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Effect of Soyghurt Fortification with *Syzygium myrtifolium* Walp. Leaves Extract on Antioxidant Activity

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Abstract: Soyghurt contains antioxidant compounds such as isoflavones which is derived from soybeans as main ingredient for making soyghurt. To increase antioxidant activity, it is necessary to add other antioxidant compounds such as anthocyanins which is found in *Syzygium myrtifolium* Walp. which also acts as a red pigment. The aim of this study was to determine the effect of adding *Syzygium myrtifolium* leaf extract to the antioxidant activity of soyghurt. It began with the making of soy milk then fermented with *Lactobacillus bulgaricus* into soyghurt. Plants were extracted by maceration in ethanol and made a dry extract. The addition of dried extract to soyghurt was carried out in 3 concentrations, 1%, 3% and 5%. The results of antioxidant activity assay using the free radical DPPH scavenging method showed that there was a decrease in the IC_{50} in soyghurt fortified with extract compared to soyghurt without extract. The higher concentration of extract, the smaller IC_{50} value. Soyghurt with 5% extract had the smallest IC_{50} (694.14 µg/mL) while the IC_{50} of soyghurt without extract was 2823.17 µg/mL. The conclusion is that fortification of *Syzygium myrtifolium* leaf extract has an effect on the antioxidant activity of soyghurt

Keywords: Antioxidant; Soyghurt; *Syzygium myrtifolium*;

1. INTRODUCTION

One of the causes of the increase in the incidence of Non-Communicable Diseases (NCDs) is an unhealthy diet or diet such as the consumption of high-fat foods. Consumption of high-fat foods can increase serum *levels of Low Density Lipopotein* (LDL), thereby causing oxidation of serum LDL which will form free radical compounds. Free radicals are unstable molecules that can interact with other compounds quickly and destructively [1]. It is this trait that causes free radicals to be able to damage the body's cell structure and cause various types of diseases, especially NCDs. To prevent the oxidation process of fat and protect cells from damage caused by free radicals, it can be done by consuming foods high in antioxidants [2].

Components in plant foods that act as antioxidants include isoflavones. Isoflavones act as antioxidants to weaken free radical reactivity, reduce LDL levels, and increase the activity and expression of antioxidant enzymes [3]. Isoflavones are abundant in nuts, especially soybeans. As many as 99% of the isoflavones in soybeans are found in the form of glycosides consisting of 64% geistin, 23% daidzin, and 13% glistin. Glycoside isoflavone compounds can be converted into aglikon compounds through a fermentation process with the help of certain bacteria. This aglikon compound has a higher bioavailability compared to glycosides, so it can be said that the fermentation process will increase the bioavailability of isoflavones in processed food products made from soybean [4]

such as *soyghurt* (*soymilk yogurt*). *Soyghurt* is the result of fermentation of soy milk by *Lactobacilus bulgaricus* and *Streptococcocus thermopilus*. These bacteria are included in Lactic Acid Bacteria (LAB) which can lower lactose levels so that they are safe to consume by lactose intolerant patients [5]. According to the previous study, advantages of *soyghurt* are lactose-free, cholesterol-free, low fat and high protein [6].

Fortification *of soyghurt* needs to be carried out, one of which is to increase antioxidant activity, one of which is by adding anthocyanins. According to previous study [7], anthocyanins are natural pigments that belong to the flavonoid group with three oxygen atoms. Anthocyanins are responsible for giving plants their orange, red, and purple colors. Anthocyanins in plants can be used as natural pigment in food, where color is an important quality factor for food [8]. In addition to being a pigment, anthocyanins are good antioxidants for the body, including reducing the risk of degenerative diseases, such as cancer, and heart [9]. *Syzigium myrtifolium* are one of the plants known to have high anthocyanins and are efficacious as antioxidants [10].

Syzigium myrtifolium are plants that are rich in phenols, flavonoids, antioxidants and rulinic acid, and have a distinctive aroma that comes from the essential oil content found in various *Syzigium* . The flavonoid contained in *Syzigium myrtifolium* is dimethyl cardamonium which is included in the chalcone group and is cytotoxic. Chalcone and its derivatives have antibacterial activity. Apart from that, *Syzigium myrtifolium* Walp also contain triterpenoids which have antibacterial activity by inhibiting their growth. The triterpenoid compound contained in *Syzigium myrtifolium* leave is betulinic acid [10]

With these data, the background for researchers in conducting research on *soyghurt* fortification with *Syzygium myrtifolium* leaf extract, because in addition to increasing antioxidant activity, the addition of these extracts can also give an attractive color to *soyghurt*. The purpose of this study is to examine the effect of the addition of *Syzygium myrtifolium* leaf extract on the antioxidant activity of *soyghurt*. The urgency of this research is to obtain a low-fat but antioxidant-rich probiotic drink that can be an alternative to plant foods to prevent Non-Communicable Diseases (NCDs).

2. MATERIALS AND METHODS

2.1. Materials

The equipments used in this study include *rotary evaporators* (Buchi®), scales (Fujitsu®), ovens (Memmert), incubators (Memmert), blenders (Philips), UV-Visible spectrophotometers (Agilent), glass tools (Pyrex). The ingredients used include *Syzygium myrtifolium* leaves obtained from Tasikmalaya, soybeans, skimmed milk, sugar, $NaHCO_3$, yogurt starter *Lactobacilus bulgaricus* (Biokul), ethanol (Merck), methanol (Merck), diphenyl-1-picrylhydrrazil or DPPH (Signa Aldrich).

2.2. Methods

2.2.1. Preparation of plant extract

The process of extracting plant is carried out by the maceration until dry extract is obtained. The maceration was carried out by soaking 300g of *Syzygium myrtifolium* leaf powder in 3L of 96% ethanol for 3x24 hours, and changing the solvent every 24 hours. All filtrates are collected and evaporated the solvent using a *rotary evaporator*, then dried in an oven with a temperature of 40-60^{o}C. Then the percentage of yield is calculated and phytochemical screening is carried out in general.

2.2.2. Preparation of soyghurt

The procedure for making soyghurt begins with making soy milk according to the previous study [11]. Soybeans are sorted and washed under running water. Then, the soybeans are soaked in water (1:3) for 12 hours, then drained. After that, the soybeans are soaked with water and 0.5% $NaHCO_3$ for 30 minutes, then rinse with running water. Next, the soybeans are separated from the skin. The soybeans are mashed with water (85°C) using a blender for 10 minutes, after which the soybean juice is filtered with a blacu cloth. After that, the soybean juice is mixed with granulated sugar and skimmed milk powder. Then, the soybean milk is heated to 80°C and cooled. Soyghurt is made by adding starter bacteria to 100-200 mL of soybean milk and then incubating in an incubator for 18 hours at a temperature of 37°C. Next, the soyghurt is cooled in the refrigerator at a temperature of 4°C to stop the fermentation process. A total of 50 mL of soyghurt is added dried extract with various concentrations of 1%, 3% and 5%. Then stirring until homogeneous. Soyghurt is then packaged in a sealed container and stored in a refrigerator [12].

2.2.3. Antioxidant activity DPPH assay

Ascorbic acid is dissolved in methanol to obtain a concentration of 1000 µg/mL. It is then diluted to obtain a variation in concentration from 0–500 µg/mL. All samples are dissolved in the same solvent and concentration. A total of 5 mg of DPPH was dissolved in 250 mL of methanol. A total of 2 ml of each sample and the raw solution is added to 3 mL of DPPH solution. Each mixture is incubated for 30 minutes at dark and room temperature. Absorbance was measured at a wavelength of 517 nm using a UV-Vis Spectrophotometer. Then the IC_{50} value is calculated.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The results of phytochemical screening showed that plant powder and extract contained secondary metabolite compounds such as alkaloids, flavonoids, saponins, polyphenols and tannins. From these results, it can be said that in addition to containing anthocyanin compounds, *Syzygium myrtifolium* also have secondary metabolite compounds that are antioxidants such as polyphenols. Phenol compounds found in plants have oxidation-reduction properties, so they can act as antioxidant compounds [13]. The antioxidant activity of phenol compounds occurs based on the reductive-oxidation reaction mechanism, namely phenol compounds function as free radical reducers which initially reactive substances will then be formed into non-reactive substances. Therefore, free radicals are inhibited in their formation and the effects of free radical attack such as damage can be repaired or inhibited. Another mechanism is that phenol compounds have hydroxy groups that can donate their hydrogen atoms and are able to neutralize the lack of electrons in free radicals [14]. The results of phytochemical screening can be seen in Table 1.

3.2. Evaluation of Soyghurt

Syzygium myrtifolium leaves are made into dried extracts with the aim of making them safer to consume because dried extracts contain almost no extraction solvents anymore. In addition, dried extracts are easier to use and store compared to liquid extracts and thick extracts. From the extraction process, it is known that the yield of extract is 15.65%, this shows that the extraction process is quite optimal because according to the Indonesian Herbal Pharmacopoeia the good yield value is not less than 7.2% [15].

Table 1. Phytochemical Screening Data of *Syzygium myrtifolium* leaves

Secondary Metabolites	*Syzygium myrtifolium*	
	Plant Powder	Extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Polyphenols	+	+
Tannins	+	+
Triterpenoids	+	+

Note: The presence of metabolite is indicated by '+' and the absence is indicated by '–' sign

In this study, soyghurt is made from soy milk made by using soybeans, skim milk and sugar. The soy milk is then fermented using starter bacteria from plain yogurt products that contain *Lactobacillus bulgaricus* that have been registered with BPOM. These bacteria is producing lactic acid, which plays an important role in creating a balance of intestinal microflora. The resulting acidity is able to inhibit disease-causing bacteria that are generally not resistant to acid [16]. In addition, *Lactobacillus bulgaricus* including LAB are given to obtain rapid acid production, form the characteristic taste of soyghurt, and prolong the shelf life of milk because microorganisms are difficult to grow in acidic and viscous atmospheres [17].

3.2.1. The organoleptic properties of soyghurt

The organoleptic of *soyghurt* of *Syzygium myrtifolium* leaves can be seen in Table 2.

Table 2. Organoleptic Test Results of *Syzygium myrtifolium* Soyghurt

Concentration of extract	Color	Aroma	Taste	Texture
1%	Brownish Pink	Typical Soybeans	Sour	Thick
3%	Brownish Pink	Typical Soybeans	Sour	Thick
5%	Brownish Pink	Typical Soybeans	Sour	Thick

The pink colors in *soyghurt* are obtained from anthocyanins found in *Syzygium myrtifolium* which are pigments that can provide color. In addition to functioning as a natural *soyghurt* color, anthocyanins also have properties as antioxidants. The typical taste of soybeans in *soyghurt* is according to the initial ingredient, which is soybean milk. Meanwhile, the sour taste and thick texture are obtained from the fermentation of soy milk by the bacterium *Lactobacillus bulgaricus* which is a lactic acid bacterium. The extract does not affect the taste of soyghurt because the addition is a small amount compared to the amount of soybean milk.

3.2.2. The viscosity of soyghurt

The results showed that the higher the concentration of extract, the higher the viscosity value. This is because the higher the concentration of *Syzygium myrtifolium* extract, the more powder is dispersed in *the soyghurt*, thus increasing the viscosity of *the soyghurt*. The greater the concentration

of extracts added, the higher the viscosity value. The viscosity value of *soyghurt* can be seen in Table 3.

Table 3. The Viscosity of Soyghurt

Concentration	Viscosity (cP)	Standard Viscosity (cP)
1%	8.99 ± 0.20	
3%	10.10 ± 0.03	8.28-13.00
5%	10.56 ± 0.07	

Based on Table 4.5, it can be seen that the viscosity of *soyghurt* extract *Syzigium myrtifolium* is in accordance with the viscosity standards in the literature. The increase in viscosity was due to the availability of glucose which came from the addition of *Syzygium myrtifolium* extract. Glucose in the extract plays an important role in the fermentation process, this supports the activity of lactic acid bacteria which will affect viscosity [18], [19].

3.2.3. The pH value of soyghurt

The pH value of *soyghurt* met the SNI 2009 requirements because the standard pH value for *yogurt* ranges from 3.5 – 4.5. The acidic pH value of *soyghurt* occurs as a result of fermentation by *Lactobacillus bulgaricus* that produce acid. The pH value also affects the taste of the soyghurt to a sour taste. However, the difference in the addition of extract did not affect the pH *of soyghurt*. The results of the pH test of *soyghurt* are presented in Table 4.

Table 4. The pH value of *Soyghurt*

Concentration	pH	Standard pH
1%	4.40 ± 0.00	3.5 – 4.5
3%	4.40 ± 0.00	3.5 – 4.5
5%	4.40 ± 0.00	3.5 – 4.5

3.3. Antioxidant activity

In testing the antioxidant activity of soyghurt was carried out using the DPPH method. The mechanism of this measurement is the presence of free radicals, DPPH mixed with antioxidant compounds that have the ability to donate hydrogen so that free radicals can be suppressed. DPPH is reacted with a test solution marked as purple to yellow [20]. Measurements from the DPPH assay results used spectrophotometry and obtained the percentage value (%) of inhibition from each concentration. The results of free radical suppression of soyghurt of *Syzygium myrtifolium* leaf extract can be seen in Table 5.

Table 5. The IC_{50} Value

Sample	**IC_{50} (µg/mL)**
Ascorbic acid	1.39
Soyghurt without extract	2823.17
Soyghurt with 1% extracts	963.87
Soyghurt with 2% extracts	793.06
Soyghurt with 3% extracts	694.14

Based on Table 5, ascorbic acid has a very strong antioxidant activity with an IC_{50} value of 1.39 µg/mL, because the smaller the IC_{50} value, the higher the antioxidant activity. Ascorbic acid is an antioxidant that is able to neutralize oxidative stress through the process of donor or electron transfer [21]. From the results of the antioxidant assay, it can also be seen that there is an increase in antioxidant activity in soyghurt after fortified with extract. The IC_{50} value of soyghurt with extract is decreasing along with the addition of concentration, meaning that the greater the concentration of extract added, the stronger the antioxidant activity of soyghurt. The antioxidant activity provided by extract comes from the anthocyanin content. In the structure of anthocyanins, there is an arrangement of conjugated double bonds that are able to make anthocyanin compounds as natural free radical antibodies. The increasing number of phenolic hydroxyl groups in the anthocyanin structure can improve their antioxidant function.

Anthocyanins can prey on different types of reactive oxygen-derived free radicals, such as hydroxyl (OH*), peroxyl (ROO*), and single oxygen (O2*). These free radicals in the body are formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, nicotine, other chemicals, and air pollution [7].

4. CONCLUSION

The results of the antioxidant activity test using the DPPH assay showed that there was a decrease in IC_{50} value in soyghurt fortified with *Syzygium myrtifolium* leaf extract compared to soyghurt without extract. The higher the concentration of the extract, the smaller the IC_{50} value. The fortification of *Syzygium myrtifolium* leaf extract has an effect on the antioxidant activity of soyghurt

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