

Determination of Total Flavonoid Levels in Packaged Tea Bags Combination of Dayak Onion and Beet Root with UV Visible Spectrophotometric Method

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Abstract: The utilization of natural resources that contain antioxidants is in high demand by the community to prevent degenerative diseases such as heart disease, diabetes, stroke and cancer. Processing of Dayak onion and beet root in the form of herbal tea has great potential to be used as a functional drink because both of these plants contain flavonoids which have antioxidant properties. In 5 tea formulas made by comparing the composition of Dayak onions and beet root, a preference test was conducted on several panelists related to color, aroma and taste and obtained 3 formulas favored by the panelists are formula 1, 3 and 4. the purpose of this study was to determine the total flavonoid levels of each of the teabag formulas favored by panelists using the UV Visible spectrophotometry method with a quercetin comparison. From the research conducted obtained linear regression equation data $y = 0.0364x + 0.0028$ with $r = 0.997$. Total flavonoid levels for each teabag combination of dayak onion and beet root for formula 1; formula 3; formula 4 is 0.9309%; 0.975% and 1.841%, where the highest total flavonoid content is formula 4, which is 1, 841%.

1 INTRODUCTION

Bukittinggi is one of the tourism city in West Sumatra, which is famous for its various culinary delights and delicious flavors. Many culinary preparations using spices and coconut milk as the basic ingredients. The pattern of life of the people of Bukittinggi who often consume foods containing coconut milk and fatty foods causes the people of Bukittinggi to have great potential to get a degenerative diseases such as heart disease, diabetes and stroke. The high price of modern medicines encourages consumers to try other alternatives by using the trend *back to nature* to maintain their health. one of the natural product that have the potential to overcome these degenerative diseases is plants that contain antioxidant compounds.

Utilization of natural resources that contain antioxidants is in high demand by the community to prevent degenerative diseases. Antioxidants are substances that at low concentrations can prevent or slow down the oxidation process by binding to free radicals and highly reactive molecules so that cell damage can be inhibited. This compound has a small molecular weight but is able to inactivate the development of oxidation reactions by preventing the

formation of radicals (Dimitrios, 2006). Antioxidants are generally present naturally in an important role for the protection of body health. This compound can prevent oxidative damage and reduce the risk of disease.

Among the plants that contain lots of antioxidants are Dayak onions (*Eleutherine Palmifolia*) and beetroot (*Beta vulgaris*). Both of these plants have identical colors that are purplish red which contain bioactive compounds such as phenols, flavonoids, taanin, glycosides, steroids and alkaloids (Claudea, 2013, Puspawati, 2013). purplish red color in both plants is caused by anthocyanin pigment content. Anthocyanins are a group of pigments that cause reddish color, located in water soluble cells in water (Jaya 2013). Besides functioning as an antioxidant, anthocyanin has other uses including as a natural indicator, and as a coloring agent textile and food industries (Hendrawan 2011). Several studies have been carried out using anthocyanin pigments from beetroot and dayak onions for natural dyes in food or natural dyes for cosmetics such as lipsticks, but no studies have been reported regarding the manufacture of functional drinks from these two plants.

Diversification of natural products that are traditionally processed can be done one of them by

making herbal tea drinks that are packaged in dyed containers like tea drinks in general. Herbal Tea is one of the tea beverage products from herbal plants that has properties in helping the treatment of an illness or as a body refreshing drink (Hambali *et al.*, 2005, Mun'in, 2008). This product is a form of change in health products.

Processing of Dayak onions and beetroot in the form of herbal tea has great potential to become a functional beverage, because it is related to its natural antioxidant content, both plants can be utilized to protect the body from free radical attack which results in degenerative diseases.

Research conducted by Yulia *et al.*, 2019, regarding the formulation of tea bags with a combination of dayak onions and beetroot with five formulas containing different composition of dayak onions and beetroot. After the hedonic test related to the color, smell, taste and shape of the 5 formulas and obtained three formulas favored by the panelists namely formulas 3, 1 and 4.

Three formulas are preferably carried out quantitative analysis of total flavonoid levels to find out how much flavonoid levels are measured in the tea packaging combination of dayak onions and beetroot. So that it can be chosen among the three formulas that have the highest flavonoid content so that it can be developed into a health functional beverage product. Determination of total flavonoid levels was carried out by the method of chang et al 2002 using UV Visible Spectrophotometry.

2 MANUSCRIPT PREPARATION

2.1 Material

The Material used include five packaged teabag formulas that have been studied by Yulia, *et. al* (2019), Ethanol 95%, Methanol, Pure Quercetin, Aquadest, 10% AlCl₃ buffer solution, Sodium Acetate buffer solution, Ethyl acetate, 4N HCl solution.

2.2 Tool

The tools used in this study include a funnel, beaker glass (pyrex), erlenmeyer (pyrex), stir bar, measuring flask (pyrex), volume pipette (pyrex), test tube, measuring cup (pyrex), UV-Vis spectrophotometer 1800 (pyrex) Shimadzu, pH meter.

2.3 Research Stages

There are three stages of research in this study the first is the preparation and formulation of tea bags (Yulia, *et.al.* 2019). The second stage was the packaging of tea bags using 95% ethanol and the preparation of standardized quercetin solutions and test solutions, and the third step was the determination of total Flavonoid and Anthocyanin levels using a UV-Vis spectrophotometer.

2.3.1 Formula Teabag Combination of Beet root-Dayak Onion

There are five Dayak onion-beetroot teabag formulas made by comparison of different combination ingredients (Yulia *et al.* 2019) as shown in Table 1.

Table 1. Beetroot - Dayak Onions Teabag Formulas

Formula Code	Beet Root (gram)	Dayak Onion (gram)	Stevia Leaves (gram)
F1	1,5	0,5	0,15
F2	1	1	0,15
F3	0,75	1,25	0,15
F4	0,5	1,5	0,15
F5	0,25	1,75	0,15

2.3.2 Manufacture of Extractions, Standard Solutions and Test Solutions

Extraction

Weigh each sample of tea bags as much as 5 grams. Enter each sample that has been weighed into each 100 ml measuring flask. Add 100 ml of 95% ethanol, cover. Figure 1 is Maceration for 24 hours. The first 6 hours shake it many times. And leave it for 18 hours. Then filter the results of maceration.



Figure 1: maceration process of sample.

Quercetin Standard Solution

Pure quercetin weighed as much as 25 mg, dissolve with methanol in a 100 ml volumetric flask to mark the mark. This result is used as a standard solution. The standard solution is then diluted with methanol in 6 different concentrations (Figure 2). Each concentration was pipetted 2 ml, then 0.1 ml AlCl₃

10% reagent was added, 0.1 ml sodium acetate, and 2.8 ml Aquadest, homogeneous and incubated for 30 minutes at room temperature. The absorbance was measured on a UV-Vis 415 nm spectrophotometer using blank solution without quercetin and $AlCl_3$.



Figure 2. Quercetin Standard Solution

Sample Solutions

The extract that was made was weighed as much as 1 gram, then hydrolyzed with 4 ml HCl as much as 2 ml for 30 minutes in a measuring flask. The solution is then filtered and concentrated. Then the extract was filtered with 15 ml of ethyl acetate, 3 times, the ethyl acetate fraction was collected and concentrated. The results of the ethyl acetate extract were put in a 25 ml measuring flask and dissolved with methanol to the limit mark (test solution). Do the same for the other 2 samples.

2.3.3 Determination of Total Flavonoid Levels and Determination of Anthocyanin Levels in Five Formulas Was Carried out by the Chang Method

Determination of Total Flavonoid Levels

The test solution was pipetted as much as 0.5 ml, then dissolved with 1.5 ml of methanol in the test tube, then add a reagent consisting of 0.1 ml of 10% $AlCl_3$, 0.1 ml of Sodium Acetate and 2.8 ml of distilled water, homogeneous and incubation for 30 minutes at room temperature. The absorption solution was measured on a UV-Vis 415 nm spectrophotometer using blank solution without the addition of $AlCl_3$ replaced with distilled water. Measurements were made three times, levels were calculated as averages. The total flavonoid content is expressed by comparison equivalents of quercetin. Do the same thing in the other 4 samples.

Determination of Anthocyanin Levels of Dayak Onions – Beet root teabag

Determination of anthocyanin levels was done by UV-Vis Spectrophotometer. The test solution was made 5 grams of a mixture of tea combination of dayak onions and beetroot extracted with 250 ml of water for 5 minutes and 10 minutes. The test solution

was taken 1 ml, then two samples were measured against 5 ml of pH solution 1.0 (buffer solution of Potassium Chloride) and 4.5 (buffer solution of Sodium Acetate). Furthermore, it was analyzed with a UV-Vis spectrophotometer at a wavelength of 520-700 nm.

3 RESULTS AND DISCUSSION

Beetroot is the taproot portion of a beet plant, usually known in North America as the beet, and also known as the table beet, garden beet, sugar beet, red beet, dinner beet or golden beet. It is one of several cultivated varieties of *Beta vulgaris* grown for their edible taproots and leaves (called beet greens), they have been classified as *B. vulgaris* subsp. *vulgaris* 'Conditiva' Group. Besides being used as a food, beets have uses as a food colouring and as a medicinal plant. Many beet products are made from other *Beta vulgaris* varieties, particularly sugar beet.

In preliminary research, beetroot juice reduced blood pressure in hypertensive people. Tentative evidence has found that dietary nitrate supplementation, such as from beets and other vegetables, results in a small improvement in endurance exercise performance. The red colour compound betanin is not broken down in the body, and in higher concentrations may temporarily cause urine or stools to assume a reddish colour, in the case of urine a condition called beeturia. Although harmless, this effect may cause initial concern due to the visual similarity to what appears to be blood in the stool, hematochezia (blood passing through the anus, usually in or with stool) or hematuria (blood in the urine). Nitrosamine formation in beet juice can reliably be prevented by adding ascorbic acid.

Dayak onions are small bulbs that produce several long, ribbon-like leaf stalks with a single flowering stem. The smooth, dark red bulbs are shaped like rounded diamonds with small, wispy brown roots extending from the ends.

Research on Dayak onions has been carried out, including plant bulbs of the genus *Eleutherine* (*Eleutherine bulbosa* and *Eleutherine Americana*), which are known to contain secondary metabolites of the naphthoquinone group (elecanacin, eleutherin, eleuthero, eleuthero). Onion Dayak has anti cancer and anti oxidants, which are usually found in vacuole cells in the form of glycosides. Some studies also state the content of the active compounds in Dayak onions is extensive, so it is very reasonable for various properties. These compounds include

alkaloids, steroids, glycosides, flavonoids, phenolics, tannins, and saponins. One of these compounds, namely flavonoids, can be efficient as anticancer, antiviral, anti-inflammatory, reduce the risk of cardiovascular disease, and free radical catchers.

According to D. Lestari *et al.*, 2018, dayak onion/tiwai onion contain secondary metabolites such as alkaloids, flavonoids, tannins, and quinones. These compounds are known to have a very broad biological activity including antioxidants and anticancer. Flavonoids are known to be good antioxidants because they have at least two hydroxyl groups in ortho positions and para which can capture free radicals by freeing hydrogen atoms from their hydroxyl groups. Flavonoids as antioxidants have a higher potential as anticancer drugs than vitamins and minerals. Flavonoid compounds can prevent the reaction of carcinogen molecules joining cell DNA so as to prevent cell DNA damage; the bioactive components of flavonoids can prevent the initial process of cancer cell formation. Flavonoids can stimulate the regeneration process of mutated DNA cell so that the cells become normal again. In addition, quinone compounds have also been reported to have antioxidant activity. It is likely that quinone originates from the oxidation of the corresponding phenols namely catechol to form ortho-quinones and quinol producing para-quinones.

Beetroot and dayak onions in this research obtained from palano stone plots in Agam Regency, West Sumatra Province can be seen in Figures 1 and 2. Beetroot are used which have ± 10 weeks old. Dayak onions used are ± 4 month old, onions or plants have flowered, this is because the quality of the Dayak onions is in an optimal state. Simplicia is cleaned with running water to remove impurities (Puspawati, 2013). After the simplicia is selected and cleaned, then thinly sliced to be dried using an oven at $45 \pm 2^\circ\text{C}$ until perfect drying is obtained, because the simplicia cannot be heated at high temperatures because it will cause damage or loss of secondary metabolites. This drying process intended to reduce water content contained in the sample, so it can prevent spoilage by bacteria. After the dried simplicia is then made into powder using a blender, the powder is weighed for each formula and packaged into a tea bag.



Figure 3: Dayak Onion



Figure 4: Beetroot

The packaging of tea bags is carried out with five different formulations namely F1, F2, F3, F4 and F5 formulas. The results of the tea bag can be seen in Figure 5.



Figure 5: Forms of Steeping five combination teabag formula (Yulia,*et.al.*2019).

This Dayak onion-beetroot combination teabag is one of the more practical forms of preparation, preferred by the public and has antioxidant properties, because each of the simplicia that has been previously studied (Melisa, 2015 and Puspawati, 2013) has a compound content. Compounds that act as antioxidants in simplicia include Flavonoids and Anthocyanins. To determine the content of these compounds in this teabag, the flavonoid content was tested by UV-Vis spectrophotometer by Chang (2002). Before conducting the test for determining the level of flavonoids carried out on this teabag extract based on the Chang method. The extraction process is aimed at take chemical compounds contained in the sample. The principle of extraction is based on displacement the mass of the components of the substance dissolved into the solvent resulting in displacement in the interface layer and diffuses into the solvent (Harborne, J.B 1987).

To get chemical compounds that are the desired extraction method is used is a method of extracting nutritious substances or substances active parts of the plant by using solvents appropriate (Yuliani & Satuhu, 2012). The extraction method used on this research is maceration, because of this method simpler, easier and without heating. Because heating can make flavonoid levels reduced. The calibration curve with a standard solution of Quercetin which is made with 6 concentrations shown in Table 2.

Table 2. Concentrations of quercetin standard solutions on calibration curves

Concentrations (ppm)	Absorbant (A)
6	0,215
10	0,360
14	0,533
18	0,659
22	0,792

Quercetin used (Figure 6) as a standard solution because quercetin is flavonoid flavonol group which has a group keto at C-4 and has a hydroxyl group on neighboring C-3 or C-5 atoms from flavones and flavonols (Azizah and Faramayuda 2014)

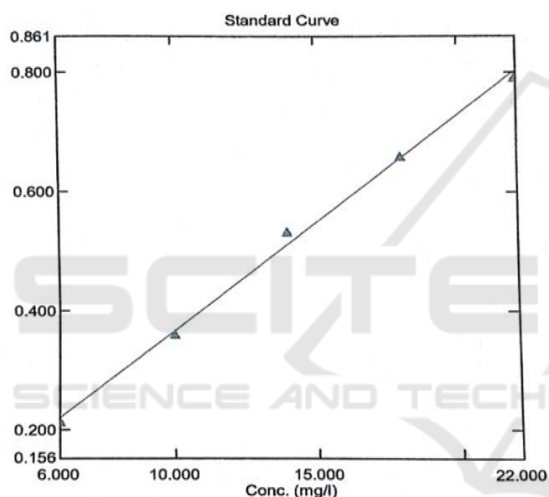


Figure 6: Quercetin calibration curve

The calibration curve obtained is $y = 0.0364x + 0.0028$, with $r = 0.997$. A value of r close to 1 indicates a linear calibration curve and there is a relationship between the concentration of quercetin solution and the absorption value, Azizah (2014). This method was chosen because it is easier and simpler, faster, economical, and is known to be more specific to the flavonoid and flavonol groups. Aluminum (III) chloride reagents are used to form acid-resistant complexes with C-4 ketone groups and C-3 or C-5 hydroxyl groups in flavones and flavonols, and form acid-resistant complexes with ortho-hydroxy groups in the ring A or B in flavonoids (Chang *et al.*, 2002 & Humadi,Istudor,2008). In the measurement of total flavonoid compounds, the sample solution is added $AlCl_3$ which can form complexes, resulting in a shift in wavelength towards

the visible which is indicated by the solution producing a more yellow color. And the addition of potassium acetate which aims to maintain wavelengths in visible areas (Chang *et al.*, 2002). The addition of aluminum chloride aims to form complexes with quercetin (Indrayani, 2008). The incubation treatment for 1 hour before the measurement is intended so that the reaction runs perfectly, so that the resulting color intensity is more maximal (Azizah and Faramayuda 2014). Total flavonoid levels are calculated as equivalence of quercetin raw materials (Humadi, Istudor, 2008).

In general, the determination of total flavonoid levels in a plant sample is based on the formation of aluminum complex compounds (Al-Flavonoids), in the form of a yellow solution. The addition of acetate salts to the determination of flavonoid levels is intended to produce shifts and stronger peak absorbance intensities (Pekal & Pyrzynska, 2014). Al-Flavonoid chelate complexes are formed in ketone groups and hydroxyl groups of flavonoids (Sepahpour, Selamat, Manap, & Razis, 2018), the reactions that occur can be seen in Figure 7.

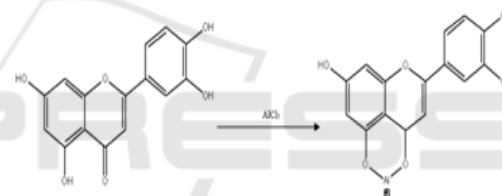


Figure 7: Chemical reaction of Flavonoid with $AlCl_3$

From this method, the results of determining the total Flavonoid levels are obtained in Table 3.

Table 3: Determination of total flavonoid levels of teabag combination with beetroot and dayak onions.

Extract weight	Absorbant at λ 436,5 nm (y)	Total flavonoid levels (%)	Average total flavonoid levels (%)
0,175 g	2.372	0,9309	1,249
0,146 g	2.072	0,975	
0,105 g	2,815	1,841	
the range of total flavonoid levels = 0.9307-1.841%			

Total flavonoid levels obtained for formula 1 teabag combination of beetroot and dayak onions is 0.9303%, for formula 3 is 0, 975% and total flavonoid level for formula 4 is 1.841% with an average total flavonoid level is 1, 249%

Determination of anthocyanin levels was carried out on the three best formulas from the results of the study (Yulia, *et.al.*2019). Determination of the levels

(Figure 8) of anthocyanin extracted in the same way according to the way the tea is served, which is brewed for a few minutes in accordance with the way tea is served in general, in the experiment 5 minutes and 10 minutes are chosen to see the levels of anthocyanin with different extraction times. Figure 8 explains the sample solution for determining anthocyanin levels.



Figure 8: Sample solution for determination anthocyanin levels.

Anthocyanins are included in the class flavonoid compounds, constitute a group the biggest natural pigment in plants which dissolve in ak which is responsible for give color to flowers, fruit and veegetables. Anthocyanins can be beneficial for health as a source of antioxidants. This is due to the compound This polyphenolic is a derived glycoside polyhydroxy and polymethoxy from, 2-phenilbenzopirilium or flavilium salt.

Anthocyanins are in several equilibrium form. Kinbtika study and thermodynamics that are studied in general accepting the transformation of differences (proton transfer, isomerization and tautomerization) flavilium cation from simple anthocyanin under various pH conditions. In acidic solution stronger (under pH 2) more flavilium cations dominant and provides anthocyanin solutions the Red one.

The anthocyanin stability is not only influenced by the heating temperature in the processing only, but also influenced by intrinsic factors and extrinsic in the product, such as pH, temperature storage, chemical structure and concentration existing anthocyanins, the presence of light, oxygen, enzymes, proteins, and metal ions. to know the stability of anthocyanin is needed initial data on anthocyanin levels from the starting material contain these substances.

Determination of anthocyanin is done with the pH difference method of pH 1.0 and pH 4.5. At pH 1.0 anthocyanin is formed oxonium compound. The increasingly situation especially if it is nearing pH I will cause more pigment anthocyanins are in the form of cations colored flavilium or oxonium and absorbance measurement will show an increasingly large amount of anthocyanin. At pH 4.5, it is on weak

acids Flavilium cations change into more forms stable colorless hemiketal and shape calchont (Figure 9).

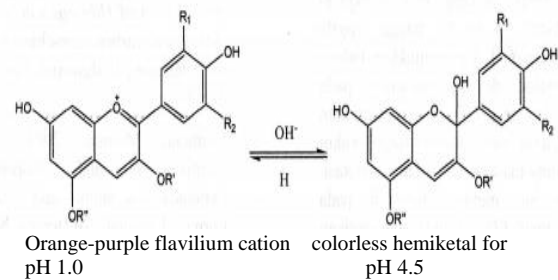


Figure 9: Flavilium cation structure and hemiketal shape R=H Glicocyde substitutens.

Measurement of anthocyanin levels using UV-Vis Spectrophotometry, as previous studies anthocyanin levels were calculated using the general equation :

$$\text{Concentration (mg/ml)} = \frac{A \times MW \times FD \times 1000}{(\epsilon \times l)}$$

Information :

A = absorbance

= A 535.2 (pH 1.0) - A535.2 (pH 4.5)

MW = molecular weight = 433.2 g / mol

FD = dilution factor

ϵ = molar absorption = 31600 L / cm mol

l = cuvette width (1 cm)

Determination of anthocyanin levels was carried out three times (Triplo) at the maximum wavelength obtained which is 518 nm, the maximum wavelength is then used to measure the absorption of the calibration curve and extract sample (Azizah, 2014). It is known that the main wavelength of anthocyanin is in the range of 475-560 nm (Harborne, 1987). From the results of determining the level of anthocyanin obtained it can be said that the brewing of tea for 5 minutes and 10 minutes is almost the same so that the brewing time can be recommended for only 5 minutes. Anthocyanin levels were determined using UV-Vis spectrophotometry, based on the ability of anthocyanins to produce colors at pH 1.0 (buffered potassium chloride solution) and pH 4.5 (sodium acetate buffer solution). The measurement data using UV-Vis spectrophotometer is shown in Table 4.

Table 4. Determination of anthocyanin levels of teabag extract combined with beetroot and dayak onions

Kode ekstrak	Berat simplisia (gram)	Waktu ekstraksi (menit)	Serapan pada $\lambda_{535.2}$ nm (y)		Kadar antosianin (mg/ml)	kadar antosianin rata-rata (mg/ml)
			pH 1,0	pH 4,5		
F1	4,3	5	0,701	0,360	5,849	3,818
F3	4,3	5	0,330	0,144	3,199	
F4	4,3	5	0,227	0,087	2,405	
Kisaran kadar antosianin dengan waktu ekstraksi 5 menit = 2,405 - 5,849						
Kode ekstrak	Berat simplisia (gram)	Waktu ekstraksi (menit)	Serapan pada $\lambda_{535.2}$ nm (y)	Kadar antosianin (mg/ml)	kadar antosianin rata-rata (mg/ml)	kadar antosianin rata-rata (mg/ml)
F3	4,3	10	0,429	0,164	4,535	4,699
F4	4,3	10	0,263	0,103	2,736	
Kisaran kadar antosianin dengan waktu ekstraksi 10 menit = 2,736 - 6,826						

This characteristic depends on the transformation of the chromophore structure. The color of the anthocyanin ion stands out at pH 1.0. While the colorless hemiketal structure at pH 4.5 (Herrera, 2004)

4 CONCLUSIONS

Determination of total flavonoid and anthocyanin levels. The results obtained for total flavonoid levels were tested on the three best formulas obtained in previous studies (Yulia, *et.al.* 2019), namely the F1, F3 and F4 formulas respectively were obtained 0.9309%, 0.975%, 1.841%, Whereas for anthocyanin levels during 5 minutes, 5,849 mg / ml, 3,199 mg / ml, 2,405 mg / ml were obtained. And for 10 minutes, 6.826 mg / ml, 4.535 mg / ml, 2.736 mg / ml were obtained.

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REFERENCES

Azizah, D.N., Endang, K., dan Fahrauk, F. "Determination of flavonoid levels of AlCl3 method in the cocoa peel methanol extract (Theobroma cacao L.) . 2014 Vol. 2(2): 45-49.
 Azizah, D.N. dan Faramayuda, F., 2014. "Determination of AlCl3 Method Flavonoid Levels in Methanol Extract of

The Skin of Cocoa Fruit (Theobroma Cacao L.). Kartika Pharmaceutical Scientific Journal, 2 (2)
 Chang,C.C., Ming-Hua Yang, Hwei-Mei Wen dan Jiing-Chuan Chern. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal Of Food And Drug Analysis. 2002. Vol. 10. No. 3. 178-182
 Claudea, Nusa., 2013., " Antioxidants Extraction of Bawang Dayak (*eleutherina palmifolia*) with Ultrasonic Bath (Study Type of Solvent and Extraction Time) "., Jurnal Pangan dan Agroindustri Vol. 5. No. 1, Malang : Jurusan Teknologi Hasil Pertanian, FTP Brawijaya University.
 Dimitrios, B., 2006., " Source of Natural Phenolic antioxidants Laboratory of Food Chemistry and Technology", School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, J. Food Sci 17 : 505 – 512
 D Lestari et al, 2019, *Antioxidant and anticancer activity of Eleutherine bulbosa (Mill.) Urb on leukemia cells L1210* J. Phys.: Conf. Ser. 1277 012022
 Hambali, *et al.*, 2005., " Membuat Aneka Herbal Tea ", Penebar Swadaya, Jakarta
 Harborne, J.B. The phytochemical method, guiding the modern way of analyzing plants, is the second issue. Occurring from Phytochemical methods, by Padmawinata, K., dan Soediro, I. Bandung : Penerbit ITB Bandung. 1987. 13-15,69-102.
 Herrera-Arellano, A., Flores-Romero, S., Chavez-Soto, M.A. and Tortoriello, J. "Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial". Phytomedicine. 2004. 11(5), 375-382.
 Humadi, S.S., dan Viorica Istudor. Quantitative analysis of bio- active compound in Hibiscus sabdariffa L. extracts. Note I quantitative analysis of flavonoids. Farmacia. 2008. Vol. 56(6), 699-707.
 Indrayani, S." Validation of coloration of quercetin in cream preparations by colorimetric with AlCl3 reagent ". Thesis. Yogyakarta: Sanata Dharma University. 2008. Hal:7,8,25.
 Mun'in et al., 2008., " Pembuatan Herbal Campuran Kelopak Bunga Rosella dan Herba Seledri", Depok : University of Indonesia. Journals
 Puspawati, R., Adirestuti, P., & Menawati, R. Efficacy of Dayak bulbs (Eleutherine palmifolia (L.) Merr.) As an antimicrobial skin herb. Kartika: Pharmaceutical Scientific Journal. 2013. 1(1), 31-37.
 Pekal, A., & Pyrzynska, K. "Evaluation of aluminium complexation reaction for flavonoid content assay". Food Anal. Methods. 2014. Vol. 7, DOI 10.1007/s12161-014-9814-x, 1776-1782.
 Puspawati, R., Putranti Adirestuti, Rizka Menawati. Efficacy of Dayak bulbs (Eleutherine palmifolia (L.) Merr.) As an antimicrobial skin herb. Kartika Pharmaceutical Scientific Journal. Faculty of Pharmacy, Jenderal Achmad Yani University. 2013. Vol.1.No.1, 31-37.

- Satheesh N.V.M, Kirti Singh. Smart unguai biopenetrant from the roots of beta vulgaris. *Journal of Applied Pharmaceutical Research*. 2017.5(2): 21–26.
- Sepahpour, S., Selamat, J., Manap, M.Y., & Razis, A. F. “Comparative analysis of chemical composition”. *Journal Molecules*. 2018. Vol. 23 Ed 402, 2-17.
- Yulia.R.& Putra.A “Teabag Formulation Packaging Combination of Beet and Onion Bulbs ”Research Report. Bukittinggi: Mohammad Natsir University. 2019
- Yuliani, S. dan Satuhu, S., 2012. A complete guide to essential oils. Self-help spreader: Jakarta. Hal, 46.

