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IN-VITRO ANTI-STAPHYLOCOCAL COMPARISON ACTIVITY OF APPLE (*PYRUS MALUS* L.) FRUIT JUICES AND RED ROSELLE (*HIBISCUS SABDARIFFA* L.) CALYX DECOCTION

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ABSTRACT

Objective: The antibacterial activities of aqueous extracts of apple (*Pyrus Malus* L.) fruit juices and red roselle (*Hibiscus Sabdariffa* L.) calyx decoction against *Staphylococcus aureus* were investigated. **Methods:** The correlation between bioactive components of plant extracts and inhibitory activity was investigated in vitro using a microdilution method. Their antibacterial potency was compared by determining minimum inhibitory concentration (MIC) values of the both samples. Total antibacterial metabolites content was extracted by a maceration method and used for phytochemical assay. **Results:** The roselle decoct showed significant antibacterial activity with low susceptibility MIC at the range of 3.9- 7.81 µg/mL and 250–500 µg/mL for the apple juices. Alkaloids, flavonoid, polyphenols, tannins and quinones were found in the roselle decoct. Meanwhile, detected secondary metabolites found in the apple juices was saponin, flavonoids, tannins and steroids. **Conclusion:** The infections caused by *S. aureus* can be developed as alternative novel herbal formulations with rich bioactive compounds from roselle decoct.

KEYWORDS: *Pyrus Malus* L., *Hibiscus Sabdariffa* L., juices, decoct, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is one of the most frequent causes of skin and soft tissue infections (SSTIs) and raises the risk of more severe systemic infections such bacteremia, septicemia, and osteomyelitis.[1,2] The prevalence of *S. aureus* bacteremia and accompanying consequences has sharply grown in recent years as a result of the rise in invasive operations, which has resulted in a large number of immunocompromised individuals and antibiotic-resistant *S. aureus* strains. Global epidemiological data have indicated that *S. aureus* biofilm frequently exacerbates skin infection, leading to widespread antibiotic resistance and reducing the number of effective treatments.[1,3] Because polysaccharide intercellular adhesin, a key component of staphylococci biofilm, is secreted, therapeutic medication actions are minimized and colonization is enhanced.[4,5] There is an urgent need for new methods and better medications to prevent and treat *S. aureus* bacteremia due to the evolving epidemiology of the disease and the pathogen's intrinsic aggressiveness.[6,7] Findings from research into the antibacterial properties of several native plants might be helpful. As a result, there is now more interest in and demand for antimicrobials made from plants.[8] Natural goods provide unparalleled prospects for novel medication leads due to the extraordinary chemical variety of naturally produced molecules, whether as pure compounds or as standardized plant extracts.[9,10] In this study, we evaluated the anti-staphylococcal of red roselle calyx and apple, which are known possessed important metabolites compound with antibacterial mechanism.

The Indonesian people consume roselle calyxes often and employ them in a number of complementary medical practices. One of the most well-known traditional medicines used by Indonesians, the roselle calyx decoct may be taken as a hot or cold beverage and has been shown to be effective in the treatment of Rosella possesses antibacterial, anthelmintic, antiplasmodic (anticonvulsant), anticancer, antihypertensive, antidiabetic, and antihypertensive properties.[11] Alkaloids, tannins, and flavonoids are a few secondary metabolites found in plants that have been investigated for their potential as antibacterial agents.[12] The anthocyanin coloring, polyphenolic components including flavonoids, and phenolic acids like gallic and protocatechuic acid were all found in the red Hibiscus calyxes.[13] As a result, this plant has the potential to be tested for its ability to combat

staphylococci species. Apples are well-known fruits that are consumed all over the world and have several health advantages[14], largely as a result of their phenolic components.[15,16] Fruits' antimicrobial qualities are linked to bioactive substances like flavonoids and phenolic compounds. Those beneficial chemicals' concentrations can change according on the type, cultivar, and processing technique.[17,18] In this study, we used apples that were grown in Indonesia, which may have different antibacterial activity than crops grown in other nations. We prepared the fruit juice using a juicing process without peeling the fruits in order to preserve the bioactive elements of the fruits. Utilizing locally grown fruit from Indonesia can increase the region's potential as a source for the creation of natural medicines.

MATERIALS AND METHODS

Samples

The *Hibiscus sabdariffa* L. fresh roselle calyxes were collected from Cihideung, West Java, Indonesia and the apples (*P. malus* L.) were bought at a local grocery in Jatinangor, West Java, Indonesia. The fruit is grown regionally in West Java, Indonesia.

Roselle Calyxes Extraction

The roselle calyxes were washed, dried, and crushed into a coarse powder before being weighed. A weight of 100 g of calyx coarse powder were soaked in distilled water at a volume-to-volume ratio of 1:4. The calyxes were then cooked for 30 min at 90^0 C. The macerates were then aseptically filtered, and their staphylococci species-specific antibacterial activity was assessed.

Apple Juices Preparation

The fresh apples were prepared to be processed as fruit juices. The fruits were cleaned with running tap water, then the surfaces of the fruits were decontaminated by 0.1% potassium permanganate for 15 min, then rinsed using a sterile distilled water and sliced off. The fruit was juiced using a commercial juicer without water addition. After the juice was obtained, they were centrifuged for 30 min at 4000 rpm. The supernatant was isolated from the crude and then pasteurized at 65°C for 30 min. Fresh juices of both fruits were conducted in the laboratory just before the anticaries activity test.

Phytochemical Screening Analysis

According to established protocols, qualitative phytochemical analysis was done on the decoct and the juice to detect for the presence of alkaloid, flavonoids, phenols, quinones, saponins, tannins, and steroids.[19]

MIC Determination

Using a microdilution technique, different concentrations of each fruit juice and roselle decoct were evaluated against *S. aureus* clinical isolates. The concentrations ranged from 500 to 7.8125 µg/mL. Comparing the MIC values of the two juices allowed us to assess their potential as anticaries. The sample concentration at which microorganism growth is least inhibited is known as the MIC value. In 96 well microtiter - plates, the MIC of samples was assessed using the microtiter dilution assay. 100 L of sterile Mueller-Hinton Broth (MHB) was added to each microtiter plate well. The first well served as the negative control, after which 100 µL of each sample (from a stock of 1000 µg/mL of sample) were added to the second well in the same row. Then pipetted into homogeneity to achieve a measured concentration of 500 µg/mL. To attain 250 µg/mL, 20 µL of the solution from the second well was pipetted into the third well. The same process was used to remove a volume of 1 mL from each well until the lowest measured concentration (7.8125 µg/mL) was attained. As a result, all wells had the same end volume. The well with the lowest concentration of sample came following the one where the control positive was discovered. The positive control was designated as the well containing MHB broth and inoculated with the bacterial suspension. With the exception of the first well, all other wells received a 100-µL inoculation of the bacterial solution. For 24 h, the microtiter plate was incubated at 37°C. Cell pellets were observed in the bottom of the well following the incubation period. The formation of a clear solution in the well indicates the material's capability for inhibiting the bacterial cell. A volume of 10 µL of the sample in its MIC value was sub-cultured on the surface of the MHA, and after 24 h of incubation at 37 ^{0}C, the MBC value was calculated.

RESULTS AND DISCUSSION

Phytochemical Analysis Result

The roselle decoction contained saponins, flavonoids, tannins, and steroids. Meanwhile, detected secondary metabolites found in the apple juices was saponin, flavonoids, tannins and steroids. Flavonoids, alkaloids, tannins, and terpenoids are the phytochemicals found in

medicinal plants that have antibacterial and antioxidant activities.[20] Numerous studies have been conducted on certain plant species' antibacterial properties.

MIC Determination Results

In the past, many medicinal plant components, including as the root, stem, flower, fruit, and twigs, were widely employed to cure a variety of human ailments.[21] The outcomes of this study showed that *S. aureus* are effectively suppressed by the apple juice and decoct of roselle, as stated in Table 1. MIC values of each plant against *S. aureus* indicates significant antibacterial effect. The roselle decoct showed significant antibacterial activity with low susceptibility MIC at the range of 3.9- 7.81 µg/mL and 250–500 µg/mL for the apple juices.

Table 1: Comparison of The MIC Values.

Concentration (µg/mL)	*S. aureus* Growth	
	Roselle	***Apple***
500	-	-
250	-	+
125	-	+
62.5	-	+
31.25	-	+
15.625	-	+
7.8125	-	+
3.90625	+	+
1.9531	+	+
0.9765	+	+
0.488	+	+

The MIC of each plant extract against the test for significant antibacterial effects. According to Mostafa et al. (2018), the variety in chemical contents and volatile nature of the components of plant extracts the main causes of the variability in MIC of the extracts.[22] The MIC values of the roselle decoct were typically lower than those of the apple juice.

CONCLUSION

This invention study confirmed that the anti-staphylococcal of *roselle decoct* more potential than apple juice for *S. aureus* infection.

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