Development of probiotic drink yogurt from *Dillenia serrata* thunb: antioxidant and antibacterial potencies

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**Abstract**

Yogurt is a functional drink that has been known to have health benefits. Yogurt has higher antioxidant activity than whole milk. This study aimed to determine the ability of antioxidant and antibacterial activity of Songi fruit extract yogurt. The antioxidant activity was assayed using DPPH (1,1-diphenyl-1-picrylhydrazyl) and the antibacterial activity using the agar diffusion method with the well-technique against Gram-negative bacteria *Escherichia coli* ATCC 35218. The test results of secondary metabolites of Songi fruit extract contain tannins, saponins, and flavonoids. The Songi fruit extract was formulated into 4 formulas, which were Formula I (0%), Formula II (10%), Formula III (15%), and Formula IV (20%). The results of the antioxidant activity test obtained with IC$_{50}$ values of formulas were 121, 75.38, 50.50, and 36.58 mg/mL respectively, and 84.82 mg/mL for commercial yogurt. Test results of antibacterial activity against bacteria *E. coli* ATCC 35218 with inhibition zone diameters were 0, 0, 1.5, and 3.5 mm, respectively for Formula I-IV, and the commercial yogurt was 0 mm. Formula IV (20% fruit extract yogurt) possessed the highest antioxidant and antibacterial activity with IC$_{50}$ 36.58 mg/mL and an inhibition zone diameter of 3.5 mm. In conclusion, the Songi fruit extract yogurt has antioxidant and antibacterial activity against *E. coli*.

1. **Introduction**

Songi fruit (*Dillenia serrata* Thunb.) is an endemic plant of Sulawesi which mainly utilized by people as a fruit and it is commonly used as traditional medicine by the people of Muna, especially in terms of medicine for digestive disorders and the treatment of diarrhea (Windadri et al., 2006). Songi belongs to the Dilleniaceae family which contains triterpenoid compounds, flavonoids, saponins, flavonoid glycosides, and phenolic compounds. It has several pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant activities (Jalil et al., 2015).

Yogurt is one type of functional drink that has been known to have health benefits. It can be used as a substitute for people with lactose intolerant, fighting the growth of pathogenic bacteria in the digestive tract, reducing digestive cancer or tumors, reducing blood cholesterol levels, and stimulating the nervous system (Legowo et al., 2009). The yogurt is produced from milk fermentation by lactic acid bacteria, therefore has a sour taste (Adolfsson et al., 2004).

Yogurt has been known to have higher antioxidant activity than whole milk (Liu et al., 2005). Yogurt production through fermentation is involving *Lactobacillus* bacteria. The previous study showed that yogurt has high antioxidant activity, providing benefits of safe and effective natural antioxidants for consumers (Zhang et al., 2011). In addition, it showed antioxidant activity in plain yogurt of 47.85-60.67 mg/mL against DPPH (2,2'-diphenyl-1-picrylhydrazyl) (Pereira et al., 2013).

Fermentation by using lactic acid bacteria produces acid, thus lowering the pH of the environment around the bacteria. As a result, other bacteria will have difficulties growing (Fitrianarni et al., 2014). In addition, several lactic acid bacteria are also known to produce

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antagonistic compounds (Rahayu, 2008). These compounds include hydrogen peroxide, diacetyl, and bacteriocin. A study researched by Aslam et al. (2011) has successfully isolated bacteriocin from Streptococcus thermophilus bacteria, inhibiting Gram-positive and Gram-negative bacteria. The addition of natural ingredients to yogurts, such as fruit and vegetables, is usually added to increase their antioxidant and functional activity (Pereira et al., 2013). In addition, the fruits and vegetables in yogurt can act as prebiotics, flavoring, and coloring agents (Allgeyer et al., 2010). Research in Portugal proved that the addition of fruit pieces in yogurt could increase antioxidant activity and increase consumer protection against diseases related to free radicals and oxidative stress (Salem et al., 2006). Thus, this study aimed to determine the antioxidant and antibacterial activity of yogurt from Songi fruit juice.

2. Materials and methods

2.1 Materials

Songi fruit (Dillenia serrata Thunb.) was obtained from Laeya, Kulisusu, North Buton Regency, Southeast Sulawesi Province. The plant was identified at the Botanical Laboratory, Faculty of Mathematics and Natural Sciences, Halu Oleo University. Skimmed UHT milk, sugar, phenolphthalein indicator (Merck), yogurt bio culture, 0.1 N NaOH, 95% alcohol, distilled water, DMSO, Nutrient Borth (Beef Extract, peptone, NaCl), Nutrient Agar (Beef Extract, peptone, NaCl, agar), DPPH (2,2’-diphenyl-1-picrylhydrazyl), 95% ethanol, and tetracycline were used as materials in this study.

2.2 Sample preparation

Songi fruit (Dillenia serrata Thunb.) was cleaned from the fruit wrapper, washed and 400 g was weighed, mashed using an extractor machine, and then pasteurized at 80°C for 15 mins (Kartikasari et al., 2014).

2.3 Phytochemical screening

Phytochemical screening was performed to identify the secondary metabolites' content in Songi fruit extract using modified methods (Farnsworth, 1966; Harborne, 1996). The secondary metabolite compounds that will be detected include alkaloids (Dragendorff), flavonoids (Mg and HCl), steroids (chloroform and H2SO4), tannins (FeCl3), and saponins (cooled hot water) (Farnsworth, 1966; Harborne, 1996).

2.4 Preparation of Songi fruit extract yogurt

The milk was heated to 45°C, then added with a yogurt culture starter containing a mixture of Lactobacillus acidophilus, S. thermophilus, and Bifidobacterium as much as 3%, Incubated at 45°C for 4-5 hrs. Then, Songi (Dillenia serrata Thunb.) extract was added and mixed well. Incubation was continued at 45°C for 2 hrs (Harjiyanti et al., 2012). The yogurt formulas are presented in Table 1. The yogurt sample was then tested for acidity, total acid titration, antioxidant activity test using the DPPH method, and antibacterial activity testing.

Table 1. Yogurt formula in 200 mL

| Ingredients       | I Formula | II Formula | III Formula | IV Formula |
|-------------------|-----------|------------|-------------|------------|
| Songi Extract     | 0%        | 10%        | 15%         | 20%        |
| Sugar             | 6%        | 6%         | 6%          | 6%         |
| Bacteria mixture  | 3%        | 3%         | 3%          | 3%         |
| UHT Milk          | Up to 200 mL | Up to 200 mL | Up to 200 mL | Up to 200 mL |

2.5 Data analysis

The IC50 value calculated the data obtained from the antioxidant activity test. At the same time, the antibacterial activity test was measured by measuring the diameter of the inhibition zone.

3. Results and discussion

3.1 Sample determination

Determination of plants was carried out to identify and ensure that the plant material used in the research is D. serrata (Songi). The identification results at the Botanical Laboratory of the Biology Department, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia, showed that the sample used in this study was Songi fruit with the species Dillenia serrata Thunb.

3.2 Characteristics of Songi fruit extract

Songi extracts 400 g was extracted using an extractor instrument and yielded 190 mL (47.5%) of extract. The characteristic of the extract was yellow, has a distinctive odor, sour taste with a pH value of 3.

3.3 Songi fruit extract phytochemical screening

The results of phytochemical screening exhibited that extract of Songi fruit (Dillenia serrata Thunb.) was positive for alkaloids, tannins, saponins, steroids, and flavonoids (Table 2).

Table 2. Phytochemical screening results of songi extract

| Compounds     | Results |
|---------------|---------|
| Alkaloid      | -       |
| Tannin        | +       |
| Saponin       | +       |
| Steroid       | -       |
| Flavonoid     | +       |

+: positive, -: negative
3.4 Yoghurt Songi fruit extract

Songi extract yogurt was obtained through a fermentation process that was formulated into 3 formulas that contain various concentrations of Songi extract (10%, 15%, and 20%, v/v). For all formulas, they were experiencing the change in milk due to the addition of culture because lactic acid bacteria break down the lactose in milk into glucose and galactose. Furthermore, glucose is converted to pyruvic acid, lactic acid, and small amounts of acetic acid and CO₂ (Widyastuti et al., 2014). The results of yogurt made from Songi Fruit Extract are shown in Figure 1.

The addition of fruit was performed after 4 hrs of fermentation to produce yogurt with a good texture, and the fruits are evenly distributed and produce a different aroma and taste from plain yogurt (Widodo, 2002). According to research by Setianto et al. (2014), yogurt made by adding Salak Pondoh fruit extract to yogurt produces a more favorable taste and aroma. It produces higher lactic acid than plain yogurt, as well.

3.5 Yogurt acidity degree

The degree of acidity is used to measure the acidity level in yogurt. Farinde et al. (2010) state that good yogurt has a pH value between 3.8 - 4.6. The decrease in the pH of yogurt is influenced by the activity of Lactic Acid Bacteria (LAB) during the fermentation process by utilizing the existing lactose to form lactic acid, resulting in a decrease in the value of pH (Djaafar and Rahayu, 2006).

Figure 2 shows the effect of Songi extract addition on the pH of yogurt. The pH value obtained was 5.9-4.36 after 5 hrs of fermentation time. For yogurt with 0% fruit extract (Formula I), it had a pH value below the standard. It is caused by the source of nutrition that only comes from milk lactose, while yogurt fruit extract contains organic acids from fruit extract as a source of other nutrients that can be broken down into lactic acid.

The organic acids found in many fruits and vegetables are citric acid and malic acid. The citric acid in fruit extract is converted into succinic acid, while malic acid is converted into lactic acid. So that the overhaul of organic compounds also influences the decrease in pH of bacteria present in the product (Kartikasari et al., 2014). Yogurt with 20% fruit extract (Formula IV) has the best pH value with a pH value of 4.36.

3.6 Total sour yogurt Songi fruit extract

A total acid test was conducted to determine the lactate percentage in yogurt and the quality standard of total acid in yogurt fermented drinks ranges from 0.5-2.0% lactate. According to Figure 3, the total acidity of yogurt obtained for all formulas is in the range of 0.81-1.26 %. They met the standards of the range of total acid in yogurt fermented drinks. The highest total acid value was obtained from 20%, which had the best total acid value with 1.12%.
stimulating the secretion of gastric extracts, and as a source of energy in the respiration process. In addition to these advantages, lactic acid exhibits a bacteriostatic (sometimes bactericidal) effect on spoilage organisms. Lactic acid also acts as an antiseptic and helps facilitate calcium and phosphorus absorption from dairy products.

3.7 Antioxidant activity of yogurt Songi fruit extract using the DPPH method

Antioxidant activity is the ability of a which scavenging or slowing down the oxidation reaction, either with a termination or a prevention mechanism (Yu, 2008). Termination of the oxidation reaction is a primary antioxidant function, by donating hydrogen atoms to radicals. At the same time, prevention is a secondary antioxidant function that can be carried out through several mechanisms, binding metal ions, capturing oxygen, and breaking down hydroperoxides into non-radicals. The ability of antioxidant activity is generally divided into 4, powerful antioxidants with IC$_{50}$ values between 0 to 50, potent antioxidants with IC$_{50}$ 50-100 mg/mL, moderate antioxidants with IC$_{50}$ 100-150 mg/mL, and weak antioxidants with IC$_{50}$ between 151-200 mg/mL.

According to results obtained (Table 3), Formula IV possessed a powerful antioxidant with an IC$_{50}$ value of 36.58 mg/mL compared to Formula II and III, as well as the commercial yogurt used with an IC$_{50}$ value of 75.38, 50.50, and 84.82 mg/mL, respectively.

The flavonoid content in fruit extract possessed antioxidant activity due to it inhibits the oxidation process through free radical scavenging mechanisms by donating one electron to an unpaired electron. Flavonoids are strong inhibitors of lipid peroxidation and can inhibit the activity of lipoxygenase and cyclooxygenase enzymes (Rohman and Sugeng, 2005). In previous research conducted by El Samh et al. (2013), plain yogurt measured using the DPPH Radical Scavenging Activity (RSA) method, had an antioxidant activity of 28.49% after adding 1.5% strawberry, black carrot, and pumpkin jam, sequentially, the antioxidant activity increased to 1.5%. 40.12%, 31.69%, and 36.34%.

3.8 Songi fruit extract yogurt antibacterial activity

An antibacterial activity test was conducted to determine the inhibition of yogurt against Escherichia coli ATCC 35218, a pathogenic bacterium that in excess

| Sample         | Concentration (mg/mL) | Inhibition (%) | IC$_{50}$ (mg/mL) | Interpretation |
|----------------|-----------------------|----------------|-------------------|----------------|
| Formula I      | 20                    | 11.676         |                   |                |
|                | 40                    | 17.365         |                   |                |
|                | 60                    | 24.251         | 121.69            | Weak           |
|                | 80                    | 36.826         |                   |                |
|                | 100                   | 40.718         |                   |                |
| Formula II     | 20                    | 18.843         |                   |                |
|                | 40                    | 26.552         |                   |                |
|                | 60                    | 43.468         | 75.38             | Potent         |
|                | 80                    | 48.608         |                   |                |
|                | 100                   | 67.023         |                   |                |
| Formula III    | 20                    | 36.402         |                   |                |
|                | 40                    | 44.111         |                   |                |
|                | 60                    | 50.535         | 50.50             | Potent         |
|                | 80                    | 66.595         |                   |                |
|                | 100                   | 77.087         |                   |                |
| Formula IV     | 20                    | 44.111         |                   |                |
|                | 40                    | 51.391         |                   |                |
|                | 60                    | 58.886         | 36.58             | Powerful       |
|                | 80                    | 63.169         |                   |                |
|                | 100                   | 71.092         |                   |                |
| Commercial Yogurt | 20                   | 16.766         |                   | Potent         |
|                | 40                    | 30.838         |                   |                |
|                | 60                    | 35.628         | 84.82             | Potent         |
|                | 80                    | 46.407         |                   |                |
|                | 100                   | 58.682         |                   |                |

*Classified as powerful if IC$_{50}$ between 50 mg/mL; 50-100 mg/mL is classified as potent; 100-150 mg/mL is classified as intermediate; and 151-200 mg/mL is classified as weak (Lee et al., 2005).
can cause diarrhea. According to Table 4 and Figure 4, only Formula III and IV exhibited weak antibacterial potency.

Yogurt extract of 15% and 20% might inhibit the growth of \textit{E. coli} bacteria due to the content of secondary metabolites of flavonoids, saponins, and tannins in Songi extract. Tannins work as antibacterial by forming stable bonds with proteins (Simaremare, 2014). Saponins act as antibacterial by increasing membrane permeability in cells, resulting in hemolysis. Flavonoids as a phenol group tend to inhibit microbial enzyme activity, ultimately disrupting metabolic processes (Nikhman and Taty, 2012).

### 4. Conclusion

Songi extract contains secondary metabolites, namely tannins, saponins, and alkaloids. Among all the formulas used, formula IV with a concentration of 20% has potential antioxidant activity against free radicals with the highest value at IC\textsubscript{50} 36.58 mg/mL. It also provided weak antibacterial activity against Escherichia coli ATCC 35218 at a value of 3.5 mm.

### Conflict of interest

The authors declare no conflict of interest.

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