

CHAPTER I PREFACE

1.1 Background of the Research

Milk is the milking product of healthy and clean dairy cattle obtained by proper milking according to applicable regulations (Meutia, 2016). Dairy milk product is a natural food product with high nutrition that is used to meet human animal protein needs. Important compounds found in milk include carbohydrates (lactose), fat, vitamins and minerals (Safitri and Swarastuti, 2013).

The dairy cattle population in Indonesia is increasing due to the high demand for milk consumption (Safitri *et al.*, 2021). Based on data from the Directorate General of Livestock and Animal Health in 2021, the population of dairy cattle is 0.5 million, the number of domestic demand for milk is 4.3 million tons per year, while domestic fresh milk production is 0.9 million tons per year, so domestic milk products only contribute 22% of the milk demand amount and the rest is fulfilled with milk imports.

Dairy farms in Indonesia are dominated by small-scale businesses where farmers have an average of 2-4 adult dairy cattle per family (Surani, 2011). Because small-scale businesses have limited workers, this results in a lack of good cattle management and care, so the production and quality of milk is low. Good business management is important for farm development. According to Food and Agriculture

Organization (FAO) of the United Nations and International Dairy Federation (2014) in the Guide to Good Dairy Farming Practice, cattle's health, milking hygiene, animal nutrition, animal welfare implementation, good environmental and socio-economic management need to be applied in order to produce milk of good quality and safe for consumption. Due to limited funds, small-scale dairy farmers use traditional ways of managing their livestock. Other than that, poor management is also one of the causes of low dairy cattle productivity. Breeders have an important role in running milk production, lack of knowledge about hygiene management and the correct way of milking can result in disease in cattle followed by decrease in milk production. One of the common diseases that are found in dairy cattle is mastitis (Tewari, 2014).

Mastitis is an inflammatory disease of the mammary glands caused by several factors, including bacterial infection, trauma, and udder injury (Tewari, 2014). Mastitis is known to have 2 types of disease, the subclinical mastitis and clinical mastitis. Subclinical mastitis is the type that does not show clinical symptoms and is usually identified through examination of mastitis, either by California Mastitis Test (CMT) or laboratory tests. While clinical mastitis shows clinical symptoms, such as swelling and hardening of the udder followed by pain and heat, decrease in udder function, milk production and quality (Nurhayati and Martindah, 2015). The bacteria that cause mastitis are contagious and environmental bacteria which are opportunist (Tewari, 2014). Balemi *et al.*, (2021) and Keane (2019) stated that staphylococcus, streptococcus, and coliform bacteria (*Escherichia coli*, *Klebsiella spp.*, and *Enterobacter spp.*) are the most frequent bacteria that cause mastitis. According to

Bhatt *et al.*, (2011) and Ikiz (2013), *Staphylococcus aureus* and *Streptococcus agalactiae* are contagious bacteria that live on the teat's skin and inside the udder. In addition, the bacteria such as *Escherichia coli*, *Klebsiella sp.*, *Enterobacter spp.*, *Streptococcus uberis* are considered as environmental pathogens (Cobirka, 2020). Treatment used for mastitis are commonly antibiotics such as streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline, but it also causes antibiotic resistance in pathogenic bacteria (Yang *et al.*, 2019). Benic *et al.*, (2018) also stated that toxins produced by *Staphylococcus aureus* cause abscesses and within it, the bacteria are protected from antibiotic activity by living within the neutrophilic leukocytes and other immune cells include phagocytes, so the treatment result using antibiotic is poor. Plants with bacteriostatic qualities could be used to treat mastitis in dairy cattle as an alternative to antibiotics because they do not cause bacteria to develop resistance, allowing them to be used for a long period (Radzikowski *et al.*, 2020). The plants used as an alternative to antibiotics are rich in natural compounds such as alkaloids, terpenoids, tannins, steroids, coumarins and flavonoids, also essential oils that can increase bacterial cell permeability, leakage of cell constituents, intracytoplasmic damage (Al AlSheikh *et al.*, 2020). Based on the background, this research aims to find alternative prevention and treatment for bovine mastitis with natural plant using the leaves of iler (*Plectranthus scutellarioides* (L.) R. Br.) plant.

Iler (*Plectranthus scutellarioides* (L.) R. Br.) plant is an ornamental plant that comes from the Lamiaceae family and has several other scientific names such as *Coleus scutellarioides* (L.) Benth., *Plectranthus blumei* (Benth.) launert, *Coleus pumilus*

Blanco, and *Coleus blumei* Benth. Iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf contains compounds such as saponins, flavonoids, eugenol, polyphenols, steroids, tannins, carvacrol, ethyl salicylate, alkaloids, methyl eugenol, rosmarinic acid, thymol, and camphor (Wasiah, 2014). Because it contains those compounds, iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf act as antibacterial, antioxidant, antidiabetic, anti-inflammatory, immunomodulatory, antihistamine, and anthelmintic (Anita *et al.*, 2019; Novanti and Susilawati, 2017). This research aims to examine the antibacterial effect of iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf extract against mastitis-causing bacteria in cattle.

1.2 Problem Statement

Does iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf extract show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* isolated from the dairy cattle with subclinical mastitis?

1.3 Theoretical Basis

Mastitis is the inflammation of the mammary gland in dairy cattle and is often caused by bacteria resulting in changes of milk chemical compounds and decreased quality milk produced. Dairy products, specifically milk, are nutrient-dense, consisting of proteins, minerals, vitamins, and energy-dense fats; as a result, milk provides a perfect environment for a wide range of food-borne bacteria and zoonotic pathogens to grow (Owusu-Kwarteng *et al.*, 2020). Mastitis also causes economic loss because of decreased quantity of milk. Benic *et al.*, (2018) stated that fungus, molds, algae can cause infectious mastitis other than bacteria; meanwhile, mechanical factors such as

being struck by a horn, udder injuries also causes non-infectious mastitis. *Staphylococcus aureus* and *Streptococcus agalactiae* are more prevalent than *Mycoplasma* species because *M. bovis* has low sensitivity and long incubation period in culture-based approaches (Dorman *et al.*, 1983; Sachse *et al.*, 2010; Gioia *et al.*, 2016; Timonen *et al.*, 2017), nonetheless, it causes gland fibrosis, abscesses, and lymphatic nodule fibrosis by damaging secretory tissue (Cheng and Han, 2020). Those bacteria are considered as contagious pathogens and are commonly found on the infected cattle's udder or teat surface and transmitted to uninfected cattle during milking (Kibebew, 2017). According to Cheng and Han (2020), bacteria such as coliforms (*Eschericia coli*, *Klebsiella spp.*, *Enterobacter spp.*), *Pseudomonas spp.*, *Streptococcus uberis*, are do not live in cattle's udder or teat skin; instead they live in the environment around it like the bedding and housing, and they are also considered as opportunistic bacteria because they wait for opportunities to infect. According to Lakew *et al.*, (2019), mastitis is more common in older cattle (29.69%) than in young adults (13.02%) or adults (20.31%). Mastitis is commonly treated with antibiotics such as streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline, but antibiotics can cause antibiotic resistance on pathogenic bacteria (Yang *et al.*, 2019).

Iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf contains polyphenols and flavonoids which are derivatives of phenolic compounds and are capable of damaging the cell walls of bacteria by the reaction between alcohols in phenolic compounds with lipids and amino acids in bacterial cell walls, then destroys the lipid structure of bacterial DNA and ends with bacterial lysis; tannins which contain hydroxyl groups

and are complex phenolic compound, inhibit bacterial growth by interfering cell permeability; alkaloids and essential oils containing carvacrol, eugenol and methyl salicylate, are disrupting the formation of bacterial cell walls which end in bacterial death (Darwis *et al.*, 2013). Polyphenols are organic compounds with hydroxyl groups bonded to a phenyl ring that are biosynthesized from phenylalanine or tyrosine, and also play an important role in plant defense mechanisms against viruses, bacteria, and fungi (Papuc *et al.*, 2017). Flavonoids are a group of low-molecular weight compounds formed from the combination of derivatives synthesized from phenylalanine and acetic acid (Coppo and Marchese, 2014). They have C6-C3-C6 carbon structures consisting of two phenyl rings (A and B) and a heterocyclic ring (C) (Xie *et al.*, 2017). Flavonoids are divided into numerous subcategories, including flavones, flavonols, flavanones, and isoflavonoids (Xiao, 2017 in Xie *et al.*, 2017). Tannins are substances which bind to proteins, amino acids, alkaloids and bend them, they are large polyphenolic compound having enough hydroxyls and other groups, such as carboxyls, to form strong complexes with different macromolecules (Kurhekar, 2016). Alkaloids are nitrogen-containing heterocyclic structures, and are divided into two groups based on the chemical structure: non heterocyclic or atypical alkaloids which known as protoalkaloids or biological amines) and heterocyclic or typical alkaloids, such as hygrines and quinine (Othman, 2019). Essential oils are lipid soluble and soluble in organic solvents, and are liquid volatile, clear, and seldom colored (Shaaban, 2020). Essential oils with large amounts of phenolic compounds, such as thymol and carvacrol from oregano and eugenol from clove, have the most antibacterial activity (Seow *et al.*,

2014). Essential oils have the ability to react with lipids on the bacterial cell membrane because of their hydrophobicity (Seow *et al.*, 2014).

1.4 Aims of Research

This research aims to determine the antibacterial activity of the extract of iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf against *Staphylococcus aureus* and *Escherichia coli* isolated from dairy cattle with subclinical mastitis.

1.5 Outcomes of Research

1.5.1 Theoretical Outcome

This research can be used as scientific data reference regarding the antibacterial activity of iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf extract as natural antibacterial against *Staphylococcus aureus* and *Escherichia coli* isolated from dairy cattle with subclinical mastitis for further research also development of prevention agent and treatment.

1.5.2 Practical Outcome

The result of this research can be applied as alternative prevention and treatment of bovine mastitis using extract of iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf.

1.6 Hypothesis

The extract of iler (*Plectranthus scutellarioides* (L.) R. Br.) leaf shows antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* isolated from dairy cattle with subclinical mastitis.