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The Effect of Kersen (*Muntingia calabura*) Fruit Extract Addition to The Yogurt Antibacterial Activity on *Escherichia coli*

(Pengaruh Penambahan Ekstrak Buah Kersen (Muntingia calabura) terhadap Aktivitas Antibakteri Yogurt pada Escherichia coli)

Annisa Krisridwany*, Putri Ayu Lestari, Aji Winanta, Sabtanti Harimurti, Vella Lailli Damarwati

School of Pharmacy, Faculty of Medicine and Health Science, University of Muhammadiyah Yogyakarta, Bantul, Indonesia.
**E-mail:* akrisridwany@umy.ac.id

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Corresponding Author:
Annisa Krisridwany
School of Pharmacy
Faculty of Medicine and Health Science
University of Muhammadiyah Yogyakarta
Bantul
55183
Indonesia
email: akrisridwany@umy.ac.id

ABSTRACT

Background: Yogurt, completed with lactic acid bacteria, can inhibit the growth of *Escherichia coli*. The flavonoids, tannin, and saponin of the *Muntingia calabura* L. plant showed antibacterial activity. **Objectives:** The present study aims to observe the effect of the addition of *Muntingia calabura* L. fruit extract to yogurt on the activity of *Escherichia coli*. **Material and Methods:** The study was an experimental study with the treatment of addition of Muntingia fruit extract infusion with various concentrations (12.5 %, 25%, 50%, 75%, and 100% with three times replications) to the cow-milk-based yogurt. The staining reagents were used for the phytochemical screening. The mixed muntingia yogurt (MMY) was then centrifuged to obtain supernatant. The antibacterial activity was tested using the disc diffusion method by observing the diameter of the inhibition zone. Plain yogurt was used for the negative control and Cefotaxime for the positive control. **Results:** In Muntingia fruit extract, flavonoids, tannin, steroid, phenol, and saponin have been observed. The MMY showed antibacterial activity toward *E. coli* as indicated by the various diameter of inhibition zones of 5.03± 2.66 mm, 4.95± 0.39 mm, 5.68± 0.84 mm, 8.02± 1.45 mm, and 15.73± 1.60 mm for the Muntingia addition concentration of 12.5 %, 25%, 50%, 75%, and 100%, respectively. The diameter of the inhibition zone of negative control was 3.95 mm, while positive control was 45.60 mm. **Conclusions:** The addition of *Muntingia calabura* L. fruits extracts to yogurt at concentrations of 75% and 100% could inhibit the growth of *E. coli* bacteria as categorized for medium and strong inhibitory, respectively.

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ABSTRAK

Yogurt merupakan produk pangan fungsional yang mengandung bakteri asam laktat yang dapat menghambat pertumbuhan bakteri *Escherichia coli*. Tanaman *Muntingia calabura* L atau yang dikenal dengan kersen mengadung senyawa seperti flavonoid, tannin, dan saponin yang memiliki aktivitas antibakteri. Penelitian ini dilakukan untuk mengamati efek penambahan ektrak buah *Muntingia calabura* L ke dalam yogurt berbasis susu sapi terhadap pertumbuhan bakteri *Escherichia coli*. Buah Muntingia diekstraksi dengan metode infundasi dan dibuat berbagai konsentrasi yaitu 12.5%, 25%, 50%, 75%, dan 100%. Skrining fitokimia dilakukan berdasarkan uji reagen. Ekstrak buah dicampur kedalam yogurt untuk menghasilkan campuran Muntingia-Yogurt (MMY). Uji aktivitas antibakteri dilakukan dengan metode difusi cakram berdasarkan terbentuknya zona hambat dengan yogurt tanpa ekstrak sebagai kontrol negatif dan antibiotik Cefotaksim sebagai kontrol positif. Senyawa flavonoid, tannin, steroid, fenol dan saponin terdapat di dalam ekstrak buah Muntingia. Hasil uji aktivitas antibakteri, MMY menghasilkan zona hambat sebesar 5.03± 2.66 mm, 4.95± 0.39 mm, 5.68± 0.84 mm, 8.02± 1.45 mm, and 15.73± 1.60 mm untuk konsentrasi 12.5%, 25%, 50%, 75%, 100% sedangkan kontrol negatif sebesar 3.95 mm dan kontrol positif 45.60 mm. Penambahan ekstrak buah Muntingia 75% dan 100% pada yogurt menunjukkan penghambatan pertumbuhan bakteri dengan kategori medium untuk kadar 75% dan kuat untuk kadar 100%.

Kata kunci: Aktivitas antibakteri, *Escherichia coli*, *Muntingia calabura* L, Yogurt

INTRODUCTION

Diarrhea is defecation with a soft to liquid consistency with a higher frequency than usual (three or more times) in one day (KEMENKES RI, 2011). Globally, diarrhea cases happen in all of the age and was the eight leading cause of death and the fifth leading cause of death in children under 5 years old (GBD, 2016). Diarrhea caused by bacteria such as *V. cholerae, C. botulinum, Shigella, C. jejuni, E. coli, Salmonella*, and *S. aureus* is prevalent worldwide (Ugboko et al., 2020). Prevention of diarrhea caused by bacteria is essential, apart from good sanitation. One of the food products that can be used as a prevention against bacteria is yogurt (Abd El Gawad,2014). Yogurt is fermented milk that contains beneficial live bacteria for digestive health. The bacteria used in yogurt are usually *Lactobacilli, Streptococcus thermophilus*, and *Bifidobacteria* (Dahlan, 2017). These bacteria produce lactate or lactic acid bacteria (LAB) after overhauling complex compounds (Trachoo, 2002). Lactic acid bacteria (LAB) can inhibit the growth of pathogenic bacteria such as *Shigella flexneri* and *Escherichia coli* bacteria by forming colonies (Hanum et al., 2017). LAB activity is characterized by increasing lactic acid levels, increasing yogurt acidity. The addition of fruit extract to yogurt can increase the acidity of yogurt so that it can also increase the inhibition of pathogenic bacteria (Jung et al., 2016). Several studies on adding fruit flavors, fruit, fruit oil, and other nutrients to yogurt have been carried out (Gonzalez et al., 2011; Hanson & Metzger, 2010; Perina et al., 2015).

Kersen is a plant that is easily found in the environment around Indonesian society. It is usually planted by the roadside as a shade tree because it can grow quickly and is shady (Yuzammi et al., 2009). This tree is easy to grow, easy to maintain, short fruiting time, and is season-free. In Indonesia, Kersen fruit is considered to have no selling value, so it is very affordable for all people (Rosandari et al., 2011). Kersen, usually called talok or cherries, is usually found in tropical areas, including Indonesia. In some

other countries, it is called datiles, aratiles, manzatinas (Philippines), khoom, somz, takhob (Laos), krakhob barang (Cambodia), kerup siam (Malaysia), capulin blanco, cacaniqua, nigua, iguito (Spain), jamaican cherry, panama berry, cherry fruit (England), and Japanese kers (Netherlands) (Kosasih et al., 2013). Ripe cherry fruit has red skin, small fruit, sweet taste, and is slightly sticky. Cherry fruit contains many compounds, such as water, carbohydrates, fiber, protein, vitamins, and flavonoids (Rahman et al., 2010). Cherry fruit (*Muntingina calabura*) has been studied to have the potential as an antibacterial that can inhibit the growth of gram-negative and gram-positive bacteria (Gorripati et al., 2018; Cuadro et al.,2018). Research conducted by Hadi and Permatasari (2019) stated that the antibacterial compounds in cherry fruit are flavonoids, saponins, tannins, and alkaloids. This fruit has potential as medicine because the ethanol extract of cherry fruit contains flavonoids 32.82 g/kg, saponins 5.07 g/kg, tannins 14.64 g/kg, and polyphenols 69.78 g/kg. These bioactive have antibacterial properties (Ami, 2016). The antioxidant content of cherry fruit is also relatively high because it contains 80.5 mg of vitamin C per 100 g of cherry fruit (Kosasih et al., 2013).

Based on the previous studies stated above, the kersen fruit was predicted to increase the yogurt's ability to inhibit the bacteria due to its antibacterial activity. Furthermore, it can be used to reduce the diarrhea problem because yogurt has been commonly consumed. This research was conducted to see the ability of the combination of cherry fruit extract yogurt to inhibit the growth of *Escherichia coli* bacteria as one of the bacteria that caused diarrhea.

MATERIAL AND METHODS

Materials

The materials used in this study were cherry fruit (*Muntingia calabura* L.) which taken from Bangunharjo, Sewon, Bantul, yogurt starter (mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophillus*), water, fresh cow milk from Kaliurang Yogyakarta, Cefotaxime antibiotic (Hexpharm Jaya®), 0.9% NaCl solution (Otsuka®), Eosin Methylene Blue Agar 27 media (EMBA) (Merck®), Mueller Hinton Agar (MHA) (Merck®), $BaCl_2$, H_2SO_4,(Merck®), HCl (Mallinckrodt®), Mg powder (Bratachem®), NaOH (Merck®), $FeCl_3$, Meyer's reagent, chloroform (Merck®), and anhydrous acetic (Merck®).

Methods

Preparation of Cherry Fruit Infusion

The cherry fruit obtained was wet sorted. The selected cherry fruit should be ripe, red, fresh, and not rotten. It was then cleaned of dirt with running water and drained. It was weighed as much as 250 g and then blended until smooth. After that, 500 mL of water was added, and an infusion was made. The

boiling process was carried out for 15 minutes, calculated at 90°C with occasional stirring. The infusion results were filtered with flannel (Adriyan & Aminah, 2012).

Phytochemical Screening

Phytochemical screening test for flavonoids, saponins, tannins, alkaloids, phenols, and steroids compounds in cherry fruit infusions was carried out qualitatively with color reagent test (Hadi & Permatasari, 2019). The extract was put in 3 test tubes in the flavonoid test, with each test tube containing 7 mL of cherry fruit extract. In tube 1, 5 mL of concentrated H_2SO_4 was added. In tube 2, 5 mL of concentrated HCl and a little Mg powder were added. The color change of tubes 1 and 2 to red indicates a positive result of flavonoids. In tube 3, 5 mL of NaOH was added. It showed a positive result for flavonoids if the color changed to yellow. In the Saponin test, 5 mL of cherry fruit extract was taken, shaken vigorously, and then allowed to stand for 30 seconds. A stable foam showed a positive result of saponins within 30 seconds. In the Tannin test, 5 mL of cherry fruit extract was taken, and $FeCl_3$ was added. A change in green, blue-green, or blue-black color or the presence of a precipitate indicates a positive result for tannins. Tannins is one of the phenolic compounds that contain phenol group that react with the $FeCl_3$ to form a blue-back color (Desinta, 2015). In the Alkaloid test, 5 mL of cherry fruit extract was taken and added with Mayer's reagent. Changes in color and the formation of a precipitate indicated a positive result for alkaloids. In the phenol test, 2 mL of cherry fruit extract was taken, put in a test tube, and added 10 drops of hot water and 3 drops of 3% $FeCl_3$ reagent. A color change to a bluish-green or dark blue indicates a positive phenol result (Harborne, 2006). In the steroid test, 5 mL of cherry fruit extract was taken and then put in 2 test tubes. In the first tube, the Libermann-Burchard test was carried out by adding 2 mL of chloroform, 10 drops of acetic anhydride, and 2 drops of concentrated sulfuric acid. The color changes to bluish red like cherry red on the chloroform layer, indicating a positive steroid test. The Salkowski test was carried out in the second tube by adding 5 drops of chloroform extract and 5 drops of sulfuric acid. If a bluish red color such as cherry red was formed, it indicates a positive steroid test (Krishnaveni & Dhanalakshmi, 2014).

Preparation of Mixed Muntingia Yogurt (MMY)

Whole cow's milk and 10% skim milk were pasteurized first at 61°C for 30 minutes to kill microorganisms in milk so that the development of lactic acid bacteria would not be disturbed due to the presence of other microorganisms. The milk then waited for cooling until its temperature reached 45°C so that the yogurt starter could develop properly. The warm milk that has been mixed with the yogurt starter was taken as much as 50 mL, put in a glass bottle, and added by 50 mL of cherry fruit extract (Setia & Wijayanti, 2019). The extract concentration variations were 12.5%, 25%, 50%, 75%, and 100% v/v, which were made by dissolving the infusion with distilled water (Jung et al., 2016). After

all the yogurt was mixed, it was incubated at 37°C for 20-24 hours in a tightly closed state. After incubation, yogurt can be removed and stored in the refrigerator (Fatmawati et al., 2013). Organoleptic tests were carried out for the physical parameters, texture, taste, aroma, and pH of yogurt.

Table 1. Composition of Mixed Muntingia Yoghurt (MMY)

MMY Concentration (v/v)	**12.5%**	**25%**	**50%**	**75%**	**100%**
Muntingia extract (mL)	6.25	12.5	25	37.5	50
Aquadest (mL)	43.75	37.5	25	12.5	0
Cow milk and 10% skim milk (mL)	50	50	50	50	50
Starter yogurt (mg)	50	50	50	50	50

Antibacterial activity test

Antibacterial activity test was carried out by disc diffusion method, which was determined by the size of the inhibition zone of the produced bacterial growth in millimeter. The diameter of the paper disc was 6 mm, and was used to calculate the zone of inhibition. *Escherichia coli* cultures were taken with heated wire, implanted on EMBA media through scratches, and then incubated at 37°C for 24 hours in an aerobic atmosphere (Setia & Wijayanti, 2019). Each group of yogurts was centrifuged at a speed of 5000 rpm for 15 minutes, and then the formed supernatant will be the sample used in the yogurt activity test (Setia & Wijayanti, 2019). In order to ensure the final product was sterile, physical filtration was conducted by using a 0.22 m syringe filter. Each sterile disc paper (the diameter of the disc 6 mm) was given a sample of yogurt prepared by immersion until it completely wet. It was then incubated at 37°C for 24 hours in an inverted position (Purnomo et al., 2019). The test was carried out with three replications. Yogurt without cherry extract was used as a control, while the antibiotic Cefotaxime 30 mg/mL was used as a positive control. The inhibition zone formed around the paper disc was measured for its vertical and horizontal diameter using a caliper from the edge of the inhibition zone and expressed in millimeters (mm), described in the following equation (Paliling et al., 2016). The observed inhibition zones, were further be classified as the inhibition ability of bacteria refers to Table 2.

$$\text{Diameter of inhibition zone (mm)} = \frac{(Dv-Dc) + (Dh-Dc)}{2}$$

Note:
Dv: vertical diameter (mm)
Dc: paper disc diameter (6 mm)
Dh: horizontal diameter (mm)

Table 2. Category of Inhibition Zone (Indriani, et al, 2020)

Category	Diameter of inhibition zone (mm)
Very Strong	>20
Strong	10-20
Medium	5-10
No activity	<5

RESULTS AND DISCUSSION

Cherry Fruit Infusion

Figure 1. Cherry Fruit

The selected cherry fruit should be ripe, red, fresh, and not rotten (Figure 1). The infusion method produces a liquid fruit extract dissolved in water, attracting polar compounds. Extraction by infusion is an easy, inexpensive, and simple method and can be easily mixed with other solutions, such as milk in the making yogurt process. The results of the cherry fruit infusion were then carried out with phytochemical tests.

Phytochemical Screening

Phytochemical screening was performed to discover the secondary metabolites present in the sample. Based on a qualitative test using color reagents, cherry fruit infusion contained secondary metabolites listed in Table 3. Some of the compounds contained in this infusion or extract of cherry fruit have antibacterial activity. They were saponin, flavonoid, phenol, steroid, and tannin.

Table 3. Phytochemical Screening Result of Cherry Fruit Infusion

Secondary Metabolite	Flavonoid	Saponin	Tanin	Alkaloid	Phenol	Steroid
Result	+++	++	+++	-	+++	+++

Notes: - means that compound did not present in the extract, + means that compound present in the extract

Saponin compounds have antibacterial activity by damaging membrane permeability. It happens because the properties of saponins are similar to detergents. It reduces the surface permeability of the cell wall and causes damage to the permeability of the cell membrane. Damaged cell membranes significantly interfere with the survival of bacteria (Harborne, 2006). This fragile membrane and cell wall make it easier for saponins to diffuse into the cytoplasm, causing cytoplasm to leak and resulting in bacterial death (Cavalieri et al., 2005).

Tannin compounds have antibacterial activity through reactions with cell membranes, inactivation of enzymes, and inactivation of the function of genetic material. It can make the formation of cell walls occur imperfectly because of the interaction between tannins and cell wall polypeptides so that the bacteria will be susceptible to lysis (Sari & Sari, 2011). The color change to dark blue occurs due to the formation of complex compounds with $FeCl_3$. The formation of tannin and $FeCl_3$ complex compounds due to Fe^{3+} ions as the central atom and tannin compounds have O atoms which have lone pairs of electrons that can coordinate to the central atom as the ligand. The Fe^{3+} ion can bind three tannins with two donor atoms, namely O atoms, so six lone pairs of electrons can be coordinated to the central atom. The O atoms at the 4‟ and 5‟ dihydroxy positions have the lowest energy in forming complex compounds, making them possible to become a ligand (Asrilya, 2014). The presence of saponin and tannin in the muntingia extract infusion were correspond to the study conducted by Saraswati et al (2021) that showed the experiment of juice from muntingia extract gave neuroprotective effect on mice due to the presence of the phytochemisty of the muntingia extract.

Phenol compounds have antibacterial activity by denaturing cell proteins where the hydrogen bonds formed between phenol and protein will damage the protein structure. The hydrogen bonds will affect the permeability of the cell wall and cytoplasmic membrane because both are composed of proteins. Permeability of cell walls and cytoplasmic membranes that are disturbed can cause an imbalance of macromolecules and ions in the cell to become lysed (Pelczar et al., 1988). Based on study conducted by Senet et al at 2017, Muntingia fruits ethanolic extract and ethyl acetate extract contain phenolic compounds that exhibited antioxidant activity.

Steroid compounds have antibacterial activity by interacting with cell membrane phospholipids which are permeable to lipophilic compounds. It decreases membrane integrity and changes cell membrane morphology, which causes cells to become brittle and lyse (Ahmed, 2007).

Study from Krishnaveni et al in 2014 reported that the aqueous exctract of Muntingia fruits from India contains alkaloid, steroids, tannins, phenolic compounds but did not contain saponin and flavonoids, however in the present study, the aqueous extract did not contain alkaloids but contain saponin and

flavonoids. These differences might be due to the different location of growth and the state of fruit that it was collected (Khrisnaveni 2014)

Mixed Muntingia Yogurt (MMY)

Based on the organoleptic test results, yogurt made without cherry fruit infusion (control) has a thick texture, white color, has a characteristic sour taste of yogurt, and has a pH of 4.625. Meanwhile, yogurt made with the addition of cherry fruit infusion (MMY) has a thick texture, slightly yellowish-white color, has a sour taste typical of yogurt, has a sweet aroma typical of cherry fruit, has a slightly sour taste, and has a pH of 4.625-4.654 as shown in Figure 2 and Table 4. This result met the yogurt quality requirements (without heat treatment after fermentation) based on the Indonesian National Standard (SNI) 2981: 2009, as follows: 1. The appearance of liquid: thick-solid 2. Odor: normal/typical 3. Taste: Sour 4. Consistency: Homogeneous.

Figure 2. The appearance of MMY (A: 12,5%; B:25%, C: 50%; D: 75%, E:100%v/v)

Table 4. The acidity (pH) of MMY

Concentration of MMY	**12.5 % v/v**	**25 % v/v**	**50% v/v**	**75% v/v**	**100% v/v**
pH	4.625	4.633	4.649	4.654	4.654

Yogurt is a dairy product produced from symbiotic fermentation of lactic acid bacteria (LAB), namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Lactic acid bacteria will convert lactose in milk into lactic acid to increase the acidity of milk and causes yogurt to have a distinctive sour taste (Jannah et al., 2014). The decrease in pH value during the fermentation process resulted in a characteristic sour taste of yogurt, where an acidic pH close to 4.5 is essential as a bactericidal in yogurt preparations (Kotz et al., 1990). Food and drug administration (FDA) stated that the acidity of yogurt is 4.5 due to the amount of lactic acid bacteria in yogurt (Weerathilake et al, 2014). The present study, pH of the plain yoghurt is around 4.6 as reported from Ihemeje et al 2016. The addition of the Muntingia extract did not alter the pH value.

Lactic acid bacteria in yogurt can increase the nutritional content of yogurt and as a good microflora in digestion so that it can improve the digestive process by inhibiting the growth of pathogenic bacteria in the digestive tract. Yogurt can also help people with lactose intolerance, prevent diarrhea, reduce the risk of cancer or gastrointestinal tumors, and lower blood cholesterol levels (Legowo et al., 2009). In making yogurt, the sugar contained in milk and fruits can stimulate the growth of lactic acid bacteria and increase the activity of lactic acid formation.

Antibacterial Activity Test

The inhibitory activity against gram-negative bacteria was performed due to the antibacterial compounds in organic acids (lactic acid, acetic acid, and formic acid), which are present in the fermented milk (Davidson, 1993). Lactic acid and acetic acid produced by lactic acid bacteria are essential antimicrobials with high activity and a broad spectrum of inhibition (Rahayu et al., 2013; Šušković et al., 2010). In addition, lactic acid can kill gram-negative bacteria by damaging the outer membrane of gram-negative bacteria cells. Lactic acid can damage the permeability of the outer cell membrane because it is water-soluble so that it can penetrate the periplasm of gram-negative bacteria through the porin protein on the outer membrane. This damaged outer membrane causes antibacterial compounds from yogurt in the form of bacteriocin, hydrogen peroxide, and diacetyl to penetrate the cell membrane to enter the cytoplasm. This unstable bacterial intracellular activity makes bacterial cells die (Alakomi et al., 2000).

Table 5. Diameter zone of inhibition (mm)

Sample (% v/v)	Inhibition Zone (mm) ± SD
MMY 12,5	5.03± 2.66
MMY 25	4.95± 0.39
MMY 50	5.68± 0.84
MMY 75	8.02± 1.45
MMY 100	15.73± 1.60
Yogurt without muntingia fruits extract	3.95
Positive control (Cefotaxime 30 mg/mL)	45.60

Based on the observations of the clear zone (inhibition zone) on agar media (Table 5), it was shown that yogurt without the addition of cherries showed an inhibition zone diameter of 3.95 mm. It means that fermented milk or yogurt has antibacterial activity against *Escherichia coli*. This result was also supported by research conducted by Danu Purnomo et al. (2019), showing the results of the wide inhibition zone of fermented milk as an antibacterial against *Escherichia coli* of 8.45 ± 0.388 mm. In

this study, the MMY 25 % v/v did not show wider zone of inhibition, might be due to the volume for pippeting the extract. For the MMY 12.5% v/v showed big standard deviation (2.66) and based on the value, it was close to the zone of inhibition of MMY 25 %v/v. With the addition of cherry fruit extract to yogurt, it was found that the greater the concentration of cherry fruit extract, the wider the inhibition zone produced. It means that the increase of cherry fruit concentration will improve the yogurt performance in inhibiting the growth of *Escherichia coli* bacteria. Based on the category of bacterial activity in Table 2, MMY 75%v/v categorized as medium activity, whereas MMY 100% was strong acitivity. This result means that yogurt with cherry fruit extract gave better antibacterial activity than yogurt without cherry fruit extract. Research conducted by Jung et al. (2016) stated that the addition of extracts or fruit in yogurt could increase the acidity of yogurt so that it can also increase the inhibition of pathogenic bacteria. However, the average pH of MMY in this study was similar to the pH of the control yogurt and did not correspond to the result reported by Jung et al (2016), hence it is not significantly different overall. The formation of a bacterial inhibition zone was possibly occurred due to the content of secondary metabolites in cherry fruit extract. For the future study, the antibacterial test should be done for the cherry extract infusion without yogurt and it is better to compare the antibacterial activity within leaves and fruits of Muntingia extract in yogurt.

CONCLUSION

The cherry fruit extract was made through the infusion method. Phytochemistry screening results showed that this extract contained saponin, flavonoid, phenol, steroid, and tannin. The addition of cherry fruit extract to yogurt can increase the ability of yogurt to inhibit the growth of *E. coli* bacteria. The addition of *Muntingia calabura* L. fruits extracts to yogurt at a concentration of 100% gave strong inhibition of the growth of *E. coli* bacteria. It was predicted that the formation of a bacterial inhibition zone was occurred due to the content of secondary metabolites in cherry fruit extract. Further study was needed to explore the antibacterial mechanism of *Muntingia calabura* L. fruits extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Abd El-Gawad IA, El-Sayed EM, El- Zeini HM, Hafez SA, Saleh FA (2014) Antibacterial Activity of Probiotic Yoghurt and Soy-Yoghurt against *Escherichia coli* and *Staphylococcus aureus*. *J Nutr Food Sci* 4: 303. doi: 10.4172/21559600.1000303

Adriyan, & Aminah, S. (2012). Karakteristik Fisik, Kimia, Dan Sifat Organoleptik Yoghurt Dengan Campuran Berbagai Konsentrasi Sari Lidah Buaya (Aloe Vera). *Jurnal Pangan Dan Gizi*, *03*(06), 8.

Ahmed, B. (2007). *Chemistry of Natural Products.* Department of Pharmaceutical Chemistry Faculty of Science Jamia Hamdard.

Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, *66*(5), 2001–2005. https://doi.org/10.1128/AEM.66.5.2001-2005.2000

Ami, P. (2016). Antimicrobial Resistance of Fermented Food Bacteria. In *Fermented Foods, Part I* (0 ed., pp. 258–276). CRC Press. https://doi.org/10.1201/b19872-16

Asrilya, N. J. (2014). Ekstraksi Tanin Dari Tanaman Stevia (Stevia Rebaudiana) Pada Variasi Pelarut Dan Suhu. *UNS-F.MIPA Jur.Ilmu Kimia-M0310034-2014*.

Cavalieri et al., S. J. (2005). *Manual of antimicrobial susceptibility testing.* American Society for Microbiology.

Cuadro Mogollón, Omar & Gonzalez-Cuello, Rafael & González López, July. (2018). In vitro antibacterial and antioxidant activity of *muntingia calabura* fruits extract. *Contemporary Engineering Sciences*. 11. 881-890. 10.12988/ces.2018.8255.

Dahlan Ha, Sani Na. The Interaction Effect Of Mixing Starter Cultures On Homemade Natural Yogurt's Ph And Viscosity. *International Journal of Food Studies.* 2017;6:152–158. Doi: 10.7455/Ijfs/6.2.2017.A3.

Davidson, P. M. (1993). *Antimicrobials In Food*. 721.

Desinta, T. (2015). Penentuan Jenis Tanin Secara Kualitatif Dan Penetapan Kadar Tanin Dari Kulit Buah Rambutan (*Nephelium Lappaceum* L.) Secara Permanganometri.*Jurnal Ilmiah Mahasiswa Universitas Surabaya* Vol. 4 No.1

Fatmawati et al., U. (2013). *Karakteristik Yogurt yang Terbuat dari Berbagai Jenis Susu Dengan Penambahan Kultur Campuran Lactobacillus bulgaricus dan Streptococcus thermophillus*. 9.

GBD 2016 Diarrhoeal Disease Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases*. 19 September 2018. doi:10.1016/S1473-3099(18)30424-9.

Gonzalez, N. J., Adhikari, K., & Sancho-Madriz, M. F. (2011). Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and synbiotics. *LWT - Food Science and Technology*, *44*(1), 158–163. https://doi.org/10.1016/j.lwt.2010.06.008

Gorripati, S., Rajashekar, K., Dasu, D., Jupaka, A., & Thupurani, M. K. (2018). Bactericidal activity of Flavonoids isolated from *Muntingia calabura*. *International Journal of Life-Sciences Scientific Research*, *4*(3). https://doi.org/10.21276/ijlssr.2018.4.3.14

Hadi, K., & Permatasari, I. (2019). Uji Fitokimia Kersen *(Muntingia calabura .L) Dan* Pemanfaatanya Sebagai Alternatif Penyembuhan Luka. *Prosiding SainsTekes Semnas MIPAKes UMRi*,1, 10.

Hanson, A. L., & Metzger, L. E. (2010). Evaluation of increased vitamin D fortification in high-temperature, short-time–processed 2% milk, UHT-processed 2% fat chocolate milk, and low-fat strawberry yogurt. *Journal of Dairy Science*, *93*(2), 801–807. https://doi.org/10.3168/jds.2009-2694

Hanum, Z., Rastina, R., & Wanniatie, V. (2017). Kemampuan Antibakteri Susu Fermentasi terhadap Escherichia coli dan Shigella. *Jurnal Agripet*, *17*(1), 24–30. https://doi.org/10.17969/agripet.v17i1.6572

Harborne, J. B. (2006). Metode fitokimia: Penuntun cara modern menganalisis tumbuhan. Edisi IV. Kokasih P. dan I. Soediro. (Penterjemah). *Institut Teknologi Bandung*.

Ihemeje, A & Nwachukwu, C & Ekwe, Chiwundu. (2015). Production And Quality Evaluation Of Flavoured Yoghurts Using Carrot, Pineapple, And Spiced Yoghurts Using Ginger And Pepper Fruit. *African Journal Of Food Science*. 9. 163-169. 10.5897/Ajfs2014.1244.

Indriani, V., Chiuman, L., Wijaya, L.L., Lister, G., & Grandis, L. (2020). Antibacterial Effect of Curcuma zedoaria Extract on *Bacillus cereus* and *Staphylococcus epidermidis*. *Althea Medical Journal, 7*, 6-10.

Jannah, A. M., Legowo, A. M., & Y.B Pramono. (2014). Total Bakteri Asam Laktat, pH, Keasaman, Citarasa dan Kesukaan Yoghurt Drink dengan Penambahan Ekstrak Buah Belimbing. *Jurnal Aplikasi Teknologi Pangan*, *3*.

Jung, J., Paik, H.-D., Yoon, H. J., Jang, H. J., Jeewanthi, R. K. C., Jee, H.-S., Li, X., Lee, N.-K., & Lee, S.-K. (2016). Physicochemical Characteristics and Antioxidant Capacity in Yogurt Fortified with Red Ginseng Extract. *Korean Journal for Food Science of Animal Resources*, *36*(3), 412–420. https://doi.org/10.5851/kosfa.2016.36.3.412

KEMENKES RI. (2011). *Pedoman Umum Penggunaan Antibiotik*. Kementerian Kesehatan RI.

Kosasih, E., Ana, E., & Encun. (2013). *Talok/kersen (Muntingia culabura L)*. Badan Litbang Kehutanan Jakarta.

Kotz, C. M., Peterson, L. R., Moody, J. A., Savaiano, D. A., & Levitt, M. D. (1990). In vitro antibacterial effect of yogurt onEscherichia coli. *Digestive Diseases and Sciences*, *35*(5), 630–637. https://doi.org/10.1007/BF01540412

Krishnaveni, M., & Dhanalakshmi, R. (2014). Qualitative and Quantitative Study of Phytochemicals in *Muntingia calabura* L. Leaf and fruit. *World Journal of Pharmaceutical Research*, *3*.

Legowo, A. M., Mulyani, S., & Kusrahayu. (2009). TEKNOLOGI PENGOLAHAN SUSU. *Universitas Diponegoro*, 27.

Paliling, A., Posangi, J., & Anindita, P. S. (2016). Uji daya hambat ekstrak bunga cengkeh (Syzygium aromaticum) terhadap bakteri *Porphyromonas gingivalis*. *E-GIGI*, *4*(2). https://doi.org/10.35790/eg.4.2.2016.14159

Pelczar, M. J., Chan, E. C. S., & Pelczar, M. F. (1988). *Elements of microbiology*. McGraw-Hill.

Perina, N. P., Granato, D., Hirota, C., Cruz, A. G., Bogsan, C. S. B., & Oliveira, M. N. (2015). Effect of vegetal-oil emulsion and passion fruit peel-powder on sensory acceptance of functional yogurt. *Food Research International*, *70*, 134–141. https://doi.org/10.1016/j.foodres.2015.01.014

Purnomo, D., Apridamayanti, P., & Sari, R. (2019). Uji Aktivitas Antibakteri Minuman Yoghurt dengan Starter Lactobacillus Casei Terhadap Bakteri *Stapylococcus aureus* dan *Escherichia coli*. 9.

Rahayu, S. E., Yogeswara, A., & Utami, P. (2013). Bakteri Asam Laktat Indigenous Berpotensi Probiotik dan Aplikasinya Untuk Produksi Susu Fermentasi. *Prosiding Seminar Intensif Riset Sinas, Jakarta*.

Rahman, M., Fakir, S. A., & Rahman, M. (2010). Fruit Growth of China Cherry (*Muntingia calabura*). *IDOSI Publications*.

Rosandari, T., Thayib, M. H., & Krisdiawati, N. (2011). *Variasi Penambahan Gula dan Lama Inkubasi pada Proses Fermentasi Cider Kersen (Muntingia calabura L)*.

Saraswati, M, Oeleu, K.Y., Dwi N,D.,2021, The Effect Of Cherry (*Muntingia Calabura*) Juice On Increased Memory In Mice (*Mus Musculus*) By Morris Water Maze Method, Vol 1 No.1, *Journal Of Science And Pharmacy*

Sari, F. P., & Sari, S. M. (2011). Ekstraksi Zat Aktif Antimikroba dari Tanaman Yodium (*Jatropha multifida* Linn) Sebagai Bahan Baku Alternatif Antibiotik Alami. *Fakultas Teknik Universitas Diponegoro*.

Senet, M., Parwata, I., & Sudiarta, I. (2017). Kandungan Total Fenol Dan Flavonoid Dari Buah Kersen (*Muntingia Calabura*) Serta Aktivitas Antioksidannya. *Jurnal Kimia (Journal Of Chemistry),*187-193. Doi:10.24843/Jchem.2017.V11.I02.P14

Setia, R. & Wijayanti, E. D. 2019. Aktivitas Antibakteri Yoghurt Daun Kelor (Moringa oleifera) dan Lidah Buaya (*Aloe vera*) Terhadap *Escherichia coli*. *Akademi Farmasi Putera Indonesia Malang*.

Šušković, J., Kos, B., & Beganović, J. (2010). Antimicrobial Activity – The Most Important Property of Probiotic and Starter Lactic Acid Bacteria. *FTB Journal*. https://www.ftb.com.hr/archives/60-volume-48-issue-no-3/138-antimicrobial-activity-the-most-important-property-of-probiotic-and-starter-lactic-acid-bacteria

Trachoo, N. (2002). Yogurt: The fermented milk. *Songklanakarin J. Sci. Technol. 24*(4), 11.

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., & Oyewale, J. O. (2020). Childhood diarrhoeal diseases in developing countries. *Heliyon*, *6*(4), e03690. https://doi.org/10.1016/j.heliyon.2020.e03690

Weerathilake, W.A.D.V., Rasika, D.M.D., Ruwanmali, J.K.U. And Munasinghe, M.A.D.D. (2014). The Evolution, Processing, Varieties And Health Benefits Of Yogurt. *International Journal Of Scientific And Research Publications*, 4(4), 1-10

Yuzammi, Triono, T., & Handayani, T. (2009). *Ensiklopedia Flora* (Vol. 1).PT Kharisma Ilmu, Bogor, Indonesia