The Application of Three Herbs; *Chrysanthemum indicum*, *Centella asiatica*, and *Andrographis paniculata* to Reduce Bacteria in Cow Milk

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Abstract

Cow milk is a kind of nutritious food that is easy to be contaminated by bacteria. The classic cow milk preservatives methods are heating and cooling in order to get rid of milk spoilage by milk pathogenic microorganisms for safety of consumer. Some of food-borne pathogenic bacteria in milk have extraordinary ability to survive, grow, and proliferate in these classic preservation methods. Based on that reason, a new choice of milk preservation method by using the natural edible antibiotic might be an alternative way to solve this problem.

Three local herbs; *Chrysanthemum indicum*, *Centella asiatica*, and *Andrographis paniculata*, are chosen in this study for their antibacterial activity on bacteria in cow milk. All of the herbs are extracted by soaking the dried ones in 95% ethanol, getting the liquid part only, dried it in water bath until it becomes slurry, and diluted it for 10 mg/ml in 95% ethanol. The extracted herbs are then added to cow milk.

*Chrysanthemum indicum*, *Centella asiatica*, and *Andrographis paniculata* contain many bioactive compounds that have antibacterial activity. *C. indicum* has flavonoid that can kill *Escherichia coli* and *Staphylococcus aureus*, *C. asiatica* contains triterpenoid acids for the most bioactive compound inside that can kill *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella*, and andrographolide is the main contain of *A. Paniculata* that can kill so many bacteria. All of the three herbs extract also can reduce the amount of *Lysteria monocytogenes* and *Bacillus cereus* in milk.

Many lower-middle class communities in Indonesia take advantage from cow milk as their income source. The small traders put value added to cow milk by process the cow milk in sachet and then sell it to family or children as end customers. The processing costs will be cheaper if the traders can process in many sachets, but the barrier is the short shelf life because undesirable bacteria infect the milk. Some traders face lost because of this short shelf life. The advantage of this treatment for cow milk is to extend its shelf life as an inventory, thus can minimize the processing costs. Aim of this study is to compare the effectiveness of antibacterial activity among these three herbs.
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and extend the shelf life and decrease the production cost of cow milk.

Keywords- Andrographis paniculata, Centella asiatica, Chrysanthemum indicum, cow milk, herbs, shelf life

I. INTRODUCTION

Essential oils are highly concentrated substances extracted from various parts of aromatic plants and trees. The aromatic substances from parts, such as petals, leaves, roots, barks, fruits, peels, roots or the whole plant are usually captured by distillation or solvent extraction method. The chemistry components of pure essential oil are very complex. Most consist of hundreds of components, such as terpenes, alcohols, aldehydes and esters. Essential oil has any benefits which are analgesic, anticoagulant, antidepressant, antiseptic, astringent, cicatrisant, cytophylatic and insecticide [1].

Chrysanthemum indicum has been used as a herbal medicine, which is prescribed for anti-inflammatory, analgesic, antipyretic purposes and the treatment of eye disease in Chinese traditional preparations. Chrysanthemum indicum flower has a strong aroma and many of the previous studies focused on the essential oil of this plant. Chrysanthemum indicum flower is a traditional Chinese medicine with strong aroma and many previous studies focused on its essential oil, flavonoids and flavonoid glycosides. Flavonoids are proved to be important bioactives in herbal plants. Several flavonoids and sesquiterpenes have been isolated from the Chinese natural medicine [2].

Centella asiatica belongs to the family of Apiaceae (Umbelliferae). In common with most traditional phytotherapeutic agents, C. asiatica is claimed to possess a wide range of pharmacological effects, being used for human wounds healing, mental disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes. C. asiatica has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid. In addition, numerous clinical reports verify the ulcer-preventive and antidepressive sedative effects of C. asiatica preparations, as well as their ability to improve venous insufficiency and microangiopathy. Previously triterpenoid acids, volatile and fatty oils, alkaloids, glycosides, flavonoids, and steroids have been isolated from the different parts of the plant [3].

Andrographis paniculata grows abundantly in southeastern Asia: Indonesia, India (and Sri Lanka), Pakistan and it is cultivated extensively in China and Thailand. The primary medicinal component of Andrographis is andrographolide. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone". The other medicinal chemicals are also bitter principles: diterpenoids viz. deoxyandrographolide, -19ß-D-glucoside, and neo-andrographolide, all of which have been isolated from the leaves. Besides the related bitters mentioned, other active components include 14-deoxy-11,12-didehydroandrographolide (andrographolide D), homoandrographolide, andrographan, andrographon, andrographosterin, and stigmasterol - the last of which was isolated from an Andrographis preparation. The leaves contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest. It has an antibacterial ability, fights bacterial activity [4].

Milk and its products are kind of perishable food [5]. It contains many high nutrition that is suited ideally for the growth of undesirable bacteria [6]. Cow milk is considered as the most perishable among all because of its form fluid and good nutritive composition.
This kind of milk comes from cow, so it provides a good medium for the growth of bacteria. Cow milk is a kind of nutritious food that is easy to be contaminated [7].

Many preservation methods like heating and cooling like people usually use nowadays cannot guarantee the death of bacteria in milk. There is several bacteria that have a high resistant can contaminate this kind of food. Those bacteria can survive in the very extreme condition like high and low temperature, for example *Listeria monocytogenes* and *Bacillus cereus*. The using of very high or very low temperature to kill those kinds of bacteria can cause the change in the milk quality. It can decrease the nutritional compounds inside and change its flavor and color.

The application of natural edible antibiotic or antibacteria into the cow milk is being a new alternative to decrease the amount of bacteria in milk without change the quality of food. With the less amount of bacteria, the shelf-life of cow milk can be longer because the critical point can be handled. All of low-middle seller cannot apply a modern technology to make the cow milk become very sterile and safe, so they have to sell all of the cow milk out before they get rancid. It just took a day or two to wait until they get rancid. With this application of natural antibiotic from herbs extract, the shelf-life of cow milk can be longer and it gives many advantages to the low-middle seller because they can reduce the number of waste products and also they can sell more products than before and get more benefit from them.

### II. MATERIALS AND METHODS

*Chrysanthemum indicum*, *Centella asiatica*, and *Andrographis paniculata* herbs are cut into small pieces and dried for 45°C in oven overnight and blended into powder then stored in refrigerator 6°C until use. 20 grams of each herb is soaked in 95% ethanol 180 ml for 48 hours and stirred every 12 hours. After that, the liquid is separated by thin cloth and centrifugated at 5000 rpm for 5 minutes. The clear solution is placed in 250 ml beaker glass and dried in water bath at 45°C or rotary evaporator until it becomes slurry. The crude extract of each herb is diluted 100 mg/ml by ethanol 95% and then sterilized by single use filter unit 0.2 µm and placed in the Appendorf tubes aseptically. After that, they are kept in freezer which the temperature is -21°C until use.

All of three herb extract are given into the cow milk with certain concentration. The method of this study is literature review and field survey. There is no certain data because of the limitation of time and equipment. This study has to be further investigated.

### III. RESULTS AND DISCUSSION

Milk is the main source of nutrition. It provides all the essential nutrients for growth and development, e.g. proteins, minerals, carbohydrates, fatty acids, growth factors, immune modulators, etc. [8]. Milk is a good medium for several bacteria to develop. The growth of bacteria in milk depends mainly on temperature and the presence of other bacteria [9]. It is important to know what kinds of bacteria that can grow in milk and make the milk become rancid. The undesirable bacteria found in milk are *Bacillus cereus*, *Brucella*, *Campylobacter jejuni*, *Coxiella burnetii*, *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella*, *Staphylococcus aureus*, and *Yersinia enterocolitica*.

*Bacillus cereus*: These bacteria produce a toxin that can cause diarrhea and another that causes vomiting. *Bacillus cereus* spores are heat-resistant and may survive pasteurization. There have even been very rare cases linked to dried milk and dried infant formula.

*Brucella*: *Brucella* is a bacterial microbe that is found in unpasteurized dairy products.
**Brucella** infection, or Brucellosis, has also been called “Undulant Fever” because of the regular recurrence of fever associated with the disease.

**Campylobacter jejuni**: *Campylobacter jejuni* is the most common bacteria to cause diarrheal disease in the U.S. and is found in raw milk and poultry. It has an increased chance of causing disease when consumed in milk, because the basic pH of milk neutralizes the acidity of the stomach.

**Coxiella burnetii**: *Coxiella* infects a variety of animals, including livestock and pets. The microbe can be found in cow’s milk and is resistant to heat and drying. Infection by *Coxiella* results in Q fever, a high fever that may last up to 2 weeks.

**E. coli**: *E. coli* has been associated with a number of food-borne outbreaks and is the cause of bloody diarrhea. Frequently associated with dairy cattle, microbial contamination of raw milk and soft cheeses can result in disease.

**Listeria monocytogenes**: *Listeria* is a common bacterial pathogen that is found in soft cheeses and unpasteurized milk. It can even survive below freezing temperatures and can therefore withstand refrigeration. It is particularly dangerous to individuals who have weakened immune systems, including pregnant women, AIDS patients, and the very young and very old.

**Mycobacterium tuberculosis**: The cause of “consumption,” a horrific wasting disease that first affects the lungs, *Mycobacterium bovis* is associated with consumption of raw milk and was one of the most common contaminants prior to the practice of pasteurization. *M. bovis* causes tuberculosis in cows and can be passed to humans via unpasteurized cow’s milk, causing a disease that is very similar to *M. tuberculosis*.

**Salmonella**: *Salmonella* contamination of raw milk and milk products has been the source of several outbreaks in recent years. Symptoms include diarrhea and high fever.

**Staphylococcus aureus**: *S. aureus* produces a toxin that causes explosive vomiting. The disease may be considered a consequence of actual food poisoning from simply consuming the toxin, rather than from an actual infection.

**Yersinia enterocolitica**: Associated with raw milk and ice cream, among other foods, contamination is believed to be a consequence of a breakdown in sanitization and sterilization techniques at dairy processing facilities [10].

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Flavonoids that is contained in *C. indicum* are ubiquitous in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Increasingly, this is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity [11]. This kind of bioactive compound can reduce the amount of certain bacteria, for example *Escherichia coli* and *Staphylococcus aureus* [12].

Terpenoids are plant components that have a smell and can be isolated from plant materials called essential oils by distillation. Triterpenoids are terpenoid derivatives of...
triterpene molecules. They may have useful antibacterial activity. Triterpenoid can be applied against *Staphylococcus aureus*, *Shigella flexneri*, *Pasteurella multocida*, *Escherichia coli*, and *Salmonella*. Three of them, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* are kind of bacteria found in milk [13].

Andrographolide is an extract from the stem and leaves of the *Andrographis paniculata*. Andrographolide is an extremely bitter substance, and a natural bitterant. The plant is grown for medicinal purposes in China and India, and is being used as a homeopathic treatment for stomach disorders in the United States. Andrographolide has been shown to be effective against certain bacteria from its antibacterial activity [14]. It were investigated for their antimicrobial activity against nine bacterial species including *Salmonella Typhumurium*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Pseudomonas Aeruginosa*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Legionella pneumophila* and *Bordetella pertussis*. Several of them can be found in cow milk also [15].

For the former study, it can be known that *Chrysanthemum indicum*, *Centella asiatica*, and *Andrographis paniculata* are effective to kill pathogenic bacteria that can survive in the extreme condition like high or low temperature called *Lysteria monocytogenes* and *Bacillus cereus*. But all of the herb extract can reduce *L. monocytogenes* more effectively than *B. cereus* because *B. cereus* has wider pH range of media where they can grow [16].

IV. CONCLUSIONS

Milk is a nutritious food so that it becomes a good media for many bacteria to grow. With these bacteria included, the milk will be faster to be rancid and the shelf-life is shorter. Many preservation methods like heating and cooling with extreme condition to kill many high resistant bacteria will cause the quality change of the milk, so that another application of preservation is needed. One of the methods is applying natural edible antibiotic from herbs extract.

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With reducing the bacteria in it, cow milk can have a longer shelf-life because it is not easy to get rancid. It is an advantage for the low-middle scow milk seller that cannot afford many modern technologies to process their product aseptically. With adding this natural edible antibiotic into the milk, they can reduce the number of waste product and can get more benefit from it.

REFERENCES


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