Potency of salt from old mangrove leaves as an antibacterial against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*

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Abstract. Salt from old mangrove leaves can be a functional salt containing antioxidants and bioactive compounds with antibacterial potential to be used as a natural preservative. This research aimed to determine the potential of salt from old mangrove leaves as an antibacterial for *Staphylococcus aureus* and *Bacillus cereus*. The research method involved the extraction of salt from old mangrove leaves with distilled water. The tests carried out were the determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and analysis of antibacterial activity at various salt concentrations, including 1.56%, 3.13%, 6.25%, and 12.5%. The bacteriostatic properties of mangrove salt were indicated by the MIC of 3.13% from Gram-positive bacteria *S. aureus* and *B. cereus*, as determined by MIC observations. In the interim, bacterial growth was observed in MBC at all salt concentrations, suggesting that it lacked bacteriocidal properties. The antibacterial activity of mangrove salt against *S. aureus* bacteria was 14.05 mm, 8.10 mm, 5.04 mm, and 3.80 mm at concentrations of 12.5%, 6.25%, 3.13%, and 1.56%, respectively. The corresponding values for *B. cereus* bacteria were 14.93 mm, 8.73 mm, 6.14 mm, and 3.94 mm, respectively. The old mangrove leaf salt's bacterial inhibitory capability increased as the salt concentration increased.
Key Words: bacteriostatic, bioactive, inhibition zone, preservative, salt.

Introduction. Mangroves are plants that live in coastal areas as marine resources that have enormous potential. Mangroves can be used as a medical ingredient to cure muscle pain, back pain, malaria, wounds, tuberculosis (TB) (Dwilestari et al 2015), asthma, diabetes, fever (Vinoth et al 2019). Furthermore, mangroves have the potential to function as both an antibacterial and an antifungal (Pringgenies et al 2023). Mangroves can be used as medical ingredients because they contain alkaloids, phenols, flavonoids and saponins (Bandaranayake 2002) produced from secondary metabolites or originate from mangrove symbiont microorganisms (Maria et al 2005; Joel & Bhimba 2013; Ramasubburayan et al 2015). This potential of mangroves can be obtained from fruit, leaves, bark and mangrove roots (Noor et al 2006).

The research results stated that the leaves of the mangrove *Sonneratia* sp. have bioactive components like phenols, flavonoids, steroids, saponins, triterpenoids, tannins and alkaloids, which have the potential as natural antioxidants (Dotulong et al 2018; Dotulong et al 2020). Mangrove leaves are also known to contain NaCl salts and minerals in macro and micro forms consisting in potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn), phosphate (P), iodine (I), and iron (Fe) (Naiym et al 2019; Ardhanawinata et al 2020).

This is the basis for valorizing mangrove leaves as a salt preparation. The research results of Sari et al (2023) showed that salt produced from old mangrove leaves has potential as a functional salt preparation with antioxidant activity characteristics of 21.12 $\mu g\ mL^{-1}$, minerals dominated by Na (5.63%) and K (4.54%), Na:K ratio in old mangrove leaf salt being 2.33, and the NaCl content of old mangrove leaf salt met the standard below 60%, which was 27.01%.

Dietary salt is the recommended salt intake for hypertensive patients. Functional salt produced from seaweed has a NaCl content of 49.94%, and lindur (*Bruguiera gymnorrhiza*) leaves have a NaCl content of 12.76%. The fact that it contains NaCl levels derived from dietary salt as well as bioactive components that have the potential to be beneficial antioxidants for health is the reason why it is suggested for salt intake in hypertension patients (Ardhanawinata et al 2020; Nurjanah et al 2020).

Apart from that, the bioactive components of mangrove leaves also have antibacterial activity in inhibiting Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) (Mardiansyah & Bahri 2016; Ibrahim et al 2019; Linggama et al 2019; Manuhuttu & Saimima 2021), so they can be used as natural preservatives. The valorization of *Sonneratia* sp. mangrove leaves into old mangrove leaf salt, which has the potential to operate as an antibacterial against *Staphylococcus aureus* and *Bacillus cereus* bacteria, is something that has to be researched so that it may be utilized based on this information. This research aims to determine the potential of old mangrove leaf salt as an antibacterial for *Staphylococcus aureus* and *Bacillus cereus*.

Material and Method

Materials. The main ingredients used in this research were old mangrove (*Sonneratia* sp.) leaves obtained from Meranti Islands Regency, Riau Province, Indonesia and distilled water. The criteria for mangrove leaves to be old were that the leaves were located near the base of the branches, were shiny dark green on top and paler on the bottom, had a harder and rougher texture, had 1 to 5 inches long, and are wide and blunt at the tip (Fadilla et al 2019). Other materials include *Bacillus cereus* and *Staphylococcus aureus* bacteria, Mueller Hinton Agar media, Nutrien Broth media, Tryptic Soy Broth media, and the antibiotic amoxicillin.

Instruments*.* Materials used in mangrove leaf preparation, mangrove leaf flour production, and mangrove leaf salt production include blenders, sieves, digital scales, porcelain cups, measuring cups, water baths, thermometers, and ovens. The instruments used for analysis are Petri dishes (CMSI Normax), tube needles, incubators (Precision Scientific), autoclaves (Hiclaveb HVE-50), Erlenmeyer (Iwaki pyrex), test tubes (Iwaki), analytical scales, Bunsen, water bath, filter paper, calliper and hot plate (Maspion), aluminium foil, and stainless steel pipette.

Salt extraction*.* Initially, old mangrove leaves were dried at room temperature, then mashed using a blender and sieved using an 80 mesh sieve to obtain mangrove leaf powder. The process of making mangrove leaf salt was done by mixing young and old mangrove leaf flour with aquadest at a ratio of 1:5. Then it was heated using a water bath at 40°C for 10 minutes. The resulting mixture was filtered using a nylon filter cloth and filter paper. Furthermore, drying was carried out using an oven at a temperature of 65°C for 120 hours or until the dried filtrate (Sari et al 2023).

Test parameters*.* The test parameters performed consisted of an analysis of antibacterial activity using the well diffusion method. The diffusion method aims to see the inhibition zone of antibacterial substances. The diffusion method uses a well technique using the pour plate method (Prayoga 2013). Determination of minimum inhibitory concentration (MIC) used the liquid dilution method, while determination of minimum bactericidal concentration (MBC) was carried out using the solid dilution method (Rudiansyah et al 2021). Gram-positive antibacterial activity tests were conducted on 4 different concentrations of old mangrove leaf salts: 1.61%, 3.15%, 6.25% and 12.5%, as well as positive control (amoxicillin) and negative control (distilled water).

Statistical analysis*.* Gram-positive antibacterial activity data were analyzed using IBM SPSS Software with ANOVA statistical analysis (Analysis of Variance) at a confidence level of 95%, and Duncan's follow-up test was carried out.

Results and Discussion

MIC and MBC of Staphylococcus aureus. The MIC determination of *Sonneratia* sp. leaf salt against *S. aureus* bacteria was determined by looking at the clear test tubes in the MIC liquid media with the smallest concentrations of 1.56%, 3.13%, 625%, and 12.5% of aged mangrove leaf salt. A tube that looks clear indicates that the tube shows no bacterial growth. Next, the MBC of old mangrove leaf salt against *S. aureus* bacteria was determined by looking at the growth of bacterial colonies on MHA solid media with the smallest concentration detected by the MIC ability. The results of observations of the MIC and MBC of old mangrove leaf salt against *S. aureus* bacteria are presented in Table 1.

Table 1

Results of observations of MIC and MBC of old mangrove leaf salt against *S. aureus* bacteria

Concentration of salt from old mangrove leaves (%)	*Staphylococcus aureus*	
	MIC	*MBC*
12.5	-	+
6.25	-	+
3.13	-	+
1.56	+	Not observed

Description: (-): no bacterial growth, (+): bacterial growth.

Table 1 showed that the results of MIC observations of *S. aureus* bacteria at concentrations of 12.5%, 6.25% and 3.13% showed no bacterial growth in each of the tested bacteria, and a concentration of 1.56% showed that there was bacterial growth. The results of these observations stated that a concentration of 3.13% was the MIC of salt from old mangrove leaves against *S. aureus* bacteria. Old mangrove leaf salt could inhibit *S. aureus* bacteria. Meanwhile, when observing MBC, it can be seen that at each concentration of 3.13%, 6.25%, and 12.5%, bacterial colonies grow. This indicates that mangrove leaf salt at this concentration is unable to kill *S. aureus* bacteria.

Antibacterial activity of Staphylococcus aureus. The antibacterial activity of *S. aureus* was determined by calculating the diameter of the clear zone produced around the paper disc. The inhibition zone of old mangrove leaf salt against *S. aureus* bacteria can be seen in Figure 1. The antibacterial activity of old mangrove leaf salt against *S. aureus* bacteria is presented in Table 2.

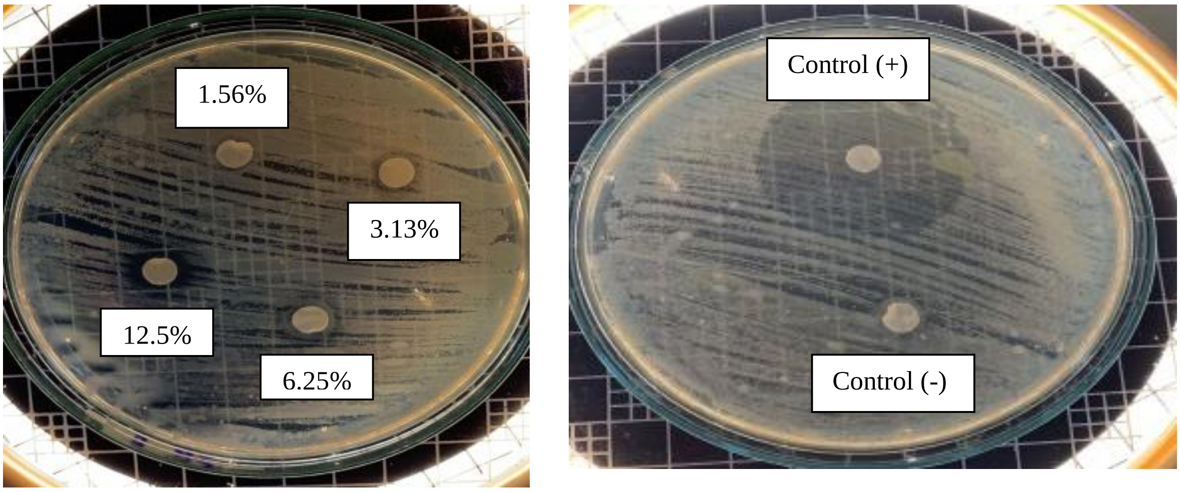

Figure 1. Inhibitory zone of salt from old mangrove leaves against *S. aureus* bacteria.

Table 2

Antibacterial activity of salt from old mangrove leaves against *S. aureus* bacteria

Treatments	*Inhibition zone diameter (mm)*
1.56%	3.80 ± 0.10^{b}
3.13%	5.04 ± 0.10^{c}
6.25%	8.10 ± 0.30^{d}
12.5%	14.05 ± 0.21^{e}
Positive control	18.69 ± 0.19^{f}
Negative control	0^{a}

Notes: Values followed by different superscript letters (a, b, c, d, e, f) in the column indicate significant differences ($p < 0.05$).

Table 2 showed that old mangrove leaf salt can inhibit the growth of *S. aureus* bacteria. The diameter of the inhibition zone in *S. aureus* bacteria has an average of 14.05 mm, 8.10 mm, 5.04 mm, and 3.80 mm at concentrations of 12.5%, 6.25%, 3.13%, and 1.56% respectively. Different salt concentrations can provide different inhibitory powers on bacterial growth. The use of high concentrations can increase the diameter of the inhibition zone. Substances that have high antibacterial properties can produce a wide inhibition zone (El Ramadhani et al 2022).

The antibacterial activity of old mangrove leaf salt against *S. aureus* bacteria is weak to strong because the inhibition zone produced in the *S. aureus* bacteria medium is 3.80 mm to 14.05 mm. The criteria for antibacterial inhibitory power are as follows: an obstacle area of ≥ 20 mm is considered very strong, an obstacle area of 10-20 mm is in the strong category, an obstacle area of 5-10 mm is in the medium category, and an obstacle area of 5 mm or less is in the weak category (Davis & Stout 1971).

MIC and MBC of Bacillus cereus. Determination of MIC and MBC from mangrove leaf salt against *B. cereus* bacteria is presented in Table 3.

Table 3

Results of observations of MIC and MBC of old mangrove leaf salt against *B. cereus*

Concentration of salt from old mangrove leaves (%)	*Bacillus cereus*	
	MIC	*MBC*
12.5	-	+
6.25	-	+
3.13	-	+
1.56	+	Not observed

Description: (-): no bacterial growth, (+):bacterial growth.

Table 3 showed the results of MIC observations of *B. cereus* bacteria, showing bacterial growth at a concentration of 1.56% old mangrove leaf salt. Meanwhile, old mangrove leaf salt showed no bacterial growth at concentrations of 12.5%, 6.25%, and 3.13%. The results of these observations stated that a concentration of 3.13% was the MIC of mangrove salt against *B. cereus* bacteria.

Based on the research of El Ramadhani et al (2022), *S. alba* leaf extract has antibacterial activity against *B. cereus* bacteria because *S. alba* leaf extract contains secondary metabolite compounds consisting of tannins, triterpenoids, flavonoids and saponins. Based on their toxicity, antibacterial can be bacteriostatic and bactericidal. The results of the MIC test on *B. cereus* used in this research showed that *Sonneratia* sp. leaf salt has a bacteriostatic mechanism of action.

This is supported by the research of El Ramadhani et al (2022) who stated that mangrove (*S. alba*) leaf extract has bacteriostatic properties. Mangrove leaf extract, known as bacteriostatic, has demonstrated the ability to inhibit bacterial growth at concentrations ranging from 25 to 100%. In the interim, the MBC observations of old mangrove leaf salt were unable to eliminate *B. cereus* bacteria, which was indicated by the growth of bacteria in the test media. This is because the bioactive compounds contained in salt can only damage the cell walls of bacteria.

Antibacterial activity of Bacillus cereus. The inhibition zone of old mangrove leaf salt against *B. cereus* bacteria can be seen in Figure 2. The antibacterial activity of old mangrove leaf salt against *B. cereus* bacteria is presented in Table 4.

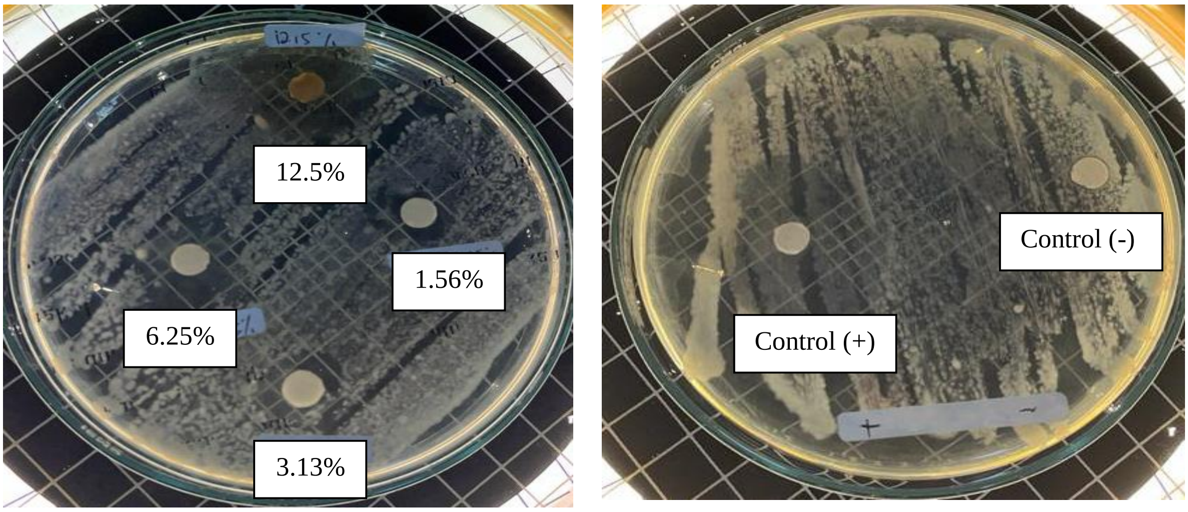

Figure 2. Inhibitory zone of salt from old mangrove leaves against *B. cereus* bacteria

Table 4

Antibacterial activity of salt from old mangrove leaves against *B. cereus* bacteria

Treatments	*Inhibition zone diameter (mm)*
1.56%	3.94 ± 0.17^{b}
3.13%	6.14 ± 0.57^{c}
6.25%	8.73 ± 0.22^{d}
12.5%	14.93 ± 0.38^{e}
Positive control	17.33 ± 0.39^{f}
Negative control	0^{a}

Notes: Values followed by different superscript letters (a, b, c, d, e, f) in the column indicated significant differences ($p < 0.05$).

Table 4 showed that old mangrove leaf salt can inhibit the growth of *B. cereus* bacteria. The diameter of the inhibition zone in *B. cereus* bacteria has an average of 14.93 mm, 8.73 mm, 6.14 mm, and 3.94 mm at concentrations of 12.5%, 6.25%, 3.13%, and 1.56% respectively. The ability of old mangrove leaf salt as an antibacterial can be categorized as weak to strong in inhibiting the growth of *B. cereus* bacteria. However, the results of this research showed higher antibacterial activity when compared to the results of research conducted by El Ramadhani et al (2022) on mangrove leaf extract against *B. cereus* bacteria, namely at a concentration of 12.5%, mangrove leaf extract has no inhibitory effect. Subsequent, the inhibitory power on *B. cereus* bacteria was detected at a concentration of 25% with an inhibitory zone diameter of 7.10 mm.

Gram-positive bacteria do not have a lipopolysaccharide layer, so antibacterial compounds that are both hydrophilic and hydrophobic can pass through. The concentration of antibacterial compounds given is thought to increase the penetration of antibacterial compounds into bacterial cells, which will damage the cell's metabolic system and can result in cell death (Manuhuttu & Saimima 2021). Gram-positive bacteria have thoracic acid (polysaccharide), which makes up the cell wall and is soluble in water, which plays a role in facilitating the process of transporting positive ions in and out. Therefore, this water-soluble property indicates that Gram-positive bacteria have polar cell walls. Metabolite compounds in *S. alba* leaf salt are polar compounds and easily enter the peptidoglycan layer, resulting in an inhibitory zone (Rajagopal & Walker 2017).

Conclusions. Salt from old mangrove leaves has the potential to act as an antibacterial against *S. aureus* and *B. cereus*, having bacteriostatic properties. The bacterial inhibitory ability of old mangrove leaf salts increased as the salt concentration increased. The highest antibacterial activity was found in a concentration of 12.5% old mangrove leaf salt with a diameter of the inhibition zone for gram-positive bacteria of 14.05 mm for *S. aureus* bacteria and 14.93 mm for *B. cereus* bacteria.

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Conflict of interest. The authors declare that there is no conflict of interest.

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