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Moringa pods (*Moringa oleifera*) and katakataka leaves (*Kalanchoe pinnata*) extract as a natural-derived medical patch against *Staphylococcus aureus*

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Abstract

This study investigated the effects of Moringa pods (*Moringa oleifera*) and Katakataka leaves (*Kalanchoe pinnata*) extracts on *Staphylococcus aureus* (*S. aureus*), a bacterium often causing skin infections. The researchers prepared ten treatments using a 95% ethyl alcohol solution and dried plants, which were then tested for their inhibitory effects on *S. aureus* growth. The results showed that treatments with Moringa pods extract, both alone and combined with a medical patch, significantly inhibited *S. aureus* growth, with zones of inhibition measuring 2.4cm and 2.7cm respectively. Conversely, Katakataka leaves extract showed little to no inhibition and even seemed to facilitate *S. aureus* growth. Statistical analysis using One-way ANOVA and Tukey's HSD test revealed significant differences between treatments, with those containing a higher percentage of Moringa pods extract proving more effective. This suggests that Moringa pods extract could be a potent natural-derived medical patch against *S. aureus*.

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Introduction

In order to keep the skin's physiological functions working, wound healing is a necessary procedure. Thus, if bacteria enter a wound and begin to multiply, an infection may develop, impeding the healing process of the wound (Leonard, 2023). The best technique for wound healing and infection control is dressing application. This lessens discomfort and improves the hypoxic environment's ability to promote healing (Nguyen *et al.*, 2023). It also helps to maintain the environment's moisture content and temperature. Herbs have been utilized by humans for wound care since the dawn of time. According to a 2019 study by Doña *et al.*, alternative medicine is becoming more popular due to the high cost of synthetic drugs and the possibility of genetic resistance developing in some microorganisms. Because of its uses in conventional medicine, the medicinal plant *Kalanchoe pinnata* is known to demonstrate antibacterial efficacy against a variety of diseases and their mechanisms of action, according to Tajudin *et al.* (2022).

The amazing tree of Moringa oleifera is rich in bioactive substances and a good source of pharmaceutical compounds including flavonoids, phenolic acid, and polyphenols, it is thought to have medical benefits like antioxidant, tissue protection, anti-inflammatory, and analgesic (Chis *et al.*, 2024). Natural polymers represent a potential class of materials for the development of skin wound dressings that can hasten the healing process and boost defense against infections (Ansari and Darvishi, 2024). Moreover, skin has extraordinary regenerating capabilities. When this regeneration process is occasionally disrupted and wounds heal slowly, patients face serious health risks because the available patches, dressings, and gauzes are insufficient to initiate a physiological wound healing process, which can lead to the formation of new lesions.

The purpose of this study is to investigate the possible application of Katakataka leaves *(Kalanchoe pinnata*) and Moringa pods (*Moringa oleifera*) extract as a naturally derived antibacterial medical patch.

Materials and methods

This study is quantitative research, specifically, experimental design and utilized *Staphylococcus aureus* (*S. aureus*) for its population, and used post-test randomized block design.

Procedure 1 (Instrument)

The researchers had laboratory testing of the Moringa pods (*Moringa oleifera*) and Katakataka leaves (*Kalanchoe pinnata*) extract and utilized the following:

1. Autoclave is a machine used to sterilize and disinfect laboratory materials.
2. Water bath is a vessel that incubates water with a constant temperature for a long time.
3. Blender for mixing samples into smaller pieces.
4. Top-loading balance used to measure mass or weight of a substance or object.
5. Rotary evaporator is a device that removes the liquid through the process of evaporation.
6. Caliper to measure the diameter of the zone of inhibition.

Procedure 2 (Data gathering)

Obtaining the katakataka leaves extract and moringa pods extract, fresh and clean Katakataka leaves and Moringa pods were air-dried when preparing the ethanolic extract. 2kg of Katakataka leaves and 670g of Moringa pods were blended into smaller particles and then macerated in the solvent 5L of 95% ethyl alcohol for the Katakataka leaves while 7L of 95% ethyl alcohol for the Moringa pods, for 48 hours with occasional stirring. Both mixtures were then filtered, and the filtrate was concentrated using a water bath at 60°C to obtain a semi-solid extract. The concentrated crude extract was then collected in an amber bottle and stored in a refrigerator (2-4°C).

Preparing the agar solution

The materials were prepared for the agar solution. The following materials were used including distilled water, a weighing scale, a sterile loop, clean paper, foil, a rubber band, a 500mL Erlenmeyer flask, and the brain heart agar itself. The researchers wore gloves, and 500mL of distilled water was poured into the Erlenmeyer flask. The 20 grams, which is supposed to be 14g of agar, were added to the flask because the agar sticks in the paper easily. The mouth of the flask was sealed with foil and a rubber band and then swirled until the agar dissolved. The Erlenmeyer flask with agar solution was then placed in the autoclave and poured with distilled water. The autoclave was set for about 20 minutes. After 20 minutes, the researchers slowly opened the autoclave, and then the agar solution was taken out. The solution was swirled while still hot.

Procedure 3 (Ethical considerations)

For the experiment's validity, the researchers consulted an expert in the field in line with the topic to check and approve the cogency and reliability of the study procedure. Working with bacteria was done with standard precautionary measures because it can be harmful to both the environment and the people implementing the research. Proper disinfection and sterilization of the equipment and the working area used before and after the procedures was observed, to avoid the spread of the microorganisms that may turn into pathogens which could harm its 19 surroundings. The researchers also followed the laboratory guidelines while doing the procedures and wore the proper laboratory gears and suits which protected them from acquiring the microorganism. After the experiment, decontamination of the plates used was followed. The autoclave was utilized to sterilize the plates properly. The plates were disposed of in a yellow bag after sterilization.

Procedure 4 (Presentation and delivery of tools)

In vitro testing

The name of the bacterial species, the time, and any other pertinent details were written on the agar plates. The researchers collected a small 21 number of bacteria from a dependable source or culture using a sterile loop. The medical patch pieces soaked in the extract on the agar plates' surface, using the sterile forceps, was then be placed then placed. The pieces were given enough room so the antimicrobial agent could diffuse. The bacterial suspension was spread evenly across the surface of the agar plate using a sterile loop. The plates were incubated for 11 hours at 35°C while inverted to prevent condensation from collecting on the agar surface. After incubation, the diameter of the inhibition zones was measured using a caliper in centimeters.

Decontamination

Before handling the decontaminated equipment and materials, proper PPE attire including laboratory gown, goggles, gloves, and surgical mask for personal protection were worn. The contaminated agar plates were put into a yellow biohazard bag with disinfectant bleach, and then sealed. The yellow biohazard bag was then stirred to separate the agar from the petri dish and the agar was then dissolved. The disposable petri dishes were also disposed inside the yellow biohazard bag for it is meant for single-use only. For the equipment that is not for single usage, it was decontaminated with disinfectant bleach and let sit for 5-10 minutes. The contaminated bleach was disposed of in a yellow biohazard 22 bag and then put in the proper trash bin. The equipment were rinsed with distilled water then with a 70% mix of isopropyl alcohol and distilled after. The washed equipment was then placed inside the autoclave to make sure that the bacteria were killed.

Data analysis

The study utilized One-way ANOVA to analyze if there is a significant difference between the presence of multiple concentrations. To determine which concentration has a significant difference between the groups of the Katakataka leaves (*Kalanchoe pinnata*) and Moring pods (*Moringa oleifera*) extract, the Tukey test was utilized.

Results and discussion

The table of treatments includes the volume of extracts in percentage. This data is crucial to this analysis due to it aiding the researchers in achieving the scope and limitations.

Table 1. Conversion of volume percentage to ml

Treatments	
T_1	Moringa Extract (100%)
T_2	Medical Patch w/ Moringa Extract (100%)
T_3	Katakataka Extract (100%)
T_4	Medical Patch w/ Katakataka Extract (100%)
T_5	Moringa Extract (50%) and Katakataka Extract (50%)
T_6	Medical Patch w/ Moringa Extract (50%) and Katakataka Extract (50%)
T_7	Moringa Extract (75%) and Katakataka Extract (25%)
T_8	Medical Patch w/ Moringa Extract (75%) and Katakataka (25%)
T_9	Moringa Extract (25%) and Katakataka Extract (75%)
T_{10}	Medical Patch w/ Moringa Extract (25%) and Katakataka Extract (75%)

Volume of extract in percentage	Equivalent to mL
100%	5ml
75%	3.75ml
50%	2.5ml
25%	1.25ml

Table 1 shows the conversion of the extract volume percentage to mL. For the treatments with 100% the total volume of extract was multiplied by the percentage and then divided by 100 which equal to 5mL. The 5mL was used as a constant to get the remaining equivalents. The 3.75mL was the product of 5 multiplied by 75%. The same equation was used with the 50% multiplied by 5 equating to 2.5ml then 5 multiplied by 35% is 1.25ml.

Table 2. The treatments and its zone of inhibition

Treatments	Replicate 1	Replicate 2	Replicate 3	Replicate 4
T_1	1.3cm	1.4cm	2.2cm	2.7cm
T_2	2.1cm	1.8cm	2.2cm	2.4cm
T_3	0cm	0cm	0cm	0cm
T_4	0cm	0cm	0cm	0cm
T_5	1.6cm	1.5cm	1.8cm	1.3cm
T_6	0cm	0cm	0cm	0cm
T_7	1.4cm	1.1cm	1.8cm	1.5cm
T_8	1.5cm	1.5cm	1.5cm	1.5cm
T_9	0.5cm	0.5cm	0.6cm	0.6cm
T_{10}	0cm	0cm	0cm	0cm

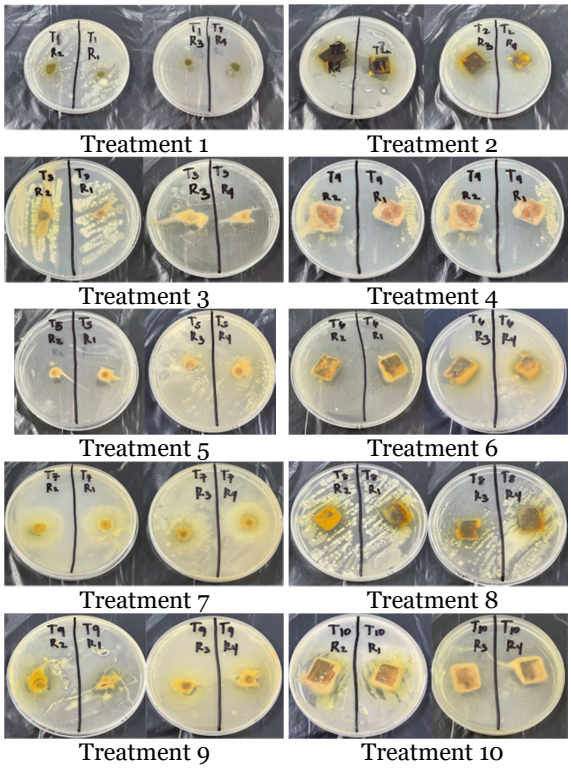

Fig. 1. Zone of inhibition of different treatments

Table 2 contains the measured zone of inhibition of each treatment used. The treatment which resulted in the largest measured zone of inhibition is treatment 1 which contains 100% Moringa pods *(Moringa oleifera)* extract with a diameter of 2.7cm/27mm. The smallest measured zone of inhibition is treatment 9 comprising 25% of Moringa pods extract *(Moringa oleifera)* and 75% of Katakataka leaves *(Kalanchoe pinnata)* extract of each extract with a diameter of 0.5cm/5mm. Four of the treatments showed no inhibition which are T3, T4, T6, and T10 (Fig. 1).

Table 3. ANOVA results

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.82875	5	1.16575	10.94316	5.9E-05	2.772853
Within Groups	1.9175	18	0.106528			
Total	7.74625	23				

One-way ANOVA was performed to compare the effect of the 10 different treatments on the *Staphylococcus. aureus*; the p-value corresponding to the F-statistic of One-way ANOVA is lower than 0.05, suggesting that the one or more treatments are significantly different. The result revealed that there was a statistically significant difference in the treatments between five groups (F (5, 18) = [10.943], p =5.9E-05) (Table 3).

Table 4. Post-hoc Tukey HSD test result

Treatment pair	Q statistic	p-value	Inference
T1 vs T2	1.3787	0.8999947	insignificant
T1 vs T5	2.1447	0.6418035	insignificant
T1 vs T7	2.7575	0.4078444	insignificant
T1 vs T8	2.4511	0.5260139	insignificant
T1 vs T9	8.2724	0.0010053	significant
T2 vs T5	3.5234	0.1783118	insignificant
T2 vs T7	4.1362	0.0818303	insignificant
T2 vs T8	3.8298	0.1222529	insignificant
T2 vs T9	9.6512	0.0010053	significant
T5 vs T7	0.6128	0.8999947	insignificant
T5 vs T8	0.3064	0.8999947	insignificant
T5 vs T9	6.1277	0.0045679	significant
T7 vs T8	0.3064	0.8999947	insignificant
T7 vs T9	5.5149	0.0113995	significant
T8 vs T9	5.8213	0.0072216	Significant

Multiple comparisons

The post-hoc test identifies which of the pairs of treatments are significantly different from each other. The Tukey's HSD test for multiple comparison found that the mean value of the diameter of the zone of inhibition was significantly different between treatment 1 and 9, treatment 2 and 9, treatment 5 and 9, treatment 7 and 9, or treatment 8 and 9, and no statistically significant difference in the diameter of zone of inhibition between treatment 1 and 2, treatment 1 and 5, treatment 1 and 7, treatment 1 and 8, treatment 2 and 5, treatment 2 and 7, treatment 2 and 8, treatment 5 and 7, treatment 5 and 8, or treatment 7 and 8.

Discussion

In 2023, Keim *et al.* released an article describing *Staphylococcus aureus (S. aureus)* as an opportunistic Gram-positive bacterium that is primarily linked to infections of the skin and soft tissues. The highly cross-linked murein architecture of *Staphylococcus aureus* is attributed to the orientation of peptide bridges. The glycan and oligopeptide chains that make up the staphylococcal cell wall murein run in a plane perpendicular to the plasma membrane, with the oligopeptide chains adopting a zigzag conformation and zippering adjacent glycan strands along their lengths (Dmitriev, 2004). *Staphylococcus aureus* absorbs fatty acids from its environment to strengthen its phospholipid bilayer. Low-density lipoprotein particles are seen in the tissues where *Staphylococcus aureus* has occupied residence. In life, these particles provide an exceptionally abundant supply of fatty acids. These results suggest that *Staphylococcus aureus (S. aureus)* can use the fatty acids found in low-density lipoproteins to circumvent the pharmacological and genetic suppression of fatty acid production. They target LDLs as a source of fatty acids for development during pathogenesis (Delekta, *et al.* 2018). Most species, including the *Staphylococcus aureus* in this instance, have somewhat complex dietary requirements, according to a study by Kloos and Schleifer (1986), which Wilkinson quoted in 1997. But generally speaking, these biomes need an organic source provided by five to twelve important amino acids, such as nicotinamide, arginine, thiamine, riboflavin, and valine. As a result, the alkaloids found in katakataka *(Kalanchoe pinnata)* include the fatty acid fraction, which contains 10.7% stearic acid and 89.3% palmitic acid, as well as oxalic acid, citric acid, and isocitric acid, as well as vitamins and amino acids like pyridoxine, glycine, cysteine, and ascorbic acid. These alkaloids facilitate the growth of the

Staphylococcus aureus (S. aureus) and its accessory growth factors (National Institute of Open Schooling, India, pg. 34). The ratio of the fatty acids, minerals, and vitamins present is higher than the antibacterial alkaloids in Katakataka *(Kalanchoe pinnata)*, despite the plant's antibacterial properties like saponins and quercetin (Pattewar, 2012). This led to a 0.5 to zero (0) inhibition of treatments 3,4,6,9 and 10.

Moreover, an aqueous extract of the plant known as Katakataka *(Kalanchoe pinnata)* that inhibited the humoral and cell-mediated immune response to mice revealed that the leaves of the plant have a phytotoxic activity of immunosuppression in-vivo. Pattewar *et al.* (2012) also cited a study by RossiBergmann *et al.* that found the animal spleen cells' capacity to proliferate in-vitro was found to be reduced in both mitogen and antigen responses. The Moringa pods *(Moringa oleifera)* showing the highest measured zone of inhibition of 2.7cm/27mm diameter is due to the phytochemical analysis of the plant composing tannins, saponins, flavonoids, terpenoids, such as quercetin at concentrations of 100mg/g (Jimenez *et al.*, 2017), which are active compounds found in the plant that can inhibit the formation of *S. aureus (Staphylococcus aureus)* as stated by the study conducted by Ervianingsih *et al.*, 2019. Yan *et al.,* 2021 said in a study they conducted that the antibacterial mechanism of natural alkaloids shows that they can disrupt the bacterial cell membrane, affect the DNA function, and inhibit protein synthesis.

Terpenoids mainly use their lipophilicity to destroy the cell membrane of bacteria. The mechanism of action of the terpenoids that the Moringa *(Moringa oleifera)* possess "can pass through the phospholipid bilayer of bacteria and diffuse inward, showing antibacterial or bactericidal effects. Since the integrity of the cell membrane is very important for the normal physiological activities of bacteria, the damage of terpenoids to the membrane will affect the bacteria's basic physiological activities. The cell's important substances such as proteins and enzymes will be lost, finally achieving the antimicrobial effect (Huang *et al.*, 2022)". Huang *et al.,* 2022 also said that the most direct energy source in living things, ATP, is essential for microbes to continue functioning normally. The terpenoids can operate on the cell membrane, causing a difference in the concentration of ATP inside and outside the cell, which can cause the membrane to become disordered and carry out antibacterial activity. Bacterial physiological activity and protein production are inextricably linked. Terpenoids are protein synthesis inhibitors that can inhibit every pathway step, thereby having an antimicrobial impact.

Conclusion

Therefore, the researchers conclude that the treatments composed of Moringa pods (*Moringa oleifera*) extract significantly affect treatments. The most effective treatment composed of 100% Moringa pods *(Moringa oleifera*) extract is treatment 1 tested against *Staphylococcus aureus* with the largest zone of inhibition of 2.7cm. The second most effective is treatment 2, which is the 100% Moringa pods (*Moringa oleifera*) extract with a medical patch with a zone of inhibition of 2.4cm. Third is the treatments 7 with 75% of Moringa pods (*Moringa oleifera*) extract and 25% of Katakataka leaves (*Kalanchoe pinnata*) extract, and treatment 9 with 25% of Moringa pods (*Moringa oleifera*) extract and 75% Katakataka leaves (*Kalanchoe pinnata*) extract with the measured zone of inhibition of 0.5cm. Treatment 5 with 50% of Moringa pods (*Moringa oleifera*) extract and 50% of Katakataka leaves (*Kalanchoe pinnata*) extract have a zone of inhibition of 1.8 cm. The remaining, treatment 3 with 100% of Katakataka leaves (*Kalanchoe pinnata*) extract, treatment 4 with 100% of Katakataka leaves (*Kalanchoe pinnata*) extract and medical patch, treatment 6 which is the medical patch w/ 50% Moringa pods (*Moringa oleifera*) extract and 50% Katakataka leaves

(*Kalanchoe pinnata*) extract and the treatment 10 with 25% of Moringa pods (*Moringa oleifera*), 75% of Katakataka leaves *(Kalanchoe pinnata*) extract and medical patch had no zone of inhibition. With these results, the study was able to prove that the Moringa pods (*Moringa oleifera*) extract is an effective antibacterial agent against *Staphylococcus aureus* while the Katakataka leaves (*Kalanchoe pinnata*) extract is a better nutrient supply for the *S. aureus* to thrive on.

The researchers also concluded that there is a significant difference between treatments 1, 2, 5, 7, and 8, using One-way ANOVA and Tukey's HSD test. These treatments have a higher percentage of Moringa pods (*Moringa oleifera*) extract than the treatments with a higher concentration of Katakataka leaves (*Kalanchoe pinnata*) extract (treatments 3, 4, 6, 9, and 10).

Recommendations

According to research, Katakataka leaves, or the *Kalanchoe pinnata* have elements that can be used as analgesic agents. They can be tested for this purpose. Moringa pods (*Moringa oleifera*) extract has been proven to possess antibacterial elements. Since Moringa pods have fibers, they can possibly be created as medical patches. Moringa pods (*Moringa oleifera*) extract can also be tested with other types of bacteria to know their extent of effectiveness. The wound healing time could also be tested in vivo using albino mice. The Katakataka leaves (*Kalanchoe pinnata*) extract could also be tested using the Kirby-Bauer technique on different culturing time frames to see how long it takes to achieve its therapeutic or medicinal effect.

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