Food Research 6 (5) : 124 - 129 (October 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

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

[1]Nuralifah, [2]Susilowati, P.E., [1,3]Parawansah, [1]Ernisa, A., [4]Purnama, L.M.J., [4]Asasutjarit R. and [1,4,*]Fristiohady, A.

[1]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Halu Oleo University, Indonesia*
[2]*Department of Chemistry, Faculty of Math and Natural Science, Halu Oleo University, Indonesia*
[3]*Department of Biomedical, Faculty of Medicine, Halu Oleo University, Indonesia*
[4]*Thammasat University Research Unit in Drug, Health Product Development and Application, Faculty of Pharmacy, Thammasat University, Thailand*

Article history:
Received: 27 July 2021
Received in revised form: 30 August 2021
Accepted: 19 December 2021
Available Online: 16 September 2022

Keywords:
Yoghurt,
Dillenia serrata Thunb.,
Songi extract,
Antioxidants,
Antimicrobial

DOI:
https://doi.org/10.26656/fr.2017.6(5).559

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-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

*Corresponding author.
Email: *adryanfristiohady@uho.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

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 H_2SO_4), tannins ($FeCl_3$), 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	Formula I	Formula II	Formula III	Formula IV
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 to200 mL

2.5 Data analysis

The IC_{50} 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 CO_2 (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.

Figure 1. Songi extract yoghurt (a) Formula II, (b) Formula III, (c) Formula IV

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.

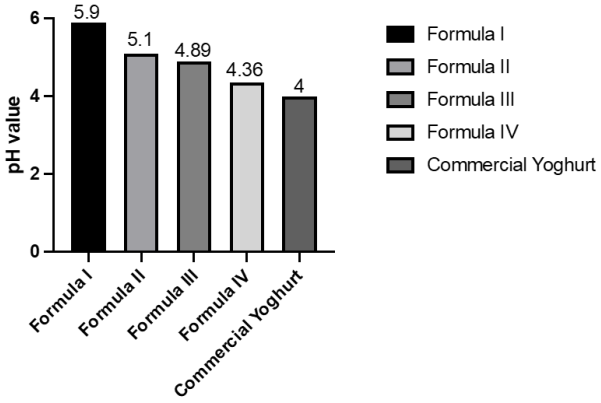

Figure 2. The acidity of formulas

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%.

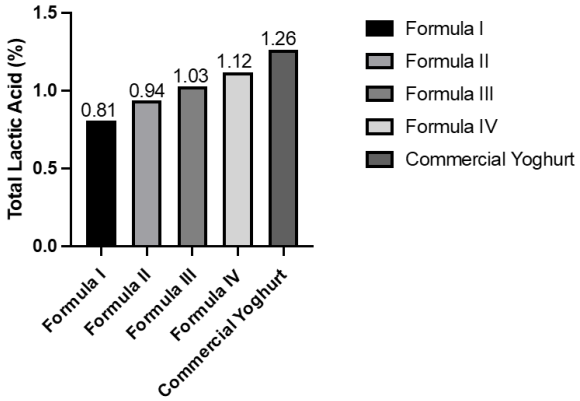

Figure 3. The total lactic acid

Lactic acid produced as a product also affects the pH value because it will dissociate to produce H^+ and $CH_3CHOHCHOO^-$. It means the higher the lactic acid, the higher the H^+ ions to be liberated in the medium, thereby lowering the pH value (Primurdia and Kusnadi, 2014). According to Nahaishi (1985), lactic acid has several benefits, such as better digestibility of milk protein. Lactic acid is deposited into fine curd particles, increasing the use of calcium, phosphorus, and iron,

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

Table 3. Antioxidant activity of songi extract yogurt

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

Table 4. Zone inhibition diameter against *E. coli* ATCC 35218

Bacteria	Sample	Zone inhibition Diameter	Results
E. coli	Songi Extract	6	Intermediate
	Commercial Yogurt	0	-
	Formula I	0	-
	Formula II	0	-
	Formula III	1.5	Weak
	Formula IV	3.5	Weak
	Control (+) Tetracyclin (30 µg/mL)	7	Intermediate
	Control (-) Milk	0	-
	Control (-) Solvent	0	-

If the inhibition zone diameter is >20 mm, it is categorized as powerful; 10-20 mm diameter is categorized as potent; 5-10 mm is categorized as intermediate; and <5mm is categorized as weak (David and Stout, 1971).

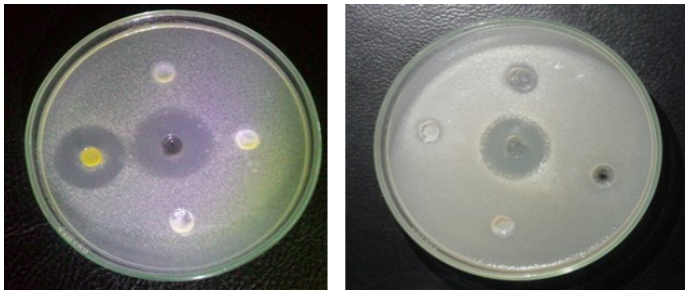

Figure 4. Antibacterial activity test. (+) positive control (Tertracylin 30 g/mL) (a) songi extract, (b) commercial yogurt, (c) formula I, (d) Formula II, (e) formula III, (f) formula IV, (g) negative control I (drug solvent), (h) Negative control II (milk)

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_{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.

Acknowledgments

AF was funded by Thammasat University Postdoctoral Grant no. TUPD 18/ 2021.

References

Adolfsson, O., Simin, N.M. and Robert, M.R. (2004). Yogurt and Gut Function. *Journal of Clinical Nutrition*, 80(2), 245-56. https://doi.org/10.1093/ajcn/80.2.245

Allgeyer, L.C., Miller, M.J. and Lee, S.Y. (2010). Sensory and Microbial Quality of Yogurt Drinks with Prebiotics and Probiotics. *Journal of Dairy Science*, 93(10), 4471-4479. https://doi.org/10.3168/jds.2009-2582

Aslam, M., Shahid, M., Rehma, F.U., Naveed, N.H., Al Batool, Sharif, S. and Asia, A. (2011). Purification and Characterization of Bacteriocin Isolate from *Streptococcus thermophilus*. *African Journal of Microbiology Research*, 5(18), 2642-2648. https://doi.org/10.5897/AJMR11.225

Djaafar, T.F. and Rahayu, E.S. (2006). Karakteristik yogurt dengan inokulum Lactobacillus yang diisolasi dari makanan fermentasi tradisional. *Agros,* 8(1), 73-80.

Farinde, E.O., Obatolu, V.A., Oyarekua, M.A., Adeniran, H.A, Ejoh, S.I. and Olanipekun, O.T. (2010). Physical and Microbial Properties of Fruit Flavoured Fermented Cowmilk and Soymilk (Yoghurt-Like) Under Different Temperature of Storage. *African Journal of Food Science and Technology*, 1(5), 120-127.

Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. *Journal of Pharmaceutical Science,* 55(3), 225-276. https://doi.org/10.1002/jps.2600550302

Fitrianarni, D., Muslimin, I. and Guntur, T. (2014). Aktivitas Antibakteri Yoghurt Susu Sapi dan Yoghurt Susu Kedelai terhadap *Shigella dysenteriae* secara *In Vitro. Lentera Bio,* 3(1)*,* 97–102. [In Bahasa Indonesia].

Harborne, J. (1996). Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan. 2nd ed. Translator: Padmawinata, K. and I. Soediro. Bandung, Indonesia: Penerbit ITB. [In Bahasa Indonesia].

Harjiyanti, M.D., Pramono, Y.B. and Mulyani, S. (2012). Total Asam, Viskositas dan Kesekuaan pada Yoghurt Drink dengan Sari Buah Mangga (*Magifera indica*) sebagai Perisa Alami. *Jurnal Aplikasi*

Teknologi Pangan, 2(2), 104-107

Jalil, J., Carla, W.S., Norizan, A., Jamia, A.J., Brahim, J., Nor-Ashila, A., Kartiniwati, M., Fhataheya, B, Hazni, F.M. and Idin, S. (2015). Inhibitory Effect of Triterpenoids from *Dillenia serrata* (Dilleniaceae) on Prostaglandin E2 Production and Quantitative HPLC Analysis of Its Koetjapic Acid and Betulinic Acid Contents. *Molecules*, 20(2), 3206-3220. https://doi.org/10.3390/molecules20023206

Kartikasari, D.I. and Fithri C.N. (2014). Pengaruh Penambahan Sari Buah Sirsak dan Lama Fermentasi Terhadap Karakteristik Fisik dan Kimia Yoghurt. *Jurnal Pangan dan Agroindustri,* 2(4), 239-248. [In Bahasa Indonesia].

Lee, J., Hwang, K., Chung, M.Y., Chao, D.H. and Park, C.S. (2005). Resistance of *Lactobacillus casei* KCTC 3260 to reactive oxygen species (ROS): Role for a metal ion chelating effect. *Journal of Food Sciences*, 70(8), 388-391. https://doi.org/10.1111/j.1365-2621.2005.tb11524.x

Legowo, A.M., Kusrahayu, and Mulyani S., (2009). Teknologi Pengolahan Susu. Semarang, Indonesia: Universitas Dipenogoro. [In Bahasa Indonesia].

Liu, J-R, Yuh-Yih, L., Ming-Ju, C., Li-Ju, C. and Chin-Wen, L., (2005). Antioxidative activities of kefir. *Asian-Australasian Journal of Animal Sciences*, 18 (4), 567-573. https://doi.org/10.5713/ajas.2005.567 [In Bahasa Indonesia].

Nahaishi, M.H. (1986). Lactobacillus acidophilus therapeutic properties, products, and enumeration. In Robinson, R.K. (Ed.) Developments in Food Microbiology. London, UK: Elsevier Applied Science Publishers

Nikhman, and Taty E.B. (2012). Uji Bahan Baku Antibakteri dari Buah Mahkota Dewa (*Phaleria macrocarpa)* Hasil Iriadiasi Gamma dan Antibiotik terhadap Bakteri Patogen, presented at Pertemuan Ilmiah Ilmu Pengetahuan dan Teknologi Bahan 2012*,* 3 Oktober 2012. Serpong, Indonesia. [In Bahasa Indonesia].

Pereira, E., Barros, L. and Ferreira, I. (2013). Relevance of The Mention of Antioxidant Properties in Yoghurt Labels: *In Vitro* Evaluation and Chromatographic Analysis. Antioxidants. *Journal Antioxidant,* 2(2), 62 -76. https://doi.org/10.3390/antiox2020062

Primurdia, E.G. and Kusnadi, J. (2014). Aktivitas Antioksidan Minuman Probiotik Sari Kurma (*Phoenix dactilyfera* L.) Dengan Isolat *L. plantarum* Dan *L. casei***.** *Jurnal Pangan dan Agroindustri,* 2(3), 98-109. [In Bahasa Indonesia].

Rahayu, E.S. (2008). Bakteri Asam Laktat – Laporan Inhernt. Yogyakarta, Indonesia: UGM and Udayana. [In Bahasa Indonesia].

Rohman, A. and Riyanto, S. (2005) Daya Antioksidan Ekstrak Etanol Daun Kemuning (*Murayya paniculata (L) Jack)* Secara in vitro. *Majalah Farmasi Indonesia,* 16(3), 136-140.

Salem, A.S., Gafour, W.A. and Eassawy, E.A. (2006). Probiotic Milk Beverage Fortified with Antioxidants as Functional Ingredients. *Egyptian Journal of Dairy Sciences*, 34, 23-32.

El Samh, M.M.A., Sherein, A.D.D. and Essam, H.H. (2013). Properties and Antioxidant Activity of Probiotic Yoghurt Flavored with Black Carrot, Pumpkin, and Strawberry. *International Journal of Dairy Science,* 8(2), 48-57. https://doi.org/10.3923/ijds.2013.48.57

Simaremare, E.S. (2014). Skrining Fitokimia Ekstrak Etanol Daun Gatal (*Laportea decumana* (Roxb.) Wedd). *Pharmacy*, 11(1), 98-107. https://doi.org/10.14710/jnc.v3i4.6843 [In Bahasa Indonesia].

Widodo, W. (2002). Fermentasi Susu, Pusat Pengembangan Bioteknologi, Malang, Indonesia: UMM. [In Bahasa Indonesia].

Widyastuti, Y., Rohmatussolihat and Febrisiantosa, A. (2014). The Role of Lactic Acid Bacteria in Milk Fermentation. *Food and Nutrition Sciences*, 5(4), 435-442. https://doi.org/10.4236/fns.2014.54051

Windadri, F.I., Rahayu, M.T. and Rustiami, H. (2006). Pemanfaatan Tumbuhan sebagai Bahan Obat oleh Masyarakat Lokal Suku Muna di Kecamatan Wakarumba, Kabupaten Muna, Sulawesi Utara. *Biodiversitas* 7, 333–339. https://doi.org/10.13057/biodiv/d070407 [In Bahasa Indonesia].

Yu, L. (2008*).* Wheat Antioxidants, United States of America: John Wiley and Sons, Inc

Zhang, S., Lu, L., Su, Y., Li, H., Sun, Q., Liang, X. and Lv, J. (2011). Antioxidative activity of lactic acid bacteria in yogurt. *African Journal of Microbiology Research*, 5(29), 5194-5201. https://doi.org/10.5897/AJMR11.997

