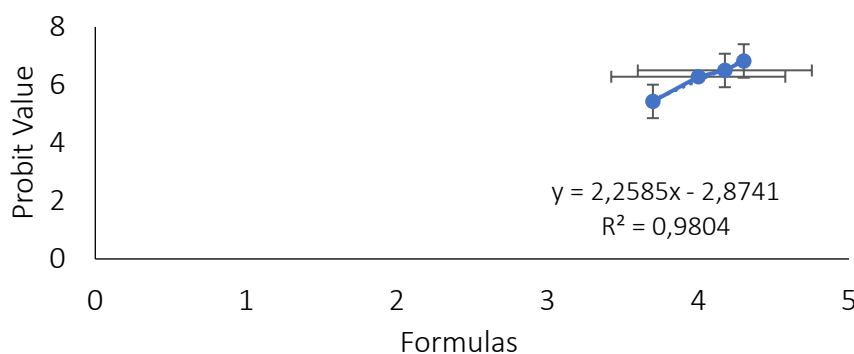


Figure 1. Toxicity testing results of MLS teabags

metabolites in plants, namely flavonoids (Supriatna et al., 2019).

Toxicity Testing with BSLT Method

After obtaining the percent mortality of *Artemia salina* Leach larvae, then it can be seen in the probit table from each value of the percent mortality obtained, the probit value is known by using the regression equation, were the value $y = 2.2585x - 2.8741$ (Figure 1). Then the concentration that causes 50% death of experimental animals is obtained $LC_{50} = 8.74 \mu\text{g/mL}$ with a very strong category. With the following calculation :

$$y = ax + b$$

$$50 = 4,8708x + 42,099$$

$$50 - 42,099 = 4,8708x$$

$$IC_{50} = 5,06 \mu\text{g/mL}$$

The mechanism of action of the compounds found in tea is to act as stomach poisoning. Therefore, if the compound enters the larval body, it will cause damage to the digestive system. The compounds can also inhibit taste receptors in the larval mouth area, preventing the larvae from receiving taste stimuli and recognizing food, causing

them to starve. Toxic compounds in the extract can be absorbed into the gastrointestinal tract through the mouth of *Artemia salina* Leach and the absorption process occurs through the cell membrane. After the absorption process, the distribution process of toxic compounds in the body of *Artemia salina* Leach continues and metabolic reactions occur which are destructive processes. The body anatomy of *Artemia salina* Leach at the naupliary stage is still very simple, consisting of the skin layer, mouth, petals, simple digestive tract and prospective thoracopods. The dramatic change in the concentration gradient between the inside and outside of the cell allows toxic compounds to spread well within the body of *Artemia salina* Leach. The resulting metabolic damage occurs rapidly, is detected within 24 hours and results in the death of 50% of *Artemia salina* Leach (Jelita et al., 2020).

The high toxic level of an extract is caused by the presence of alkaloid, terpenoid, tannin and steroid compounds (Kurniawan et al., 2022). Flavonoids

are one of the natural compounds found in many plants and foods (Arifin & Ibrahim, 2018).

Flavonoids belong to polyphenolic compounds, secondary metabolites of plants and have anticancer activity. Flavonoids contain quercetin which comes from the flavonol subclass. Quercetin, genistein or flavopiridol can be used as ingredients for cancer drugs (Ravishankar et al, 2013). Flavonoid, alkaloid and triterpenoid compounds are also said to be anticancer by inhibiting the division mechanism and activating the cancer cell apoptosis pathway (Gusungi et al., 2020). According to research conducted by Egra et al (2023) *Bruguiera parviflora* has antioxidant activity based on its activity. In addition, based on phytochemical tests conducted, *Bruguiera mangrove* plants contain secondary metabolite compounds such as tannins, flavonoids and saponins. According to research conducted Hesturini et al (2023) The results obtained from stevia extract contain alkaloids, flavonoids, tannins. Natural antioxidants come from secondary metabolites in plants, namely flavonoids (Supriatna et al, 2019).

Stabilituy Level of MLS Teabags

Stability of teabag preparations consisting of lenggadai leaves and stevia leaves after going through organoleptic tests for 2 weeks (14 days). The results of the research obtained by the preparation

of mangrove leaf tea lenggadai and stevia from all formulas are stable during storage in closed and open packaging both at room temperature (15-30°C) and cool temperature (8-15°C) both in taste, color, odor and dosage form, from all formulas no changes occur.

Determining the stability of herbs in the formula is important. Stability aims to ensure that the herbal drug/product remains within the specifications set to guarantee its identity, strength, quality and purity. It can be defined as the length of time under certain conditions and storage that a product will remain within predetermined limits for all its important characteristics. Any ingredient, whether therapeutically active or inactive, in a dosage form can affect stability. Environmental factors such as temperature, light, air (particularly oxygen, carbon dioxide and water vapor) and humidity can affect stability. Similarly, factors such as particle size, pH, the nature of water and other solvents used, the nature of the container and the presence of other chemicals resulting from contamination or from intentional mixing of different products can affect stability (Thakur et al., 2011).

MLS Teabag Hedonics

Food flavor is an assessment factor for the results of combining ingredient formulas in the manufacture of a food

Table 6. Hedonic of MLS teabag

Formula	Taste	Color	Smell
F1	3.28	3.96	4.00
F2	3.44	3.96	4.08
F3	3.84	4.04	4.08
F4	4.08	4.12	4.16
F5	3.32	4.00	4.28

product. Taste is a determinant of the level of liking in food products assessed by the tongue, by measuring sweetness, sourness, bitterness or other combinations (Yuli et al., 2020). The average results of hedonic testing of the taste category of mangrove leaf lenggadai and stevia tea bags can be seen in Table 6.

The highest panelist liking value was found in MLS F4 teabag at 4.08 (like). While the lowest panelist liking value was found in F1 MLS teabag of 3.28 (neutral). F2 scored 3.44 (neutral), F3 by 3.84 (neutral) and F5 by 3.32 (neutral). In Table 6, it can be seen that each formula has different score results, this is because each formula has a different concentration of lenggadai mangrove leaves and stevia, causing different taste effects. The results of the one-way ANOVA (Analysis Of Variance) test showed that the MLS teabag formula had a significant effect ($p > 0.05$), namely 0.009. The results of the analysis tested further using Duncan's Multiple Range Test (DMRT) showed that MLS teabags F1, F2, F3 and F5 had significant differences. Teabags of mangrove leaves lenggadai and stevia F3 and F4 have significant

differences with F1, F2 and F5. This is in accordance with research conducted by Desy et al (2020) that the addition of stevia leaves to tin leaf teabags has a significant effect on the taste produced ($P < 0.05$). Research conducted by Asmono et al (2021) showed that arabica coffee drinks with the highest addition of stevia powder (2 g) had the lowest level of liking. The sweetness in stevia leaves comes from stevioside compounds. The high level of sweetness of steviosida causes the use of stevia in large quantities to give a taste that is too sweet.

Color is the first impression that panelists get before recognizing other stimuli. Color is very important for every product so that attractive colors will affect consumer acceptance (Aquastini & Rita, 2019). The average results of hedonic testing for the color category of MLS teabags can be seen in Table 6.

The highest panelist liking value was found in MLS F4 teabag at 4.12 (like). While the lowest panelist liking value was found in F1 and 2 MLS teabags of 3.96 (neutral). F3 is 4.04 (like) and F5 is 4.00 (like). In this table, it can be seen that each formula has different score results, this is because each formula has a different concentration of lenggadai mangrove leaves and stevia, causing different color effects. The results of the one-way ANOVA (Analysis Of Variance) test of the MLS teabag formula

did not have a significant effect ($p > 0.05$), namely 0.967. The results of the analysis were further tested using Duncan's Multiple Range Test (DMRT) MLS teabags F1, F2, F3, F4 and F5 did not have significant differences. This is in accordance with research conducted by Arumsari et al (2019) The results of statistical analysis of panelists' liking for tea color with a combination of kecombrang flower and mint leaf formulas have no effect on the level of panelist acceptance of color. The results of the nonparametric statistical test with the Friedman test with a 95% confidence level obtained a p value of 0.086 ($p > 0.05$) so it can be concluded that there is no influence between treatments.

Smell is one of the important parameters because it can directly affect the perception of good taste of a food or drink Asmono et al (2021). The average results of hedonic testing for the smell category of lenggadai mangrove leaf and stevia tea bags can be seen in Table 6.

The highest panelist liking value was found in the MLS F5 teabag of 4.28 (like). While the lowest panelist liking value was found in MLS F1 teabag at 4.00 (like). F2 and 3 got a score of 4.08 (like) and F4 of 4.16 (like). In the table 6 it can be seen that each formula has different score results, this is because each formula has a different concentration of lenggadai mangrove

leaves and stevia so that it causes a different smell effect.

The results of the one way ANOVA (Analysis Of Variance) test in Appendix 25 show that the MLS teabag formula does not have a significant effect ($p > 0.05$), which is 0.884. The results of the analysis tested further using Duncan's Multiple Range Test (DMRT) which can be seen in appendix 25 show that MLS teabags F1, F2, F3, F4 and F5 have no significant difference. This is in accordance with research conducted by (Desy et al., 2020) on hedonic sensory testing, it is known that the difference in the addition of stevia leaf concentration does not have a significant effect ($P > 0.05$) on the smell of tin leaf tea. The average hedonic quality value of tea smell is in the range of 3.52-3.72 (like)

CONCLUSION

Water Teabags of lenggadai mangrove leaves (*Bruguiera parviflora*) and stevia leaves (*Stevia rbaudiana bertonii*) have characteristics in accordance with the quality requirements of SNI 4324: 2014, only the ash content insoluble in water does not meet the requirements. Dyed tea of mangrove lenggadai leaves (*Bruguiera parviflora*) and stevia leaves (*Stevia rbaudiana bertonii*) has antioxidant activity with an IC₅₀ value of 13.38 µg/mL which is included in the category of very strong antioxidants. Dyed tea of mangrove lenggdai leaves (*Bruguiera parviflora*) and

stevia leaves (*Stevia rbaudiana bertonii*) has toxicity activity against *Artemia salina* Leach, which shows an LC50 value of 8.74 µg/mL which is included in the highly toxic category. Further research directly on cancer cells is expected to prove the anticancer activity of lenggadai mangrove leaves (*Bruguiera parviflora*).

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