



(RESEARCH ARTICLE)



Effect of flower extract of Roselle (*Hibiscus sabdariffa* L) on microbial quality of raw milk of Etawa goats

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Abstract

Etawa goat milk is the name for dairy products from a hybrid goat named Etawa. In Indonesia, goat milk is usually marketed and consumed fresh, without proper sterilization in the production process. As a raw product, Etawa goat milk has the potential to be contaminated with various microbes. In order to improve the microbial quality of Etawa's raw milk, we conducted a study aimed to evaluate the effect of adding aqueous extract of Roselle flower *Hibiscus sabdariffa* L on the milk content of bacteria. Five groups of fresh milk samples, each 25 ml, were given roselle flower extract sequentially of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml with a repetition of 5 times. Before adding the extract, the type of bacteria identified was *Escherichia coli*, *Lactobacillus* sp, and *Staphylococcus* sp with a total bacterial content of 1,569,586 CFU/ml. After adding extracts of 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.2 ml, the total number of bacteria decreased to 1,078,660 CFU/ml, 1,010,423 CFU/ml, 938,602 CFU/ml, 882,873 CFU/ml, and 874,238 CFU/ml respectively. Thus it can be concluded that roselle flower extract has the potential to be used as an ingredient to improve the microbial quality of fresh milk of Etawa goats.

Keywords: Etawa Goats; Etawa Goat Milk; Roselle Plant; *Hibiscus sabdariffa* L; Microbial Quality of Raw Milk

1. Introduction

Etawa goat milk is the name for dairy milk products from a hybrid goat named Etawa. This goat is the result of cross-breeding of the Etawah goat from India and a local Indonesian goat called the Kacang goats. Fresh goat milk is quite popular in Indonesia because it is believed to have many health benefits and the price is relatively cheap compared to cow's milk. In other extent, goat milk is known to have unique characteristics in comparison to cow milks including higher in digestibility, alkalinity, buffering capacity; lower heat stability; and good for individuals with cow milk allergies and lactose sensitivity [1-3].

However, here the goat milk is usually marketed and consumed fresh, without proper sterilization in the production process. The fresh milk was not processed further because it is considered to reduce the freshness of milk. As a raw product, Etawa goat milk has the potential to be contaminated with various microbes. As a consequence, consumers' expectations of consuming fresh goat's milk in order to obtain its properties cannot be fulfilled [4,5].

To ensure the quality of raw milk from dairy goats, all dairy goat farmers must implement a process of milking, processing and storage of products that meet hygiene standards. In Indonesia, the quality standards for fresh milk are regulated by National Standardization Agency of Indonesia (*Badan Standardisasi Nasional Indonesia* or BSN). In term of raw Etawa goat milk, the quality standard is regulated in SNI (*Standar Nasional Indonesia*) 3141.1:2011 Susu Segar - Bagian 1: Sapi. One of the quality parameters of fresh milk according to the SNI is total bacterial contamination

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(total plate count) not more than 1×10^6 CFU/ml, the total count of *Staphylococcus aureus* less than 1×10^4 CFU/ml, and the Enterobacteriaceae count less than 1×10^3 CFU/ml [6].

To improve the quality of raw milk, various efforts to inhibit bacterial growth have been carried out by researchers in many countries, for example by utilizing herbal plants. *Vernonia amygdalina* is an example of plant that identified as effective to be used for improving the quality and microbial safety of milk [7]. A study reported from Algeria indicated that plant from genera of *Rosmarinus* and *Origanum* is effective in limitation of microbial growth [8]. Previously, from Egypt it has also reported that ethanolic extracts of *Rhus coriaria*, *Tamarindus indica*, *Rosmarinus officinalis*, *Hibiscus sabdariffa* and *Citrus limon* can be used as natural preservatives in cow milk [9].

The three studies conducted in Africa above all used cow's milk as samples. In this study, we re-examined whether the roselle plant, which has been proven to be efficacious as a preservative for cow's milk, can also be used to improve the microbial quality of goat's milk. We chose roselle (*Hibiscus sabdariffa* L.) because this plant is known to have many medicinal benefits including antioxidant, antihypertency, antidiabetes, and antibacterial properties. The antioxidant properties of roselle flowers are possible because of the high content of active compounds such as gossypetin, anthocyanin, phosphorus, iron, organic acid, essential amino acids (lysine and arginine), polysaccharide and omega-3 [10].

Antibacterial properties of roselle plant have been reported in some previous studies. Quercetin (the main flavonoid of this plant), for example, has been shown to have bactericidal properties against Gram positive bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, and *Propionibacterium acne*, as well as Gram negative bacteria such as *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* [11]. Other studies have also succeeded in showing that roselle plants have inhibitory properties against *Streptococcus pyogenes* and *Escherichia coli* [12-14].

2. Material and methods

2.1. Roselle flower and extraction

The roselle plant samples used in this study were the dried flower calyx, labeled as “Teh Bunga Rosella Merah LAM 88”, bought from Vivi Agung Mandiri, Sidoarjo, East Java, Indonesia. Extraction was carried out by decoction technique with an initial ratio of dry calyx and water was 1:4. Boiling (decoction) is considered complete after the volume has shrunk to one-fourth of its original volume. The concentrated extract was then filtered and the filtrate obtained was used as a stock solution.

2.2. Goat's milk samples

Etawa fresh milk samples were taken from a dairy goat farm in Sungai Langka Village, Gedong Tataan District, Pesawaran Regency, Lampung Province, Indonesia. Sampling was carried out in the morning at 5:30. The fresh milk was put into a thermos filled with ice cubes and then taken to the Bacteriology Laboratory at the Department of Health Analyst at the Politeknik Kesehatan Kemenkes Tanjungkarang for further analysis.

2.3. Experimental design and treatment

By using a Completely Randomized Design the etawa goat milk samples were grouped into six with five repetitions, 25 ml each. The first group was not given roselle extract and was used as the control group (base line). Stock solution was divided into five groups with different concentrations, namely: Groups 2 to 5 were each given roselle flower extract of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml respectively. For each of these experimental units (30 units) an examination of the type of bacteria and the total number of bacterial contaminants in the fresh milk sample was carried out.

2.4. Bacterial identification test

To identify the type of microbial contaminants in the goat milk samples, morphological and biochemical tests were carried out. Morphological test performed using MacConkey Agar (MCA, Oxoid), Blood Agar Plate (BAP, Oxoid), and Nutrient Agar Plate (NAP, Merck). After inoculation and incubated for 24 h the cultures were observed and Gram stained. Biochemical test were carried out using Triple Sugar Iron Agar (TSIA Merck), Sulfur Indol Motility (SIM, Merck), Simmons Citrate Agar (SCA, Merck), Methyl Red Voges Proskauer (MRVP, Merck) and Mannitol Salt Agar (MSA, Merck).

2.5. Total plate count

The total bacterial content was calculated using the plate count method. A 1 mL milk sample was taken and then diluted gradually up to 10^{-5} in 0.1% Buffered peptone water (BPW). Then 1 mL of each solution was taken into a sterile petri dish and then poured with liquid Plate Count Agar (PCA, Oxoid CM 325)) and homogenized. The agar is allowed to freeze and incubated at 37 °C for 24 - 48 hours. All growing microbial colonies were counted under the colony counter and determined as the total plate count (TPC) value.

2.6. Data analysis

One way ANOVA was applied to analyzed the difference in mean values of independent variables. Furthermore, Least Significant Difference (LSD) was used in the post hoc analysis.

3. Results and discussion

3.1. Types of bacterial Identified

There are three types of bacteria found in the raw etawa goat milk samples evaluated in this study as presented in Table 1.

Table 1 Types of bacteria contaminating Etawa fresh milk

No	Bacterial species	Type
1	<i>Escherichia coli</i>	Gram-Negative
2	<i>Lactobacillus sp.</i>	Gram-Positive
3	<i>Staphylococcus aureus</i>	Gram-Positive

The presence of the three types of bacteria in Table 1 indicates that the raw milk of the Etawa goat targeted in this study has potential health problems for its consumers. *Escherichia colifor* one instance, although it is known as a harmless bacterium and normally live in human intestine, but certain strains such as *E. coli* O157:H7 may lead to serious illnesses such as severe stomach cramps, bloody diarrhea and vomiting [15, 16].

Next, *Staphylococcus aureus*. This bacterium does not normally cause infection on healthy skin, however if it is allowed to enter the internal tissues or bloodstream, these bacteria may cause a variety of clinical infections. It is a major cause of bacteremia and infective endocarditis as well as osteoarticular [17, 18]. Several infectious diseases caused by *Staphylococcus sp* are boils, pimples, impetigo, and wound infections. More severe infections include pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis and endocarditis [19]. *Staphylococcus sp* is also a major cause of nosocomial infections, food poisoning and toxic shock syndrome [20].

The positive thing in term of bacterial contamination found in fresh Etawa goat milk is the presence of *Lactobacillus sp*. These bacteria are known as the probiotic microbes due to their positive roles in maintaining our health. The bacteria help break down fiber from our diet and able to compete with pathogenic microbes our digestive system. Additionally, lactic acid bacteria also able to ferment our food to simple by products that are easier to be metabolized further [21]. Many species of *Lactobacillus* have excellent decomposing ability of plant matter. The production of lactic acid makes the environment acidic and interferes with the growth of some harmful bacteria [22].

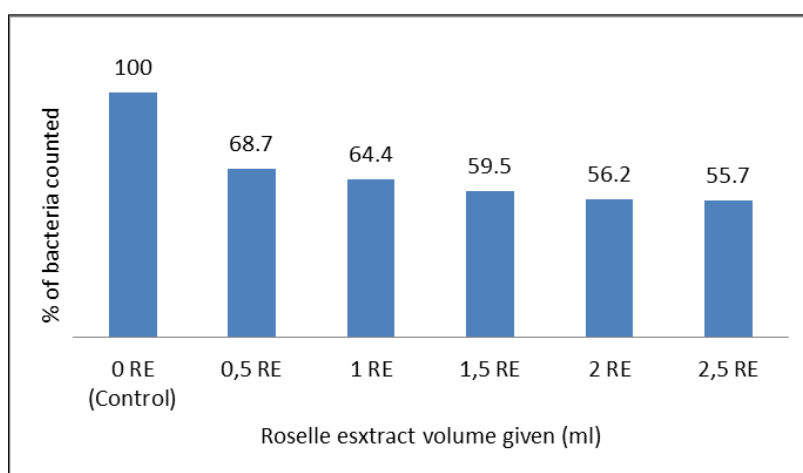
3.2. Total plate count

The effect of giving roselle calyx extract on the total microbial content detected in fresh milk of Etawa goats, expressed in CFU/ml, can be seen in Table 2. If the mean TPC values in Table 1 are compared with each other using the control TPC values as the base line, then the percent change pattern in the number of bacteria in each treatment can be seen in Figure 1.

Table 2 Total plate count (TPC) of microbial contaminant in fresh milk of Etawa goats

No. Trial	Bacterial number by roselle extract (RE) volume given (CFU/ml)					
	0 ml RE (Control, base line)	0.5 ml RE	1 ml RE	1.5 ml RE	2 ml RE	2.5 ml RE
1	1,175,780	1,114,100	1,064,667	963,061	921,289	937,308
2	1,287,575	1,083,175	973,925	968,275	908,075	915,325
3	1,891,875	971,325	905,725	879,625	874,025	899,025
4	1,894,450	1,172,550	1,111,950	956,525	890,725	903,225
5	1,598,250	1,052,150	995,850	925,525	820,250	845,250
Mean	1,569,586^a	1,078,660^b	1,010,423^b	938,602^{bc}	882,873^{bc}	874,238^c

Mean values followed by the same superscript are not statistically different at $\alpha=0.05$

**Figure 1** Percent change in the number of contaminating bacteria in fresh milk of Etawa goats given roselle flower extract (RE) at different concentrations

Based on the microbial TPC values obtained in the control group, as presented in Table 1, it can be established that unsterilized fresh milk of etawa goats does not meet the quality standards required by SNI 3141.1:2011. According to SNI the maximum number of microbes allowed is 1×10^6 CFU/ml, while the TPC value in the fresh etawa goat milk sample we tested reached 1.6×10^6 .

Fortunately, still basing on the data in Table 1, the bacterial contamination in fresh Etawa goat milk can be reduced by adding roselle calyx extract. It was depicted by the significant effects of giving roselle flower extracts of 1.5 - 2.5 ml into 25 ml of milk samples. (F value = 9.759, $P=0.000$). The decrease in TPC values after administration of roselle flower extract, as shown in Figure 1, can reach 44.3%.

These findings confirm the results of previous studies that roselle flower extract does have anti-microbial properties such as against *Streptococcus pyogenes* and *Staphylococcus aureus* [13, 23]. On multidrug-resistant *Salmonella* strains and pathogenic *Escherichia coli* bacteria, the minimum inhibitory concentration of the acetone extract of roselle calyx was 7 mg/mL, and the minimum bactericidal concentration was 10 mg/mL [24].

As it is known that roselle plant flower rich in organic acids, phenolic compounds, flavonoids and anthocyanins and antioxidant [25]. Amongst these, flavonoids, known as polyphenolic compounds, show antibacterial property against a wide range of pathogenic microorganisms, including multidrug resistant bacteria due to their tendency to retard the microbial growth [26].

Flavonoid, especially quercetin, has been at least partially attributed to inhibition of DNA gyrase *Sophoraflavone G* and (-)-*epigallocatechin gallate* proposed to could inhibit cytoplasmic membrane function, whereas licochalcones A and C inhibit energy metabolism. Other flavonoids include robinetin, myricetin, apigenin, rutin, galangin, 2,4,2'-trihydroxy-5'-methylchalcone and lonchocarpol A also have been investigated for their mechanisms of action. These compounds are thought to be feasible to be developed as pharmacologically antimicrobial agent or class of agents [27]. In *Staphylococcus aureus* flavonoid (chalcone) induced a significant membrane permeabilization and lead to cells leakage. In addition, not only bacteria are affected by flavonoid compounds but also fungi [28].

4. Conclusion

The findings of our study show that Etawah goat raw milk without proper treatment and pasteurization does not meet the quality standards set out in SNI 3141.1:2011. The total bacterial content in Etawa raw milk samples can be reduced by up to 43% by adding roselle calyx flower extract. Thus it can be concluded that roselle calyx extract has the potential to be developed as an ingredient in enhancing the microbial quality of fresh milk of Etawa goats.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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