e-ISSN: 2987-0836 DOI: https://doi.org/10.59585/ijhs

Publish: Association of Indonesian Teachers and Lecturers
International Journal of Health Sciences (IJHS)
Journal Homepage: https://jurnal.agdosi.com/index.php/IJHS/index
Volume 2 | Number 3 | September 2024 |

Innovation of Benalu Jeruk Leaf Antioxidant Biscuits with a Combination of Sweet Potato Leaf in the Gen-Z Era

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Abstract

Free radicals are toxic by-products of aerobic metabolism and can cause oxidative damage and tissue dysfunction. Antioxidants can play a role in overcoming excess free radicals in the body by capturing free radicals and preventing chain reactions. Orange bitter melon leaves and sweet potato leaves are considered to contain flavonoids and anthocyanins that act as antioxidants. Therefore, a biscuit innovation was made with orange and sweet potato benalu leaf powder with antioxidants. This study aimed to determine the antioxidant content of biscuits with a combination of orange and sweet potato leaf powder. The biscuits were tested for water content and hedonic and antioxidant activity test using the DPPH method. The antioxidant test results showed that the IC50 value was 179.753, with a moisture content value of 2.21%. Hedonic test results show that the most preferred taste is in F1 because the use of the right simplisia powder ratio can provide an alluring taste. Things that must be considered from this study are that further experiments need to be carried out regarding the effect of giving biscuits containing antioxidants.

Keywords: Antioxidant, Simplisia Powder, Orange Benalu Leaf, Sweet Potato Leaf, DPPH Method

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1. Introduction

Free radicals are chemical species that contain one or more unpaired electrons in the outermost orbit and can generally show reactivity. Free radicals have a dual role in

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living systems, namely toxic by-products derived from aerobic metabolism that can cause tissue dysfunction and oxidative damage [1].

One compound that can overcome the excess of free radicals is antioxidants. Antioxidants are stable enough to donate electrons or hydrogen to free radical compounds and neutralize them, reducing their ability to carry out free radical chain reactions. Antioxidants function to overcome excess free radicals in the body that work by capturing free radicals and preventing chain reactions[2]. Antioxidants are grouped into 2, namely natural and synthetic antioxidants. Natural antioxidants are antioxidants obtained from natural materials, resulting from secondary metabolites of plants that produce active compounds such as flavonoid group compounds[3]. Synthetic antioxidants are obtained from synthesizing chemical reactions produced for commercial purposes[4]. There are concerns about the side effects of using synthetic antioxidants that can increase carcinogenesis, which encourages using natural antioxidants as an alternative source of antioxidants. One of the plants as a natural antioxidant is benalu jeruk and sweet potato benalu leaf.

The benalu jeruk leaf (*Dendrophthoe glabrescens* (Blakely) Barlow) are one of the plants included in the Loranthaceae family that lives on its host plant, namely citrus plants, as a parasite on plants and is widely used by the community with various properties [5]. Benalu jeruk is one of the traditional medicinal plants in Indonesia that is used as an anticancer [6]. Benalu jeruk is known to contain flavonoid compounds that can act to capture free radicals or function as natural antioxidants. In addition to containing flavonoid compounds, benalu jeruk leaf have chemical content, namely alkaloids, saponins, tannins and phenols [6]. The study states that the extract of benalu jeruk leaf has an IC50 of 54.490 ppm, indicating that the antioxidant activity is quite strong [7].

In addition, sweet potato (Ipomoea batatas L.) leaf are one of the natural sources of antioxidants containing phenolic acids, tannins, polyphenols, anthocyanins, and beta-carotene. Sweet potato belongs to the Convolvulaceae family and contains protein, zinc, vitamin B, iron, and calcium. Anthocyanins in sweet potatoes act as antioxidants, antihyperglycemic, antihypertensive, and anticancer. The study found that sweet potato leaf

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have an IC50 of 29.2631, indicating that the antioxidant activity is very strong. Using sweet potatoes in other forms will be more beneficial, such as saving storage, always being available regardless of the season, and being more flexible[8]. Compounds that can be said to be very strong antioxidants if the value is less than 50 ppm, strong 50-100 ppm, medium 100-150 ppm, and weak 151-200 ppm. The smaller the value, the higher the antioxidant activity[4].

Biotechnology is a branch of biological science about utilizing living things or products from living things to produce goods or services for human use. Biotechnology can increase the utilization of living things and preserve Indonesia's natural wealth and environment[9]. One application of biotechnology is utilizing benalu jeruk leaf and sweet potato leaf as antioxidant biscuits that have various health benefits for humans. Biscuits are one of the bread products that have low water content. The crispness of biscuits is related to the low water content and is related to the thickness of the biscuits. Biscuits are most preferred because they are ready to eat, cheap, have a longer shelf life than other breads, and contain many nutritional qualities[10]. By making innovative biscuit preparations with a combination of two types of plants containing antioxidants, it is hoped that it can produce biscuit preparations that are useful and rich in antioxidants.

2. Research Method

Tools and Materials

This study used an oven, analytical balance, baking pan, cake mold, container, filter paper, spoon, 80 mesh sieve, blender, parchment paper, metal spatula, horn spoon, stirring rod and other glassware for analysis. The main materials used for this study were benalu jeruk (Dendrophthoe glabrescens (Blakely) Barlow) leaf powder and sweet potato (Ipomoea batatas L.) leaf powder obtained from Manikliyu Village, Kintamani District, Bangli Regency, Bali Province. Other additional ingredients used were egg yolk, bogasari wheat flour, koepoe koepoe baking soda, blueband margarine, vanilla powder, tropicana slim stevia sugar, greenfields skim milk, and chemicals used for analysis, namely, DPPH powder (2,2-Diphenyl-1-Picrylhydrazyl), methanol p.a., and ascorbic acid.

Preparation of simplisia

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Simplisia of benalu jeruk leaf and simplisia of sweet potato leaf are made by cleaning benalu leaf and fresh sweet potato leaf from dirt, then washing them with clean running water. Then, the leaves are dried in an oven at 50ºC until the simplisia dries[11].

Preparation of citrus paradise leaf powder and sweet potato leaf powder

Benalu jeruk leaf powder and sweet potato leaf powder can be made from benalu jeruk leaf simplisia and sweet potato leaf simplisia, which are pulverized and sieved using an 80 mesh sieve.

Preparation of citrus paradise leaf biscuits and sweet potato leaf biscuits

Biscuits were made using several modified formulations from the research of Zaddana, Miranti, Almasyhuri, and Tanzila (2018)[12].

Table 1.
Formulation of biscuit preparation

Material Composition	Material Weight (grams)	
	F0	F1
Orange bitter melon leaf powder		10
Sweet potato leaf powder		20
Wheat flour	35	35
Egg yolk	15	15
Margarine	13,2	13,2
Vanilla	0,4	0,4
Baking soda	0,4	0,4
Skim milk	2,8	2,8
Stevia sugar	11,2	11,2

Making biscuits is done through several mixing stages. The first stage of mixing stevia sugar, margarine, and milk, then stirring using a mixer for about 5 minutes. In the second mixing stage, vanilla and egg yolk were homogenized using a mixer for approximately 10 minutes. The third mixing is in a container with orange or sweet potato leaves, flour, and baking soda until homogeneous. The fourth mixing is to put the flour mixture into the first container and then stir until homogeneous. The smooth dough is molded and placed in the oven at 100oC for 20-30 minutes[12].

Moisture content test of biscuits

Moisture content analysis in biscuits was done using the oven method (AOAC 2005). In the first stage, the cup was dried using an oven at 105^0C for 1 hour. In the second

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stage, the cup was cooled in a desiccator for 15 minutes and weighed until the weight was constant. In the third stage, samples that have been weighed initially are placed in a cup and then dried using an oven at 105^0C for 24 hours. The dried sample was cooled for 15 minutes in a desiccator and weighed. The formula for calculating water content is as follows [13]:

$$Cw = \frac{w1-w2}{w1} \text{ x } 100\% \text{ [14]}$$

Cw= moisture content (%); W1= weight of cup with sample before drying (g); W2= weight of cup and sample after drying (g).

Hedonic test of biscuits

The hedonic test was conducted by a 50-person untrained panel of 25 men and 25 women aged 20-45, with the condition that panelists are willing to volunteer and do not have allergies to certain ingredients, so that panelists can determine the acceptability of a product and to assess the quality of food [15].

Acceptability assessment includes the aroma, taste, color, and texture of the biscuits. Performed based on the level of liking, namely: 1 (like), 2 (somewhat like), 3 (neutral), 4 (somewhat dislike), and 5 (dislike). The hedonic test is spontaneous, where panelists rate the biscuits directly without comparing them with the previous biscuits.

Antioxidant activity using the DPPH method

Making blanks

DPPH powder 0.1 mM was weighed as much as 3.9 mg and dissolved in methanol p.a to exactly 100.0 mL (0.1 mM).

Preparation of vitamin C comparator

A total of 0.5 mg of vitamin C was added to 50.0 ml of water to obtain a level of 1%. From this level, a concentration series of 60, 80, 100, 120, and 140 µg/mL was made[16].

Determination of the maximum wavelength of 0.1 mM DPPH solution

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Determination of wavelength (λ) was done by measuring 4.0 mL of 0.1 mM DPPH solution on a spectrophotometer at a wavelength of 400-600 nm to obtain an absorbance of ± 0.2-0.8.

Determination of the operating time of 0.1 mM DPPH solution

Determination of operating time is done by reacting 50 µl of vitamin C comparator standard plus 4.0 mL of 0.1 mM DPPH solution, homogenized with a stirrer for 1 minute and then measuring the absorbance at minutes 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 at the maximum λ that has been obtained.

Preparation of sample solution.

A stock solution of 500 ppm was made by weighing 5 mg of extract, then dissolved in absolute methanol while stirring, homogenizing, and then increasing the volume to 10 ml. Furthermore, a concentration variation of 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm was made.

Antioxidant Activity Measurement

The test was carried out by pipetting 2 ml of sample solution from various concentrations (10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm). Then, each was added 2 ml of DPPH. Then, Divortex and incubated at 37ºC in a dark room. The absorbance was measured at a wavelength of 517 nm.

3. Results And Discussions

Biscuits with a combination of citrus paradise leaves and sweet potato leaves can be seen in Figure 1.

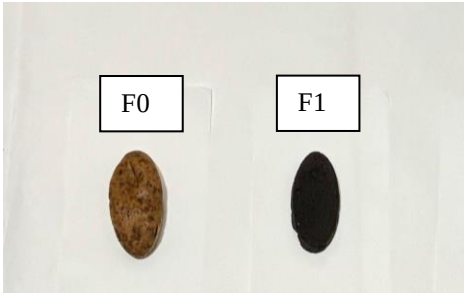

Figure 1.
Combination biscuits of orange bitter melon leaves and sweet potato leaves

Moisture content test

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Water content testing aims to determine the percentage of water content contained in biscuits. The results of the water content test can be seen in Figure 2.

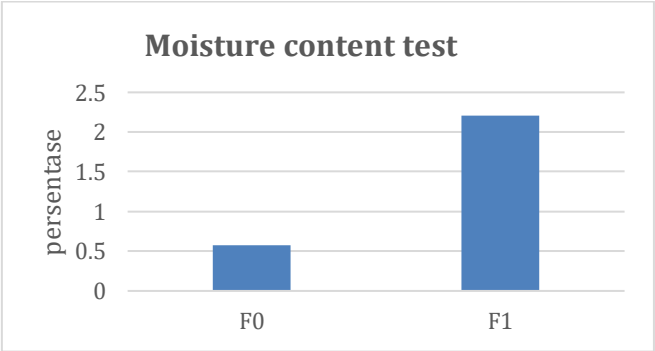

Figure 2.
Water content test graph

F0 biscuits have a moisture content of 0.58% and F1 of 2.21%. This shows that adding citrus paradise leaf simplisia powder can increase the moisture content because citrus paradise leaves can bind water so that the moisture content becomes high. Both biscuit formulas meet the requirements for moisture content according to the World Health Organization (WHO) and Food and Agriculture Organization (FAO), which is below 4.5%. In addition, both biscuit formulas meet the Indonesian National Standard (SNI) requirements, below 5%[17].

Hedonic test

The hedonic test is a test in organoleptic sensory analysis used to determine the magnitude of a difference in the quality of similar products by assessing certain properties of a product and determining the level of liking of a product[18]. The level of favorability includes like, somewhat like, neutral, somewhat dislike, dislike, and dislike.

The color value is used in this assessment because a product's color can determine the level of product acceptance by consumers visually, and color is the first thing consumers notice in purchasing and consuming a product[19]. Biscuits without simplisia are light brown, while biscuits with orange balu leaves and sweet potato leaves are black. The black color on the orange and sweet potato leaf biscuits is due to simplisia. The analysis of color parameters from 50 panelists showed significant hedonic test differences between F0 and

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F1. Based on the data obtained, the color criteria most preferred by panelists is the color of the F0 biscuit sample (negative control), which is light brown. This is by research according to the literature which states that panelists prefer biscuits with brighter colors compared to darker colors[20].

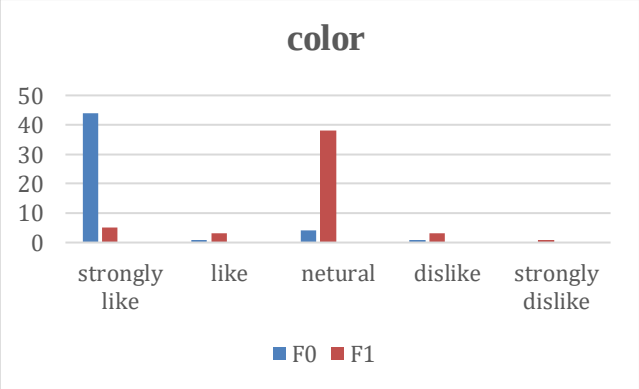

Figure 3.
Color hedonic test graph

Taste testing is a test that involves the sense of taste, namely the tongue. Taste is a very determining factor in whether a product can be accepted or not by consumers[19]. The results of sensory testing on biscuits without simplisia produce a sweet taste. In contrast, biscuits with benalu jeruk and sweet potato leaves produce a sweet biscuit preparation with a slightly bitter final taste. The senses of taste can accept them. The results of the analysis of flavor parameters from 50 panelists showed significant hedonic test differences between F0 and F1. Based on the data obtained, the most preferred taste criteria by panelists is the taste of the F1 biscuit sample, which has a good taste because it uses the right simplisia ratio to provide an alluring taste and become a favorite, according to the panelists.

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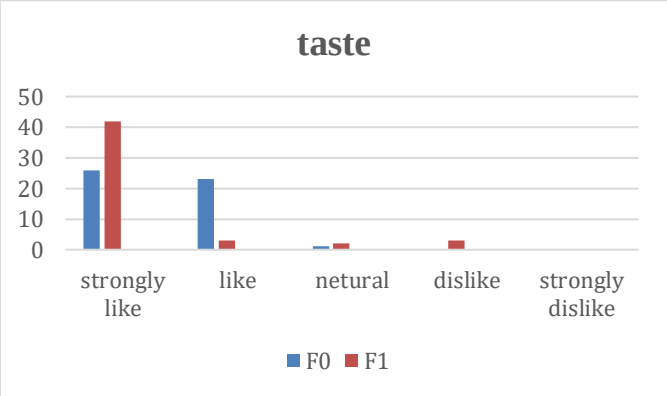

Figure 4.
Taste hedonic test graph

Texture is a sensation of pressure that can be felt with the mouth (when bitten, chewed, and swallowed) or even with fingers [19]. One of the quality parameters that is very instrumental in displaying the characteristics of a food is texture. From the test results, the biscuit sedian was found to be crispy. The results of the analysis of texture parameters from 50 panelists showed similarities in the F0 and F1 hedonic tests. Based on the data obtained, the texture criteria most favored by panelists is the texture of biscuit samples F0 and F1, which has a crispy texture.

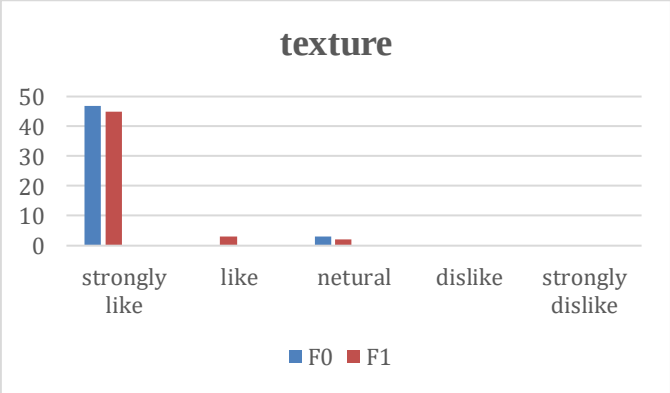

Figure 5.
Texture hedonic test graph

Aroma is an attraction in determining the good taste of a product[19]. Testing the aroma of a product is done using the five senses, namely the nose. In biscuits without simplisia, the aroma of vanilla is produced. In contrast, biscuits with orange and sweet potato leaves produce biscuit preparations that have a distinctive aroma of orange and sweet

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potato leaves. The analysis of aroma parameters from 50 panelists showed significant hedonic test differences between F0 and F1, F2, and F3. Based on the data obtained, the aroma criteria most favored by panelists is the aroma of the biscuit sample containing simplisia, namely F1. Panelists like the aroma because the simplisia used has a distinctive aroma.

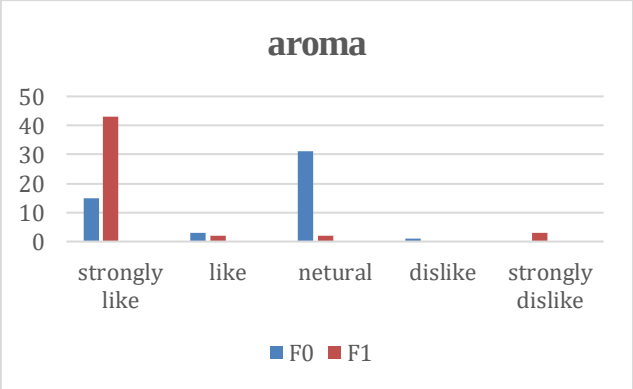

Figure 6:
Aroma hedonic test graph

Antioxidant activity test

The antioxidant activity of biscuits with Vitamin C as a positive control can be seen in Table 2. The highest antioxidant activity was obtained in biscuits with formula 1. From the table, it can be seen that the addition of citrus paradise leaf powder and sweet potato leaves increases the antioxidant activity of the biscuits. The absorbance value decreases along with the addition of concentration. This is by research, which states that the greater the concentration of the material, the smaller the absorbance value read, which means that the activity of the test material in capturing DPPH radicals is greater[21].

Testing of biscuit samples with a combination of orange balu leaves and sweet potato leaves was replicated three times. Absorbance data and % inhibition as a percentage of observation results against free radicals can be seen in Table 2.

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Table 2:
Antioxidant activity of biscuits

Sample	Concentration (ppm)	Sample absorbance	%Inhibition	IC50
F0	60	0,464	4,850	
	80	0,457	6,284	
	100	0,450	7,855	648,641
	120	0,442	9,358	
	140	0,434	10,997	
F1	60	0,353	27,664	
	80	0,327	33,060	
	100	0,318	34,836	179,753
	120	0,300	38,525	
	140	0,277	43,169	

From the data above, a linear equation is obtained to calculate the test sample's IC50 value, which is the concentration that can overcome 50% of DPPH free radicals. The linear equations of F0 and F1 can be seen in Figure 7 and Figure 8.

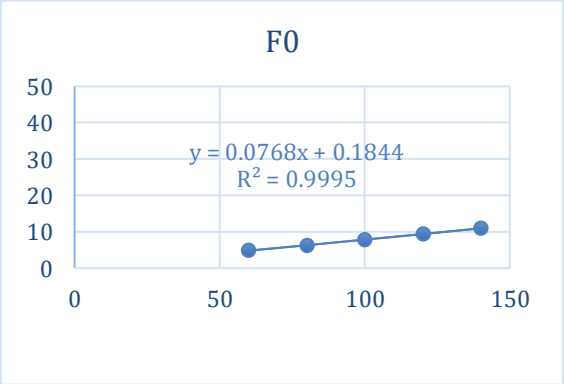

Figure 7.
Linear regression curve of F0

e-ISSN: 2987-0836 DOI: https://doi.org/10.59585/ijhs

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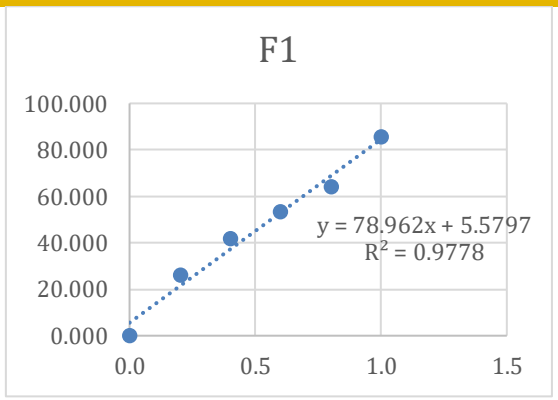

Figure 8.
F1 linear regression curve

Testing of biscuit samples of a combination of orange and sweet potato leaves showed that the concentration increased along with the percent inhibition. This is by research which states that the higher the IC50 value, the less antioxidant activity and vice versa. Antioxidant properties are considered very strong when the IC50 value is less than 50 ppm, rather strong when the IC50 value is between 50 to 100 ppm, moderate when the IC50 value is 100 to 150 ppm, weak between 150 and 200, and very weak when the IC50 value is above 200 ppm[7].

The IC50 value of F0 showed weak antioxidant activity because it was less than 200 ppm, while F1 showed weak antioxidant activity in the 151-200 ppm range. The test results of the antioxidant activity of F1 compared to F0 showed that F1 has better antioxidant activity compared to F0; this is because F1 is a biscuit with the addition of powdered citrus paradise leaves and sweet potato leaves, while F0, without the addition of powdered citrus paradise leaves and sweet potato leaves. The antioxidant activity found in F1 is thought to be because the powder contains flavonoids, phenols, and tannins, which are chemical compounds with antioxidant activity[7] [22]. In addition, sweet potato leaves contain flavonoids and anthocyanins that work as antioxidants[23].

One compound with antioxidant activity is phenol, which consists of lignans (tocopherols), flavonoids, and phenolic acids (phenylpropanoids, flavonoids, and quinones). Phenolic compounds are known to have antioxidant activity because they can donate hydrogen atoms. It can also substituent the aromatic ring, which affects the stability

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of the structure and consequently affects radical quenching. Hence, different phenolic acids have different antioxidant activities. The antioxidant activity of free, esterified, glycosylated, and nonglycosylated phenols is different[24].

Flavonoid compounds have molecular mechanisms in antioxidant action, namely as follows: 1) scavenging ROS directly through the arrangement of hydroxyl groups on flavonoids where ring B with its ortho-dihydroxy structure allows flavonoid phenoxyl radicals to participate in electron delocalization, thus showing antioxidant activity. 2) Antioxidant enzyme activity. Flavonoids can activate antioxidant enzymes, including phase II detoxification enzymes (such as NAD(P)H-quinone oxidoreductase, glutathione S-transferase, and UDP-glucuronosyltransferase), which are key defense enzymes against electrophilic toxicants and oxidative stress. 3) Oxidation inhibition such as xanthin oxidase (XO) and protein kinase C that catalyze the production of superoxide anion, cyclooxygenase, NADH oxidase, microsomal monooxygenase, and lipoxygenase. 4) Metal chelating activity as catechins chelate a large number of metal ions such as Mn(VI), Fe(II), Fe(III), Cu(II), Zn(II), and Al(III). 5) Increases the level of α-tocopherol radicals. Flavonoids act as hydrogen donors of α-tocopherol radicals and can potentially delay LDL oxidation via α-tocopherol radical donors. 6) Mitigation of oxidative stress caused by nitric oxide. Nitric oxide (NO) plays an important role in maintaining blood vessel dilation, and NO loss causes oxidative stress in blood vessels[25].

The results of the antioxidant activity test of the biscuit combination of citrus fruit leaves and sweet potato leaves, when compared to the vitamin C comparison solution, showed that the biscuit combination of citrus fruit leaves and sweet potato leaves had lower antioxidant activity than the comparison. Vitamin C is a natural antioxidant with a very strong IC50 value, so vitamin C is most commonly used as a comparison compound in testing the antioxidant activity of plants. At the same time, biscuits are a form of compound mixture that may have different properties[26].

The use of additives on antioxidant activity in biscuits has yet to be seen. However, the processing of biscuits, namely roasting, can reduce flavonoids, phenols, and tannin compounds that have antioxidant activity in benalu jeruk leaf and sweet potato leaf.

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4. Conclusion

In F1 biscuits, the combination of powdered orange bitter melon leaves and sweet potato leaves has a moisture content value of 2.21%, IC 50% of 179.753. Based on the hedonic test of biscuit color in F1, the panelists did not like it. The taste of F1 biscuits is the most preferred. F1 biscuits have the most favorable texture, and the most favorable aroma is F1. From the results of this study, it is necessary to increase the concentration of simplisia powder or use extracts.

5. Acknowledgements

Thank you to the Faculty of Pharmacy, Universitas Mahasarswati Denpasar, for providing laboratory facilities for conducting our research.

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