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THE UTILIZATION OF MORAGE LEAF FOR MAKING ENVIRONMENTALLY FRIENDLY HAND WASHING SOAP IN THE ERA OF THE COVID PANDEMIC 19 IN SMA NEGERI 2 PADALARANG

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ABSTRACT

THE UTILIZATION OF MORAGE LEAF FOR MAKING ENVIRONMENTALLY FRIENDLY HAND WASHING SOAP IN THE ERA OF THE COVID PANDEMIC 19 IN SMA NEGERI 2 PADALARANG

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Health is an important thing that must be considered, especially hand hygiene before and after carrying out activities in the era of the Covid 19 pandemic. According to WHO (2013) the spread of Staphylococcus aureus and Escherichia coli bacteria is most often transmitted from hand to hand. Staphylococcus aureus is a gram-positive micrococcal bacterium that is often considered the main pathogen for humans, while Escherichia coli is a gram-negative bacterium that has less peptidoglycan content and more lipid content. This study aims to compare the concentration of Moringa leaf extract which has the greatest inhibition against Staphylococcus aureus and Escherichia coli bacteria in Moringa leaf handwash. The study used a completely randomized design with three repetitions on the effect of the concentration of Moringa leaf extract from the powder and from the dried leaves at various concentrations ie test 1: 2.5%, 5%; 10%; 20%; 40%, and 80% b/v, and test 2: 2.5%, 5%; 7.5%; 10%; 12.5%, and 15% b/v were then compared with other soaps that did not contain moringa as a control, and involved 10 students who were members of the Research and Development (RnD) team of SMA Negeri 2 Padalarang. Extraction was carried out using the soxhlet method with 96% ethanol solvent for 3 hours, followed by a qualitative test for the phytochemical content of tannins, flavonoids, polyphenols, and saponins, and a quantitative test for the tannin content in Moringa handwash using the HPLC method, as well as the inhibition against Staphylococcus aureus and Escherichia coli bacteria.

The results of the quantitative test for measuring tannin levels in each formula, using the HPLC method, showed that hand soap with a concentration of 2.5% Moringa leaf extract had a tannin content of 28.2659ppm or 0.0028266%, a 5% concentration of Moringa leaf extract had a tannin content of 46.4910ppm or 0.0046491%. , the concentration of moringa leaf extract 10% has a tannin content of 128.9282ppm or 0.01289282%, a concentration of 20% moringa leaf extract has a tannin content of 306.2910ppm or 0.0306291%, a concentration of 40% moringa leaf extract has a tannin content of 900.5194 ppm or 0.09005194%. The optimum inhibition against Staphylococcus aureus was achieved at a concentration of 2.5% Moringa leaf extract, while the optimum inhibition against Escherichia coli bacteria was at a concentration of 10% and 80%, respectively. Staphylococcus aureus is a Gram positive bacterium that has a more complex peptidoglycan structure and a lower lipid content, while Escherichia coli is a Gram negative bacterium that has less peptidoglycan/thin content and more lipid content, so that the cell wall of

Staphylococcus aureus is more easily damaged by compounds. active Moringa leaf extract from Escherichia coli.

Keywords: *Moringa (Moringa oleifera) handwash, optimum inhibition, HPLC*

1. Introduction

1.1. Background

Currently the world is facing the spread of Corona Virus Disease 2019 (COVID-19) so that the World Health Organization (WHO) has declared it a pandemic status. Currently, the spread of COVID-19 in Indonesia is increasingly widespread and reaches almost all regions. Based on Presidential Decree No. 12 of 2020, the spread of COVID-19 is declared a National Disaster. The Covid-19 pandemic has had an impact on several sectors of life, including the education sector. To limit the spread and transmission of the Covid-19 virus widely in education units, the Ministry of Education and Culture of the Republic of Indonesia adopted a policy of implementing Learning from Home (BDR). Similar policies are also implemented in more than 180 countries around the world.

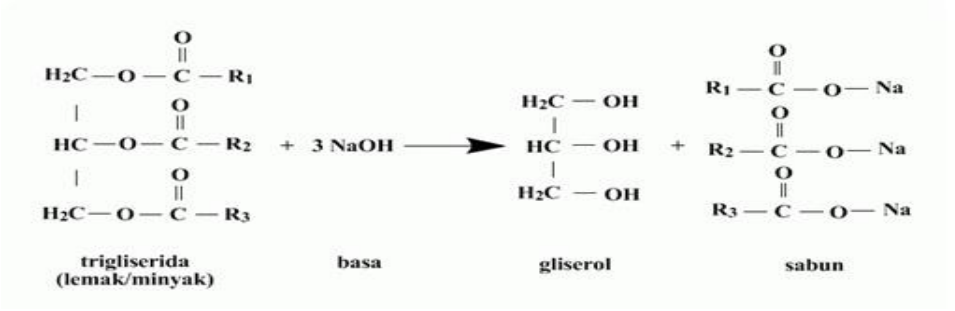
In Indonesia, various socio-economic conditions, access to technology, and conditions in the Covid-19 distribution area have caused the implementation of BDR and student learning outcomes to vary. However, learning needs to be prepared and prepared to face the challenges of the 21st century. Science/science education as part of education plays an important role in preparing students who have scientific literacy, namely those who are able to think critically, creatively, logically, and take the initiative in responding to issues in society. caused by the impact of changing environmental situations and conditions due to various natural events, chronic disease outbreaks, which lead to changes in lifestyle globally. Students are expected to be able to study themselves and the environment, understand and understand the relationship between the knowledge learned in school and real life, apply their knowledge to find solutions to problems, as well as prospects for further development in real life.

One thing that needs to be understood is that the Covid-19 pandemic situation has brought many changes to people almost all over the world. One of the changes that occur is that people are increasingly concerned about health, personal hygiene, and the environment. Soap and hand sanitizer are vital items that are very familiar to us, available in every corner of the place, and become a necessity at all times. However, the use of the wrong type of soap can cause other unexpected effects. For example, dish soap, produced to remove grease on dishes, then used inappropriately for washing hands, The chemical content in it, can strip the skin of natural oils,

irritation, dry, cracked, hot, and rough skin. The raw material for making soap is also often the biggest contributor to water pollution.

Water pollution that occurs in various regions in Indonesia has resulted in a clean water crisis. Weak government supervision and reluctance to enforce the law properly make the problem of water pollution a chronic problem that is getting worse and worse. Therefore, as humans who care about the survival of all living things, it is our obligation to prevent and overcome the problem of water contamination by hazardous substances, especially from household waste, one of which is used soap washing water, which is a potential source of organic pollutants. .

Saponification is the reaction between an oil or fat with an alkaline base to form soap and glycerin. The principle of saponification in fats is that the oil or fat will be hydrolyzed by alkaline bases to form glycerin and crude soap. Triglycerides can be esters of fatty acids to form carboxylic salts. The reaction is as follows:



Soap is a compound salt of high fatty acids, such as sodium stearate, $C_{17}H_{35}COO^{-}Na^{+}$, where one soap molecule contains a long hydrocarbon chain plus an ionic end. The hydrocarbon part of the molecule is hydrophobic which is soluble in non-polar substances, while the ionic end is hydrophilic which is soluble in water. Due to the presence of hydrocarbon chains, a soap molecule as a whole is not completely soluble in water. However, soap is easily suspended in water because it forms micelles, which are collections (50 – 150) of soap molecules whose hydrocarbon chains are clustered with the ends of the ions facing the water. Potassium soap (RCOOCK) is a soap produced from the reaction between triglycerides and potassium hydroxide (KOH) base. This soap is soft, commonly used for liquid bath soap, laundry soap or household soap (Riswiyanto, 2009).

The washing action of soap is largely due to the emulsifying strength and surface tension lowering ability of water. This concept can be understood by considering the two properties of the soap ion. An image of the stearate consists of the carboxyl ion as the "head" with the long hydrocarbon as the "tail". In the presence of

oils, fats and other water-insoluble organic matter, the tendency for the "tails" of the anions to dissolve in the organic matter, while the "heads" remain in aqueous solution. Therefore soap emulsifies or suspends organic matter in water.

Soap raw materials generally consist of:

1. Surfactants (*surface active agents*) are surface active substances that have different ends, namely hydrophilic (like water) and hydrophobic (like fat). This active ingredient functions to lower the surface tension of the water so that it can release dirt that sticks to the surface of the material. The rest of the surfactant can form chlorbenzene in the chlorinization process of PDAM drinking water treatment. Chlorbenzene is a chemical compound that is toxic and dangerous to health. The used soapy water that is thrown into the river / river causes the contact of water and air to be limited so that it reduces the process of dissolving oxygen into the water. This causes organisms in the water to lack oxygen, which can lead to death.
2. *Builder* functions to increase the washing efficiency of surfactants by deactivating minerals that cause water hardness. Examples include Phosphates (Sodium Tri Poly Phosphate/STPP), Acetate (Nitrile Tri Acetate/NTA), Ethylene Diamine Tetra Acetate (EDTA), Silicates (Zeolite), and Citrate (citric acid). In too many quantities, builders can cause excessive nutrient enrichment (eutrophication) in the water, so that the water lacks oxygen due to the rapid growth and development of algae (*phytoplankton*). Algae are also food for bacteria, so their development triggers an overpopulation of bacteria.
3. *Filler* is a soap additive that does not have the ability to increase washing power, but increases the quantity or can solidify and solidify so as to reduce the selling price, for example: sodium sulfate.
4. *Additives* are supplements/additives to make products more attractive, such as fragrances, solvents, bleaches, dyes and so on that are not directly related to the washing power of soap. Additives are added to support the appearance or commercialization of the product. Although its existence does not have to be in soap products, it is now a must to be able to compete with competitors. In recent years, a lot of research has been done on the use of renewable natural resources as additives in liquid soap. Good hand soap must meet quality requirements based on SNI Number 06-4085-2017 which is presented in table 1.

Table 1. Quality requirements for liquid bath soap (National Standardization Agency, 1996)

No	Test Criteria	Unit	Requirements	
			Types of Surfactants	Type of Detergent
1	Circumstances:			
	Form		Homogeneous liquid	Homogeneous liquid
	Smell		Typical	Typical
	Color		Typical	Typical
2	pH 25°C		8-11	6-8
3	Free Alkali (as NaOH)	%	≤ 0,1	Not required
4	Active Ingredients	%	≥ 15	≥ 10
5	Specific Gravity, 25°C		1,01-1,10	1,01-1,10
6	Microbial contamination: Total plate number	Koloni/g	$\leq 1x10^5$	$\leq 1x10^5$

In this study, the soap quality test or soap preparation parameters only focused on its antibacterial properties. Soap that enters sewage or an aquatic system usually precipitates immediately as calcium and magnesium salts. Therefore, some of the effects of the soap in the solution may be removed. But the effect will be different on the organs of humans, animals and plants. Therefore, new innovations in soap making are needed, besides being environmentally friendly, they are also friendly to living organs, especially humans, generally animals and plants.

Currently, liquid soap is more in demand by consumers because its use is more practical, easy to carry around, and if used together it will be more hygienic than using solid soap alternately (Yulianti et al., 2015). The part of the body that is moist and prone to contact with germs that cause and spread disease is the hands. According to WHO (2013) the spread of *Staphylococcus aureus* and *Escherichia coli* bacteria is most often transmitted from hand to hand. *Staphylococcus aureus* is a Gram-positive micrococcal bacterium that is often considered a major pathogen for humans, while Escherichia coli is a Gram negative bacterium that has less peptidoglycan content and more lipid content. Besides being very pathogenic, *Staphylococcus aureus* is a bacterium that is often found on the palms of the hands.

Epidemiological studies show that infections due to *Staphylococcus aureus* in the world have increased in the last two decades. Data in the United States and Europe show that *Staphylococcus aureus* is the most common bacterial pathogen causing infection with a prevalence of 18-30%, while in the Asian region, *Staphylococus aureus* and *Pseudomonas aeruginosa* have almost the same incidence of infection.

Hand washing is a simple activity that aims to remove dirt and reduce the number of germs or bacteria on the hands and palms. Hand washing can be done using

soap and water, as well as an antiseptic gel. In a study showed that by washing hands, we can reduce the number of germs by about 58% on the palms of the hands. The decline in this figure is related to the health of the individual. As in the study of Dorson (2000) states that by washing hands can reduce the death rate of one million per year caused by various diseases caused by various kinds of bacteria and viruses.

Microbes are very small organisms, such as bacteria and fungi. In soap products, antibacterial is needed to keep the levels of bacteria in the soap in accordance with the provisions of SNI and also kill germs attached to the body so as to provide a positive psychological effect for consumers. The antimicrobial mechanism in killing bacteria can occur through inhibition of nucleic acid synthesis, inhibition of cell membrane function, cell wall synthesis, and protein synthesis (Jawetz, 2007). One of the synthetic antibacterials commonly used in soap products is triclosan. This synthetic antibacterial position can be replaced using natural antibacterials found in various plants.

In Indonesia, there is a high biodiversity and many plants that are useful and also efficacious for health, one type of plant that can be utilized and explored for its potential is Moringa leaf (*Moringa oleifera*). Moringa leaves contain active ingredients as a result of secondary metabolism in plants that are efficacious as anti-cancer, hypotensive, inhibiting bacterial and fungal activity. Moringa leaves have active compounds that can act as antibacterial substances such as saponins, flavonoids, alkaloids, and tannins. These compounds have a mechanism of action by damaging the bacterial cell membrane. Natural ingredients such as Moringa leaves can be used as natural antibacterials as an alternative to synthetic materials in preventing bacterial infections (Busani et al., 2020).

Moringa oleifera Lam (synonym: *Moringa pterygosperma Gaertner*) which we know as Moringa is the most famous species of the thirteen species of the genus *Moringacae*. Although it is native to the southern foothills of the Himalayas, Moringa is present in all tropical countries (Krisnadi, 2015). In Indonesia, the Moringa plant is known by various names. The people of Sulawesi call it kero, wori, kelo, or Keloro. The Madurese call it maronggih. In Sundanese and Malay it is called Kelor. In Aceh it is called murong. In Ternate it is known as kelo. In Sumbawa it is called kawona. While the Minang people know him by the name mungai.

Moringa (*Moringa oleifera*) grows in the form of a tree, long-lived (perennial) with a height of 7 - 12 m. Stem woody (*lignosus*), erect, dirty white, thin skin, rough

surface. Sympodial branching, the direction of the branch is upright or oblique, tends to grow straight and elongated. Propagation can be generative (seeds) or vegetatively (stem cuttings). It grows in the lowlands and highlands up to an altitude of ± 1000 m above sea level, widely planted as a boundary or fence in the yard or field (Krisnadi, 2015). Moringa is a plant that can tolerate a variety of environmental conditions, so it is easy to grow even in extreme conditions such as very high temperatures, in the shade and can survive in areas of light snow. Moringa is resistant to long dry seasons and grows well in areas with annual rainfall ranging from 250 to 1500 mm. Although it prefers dry sandy loam or loam soil, it can live in soils that are dominated by clay.

The Moringa plant has been known for centuries as a multi-purpose, nutrient-dense and medicinal plant. All parts of the plant can be used, the leaves are made into vegetables such as spinach or kale, the young fruit is cooked in a variety of different ways, the young seeds are used like peas or made into pulp like green beans, the oil extracted from the seeds is used for cooking and cosmetic ingredients, especially in care. skin as skin nutrition, antiaging, moisturizer and sunscreen.

Ann Hirsch, PhD, in Krisnadi (2015) states that Moringa is one of the very few plants that contain all (eight) essential amino acids. To be healthy the body needs small biomolecules called amino acids. Moringa plants naturally contain 46 powerful antioxidants that protect the body from free radicals, contain 18 amino acids (8 of which are essential) that the body needs to build new cells, 36 anti-inflammatory compounds, and 90 natural nutrients such as vitamin A (Alpha & Alpha). Beta-carotene), Moringa has Vitamin A (Beta Carotene), Vitamin B1 (thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6 (Pyrodixine), Vitamin B7 (biotin), Vitamin C (ascorbic acid) , Vitamin D (cholecalciferol), Vitamin E (Tocopherol), vitamin K, folic acid, and biotin, and other minerals in abundance.

Moringa leaves contain phytochemicals that make plants able to carry out self-defense mechanisms. Phytochemicals in plants are divided into primary and secondary metabolites. Secondary metabolites are produced in small amounts but have important significance in plants. Secondary metabolite compounds such as flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids are known to function as antimicrobials produced by plants (Aguinaldo, 2004). These compounds have the ability as medicine, the benefits are as detoxification and water purification, antibiotics, skin care, anti-inflammatory, ulcers, blood pressure, diabetes and anemia (Mardiana, 2012).

Moringa leaf extraction can be done by maceration method, which is a simple way to extract phytochemical compounds found in plants. The first step, making Moringa leaf powder, the leaves are dried using an oven at 40 C until the water content is <10% then blended and sieved with a 60 mesh sieve with the aim of reducing the surface area so that the ingredients in the powder can be more easily extracted. The extraction process lasts for +/- 24 hours, after that the extract obtained in a water bath at a temperature of 70 C for 2 hours so that the active compound can be extracted optimally (Anwar, 2016).

Sally in 2014 stated that Moringa leaves contain phytochemical compounds, namely active ingredients such as flavonoids, saponins, tannins, and polyphenols, which are produced by these plants. The content of the active ingredient functions as an antimicrobial. Therefore, it is necessary to ascertain the presence of the active ingredients in Moringa leaves through a qualitative phytochemical test in handwash recipes with the active ingredients of Moringa leaves. The results of the phytochemical tests that have been carried out are obtained as follows:

Table 2. Results of Phytochemical Maceration of Moringa Leaves

Compound	Positive Results	Hasil Uji	Information
Flavonoid	Yellow	Yellow shadows	+++
Saponin	Stable foam (< 7 minutes)	Stable foam (< 7 minutes)	+
Tanin	Blackish green	Blackish green	++++
Polifenol	Dark green color	Dark green color	+++

Description: + less clear; ++ is rather self explanatory; +++ clear; ++++ very clear

Based on previous research literature studies on qualitative tests of phytochemical compounds, the total tannin content was thought to be the most abundant in maceration of Moringa leaves, quantitative tests were carried out by spectrophotometry with a standard solution of tannic acid and *Folin Ciocalteau* reagent, which was based on the formation of a complex from molybdenum-tungsen blue so that easier to detect using spectrophotometry. Tannic acid is used as a comparison because it has a phenol group, a compound that is stable, pure, and cheaper. (Waterhouse, 1999). Based on research conducted by Ojiako (2014), Moringa leaf extract with various solvents namely ethanol, N-hexane, and ethyl acetate contains tannin levels of 8.22%.

The growth inhibition of *Staphylococcus aureus* and *Escherichia coli* (w/v%) at maceration concentration of Moringa leaves was compared to the growth inhibition of *Staphylococcus aureus* and *Escherichia coli* (w/v%) liquid handwashing soap produced by SMAWA toiletries without Moringa leaves. The hope of this research is that the maceration of Moringa leaves can naturally inhibit the growth of microorganisms due to its tannin content, while the liquid hand soap produced by SMAWA toiletries without Moringa leaves has the ability to inhibit microorganisms due to the chemicals contained in its composition.

Martha (2017) in her research on the Effectiveness of Moringa Leaf Extract as a Hand Bio-Sanitizer and Lettuce Leaf (*Lactuca sativa*) obtained the following data:

Table 3. Effect of Maceration Concentration of Moringa Leaves on Reduction of *Staphylococcus aureus* Bacteria (%) on Hands (Source: Martha, 2017)

Application	Moringa Leaf Maceration Concentration				Control
	100% b/v	80% b/v	60% b/v	40% b/v	
Hand	$70,14^{a}$	$38,75^{ac}$	$36,75^{bc}$	$15,55^{b}$	$38,88^{bc}$

Based on the tests that have been carried out on MSA medium regarding the effect of maceration concentration of Moringa leaves on the reduction of bacteria (%) *Staphylococcus aureus* on the hands of the research object, the results were significantly different. Maceration of Moringa leaves with a concentration of 40% gave the lowest yield of 15.55%. The maceration concentration of Moringa leaves 100% gave the best reduction result of 70.14%. The maceration concentration of Moringa leaves 80%, 60%, 40%, and control were not significantly different but significantly different with 100% concentration.

Based on research conducted by Hudaya (2010), regarding the antibacterial water extract of kecombrang flowers that can inhibit *Staphylococcus aureus* bacteria with a concentration of 20% while Escherichia coli bacteria with a concentration of 60%. This is because the bacteria used in the study were different. *Staphylococcus aureus* is a Gram positive bacterium that has a more complex peptidoglycan structure and lower lipid content, while *Escherichia coli* is a gram negative bacterium that has less peptidoglycan content and more lipid content, so that the cell wall of *Staphylococcus aureus* is more easily damaged by the active compounds of the extract. kecombrang flower water from *Escherichia coli*.

The mechanism of microorganism reduction using maceration of Moringa leaves is caused by the presence of phytochemicals that act as antibacterial, namely flavonoids, tannins, saponins, and polyphenols with a bacterial inhibition mechanism. Flavonoid compounds are one of the chemical compounds in Moringa leaves that are bacteriostatic. The mechanism of action is by denaturing bacterial cell proteins and damaging the cytoplasmic membrane (Posangi et al., 2011).

Tannins inhibit the growth of bacteria by shrinking the cell wall so that cell permeability is disturbed. Cells cannot carry out living activities and their growth becomes inhibited or dies, if cell permeability is disturbed. Tannins can inhibit the growth of bacteria by precipitation of protein because tannins are thought to have the same effect as phenolic compounds as antibacterial. The antibacterial effect of tannin compounds is through reactions with cell membranes, inactivating enzymes, and destroying the function of genetic material.

Saponins are antibacterial by damaging cell membranes. Damage to the cell membrane can cause important substances to leave the cell and prevent important materials from entering the cell. If the function of the cell membrane is damaged, it can cause cell death. Saponins are polar compounds whose presence in plants can be extracted with polar or semipolar solvents (Lehninger, 2008).

Polyphenols inhibit bacteria by poisoning the protoplasm, penetrating and damaging cell walls, causing cell leakage and by precipitating bacterial cell proteins at high concentrations while at low concentrations can inhibit enzyme synthesis. Polyphenol compounds are able to break the peptidoglycan cross-links to penetrate the cell wall. Polyphenol compounds can cause cell nutrient leakage by damaging the hydrophobic bonds that make up cell membranes such as proteins and phospholipids. Damage to cell membranes can cause the activity and biosynthesis of specific enzymes to be inhibited for metabolic reactions (Lehninger, 2008).

Saponification material (saponification reaction) has very contextual characteristics, the use of which is very closely related to people's daily lives. Because this material is used as a research topic in schools, the lessons that should be delivered in the even semester of class XII at the high school education unit level, are drawn in the odd semester of class XI as a slice of hydrocarbon and petroleum material. In addition, innovation to overcome environmental problems due to the use of soap, has long been carried out at SMA Negeri 2 Padalarang, through the SMAWA Toiletris Production Unit, for the manufacture of environmentally friendly soap, with soap raw

materials that are relatively safe for the body and the environment, and become one of the programs school excellence.

On this occasion, through the theme of sustainable use of tropical biodiversity, the author plans to make other innovations in the manufacture of handwashing soap using Moringa leaves which are widely planted by the community around the school, but its use is still very lacking, as one of the raw materials. soap making. Moringa leaf processing is the first and decisive step in the quality of Moringa-based products. To extract Moringa leaves, maceration can be carried out using water solvents that are applicable to the community and can be made with simple equipment without having to be in a laboratory or industry.

The relevant previous studies are as follows:

1. Anwar S.dkk. (2014). In the toxicity test of extracts of distilled water (room temperature) and hot aquadest (70°C) of Moringa leaves (*Moringa oleifera Lamk*.) on *Artemia salina* shrimp larvae, it was stated that Moringa Leaf Extraction can be carried out by maceration method, which is a simple way to extract phytochemical compounds that found in plants.
2. Sally, et al (2014). in Harvesting time and temperature relationship with antimicrobial activity of *Moringa oleifera Lam (dum stick*), stated that: Moringa leaves contain active ingredients such as flavonoids, saponins, tannins, and polyphenols. The active ingredient in Moringa leaves can function as an antimicrobial.
3. Aguinaldo, A.M. (2007). in Selected *Zingiberaceae* Species Exhibiting Inhibitory Activity Against *Mycobacterium tuberculosis* H37Rv: Phytochemical Profile, states that phytochemicals in plants are divided into primary and secondary metabolites. Secondary metabolites such as flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids are produced in small quantities but have important significance in plants that function as antimicrobials produced by plants.
4. Martha (2017) in the Effectiveness of Moringa Leaf Extract as a Hand Bio-Sanitizer and Lettuce (*Lactuca sativa*) obtained the results that the effect of maceration concentration of Moringa leaves on the reduction of *Staphylococcus aureus* bacteria (%) on the hands of the object of research on the medium concentration of Moringa leaf maceration 100 % gives the best reduction result that is equal to 70.14%.

1.2. Aim

1. To determine the antibacterial activity of Moringa leaf extract against *Staphylococcus aureus* and *Escherichia coli* bacteria.
2. Knowing the concentration of Moringa leaf extract which has the greatest inhibition against *Staphylococcus aureus* and *Escherichia coli* bacteria
3. Comparing the growth inhibition of *Staphylococcus aureus* and *Escherichia coli* bacteria at handwash of Moringa leaves with the growth inhibition of *Staphylococcus aureus* and *Escherichia coli* bacteria produced by SMAWA toiletries without Moringa leaves.
4. Cultivate students' creativity in creating designs, methods, and prototypes for the installation of handwashing soap (handwash) made from Moringa leaves that are safe, inexpensive and friendly to organs, especially the skin and the environment.

1.3. Expected results

1. Making Moringa leaf natural ingredients as an alternative natural antibacterial substitute for synthetic materials in the manufacture of liquid hand soap (handwash).
2. The growth of scientific ethos among students so that they are not only consumers of knowledge, but are also capable of becoming producers of scientific thinkers and producers of innovative products.

2. The benefits and importance of conducting research

The benefits of this research are expected to be able to contribute to the world of education and the wider community, including:

1. Development of knowledge in the use of Moringa leaves as antibacterial soap that is friendly to the body and the environment, in order to improve health services for school residents, can also be used as a source of information to the public about the use of Moringa leaves as an anti-bacterial.
2. Developing the ability of students to design and produce alternative products that have potential, have higher practical and economic values, which can be used by the wider community as a form of awareness to participate in protecting the environment.

3. Adding skills and creating an entrepreneurial spirit based on science and technology in order to make the JUS (School Age skipper) program a success, which is the flagship program of SMA Negeri 2 Padalarang as a reference school.
4. This research can be used as a consideration or reference to conduct other similar research.

3. Methodology

The type of research used in this study is experimental research, which was conducted at SMA Negeri 2 Padalarang and SMK N 13 Kimia Analyst Bandung, involving 10 students who are members of the Research and Development (RnD) team of SMA Negeri 2 Padalarang.

The study used a completely randomized design the effect of concentration of Moringa leaf extract from powder and from dried leaves in various concentrations %: 2.5%, 5%; 10%; 20%; 40%, and 80% w/v were then compared with other soaps that did not contain moringa as a control. Each treatment was repeated 3-4 times. The stages of this research include:

A. Extraction of phytochemical compounds in Moringa leaves

The highest concentration of phytochemical compounds was found in young Moringa leaves, because the concentration and composition of plant chemical and phytochemical compounds changed during the growth process. The extraction steps are as follows:

1. Making Moringa leaf powder, the leaves are dried using an oven at 40 C until the water content is less than 10%, then
2. Blended and sieved with a 60 mesh sieve with the aim of reducing the surface area so that the material in the powder can be more easily extracted. The extraction process lasts for approximately 24 hours, after that
3. The extract obtained in a water bath at a temperature of 70 C, for 2 hours so that the active compound can be extracted optimally (Anwar, 2016).

B. Qualitative test of phytochemical compound content

1. Saponin test

   0.5 grams of Moringa extract, put in a test tube, added 10 ml of hot water and shaken for 10 minutes, until foam or more is formed and then dripped with 2N HCl, if the foam does not disappear with the addition of 2N HCl then the extraction is positive for saponins

2. Tannin Test

The extract was put in a test tube, added 10 ml of hot water and shaken, then added 20 ml of 10% NaCl and filtered. The resulting filtrate is added with FeCl3 and if there is a dark blue or black color change, it is positive that it contains tannins

3. Flavonoid Test

   The extract was weighed as much as 0.5 grams added with ethanol. Then 5-6 drops of concentrated HCl are added, forming a red color which indicates the presence of flavonoids

4. Polyphenol Test

   The test extract solution as much as 1mL was reacted with 10% FeCl3 solution, if dark blue, blackish blue or greenish black color occurred, it indicated the presence of polyphenol compounds.

C. Qualitative phytochemical test on the recipe for handwashing liquid (handwash) with the active ingredient of Moringa leaves, in the same way in step B.

D. Quantitative tests were carried out to analyze Tannin levels in Moringa extract and Moringa leaf soap with various concentrations, using High Performance Liquid Chromatography (HPLC). The working principle of HPLC is as follows: the liquid mobile phase is flowed through the column to the detector with the help of a pump. The sample is introduced into the mobile phase stream by injection. In the column there is a separation of the components of the mixture. Due to the difference in the strength of the interaction between the solutes and the stationary phase.

E. The inhibition of growth of *Staphylococcus aureus* and *Escherichia coli* (w/v%) bacteria at the maceration concentration of Moringa leaves on students' hands was compared with the growth inhibition of Staphylococcus aureus (w/v%) liquid handwashing soap without Moringa leaves by using the diffusion method so that the wells , and the results of the measurement of the average diameter of the inhibition zone of Moringa leaf extract against Staphylococcus and *Escherichia coli* bacteria. The steps of the bacterial inhibition test are as follows:

1. Equipment Sterilization

   The tools used in this antibacterial activity study were sterilized first. Glass utensils and media are sterilized in an autoclave at 121^{o}C for 15-20 minutes, while for ose needles and tweezers are sterilized by burning over a direct fire using a spirit.

2. Preparation of Negative Control Solution

   The negative control solution was made from hand washing soap without Moringa according to the formula dose by dissolving NaCl/salt into water in a ratio of 2:3, SLS, Na_2SO_4 , EDTA, mixed in a small basin, then stirred until homogeneous, then add salt water and stir mix well until homogeneous, enter the ckp foam mix well until homogeneous, then add water while stirring well (do the same thing until the water runs out/liquid thickened soap). The last step is to add the BKC solution, glycerin, PG, and essens oil, mix well until homogeneous, and dissolve the dye in enough water, then pour it into the dough to taste, stir until homogeneous.Positive Control Creation

   Positive control solution was made from 3 preparations, namely Ciprofloxacin 500 mkg tablet. One Ciprofloxacin tablet was crushed, then weighed and equalized with 500 Mg, 70% alcohol solution, and 6N CuSO4.

3. Preparation of Test Solution

   The test solution was prepared with w/v% ie test 1: 2.5%, 5%; 10%; 20%; 40%, and 80% w/v, and test 2: 2,5%, 5%; 7,5%; 10%; 12,5%, dan 15% b/v by weighing of Moringa leaf extract.

4. Making Agar Media

   A total of 0.4 grams of Nutrient Agar (NA) was dissolved in 20 ml of distilled water using an erlenmeyer. After that it is homogenized with a stirrer over a water bath until it boils. A total of 5 ml each was poured into 2 sterile test tubes and covered with aluminum foil. The media was sterilized in an autoclave at 121^oC for 15 minutes, then left at room temperature for ± 30 minutes until the media solidified. Agar medium is used for inoculation of bacteria.

5. Inoculation of Bacteria on Agar Media

   The test bacteria were taken with a sterile ose needle, then implanted on the agar medium by scraping. It was then incubated in an incubator at 37^oC for 24 hours.

6. Preparation of Standard for Turbidity Solution (Mc. Farland's Solution)

   99.5 ml of 0.36 N H_2SO_4 solution was mixed with 0.5 ml of 1.175% $BaCl_2.2H_2O$ solution in an erlenmeyer. Then shaken until a cloudy solution is formed. This turbidity is used as a standard for the turbidity of the test bacterial suspension).

7. Preparation of Test Bacteria Suspension

   The inoculated test bacteria were taken with sterile ose wire and then suspended into a tube containing 2 ml of 0.9% NaCl solution until the turbidity was the same as the standard turbidity of Mc. Farland.

8. Making Media Testing Media

   The test was made using the agar diffusion method by pouring 10 mL of NA into 6 petri dishes for the base layer after the first layer had solidified, after which a well was made.

9. Bacterial suspension is inserted in the well

10. Observations were made after 1x24 hours of incubation.

    The clear area is an indication of the sensitivity of bacteria to the antibacterial material used as a test material which is expressed by the width of the inhibition zone diameter. The diameter of the inhibition zone was measured in millimeters (mm) using a caliper by means of the overall diameter minus the well diameter of 7 mm. Then the diameter of the inhibition zone was categorized for its antibacterial power based on the Davis and Stout classification.

4. Hasil dan Pembahasan

A. Extraction of Phytochemical Compounds in Moringa Leaves

The highest concentration of phytochemical compounds was found in young Moringa leaves, because the concentration and composition of plant chemical and phytochemical compounds changed during the growth process. Phytochemical compounds were extracted from dried leaves and Moringa powder.

The extraction steps are carried out as follows:

1. Drying Moringa leaves in the oven

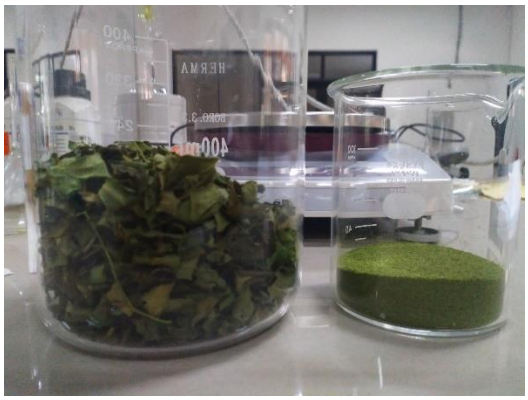


Figure 1. Moringa leaves and dried Moringa powder

2. Making Moringa leaf powder, the leaves are dried using an oven at 40 C until the water content is less than 10%, then
3. Blended and sieved with a 60 mesh sieve with the aim of reducing the surface area so that the material in the powder can be more easily extracted. The extraction process lasts for approximately 2x24 hours, in order to obtain the maximum extract, after that the extract obtained in a water bath at a temperature of 70 C, for 6 hours so that the active compound can be extracted optimally with 98% ethanol solvent.

Figure 2. Moringa Leaf and Powder Soxhlet Tool Set

Figure 3. Waterbathed Moringa powder extract (Left) and leaves (right)

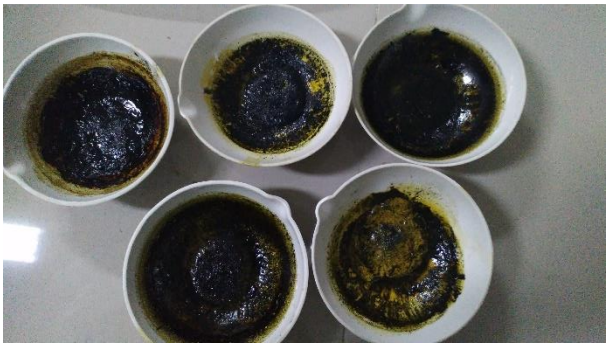


Figure 4. Results of 5 sets of Moringa powder soxhlation @ 10 grams

B. Qualitative test of the content of phytochemical compounds in Moringa leaves.

1. Saponin test

   Weighed 0.5 grams of extract, put in a test tube, added 10 ml of hot water and shaken for 10 minutes, until foam or more was formed and then dripped with 2N HCl, if the foam did not disappear with the addition of 2N HCl then the extraction was positive for saponins.

Figure 5. Saponin test results

2. Tannin test

   The extract was put in a test tube, added 10 ml of hot water and shaken, then added 20 ml of 10% NaCl and filtered. The resulting filtrate is added with FeCl3 and if there is a dark blue or black color change, it is positive that it contains tannins

Figure 6. Tannin Test Results

3. Flavonoid test

The extract was weighed as much as 0.5 grams added with ethanol. Then 5-6 drops of concentrated HCl are added, forming a red color which indicates the presence of flavonoids and the formation of an orange color indicating the presence of flavone compounds (Tiwari et al, 2011).

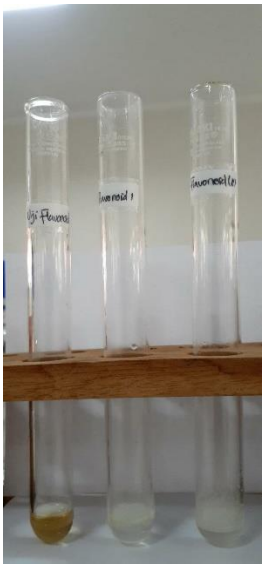


Figure 7. Flavonoid Test Results

4. Polyphenol test

1 ml of the test extract solution was reacted with 10% FeCl3 solution, if dark blue, blackish blue or greenish black color occurred, it indicated the presence of polyphenolic compounds.

Figure 8. Polyphenol . Test Results

Table 4. Phytochemical Test Results of Moringa Leaf Extract

Compound	Positive Results	Test results	Information
Flavonoids	Yellow	Light yellow	+
Saponins	Stable foam (< 7 minutes)	Stable foam (< 7 minutes)	+++
Tannins	Blackish green	Blackish green	++++
Polyphenol	Dark green color	Dark green color	++++

Description: + less clear; ++ is rather self explanatory; +++ clear; ++++ very clear

Table 5. Phytochemical Test Results of Moringa Powder Extract

Compound	Positive Results	Test results	Information
Flavonoids	Yellow	Light yellow	++
Saponins	Stable foam (< 7 minutes)	Stable foam (< 7 minutes)	++++
Tannins	Blackish green	Blackish green	++++
Polyphenol	Dark green color	Dark green color	++++

Description: + less clear; ++ is rather self explanatory; +++ clear; ++++ very clear

C. Qualitative phytochemical test on liquid handwash recipe with active ingredients of Moringa leaves, in the same way in step B. Shows the same observation results as in step A.

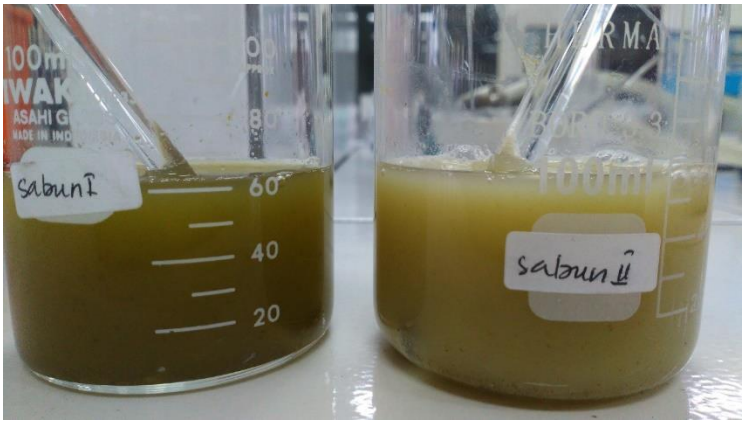
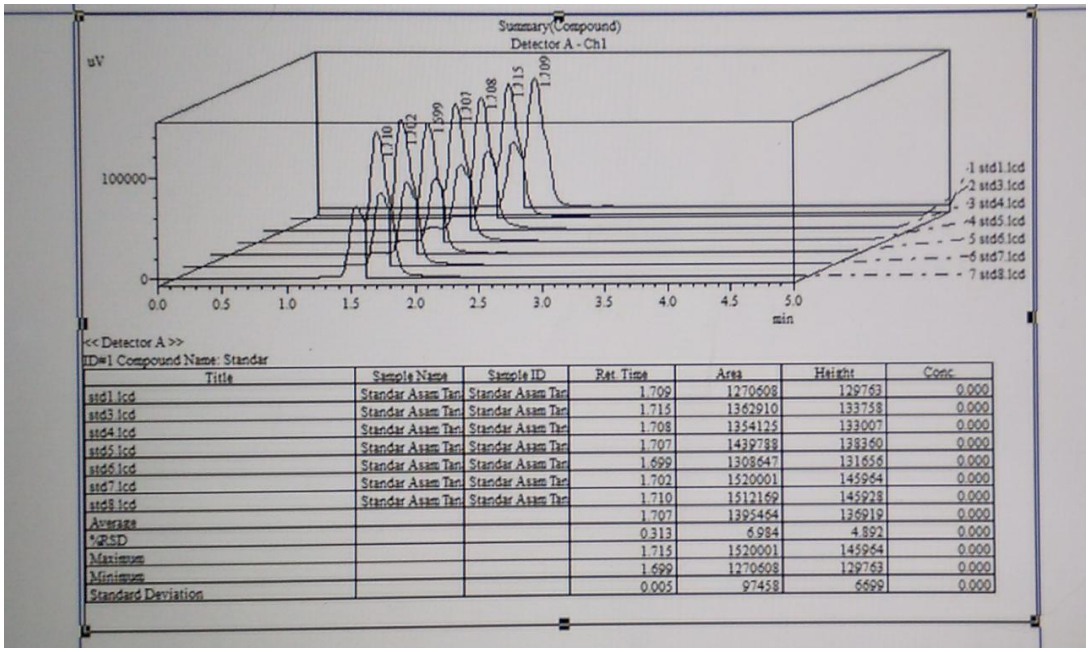


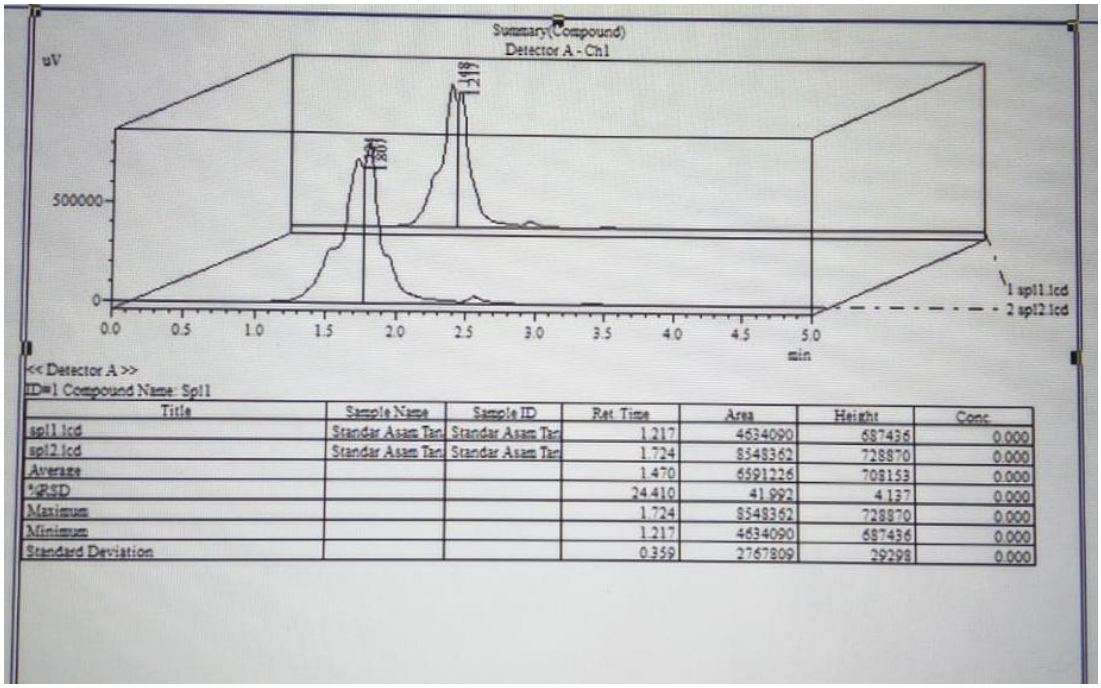
Figure 9. Soap Solution Formula for Qualitative Test

D. Quantitative tests were carried out to analyze the tannin levels in Moringa extract and Moringa leaf soap with several variations in concentration for test 1, using Chromatography High Performance Fluids (HPLC) obtained the following data:



Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
std1.lcd	Standar Asam Tan	Standar Asam Tan	1.709	1270608	129763	0.000
std3.lcd	Standar Asam Tan	Standar Asam Tan	1.715	1362910	133758	0.000
std4.lcd	Standar Asam Tan	Standar Asam Tan	1.708	1354125	133007	0.000
std5.lcd	Standar Asam Tan	Standar Asam Tan	1.707	1439788	138350	0.000
std6.lcd	Standar Asam Tan	Standar Asam Tan	1.699	1308647	131656	0.000
std7.lcd	Standar Asam Tan	Standar Asam Tan	1.702	1520001	145964	0.000
std8.lcd	Standar Asam Tan	Standar Asam Tan	1.710	1512169	145928	0.000
Average			1.707	1395464	136919	0.000
%RSD			0.313	6.984	4.892	0.000
Maximum			1.715	1520001	145964	0.000
Minimum			1.699	1270608	129763	0.000
Standard Deviation			0.005	97458	6699	0.000

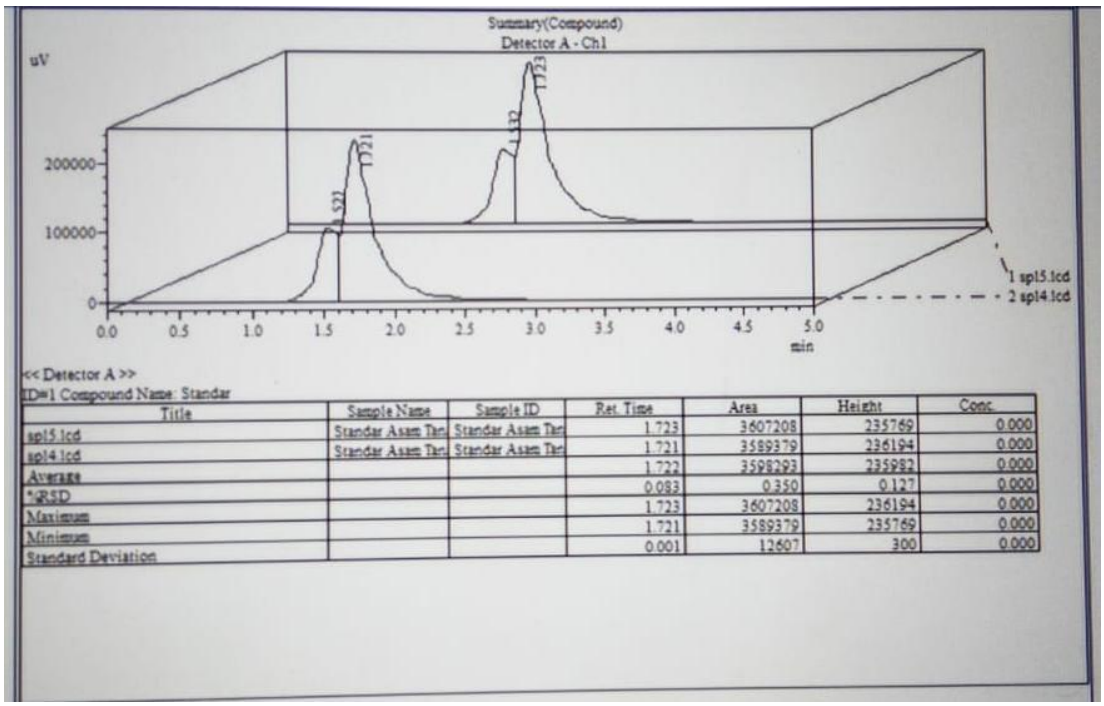
Figure 10. Tannin Standard



<< Detector A >>
ID#1 Compound Name: Spl1

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
spl1.lcd	Standar Asam Tart	Standar Asam Tart	1.217	4634090	687436	0.000
spl2.lcd	Standar Asam Tart	Standar Asam Tart	1.724	8548362	728870	0.000
Average			1.470	6591226	708153	0.000
%RSD			24.410	41.992	4.137	0.000
Maximum			1.724	8548362	728870	0.000
Minimum			1.217	4634090	687436	0.000
Standard Deviation			0.359	2767809	29298	0.000

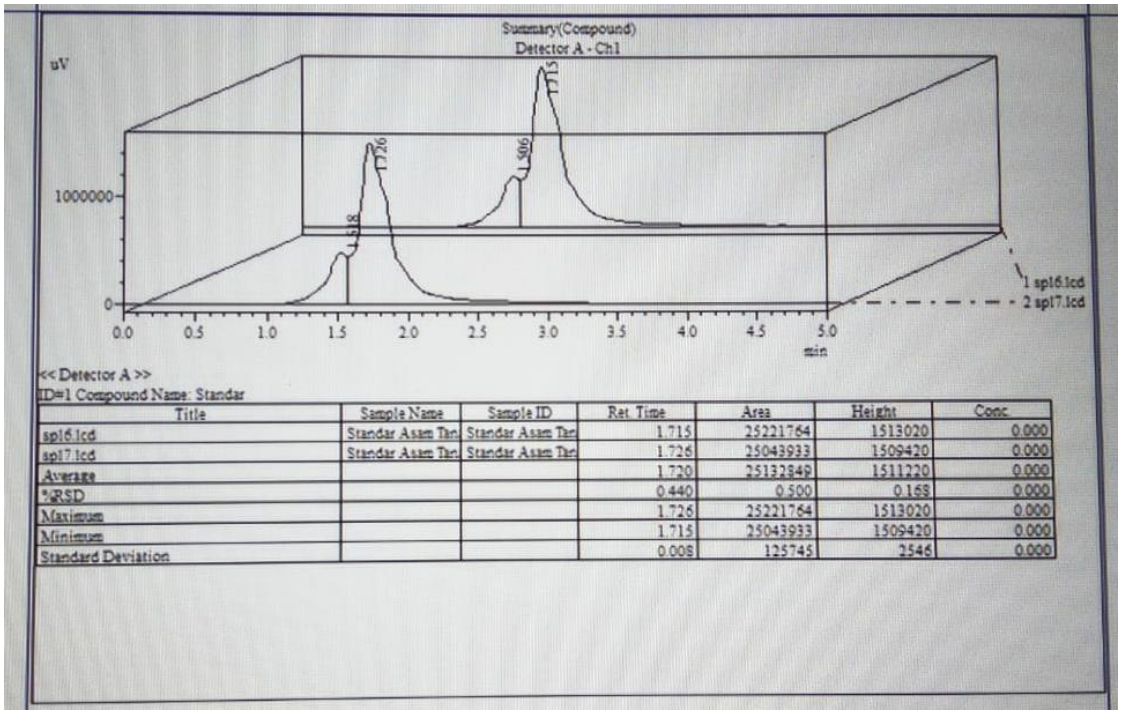
Figure 11. Sample 1 Moringa Concentration 20%



<< Detector A >>
ID#1 Compound Name: Standar

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
spl5.lcd	Standar Asam Tart	Standar Asam Tart	1.723	3607208	235769	0.000
spl4.lcd	Standar Asam Tart	Standar Asam Tart	1.721	3589379	236194	0.000
Average			1.722	3598293	235982	0.000
%RSD			0.083	0.350	0.127	0.000
Maximum			1.723	3607208	236194	0.000
Minimum			1.721	3589379	235769	0.000
Standard Deviation			0.001	12607	300	0.000

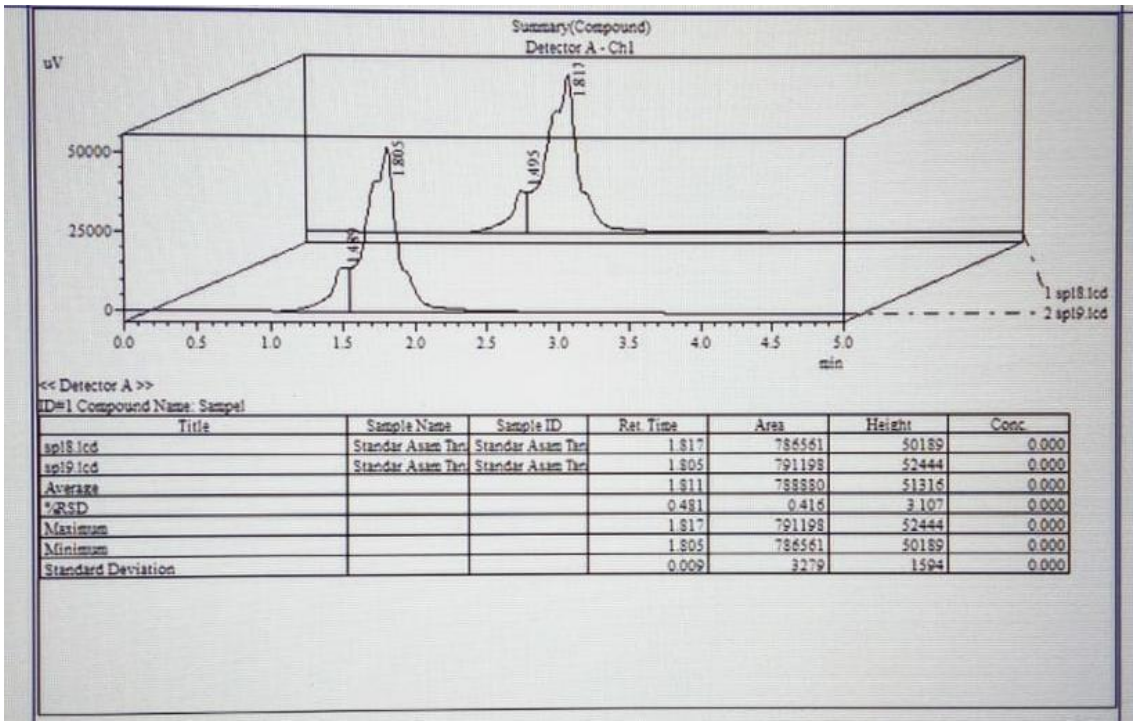
Figure 12. Sample 2 Moringa Concentration 10%



<< Detector A >>
ID#1 Compound Name: Standar

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
spl6.lcd	Standar Asam Tar	Standar Asam Tar	1.715	25221764	1513020	0.000
spl7.lcd	Standar Asam Tar	Standar Asam Tar	1.726	25043933	1509420	0.000
Average			1.720	25132849	1511220	0.000
%RSD			0.440	0.500	0.168	0.000
Maximum			1.726	25221764	1513020	0.000
Minimum			1.715	25043933	1509420	0.000
Standard Deviation			0.008	125745	2546	0.000

Figure 13 Sample 3 Moringa Concentration 40%



<< Detector A >>
ID#1 Compound Name: Sampel

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
spl8.lcd	Standar Asam Tar	Standar Asam Tar	1.817	786561	50189	0.000
spl9.lcd	Standar Asam Tar	Standar Asam Tar	1.805	791198	52444	0.000
Average			1.811	788880	51316	0.000
%RSD			0.481	0.416	3.107	0.000
Maximum			1.817	791198	52444	0.000
Minimum			1.805	786561	50189	0.000
Standard Deviation			0.009	3279	1594	0.000

Figure 14. Sample 4 Moringa Concentration 2,5%

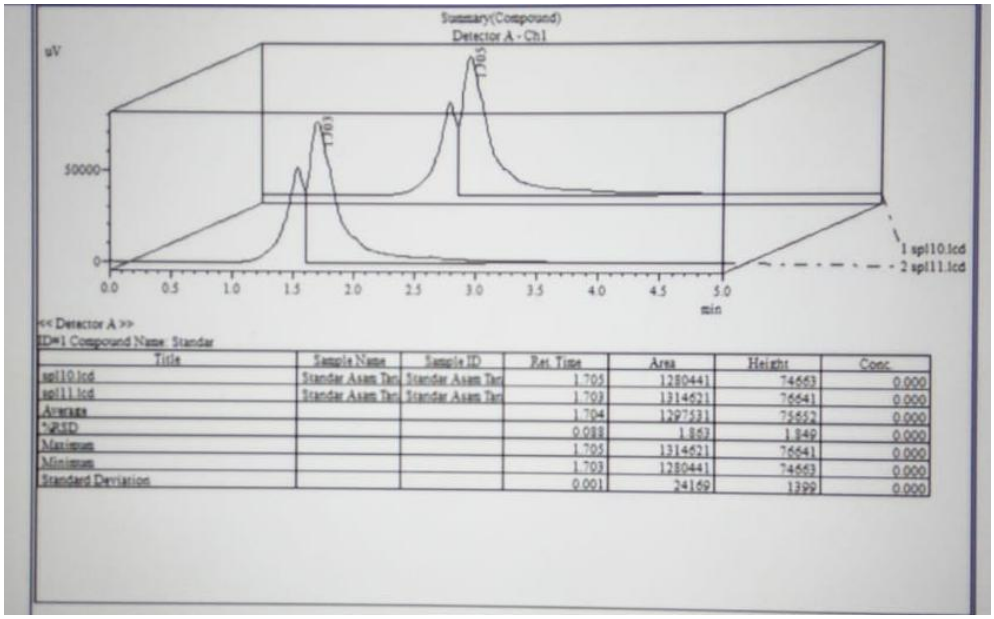


Figure 15. Sample 5 Moringa Concentration 5%

E. To test the growth inhibition of *Staphylococcus aureus* and *E.Coli* bacteria at the maceration concentration of Moringa leaves in Moringa soap and 2 samples of Moringa extract from dried leaves and powder, compared with the growth inhibition of *Staphylococcus aureus* and *E.Coli* liquid hand washing soap without leaves Moringa leaf extract using the diffuse agar agar pitting method, and the results of measuring the average diameter of the inhibition zone of Moringa leaf extract against *Staphylococcus* and *E.coli* bacteria.

The steps of the bacterial inhibition test are as follows:

1. Equipment Sterilization

   The tools used in this antibacterial activity study were sterilized first. Glass utensils and media are sterilized in an autoclave at 121oC for 15-20 minutes, while for ose needles and tweezers are sterilized by burning over a direct fire using a spirit.

Figure 16. Sterilization of tools in an autoclave

2. Preparation of Negative Control Solution

   The negative control solution was prepared by making a soap formula without Moringa leaves.

3. Positive Control Creation

   Positive control solution was made from 3 preparations, namely Ciprofloxacin 500 mkg tablet. One Ciprofloxacin tablet was crushed, then weighed and equalized with 500 Mg, 70% alcohol solution, and 6N CuSO4 solution.

Figure 17. Positive Control Solution

4. Preparation of Test Solution

   - Test Solution 1 was prepared with w/v%: 2.5%: 5%; 10%; 20%; 40%: and 80% w/v by weighing 0.025g: 0.05g; 0.1 g; 0.2 g; 0.4 g; and 0.8g of Moringa leaf extract and Test solution then each dissolved in 10 ml of soap solution

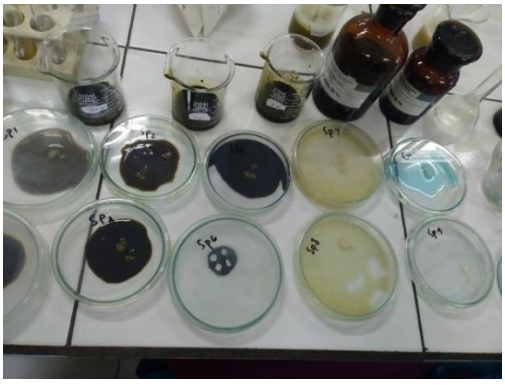


Figure 18. Test Solution Moringa Extract Soap, Positive Test Solution, and Negative Test Solution

- Test Solution 2 was prepared with w/v%: 2.5%, 5%; 7.5%; 10%; 12.5%; and 15% w/v by weighing 0.025, 0.05 g; 0.075 g; 0.1 g; 0.125 g; and 0.150 g of Moringa leaf powder and Test solution then each dissolved in 10 ml of soap solution.

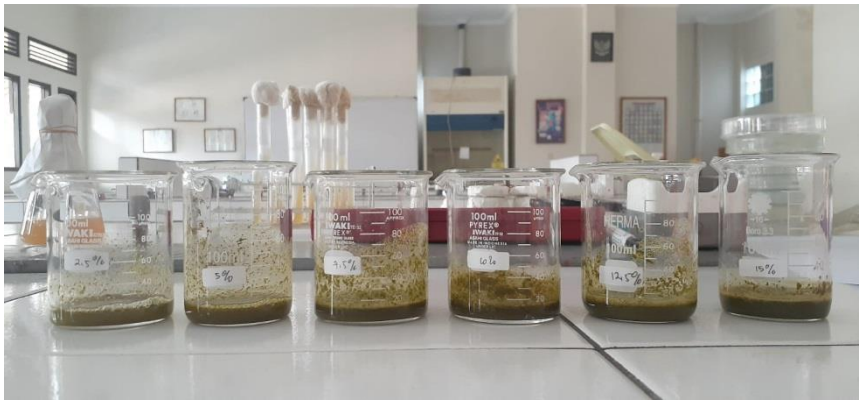


Figure19. Moringa Powder Soap Test Solution

5. Making Agar Media

A total of 0.4 grams of Nutrient Agar (NA) was dissolved in 20 ml of distilled water using an erlenmeyer. After that it is homogenized with a stirrer over a water bath until it boils. A total of 5 ml each was poured into 2 sterile test tubes and covered with aluminum foil. The media was sterilized in an autoclave at 121oC for 15 minutes, then left at room temperature for ± 30 minutes until the media solidified. Agar medium is used for inoculation of bacteria.

Figure 20. Nutrient Agar Media

6. Inoculation of Bacteria on Agar Media

The test bacteria were taken with a sterile ose needle, then implanted on the agar medium by scraping. It was then incubated in an incubator at 370C for 2x24 hours.

Figure 21. Pouring Bacteria into petri dish

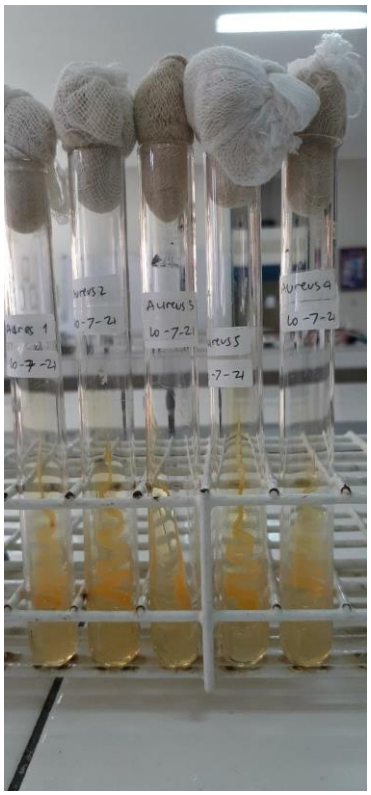


Figure 22. Culture of S. aureus

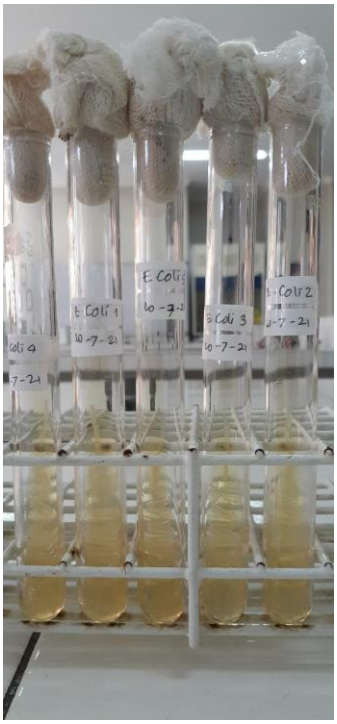


Figure 23. Culture of E.Coli

7. Preparation of Standard for Turbidity of Solution (Mc. Farland's Solution)

   99.5 ml of 0.36 N H_2SO_4 solution was mixed with 0.5 ml of 1.175% $BaCl_2.2H_2O$ solution in an erlenmeyer. Then shaken until a cloudy solution is formed. This turbidity is used as a standard for the turbidity of the test bacterial suspension).

Figure 24. Mc.Farland Solution

8. Preparation of Test Bacteria Suspension

The inoculated test bacteria were taken with sterile ose wire and then suspended into a tube containing 2 ml of 0.9% NaCl solution until the turbidity was the same as the standard turbidity of Mc. Farland.

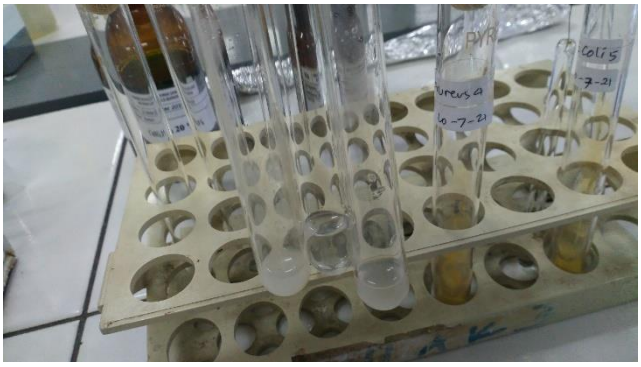


Figure 25. Test Bacterial Suspension

9. Making Media Testing Media

The test was made using the agar diffusion method by pouring 10 mL NA into 6 petri dishes for the base layer after the first layer had solidified, after which a well was made.

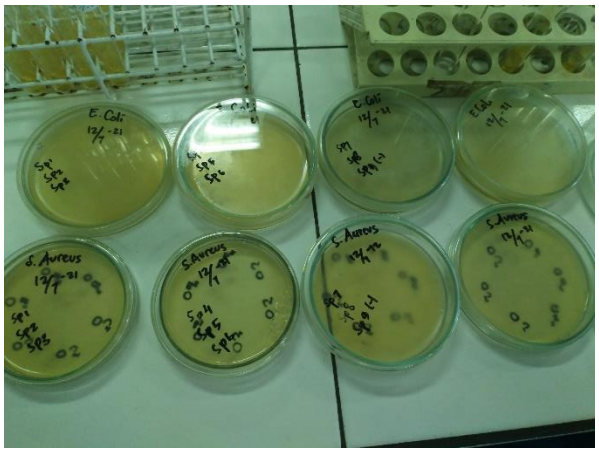


Figure 26. Media for S. aureus and E. coli . bacteria

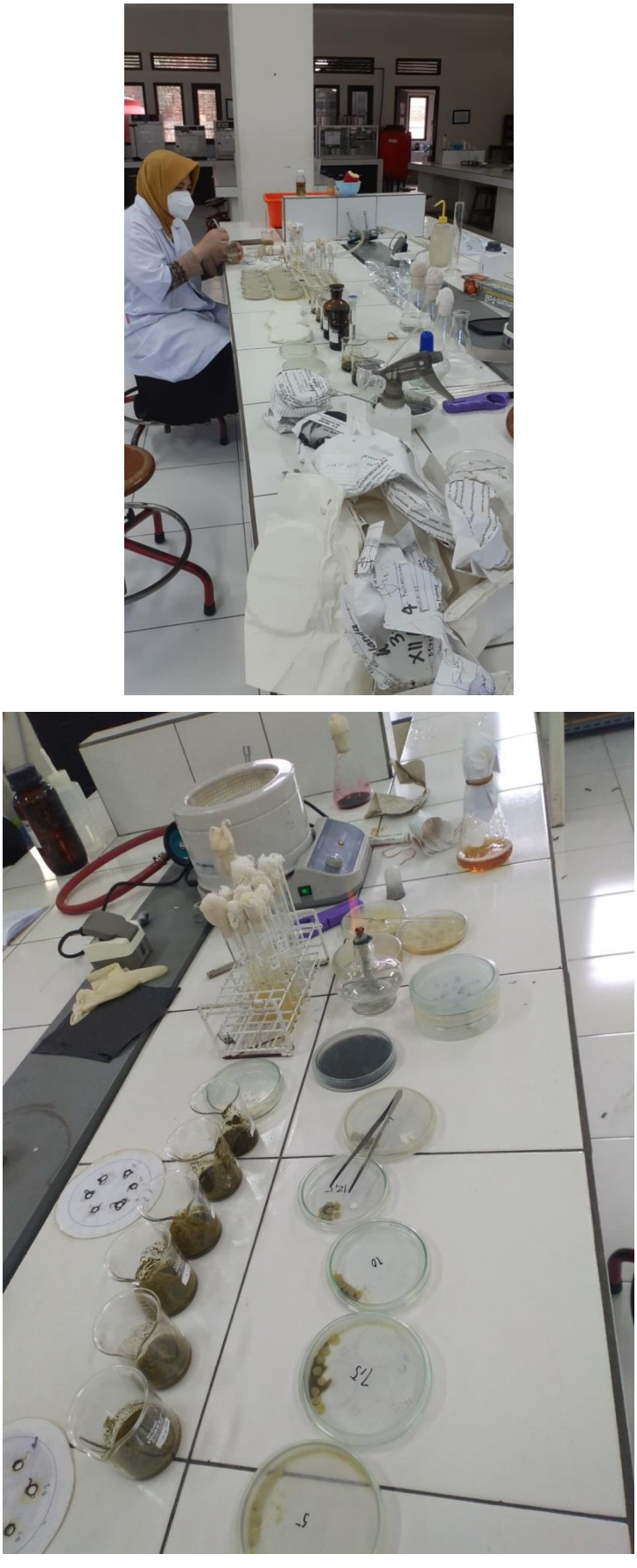


Figure 27. Preparation of Bacterial Media in Petri dishes

10. Bacterial suspension is inserted into the well

Figure 28. Bacterial suspension is inserted in the well

11. Observations were made after 2x24 hours of incubation.

Figure 29. Incubation of bacteria in an incubator at 37 ^{o}C

The clear area is an indication of the sensitivity of bacteria to the antibacterial material used as a test material which is expressed by the width of the inhibition zone diameter. The diameter of the inhibition zone was measured in millimeters (mm) using a scale ruler by means of the overall diameter minus

the well diameter of 7 mm. Then the diameter of the inhibition zone was categorized for its antibacterial power based on the Davis and Stout classification.

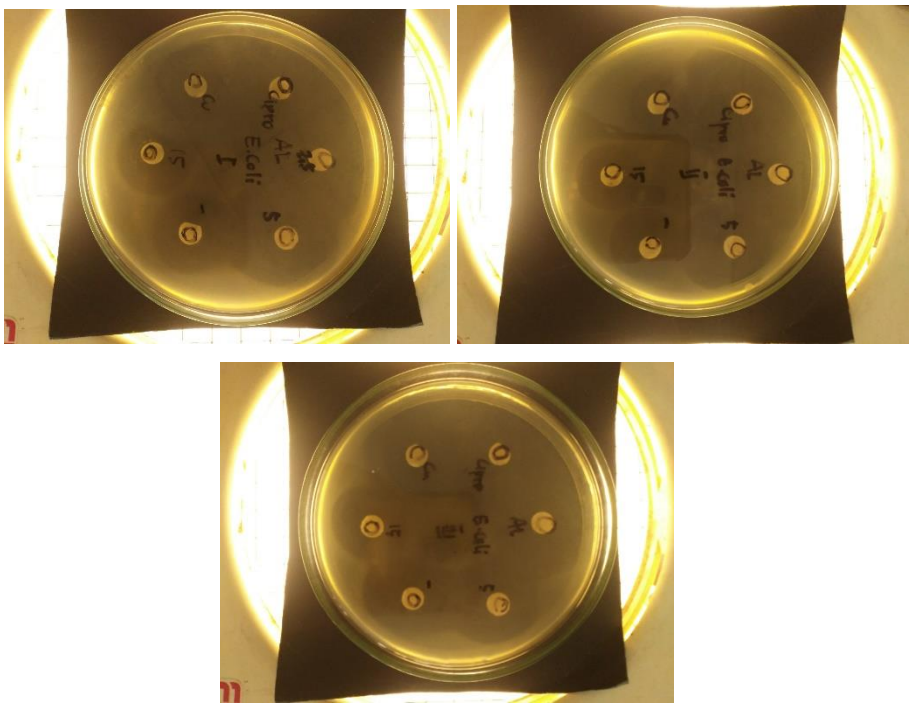


Figure 30. E.Coli Bacteria Incubation Results (3x Measurements)

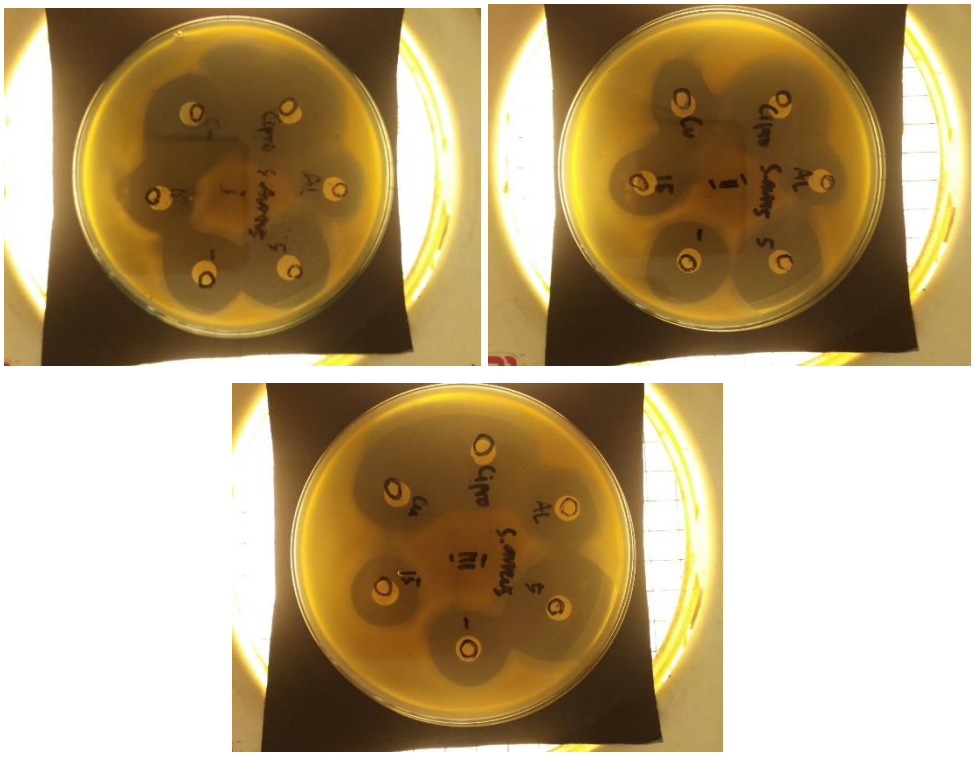


Figure 31. Results of S. aureus Bacteria Inoculation (3x Measurement)

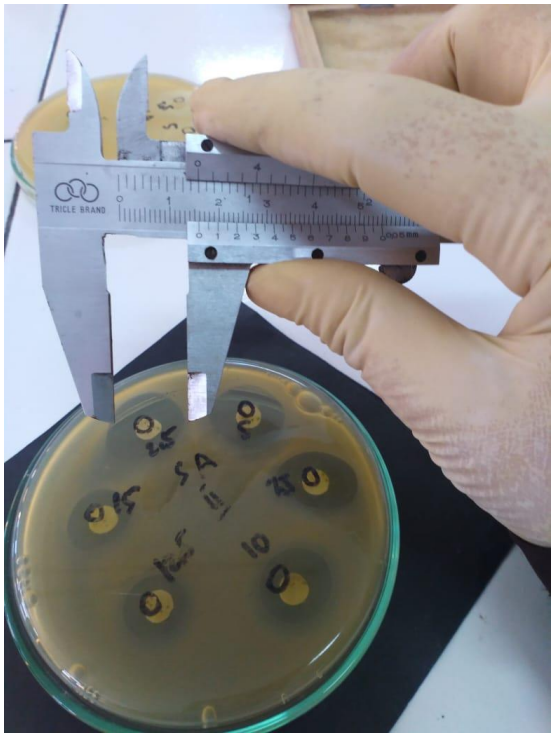


Figure 32. Measurement of Bacterial Inhibitory Diameter using a caliper

Based on research that has been done, it is proven that Moringa leaf extract has antibacterial activity against *Staphylococcus aureus* and *E.Coli* bacteria. This can be seen from the formation of the inhibition zone. Moringa leaves have secondary metabolites such as flavonoids, alkaloids, phenols which can also inhibit bacterial activity. Flavonoid compounds have an antibacterial effect. There are three kinds of flavonoid antibacterial mechanisms, namely by inhibiting energy metabolism, inhibiting nucleic acid synthesis, and inhibiting cell membrane function. Flavonoids can inhibit the movement of bacteria and prevent the formation of energy in the bacterial cytoplasmic membrane. Flavonoids are able to inhibit the use of oxygen by bacteria, causing inhibition of energy metabolism. Flavonoid compounds are one of the chemical compounds in Moringa leaves that are bacteriostatic. Flavonoids can denature bacterial cell proteins and damage the cytoplasmic membrane. Flavonoids can also damage the cytoplasmic membrane causing the release of important metabolites and the bacterial enzyme system to become inactive. This situation can cause bacterial death because the release of nucleotides and amino acids can prevent the entry of active ingredients into bacterial cells. The destruction of the cytoplasmic membrane, H^+ ions from phenol compounds and their derivatives will attack the polar group (phosphate group) which causes the phospholipid molecule to break down into

glycerol, carboxylic acid, and phosphoric acid. This causes the phospholipids to be unable to maintain the shape of the cytoplasmic membrane so that the cytoplasmic membrane leaks and bacterial growth is inhibited or dead.

Tannins inhibit the growth of bacteria by shrinking the cell wall so that cell permeability is disturbed. Cells cannot carry out living activities and their growth becomes inhibited or dies, if cell permeability is disturbed. Tannins can inhibit the growth of bacteria by precipitation of protein because tannins are thought to have the same effect as phenolic compounds as antibacterial. The antibacterial effect of tannin compounds is through reactions with cell membranes, inactivating enzymes, and destroying the function of genetic material.

Saponins are antibacterial by damaging cell membranes. Damage to the cell membrane can cause important substances to leave the cell and prevent important materials from entering the cell. If the function of the cell membrane is damaged, it can cause cell death. Saponins are polar compounds whose presence in plants can be extracted with polar or semipolar solvents.

Polyphenols inhibit bacteria by poisoning the protoplasm, penetrating and damaging cell walls, causing cell leakage and by precipitating bacterial cell proteins at high concentrations while at low concentrations can inhibit enzyme synthesis. Polyphenol compounds are able to break the peptidoglycan cross-links to penetrate the cell wall. Polyphenol compounds can cause cell nutrient leakage by damaging the hydrophobic bonds that make up cell membranes such as proteins and phospholipids. Damage to cell membranes can cause the activity and biosynthesis of specific enzymes to be inhibited for metabolic reactions.

Table 6. Test 1. Results of Measurement of Inhibitory Zone Diameter of Moringa Leaf Extract Soap against *E. Coli*

Test	**Inhibition Zone of Moringa Extract Soap (mm)**									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**Kontrol (-)**	**Kontrol (+1)**	**Kontrol (+2)**	**Kontrol (+3)**
1	15,45	18,30	19,00	17,75	18,70	19,20	13,25	23,45	23,45	20,60
2	16,00	17,70	19,75	17,70	18,70	19,00	12,75	26,50	26,50	20,70
3	16,00	17,15	19,80	17,45	18,75	19,65	13,15	24,25	26,25	20,50

Table 7. Test 1. Results of Measurement of Inhibitory Zone Diameter of Moringa Leaf Extract Soap against *Staphylococcus Aureus*

Perc	Inhibition Zone of Moringa Extract Soap (mm)									
	F1	F2	F3	F4	F5	F6	Kontrol (-)	Kontrol (+1)	Kontrol (+2)	Kontrol (+3)
1	23,95	20,20	20,15	23,00	23,90	21,35	15,25	35,05	23,60	27,05
2	23,90	20,15	20,35	22,50	23,75	19,20	15,10	34,40	23,60	27,40
3	23,20	20,30	20,20	23,30	23,90	21,60	15,15	35,50	23,45	27,05

Information:

F1 : concentrated leaf extract soap 2.5%
F2 : concentrated leaf extract soap 5%
F3 : concentrated leaf extract soap 10%
F4 : concentrated leaf extract soap 20%
F5 : concentrated leaf extract soap 40%
F5 : concentrated leaf extract soap 80%

Control (-): soap without Moringa leaf extract
Control (+1): Ciprofloxacin Standard solution
Control (+2): 70% alcohol Standard solution
Control (+3): Standard solution of CuSO4, 6N

Table 8. Test 2. Results of Measurement of Inhibitory Zone Diameter of Moringa Leaf Extract Soap against *E. Coli*

Test	Inhibition Zone of Moringa Extract Soap (mm)									
	F1	F2	F3	F4	F5	F6	Kontrol (-)	Kontrol (+1)	Kontrol (+2)	Kontrol (+3)
1	15,45	18,30	19,00	17,75	18,70	19,20	13,25	23,45	23,45	20,60
2	16,00	17,70	19,75	17,70	18,70	19,00	12,75	26,50	26,50	20,70
3	16,00	17,15	19,80	17,45	18,75	19,65	13,15	24,25	26,25	20,50

Table 9. Test 2. Results of Measurement of Inhibitory Zone Diameter of Moringa Leaf Extract Soap against *Staphylococcus Aureus*

Perc	Inhibition Zone of Moringa Extract Soap (mm)									
	F1	F2	F3	F4	F5	F6	Kontrol (-)	Kontrol (+1)	Kontrol (+2)	Kontrol (+3)
1	23,95	20,20	20,15	23,00	23,90	21,35	15,25	35,05	23,60	27,05
2	23,90	20,15	20,35	22,50	23,75	19,20	15,10	34,40	23,60	27,40
3	23,20	20,30	20,20	23,30	23,90	21,60	15,15	35,50	23,45	27,05

Information:

F1 : concentrated leaf extract soap 2.5%
F2 : concentrated leaf extract soap 5%

Control (-): soap without Moringa leaf extract
Control (+1): Ciprofloxacin Standard solution

F3 : concentrated leaf extract soap 7,5%
F4 : concentrated leaf extract soap 10%
F5 : concentrated leaf extract soap 12,5%
F5 : concentrated leaf extract soap 15%

Control (+2): 70% alcohol Standard solution
Control (+3): Standard solution of CuSO4, 6N

The antibacterial test was carried out in the microbiology laboratory of SMKN 13 Bandung. The criteria for the strength of antibacterial activity according to Davis and Stout (1971) are categorized based on the diameter of the inhibition zone formed, namely the diameter of the inhibition zone of 5 mm or less is categorized as weak, the inhibition zone of 5-10 mm is categorized as moderate, the inhibition zone of 10-20 mm is categorized as strong and the inhibition zone is 20. mm or more is categorized as very strong (Dimpudus et al, 2017).

The antibacterial ability contained in the extracts and antibiotics indicates that the growth of bacteria can be prevented. In the test agar medium, the expansion of bacterial colonies will be blocked by compounds contained in the test or treatment material. After incubation, the zone of inhibition will be identified from the presence of a transparent area. This area shows that there is no bacterial colony (Ariza et al, 2014). In this study, a negative control was used, namely the preparation of liquid hand soap without the addition of Moringa extract (0 ml) and a positive control using an antibiotic solution of ciprofloxacin, 70% alcohol, and CuSO4 6N. The results obtained from the negative control were the formation of an inhibition zone against bacteria with a clear area around the well with a diameter of 12.75-15.25 mm (categorized as strong), this is because the basic ingredients for making soap have the ability to inhibit bacteria. SLES material (Sodium Lauret Ether Sulfate) functions as a surfactant that has a hydrophilic group and a lipophilic group, so that it is able to unite a mixture consisting of water and oil. In addition, SLES is a common and basic ingredient in the manufacture of various types of soap. Meanwhile, propylene glycol and glycerin have antibacterial properties. Propylene glycol is structurally and functionally similar to glucotine as a surfactant. Glycerin has antibacterial inhibition ability with levels of less than 20%, propylene glycol has preservative properties in preparations or semisolids with levels of 15-30% (Rowe et al., 2009 in Febrianti, 2013). The results obtained from the positive control were the formation of an inhibition zone against bacteria with a clear area around the well with a diameter of 20.50-35.50 mm (categorized as very strong), this indicates that ciprofloxacin as one

type of antibiotic, alcohol 70%, and CuSO4 are sensitive to various types of bacteria. Along with the increase in the concentration of Moringa extract in liquid hand soap preparations, the ability to inhibit bacteria also increases.

Based on the results of research conducted in triples, the antibacterial of Moringa leaf extract which can inhibit *Staphylococcus aureus* with a concentration of 2.5% has reached the optimum condition, while *Escherichia coli* bacteria with a concentration of 10% and 80%. This is because the bacteria used in the study were different. *Staphylococcus aureus* is a Gram positive bacterium that has a more complex peptidoglycan structure and a lower lipid content, while *Escherichia coli* is a Gram negative bacterium that has less peptidoglycan/thin content and more lipid content, so that the cell wall of *Staphylococcus aureus* is more easily damaged by compounds. active moringa leaf extract from *Escherichia coli*.

Quantitative test of measurement of tannin levels in each formula, using the HPLC method. Standard tannic acid, injected 7 times with an average area measurement of 1395464. Sample 4 with a concentration of 2.5% Moringa leaf extract, the results of the measurement of the area of the 1st injection: 786561, 2nd: 791198. Average Average area: 78880. Tannic acid content (tanin) = 28.2659 ppm or 0.0028266%. Sample 5 with a concentration of 5% Moringa leaf extract, the results of the measurement of the area of the 1st injection: 1280441, 2nd: 1314621. Average area: 1297531. Tannic acid content (tanin) = 46.4910 ppm or 0.0046491%. Sample 2 with a concentration of 10 % Moringa leaf extract, the results of the measurement of the area of the 1st injection: 3607208, 2nd: 359379. Average area: 3598293. Tannic acid content (tanin) = 128.9282 ppm or 0 0.01289282%. Sample 1 with a concentration of 20% moringa leaf extract, the results of the measurement of the area: 8548362. Tannic acid content (tanin) = 306.2910 ppm or 0.0306291 %. Sample 3 with a concentration of 40% Moringa leaf extract, the results of the measurement of the area of the 1st injection: 25221764, 2nd: 25043933. Average area: 25132849. Tannic acid (tanin) content = 900.5194 ppm or 0.09005194%. Meanwhile, with 80 % concentration of Moringa leaf extract, it was not tested because the equipment was damaged. However, based on previous data, tannic acid levels will be the highest.

Table.10 Tannin content in Moringa Handwash

No	Sample Description	Extract Concentration (%)	Levels of Tannic Acid (tannins) (ppm)	Content of Tannic Acid (tannins) (%)
1	Sample 4	2,5	28,2659	0,0028266
2	Sample 5	5	46,4910	0,0046491
3	Sample 2	10	128,9282	0,01289282
4	Sample 1	20	306,2910	0,0306291
5	Sample 3	40	900,5194	0,09005194
6	Sample 6	80	* didn't have time to test	

The implementation of this research was carried out in class XI MIPA 1 to XI MIPA 7 with an average number of 36 students, through project-based learning (PjBL) using the STEM approach (Science, Engineering, Engineering, and Mathematics). Where 4 subject teachers (Mathematics, Physics, chemistry and Biology) collaborate on a STEM project with the topic Handwash Moringa Ol. Students work in 1 group of 3-4 people, 1 class consists of 9 groups. In July-September, each subject provides prerequisite materials that support the Moringa OL Handwash Project, and the implementation of the project starts from October to November, accompanied by 4 subject teachers and assisted by 10 research team students who have participated in the early research either carried out at SMAN 2 Padalarang and at SMKN 13 Bandung. This activity is also one of the implementations of the Independent Learning School program In addition to the assessment of the project, and the resulting product, for individual assessment, students are also given an integrated Final Semester Assessment (PAS) related to the Moringa OL Handwash project in the last week of November. The PjBL Learning Guide for class XI students, the STEM Learning Implementation Plan (RPP), preliminary material PPt, Moringa OL Handwash project material, and The end of semester assessment questions are listed in the appendix.

5. Conclusion

Moringa leaf extract has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria which can be seen from the formation of inhibition zones. Phytochemical compounds which are one of the secondary metabolites

contained in the Moringa leaf extract studied include flavonoids, saponins, tannins, and polyphenols that each have their respective roles in inhibiting bacteria. Flavonoids have three kinds of mechanisms in inhibiting bacteria, namely by inhibiting energy metabolism, inhibiting nucleic acid synthesis, and inhibiting cell membrane function. Tannins inhibit the growth of bacteria by shrinking the cell wall so that cell permeability is disturbed. Saponins are antibacterial by damaging cell membranes. Meanwhile, polyphenols inhibit bacteria by poisoning the protoplasm, penetrating and damaging cell walls, causing cell leakage and by precipitating bacterial cell proteins at high concentrations, while at low concentrations they can inhibit enzyme synthesis.

Quantitative test of measuring tannin levels in each formula, using the HPLC method, showed that hand soap with a concentration of 2.5% moringa leaf extract had a tannin content of 28.2659ppm or 0.0028266%, a 5% concentration of moringa leaf extract had a tannin content of 46.4910ppm or 0.0046491%, moringa leaf extract concentration 10% has a tannin content of 128.9282ppm or 0.01289282%, a concentration of 20% moringa leaf extract has a tannin content of 306.2910ppm or 0.0306291%, a concentration of 40% moringa leaf extract has a tannin content of 900.5194 ppm or 0.09005194%. Meanwhile, with a concentration of 80% oringa leaf extract, it was not tested because the equipment was damaged. However, based on previous data, tannic acid levels will be the highest.

Liquid hand soap with the addition of Moringa leaf extract has a growth inhibition of *Staphylococcus aureus* and *Escherichia coli* bacteria which is greater than the liquid hand soap produced by SMAWA toiletries without Moringa leaves. The optimum inhibition against *Staphylococcus aure*us was obtained at a concentration of 2.5% Moringa leaf extract, while the optimum inhibition against *Escherichia coli* bacteria was at a concentration of 10% and 80%, respectively. *Staphylococcus aureus* is a gram positive bacterium that has a more complex peptidoglycan structure and a lower lipid content, while *Escherichia coli* is a Gram negative bacterium that has less peptidoglycan/thin content and more lipid content, so that the cell wall of *Staphylococcus aureus* is more easily damaged by compounds. active Moringa leaf extract from *Escherichia coli*.

6. Team Leader and Research Member

Research Team Leader : Yulvianah, S.Pd

Research Team Member : Elsa Mahardika, S.Pd

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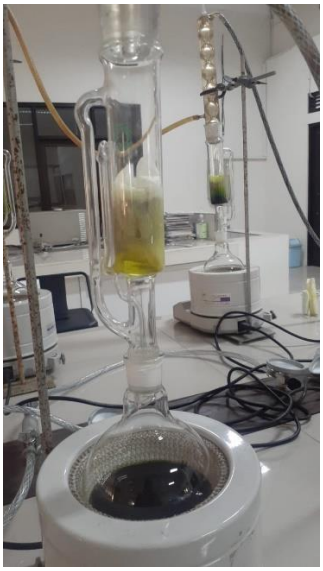
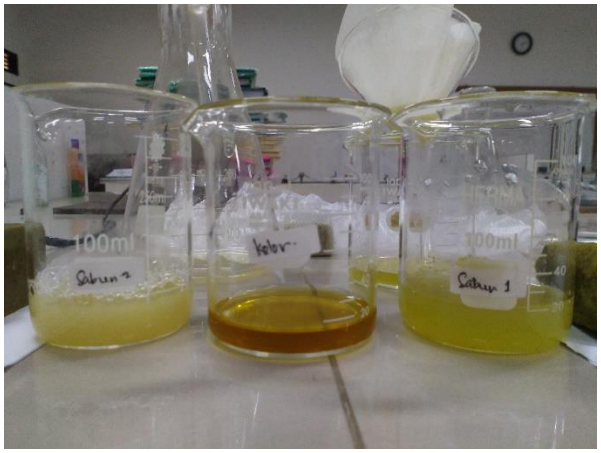
Attachment

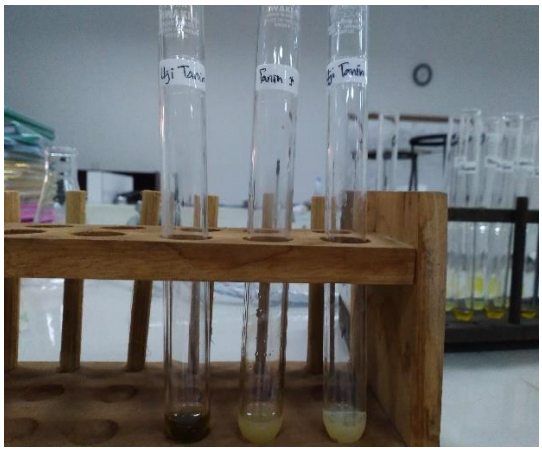
Documentation of Research and Implementation in PjBL Learning Using the STEM Approach

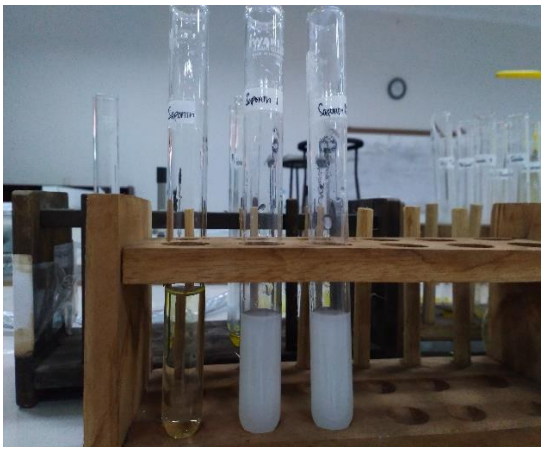


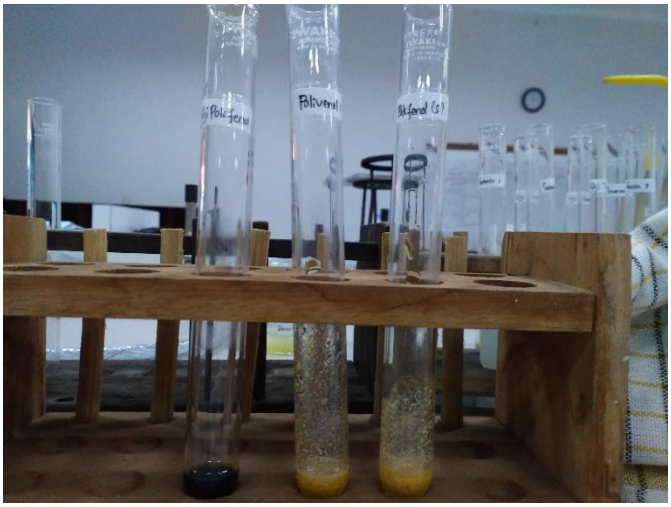



Moringa Leaf Extraction Process Documentation

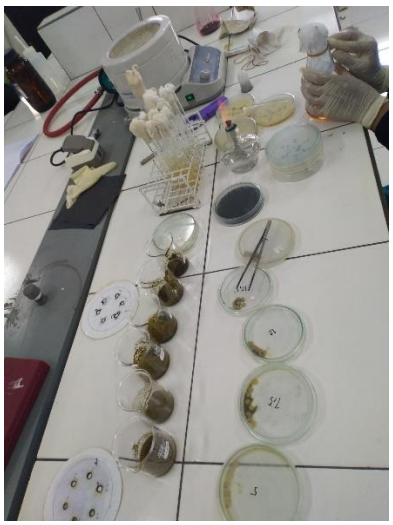


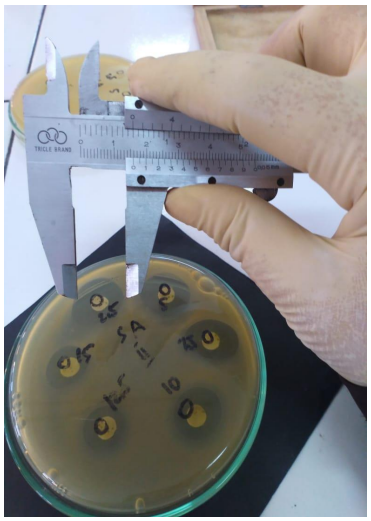




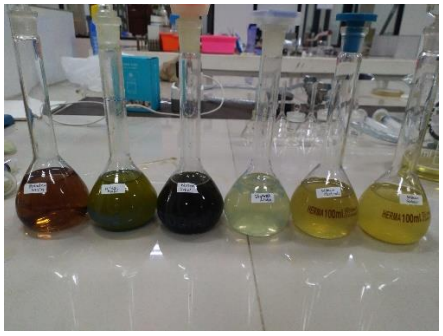


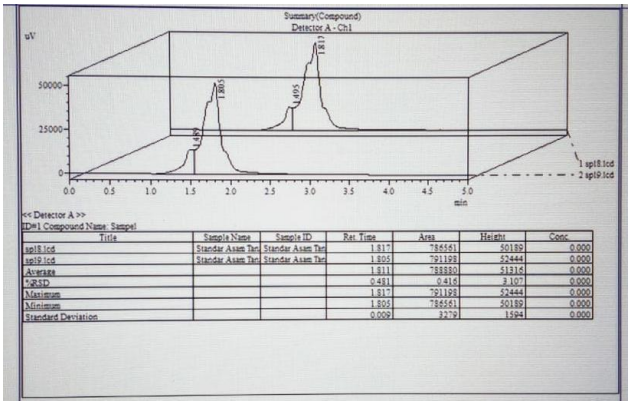
Moringa Qualitative Test Documentation and Moringa Handwash





Bacterial Inhibitory Test Documentation





Documentation of Quantitative Test of Tannin Levels in Moringa Extract and Moringa Handwash

Documentation of Learning Implementation in the classroom

Moringa Handwash Project Implementation Documentation

at school (SMAN 2 Padalarang)

Kolaborasi Riset *Handwash* Kelor SMAN 2 Padalarang di SMKN 13 Bandung

Documentation of the Implementation of the Independent Learning School Cross-School Learning Collaboration
SMAN 2 Padalarang and SMKN 13 Bandung

Environmental Friendly Moringa Ol Handwash Product Documentation