The capability of Cemba (*Albizia lebbeckoides* [DC.]) benth leaf extract in inhibiting *Staphylococcus aureus*

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**Abstract.** Cemba (*Albizia lebbeckoides* [DC.]) Benth leaf extract contains phytochemical components namely: phenolics, flavonoids, tannins, alkaloids, glycosides, and saponins that potent to be an antibacterial agent. The study aimed to evaluate the antibacterial capability of cemba leaf extract against *Staphylococcus aureus* contaminating animal products frequently. Forty grams of cemba leaves were extracted with 400 mL of distilled water for 24 h. One mL of each prepared concentration of CLE (100, 105, 110, 115, 120, 125, 130, 135, and 140 mg mL⁻¹) was cultured together with 1 mL *S. aureus* concentration of $1 \times 10^6$ CFU mL⁻¹ in MHB media, then incubated for 24 h at 37°C. Furthermore, each bacterial suspension contained in the MHB was grown on MHA media and incubated for 24 h at 37°C. The number of bacteria colonies growing was calculated for determining MIC and MBC. The time-kill test used CLE with a concentration of 1 MIC and 2 MIC. Besides, SEM was also observed to know the changes in the morphology of *S. aureus* cells after tested contact with 1 MIC CLE. The results showed that CLE was able to inhibit and kill *S. aureus* bacteria with MIC values of 120 mg mL⁻¹ and MBC of 125 mg mL⁻¹. Time-kill test results showed CLE concentrations of 120 mg mL⁻¹ (1 MIC) and 240 mg mL⁻¹ (2 MIC) were able to kill *S. aureus* bacteria with a contact length of 4—8 hours. The results of SEM observations indicated that CLE was able to kill *S. aureus* by damaging bacterial cell wallsbad been tested, our method can be used for validated method for aflatoxin analysis in EBN. Further analysis of aflatoxins in edible bird nest will be reported separately.

**1. Introduction**

Meat and its processed products are one of the food products that are easily contaminated by microbes. Microbial contamination in food products not only causes a decrease in quality but can also cause disease [1]. *Staphylococcus aureus* is Gram-positive bacteria that often contaminates food products including meat and its processed products that can cause foodborne disease [2,3].

Plants are a source of various bioactive components which among others have been used for pharmaceuticals and food additives. Plants are used as food additives because of their ability to provide color, flavor, and aroma in food processing [4]. Besides, plants that contain bioactive components such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, glycosides, and steroids have the potential as antimicrobials [5,6].
Some researchers have reported the ability of antimicrobial compounds derived from plants in inhibiting bacterial growth. Leaves of Albizia lebbeck extracted with ethyl acetate were able to inhibit *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* [7]. Sheyin et al [8] stated that using ethyl acetate extract of *A. lebbeck* at a concentration of 400 mg mL\(^{-1}\), was able to inhibit *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Shigella spp.*

One of the plants that are often used as food additives by the people of Enrekang Regency, South Sulawesi Province is the cemba plant (*Albizia lebbeckoides* (DC) Benth). Cemba leaves have long been used as cooking spices on processed beef or buffalo, which is known by the local community as "nasu cemba". The results of phytochemical screening for powder and cemba leaf extracts (CLE) obtained several bioactive components such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides which function as antimicrobials [9]. Based on the bioactive component of the cemba leaf extract and its potential as an antimicrobial, a study was conducted to evaluate the antibacterial ability of the cemba leaf extract against the Staphylococcus aureus bacteria which often contaminate livestock products.

**2. Materials and methods**

**2.1. Materials**

The used materials cemba leaf, *S. aureus* ATCC 6538 bacteria, auto-clave, shaker, laminar air flow, Eppendorf, micropipette, distilled water, dimethyl sulfoxide, Mueller–Hinton broth and Mueller–Hinton agar

**2.2. Preparation of plant extract**

The plants of *A. lebbeckoides* were obtained from Enrekang District, South Sulawesi, Indonesia. The cemba leaves were dried at room temperature for 7 days and then dried at a temperature of 40°C in an oven for 1 h. The dried leaves were milled using a kitchen blender and sieved through a 35-mesh sieve one hour. Forty grams of leaf powder macerated with 400 mL distilled water for 24 h followed the extract was filtered using Whatman No. filter paper 1. The solvent in the extracts was completely removed by using a rotary evaporator Heidolph Type Antrieb-W-Micro, Germany) at 40°C to obtain a semi-solid mass before drying using a freeze dryer. Cemba leaf extract (CLE) obtained was stored at 4°C until assayed

**2.3. Antibacterial susceptibility assays**

The determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CLE determined extract using macro-dilution[10]. The CLE was dissolved in Mueller-Hinton Broth (MHB; Himedia, India) and 5% DMSO with a concentration of 100–140 mg mL\(^{-1}\). About 1 mL of each diluted CLE was put into a tube, then 1 mL of bacterial culture was added with a density of 1×10\(^6\) CFU mL\(^{-1}\) and incubated at for 24 h at 37°C. Furthermore, each bacterial suspension contained in the MHB was grown onto Mueller-Hinton Agar (MHA, Oxoid) and incubated for 24 h at 37°C. The number of growing colonies was calculated for the determination of MIC and MBC extracts

**2.4. Time kill study**

The method was modified from Chen’s method [11]. The CLE diluted using MHB media and 5% DMSO with 1 MIC and 2 MIC dilution series. Furthermore, media containing CLE were 1 MIC (120 mg mL\(^{-1}\)) and 2 MIC (240 mg mL\(^{-1}\)), inoculated with *S. aureus* bacteria to the bacterial density of 1x10\(^6\) CFU mL\(^{-1}\), then incubated for 48 h at 37°C. The contact time of bacteria with EDC was evaluated for 48 h at intervals every 4 h. Samples were grown on MHA media by the spread method and incubated for 36 h at 37°C. Furthermore, the number of bacterial colonies growing is calculated and expressed as log CFU mL\(^{-1}\).
2.5. Scanning electron microscopy (SEM) observations.
To determine the efficacy of CLE on the morphological changes of the *S. aureus*, SEM observations were performed on the *S. aureus*. The method of SEM was modified from Su’s method [12]. *S. aureus* bacterial suspension with a density of $1 \times 10^6$ CFU mL$^{-1}$ was contacted with CLE at concentrations of 0 and 1 MIC for 6, 12 and 24 h. The bacterial suspension was centrifuged at a speed of 2510 x g for 10 minutes, the liquid was discharged to obtain bacterial cells. Bacteria cells are washed with phosphate buffer solution and then centrifuged, the liquid was again discharged and pellets are washed with phosphate buffer. The washing process is done 2 times. The bacterial cells were fixed in 2.5% glutaraldehyde solution (0.1 M sodium cacodylate buffer (pH 7.2) for 12 h at 4°C and then fixed 1% osmium tetroxide (OsO$_4$). Cells are dehydrated with acetone and dried, then coated with gold for observation using a JSM-5310LV (JEOL, Tokyo, Japan).

3. Results and discussion

3.1. MIC and MBC of cemba leaf extract
The MIC value of leaf extract with water solvent against *S. aureus* bacteria was 120 mg mL$^{-1}$ and MBC value was 125 mg mL$^{-1}$. MIC value of cemba leaf extract obtained is in line with MIC value of *A. lebbeck* leaf extract with ethyl acetate solvent that is 50─140 mg mL$^{-1}$ [8], but higher than the results of the study of Acharyya et al.[13] who extracted *Albizia lebbeck* using ethanol solvents with MIC values ranging from 16─24 mg mL$^{-1}$. Differences in MIC and MBC values are not only due to differences in the levels of bioactive components produced from each type of solvent but also due to differences in morphological structure and composition of the test bacterial cells used [12].

3.2. Time Kill
The ability of bactericidal and bacteriostatic CLE against *S. aureus* bacteria is determined by a time-kill curve (fig 1). Bactericidal and bacteriostatic activity is the antibacterial ability to reduce $\geq 3$ and $< 3$ log$_{10}$ CFU mL$^{-1}$ of bacteria compared with the starting inoculum$^{14}$. 

![Figure 1](image.png)

Figure 1. Growth profile of *S.aureus* after exposure to the cemba leaf extract.

Based on the growth chart of *S. aureus* bacteria that were tested in contact with CLE (Figure 1.), it appears that CLE has bactericidal ability against *S. aureus* bacteria. The addition of 1 MIC CLE was able to reduce the total bacteria of *S. aureus* by 3log at the 8th-hour, while the addition of 2 MIC CLE was able to reduce the total bacteria by 3log at the 4th-hour observation. While in the treatment without the addition of CLE, the population of *S. aureus* bacteria increased until the 20th hour. The difference in CLE concentration is highly correlated with the ability to inhibit and kill test bacteria. Alwash *et al*
states that an antibacterial material is bactericidal if it can reduce the bacterial population by $3\log_{10}$ CFU mL$^{-1}$ compared to the number of initial bacteria.

3.3. SEM

The ability of CLE to inhibit and kill S. aureus bacteria was also confirmed based on changes in S. aureus cell morphology after exposure to CLE which was observed using SEM as in Figure 2. S. aureus bacteria without the addition of CLE (fig. 2.A) appeared with normal, round shape and smooth surfaces. Regarding CLE treatment, it is observable that after 6 h of exposure (2.B), the bacterial treated cells began to lyse and combine with each other. Observation of the 12th hour shows the shape of the S. aureus cell wall is irregular and begins to disintegrate. As for the final incubation period (fig. 2.D), dramatic lysis was apparent on the cell. This phenomenon shows that the phytochemical component of CLE was capable of damage the cell wall membrane of S. aureus bacteria.

Some research results report that plants containing phenolic compounds can inhibit the growth of S. aureus bacteria [12,15]. The results of SEM observations by Zhang et al. [16] showed that the antibacterial component of Ginkgo biloba leaf extract was able to destroy the bacterial cell membrane Shewanella putrefaciens and Staphylococcus saprophytic by changing the permeability of cell membranes resulting in the loss of intracellular components on the cell surface. The same phenomenon was observed on S. aureus bacteria after exposed to Aloe vera extract [17].

![Figure 2. Scanning electron micrographs of S. aureus before (A) and after (B) 1 MIC cemba leaf extract for 6 h, (C) 12 h, and (D) 24 h of exposure to extracts. (10 000 ×).](image)

Polyphenol compounds, especially flavonoids, can function as an antibacterial by inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function, inhibiting energy metabolism, inhibiting adhesion and biofilm formation, changing the permeability of bacterial cell membranes, and weakening bacterial pathogenicity [18]. When inhibiting the function of cell membranes, flavonoids form complex compounds with extracellular proteins that can damage bacterial cell membranes, then followed by the release of intracellular components of these bacteria. Flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria [19].

Saponins are secondary metabolites that contain steroidal aglycones and triterpenoids that are attached to one or more sugar chains, these chemical structures determine biological properties as
antimicrobials [20]. Saponins can suppress the growth of bacterial chains by lowering the surface tension of bacterial cell walls so that antibacterial substances will easily enter the cell and disrupt metabolism until bacterial death occurs [21].

Tannin compounds can inhibit bacterial growth by coagulating proteins and chelating iron so that cell wall formation will be inhibited [21,22]. Tannins also have the ability to activate bacterial enzymes, suppression of oxidative phosphorylation or interfere with transport proteins in the inner layer of cells [3].

4. Conclusion
Cemba leaf extract (CLE) has the ability to inhibit and kill S. aureus bacteria with MIC values of 120 mg mL$^{-1}$ and MBC around 125 mg mL$^{-1}$. Time-kill test results showed EDC 1 MIC and 2 MIC extracted with water were able to kill S. aureus bacteria with a contact time of 4-8 hours. Morphological observations of S. aureus with SEM showed that CLE was able to kill S. aureus by damaging bacterial cell walls.

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