Chemistry and Materials

The Center for Science Innovation

Research Article

Chem. Mater. 3 (2) 2024, 64-70

Utilization of Used Cooking Oil in Making Liquid Soap with the Addition of Water Hyacinth (*Eichhornia crassipes*) Leaf Extract as an Antibacterial

Bella Pricilya Nababan*, Rinda Mulmeyda, Navisya Putri Insyani

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jl. Rawamangun Muka, Jakarta 13220, Indonesia

*Corresponding author: bellanababan42@gmail.com

Received
2 May 2024

Received in revised form
24 June 2024

Accepted
26 June 2024

Published online
30 June 2024

DOI
https://doi.org/10.56425/cma.v3i2.76

Abstract

The frequent disposal of used cooking oil into waterways, along with the proliferation of water hyacinth plants that become invasive in water bodies, contributes to environmental pollution and compromises ecosystem health. One of the effective solutions against various diseases is thorough handwashing with antibacterial soap to eliminate germs and dirt. Therefore, this study aims to investigate the impact of water hyacinth leaf extract addition on the antibacterial activity of cooking oil-based liquid soap. The research employs an experimental approach. Results from pH testing indicate that all liquid soap formulations produced meet the criteria for high-quality liquid soap. Among the variations tested, the formula containing 5% water hyacinth extract exhibited the best foam stability. Regarding antibacterial efficiency, the results suggest that all three samples exhibited weak inhibition of bacterial growth. However, the formula with 10% water hyacinth concentration showed the highest average inhibition zone of 3.46 mm.

Keywords: antibacterial, liquid soap, used cooking oil, water hyacinth leaf.

1. Introduction

Palm oil is the most widely consumed vegetable oil globally, accounting for 35% of the global vegetable oil supply [1,2]. According to United States Department of Agriculture (USDA), palm oil consumption in 2021-2022 is 73.87 million metric tons. It is estimated that 85% of global palm oil produced is used for food consumption [3]. Fried food has become a popular dish in all walks of life, as it has a savoury and crunchy taste, at an affordable price. Generally, fried foods are sold by street vendors on the side of the road or in markets that are the centre of the crowd.

In fried foods, achieving perfect crispiness often requires deep frying. However, this technique increases the levels of unsaturated fatty acids in the cooking oil, leading to its deterioration [4]. Repeated use of cooking oil results in hydrolysis and oxidation processes, which further degrade its quality. Signs of deteriorated cooking oil include a less appetizing aroma and darkening colour [5]. Consuming used cooking oil can lead to health issues and potentially cause various diseases. This poses both health and environmental concerns, as used cooking oil, known commonly as waste cooking oil, can have detrimental effects on the environment.

Oil is one of the groups that belong to the lipid group. One property that is typical and characterizes the lipide group is its solubility in organic solvents such as ether, benzene, chloroform or vice versa its insolubility in water solvents [6]. Dyes in oil consist of two classes, such as natural dyes and colours from the degradation of natural dyes. These dyes consist of α and β carotene, xanthophyll, chlorophyll and anthocyanin. These dyes cause the oil to have a yellow, brownish-yellow and reddish colour [7].

The rampant disposal of used cooking oil into waterways without prior treatment can pollute the environment. Used cooking oil is a very dangerous waste for health, because it contains carcinogenic compounds, which can cause various diseases, such as kidney, cancer, heart disease, and so on [8]. It was observed that the reused cooking oil and fumes present in the atmosphere of the kitchen increased the incidence of aberrant cells, including breakage, fragmentation, exchange, and damage to the chromosomes and micronuclei, in a dose-dependent manner [9]. Used cooking oil is considered waste and is often disposed of directly into waterways or the ground. This practice results in the emission of a rancid odour, the pollution of coastlines, the clogging of water treatment plants, and the clogging of household kitchen pipes [10]. Although used cooking oil is one of the wastes that is very harmful to the environment and health, it can be processed into soap products [11].

On the other hand, there are many infectious diseases if you do not wash your hands properly, such as diarrhea, cholera, dysentery, typhus, helminthiasis, skin diseases, acute respiratory infections [12]. This action is very important because of the function of human hands which tend to meet their own bodies or other people, either directly or indirectly. The danger will be present if contact is made with dirty hands, because it can cause the spread of disease by transferring viruses, bacteria and parasites from one individual to another without realising it.

Soap is one of the media to clean our hand from germs, dirt, and other things that can bring dirt [13]. Soap comes from a mixture of basic compounds with fatty acids that are used as body cleaning agents. Soaps are sodium (Na) or potassium (K) salts of long-chain fatty acids linked by carboxylic acid functional groups. Soap can be both solid and liquid depending on the ingredients used for preparation. For example, solid soaps usually consist of sodium salts of fatty acids and liquid soaps consist of potassium salts of fatty acids. Soap is a compound of sodium or potassium with fatty acids from vegetable oils or animal fats in solid form, soft or liquid, and foaming. Soap is produced by the process of saponification, which is the hydrolysis of fats into fatty acids and glycerol under alkaline conditions. glycerol under alkaline conditions. Commonly used alkaline conditioners are Sodium Hydroxide (NaOH) and Potassium Hydroxide (KOH) [14]. Fatty acids are the main components that make up fats and oils. Used cooking oil that has been processed beforehand will be the type of oil used in this study, with the addition of antimicrobial substances from water hyacinth plants. Thus, making soap from used cooking oil can be one of the community's alternatives in reducing household waste, while preventing infectious diseases through hand washing from soap that can be produced at home.

In previous studies, sodium hydroxide soap was made with 40% NaOH concentration and 450ºC saponification process temperature from used cooking oil. For the purification process, neutralization is carried out by adding 15% NaOH and the bleaching process using activated charcoal from coconut shell charcoal as much as 7.5% of the weight of the cooking oil used and it is found that the concentration of NaOH and the temperature of the soap making process have an important influence on the quality of the soap produced, namely if the concentration of NaOH used more than 40%, the soap produced is a hard soap that can cause irritation to the skin. It should be noted that if the sodium hydroxide concentration employed is below 40%, the resultant soap is of a quality that renders it challenging to lather and difficult to form a solid bar [15].

The next research examines the manufacture of liquid soap from waste cooking oil using KOH concentration consisting of 3 levels, namely 25%, 30%, and 35%. The characteristics of liquid soap observed were viscosity, free alkali content, as well as pH and foam height. The results show that the most optimal KOH concentration is 25% with a pH value of 10.3 and a free alkali value of 0.0847%. Based on these results, the results of the soap characteristics obtained are close to the maximum limit of soap quality provisions [16].

Water hyacinth plants are aquatic weeds that are often found in various fresh waters such as in rivers. Water hyacinth growth is very fast, because one water hyacinth plant can reproduce up to 1000 times in one month. Water hyacinth obstructs water flow, inhibits aquatic ecosystems, and becomes a habitat for disease-carrying animals, such as the liver fluke-carrying molluscs Bithynia and mosquito larvae [11]. The water hyacinth plant belongs to the Pontederiaceae family which has various secondary metabolite compounds, such as alkaloids, tannins, flavonoids, and phenols. These compounds have potential as anti-functional, antibacterial, antioxidant, anticancer, and antiaging [17] Water hyacinth plants are also one of the plants that produce cyclic acid compounds (shikimic acid) which play an important role in the creation of aromatic compound components (tyrosine, tryptophan, and phenylalanine) in plant or microorganism [18]. Then, the tannin content in plant extracts shows antimicrobial activity through protein binding which results in inhibition of pathogenic microbial protein cell synthesis [19]. Since the water hyacinth population is large, utilizing it for other purposes is an environmentally friendly option to reduce the number of water hyacinths. Water hyacinth stems contain high cellulose content [20]. It consists of 25% cellulose, 10% lignin and 33% hemicellulose. The antimicrobial activity in water hyacinth plants can be used to improve into used cooking oil-based antibacterial soap. [21].

2. Materials and Method

2.1 Materials

Used cooking oil, water hyacinth leaves, aquades (H_2O), sodium hydroxide (NaOH), nutrient agar, and activated carbon, ethanol, texaphone, potassium hydroxide (KOH).

2.2 Procedure

The method design used in this research is true experimental design. To obtain the extract, water hyacinth leaves were first cleaned, then dried and pulverized into powder. The results obtained were then weighed as much as 200 grams for maceration using 96% ethanol as much as 1 L for 3 × 24 hours.

In the first step of the liquid soap production process, 100 mL of refined used cooking oil is placed in an Erlenmeyer flask and heated to 70℃. Then a solution of 30 mL KOH, 30 mL Texapon and 40 mL 96% ethanol was added and stirred for 60 minutes until homogeneous. After becoming solid and pasty, the soap paste was dissolved with 100 mL of distilled water and stirred again until it became a liquid soap solution. The liquid soap solution was then cooled and water hyacinth leaf extract was added with a concentration variation of 5%, 7% and 10%.

3. Results and Discussion

3.1 Soap Production

Three liquid soap formulations, each totalling 200 ml, were prepared. The ingredients in these formulations included used cooking oil that had undergone purification, neutralization, and bleaching, along with the addition of water hyacinth (*Eichhornia crassipes*) leaf extract as an antibacterial agent.

The liquid soap solution that has not been augmented with water hyacinth leaf extract exhibits a milky white and thick consistency. In contrast, the liquid soap that has been combined with water hyacinth leaf extract undergoes a colour change from white to light brown to dark brown. The higher the concentration, the more intense the colour of the soap obtained, and the texture is slightly thicker than before the addition of water hyacinth leaf extract. The liquid soap produced in this study is of a liquid consistency, with a typical odor of water hyacinth leaves. The brown coloration of the liquid soap indicates the presence of water hyacinth leaf extract, which differs from the white liquid soap base prior to the addition of the extract.

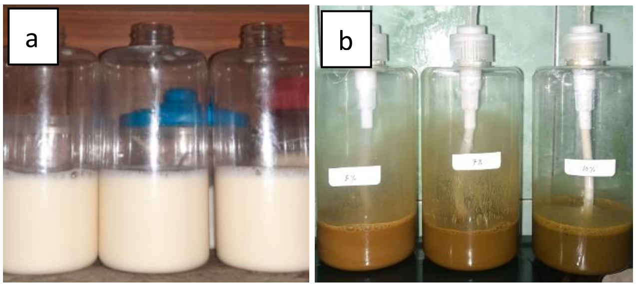

Figure 1. The liquid soap is made from used cooking oil (a) before the addition of water hyacinth leaf extract, (b) after the addition of water hyacinth leaf extract with various concentrations.

3.2 Chemical and physical parameter test

3.2.1 pH test

The pH test is one of the quality requirements of liquid soap. This is because liquid soap is in direct contact with the skin and can cause problems if its pH does not match the pH of the skin. According to SNI 2588:2017, the pH of liquid soap is allowed between 4-11. In testing the pH value, it is repeated 3 times to determine the accurate pH value.

After performing a pH test with a repetition of 3 times for each liquid soap sample using a universal pH indicator paper, the results show that the liquid soap base has a pH of 10, 5% concentration liquid soap has a pH of 9, 7% concentration has a pH of 9, 10% concentration has a pH of 8. The results show that all liquid soap formulas produced meet the criteria for good liquid soap.

3.2.2 Foam height and stability measurement test

The foam height test was carried out to see the foam power produced by liquid soap made in accordance with the soap foam height standard set by the Indonesian National Standard (SNI) which is 13-220 mm. The results of the foam height test can be seen in the following table.

Table 1. The result of foam height measurement test.

Formulas	Foam Height Measurement (mm)			
	Repetition			Average
	1	2	3	
0%	26	25	23	24,6
5%	20	24	21	21,6
7%	22	19	19	20
10%	19	19	17	18,3

Based on SNI, the required foam height of liquid soap is 13-220 mm. From the observations, it was found that the average foam height of the liquid soap base was 24.6 mm, the 5% concentration liquid soap had an average foam height of 21.6 mm, the 7% concentration had an average foam height of 20 mm, and the 10% concentration had an average foam height of 18.3 mm. From these results it can be seen that the largest foam height value is achieved by the liquid soap without the addition of water hyacinth leaf extract and based on observations, the higher the concentration of extract added to the liquid soap formula, the less foam is produced with the smallest foam height value at the highest extract concentration, namely 10% concentration. This is in agreement with the results of research by Schramm (2005), which states that foam stability is influenced by the concentration and viscosity of the formulation. From the results obtained, all concentration variations meet the soap standards according to SNI.

In soap products, foam content is one of the attractions. Foam stability is expressed as the resistance of a bubble to maintain the size or break the film layer of the bubble. Based on good foam stability standards, which range from 60-90%.

Table 2. Foam stability measurement test.

Formulas	Foam Stability (%)			
	Repetition			Average
	1	2	3	
0%	86,6	80,6	79,3	82,16
5%	76,9	80	77,7	78,2
7%	73,3	67,8	65,5	68,8
10%	70,3	67,8	65,3	67,8

The observation results show that the foam stability of liquid soap samples for all concentration variations meets the standards of good foam stability, that is, the foam content has good stability, which ranges from 60-90%. The average foam stability of liquid soap without the addition of extract or soap base is 82.16%, at 5% water hyacinth concentration the average foam stability is 78.2%, at 7% water hyacinth concentration the average foam stability is 68.8%, and at 5% water hyacinth concentration the average foam stability is 67.8%. This proves that the addition of water hyacinth extract to the soap formula affects the stability of the foam formed, i.e. the higher the concentration of the extract added, the lower the foam stability value. The percentage of foam stability is directly proportional to the height of the foam. The best foam stability value among the variations of water hyacinth extract addition is at 5% concentration.

3.4 Organoleptic test

This organoleptic test was conducted to determine the level of acceptance and consumer interest in edible oil-based soap products and water hyacinth leaf extracts produced from the 5%, 7% and 10% concentration formula. The determination scale in this organoleptic test consists of 5 scales, namely very dislike, dislike, enough, like, very like. The panelists used in this organoleptic test were untrained panelists with a quantity of 30 people. The general liking of the panelists for this liquid soap can be seen in the following graph.

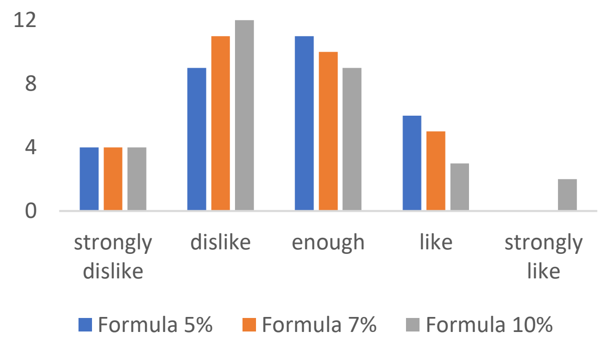

Figure 4. Organoleptic test on aroma.

Based on the graph of preference test for aroma above, it can be concluded that the three formulas have equal votes on scale 1 (strongly dislike). In scale 2 (dislike), the 10% Concentration Formula obtained the most votes with a total of 12 votes. Meanwhile, for scale 4 (like) the most votes were obtained by the 5% Concentration Formula. This shows that the most preferred soap from the aroma indicator is the 5% Concentration Formula, while the least preferred is the 10% Concentration Formula.

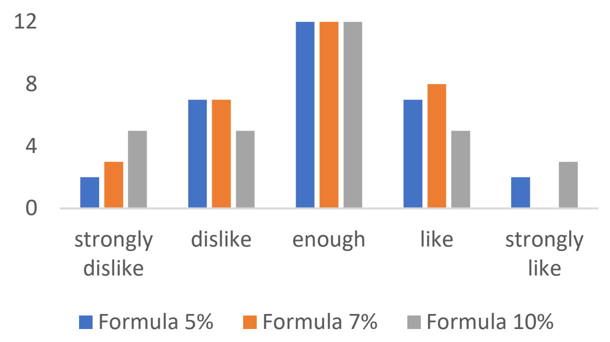

Figure 5. Organoleptic test on colour.

Based on the color preference test graph above, it can be seen that the 10% concentration formula has the most votes on scale 1 (strongly dislike) with a total of 5 votes, while the 5% concentration formula has 2 votes and the 7% concentration formula has 3 votes. Then, the 5% concentration formula has the most votes for the preferred soap with a total of scale 3, scale 4, and scale 5 which exceeds the other formulas, with a total of 21 votes. Therefore, the soap that is highly preferred in terms of color is the 5% concentration formula, while the least preferred soap is the 10% concentration formula.

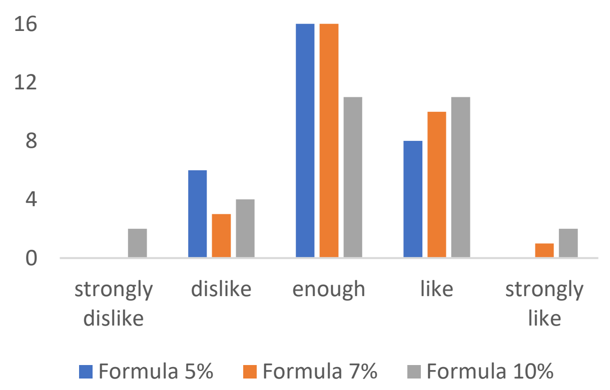

Figure 6. Organoleptic test on texture.

Based on the graph of the organoleptic test for texture above, it can be concluded that scale 2 (dislike) is mostly obtained by the 5% concentration formula with a total of 6 votes. This shows that the 5% concentration formula tends to be disliked in terms of texture than other concentration formulas. Meanwhile, the 10% concentration formula has the most votes on a scale of 4 (like) and 5 (strongly like) with a total of 11 votes and 2 votes. This shows that the soap that is very liked from the texture is the 10% concentration formula.

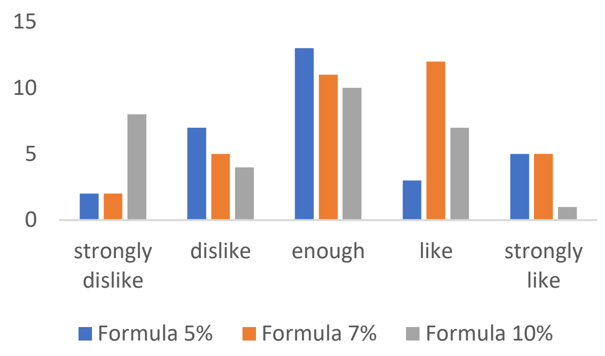

Figure 7. Organoleptic test on amount of foam.

Based on the organoleptic test graph of the amount of foam above, it can be seen that the 10% concentration formula has the most votes in scale 1 (strongly dislike), which is 8 votes. Meanwhile, the 7% concentration formula obtained the most votes on a scale of 4 (like) and 5 (strongly like) with a total of 12 votes and 5 votes. This shows that the most preferred soap in terms of a lot of foam is the 7% concentration formula, while the least preferred soap is obtained from the 10% concentration formula.

3.5 Antibacterial test

The antibacterial activity of water hyacinth leaf extract added to the used cooking oil-based liquid soap formula was evaluated using the agar diffusion method with wells. In this antibacterial test, four samples were used: one sample of liquid soap formula without the addition of extracts, which served as the negative control, and three other samples of liquid soap formula with the addition of water hyacinth leaf extract at three different concentration variations, namely 5%, 7%, and 10%. The antibacterial activity of the prepared soap formula was determined by measuring the inhibition zone around the wells after incubation for 24 hours.

After conducting antibacterial tests on the used cooking oil-based liquid soap formula with various concentrations of water hyacinth leaf extract, the test carried out three times over 24 hours, the results showed the diameter of the inhibition zone of bacterial growth formed around the well. These results, which respond to antibacterial test from different concentrations of water hyacinth leaf extract, are summarized in the following table.

Table 3. Antibacterial test on liquid soap formula with various water hyacinth leaf extract.

Formula	Inhibition zone (mm)				
	Repetition			Average	Interpretation
	I	II	III		
0%	-	-	-	-	No inhibition
5%	3	2,8	3	2,93	Weak
7%	3,3	3,4	3,2	3,3	Weak
10%	3,5	3,4	3,5	3,46	Weak

According to Davis & Stout [18], the response to inhibition of bacterial growth can be classified based on several interpretations, namely, if the inhibition zone <5 mm is said to be weak, 5-10 mm is said to be moderate, 11-20 mm is said to be strong, and >20 mm is said to be very strong. Based on the observation and measurement of the inhibition zone formed around the well after 24 hours of incubation, the interpretation of the results shows that the three soap formulas with water hyacinth leaf extract added have weak antibacterial activity. While the soap formula without the addition of water hyacinth leaf extract did not show any inhibition zone around the wells.

It can be concluded that the addition of water hyacinth leaf extract to liquid soap formulas made from used cooking oil can provide antibacterial properties and increases its effectiveness in inhibiting bacterial growth compared to soap without the addition of water hyacinth leaf extract. The antibacterial activity may be influenced by the presence of chemical compounds contained in the extract. Research by Rufchaei et al. [19] indicates that water hyacinth leaves contain phenolic compounds, including alkaloids, tannins, and flavonoids, which act as antibacterial agents. The alkaloids present in water hyacinth leaf extract exhibit antibacterial biological activity, disrupting the components that comprise bacterial cells, preventing the formation of cell walls, and facilitating their lysis [20]. Flavonoids, which are antibacterial compounds, have a mechanism of action that interferes with the synthesis of bacterial membranes. They can disrupt bacterial reproductive pathways and damage bacterial membrane walls, ultimately leading to bacterial death [21]. Tannins are known to have antibacterial activity related to their ability to form hydrogen bonds, which result in denaturation of proteins in membranes, so that cells experience damage, which prevents the bacteria from fulfilling their nutritional needs and reduces their energy [22].

In this study, the antibacterial activity of three soap formulations with varying concentrations of water hyacinth extract was evaluated. The results demonstrated that the soap formulation with a 10% concentration of water hyacinth extract exhibited the greatest antibacterial activity, as indicated by the average inhibition zone formed. These findings suggest that the antibacterial efficacy of the soap formulations increased with increasing extract concentration. This observation is consistent with the results of a study by Afidat et al. [23], which demonstrated that the antibacterial activity of water hyacinth leaf extract against the bacteria *A. actinomycetemcomitans* increased with higher concentrations.

4 Conclusion

Based on the results of the research conducted, it can be concluded that incorporating water hyacinth leaf extract into the cooking oil-based liquid soap formula significantly impacts both its physical and antibacterial properties. The pH test results indicate that all liquid soap formulations meet the criteria for high-quality liquid soap: the soap without extract has a pH of 10, while those with 5%, 7%, and 10% concentrations exhibit pH levels of 9, 9, and 8 respectively. Among the variations tested, the formula containing 5% water hyacinth extract demonstrated the best foam stability. Organoleptic testing, conducted through questionnaires distributed to 30 panelists, revealed that the 5% concentration received the highest ratings for aroma, colour, texture, and foam characteristics. In terms of antibacterial efficacy, although all three samples exhibited weak inhibition of bacterial growth, the formula with 10% water hyacinth extract showed the highest average inhibition zone of 3.46 mm.

Acknowledgement

The author would like to express our profound gratitude to Kelompok Peneliti Muda (KPM) at Universitas Negeri Jakarta for their invaluable support of our research through the P3KPM funding program 2023.

References

[1] F. Taheripour, T. W. Hertel, N. Ramankutty, Market-mediated responses confound policies to limit deforestation from oil palm expansion in Malaysia and Indonesia. *Proceedings of the National Academy of Sciences*, **116** (2019), 19193-19199.

[2] S. M. Downs, K. Nicholas, K. K. Linn, J. Fanzo, Examining the trade-offs of palm oil production and consumption from a sustainable diets' perspective: lessons learned from Myanmar. *Public health nutrition*, **25** (2022), 964-976. https://doi. org/10.1017/S1368980021004353

[3] C. Y. May, K. Nesaretnam, Research advancements in palm oil nutrition. *European journal of lipid*

science and technology, **116** (2014), 1301-1315. https://doi.org/10.1002/ejlt.201400076

[4] S. Mucti, R. Purwasih, I. D. Destiana, Analisis Mutu Minyak Goreng yang Dipakai oleh Pedagang Gorengan di Pasar Pujasera Subang, *EDUFORTECH,* **8** (2021). https://doi. org/10.17509/edufortech.v8i2.54642

[5] N. E. Setyaningsih, W. S. Wiwit, Pengolahan Minyak Goreng Bekas (Jelantah) Sebagai Pengganti Bahan Bakar Minyak Tanah (Biofuel) Bagi Pedagang Gorengan Di Sekitar Fmipaunnes. *Rekayasa: Jurnal Penerapan Teknologi dan Pembelajaran*, **15** (2018) 89-95.

[6] A. S. Afrozi, D. Iswadi, N. Nuraeni, G. I. Pratiwi, Pembuatan sabun dari limbah minyak jelantah sawit dan ekstraki daun serai dengan metode semi pendidihan. *Jurnal Ilmiah Teknik Kimia UNPAM*, **1** (2017).

[7] J. Li, Evaluation of fatty tissue representative solvents in extraction of medical devices for chromatographic analysis of devices extractables and leachables based on Abraham general solvation model. *Journal of Chromatography A*, **16** (2022). https://doi.org/10.1016/j.chroma.2022.463240

[8] A. A. Adeyemo, I.O. Adeoye, & O.S. Bello, Adsorption Of Dyes Using Different Types Of Clay: A Review. *Appl Water Sci* 7, **5** (2017). https://doi.org/10.1007/s13201-015-0322-y

[9] K. Ganesan, K. Sukalingam, B. Xu, Impact of consumption of repeatedly heated cooking oils on the incidence of various cancers-A critical review. *Critical reviews in food science and nutrition*, **59** (2019) 488-505. https://doi.org/10.1080/10408398.2017.1379470

[10] I. Thushari, S. Babel, S. Comparative study of the environmental impacts of used cooking oil valorization options in Thailand. *Journal of Environmental Management*, **310** (2022). https://doi.org/10.1016/j.jenvman.2022.114810

[11] S. N. K. Azme, N. S. I. M. Yusoff, L. Y. Chin, Y. Mohd, R. D. Hamid, M. N. Jalil, H. M. Zaki, S. H. Sales, N. Ahmat, M. A. F. A. Manan, N. Yury, N. N. F. Hum, F. A. Latif, Z. M. Zain, Recycling waste cooking oil into soap: Knowledge transfer through community service learning. *Cleaner Waste Systems*, **4** (2023). https://doi.org/10.1016/j.clwas.2023.100084

[12] K. Handayani, M. Kanedi, S. Farisi & W. A. Setiawan, Pembuatan sabun cuci dari minyak jelantah sebagai upaya mengurangi limbah rumah tangga. *Jurnal Pengabdian Kepada Masyarakat (JPKM) TABIKPUN*, **2** (2021). 55-62 https://doi. org/10.23960/jpkmt.v2i1.25

[13] A. Adhani, F. Fatmawati, Pelatihan Pembuatan Lilin Aromaterapi Dan Lilin Hias Untuk Meminimalisir Minyak Jelantah Bagi Masyarakat Kelurahan Pantai Amal. Jurnal Pengabdian Masyarakat Borneo, **3** (2019), 31-40.

[14] A. Gusviputri, N. M. PS & N. Indraswati, Pembuatan sabun dengan lidah buaya (*Aloe vera*) sebagai antiseptik alami. *Widya Teknik*, **12** (2017). 11-21. https://doi.org/10.33508/wt.v12i1.1439

[15] S. Hartini, Y. Widharto, S. R. Indarto, G. Murdikaningrum, Eco-efficiency analysis of waste cooking oil recycling into liquid dish soap using life cycle assessment. *In IOP Conference Series: Earth and Environmental Science*, **896** (2021). https://doi.org/10.1088/1755-1315/896/1/012066

[16] N. A. Dalimunthe, Pemanfaatan Minyak Goreng Bekas Menjadi Sabun Mandi Padat. *Jurusan Teknik Kimia. Tesis : Universitas Sumatera Utara* (2009)

[17] X. Y. Gao, X. Y. Li, C. Y. Zhang, C. Y. Bai, Scopoletin: a review of its pharmacology, pharmacokinetics, and toxicity. *Frontiers in Pharmacology*, **15** (2024). https://doi.org/10.3389/fphar.2024.1268464

[18] W.W. Davis, T. R. Stout, Disc Plate Methods of Microbiological Antibiotic Assay, *Microbiology,* **22** (1971) 659-665.

[19] R. Rufchaei, M. Abbas-Mohammadi, A. MirzajaniS. Nedaei, Evaluation of the chemical compounds and antioxidant and antimicrobial activities of the leaves of *Eichhornia crassipes* (water hyacinth). *Jundishapur Journal of Natural Pharmaceutical Products*, **17** (2022). https://doi.org/10.5812/jjnpp.101436

[20] T. T. Cushnie, B. Cushnie, A. J. Lamb, Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International journal of antimicrobial agents*, **44** (2014), 377-386. https://doi. org/10.1016/j.ijantimicag.2014.06.001

[21] Y. Xie, W. Yang, F. Tang, X. Chen, L. Ren, Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current medicinal chemistry*, **22** (2015), 132-149. https://doi.org/10.2174/092986732166614091611 3443.

[22] G. Sofiane, N. Wafa, D. Ouarda, Antioxidant, antimicrobial and anti-inflammatory activities of flavonoids and tannins extracted from Polypodium vulgare L. *Asian Journal of Biochemical and Pharmaceutical Research*, **5** (2015) 114-122.

[23] Y. I. Afidati, I. J. Savitri, A. Krismariono, Inhibition activity of water hyacinth leaf extract (*Eichhornia crassipes*) against *Aggregatibacter Actinomycetemcomitans*. *Asian Journal of Pharmaceutical and clinical research*, **12** (2019), 122-125.