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FORMULATION AND TEST OF ANTIOXIDANT ACTIVITY OF TEA BAG COMBINATION OF BLACK (Camellia sinensis OKvar. assamica (Mast) AND MANALAGI APPLE (Malus sylvestris (L.) Mill.)

Ronal Tolkhah[1)], Indra Kurnia Setyani[2)], Ricka Prasdiantika[3)], Safna Anggita Widiastuti[4)]

[1)]Anafarma Lecturer, [2)]Anafarma Lecturer, [3)]Anafarma Lecturer, [4)]Anafarma Department Student
Anafarma Diploma III Study Program, Health Polytechnic, Ministry of Health, Surakarta

Email:rickaprasdiantika@gmail.com

Abstract: Tea bags are a single dry tea product or a mixture of several types of tea or with the addition of other food ingredients that are permitted according to applicable regulations and are packaged and ready to be brewed. This research created a tea bag formulation from black tea (*Camellia sinensis* OK var. assamica (Mast)) and manalagi apple (*Malus sylvestris (L.) Mill*.) which has specific properties, namely antioxidants with a ratio of F1 (25:75), F2(50:50), and F3(75:25). This research aims to determine the quality test and antioxidant activity of tea bags combining black tea and manalagi apples. This research uses a quantitative descriptive method with parameters including organoleptic tests, water content, ash content and antioxidant activity. The organoleptic results of the tea bag have a brown tea color, smell typical of tea and taste bitter, slightly sweet. The moisture content test results for F1, F2, and F3 were respectively 5.426%; 4.476%; 3.686 %. The ash content test results for F1, F2, and F3 respectivelywere 3.611%; 5.537 %; 6.507 %. The quality test results are in accordance with SNI standards. The IC50 value of tea bag preparations in F1, F2, and F3 respectively was 61.91 ppm; 84.21 ppm; and 96.43 ppm. F1 has the strongest antioxidant activity due to the addition of more manalagi apples. The results showed that the antioxidant activity of the black tea and manalagi apple formulations was included in the strong category.

Keywords :antioxidants, tea bags, black tea, manalagi apples

INTRODUCTION

Along with changes in lifestyle, many people want a product that is practical, easy to carry, easy to consume, has a high taste and is beneficial for the body. One type of product is a functional drink. Functional drinks are drinks which, when consumed, can have a positive impact on body health because they contain nutritional or non-nutritional elements, which play a role in protecting or preventing disease, increasing optimal performance of body functions, and increasing immunity or the body's immune power, use of plants which contain various compounds are more beneficial than single compounds for obtaining specific properties, one of which is antioxidants (Komsatun, 2022; Ryadha et al., 2021).

One of the functional drinks is tea in bag form. Tea bags are very popular with people around the world because tea bags can be enjoyed cold, hot and warm (Arumsari and Aminah, 2019). Tea bags are tea that is brewed using small bags. After the water turns brownish, the tea bag is removed and thrown away. The process of making this tea is very practical and clean without dregs, so consumers prefer tea preparations in the form of tea bags (Dewitayani et al., 2019). Tea bags can be made by combining black tea leaves and

manalagi apples.

According to Hakim et al (2022), black tea (Camellia sinensis OK var. assamica (Mast)) is the type of tea that is most widely produced in Indonesia and makes Indonesia the 5th largest black tea exporting country in the world. The main polyphenols in black tea are tannins and flavonoids which function as anti-free radicals or antioxidants. The tannins in tea are mostly composed of catechin, epicatechin, epicatechin gallate, epigalo catechin, epigalo catechin gallate and, gallocatechin (Fadhilah et al., 2021). The flavonoids in black tea are flavonols, namely quercetin, kempferol and myricetin (Utomo et al., 2015). According to research by Leslie and Gunawan (2019), black tea has antioxidant activity of 137.6 ppm. As a result of the processing process, the epigalo catechin gallate content in black tea is only 2.21%, the smallest compared to green tea or oolong tea, therefore its antioxidant activity needs to be increased and its stability maintained by adding manalagi apples (Bartosikova and Necas, 2018).

Manalagi apples (Malus sylvestris (L.) Mill.) are one of the most popular types of fruit and are liked by the public because of their sweet, slightly sour taste, lots of nutrients and various chemical compounds that are efficacious as antioxidants, including tannins, flavonoids (quercetin), and vitamin C (Pertiwi et al., 2016). Apple skin is often not consumed when consuming apples and is a waste product that is only used as a substitute for animal feed and plant fertilization. Apple skin is useful as an antibacterial, antioxidant and antiproliferative (Fadilah, 2020). Manalagi apples show strong antioxidant activity, namely 95.15 ppm (Putri et al., 2022).

The addition of apple flesh and skin to black tea not only adds taste and aroma, but can increase the antioxidant activity and stability of the tea preparation.

Tea bag preparations are tested based on quality requirements which include organoleptic tests, water content tests and ash content tests. According to SNI No. 01-3836-2013, it explains the quality requirements for tea bags which are organoleptically tested by observing the smell, taste, color, maximum water content of 8.0% and maximum ash content of 8.0% (BSN, 2013). Antioxidant activity can be measured using the DPPH method using a UV-Vis spectrophotometer tool. The DPPH method is used to determine how much antioxidant potential the teabag has, which gives the sample a distinctive purple color and uses small amounts of sample (Chandra et al., 2019).

Based on this background description, tea bags combined with black tea and manalagi apples can be used as a functional drink regarding the content and benefits of the ingredients used. This research was carried out with the aim of knowing the results of the formulation and antioxidant activity test of a combination of black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apple (Malus sylvestris (L.) Mill.) using the DPPH method.

METHODS

-This type of research is quantitative research. The definition of quantitative research is research aimed at processing numbers and used to examine a certain population or sample, as well as the use of research instruments in collecting data. The aim of this research is to determine the number of components that can be measured in numerical form (Sugiyono, 2018). This research will calculate a number of qualities including organoleptic tests, water content tests and ash content tests, as well as the antioxidant activity of teabags combining black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apples (Malus isylvestris (L.) Mill.)

The design of this research uses a descriptive design. This type of design is carried out to provide a more detailed description of a phenomenon, namely a natural phenomenon or man-made phenomenon, or is used to analyze or describe the results of a subject, but is not intended to provide wider implications (Adiputra et al., 2021). This research was conducted to describe the quality and antioxidant activity of teabags combining black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apples (Malus sylvestris (L.) Mill.)

This research uses a single variable, which only describes the elements and factors in each symptom. The variable in the research was a tea bag formulation combining black tea

(Camellia sinensis OK var. assamica (Mast)) and manalagi apple (Malus sylvestris (L.) Mill.) with sub variables, namely quality test and antioxidant activity test.

The tools used in this test are glass bottles, knives, cutting boards, basins, ovens (Sense), baking sheets, analytical scales (Labex), 40 mesh sieves, stirring rods (Pyrex), beakers (Pyrex, pipettes, spatulas, test tubes (Pyrex), measuring cup (Pyrex), vortex (Thermo scientific), measuring flask (Pyrex), cuvette, micropipette (Socorex), porcelain cup, crucible, desiccator, and UV-Vis Spectrophotometry (Raptor).

The ingredients used in making tea bags are gambyong kemuning black tea, manalagi apples. Materials used in the test included distilled water (Brataco), methanol pa (Merck), ascorbic acid, filter paper, DPPH (1,1-diphenyl-2-picrylhydrazil) (Aldrich), aluminum foil (Klinpak).

Ways of working

1. In this stage, plant determination is carried out and preparation of raw materials in the form of gambyong kemuning black tea and manalagi apples is carried out by drying which can be tested for organoleptics, water content tests, ash content tests and antioxidant activity tests based on the dosage form using the DPPH method on tea bags with a combination of black tea and fruit. manalagi apple.
   a. Plant determination
      Determination is carried out with the aim of finding out the truth of the plant to be studied, avoiding errors in collecting material and avoiding the possibility of mixing the plant to be studied with other plants. Determination of green tea leaves (Camellia sinensis (L.) Kuntze) and manalagi apples (Malus sylvestris (L.) Mill.) was carried out at the UPFe. Traditional Health Service, RSUP Dr. Sardjito which is located in the Tlogodringo Aromatic Garden, Tawangmangu, Kab. Karanganyar, Central Java.
   b. Preparation of raw materials
      The research sample was black tea Camellia sinensis OK var. assamica (Mast)) with the market name gambyong kemuning black tea which was purchased in the Karanganyar area and manalagi apples which were purchased at Bunder Market Sragen by choosing yellowish green fruit, smooth skin with no spots, bruises or holes.
   c. Making simplicia powder
      Manalagi apples are washed with running water. Then dry it using a tissue so that the remaining dirt sticks to the tissue. Chopping raw materials is done by chopping crosswise and to the appropriate size using a knife. Material that has gone through the chopping process can be dried using an oven at a temperature of 75 °C until dry. Dried black tea simplicia and manalagi apples are powdered using a blender. The simplicia powder was sieved using a 40 mesh sieve and then weighed (Fadilah et al., 2021).
   d. Making tea bags
      The powdered ingredients are weighed with a total weight of 2 grams for each tea bag and then put into a tea bag measuring 5 x 5.7 cm. Tea bags are brewed in 200 mL of hot water at a temperature of 90-100°C and steeped for about 5 minutes. (Fikri, 2021). The tea bag formulation is presented in Table 3.2.

Table 3. 2 Tea Bag Formulation

Formulation	Dry black tea (g)	Manalagi apples dry (g)	Total formulation/ packaging (g)
F1	0.5	1.5	2
F2	1	1	2
F3	1.5	0.5	2

Source: Atmadja and Yunianto Modification (2019)

e. Quality test
   Tea is tested based on quality requirements including organoleptic tests, water content tests and ash content tests (Rohmayanti et al., 2019).
   1) Organoleptic test
      Samples were tested using human senses including organoleptic elements, namely aroma, taste and color (Suryono et al., 2018). Based on BSN (2013) regarding packaged dry

tea, organoleptic tests in the form of smell, taste and color of the tea brewing water must be unique to the product.

2) Test water content
   The porcelain cup is first placed in the oven for 15 minutes at 105 ℃, then cooled and weighed. A sample of 2 grams was put into a cup of known weight, then put in the oven for 3 hours at a temperature of 105℃. After that, it is cooled in a desiccator for 15 minutes, after cooling it is weighed until the weight is constant. If the weight is not constant, you can carry out the same treatment until it is constant and the water content obtained is calculated (Handayani et al., 2019). A good water content for tea is a maximum of 8% w/w (BSN, 2013).
3) Ash content test
   The crucible was placed in the oven at a temperature of 100-105 ℃ for 15 minutes, then cooled in a desiccator and weighed until constant. The sample was weighed as much as 2 g in a crucible. After that, ash is carried out on a stove heater at a stable temperature until the color changes to whitish ash. The crucible was then removed, cooled in a desiccator, weighed until the weight was constant and replicated (Tahar et al., 2017). Ash content not more than 8% w/w (BSN, 2013).

f. Antioxidant activity test

1) Preparation of 50 ppm DPPH solution
   Making a 50 ppm DPPH solution is done by carefully weighing 2.5 mg of DPPH then putting it in a beaker, dissolving it with a little methanol, then putting it in a measuring flask and adding methanol to the 50 mL mark (Fadilah et al., 2021).
2) Preparation of blank solution and determination of maximum wavelength
   The blank solution was prepared by taking 2 mL of 50 ppm DPPH solution plus methanol PA to a limit of 5 mL. The solution was then incubated in a dark room for 30 minutes, covered with aluminum foil to avoid exposure to sunlight so as not to damage the free radical reduction process. Next, measure the absorbance at a wavelength of 500-540 nm at intervals of 2 using a UV-Vis spectrophotometer to obtain the maximum wavelength (Fadilah et al., 2021)
3) Preparation of quercetin solution as a positive control
   A total of 1.25 mg of quercetin was dissolved in methanol pa in a 5 mL volumetric flask, then homogenized to obtain a solution with a concentration of 25 ppm. The quercetin solution was then diluted to obtain concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm (Tahir et al., 2020).
4) Preparation of sample solution
   The sample solution was made by brewing 2 g of each formulation in 200 ml of hot water at a temperature of 90-100℃ for approximately 5 minutes (Fikriyah & Nasution, 2021). Then 2.5 mL of the sample solution was taken and put into a 5 mL measuring flask after which methanol was added until the limit mark (5000 ppm). Test solutions from the three formulations were made with concentrations of 20, 40, 60, 80, and 100 ppm put into a 5 mL volumetric flask and diluted using methanol pa to the limit mark (Tahir et al., 2020).
5) Test antioxidant activity using the DPPH method
   Antioxidant activity testing was carried out by measuring the absorbance of quercetin and 2 mL of tea bag sample solution was taken from each concentration and 3 mL of 50 ppm DPPH solution was added and then methanol was added to a limit of 5 mL. The solution was then incubated in a dark room for 30 minutes, covered with aluminum foil to avoid exposure to sunlight so as not to damage the free radical reduction process (Fadilah et al., 2021). Absorbance measurements for each control and sample were replicated for each concentration.
6) Data analysis
   Data from absorbance measurements were analyzed for the percentage of antioxidant activity and expressed in % inhibition which was determined using the equation:

   % Inhibition = standard absorbance / sample absorbance x 100%

   IC value50 is a number that indicates the concentration of the test sample which provides 50% immersion (capable of inhibiting or soaking the oxidation process by

Formulation	Dry black tea formulation (g)	Manalagi apples dry (g)	Total formulation / packaging (g)	
F1	F1 1.5	Light brown	Typical tea smell	Bitter a little sweet
F2	1	1	2	
F3	F2 1.5	0.5	2	
	F3	Dark brown	Typical tea smell	Bitter

50%). The IC50 value is determined by creating a linear curve between the concentration of the test solution (x-axis) and the % attenuation (y-axis) so that the equation y = bx + a is obtained where y is the % inhibition and x is the IC50 value (Safitri, 2022). The IC50 value can be calculated using the formula:

I.C50 = 50 – a / b

Information:

IC50 = % inhibition

a = intercept (intersection of the line on the y-axis)

b = Slope (slope)

RESULTS AND DISCUSSION

1. Plant Determination

   Green tea leaves (Camellia sinensis (L.) Kuntze) and manalagi apples (Malus sylvestris (L.) Mill.) were determined at the UPF Traditional Health Service of RSUP Dr. Sardjito which is located in the Tlogodringo Aromatic Garden, Tawangmangu, Kab. Karanganyar, Central Java. After determination, it can be confirmed that the plants used in this research are truly tea leaves used to produce black tea and manalagi apples.

2. Making Tea Bags

   Making tea bags uses a combination of black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apples (Malus sylvestris (L.) Mill.) which have been made into simplicia powder and then formulated as follows:

   Table 4. 1 Formulation for Making the Celup

   Each formulation was weighed, put into a tea bag and shaved. Based on the data above, it shows that the total weight of each tea bag formulation is 2 grams per bag.

3. Organoleptic Test

   The results of organoleptic tests on tea bag preparations can be seen in Table

   Table 4. 2 Organoleptic Test Results

   Informaton:

   F1: Black tea formulation: manalagi apple (25%:75%)

   F2: Black tea formulation: manalagi apple (50%:50%)

   F3: Black tea formulation: manalagi apple (75%:25%)

   From the data obtained, the teabag combination of black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apple (Malus sylvestris (L.) Mill.) in F1 produces a light brown color with a typical tea smell and a bitter taste that gives sweet taste at the end. In F2, the results were obtained with a brown color and a typical tea smell and a bitter taste which gave a sweet taste at the end. The dark brown F3 has a distinctive tea smell and also has a bitter taste. Based on Table 4.2, the results show that the color becomes more intense as more tea extract is added.

   The greater the ratio of black tea in the mixture, the more intense the brown color of the brew becomes, this is influenced by the tannin content in the tea. The tannin compounds in tea will undergo oxidation which causes the tannin to be broken down to produce theaflavin compounds which affect the brightness level of the tea brew and thearubigin which affects the stability of the color produced (Suseno et al., 2023). The bitter taste of the brew is influenced by the catechins in the tea leaves. According to research by Fadhilah et al (2021), catechins can influence the taste, color and aroma of tea.

4. Water Content Test

   The results of the water content test on tea bags can be seen in Table 4.3 below:

   Table 4. 3 Water Content Test Results

Formulation	Replication	% Ash content	Mean ± SD	Standard (SNI No. 01-3836-2013)
F1	I	3.153 %	3.611 % ± 0.42 %	Max 8.0 %
	II	3.992 %		
	III	3.688 %		
F2	I	5.15 %	5.537 % ± 0.37 %	Max 8.0 %
	II	5.725 %		
	III	5.891 %		
F3	I	6.337 %	6.507 % ± 0.14 %	Max 8.0 %
	II	6.609 %		
	III	6.576 %		

Water content testing was carried out 3 times in replication. Based on Table 4.3, the average value of water content in F1, F2 and F3 is 5.426%; 4.476%; and 3.686%. According to BSN (2013), the quality requirement for packaged dry tea water content according to SNI 3836:2013 is no more than 8%, in this study the water content of all formulations met the requirements.

The water content in the material determines the durability and shelf life of the product against microbes so that it is suitable for consumption. The water content increases with the greater the weight of manalagi apples and the lower the weight of black tea in the formulation so that the water content of F1 is the highest and the water content of F3 is the lowest. This is because the water content of manalagi apples is higher than the water content of black tea, in line with research by Fikriyah and Nasution (2021), namely the water content of black tea is 7.63% and the water content of manalagi apples is 24.60% (Pitriana et al., 2023)

5. Ash Content Test

The results of the ash content test on tea bags can be seen in Table 4.4 below:

Formulation	Replication	% Water content	Average ± SD	Standard (SNI No. 01-3836-2013)
F1	I	5.467 %	5.426 % ± 0.31 %	Max 8.0 %
	II	5.717 %		
	III	5.094 %		
F2	I	4.158 %	4.476 % ± 0.28 %	Max 8.0 %
	II	4.561 %		
	III	4,710 %		
F3	I	3.832 %	3.686 % ± 0.31 %	Max 8.0 %
	II	3.326 %		
	III	3.901 %		

Table 4. 4 Ash Content Test Results

Based on Table 4.4, the average percent of ash content in F1, F2, and F3 is respectively 3.611% ± 0.42%; 5.537 % ± 0.37 %; and 6.507 % ± 0.14 %. The ash content test results of a combination of black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apple (Malus sylvestris (L.) Mill.) are said to meet the quality requirements because they are not more than 8% in accordance with the parameters of SNI No. 01–3836–2013 for dry tea (BSN, 2013). The ash content increases as the amount of black tea added to each formulation increases. The mineral content of black tea is higher than manalagi apples, this content is in the form of potassium, flour, sodium, calcium, zinc, manganese and cuprum (Sahputra et al., 2018). A high ash content indicates that there is a lot of minerals contained in the sample, however, excess minerals are not recommended in the preparation, therefore a maximum limit for ash content is set. This is in line with research by Fikriyah and Nasution (2021), where the ash content of black tea is 5.8%

higher than the ash content of manalagi apples according to Sahputra et al (2018), namely 0.92%.

6. Antioxidant Activity Test using the DPPH Method

   Antioxidant activity testing was carried out by measuring the maximum DPPH wavelength between 500-540 nm. The results of measuring the maximum wavelength of the DPPH mother solution in the study were 516 nm with an absorbance value of 0.642. The IC50 values obtained from each quercetin formulation and comparison can be seen in Table 4.5 below:

Table 4.5 Antioxidant Activity Test Results

Sample	IC value50 (ppm)	Antioxidant Category
F1	61.91	Strong
F2	84.21	Strong
F3	96.43	Strong
Quercetin comparator	6.74	Very strong

Based on Table 4.5 shows the IC results50 on F1, F2, and F3 respectively 61.91 ppm; 84.21 ppm; and 96.43 ppm, including strong antioxidant activity. Based on this data, it is known that the more comparisons of manalagi apples are formulated, the resulting IC50 value increases. According to research by Leslie and Gunawan (2019), black tea has antioxidant activity of 137.6 ppm. Manalagi apples show strong antioxidant activity, namely 95.15 ppm (Putri et al., 2022). This shows that the antioxidant activity of manalagi apples is higher than black tea. According to Fauziyah et al (2022), Manalagi apples contain quercetin and vitamin C compounds which have large differences in redox potential so they can produce a synergistic effect in increasing antioxidant activity. Therefore, formulations containing more manalagi apples will produce stronger antioxidant activity, so that the % inhibition value also increases.

CONCLUSION

Based on tests carried out on a tea bag combination of black tea (Camellia sinensis OK var. assamica (Mast)) and manalagi apple (Malus sylvestris (L.) Mill.) the following conclusions can be drawn:

1. Organoleptic tests on F1 resulted in a light brown color with a typical tea odor and a slightly sweet bitter taste. In F2, the results were obtained with a brown color and a typical tea smell and a bitter, slightly sweet taste. The dark brown F3 has a distinctive tea smell and also has a bitter taste.
2. The water content tests for F1, F2 and F3 were respectively 5.426%; 4.476%; and 3.686% meets the requirements for good water content.
3. The ash content tests for F1, F2 and F3 were respectively 3.611%; 5.537 %; and 6.507% meets the requirements for good ash content.
4. The antioxidant activity test results of F1, F2 and F3 had antioxidant activity with IC50 values of 61.91 ppm respectively; 84.21 ppm; and 96.43 ppm is categorized as strong.

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