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ANTIBACTERIAL ACTIVITY OF A HAND SANITIZER BASED ON ASIAN SWEET FLAG (*Acorus calamus* L.) AND FRAGRANT PANDAN (*Pandanus amaryllifolius* Roxb. ex Lindl.) LEAVES EXTRACTS AND GC-MS PROFILING OF BIOACTIVE COMPOUNDS

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ABSTRACT

This research aimed to evaluate the antibacterial effectiveness of a herbal hand sanitizer from a combination of methanol extracts of Asian Sweet flag (Acorus calamus L.) and fragrant pandan (Pandanus amaryllifolius Roxb. ex Lindl.) leaves, and to identify the bioactive compounds by GC-MS analysis. Asian sweet flag (Dlingo in Javanese) and pandan leaves were macerated in methanol. The extracts were subjected to phytochemical screening and formulated into hand sanitizer preparations with concentrations of 25%, 50%, 75%, and 100%, including a single 100% formulation of each extract. The disc diffusion method was used to investigate antibacterial activity against Staphylococcus aureus and Escherichia coli, while bioactive compounds were identified by GC-MS. The results showed that the extraction yielded 17.75% for dlingo leaves and 18% for pandan leaves. Phytochemical analysis identified flavonoids, tannins, alkaloids, and saponins in both extracts. All formulations inhibited S. aureus and E. coli, with the highest activity in the 100% combination formulation, producing inhibition zones of 9.60 mm and 6.47 mm. GC-MS analysis identified 196 compounds dominated by phenolics, aromatic aldehydes, benzofurans, and long-chain fatty acids with antibacterial potential. The study concludes that the combination of dlingo and pandan leaf extracts has potential as a natural active ingredient in herbal hand sanitizer formulations.

INTRODUCTION

Hand hygiene is one of the most effective methods for preventing the transmission of harmful microorganisms that cause gastrointestinal, respiratory, and skin infections (Bloomfield et al., 2007). Hands come into contact with many surfaces, food, and other body parts, making them the primary route of transmission of bacteria. The simplest preventive measure is to maintain hand hygiene, either by washing hands with soap or using hand sanitizer when water is unavailable.

Currently, most hand sanitizers on the market are alcohol-based and contain high concentrations of alcohol. They are the most widely used hand hygiene products because they are practical and can quickly reduce the number of bacteria. However, the use of high concentrations of alcohol in hand sanitizers can cause dry skin, irritation, and strip the protective lipid layer on the skin (Boyce et al., 2002). In addition, alcohol acts non-selectively, potentially disrupting the balance of normal skin microbiota, which plays an important role in natural protection against pathogens. Therefore, there is a need for natural hand sanitizer formulations that are safer and gentler on the skin.

Indonesia has a high diversity of medicinal plants and has the potential to serve as a source of natural antibacterial compounds. One plant that has not been widely developed as an antiseptic is dlingo (*Acorus calamus* L.). Traditionally, the leaves of dlingo have been used as an herbal remedy for treating wounds and skin disorders. Its rhizomes and leaves are known to contain secondary metabolites, including phenylpropanoids, tannins, terpenoids, alkaloids, essential oils, flavonoids, steroids, glycosides, and resins, particularly β-asarone and α-asarone, which are known to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria (Bhuvaneswari & Balasundaram, 2009; Mardianto & Siwi, 2024). These compounds can disrupt the permeability of bacterial cell membranes and inhibit the action of enzymes in bacteria, causing leakage of intracellular components (Dasawanti, 2025).

Another plant that is commonly found in local communities is fragrant pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) Pandan is generally used as a natural dye and food flavoring. Pandan leaves contain bioactive compounds such as alkaloids, tannins, flavonoids, and aromatic volatile components that have antibacterial potential (Karima et al., 2024). Phenolic compounds and flavonoids in plants are known to inhibit bacterial enzyme activity and cause denaturation of microbial cell proteins (Cowan, 1999a).

Therefore, the combination of these two plants (dlingo leaf extract and pandan leaf extract) is interesting to study because it has the potential to produce synergistic effects. Dlingo tends to have strong antibacterial activity due to its essential oil content. At the same time, pandan acts as both an antibacterial agent and an aroma stabilizer, making it more suitable for use as a herbal hand sanitizer. Thus, the combination of dlingo leaf extract and pandan leaf extract has the potential to produce a broader antibacterial effect through different mechanisms of action.

The development of herbal hand sanitizer products requires not only testing for antibacterial activity, but also the identification of their constituent chemical compounds. This identification is important to explain how the secondary metabolite content interacts with the resulting biological activity. Secondary metabolite identification was carried out using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, because it is capable of accurately separating and identifying volatile compounds in plant extracts (Sparkman, 2011). Through GC-MS analysis, the dominant components that are thought to play an antibacterial role can be identified.

Research on the use of dlingo leaf and pandan leaf extracts as components of herbal hand sanitizer formulations and their relationship to the profile of bioactive compounds identified by GC-MS analysis is still extremely limited. Therefore, this research was conducted to test the antibacterial activity of herbal hand sanitizers based on dlingo leaf and pandan leaf extracts and to identify their bioactive compounds using GC-MS. This research is expected to provide a scientific basis for the development of safer natural hand antiseptic products that utilize local biological resources.

MATERIALS AND METHODS

Research Subject

The subjects in this research were extracts of dlingo leaves (*Acorus calamus* L.) and pandan leaves (*Pandanus amaryllifolius* Roxb. ex Lindl.) obtained from Tambakrejo Village, Pasrepan District, Pasuruan Regency, East Java. Both plant extracts were then formulated into herbal hand sanitizer preparations. The resulting preparations were evaluated for their antibacterial activity against *S.aureus* and *E.coli* bacteria using the disk diffusion method. In addition, bioactive compounds were

identified using GC-MS to identify the active chemical compounds that contribute to antibacterial efficacy.

The characteristics of the leaves used in this study were fresh green leaves with intact surfaces without spots, holes, or signs of pest damage, wilting, or tearing. Both types of leaves were taken from the middle portion of the plant to obtain leaves with relatively uniform physiological conditions, neither too young nor too old, so that the content of secondary metabolites would be more stable and optimal for use as research material.

Tools and Materials

The equipment used included test tubes, measuring cups, a Laminar Air Flow (LAF) cabinet, an analytical balance, a water bath, an Erlenmeyer flask, an oven, a grinder, a beaker, a Bunsen burner, petri dishes, a spray bottle, pH paper, a glass stirring rod, a micropipette, a vortex mixer, a 100 mesh sieve, a caliper, an autoclave, an inoculation needle, tweezers, and a hotplate stirrer. Meanwhile, the materials utilized in this research were extracts of dlingo (*Acorus calamus*) leaves, and pandan (*Pandanus amaryllifolius*) leaf extracts, Nutrient Agar (NA), *Staphylococcus aureus*, *Escherichia coli*, Nutrient Broth (NB), DMSO, Na-CMC, Glycerin, Propyl paraben, Methyl paraben, distilled water, methanol (Merck), Dragendorff's reagent, filter paper, 1N HCl, magnesium (Mg), 1% $FeCl_3$, Mayer's reagent, Bouchardat's reagent, cotton, gauze, Liebermann-Burchard's reagent and filter paper.

Sample Preparation

Samples of dlingo leaves (*Acorus calamus* L.) and pandan leaves (*Pandanus amaryllifolius* Roxb. ex Lindl.) were washed thoroughly with running water. Both samples were dried and then ground into powder using a blender. The powdered dlingo and pandan leaves samples were macerated in 96% methanol at a ratio of 1:5 w/v) for 3 × 24 (Fauziah et al., 2025; Sri Sulasmi et al., 2019). The resulting extract was then subjected to phytochemical screening. The concentrated extract obtained was weighed, and its yield was calculated using the following formula.

$$\text{Extract Yield} = \frac{\textit{Mass of the extract obtained (gram)}}{\textit{Mass of Simplisia before extraction (gram))}} \text{ x } 100\% \text{ (Wijaya \& Jubaidah, 2018)}$$

Phytochemical Screening

Alkaloid Test

One milligram (1 mg) of each dlingo leaf extract and pandan leaf extract was individually placed in separate test tubes, followed by the addition of 0.5 mL of 2% HCl solution, and then homogenization. The homogenized solution was subsequently aliquoted into two test tubes. In the first tube, 2-3 drops of Dragendorff's reagent were added, whereas in the second tube, 2-3 drops of Mayer's reagent were added. The alkaloid test was deemed positive if a brick red, crimson, or orange precipitate appeared following the addition of Dragendorff's reagent, and a white to yellowish precipitate formed after the addition of Mayer's reagent (Khafid et al., 2023).

Flavonoid Test

The extract was dissolved in 1-2 mL of 50% heated methanol. Subsequently, about 0.2 g of magnesium powder and 4-5 drops of concentrated hydrochloric acid were added. A positive indication of flavonoids was observed when the solution exhibited a red or orange coloration (Oktavia & Sutoyo, 2021).

Saponin Test

Each extract of dlingo and pandan leaves was mixed with water in a 1:1 ratio and shaken for approximately 1 minute. If foam formed, the solution was then treated with 1 N HCl. A positive saponin test was indicated by the formation of stable foam/bubbles in the solution (Razoki et al., 2023).

Steroid and Terpenoid Test

Liebermann-Burchard reagent (2-3 drops) was then added to the extract. A positive test result for triterpenoids was indicated by the formation of a brown or purple ring at the interface between the two solvents. In contrast, a color change to blue-green indicates the presence of steroid compounds (Harborne, 1987).

Tannin Test

Then, 2-3 drops of 1% $FeCl_3$ solution were added to the extract. The tannin test was positive if the solution turned blackish green or dark blue (Harborne, 1987).

Formulation of Hand Sanitizer from Dlingo Leaf Extract (Acorus calamus L.) and Pandan Leaf Extract (Pandanus amaryllifolius Roxb. ex Lindl.)

The hand sanitizer formula used in this study was made by direct mixing under hygienic conditions. Na-CMC was first dispersed in warm distilled water until a

homogeneous gel was formed. Next, methyl paraben, glycerin, and propyl paraben were added. After the mixture was homogeneous, dlingo leaf extract (*Acorus calamus* L.) and pandan leaf extract (*Pandanus amaryllifolius* Roxb. ex Lindl.) were added according to the formula variations, then stirred slowly until evenly mixed. Distilled water was added until the total volume reached 5 mL, and the preparation was placed in a sterile container and coded according to treatment. The composition of each hand sanitizer formula is presented in **Table 1**. Each formula (F1-F6) was then subjected to organoleptic testing, including observations of color, texture, pH, and flavor. The observations were performed to assess the initial stability and physical characteristics of the hand sanitizer formulations.

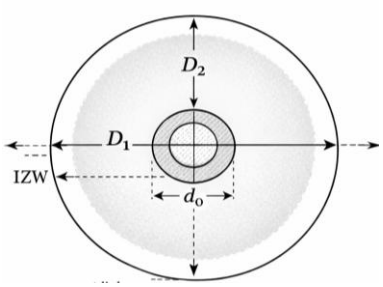
Table 1. Hand Sanitizer Formulation: Dlingo Leaf Extract (Acorus calamus L.) and Pandanus Leaf Extract (Pandanus amaryllifolius Roxb. ex Lindl.)

Material	Concentration (% b/v)						Information
	F1	F2	F3	F4	F5	F6	
Dlingo leaf and pandan leaf extract	25% (1.25 g)	50% (2.5 g)	75% (3.75 g)	100% (5 g)	100% pandan leaf extract	100% dlingo leaf extract	Active Ingredients
Na-CMC	0.005	0.005	0.005	0.005	0.005	0.005	adhesive
Glycerin	0.1	0.1	0.1	0.1	0.1	0.1	Emollient
Methyl paraben	0.005	0.005	0.005	0.005	0.005	0.005	Preservative
Propyl paraben	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	Preservative
Aquadest	Adv 5 ml	Adv 5 ml	Adv 5 ml	Adv 5 ml	Adv 5 ml	Adv 5 ml	Solvent

Antibacterial Activity Test

The antibacterial activity of hand sanitizers based on dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaf extracts was tested using the disk diffusion method. *Staphylococcus aureus* and *Escherichia coli* bacteria that had been incubated for 24 hours were evenly inoculated onto the surface of Nutrient Agar (NA) medium using a sterile cotton swab. Sterile paper discs were soaked in each formula for 3 minutes, then placed on the NA medium that had been inoculated with bacteria (Zannah et al., 2025). As a positive control, this study used commercial hand sanitizer (Antis), while 1% DMSO was used as a negative control. Nutrient agar (NA) media inoculated with *S.aureus* and *E.coli*, along with disk diffusion discs, were incubated at 37°C for 1 × 24 hours in an inverted position to allow the active compounds to diffuse and inhibit bacterial growth (Balouiri et al., 2016; Bauer et al., 1966).

This research employed a completely randomized design (CRD) with eight treatments, namely combinations of dlingo leaf and pandan leaf extracts at a ratio of 1:1 at concentrations of 25%, 50%, 75%, and 100%, as well as single extract treatments consisting of 100% dlingo leaf extract and 100% pandan leaf extract. Antibacterial activity was indicated by the presence of a clear zone surrounding the disc. The diameter of the inhibition zone was measured in two perpendicular directions using a caliper and expressed in millimeters (mm)—the calculation of the inhibition zone diameter is shown in **Figure 1.**



$$IZW = \frac{D1 + D2}{2} - d_0$$

Description :
IZW : Inhibition Zone Width
D1 : First Measurement Diameter
D2 : Second Measurement Diameter
d_0 : Disc diameter

Figure 1. Scheme of measuring the inhibition zone in the disc diffusion test

The diameter of the inhibition zone reflected the effectiveness of the antimicrobial compounds in suppressing bacterial growth (Maina et al., 2021). The strength of antibacterial activity is interpreted in **Table 2.** (Davis & Stout, 1971).

Table 2. Strength of antibacterial activity

Inhibition Zone Diameter	Category
< 5 mm	Weak
5 – 10 mm	Medium
10 – 20 mm	Strong
> 20 mm	Very Strong

GC-MS Analysis

The bioactive compounds present in the hand sanitizer were analyzed. It was made from methanol extracts of dlingo *(Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaves and was analyzed using GC–MS. A total of 1 mL of sample was diluted with methanol, then homogenized and filtered using a 0.22 µm syringe filter and placed in a GC vial. The sample was injected 1 µL into a capillary column with helium carrier gas in split mode. The oven temperature program was run in stages from a low initial temperature to a high temperature to separate volatile and semivolatile compounds. Mass detection was carried out using electron impact

ionization at 70 eV, with a scanning range of m/z 40–550. Compounds were identified based on mass spectrum matching with the NIST/Wiley library and retention time, while relative abundance was determined from the percentage of peak area in the total ion chromatogram.

RESULTS AND DISCUSSION

Characteristics of Simplisia and Methanol Extract Yield of Dlingo Leaves (Acorus calamus L.) and Pandan Leaves (Pandanus amaryllifolius Roxb. ex Lindl.)

The dried leaves of dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) obtained were in dry condition with colors characteristic of each type of leaf. The powdered simplisia was then extracted using methanol solvent to produce a thick extract from both materials. The resulting extract showed the characteristic color and aroma of the plants, indicating that the bioactive compounds were successfully extracted during the extraction process. Based on yield calculations, the methanol extract of dlingo leaves had a yield of 17.75%, while the extract of pandan leaves had a yield of 18%. These values are considered good because they exceed the minimum threshold of 10% commonly used as an indicator of successful extraction (Kementerian Kesehatan Republik Indonesia, 2017). The extraction yields of dlingo and pandan leaves in this study were higher than those reported in previous studies. A study by Nia (2018) reported a yield of 10.42% for concentrated dlingo leaf extract, while a study by Muarifah et al. (2025) showed a yield of 12.4% for fragrant pandan leaf extract. Differences in yield can be influenced by various factors, including the condition and characteristics of the plant material, the particle size of the powder, and the concentration, type, and volume of the solvent (Selviana et al., 2024).

These results indicate that methanol as a solvent is effective in extracting compounds contained in dlingo leaves and pandan leaves. The relatively high yield also indicates that a significant amount of bioactive compounds was successfully extracted from the plant material (Pawarti et al., 2023). In addition, the ability of solvents to extract bioactive compounds is greatly influenced by the solubility properties of these compounds. Solvents with appropriate polarity levels tend to be more effective in dissolving and extracting active components from plant materials (Supria et al., 2022).

This finding was consistent with previous studies reporting that polar solvents, such as methanol, were able to produce higher extraction yields compared to other solvents due to their greater efficiency in dissolving a wide range of plant bioactive compounds (Fikri et al., 2019).

Phytochemical Screening Results

Based on the results of phytochemical screening of dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaf extracts, it was found that dlingo leaf extract contains alkaloids, flavonoids, saponins, triterpenoids, and tannins. Meanwhile, pandan leaf extract contains alkaloids, flavonoids, saponins, steroids, and tannins. The screening results are presented in **Table 3** and **Figure 2.** These findings were consistent with previous studies indicating that dlingo leaves contained flavonoids, phenolics, alkaloids, saponins, triterpenoids, tannins, cardiac glycosides, and proanthocyanidins (Devi & Ganjewala, 2011; Tiwari et al., 2010). The difference in the number of compounds is due to the scope of the testing, as this study only tested for alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. Meanwhile, the results for pandan leaves are consistent with those of Muarifah et al. (2025), who identified saponins, alkaloids, tannins, and flavonoids. Steroids and triterpenoids were not reported because these two compound classes were not tested in that study. Thus, the results of this study are consistent with previous research.

Alkaloids are known to have various pharmacological activities, including antioxidant, antibacterial, analgesic, antidepressant, and anticancer properties (Adibah & Azzteena, 2019). According to Rohmawati & Amalia (2025), alkaloid compounds are known to have antibacterial activity through a mechanism that inhibits bacterial DNA synthesis by inhibiting the action of topoisomerase enzymes and disrupting the energy transport process (ATP) in bacterial cell membranes.

Table 3. Results of Phytochemical Screening of Dlingo (*Acorus calamus* L.) and Pandanus (*Pandanus amaryllifolius* Roxb. ex Lindl.) Leaf Extracts

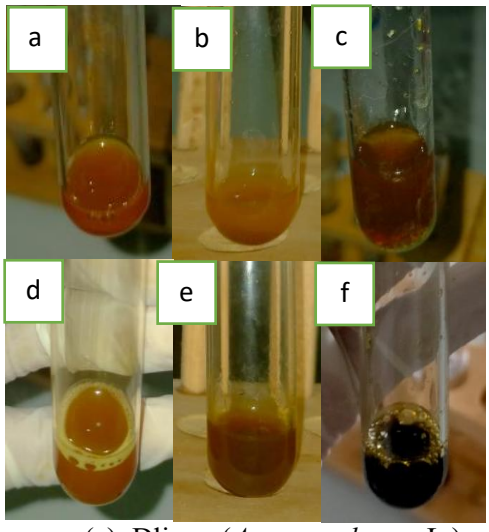
Phytochemical Test	Results	
	Dlingo Leaf Extract	Pandan Leaf Extract
Alkaloid		
Dragendofff	+	+
Mayer	+	+
Flavonoid	+	+
Saponin	+	+
Steroid	-	+

Phytochemical Test	Results	
	Dlingo Leaf Extract	Pandan Leaf Extract
Triterpenoid	+	-
Tanin	+	+

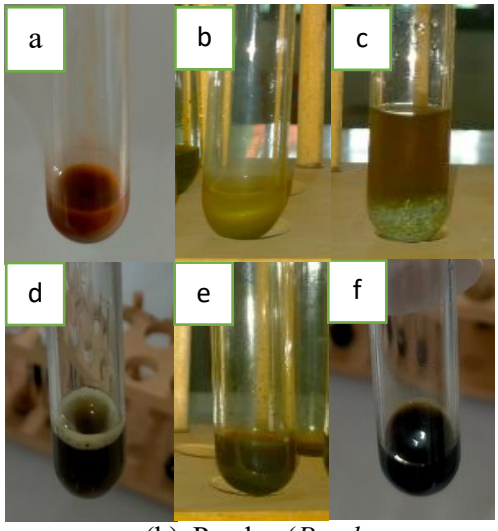
Information: (+) = contains secondary metabolites, (-) = does not contain secondary metabolites

Both extracts also tested positive for flavonoids, indicated by a color change to orange, and saponins, indicated by the formation of stable foam for 10 minutes, while steroid and triterpenoid results differed between species: pandan leaf extract was positive for steroids (bluish-green color) but negative for triterpenoids, whereas dlingo leaf extract showed the reverse pattern. Each compound contributes to antibacterial activity through a distinct mechanism: flavonoids disrupt bacterial cell wall synthesis, inhibit biofilm formation, and suppress efflux pump activity (Liu et al., 2025); saponins reduce cell membrane surface tension, increasing permeability and causing leakage of intracellular components (Hayon et al., 2023); steroids interact with the bacterial lipid membrane, causing leakage of the liposomal or cell membrane structure (Madduluri et al., 2013); and triterpenoids possess broad pharmacological activity, including antiviral, antibacterial, anti-inflammatory, and anticancer effects (Balafif & Andayani, 2013).

The results obtained from the phytochemical analysis of dlingo leaf and pandan leaf extracts can be seen visually in the image below:



(a) Dlingo (*Acorus calamus* L.) leaves



(b) Pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaves

Figure 2. Phytochemical Test Results of Dlingo Leaves and Pandan Leave). (a) Alkaloid Dragondraff; (b) Alkaloid Mayer; (c) Flavonoid; (d) Saponin; (e) Steroid dan Triterpenoid; (f) Tanin.

In the tannin test, both extracts showed positive results with the appearance of a dark greenish-black color. According to Makatambah et al. (2020), tannins are secondary metabolites that exhibit a wide range of biological activities, including antibacterial, astringent, antidiarrheal, and antioxidant activities. The antibacterial activity of tannin compounds was attributed to their ability to inhibit microbial cell adhesion, inactivate enzymes, and disrupt protein transport mechanisms within cells (Ngajow et al., 2013). Overall, the phytochemical screening results indicated that dlingo leaf and pandan leaf extracts contained a diverse range of bioactive compounds with the potential to exhibit biological activity, particularly as antibacterial agents.

Organoleptic Hand Sanitizer from Dlingo Leaf Extract (*Acorus calamus* L.) and Pandan Leaf Extract (*Pandanus amaryllifolius* Roxb. ex Lindl.)

The organoleptic test results of hand sanitizers made from dlingo (Acorus calamus L.) and pandan (Pandanus amaryllifolius Roxb. ex Lindl.) leaf extracts were characterized based on the color, texture, pH, and aroma. The organoleptic test observation results are presented in Table 4 and Figure 3. Organoleptic testing was carried out to assess the physical characteristics of the hand sanitizer formulations by observing their color, texture, and flavor. Based on the results presented in Table 4, all hand sanitizer formulations had a liquid texture. This indicates that the spray preparations produced were in accordance with the expected formulation.

Table 4. Organoleptic Test Results of Hand Sanitizer from Dlingo (*Acorus calamus* L.) and Pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) Leaf Extracts

Treatment	Color	Texture	pH	Aroma
F1 (25%)	*Transparent brown*	*Liquid*	3	*Specific*
F2 (50%)	*Light brown*	*Liquid*	3	*Specific*
F3 (75%)	*Dark brown*	*Liquid*	4	*Specific*
F4 (100%)	*Dark chocolate*	*Liquid*	4	*Typical concentrated extract of Dlingo*
F5 (100%) *dlingo leaf extract*	*Dark chocolate*	*Liquid*	5	*Typical Dlingo extract*
F6 (100%) *pandan leaf kextract*	*Light brown*	*Liquid*	5	*Pandan extract specialties*

In terms of color, there are visible differences in each formulation, which are influenced by the concentration and type of extract used. Formulations with concentrations of 25% and 50% had transparent brown to light brown colors, while

increasing the extract concentration in formulations of 75% and 100% resulted in increasingly intense colors, namely dark brown to deep brown. This indicated that increasing the concentration of the extract resulted in a darker color of the formulation, which was attributed to the higher content of active compounds present in the extract. In the 100% dlingo leaf extract formulation, the color produced was dark brown with the characteristic aroma of dlingo extract. In comparison, the 100% pandan leaf extract formulation showed a light brown color with the characteristic aroma of pandan. This variation in flavour was influenced by the presence of volatile compounds in each extract, which imparted distinctive characteristics to each formulation (Devi et al., 2021). This finding demonstrated that increasing the extract concentration led to a darker appearance of the formulation, which was associated with a higher level of active compounds in the extract. The 100% dlingo leaf extract formulation produced a dark brown color with a distinctive dlingo extract aroma. In comparison, the 100% pandan leaf extract formulation produced a light brown color with a distinctive pandan aroma.

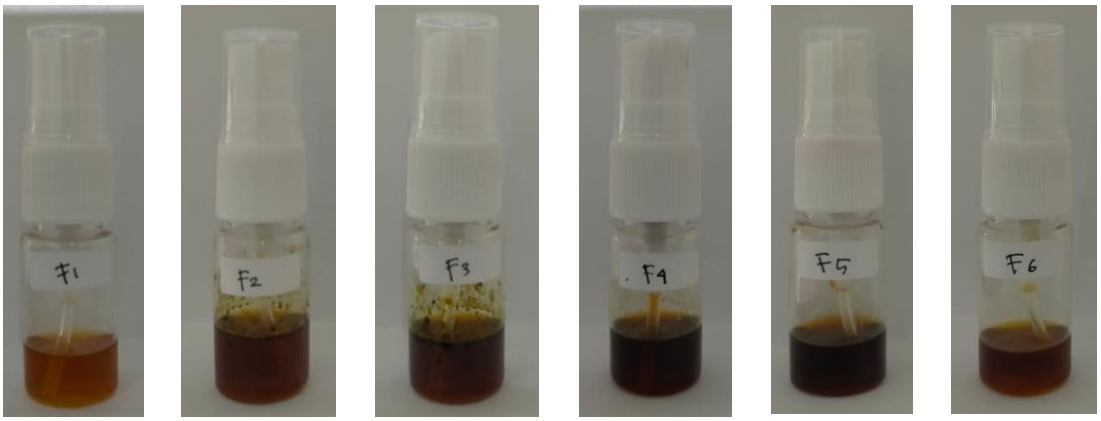


Figure 3. Results of Hand Sanitizer Extract of Dlingo Leaves and Pandan Leaves

Based on the standards set by the National Standardization Agency, the permissible pH value of hand sanitizers is in the range of 4–10 (SNI, 2017). With reference to these standards, most of the hand sanitizer formulations produced have met the pH requirements, particularly formulations F3, F4, F5, and F6. However, formulations F1 and F2 had pH values below the minimum limit, which could potentially cause skin irritation if used for a certain period of time. According to Dewi et al. (2023), pH values that are too acidic can cause skin irritation, while pH values that are too alkaline are also unsafe because they can make the skin dry and scaly.

The pH values obtained indicate a tendency for pH to increase as the concentration of the extract used increases. This finding was consistent with the view reported by Paramita et al. (2021), who stated that the higher the extract concentration, the more the pH of the preparation tends to increase. The increase in pH at each concentration variation is thought to be caused by the presence of secondary metabolites, such as flavonoids, which are alkaline and therefore contribute to the increase in hand sanitizer pH.

In addition, the higher pH values in the single extract formulations (F5 and F6) compared to the combination formulations indicated the possibility of interactions between the active compounds in the dlingo and pandan extracts, causing the pH of the combination preparations to be lower. Differences in the composition of secondary metabolites, particularly acidic and basic compounds in each extract, were considered to contribute to the observed variations in pH. Previous studies have also reported differences in pH values between plant extracts and finished herbal product formulations, indicating that the composition of the ingredients affects the final pH of the preparation (Julius et al., 2024).

Antibacterial Activity

Based on the results of antibacterial activity testing of hand sanitizers containing dlingo (Acorus calamus L.) and pandan (Pandanus amaryllifolius Roxb. ex Lindl.) leaf extracts, this product demonstrated the ability to suppress the growth of S. aureus and E. coli in nearly all formulations. The positive control (F+) showed the largest inhibition zone, which was 13.943 ± 3.51 mm for S. aureus and 10.47 ± 0.96 mm for E. coli, while the negative control (DMSO) did not produce an inhibition zone. This confirms that the antibacterial activity comes from the active compounds in the plant extracts, not from the solvent.

In the hand sanitizer formulation of dlingo leaf and pandan leaf extracts, antibacterial activity began to appear at a concentration of 25% (F1) and increased with increasing concentration. The 50% (F2) and 75% (F3) formulations showed an increase in the inhibition diameter, reaching higher values in the 100% (F4) combination, namely 9.60 ± 3.41 mm against S. aureus and 6.477 ± 1.20 mm against E. coli. The single formulation of dlingo leaves (F6) exhibited a relatively high level of inhibition, namely 9.122 ± 0.336 mm against S. aureus and 4.919 ± 1.51 mm against E. coli. In contrast, the pandan leaf extract (F5) exhibited moderate inhibition of 6.254 ± 0.77 mm against

S. aureus and 5.969 ± 1.50 mm against E. coli (Davis & Stout, 1971). This indicates that dlingo leaves have stronger antibacterial activity, but the combination of both still provides a stable inhibitory effect.

In general, the average inhibition zone against S. aureus (6.329 mm) is greater than that against E. coli (4.895 mm). This indicated that Gram-positive bacteria were more susceptible than Gram-negative bacteria, as the more complex cell wall structure of E. coli made it more difficult for antibacterial compounds to penetrate. Thus, increasing the extract concentration was directly associated with enhanced inhibitory activity, indicating that formulations with higher concentrations had the potential to be developed as natural antiseptics. In detail, the antibacterial activity against S. aureus and E. coli is presented in Table 5.

Table 5. Outcomes of Antibacterial Activity Assays of Hand Sanitizer from Dlingo Leaf and Pandan Leaf Extracts Against *S. aureus* and *E. coli* Bacteria

No	Formula	*Staphylococcus aureus*	*Escherichia coli*	Average
1	F+	13.943 ± 3.51	10.47 ± 0.96	12.20^{e}
2	F-	0.00 ± 0.00	0.00 ± 0.00	0.00^{a}
3	F1	2.626 ± 0.28	2.638 ± 0.55	2.632^{b}
4	F2	4.558 ± 0.89	4.318 ± 1.99	4.438^{bc}
5	F3	4.61 ± 0.82	4.412 ± 1.89	4.511^{bc}
6	F4	9.60 ± 3.41	6.477 ± 1.20	8.038^{d}
7	F5	6.254 ± 0.77	5.969 ± 1.50	6.11^{cd}
8	F6	9.122 ± 0.336	4.919 ± 1.51	7.02^{d}
Average		6.329	4.895	

Information: F+: Commercial hand sanitizer spray (Antis); F-: DMSO; F1: Formulation of dlingo leaf extract and pandan leaf with a concentration of 25%; F2: concentration of 50%; F3: concentration of 75%; F4: combination of dlingo leaf extract and pandan leaf with a concentration of 100%; F5: Formulation of dlingo leaf extract with a concentration of 100%; F6: Formulation of pandan leaf extract with a concentration of 100%)

Based on the classification of antibacterial strength, most hand sanitizer formulations fall into the weak (<5 mm) to moderate (5–10 mm) categories, while the positive control falls into the strong category (10- 20 mm) (Davis & Stout, 1971). These results indicate that *S. aureus* was more sensitive than *E. coli*. This difference was associated with the cell wall structure of Gram-negative bacteria, which possessed a more complex lipopolysaccharide layer that limited the penetration of antibacterial compounds (Madigan et al., 2019).

The antibacterial activity of hand sanitizer formulations from dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaf extracts was

supported by phytochemical screening results showing the presence of flavonoids, saponins, triterpenoids, alkaloids, and tannins, each contributing to antibacterial activity through the mechanisms described above. Tannins additionally act by precipitating proteins and interfering with microbial enzyme activity (Cowan, 1999). The synergy between these secondary metabolite compounds is thought to be the main factor in the formation of the inhibition zone in antibacterial activity testing.

The inhibition zones observed for the single-extract formulations in this study were considerably lower than those reported in several previous studies using the same plant species. For the pandan extract (F5), the inhibition zone (6.254 ± 0.77 mm against *S. aureus* and 5.969 ± 1.50 mm against *E. coli*) was substantially smaller than that reported by Dasopang and Simutuah (2016), who found that a 10% ethanol extract of pandan wangi (*Pandanus amaryllifolius* Roxb.) formulated into a hand antiseptic gel produced inhibition zones of 13.23 mm against *S. aureus* and 13.13 mm against *E. coli*, even at a much lower concentration. A similar pattern was observed for the dlingo extract (F6, 9.122 ± 0.336 mm against *S. aureus* and 4.919 ± 1.51 mm against *E. coli*), which was considerably lower than the values reported by Novaryatiin et al. (2018) for ethanol extracts of dlingo leaves against *S. aureus* (13.1–32.3 mm across concentrations of 1–15%) and by Anisah et al. (2014) for ethanol extracts of dlingo rhizome against *S. aureus* and *E. coli* (29.8 mm and 27.5 mm, respectively, at 100% concentration). These differences are likely attributable to several factors: (1) the solvent used, since methanol was employed in this study while ethanol was used in the comparison studies, and solvent polarity influences the type and quantity of bioactive compounds extracted; (2) the formulation matrix, as gel-based preparations may prolong the contact time between active compounds and the bacterial surface compared with liquid hand sanitizer formulations; and (3) differences in plant part and extraction technique, particularly for the rhizome-based comparison, since rhizomes generally contain a higher concentration of bioactive secondary metabolites than leaves. Nevertheless, the concentration-dependent increase in inhibition zone observed in this study is consistent with the general pattern reported in these previous studies, supporting the conclusion that dlingo and pandan leaf extracts possess genuine antibacterial activity.

Overall, increasing the extract concentration was directly proportional to the enlargement of the inhibition zone diameter. High-concentration formulations,

particularly the 100% extract combination, show the best potential as natural antiseptics, although their effectiveness is still below that of commercial products. Optimization of the formulation and active ingredient concentration was required to achieve stronger and more consistent antibacterial activity.

GC-MS Analysis: Hand sanitizer Extract of Dlingo (*Acorus calamus* L.) and Pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) Leaves

Based on the GC-MS analysis results of herbal hand sanitizers from dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaf extracts, a complex chromatogram profile was observed with peaks appearing at retention times of approximately 4–52 minutes. Based on the GC-MS results, 196 compounds were detected, consisting of various chemical groups such as phenolic compounds, fatty acids, esters, aromatic aldehydes, furans, and benzene derivatives.

Based on all identified compounds, compounds appearing at the initial retention time (±4-10 minutes) were dominated by low molecular weight polar compounds such as 1,3,5-triazine-2,4,6-triamine, benzoic acid, 2-pyrrolidinone, 4H-pyran-4-one-2,3-dihydro, 3,5-dihydroxy-6-methyl, benzofuran-2,3-dihydro, and 5-hydroxymethylfurfural. The peaks at the beginning of the chromatogram had a small to moderate relative area, indicating light, volatile components.

At medium retention times (±10-24 minutes), aromatic compounds and phenolic derivatives began to appear, including 2-methoxy-4-vinylphenol, trans-cinnamic acid, quinic acid, phenol 4-ethenyl-2,6-dimethoxy, 2-n-heptylfuran, and methoxy derivatives of benzaldehyde. The peak intensity in this section increased compared to the initial section, indicating a higher content of semi-volatile compounds. At a retention time of approximately 22-24 minutes, cis-asarone, one of the main components of dlingo leaves, was detected. This peak had a relatively large area, indicating the presence of this compound in significant amounts.

The final part of the chromatogram (±25-49 minutes) was dominated by compounds with high molecular weight and low volatility. In this range, various fatty acid derivatives and long-chain hydrocarbons were identified, including erucic acid, 8-methylnonanoic acid, hexadecanoic acid methyl ester, cis-11-eicosenoic acid, 9,12,15-octadecatrienoic acid, n-hexadecanoic acid, octadecanoic acid, octadec-9-enoic acid, eicosanoic acid,

9,12-octadecadienoic acid methyl ester, and other fatty acid ester derivatives. Squalene was also detected in this section.

The detected peak diversity indicated that methanol extracts of dlingo leaves and pandan leaves contained various secondary metabolites with different chemical properties in a single hand sanitizer formulation. Thus, the antibacterial activity of herbal hand sanitizers does not originate from a single component, but is the result of the combined action of a number of secondary metabolites contained in dlingo leaf and pandan leaf extracts. The diversity of compounds detected through GC-MS supports that this formulation has potential as a natural plant-based antiseptic. The GC-MS results are shown in **Figure 4.**

The dominant compounds detected were aldehydes and long-chain fatty acids. Fatty acids, including n-hexadecanoic acid, octadecanoic acid, and octadec-9-enoic acid, were reported to disrupt bacterial cell membranes by compromising the integrity of the lipid bilayer (Desbois & Smith, 2010). This mechanism causes an increase in cell permeability, allowing intracellular components to escape and causing cell death

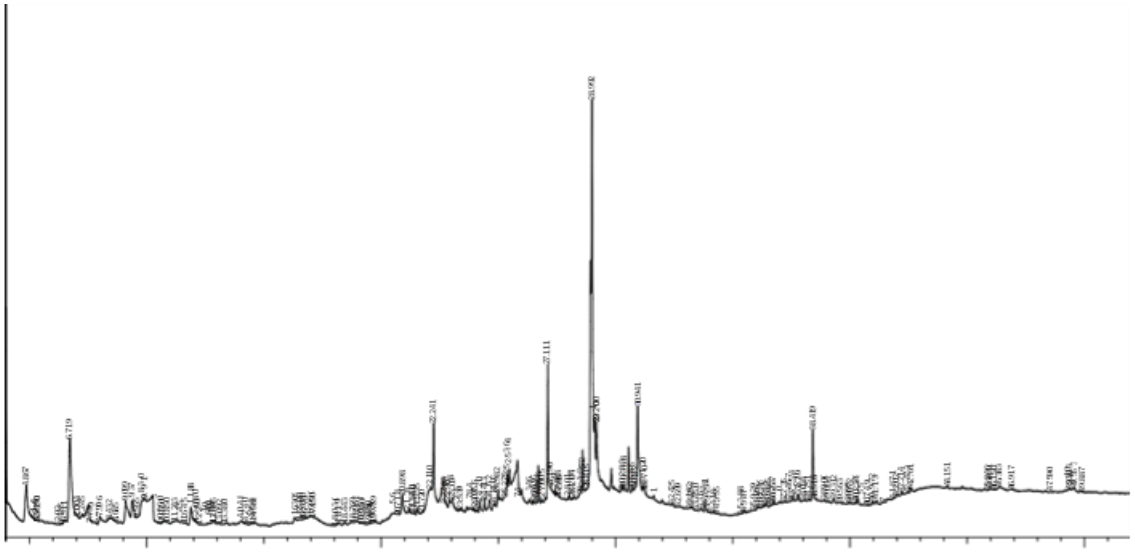


Figure 4. GC-MS Chromatogram of Hand Sanitizer from Methanol Extract of Dlingo (*Acorus calamus* L.) and Pandanus (*Pandanus amaryllifolius* Roxb. ex Lindl.) Leaves

The presence of cis-asarone as a characteristic component of dlingo leaves is also an important indicator of biological activity. This compound has been reported to have antimicrobial activity through disruption of the microbial enzymatic system (Raina et al., 2003). In addition, phenolic compounds such as 4H-pyran-4-one and other phenol derivatives act as antioxidants and antibacterials by denaturing proteins and disrupting

cell metabolism (Cowan, 1999). The compound 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was classified as a flavonoid. Flavonoids can inhibit bacterial growth by damaging cell walls, inactivating enzymes, and damaging cell membranes (Waluyo et al., 2025). Benzoic acid is an organic compound that can be used as an antimicrobial agent. Organic acids were able to disrupt the integrity of microbial cell membranes and macromolecules, as well as interfere with nutrient transport and energy metabolism, thereby exerting a bactericidal effect (Eklund, 1985).

Azelaic acid is identified as a non-phenolic saturated dicarboxylic acid containing nine carbon atoms. This compound exhibits various physiological activities, including antibacterial, anti-keratinization, anti-inflammatory, antimelanogenic, and antioxidant properties (Feng et al., 2024). Palmitic acid and stearic acid are compounds that exhibit antibacterial activity against Gram-positive and Gram-negative bacteria (Casillas-Vargas et al., 2021). Squalene is a polyunsaturated triterpenoid compound found in various organisms. Squalene has various biological activities, such as boosting immunity, resisting skin aging, detoxification effects, hypolipidemic, antioxidant, antitumor, and antibacterial effects (Cheng et al., 2024).

Overall, the GC-MS profile showed that the antibacterial activity of herbal hand sanitizers was not only influenced by a single compound, but rather by a combination of aldehydes, fatty acids, phenolics, and terpenoids. This composition supported the results of the antibacterial activity test, which demonstrated inhibitory effects against both Gram-positive and Gram-negative bacteria. Therefore, the presence of these fifteen dominant compounds further confirmed the potential of the formulation as a natural plant-based antiseptic.

Based on the percentage of peak area, the fifteen compounds with the greatest relative abundance are shown in Table 6.

Table 6. Fifteen Compounds with the Highest Peak Area Percentage Based on GC-MS Analysis in Hand Sanitizer Extracts of Dlingo (*Acorus calamus* L.) and Pandanus Leaves (*Pandanus amaryllifolius* Roxb. ex Lindl.)

No	RT (min)	Compound Name	Molecular Formula	BM (g/mol)	Peak Area %	Function
1	6.719	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	$C_6H_8O_4$	144	2.37	Natural antioxidant and antibacterial (Sato et al.,

No	RT (min)	Compound Name	Molecular Formula	BM (g/mol)	Peak Area %	Function
						2025;Waluyo et al., 2025)
2	7.519	Benzoic acid	$C_7H_6O_2$	122	0.70	*Antimicrobial* (Eklund, 1985)
3	9.375	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	0.61	*Antioxidant, antibacterial, potential as a drug for oral cancer* (Joel & Maharjan, 2021; Kong et al., 2019)
4	11.885	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.37	*Anti-inflammatory, anticancer & antibacterial* (Asami et al., 2023; Dijkman & Fraaije, 2014; Lee et al., 2025)
5	20.898	Phenol, 4-ethenyl-2,6-dimethoxy	$C_{10}H_{12}O_3$	180	0.78	*Antimicrobial, analgesic, antioxidant, anti-inflammatory, and COX-2 inhibitor* (Kuwahara et al., 2004; Sahni et al., 2022)
6	22.241	cis-Asarone	$C_{12}H_{16}O_3$	208	2.08	*Antimicrobial, antihelminthic* (Windy et al., 2021)
7	22.659	Azelaic acid	$C_9H_{16}O_4$	188	1.65	*Antibacterial, anti-inflammatory, antioxidant, antikeratinizing, and antimelanogenic* (Feng et al., 2024)
8	23.036	2-n-Heptylfuran	$C_{11}H_{16}O$	164	0.83	*antineoplastic agent*
9	25.814	Erucic acid	$C_{22}H_{42}O_2$	338	3.37	*Antibacterial and antiviral* (Galanty et al., 2023)
10	27.111	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	1.83	*Antimicrobial, Anti-inflammatory and Antioxidant* (Ashraf

No	RT (min)	Compound Name	Molecular Formula	BM (g/mol)	Peak Area %	Function
						et al., 2025;Krishnaveni et al., 2014)
11	28.532	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	294	0.40	*Antibacterial* (Soliman & Abdel-Wahhab, 2024)
12	29.108	Linolenic acid	$C_{18}H_{30}O_2$	278	1.31	*Antibacterial* (Soliman & Abdel-Wahhab, 2024)
13	29.200	Stearic acid	$C_{18}H_{36}O_2$	284	1.32	*Antibacterial & Anti-inflammatory (Casillas-Vargas et al., 2021)*
14	30.941	Oleic acid	$C_{18}H_{34}O_2$	282	2.88	*Anti-inflammatory and anticancer* (Rizki et al., 2023)
15	37.545	Squalene	$C_{30}H_{50}$	410	0.29	*Antioxidant, antibacterial, antitumor, and detoxification* (Cheng et al., 2024)

CONCLUSION

Hand sanitizer combining methanol extracts of dlingo (*Acorus calamus* L.) and pandan (*Pandanus amaryllifolius* Roxb. ex Lindl.) leaves was successfully formulated, with extraction yields of 17.75% and 18%, respectively. Both extracts contained flavonoids, saponins, alkaloids, and tannins, while steroids were found only in pandan and triterpenoids only in dlingo. The resulting preparation was a brown to dark brown liquid with a distinctive aroma and a pH of 3–5. All formulations inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, with average inhibition zones of 6.329 mm and 4.895 mm, respectively, and the strongest activity observed in the 100% combination formulation (F4: 9.60 mm against *S. aureus*, 6.47 mm against *E. coli*). GC–MS analysis identified 196 compounds, dominated by phenolics, benzofuran, aromatic aldehydes, and long-chain fatty acids such as cis-asarone, hexadecanoic acid, octadecanoic acid, and squalene. These findings indicate that dlingo and pandan leaf extracts have potential as

active ingredients in natural hand sanitizers, warranting further investigation of storage stability, skin safety, and the optimum concentration for consistent antibacterial effectiveness.

REFERENCES

Adibah, K. Z. M., & Azzreena, M. A. (2019). Plant toxins : alkaloids and their toxicities. *GCS biological and pharmaceutical*, *06*(02), 21–29. DOI: 10.30574/gscbps.2019.6.2.0003

Asami, E., Kitami, M., Ida, T., Kobayashi, T., & Saeki, M. (2023). Anti-inflammatory activity of 2-methoxy-4-vinylphenol involves inhibition of lipopolysaccharide-induced inducible nitric oxidase synthase by heme oxygenase-1. *Immunopharmacology and Immunotoxicology*, *45*(5), 589–596. https://doi.org/10.1080/08923973.2023.2197141

Ashraf, G. J., Saha, A., Dua, T. K., Paul, P., Nandi, G., Dey, S., Limbu, D., Adhikari, M. D., Kumar, A., & Sahu, R. (2025). Phytochemical profiling and evaluation of antimicrobial properties of Papilionanthe teres leaves extracts and their green synthesized silver nanoparticles. *The Microbe*, *8*, 100475. https://doi.org/10.1016/j.microb.2025.100475

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., Del Valle, R. G., Carballeira, N. M., & Sanabria-Ríos, D. J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*, *82*, 101093. https://doi.org/10.1016/j.plipres.2021.101093

Cheng, L., Ji, T., Zhang, M., & Fang, B. (2024). Recent advances in squalene: biological activities, sources, extraction, and delivery systems. *Trends in Food Science & Technology*, 146, 104392. https://doi.org/10.1016/j.tifs.2024.104392

Dasawanti, Y., & Candrika. (2025). Penentuan nilai konsentrasi hambat minimum (KHM) dan konsentrasi bunuh minimum minyak daun cengkeh (*Syzygium aromaticum*) terhadap *Propionibacterium acnes*. Journal of Pharmacy and Science, 8(2), 205–211. https://doi.org/10.36341/jops.v8i2.6001

Dasopang, E. S., & Simutuah, A. (2016). Formulasi sediaan gel antiseptik tangan dan uji aktivitas antibakteri dari ekstrak etanol daun pandan wangi (*Pandanus amaryllifolius* Roxb.). *BioLink: Jurnal Biologi Lingkungan, Industri dan Kesehatan*, *3*(1), 81–91. https://doi.org/10.31289/biolink.v3i1.815

Devi, M. A., Sahoo, D., Singh, T. B., & Rajashekar, Y. (2021). Antifungal activity and volatile organic compounds analysis of essential oils from Cymbopogon species

using solid-phase microextraction-gas chromatography-mass spectrometry. *Journal of Agriculture and Food Research*, *3*, 1. https://doi.org/10.1016/j.jafr.2021.100110

Dewi, Y. K., Nopiani, Y., Johan, V. S., Saputra, E., Idhamsyah, M., & Ayuni, P. (2023). Karakteristik gel hand sanitizer berbahan dasar ekstrak limbah kulit nanas dan serai wangi. *Jurnal Teknologi Pertanian Andalas*, *27*(1), 45–53. https://doi.org/10.25077/jtpa.27.1.45-53.2023

Fauziah, S. M., Sutama, D. K., & Nisak, Y. K. (2025). Profil fitokimia dan potensi antioksidan ekstrak bunga dari beberapa varietas tanaman krisan *(Chrysanthemum spp.)*. Berita Biologi, 24(3), 445–457. https://doi.org/10.55981/berita_biologi.2025.13439

Feng, X., Shang, J., Gu, Z., Gong, J., Chen, Y., & Liu, Y. (2024). Azelaic acid: mechanisms of action and clinical applications. *Clinical, Cosmetic and Investigational Dermatology*, *Volume 17*, 2359–2371. https://doi.org/10.2147/CCID.S485237

Fikri, M., sari, anna khumaira, & Febrianti, D. R. (2019). Pengukuran rendemen dan identifikasi senyawa metabolit sekunder pada ekstrak daun terap (*Artocarpus odoratissimus Blanco*) dengan variasi pelarut. *Jurnal Insan Farmasi Indonesia*, *2*(2), 231–240. https://doi.org/10.36387/jifi.v2i2.400

Galanty, A., Grudzińska, M., Paździora, W., & Paśko, P. (2023). Erucic acid—both sides of the story: a concise review on its beneficial and toxic properties. *Molecules*, *28*(4), 1–11. https://doi.org/10.3390/molecules28041924

Hayon, M. F. K., Supriningrum, R., & Fatimah, N. (2023). Identifikasi jenis saponin dan uji aktivitas antibakteri ekstrak metanol kulit batang sekilang (*Embelia borneensis* Scheff.) terhadap bakteri *Pseudomonas aeruginosa* ATCC 9027 dan *Streptococcus mutans* ATCC 25175. *Jurnal Riset Kefarmasian Indonesia*, *5*(2), 258–272. https://doi.org/10.33759/jrki.v5i2.368

Joel, O. O., & Maharjan, R. (2021). Effects of 5-hydroxymethylfurfural isolated from Cola hispida on oral adenosquamous carcinoma and MDR *Staphylococcus aureus*. *Journal of Medicinal Plants and Herbal Therapy Research*, *8*(1), 1–7. doi.org/10.33500/jmphtr.2021.08.001

Julius, D., Larbie, C., Kobi, G. A. A., Oppong, R. A., Emikpe, B., & Daniel, A. A. (2024). Levels of pathogens, heavy metals, essential ions and organochlorides in herbal medicines and plant species used for hepatic diseases in Ghana. *Research Journal of Phytochemistry*, *18*(1), 1–12. https://doi.org/10.3923/rjp.2023.01.12

Karima, I., Kartika, E. Y., & Rahmat, B. (2024). Aktivitas antibakteri ekstrak etanol daun pandan wangi (*Pandanus amaryllifolius* Roxb. ex. Lindl) terhadap *Staphyloccocus aureus* dan *Pseudomonas aeruginosa*. *Journal of Chemistry Sciences & Education*, *01*(01), 36–43. https://journal.pubsains.com/index.php/jcse/article/view/55

Kementerian Kesehatan Republik Indonesia. (2017). *Farmakope Herbal Indonesia Edisi II.* Kementerian Kesehatan RI.

Khafid, A., Wiraputra, M. D., Putra, A. C., Khoirunnisa, N., Putri, A. A. K., Suedy, S. W. A., & Nurchayati, Y. (2023). Uji kualitatif metabolit sekunder pada beberapa tanaman yang berkhasiat sebagai obat tradisional. *Buletin Anatomi dan Fisiologi*, *8*(1), 61–70. https://doi.org/10.14710/baf.8.1.2023.61-70

Kong, F., Fan, C., Yang, Y., Lee, B. H., & Wei, K. (2019). 5-hydroxymethylfurfural-embedded poly (vinyl alcohol)/sodium alginate hybrid hydrogels accelerate wound healing. *International Journal of Biological Macromolecules*, *138*, 933–949. https://doi.org/10.1016/j.ijbiomac.2019.07.152

Krishnaveni, M., Dhanalakshmi, R., & Nandhini, N. (2014). GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of gossypium seeds. *International Journal of Pharmaceutical Sciences Review and* Research, 27(1), 273-276. https://www.researchgate.net/publication/264549499_GC-MS_Analysis_of_phytchemicalsFatty_acid_Profile_Antimicrobial_Activity_of_Gossypium_Seeds

Lee, J., Morshidi, N. A. A. B., Lee, J., Sim, W., & Kim, J.-H. (2025). 2-Methoxy-4-vinylphenol mitigates malignancy of cholangiocarcinoma cells through the blockade of sonic hedgehog signalling. *Biochemical and Biophysical Research Communications*, *754*, 151515. https://doi.org/10.1016/j.bbrc.2025.151515

Liu, Y., Zhu, J., Liu, Z., Zhi, Y., Mei, C., & Wang, H. (2025). Flavonoids as promising natural compounds for combating bacterial infections. *International Journal of Molecular Sciences*, *26*(6), 1–20. https://doi.org/10.3390/ijms26062455

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., Stahl, D. A., & Brock, T. D. (2019). *Brock biology of microorganisms.* (Fifteenth, global). Pearson.

Makatambah, V., Fatimawali, F., & Rundengan, G. (2020). Analisis senyawa tannin dan aktifitas antibakteri fraksi buah sirih (*Piper betle* L) terhadap *Streptococcus mutans. Jurnal MIPA*, *9*(2), 75–80. https://doi.org/10.35799/jmuo.9.2.2020.28922

Mardianto, R., & Siwi, S. E. (2024). Uji aktivitas ekstrak rimpang jeringau (*Acorus calamus* L) terhadap bakteri *Escherichia coli*. *Journal Medicine And Clinical Pharmacy*, *1*(1), 30–36. https://doi.org/10.47794/medclip.v1i1.5

Muarifah, L., Hasriyani, & Arif, F. (2025). Formulasi dan uji efektivitas sediaan patch ekstrak etanol 96% daun pandan wangi *(Pandanus amarillyfolius* Roxb.) sebagai repellent terhadap nyamuk *Aedes aegypti*. Journal of Innovative & Creativity, *5*(2), 23400–23417. https://doi.org/10.31004/joecy.v5i2.3307

Nia, D. H. (2018). *Uji aktivitas antioksidan ekstrak daun dan rimpang jeringau (Acorus calamus L.) terhadap pereaksi DPPH dengan metode spektrofotometri UV-Vis* [Skripsi]. Sekolah Tinggi Ilmu Farmasi (STIFI) Bhakti Pertiwi.

Novaryatiin, S., Pratomo, G. S., & Yunari, C. (2018). Uji daya hambat ekstrak etanol daun jerangau hijau terhadap *Staphylococcus aureus*. *Borneo Journal of Pharmacy*, *1*(1), 11–15. https://doi.org/10.33084/bjop.v1i1.236

Oktavia, F. D., & Sutoyo, S. (2021). Skrining fitokimia, kandungan flavonoid total, dan

aktivitas antioksidan ekstrak etanol tumbuhan *Selaginella doederleinii*. *Jurnal Kimia Riset*, *6*(2), 141–153. https://doi.org/10.20473/jkr.v6i2.30904

Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Frontiers in Microbiology*, *10*, 1–28. https://doi.org/10.3389/fmicb.2019.00911

Paramita, H. E., Ambari, Y., & Ningsih, A. W. (2021). Formulasi dan uji stabilitas sediaan gel hand sanitizer ekstrak etanol buah mentimun (*Cucumis sativus* L.). *(J-PhAM) Journal of Pharmaceutical Care Anwar Medika*, *3*(2), 110–125. DOI: 10.36932/jpcam.v3i2.43

Pawarti, N., Iqbal, M., Ramdini, D. A., & Yuliyanda, C. (2023). Pengaruh metode ekstraksi terhadap persen rendemen dan kadar fenolik ekstrak tanaman yang berpotensi sebagai antioksidan. *Medula*, *13*(4), 590–593.

Razoki, Butar-Butar, R. G. S., Neswita, E., Sembiring, N. B., Novriani, E., Simanjuntak, N. J. P., & Pakpahan, E. H. (2023). Uji skrining fitokimia dan pengukuran kadar total flavonoid pada ekstrak paku (*Nephrolepis biserrata*) dengan fraksi n-heksana, etil asetat, dan air. *Journal of Pharmaceutical and Sciences*, *6*(3), 1142–1160. https://doi.org/10.36490/journal-jps.com.v6i3.185

Rizki, D. K., Nabila, A. Y., Salsabila, D., Anggy, F., Damayanti, F., & Nurhidayati, N. (2023). Oleic acid sebagai terapi komplementer kanker. *Unram Medical Journal*, *12*(2), 222–227. https://doi.org/10.29303/jk.v12i2.4440

Rohmawati, U., & Amalia, A. (2025). Potential of bioactive compounds of *Allium sativum* L. var. solo garlic extract in inhibiting InhA protein in *Mycobacterium tuberculosis*. *Jurnal Biologi Tropis*, *25*(2), 2103–2114. https://doi.org/10.29303/jbt.v25i2.9119

Sahni, T., Sharma, S., Verma, D., Sharma, P., & Kaur, S. (2022). Antioxidant and antibacterial evaluation of 2,6-dimethoxy-4-((phenylamino) methyl) Phenol and 2,6-Dimethoxy-4-((4′-nitrophenylimino)methyl) Phenol. *Biological Forum–An International Journal*, *14*(1), 1–7.

Sato, T., Tomizawa, M., Segawa, S., Matsuoka, M., & Aurues, T. (2025). Effect of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one-containing lactic acid bacterial beverages on skin moisture: a randomized, double-blind, placebo-controlled, parallel study. *Journal of Clinical Biochemistry and Nutrition*, *76*(2), 24–178. https://doi.org/10.3164/jcbn.24-178

Selviana, A. P., Khoirotunnisa, U., Ulandari, A. S., Rahayu, I. D., & Andrifianie, F. (2024). Pengaruh konsentrasi dan volume etanol terhadap rendemen ekstrak bunga telang (*Clitoria ternatea* L.) pada metode ekstraksi maserasi. *Jurnal Kesehatan dan Agromedicine*, *11*(2), 94–100. https://doi.org/10.23960/jka.v11i2.pp94-100

Soliman, H. M., & Abdel-Wahhab, M. A. (2024). Synthesis of antibacterial bioactive compounds using linoleic acid extracted from melon seeds oil and evaluation of its waste meal ash for fried oil regeneration. *Waste and Biomass Valorization*, *15*(1), 487–499. https://doi.org/10.1007/s12649-023-02161-0

Sri Sulasmi, E., Faiqohtun Wuriana, Z., Suryaningtya Sari, R., & Rohmawati, U. (2019). Flavonoid characterization in ferns from Baluran National Park. *IOP Conference Series: Earth and Environmental Science*, *276*(1), 012033. https://doi.org/10.1088/1755-1315/276/1/012033

Supria, R. M., Handayani, R. P., & Djamaludin, A. (2022). Pembuatan dan uji organoleptik sediaan spray antiseptik alami kombinasi ekstrak daun kirinyuh (*Chromolaena odorata*) dan sereh wangi (*Cymbopogon nardus*). *Journal Of Holistic and Health Sciences*, *6*(1), 9–16. https://doi.org/10.51873/jhhs.v6i1.170

Waluyo, J., Kuswati, K., Putri, R. W., Rosalinda, J. D., Fatmawati, Y., & Habibi, M. W. (2025). Phytochemical profiling and anti-*Staphylococcus epidermidis* activity of mango leaf extract using gas chromatography-mass spectrometry. *Edubiotik : Jurnal Pendidikan, Biologi dan Terapan*, *10*(01), 250–257. https://doi.org/10.33503/ebio.v10i01.1177

Wijaya, H., & Jubaidah, S. (2018). Perbandingan metode ekstraksi terhadap rendemen ekstrak daun rambai laut *(Sonneratia caseolaris L. Engl)*. *Jurnal Ilmiah Manuntung Sains Farmasi dan Kesehatan*, *4*(1), 79–83. https://jurnal.stiksam.ac.id/index.php/jim/article/view/148

Windy, T., Rahayu, I., & Timotius, K. H. (2021). Antimicrobial and antihelmintic activities of asarone rich herbal materials. *A Review. International Journal of Herbal Medicine, 9*(5), 01–07.

Zannah, M. R. N., Dalimunthe, G. I., Lubis, M. S., & Yuniarti, R. (2025). Formulasi paper soap ekstrak etanol dedak padi (*Oryza sativa* L.) dan uji aktivitas antibakteri terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Journal of Pharmaceutical and Sciences*, 324–345. https://doi.org/10.36490/journal-jps.com.v8i1.229