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held by Universitas Sebelas Maret

September 10th – 11th, 2019
Surakarta, INDONESIA

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PREFACE

Welcome to Surakarta (Solo) - Indonesia

Assalamualaikum wr wb.

Allahamdulillah to the almighty ALLAH SWT, thank you very much for this opportunity to hold the international conference of JCC 14 on 10-11 September 2019, by Chemistry Department, Faculty of Math and Natural Science, Universitas Sebelas Maret. This JCC conference is as annually program of the consortium of five chemistry departments in the region of Middle Java (Universitas Sebelas Maret, Universitas Diponegoro, Universitas Jenderal Sudirman, Universitas Negeri Semarang, Universitas Kristen Satya Wacana) and a guest member from Malaysia (Universitas Malaysia Sabah).

On the behalf of organizing committee of JCC 2019, I appreciate to all participants to meet in this scientific conference on chemistry 2019, 14th JCC. It was my pleasure to facilitate the ideas in development of chemistry and education chemistry within the region of middle Java also worldwide. I expect that our conference gives good impact to the chemist society not only in the region but in the world through the scientific ideas or publications outcoming from this conference. Some outstanding papers that have been presented in the venue of conference were and will be published in some different journals such as Evergreen, Open Chemistry, Indonesian Journal of Chemistry and also in AIP publishing that are indexed by Scopus.

I would like to thank to Scientific Committee for the publishing articles of the proceeding of the 14th JCC conference in PCC proceeding.

Waalaikumsalam wr. wb.

Surakarta, 6 June 2020

Dr. rer.nat. Atmanto Heru Wibowo,
Chairman of JCC 2019

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Welcome Speech from Head of Chemistry Department

Universitas Sebelas Maret

We are very pleased to introduce The 14th Joint Chemistry Conference held by Chemistry Dept. of Sebelas Maret University on behalf of the Chemistry Consortium in Central Java, Indonesia. In this year, we have guests consortium from University Malaysia Sabah (UMS), I hope that the joining of UMS can increase the quality of this conference and can be continued for the upcoming JCC.

The 14th Joint Chemistry Conference was held on the Solo Paragon Hotel and Residences (Paragon Hotel) in Solo during 10 -11th of September 2019. Solo as "The Spirit of Java," a Javanese culture and heritage center, batik capital, and tourist-friendly city. Theme this conference is “Strengthening the Foundation of Sustainable Development: Research, Practice and Education”. The conference will emphasize the multidisciplinary chemical issue and impact of today’s sustainable chemistry which covering the following topics: Electrochemistry, Polymer Chemistry, Materials Chemistry, Nanomaterials, Medicinal Chemistry, Pharmaceutical Chemistry, Green Chemistry, Computational Chemistry, Natural Products Chemistry, Surface Chemistry and Interfaces, and Educational Chemistry.

We hope that this conference can initiate UNS cooperation with various parties to contribute our science for the benefit of society. Finally, we hope this seminar can take place smoothly and successfully, and its results can be implemented and bring benefit to the wider community.

Dr. Abu Masykur, M.Si.
Head of Chemistry Department,
Universitas Sebelas Maret (UNS)

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Welcome Speech from Head of Chemistry Department

Universitas Jenderal Soedirman

Assalamualaykum warohmatullohi wabarokatuh,

Praises to Alloh SWT who give blessing to allow us to organize the 14th Joint Conference on Chemistry 2019.

I would like to greatly appreciate to the Keynote Speakers, Invited Speaker and all participant who delight to joint this international conference in chemistry. I also would like to deepest appreciation to the organizing committee of 14th Joint Conference on Chemistry who well organize this event. The great collaboration between Diponegoro University, Semarang State University, Jenderal Soedirman University, Sebelas Maret University and Satya Wacana University continuously improve this yearly scientific event in chemistry. Hopefully in the future, the collaboration would be improved in other fields such as research collaboration and lecture exchange.

In the end, I hope this event provide a scientific discussion, professional networking, research collaboration, education, and dissemination of scientific research, innovation and industrial products in order to solve the problem in Chemistry in the future life.

Amin Fatoni, Ph.D.
Head of Chemistry Department,
Universitas Jenderal Soedirman (UNSOED)

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Welcome Speech from Head of Chemistry Department

Universitas Kristen Satya Wacana Indonesia

Distinguished guest, ladies and gentlemen,

Welcome to The14th Joint Conference on Chemistry (The 14th JCC). This Year, The 14thJCC is conducted by Department of Chemistry, Universitas Sebelas Maret, Indonesia. The 14th JCC take theme "Strengthening the Foundation of Sustainable Development: Research, Practice and Education".

Chemistry Department, Faculty of Science and Mathematics of Universitas Kristen Satya Wacana (UKSW) Indonesia has been motivated to joint in Chemistry Department Central Java Indonesia Consortium to strengthen networking and collaboration in the advancement of the mastery of science and technology and the applications, to the benefits of all human kinds. Chemistry Department UKSW Indonesia works focus on applied chemistry in food, natural resources and environment research development. Working with several industries, Chemistry Department UKSW diffuses innovation through product development in health and functional food.

We truly expect that this conference can advance networking and collaboration through chemistry and related field innovation for sustainable development. We hope you will enjoy a pleasant and valuable conference at The 14th JCC.

Dr. Yohanes Martono, M.Sc.
Head of Chemistry Department, Faculty of Science and Mathematics
Universitas Kristen Satya Wacana Indonesia (UKSW)

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Welcome Speech from Head of Chemistry Department

Universitas Diponegoro

Warm greetings!!!

On behalf of the Chemistry Department Diponegoro University, I am pleased to welcome all the delegates and their guests to Surakarta, Jawa Tengah, for the 14th Joint Conference on Chemistry that will take place from September 10-11, 2019. This annual conference dedicated to the science and practice of chemistry, and it will give participants a stage to uncover novel opportunities, discuss ideas, meet fresh contacts, reacquaint with colleagues, and grow their understanding. We believe that the event, as in earlier years, will offer a medium for lively discussion among participants.

Research and innovation is the pillars of chemistry. That calls upon us to strengthen our basic research energy and our communal networks to become a global leader in chemistry. I cannot help but be astounded by the breadth and depth of the topics addressed in the program for this conference. It is an agenda that well embody the crucial roles that chemists play. By coming to this meeting, you confirm your capacity to take up and implement this knowledge as well as to transfer it so as to have impact.

To all partakers I acknowledge each of you for joining our conference and conveying your knowledge to our gathering. I also would like to thank fellow associates of the consortium who have devotedly partaken this occasion every year. Lastly, my uppermost appreciation and gratefulness goes to the entire Organizing and Scientific Program committees, for the enormous amounts of time and energy they have dedicated to guarantee that this conference is a success.

Thank you and enjoy the conference! Semarang, September 2019

Dr. Dwi Hudiyanti, MSc.
Head of Chemistry Department,
Universitas Diponegoro (UNDIP)

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Welcome Speech from Head of Chemistry Department

Universitas Negeri Semarang

The 14th Joint Conference on Chemistry (JCC) 2019 was successfully took place on the 10th -11th October 2019 at Solo, Indonesia. The JCC2019 was organized by Universitas Negeri Sebelas Maret (UNS) in collaboration with Universitas Negeri Semarang (UNNES), Universitas Jenderal Soedirman (UNSOED), Universitas Diponegoro (UNDIP) and Universitas Kristen Satya Wacana (UKSW). The JCC2019 aims to provide a platform for discussing the issues, challenges, opportunities and findings of chemistry and related field research.

The responses to the call-for-papers had been overwhelming. We would like to express our gratitude and appreciation for all of the reviewers who helped us maintain the high quality of manuscripts. We would also like to extend our thanks to the members of the organizing team for their hard work.

It is a great privilege for us to present the proceedings of the JCC2019 to the authors and delegates of the event. We hope that you will find it useful, exciting and inspiring.

Let us wish that all the participants of JCC2019 will have a beneficial, wonderful and fruitful time at the conference.

Cepi Kurniawan, PhD
Chemistry Department
Universitas Negeri Semarang (UNNES)

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Activation of Natural Zeolite and Its Application For Adsorben in Domestic Waste Water Treatment in Tembalang District Semarang City

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Abstract. Tembalang is part of one of the sub-districts in Semarang whose economic growth is fast. There are 12 subdistricts in Tembalang District, one of which is Tembalang District which is the campus area of Diponegoro University. Because the population is very dense, the problem of domestic wastewater and clean water supply is an interesting problem to study. This study aims to describe domestic wastewater in Tembalang District, Semarang City, as well as the application of activated zeolites and activated zeolites to reduce COD, pH, BOD and TSS in domestic wastewater and compare the parameters of wastewater with applicable regulations [1]. Research to improve the quality of domestic wastewater by adsorption using natural zeolite adsorbents and activated zeolites. The results showed that the adsorption treatment with natural zeolite and activated zeolite showed significant reduction results. The results of the decrease in chemical parameters of domestic wastewater are: with natural zeolite the COD reduction is 21.8%, pH 11.5%, BOD 9.2% & TSS 10.8% and with HCl activated zeolite, COD reduction 78.9% , pH 16.2%, BOD 11.8% & TSS 74.4% and COD reduction 85.5%, pH 17.9%, BOD 12.6% and TSS 89.7% for H2SO4-activated natural zeolites.

Keywords: domestic wastewater, natural zeolite, activated natural zeolite, COD, pH, BOD and TSS

1. Introduction

Management of water resources and the environment often requires multidiscipline understand groundwater-surface water (GW-SW) interactions because these interactions form a key link between land activities, aquatic ecosystems, and the integrity of water resources [2]. Fixed amount of land and the ever increasing population causes the problem of clean water and waste water sources to be interesting to study, especially with the presence of active chemicals that are used directly in the household (such as detergents, shampoo, toothpaste, cooking oil) which are directly discharged into sewers water without prior treatment, the carrying capacity of the environment is reduced due to increased waste disposal [3]. Therefore, domestic wastewater and hospital wastewater treatment before disposal in the sewer is absolutely necessary, such as conducted in Doha, Qatar, which is somewhat different from the habits of the people in the country [4]. Indonesian communities in our country generally do not treat their domestic wastewater, and are immediately disposed of in the surrounding water gutters. While the material for treating domestic wastewater by adsorption (with zeolite as an adsorbent) is still wide open. In Indonesia zeolite deposits are very abundant [5]. Zeolite is a hydrated aluminosilicate crystal which has interesting properties and structures on the surface of its mesoporous. Some names of natural zeolites are well known such as mordenite, analcime, phillipsite, chabazite, heulandite, clinoptilolite, erionite, ferrierite and laumontite [6]. Zeolite is usually used as an adsorbtion and catalyst in an industry or company. Natural zeolite in general

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has a thermal stability that is low, non-uniform pore size and low catalytic activity, so it is necessary to make modifications to improve the adsorbtion and catalytic properties [7].

The problem of clean water and domestic wastewater is very much felt in big cities in Indonesia. Semarang is one of them, and Tembalang sub-district as an example. Tembalang is part of one of the sub-districts of Semarang whose rapid economic growth. There are 12 kelurahans in Tembalang sub-district, but Tembalang kelurahan is crowded if compare with another kelurahan in Tembalang sub-district, because which is the location of the Diponegoro University. Thus, the need for clean water and disposal of domestic wastewater becomes quite complicated so it is interesting to study. Domestic wastewater in addition to causing environmental pollution, can cause discomfort and even health problems. One way to reduce the impact of domestic wastewater is to treat domestic wastewater before the environment is discharged.

This study aims to describe domestic wastewater before it is processed in Tembalang sub-district, Tembalang sub-district, Semarang city, as well as the application of natural zeolites and activated zeolites to reduce COD, pH, BOD, TSS and oil in domestic wastewater as well as comparing wastewater parameters with regulations applies [1]. Samples of domestic wastewater are taken from 3 kelurahan, Bulusan kelurahan, Tembalang kelurahan and Meteseh kelurahan. Water chemistry parameters measured were COD, pH, BOD, and TSS.

Previous studies of communal domestic wastewater treatment, using a multilevel screening process and water hyacinth bioremidiation [8], with satisfactory results but requiring a long time and a large area. Another way is to combine between Anaerobic Baffled Reactor (ABR), Anerobic Filter (AF) and Unaaerobic Sludge Banked (UASB), which is carried out by a number of elite house in Surabaya.The combination of these three methods gives very good results (above 80%), but requires time quite a long time, the cost is not small and a fairly large area [9]. The other way that is cheaper and easier to do is adsorption using zeolites with unsatisfactory results if only one treatment (25%) but if replicated up to 3 times will get a significant result (74.5%), whereas if combined with iron sand and zeolite give good results satisfactory (96.6%) [10]. Natural zeolites in general have low thermal stability, non-uniform pore size and low adsorbtion and catalytic activity. To improve thermal stability, the adsorption and catalytic activity of zeolites need to be modified to natural zeolites. Modifications made can be used chemical modification methods such as dealumination with acids or cation exchange with metals. In dealumination, the addition of acid causes aluminum to come out of the zeolite framework and increase the silica ratio [7].

Figure 1. Map of 12 villages in Tembalang sub-district

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Referring to the research and assumptions above, encouraging research on the dealumination of natural zeolites from Bayat using acids (HCl and H_2SO_4) for adsorbent domestic wastewater. This study aims to activate natural zeolites with HCl and H_2SO_4 and apply them to improve the quality of domestic wastewater in the Tembalang sub-district of Tembalang sub-district, by means of adsorption. In this domestic wastewater treatment research an adsorption method will be used by using natural zeolites and activated zeolites (HCl and H_2SO_4) as adsorbents with measurement parameters of COD, pH, BOD and TSS as indicators of the success of the adsorption process.

The results of the dealumination of natural zeolites from Bayat using acids (HCl and H_2SO_4) were characterized by IR spectroscopy and data on the increase of Si / Al ratio in natural zeolites and zeolites of acid dealumination were analyzed by Atomic Absorption Spectrophotometry (AAS). Natural zeolite, HCl activated zeolite and H_2SO_4 activated zeolite are used for adsorption in domestic wastewater treatment in Tembalang sub-district Tembalang sub-district. The results showed that adsorption treatment with natural zeolite and HCl activated zeolite and H_2SO_4 activated zeolite showed significant decreases. With natural zeolite adsorbents, HCl-activated zeolites and H_2SO_4-activated natural zeolites are averaged from the reduction of domestic wastewater in 5 sub-districts in Tembalang sub-district with chemical parameters COD, pH, BOD, and TSS are as follows. With natural zeolite the COD reduction was 21.8%, pH 11.5%, BOD 9.2% & TSS 10.8% and with zeolite activated HCl, COD reduction 78.9%, pH 16.2%, BOD 11, 8% & 44.4% TSS and 85.5% COD reduction, 17.9% pH, 12.6% BOD and 49.7% TSS for H_2SO_4-activated natural zeolite.

2. Research Method

2.1 Materials and tools

2.1.1. Material

The materials used are domestic wastewater (as much as 1 Liter taken from 5 different kelurahans in Tembalang district as samples), 250 g of (Bayat, Klaten) natural zeolite, 6 M hydrochloric acid (HCl), sulfuric acid (H_2SO_4) 6 M, potassium permanganate ($KMnO_4$)) 0.5 M, distilled water, and Argentum Nitrate ($AgNO_3$) (Merck).

2.1.2. Tool

The tools used are a pH meter (HANNA HI 8314), IR spectrometer (IR 100 Perkin Elmer Spectrometer), Atomic Absorption Spectrophotometry (AAS) (Perkin Elmer 900F), a set of titration tools, a 100-200 mesh sieve, an oven, a 250 mL beaker, a 100 mL measuring cup, a dropper, a separating funnel, furnace, flask round, magnetic stirrer, water bath, a set of reflux apparatus, measuring flask, electric scales and hot plates.

2.2 Experimental procedure

2.2.1. Sample preparation

Bayat natural zeolite is washed and soaked with distilled water overnight. Dried at a temperature of 110o C crushed until smooth and sieved with a size of 100-200 mesh.

2.2.2. Dealumination of natural zeolites

Zeolites selected were zeolites from the Klaten (Bayat) region. Bayat natural zeolite is washed and soaked with distilled water overnight and dried at 110 ^oC. Soaking with distilled water is intended to remove organic impurities present in the natural zeolite. Natural zeolite which has been dried, crushed with porcelain mortal until smooth and sieved with a 100-200 mesh size sieve. Destruction is done to increase the surface area of natural zeolites so that the catalysis ability can be more optimal. Zeolites which are free from organic matter and oxide impurities, do the dealumination process by acidifying and adding KmnO4. The acidification process is carried out by adding H_2SO_4 and HCl. Whereas $KMnO_4$ (as

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an oxidizing agent) is intended for oxidizing organic impurities present. The activation process is carried out for 4 hours at 80 ^{o}C, allowed to stand overnight at room temperature, it is expected that alumina coming out of the zeolite framework can be optimized. Zeolite (50 grams) are added with 100 ml of 6 M H_2SO_4 and 100 ml of $KMnO_4$ 0.5 M. The mixture is heated at 80 ^{O}C for 4 hours, allowed to stand for one night (48 hours) at room temperature. The mixture obtained was then filtered and washed with distilled water until it was neutral (tested with AgNO3 solution until no white precipitate appeared) and dried at 110 ^{O}C for 5 hours and calcined for 3 hours at 500 ^{O}C (CODE Z1).

Zeolite as much as 50 grams is added with 100 ml of HCl 6 M and 100 ml $KMnO_4$ 0.5 M. The mixture is heated at 80 ^{O}C for 4 hours, allowed to stand for one night (48 hours) at room temperature. The obtained mixture is then filtered and washed with distilled water until it is neutral and dried at 110 ^{O}C for 5 hours and calcined for 3 hours at 500 ^{O}C (CODE Z2).

2.2.3. Characterization of natural zeolites and HCl-activated zeolites and H_2SO_4-activated zeolites

Characterization was performed on natural zeolites, HCl-activated zeolites and H_2SO_4-activated zeolites, the Si / Al ratio using atomic absorption spectroscopy (AAS) and analysis of functional group changes was carried out by analyzing infrared spectroscopy (IR).

2.2.4. Adsorption Activity Test

2.2.4.1. Collection and treatmen of Domestic Waste Water Samples

Before conducting the activity test, it was taken in 5 villages in Tembalang sub-district, each from Sendangguwo, Sendangmulyo, Tembalang, Meteseh and Rowosari. A total of 100 mL samples of domestic wastewater, added $AgNO_3$ to remove chloride ions. A total of 1 gram of natural zeolite adsorbent was mixed into the sample, heated at 60 ^{o}C in a three-neck flask equipped with a thermometer and stirrer, then refluxed for 60 minutes. Performed on variations: natural zeolite, H_2SO_4 dealuminated natural zeolite, HCl dealuminated natural zeolite

2.2.4.2. Separation of Residues and Filtrates

Heterogeneous mixture obtained after domestic wastewater is subjected to an adsorption process, filtered with a funnel. Then the filtrate and residue are obtained. The filtrate is wastewater that is thought to have undergone a process of adsorption perfectly and the residue is a zeolite adsorbent. The filtrate is left for a day and a night in the separating funnel, then analyzed.

2.2.5. Method of analysis

Water samples (100 mL) (from various Kelurahans in Tembalang District) were given 1 gram of natural zeolite, 1 gram of chlorinated acid zeolite and 1 gram of sulfuric acid zeolite. Stirring is done for 1 hour, then allowed to stand for 24 hours. The screening process is carried out. After the separation process, an analysis is performed on the sample solution, with COD analysis, acidity (pH) analysis, BOD analysis, and TSS analysis.

3. Results and Discussion

The acidification process is carried out by adding H_2SO_4 and HCl. Where as $KMnO_4$ (as an oxidizing agent) is intended for oxidizing organic impurities present. The activation process is carried out for 4 hours at 80 ^{o}C, allowed to stand overnight at room temperature, it is expected that alumina coming out of the zeolite framework can be optimized. The addition of acid aims to exchange cations to form H-zeolite. An exchange occurs between cations in zeolites and H^+. The ion exchange between the cations in zeolites with H^+ aims to replace all the cations in the zeolites because in the zeolites there are still alkaline or alkaline earth cations such as Na^+, Ca^{2+} , Mg^{2+} which act as zeolite balances that can be exchanged with

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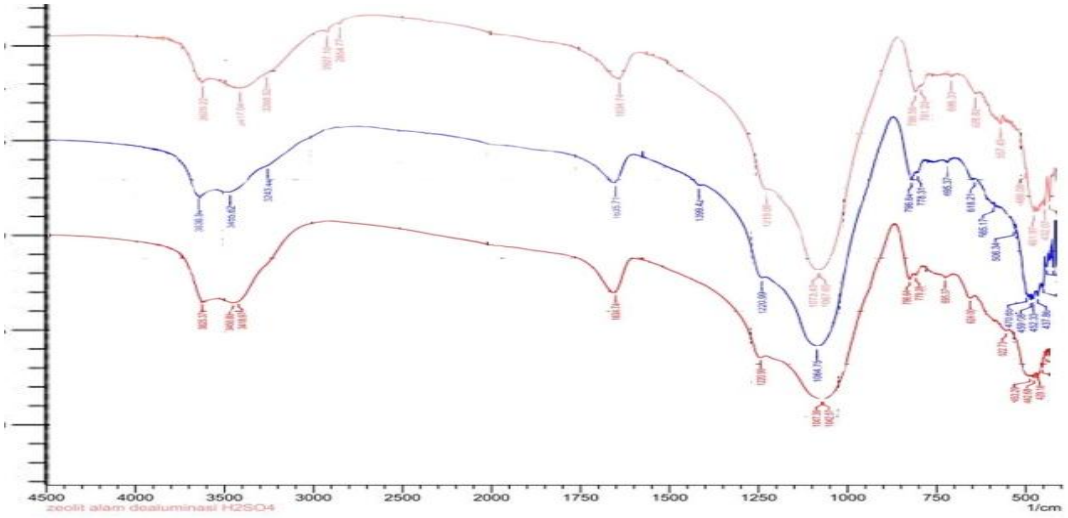
other cations so that all the cations will be exchanged to be H^+. While $KMnO_4$ needs to be added because it is able to function as an oxidizer and able to react with acids to remove impurities [7, 11].

As a result of the addition of acids, there will be interactions between acids and Zeolites. The interaction of acids with zeolite surfaces results in the release of alumina species from zeolites. H^+ ions derived from acids affect the free electrons in the O atom to form coordination bonds. Al-O group will lack electrons so that it will be more polar and not as strong as before, so Al will break from its bonds [7, 11].

In general the range of wave numbers 300-1300 cm^{-1} in the form of tetrahedral bonds, namely O-Si-O and O-Al-O. On the band 900-1250 cm^{-1} is an asymmetrical range, the symmetrical range is shown on the band 650-850 cm^{-1}, bending the Si-O / Al-O (TO) internally appears in the area of 420-500 cm^{-1} while for the external will appeared at 700-780 cm^{-1} [11-13]. The spectra in Figure 1 are the results of the FTIR (Fourier Transform Infra-Red) spectrometer analysis of natural zeolites and zumolite dealumination.



* H_2SO_4 dealuminated natural zeolite *** HCl dealuminated natural zeolite** ***natural zeolite**

Figure 1. IR spectrum of natural zeolite H_2SO_4 dealuminated, natural zeolite HCl dealuminated and natural zeolite

Interpretation of FTIR spectrum can be seen from several uptake. Dealumination occurs when internal stretching vibrations increase and external stretching vibrations decrease in wave numbers. The absorption band in the region of wave number 420-300 cm^{-1} shows the pore opening. Natural zeolite is at wave number 322.13 cm^{-1}, natural zeolite dealumination H_2SO_4 is at 313.45 cm^{-1} and HCl is at 316.34 cm^{-1}. Pore opening belongs to the zeolite external braid vibrational spectrum [12, 14-15]. From the FTIR spectra produced, it turns out that external braid vibrations have decreased which indicates a dealumination reaction in natural zeolites [12, 14-15].

Furthermore, stating that internal vibrations associated with TO4 tetrahedral is the main structural unit. The intended spectrum is in the FTIR Spectra in the area of 950-1250 cm^{-1}. These wave numbers represent the internal interwoven tetrahedral asymmetrical stretching vibration and the external interwoven asymmetric stretching vibration. This uptake increased the wave number from natural zeolites by 1042.57 cm^{-1} to 1072.47 cm^{-1} in the H_2SO_4 dealumination zeolite and 1064.75 cm^{-1} in the deaaluminated HCl zeolite [12, 14-15]. This shift in FTIR uptake is due to dealumination in natural

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zeolites, resulting in tetrahedral stretching vibrations in buckling T-O (T = Si, Al). From this increase in wave number, H_2SO_4 natural zeolite has the sharpest increase compared to HCl dealumination natural zeolite, this shows that H_2SO_4 dealumination is more effective than HCl [12, 14-15].

At wavenumber 3450.80 cm^{-1} on natural zeolite, wave number 3417.04 cm^{-1} on natural zeolite delauminated H_2SO_4, and wave number 3455.62 cm^{-1} on zeolite dealumination HCl, indicating the existence of a stretching -OH bond. This group provides information that there is water (hydrate) that is owned by zeolite crystals [12, 14-15]. Table 1 is a table of the results of the natural zeolite dealumination with the natural zeolite dealumination of hydrochloric acid and sulfuric acid.

Table 1. Ratio Si/Al

No	Sample	Ratio Si/ Al
1	Natural Zeolite	4.5662
2	Natural Zeolite HCl dealuminated	5.4098
3	Natural Zeolite H_2SO_4 dealuminated	6.4159

Unrealizedum natural zeolite has the lowest Si / Al ratio of 4.5662. It can be said that for the same time and temperature the use of H_2SO_4 with a Si / Al ratio of 6.4159 as a dealumination agent is more effective than HCl with a Si /Al ratio of 5.4098. This result has similarities with research conducted by Sriatun (2005) where dealumination with H_2SO_4 is more effective than HCl [11-14].

Adsorption activity test for zeolite dealumination in adsorption of domestic wastewater is done by mixing samples (100 mL domestic wastewater) and zeolites (1 gram) which are heated at 40^{o}C in a three neck flask equipped with a thermometer and stirrer. Stirring is carried out at optimum temperature (40 ^{o}C) because it refers to several previous studies [11, 13]. By stirring and heating at the optimum temperature, it is expected that the impact intensity between the reactants will increase and reach the optimum point.

Domestic Wastewater measurement results from the 3 villages in Tembalang sub-district (Kramas sub-district, Tembalang sub-district and Meteseh sub-district) (with 3 measurements, averaged) and the effectiveness of the adsorption performance of natural zeolites (ZA), zeolites activated by HCl (ZT 1) and zeolites activated with H_2SO_4 (ZT 2) can be seen in Table 2.

The results of the Domestic Kramas Village Domestic Waste Water analysis were compared with the Standards of Quality Standards [1] and the effectiveness of the adsorption performance of natural zeolites (ZA), zeolites activated by HCl (ZT) 1) and zeolites activated by H_2SO_4 (ZT). 2). The adsorption capacity of the three adsorbents is measured by several parameters, such as pH, temperature, COD, BOD and TSS as shown in Table 2.

Table 2. Results of Domestic Waste Water analysis in Kramas village and the effectiveness of adsorption performance from natural zeolites (ZA), zeolites activated with HCl (ZT 1) and zeolites activated with H_2SO_4 (ZT 2)

Parameter	Unit	Method	Quality Standart	Result	Adsorption Effectiveness		
					ZA	ZT 1	ZT 2
pH	-	SNI 06.6989.11.2004	6 – 9	7.4	11.5%	16.2%	17.9%
Temperature	^{o}C	SNI 06.6989.23.2005	-	27.3	-	-	-
COD	mg/L	SNI 6989.2.2009	50	70.6	21.8%	78.9%	85.5%
BOD	mg/L	SNI 06.6989.72.2009	100	47.8	9.2%	11.8%	12.6%
TSS	mg/L	SNI 06.6989.10.2004	30	38.8	10.8%	44.4%	49.7%

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The results of the Domestic Tembalang Village Domestic Waste Water analysis were compared with the Standards of Quality Standards [1] and the effectiveness of the adsorption performance of natural zeolites (ZA), zeolites activated by HCl (ZT) 1) and zeolites activated by H_2SO_4 (ZT). 2). The adsorption capacity of the three adsorbents is measured by several parameters, such as pH, temperature, COD, BOD and TSS as shown in Table 3.

Table 3. Results of Domestic Waste Water analysis in Tembalang and the effectiveness of adsorption performance from natural zeolites (ZA), zeolites activated with HCl (ZT 1) and zeolites activated with H_2SO_4 (ZT 2)

Parameter	Unit	Method	Quality Standart	Result	Adsorption Effectiveness		
					ZA	ZT 1	ZT 2
pH	-	SNI 06.6989.11.2004	6 – 9	7.5	11.5%	16.2%	17.9%
Tempertaure	^oC	SNI 06.6989.23.2005	-	27.3	-	-	-
COD	mg/L	SNI 6989.2.2009	50	86.7	21.8%	78.9%	85.5%
BOD	mg/L	SNI 06.6989.72.2009	30	59.6	9.2%	11.8%	12.6%
TSS	mg/L	SNI 06.6989.10.2004	50	56.8	10.8%	44.4%	49.7%

The results of the Domestic Meteseh Village Domestic Waste Water analysis were compared with the Standards of Quality Standards [1] and the effectiveness of the adsorption performance of natural zeolites (ZA), zeolites activated by HCl (ZT) 1) and zeolites activated by H_2SO_4 (ZT). 2). The adsorption capacity of the three adsorbents is measured by several parameters, such as pH, temperature, COD, BOD and TSS as shown in Table 4.

Table 4. Results of analysis of Domestic Wastewater Meteseh and effectiveness of adsorption performance of natural zeolites (ZA), zeolites activated with HCl (ZT 1) and zeolites activated with H_2SO_4 (ZT 2)

Parameter	Unit	Method	Quality Standart	Result	Adsorption Effectiveness		
					ZA	ZT 1	ZT 2
pH	-	SNI 06.6989.11.2004	6 – 9	7.3	11.5%	16.2%	17.9%
Temperature	^oC	SNI 06.6989.23.2005	-	27.3	-	-	-
COD	mg/L	SNI 6989.2.2009	50	71.2	26.4%	78.9%	85.5%
BOD	mg/L	SNI 06.6989.72.2009	30	49.1	9.2%	11.8%	12.6%
TSS	mg/L	SNI 06.6989.10.2004	50	46.6	11.7%	44.4%	49.7%

As observed in Table 2, Table 3 and Table 4, the measurement results for pH, COD, BOD and TSS are relatively higher in Tembalang kelurahan compared to 2 other kelurahan (kelurahan Kramas and kelurahan Meteseh). This can be understood, because the population density in Tembalang is indeed the highest. The existence of Diponegoro University students, Semarang State Polytechnic, Dental Care Academy, Pandanaran University and others as well as the existence of food and beverage stalls adjacent to the college campuses, greatly affect the population density in the Tembalang village. So that the need for clean water and discharge of domestic wastewater that is also becoming even greater.

The degree of acidity (pH) is a description of the acidic or basic state of a solution. If the pH of the solution <7 means that the solution is acidic, whereas if the pH of the solution> 7 means that the solution is basic. So that pH can describe the acidity and alkalinity of a solution [16]. The pH values in

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these 3 villages (both Tembalang and Kramas and Meteseh) still meet the standards of the Quality Standards [1]. However, domestic wastewater in Tembalang is relatively higher This may be caused by the presence of some waste water from the Toilet Washing Toilet (MCK) that has not been processed and is immediately discharged into the environment.

Chemical Oxygen Demand (COD) is the amount of oxygen needed by chemicals to neutralize organic compounds dissolved in water. In Tembalang kelurahan the measurement result of Chemical Oxygen Demand (COD) is 86.7 (mg / L) (higher than Kramas kelurahan 70.6 (mg / L) and Meteseh village 71.2 (mg / L)). This is due to the amount of population density in the Tembalang village which is more, so that the discharge of waste water from the Mandi Wash Kakus (MCK) and several food / beverage stalls also increases. The higher the COD value in a solution, the worse the quality of the water. Usually excessive organic matter content in water, resulting in turbidity, odor and the color of the solution that is no longer clear [17]. If the price of COD in a waters exceeds the specified threshold value, this can result in the destruction of ecosystems in the waters, even if this is not properly addressed will worsen the quality of the aquatic environment [18-21]. To avoid this kind of thing, it is necessary to treat / treat domestic wastewater before being discharged into sewers, so as not to pollute the environment [18, 21-22].

Biologycal Oxygen Demand (BOD) is the amount of oxygen needed by microorganisms to neutralize organic compounds dissolved in water. In Tembalang kelurahan, the measurement result of Biologycal Oxygen Demand (BOD) is 59.6 ppm (mg / L) (higher than Kramas kelurahan, 47.8 (mg/ L) and Meteseh village 49.1 (mg / L)). The higher the population density in the Tembalang village, the discharge of waste water from Mandi Cuci Kakus (MCK) and several food / beverage stalls also increased. The higher the BOD value in a solution, the worse the quality of the water. Usually excessive organic matter content in water, resulting in turbidity, odor and the color of the solution that is no longer clear [18]. Microorganisms with sufficient oxygen conditions are able to degrade organic compounds (proteins, fats, carbohydrates, etc.) dissolved in domestic wastewater into simpler molecules. If the BOD price in a waters exceeds the specified threshold value, this can result in the destruction of ecosystems in the waters, even if this is not properly addressed will worsen the quality of the aquatic environment [18-21]. To avoid this kind of thing, it is necessary to treat / treat domestic wastewater before being discharged into sewers, so as not to pollute the environment [18, 21]. However, the presence of oxygen in waters that contain lots of plants is enough to help to increase oxygen levels in these waters. The main source of oxygen is the result of plant synthesis through roots in the soil, then oxygen due to flowing water or rainwater can cause oxygen to dissolve in water [18, 22].

Total Suspended Solid (TSS) is derived from natural sources, garbage, agricultural runoff water, fisheries, urban areas and industry. In Tembalang kelurahan, the total Suspended Solid (TSS) measurement result was 56.8 (mg / L) (higher than Kramas kelurahan, 38.8 (mg / L) and Meteseh sub-district 46.6 (mg / L)). If wastewater that is channeled into drains / gutters contains high TSS levels, it will cause water to become turbid and will also cause an increase in temperature in the water, as a result it can reduce oxygen dissolved in water, thus disrupting the life (biota) of aquatic habitat. High TSS levels result in the blocking of sunlight to water bodies, so that the photosynthesis process of plants in these waters becomes disturbed [18, 22].

4. Conclusion

The results of the IR spectrogram and the zeolite Si / Al ratio determine that zeolite acid dealumination will show a significant change in wave numbers and Si / Al ratio, where the natural zeolite Si / Al ratio of 4.57 rises to 5.41 in natural zeolites dealumination HCl and become 6.42 on natural zeolite dealumination H_2SO_4. With natural zeolite adsorbent, HCl activated zeolite and H_2SO_4 activated natural zeolite are the average results of the reduction of domestic wastewater in 5 villages in Tembalang sub-

district with chemical parameters COD, pH, BOD, and TSS are as follows. With natural zeolite the COD reduction was 21.8%, pH 11.5%, BOD 9.2% & TSS 10.8% and with zeolite activated HCl, COD reduction 78.9%, pH 16.2%, BOD 11, 8% & 44.4% TSS and 85.5% COD reduction, 17.9% pH, 12.6% BOD and 49.7% TSS for H_2SO_4-activated natural zeolites.

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Characteristic Changes and Antibacterial Activities of Liquid Soap from Nyamplung Seed Oil (*Calophyllum inophyllum L*) Due to Storage

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Abstract. The storage time of antibacterial liquid soap from nyamplung seed oil (*Calophyllum inophyllum* L) has been determined. The stability of antibacterial liquid soap during storage greatly affects the quality of the product. The purpose of this study was to determine the relationship between the characteristics of antibacterial liquid soap to the time of storage and determine the limit of storage time. Characterization was determined for 12 weeks. The characteristic parameters were the total fatty acids, free fatty acids, neutral fat, pH, specific gravity and foam stability. Using correlation test analysis, the results showed correlation between storage time with free fatty acids, pH and neutral fat. Decreasing antibacterial activity during storage only 5.5%.

Keywords: Antibacterial, Nyamplung, seed oil, *Calophyllum inophyllum L*

1. Introduction

Nyamplung is a plant that is widely found on the coast of Java. Nyamplung seeds contain high levels of oil (70%) that is higher than other plants, such as jatropha (40-60%) and palm oil (46-54%) [1]. Nyamplung seed oil has been used as a basic ingredient in soap production. Nyamplung seed oil contains xanthone antibiotics that play an active role as an antibacterial that can inhibit *S. aureus, P. aeruginosa, B. subtilis, S. typhimurium*, and *K. pneumonia* [2]. Research on antibacterial soap from nyamplung seed oil states that soap from nyamplung seed oil has antibacterial activity against *S. Aureus* and *E.coli* [3]. Soap is a cleaning product to clean dirt and bacteria. The soap can be divided into antioxidant, antibacterial, antifungal and beauty soap [4] . The form of soap varies from solid, liquid, foam, cream or gel, and powder. Liquid soap is preferred because it is more hygienic in storage and more practical in use [5].

Soap has a time limit for use called storage time. It is the time between starting production until the quality of product decrease. Soap products that have a low quality cannot be used anymore, because it cause irritation, itching, and skin diseases [6]. Previous research did not examined the effect of storage time on the quality of soap. This research determine the characteristics and antibacterial activity of liquid soap from nyamplung seed oil due to storage time so that the soap products are safe to use.

2. Materials and methods

2.1. Materials

The research used nyamplung seeds oil, ammonium sulfate, potassium hydroxide, carboxy methylcellulose, sodium lauryl sulfate, hydrochloric acid, sulfuric acid, S. aureus bacteria, NA media (Nutrient Agar), NB media (Nutrient Broth), and tetracycline

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2.2. Preparation of antibacterial liquid soap from nyamplung seed oil

Nyamplung seed oil (300 g) was heated until the temperature reached 70 °C. 150 grams of 30% KOH solution (b/v) were added. The mixture were heated for 1 hour. When the temperature decline to 60 °C, deionized water were added (1:1). Stirring continues until homogeneous. Additive such as CMC (2.25 g), SLS (4.5 g), and deodorizer (8 g) were added. The stirring process used 500 rpm for 10 – 15 minutes.

2.3. Characterization of soap products

The soap was divided into 13 containers and stored based on variations in Table 1. The characterizations that carried out during storage time were the determination of the total amount of fatty acids, free fatty acids, neutral fats, specific gravity, foam stability and pH.

Table 1. Storage time variations

Storage time	Days
T0	0
T1	7
T2	14
T3	21
T4	28
T5	35
T6	42
T7	49
T8	56
T9	63
T10	70
T11	77
T12	84

2.4. Antibacterial activity test

The antibacterial activity test was carried out by pouring 15 mL of Nutrient Agar (NA) medium at ± 37 °C into sterile petri dish then allowed at room temperature until the medium solidify. A bacterial cultures in Nutrient Broth (NB) medium are taken and dispersed in Nutrient Agar (NA) medium. The volume of bacteria that was taken based on the absorbance results at 600 nm. If the absorbance value is less or equal to 0.5 then te volume is taken 100 µL bacterial and if 0,6-1,0 the volume is taken 50 µL. The suspension of the test bacteria on Nutrient Agar (NA) medium is distreaked on the spread plate using drugalsky, then allowed for 15 minutes at room temperature. After drying, a paper disc with a diameter of ± 6 mm was placed over the NA medium. Samples and controls were taken 10 µL and dripped onto paper disc then incubated for 24 hours at 37 °C. Then measured the inhibitory diameter formed around the disc paper.

3. Results and discussion

3.1. Characteristics of antibacterial soap from nyamplung seed oil

3.1.1. Total fatty acid

Total fatty acid is all fatty acid contained in soap, which have reacted or not reacted with alkalis. Total fatty acids during storage can be seen in Figure 1.

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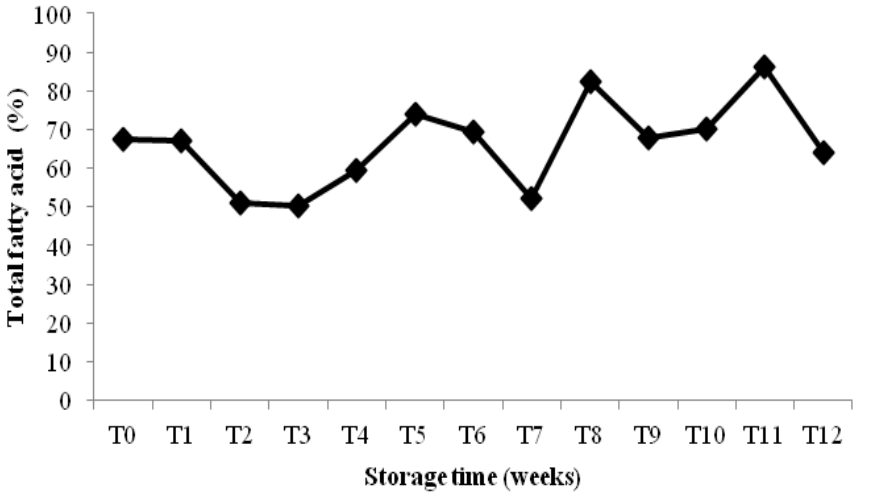

Figure 1. Total fatty acid of antibacterial soap from nyamplung seed oil during the storage

The total fatty acids showed fluctuation with values around 50.14% - 86.36%. The total fatty acids changes because the presence of oxygen which causes oxidation. The longer storage time, the higher oxygen pressure that causes increasing the rate of oxidation of fatty acids [6]. The oxidation reaction will cause the fatty acids to break into aldehydes, ketones and free fatty acids. The amount of fatty acids and free fatty acids is related to pH, that the higher fatty acids the lower pH.

3.1.2. Free fatty acid

Free fatty acid is fatty acid which have not react with potassium. Good quality soap contains a small amount of fatty acids. The results of the characterization of free fatty acids during storage time can be seen in Figure 2.

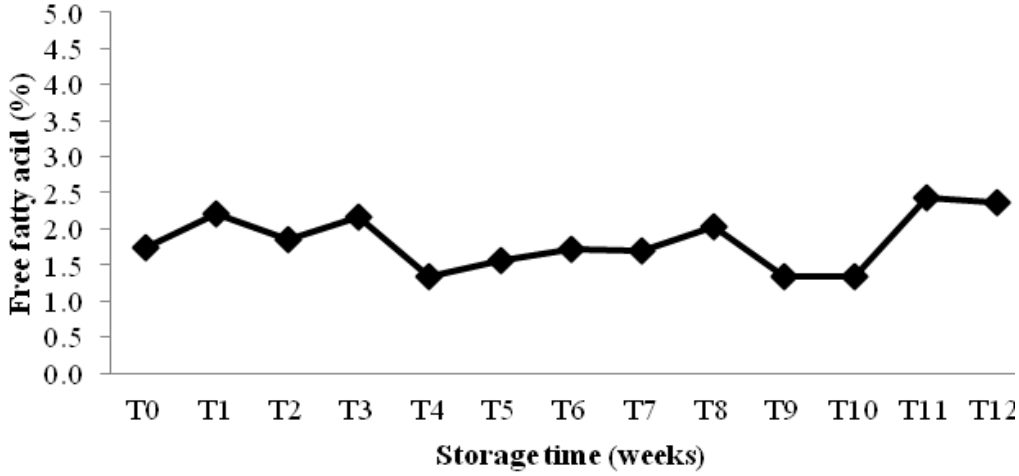

Figure 2. Free fatty acid of antibacterial soap from nyamplung seed oil during the storage

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During storage, free fatty acids show a fluctuating in the range of values of 1.34% - 2.42%. Free fatty acids have increased due to oxidation and hydrolysis reactions during storage. The hydrolysis reaction occurs because of the presence of water in the product, while the oxidation reaction in the double bond also produces free fatty acids [6]. Unsaturated fatty acids break down due to heating, because hot oil or fat comes in direct contact with air. The carbon chain in the double bond is broken so that free fatty acids increase. The effect of storage time on free fatty acids shows a correlation. High free fatty acids also cause low of pH.

3.1.3. Neutral fat

Neutral fat is fat that does not react with KOH to produce soap. A good soap must contain a small neutral fat. The results of neutral fat during storage can be seen in Figure 3.

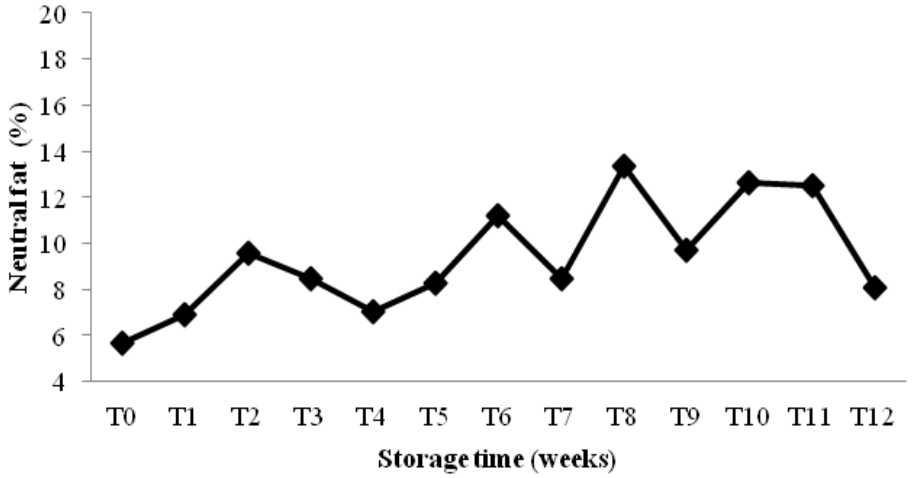

Figure 3. Neutral fatty of antibacterial soap from nyamplung seed oil during the storage

During storage, neutral fat shows fluctuating results with values 5.66% - 13.33%. The high neutral fat is due to natural nyamplung oil properties. Nyamplung oil contain high neutral fat [1]. Neutral fat can be sterols, dyes, hydrocarbons, and complex lipids. The making of this soap uses essential oil fragrance that containing terpenoid which are including one of the neutral fats.

The results of data analysis regarding the effect of storage time on neutral fat using the correlation method showed a correlation. The significance value is 0.020, which means less than 0.05, then there is a correlation between the two variables. The value of the degree of relationship is 0.634, which means the correlation is strong, and a positive sign indicates data that tends to rise.

3.1.4. pH

pH is one of the important parameters. pH is used as a parameter whether a soap is safe to use because a low pH has the potential to cause irritation such as sores, itching, or peeling of the skin. Soap generally has a pH of around 10 [7]. The results of measuring the pH of the soap during storage can be seen in Figure 4.

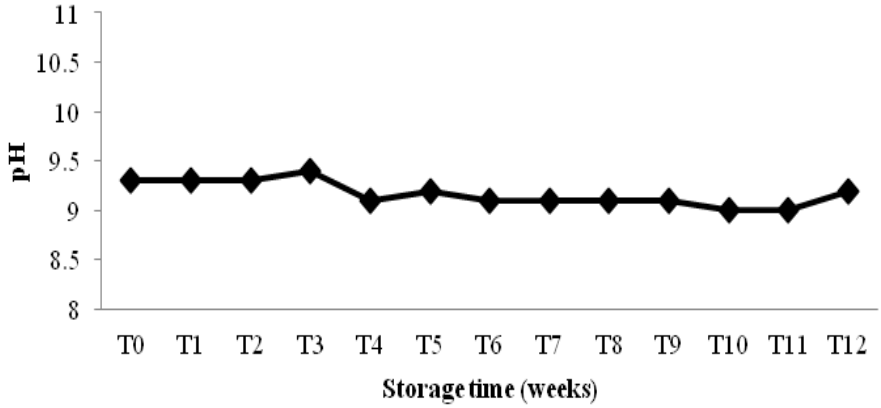

Figure 4. pH of antibacterial soap from nyamplung seed oil during the storage

Figure 4 shows that during storage time, pH decreased fluctuatively with a value of about 9.0 - 9.4. This can be caused by the increasing free fatty acids during storage time which causes soap getting more acidic. A good soap must have pH 8-11. pH which is below 8 or above 11 has a bad effect when used on the skin, it will cause irritation [8].

The results of data analysis regarding the effect of storage time with the pH generated using the correlation method shows the correlation. The significance value is 0.004, which means it is smaller than 0.05, then it is correlated. The value of the degree of relationship is -0,736 which means strong correlation, and negative sign means the data shows a decrease.

3.1.5. Spesific gravity

Specific gravity is the ratio of the weight of substances in the air at 25 °C to the weight of water with the same volume and temperature [9] . Measurement of specific gravity was conducted by comparing the weight of the sample with the weight of distilled water at room temperature using a pycnometer. The type of soap weight during storage can be seen in Figure 5.

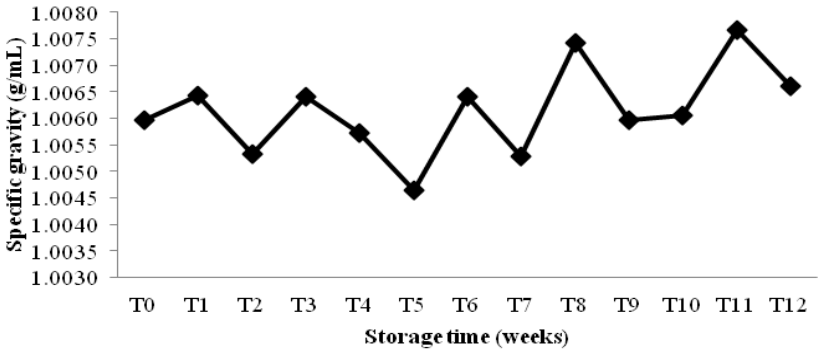

Figure 5. Spesific gravity of antibacterial soap from nyamplung seed oil during the storage

During storage, the specific gravity data showed a fluctuating increase in the range of values of 1.0053 - 1.0077 g/mL. Specific gravity is affected by the mass and the volume of the solution. The mass of soap during storage does not change because the soap is stored in a closed container and at room temperature. Based on the law of conservation of mass (Lavoiser's law), the mass of an object will remain

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constant if stored in a closed container and as long as storage is not reduced or other ingredients are added. The volume of soap during storage can be reduced due to shrinkage of foam that is still present in the soap. A fixed mass and a reduced volume cause the specific gravity during storage to increase. The results of data analysis regarding the effect of storage time with specific gravity using the correlation method showed no correlation or no significant difference. The significance value is 0.163 which is greater than 0.05, meaning that the two variables are not correlated.

3.1.6. Foam stability

Abundant and stable foam soap is preferred. Foam characteristics are influenced by active ingredients of soap or surfactants or foaming stabilizers. Foam stability during storage can be seen in Figure 6.

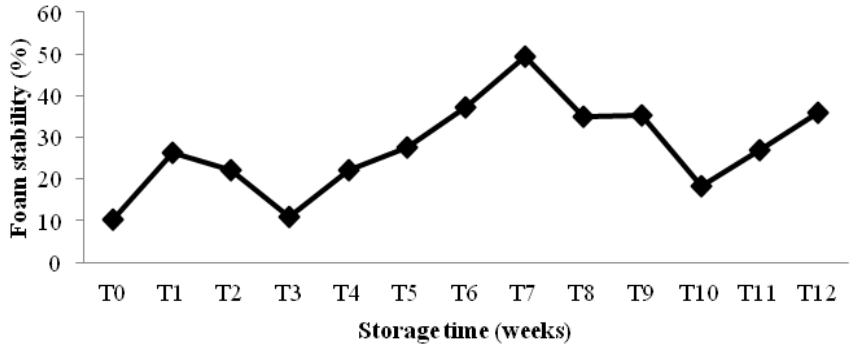

Figure 6. Foam stability of antibacterial soap from nyamplung seed oil during the storage

During storage, foam stability showed a fluctuating increase data, with a range of values of 10.31-49.30%. The stability of the foam depends on the physical and chemical properties of the surfactant used. Sooner or later the foam will break, due to the depletion of the liquid layer that forms foam [5]. The results of data analysis about the effect of storage time to foam stability using the correlation method showed no correlation or no significant effect. The significance value obtained is 0.077, it is higher than 0.05 that means it does not correlate.

3.2. Antibacterial activity

The liquid soap antibacterial activity test was conducted to determine the ability of soap to inhibit bacteria before and after storage. This test is carried out at the 0 and 12 weeks. The bacteria are S. aureus bacteria, because these bacteria usually infect the skin. Tetracycline is used as a positive control and distilled water as a negative control. Tetracycline is an antibiotic that can inhibit gram-positive and gram-negative bacteria by inhibiting protein synthesis. A distilled water is used as a negative control so that it is known that as a comparison the solvent used does not affect the results [3]. The results of the antibacterial test can be seen in Table 2.

Table 2. Antibacterial activity test results

Sample	Inhibitory diameter (mm)
Aquades (-)	-
Tetrasiklin (+)	35.08
0 week	15.68
12 week	14.81

The results showed that distilled water has no effect to the antibacterial activity. Tetracycline as a positive control produces an average inhibition diameter of 35.08 mm. This value is greater than the zero week soap and the 12 week soap. The zero week soap produced an average inhibition diameter of 15.68 mm whereas for 12 week soap was 14.81 mm. These results indicate that storage decrease the antibacterial activity about 5.5%. The decreasing caused by the presence of light and oxygen which cause oxidation process.

4. Conclusion

The storage time affects free fatty acids, pH and neutral fat of liquid soap from Nyamplung seed oil. Antibacterial activity decreased by 5.5% during storage time.

Acknowledgments

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Antioxidant Activities of Ethanol Extraction Product from Citronella Grass (*Cymbopogon nard*us) Distillation Residue

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Abstract. Indonesia's floral diversity provides huge reserve for traditional medicinal raw materials, one of which is citronella grass *(Cymbopogon nardus)*. This study aims to identify the content of secondary metabolites in its ethanol extraction product from distillation residue and determine its antioxidant activity. Ethanol extraction product of the distillation residue was blackish red in color with a yield of 8.882%. Phytochemical screening on residual powder and EDR (distillation residue ethanol extraction product) showed positive results for flavonoids, tannins, quinones, phenols and steroids. Total polyphenolcontent test obtained a concentration of 45.955 mg gallic acid equivalent/g EDR while EDR antioxidant activity test obtained IC_{50} value of 189.905 ppm.

Keywords: antioxidant, *Cymbopogon nardus*, distillation residue, ethanol extraction product (EDR)

1. Introduction

Indonesia's floral diversity burgeoning traditional medicinal raw materials. Traditional medicines derived from plants have long been widely known and used by the people of Indonesia. The potency of medicinal plants is due to the presence of secondary metabolites which are present in these plants [1]. Among them is fragrant lemongrass which possesses many benefits. Fragrant lemongrass or citronella has traditionally been known to have the ability of treating sore throat, helping digestion, medicating worm, reducing fever, shed sweat, diuretics, and ease menstruation [2]. Fragrant lemongrass extract also has antioxidant properties [3]. Fragrant lemongrass leaves are extracted for its essential oils and they are widely used in various industrial fields [4]. Citronella essential oil also has a greater insecticidal activity than ethanol extract [5].

The production of citronella oil requires very large amounts of base material; around 20 tons to produce 160 liters of oil. Abundant essential oil distillation residues have the potential to be utilized optimally. Utilizations residues from fragrant citronella essential oil distillation have been limited to the use as animal feed, distillation fuel and fertilizer [6]. Previous research on chloroform extract of fragrant citronella distillation residues showed antibacterial activity [7]. Considering the potential and abundance of fragrant citronella essential oil distillation residues there is an appeal in identifying the content of secondary metabolites in the ethanol extract of distillation residues and determining their antioxidant activity. This research is hoped to provide input to increase the utilization of distillation residues that have not been optimal up to this moment. In this study, the sample used was the residue of lemongrass leaves which had been extracted for its essential oil.

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2. Materials and Method

2.1. Materials and equipment

The materials used were 4 kg of fresh citronella leaves, 96% technical ethanol, technical methanol, petroleum ether, methanol p.a (Merck), ethanol p.a (Merck), chloroform p.a (Merck), aquadest, $_{FeCl3}$, ammonia (Merck), Mg powder, H_2SO_4 (Merck), Dragendorff reagent containing a solution of potassium bismuth iodide (Merck), Mayer reagent containing mercury chloride and potassium iodide (Merck), amyl alcohol p.a (Merck), acetone (Merck), NaOH (Merck), HCl (Merck), Folin-Ciocalteu (Merck) reagents, gallic acid (Sigma), Na_2CO_3 (Merck), DPPH (Merck) and quercetin (Merck).

The equipment used are macerator, standard laboratory glass wares, filter paper, stirrer, blender (Cosmos), water bath, vial bottle, electric heater, analytic balance (OHAUS), UV-Vis spectrophotometer (SHIMADZU UV-1280), and rotary evaporator (Buchi-B480).

2.2. Phytochemical residue screening

The distillation residue of fragrant lemongrass leaves was dried through aeration at room temperature and then turned into powder. The powder was later tested for alkaloids, saponins, flavonoids, tannins, quinones, steroids, terpenoids and phenols contents [8]. The same test was carried out on ethanol extraction product of the distillation residue [9].

2.2.1. Alkaloid test

As much as 5 gram of powdered fragrant lemongrass distillation residue is moistened with 5 mL of 25% ammonia and crushed in porcelain crucible. The resulting product was then added with 20 mL of chloroform and again crushed firmly. Next, filtering was carried out to separate the filtrate and the residue. For alkaloid examination, the filtrate was extracted using liquid-liquid extraction with HCl 2N where the two layers formed were separated. The top layer was divided into two equal amounts to be tested with Dragendorff and Mayer reagents. The portion which was tested with Dragendorff reagent would form brick red deposits as a positive test while the portion that was tested with Mayer reagent would form white deposits if alkaloids exist.

2.2.2. Saponin test

As much as 5 g of distillation residue powder was boiled in 100mL of water for 5 minutes then filtered in hot condition. Then 10 mL of the solution was shaken strongly in a vertical manner for 10 seconds. Positive results were shown through the appearance of foam whereinwith the addition of 1% HCl the foam remained stable.

2.2.3. Flavonoid test

As much as 10 grams distillation residue powder was added with 100 mL distilled water and then heated until boiling. The filtrate obtained was taken as much as 5 mL that was then added with Mg powder, 1 mL concentrated HCl, and 2 mL amyl alcohol. The mixture was shaken and left to allow separation where an amyl alcohol layer would be formed. Red, yellow or orange deposits in the amyl alcohol layer indicate positive results [10].

2.2.4. Tannin/ Phenolic compounds test

As much as 1 gram of distillation residue powder was filtered with 10 mL of distilled water. The filtrate was then diluted with distilled water until it is colorless. 2 mL of the solution was taken and added with 1 to 2 drops of iron (III) chloride reagent. Blue or blackish green color formed indicates the presence tannin.

2.2.5. Quinone Test

As much as 1 g of distillation residue powder was boiled in 10mL of water for 5 minutes, then cooled and filtered. Into 5 mL of the filtrate produced, NaOH 1N solution was added. Red would form if quinones exist.

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2.2.6. Triterpenoid and Steroid Tests

As much as 5 gram of distillation residue powder was macerated with 20 mL ether for 2 hours and then filtered. As much as 5 mL of the filtrate was evaporated in an evaporator plate to dry and was given 2 drops of acetic acid anhydride and 1 drop of concentrated sulfuric acid. The formation of blue/green color indicates the presence of steroids while the formation of red/purple indicates triterpenoid.

2.3. Extraction of distillation residue with ethanol

As much as 200 grams of distillation residue powder was macerated using 96% technical ethanol solvent until the solution turned close to clear. The solvent was replaced every 24 hours. The extract obtained was then concentrated to obtain distillation residue ethanol extraction product (EDR).

2.4. Total phenol content test of distillation residue ethanol extraction product [11, 12]

2.4.1. Gallic acid calibration curve formation using folin-ciocalteu reagent

As much as 10 mg of gallic acid was added with 10 mL of methanol p.a so that a concentration of 1000 ppm was obtained. Concentration variations of 125, 100, 75, 50 and 25 ppm were then made. From each concentration, 0.5 mL was taken and added with 0.5 mL of distilled water and 2.5 mL of Folin-Ciocalteu reagent to be homogenized and left for 15 minutes. 7.5% Na_2CO_3 solution was later added as much as 2 mL and then left for 30 minutes. The solution was measured for its absorbance with a UV-Vis spectrophotometer at 765 nm and a linear regression curve was created to represent the relationship between the concentrations of the standard gallic acid and absorbance.

2.4.2. Total Phenol Content Determination through Folin-Ciocalteu Method

A total of 10 mg of EDR extract was dissolved with 10 mL of methanol to obtain a concentration of 1000 ppm. A total of 0.5 mL of 1000 ppm EDR solution was added with 2.5 mL of aquades and 2.5 mL of Folin-Ciocalteu reagent, homogenized and left to stand for 15 minutes. It was then added with 2 mL of 7.5% Na2CO3 and left for 30 minutes. The solution was measured for absorbance with a UV-Vis spectrophotometer at a wavelength of 765 nm.

2.5. Antioxidant activity test with DPPH method [13,14]

DPPH 0.1 mM solution was made by dissolving 3.9432 mg of DPPH powder into 100 mL of methanol. The DPPH control solution was measured for its absorbance at a wavelength of 517 nm. The EDR was made with a concentration of 250, 200, 150, 100 and 50 ppm while quercetin as a comparison was made at 50 ppm, 40 ppm, 30 ppm, 20 ppm and 10 ppm. A total of 3.8 mL of the 0.1 mM DPPH solution was added to 0.2 mL of EDR samples from each concentration. The mixture was homogenized and left for 30 minutes in a dark place. Then the absorbance was measured at a wavelength of 517 nm.

$$\%\,inhibition = \frac{Control\ absorbance(DPPH) - Sample\ absorbance}{Control\ absorbance} \times 100\% \quad (1)$$

IC_{50} is concentration of sample that able to reduce 50% of DPPH radical activity.

3. Results and discussion

3.1. Distillation residue extraction and phytochemical screening

Extraction of distillation residue of citronella leaves was carried out by maceration using ethanol solvents. The solvent was removed using Buchii evaporator so that the product of ethanol extraction from the distillation residue (EDR) was produced. The ethanol extraction residue obtained a yield of 8.882%. The results of phytochemical screening tests carried out on the powdered residue and ethanol extraction product of citronella leaves distillation residue (EDR) can be seen in Table 1.

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Table 1. Phytochemical screening test of powdered residue and ethanol extraction product of distillation residue (EDR)

Test	Powdered Residue	EDR
Flavonoid	+	+
Alkaloid	-	-
Tanin	+	+
Kuinon	+	+
Saponin	-	-
Fenol	+	+
Steroid	+	+

Description: + present; - not present

From the table above it can be seen that ethanol extraction product still contain many secondary metabolites such as flavonoids, tannins, quinones, phenols and steroids. The results are in accordance with a study conducted by Verawati et al [15]. The results indicate that the distillation residue ethanol extraction product has the potential to be used as a material to produce traditional medicine.

3.2. Total EDR phenol content test

The absorbance measurement results of the gallic acid standard solution can be seen in Table 2. A graph of a linear regression curve is made (as shown in Figure 1), representing the relation between the concentration of gallic acid standard solution and the absorbance, to be used to determine the total phenol content in the sample using the regression equation. The graph of the linear regression curve of the relationship between the concentrations of the standard solution of gallic acid and absorbance is shown in Figure 1.

Table 2. Phytochemical screening test of powdered residue and ethanol extraction product of distillation residue (EDR)

No.	Concentration (ppm)	Absorbance measurements		Average Absorbance
		1	2	
1.	25	0.172	0.172	0.172
2.	50	0.265	0.267	0.266
3.	75	0.387	0.385	0.386
4.	100	0.524	0524	0.524
5.	125	0.607	0.609	0.608

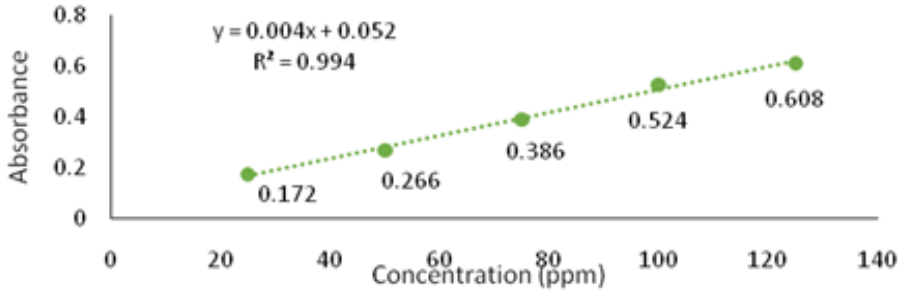

Figure 1. Graph of linear regression of the relationship between concentration of gallic acid standard solution and absorbance.

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Figure 1 shows a linear regression equation of the relation between the concentration of gallic acid standard solution and absorbance with y=0.0045x + 0.0522 and R^2 = 0.9946. Table 3 shows the results of absorbance measurements of EDR sample.

Table 3. Absorbance measurements of EDR sample using a UV vis spectrophotometer.

Concentration (ppm)	Absorbance Measurement (nm)		Average
	1	2	
1000	0.259	0.259	0.259

Analysis of total phenol content was carried out to determine the antioxidant potential of EDR as an antidote to free radicals. The chemical components that act as antioxidants are phenol and polyphenol compounds. Determination of total phenol content was carried out using the equation y = 0.0045x + 0.0522 of the linear regression curve of the relationship between the concentration of gallic acid standard and absorbance previously obtained. The total phenol content in the EDR sample obtained was 45.955 mg gallic acid equivalent/g EDR.

3.3. Antioxidant activity test with DPPH method

Antioxidant activity test of EDR sample was performed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method with a known antioxidant quercetin as a comparison. The DPPH test was done by measuring the absorbance and wavelength of DPPH solution in methanol. The DPPH solution in methanol is dark purple in color. The intensity of the color can be reduced or turn pale yellow when DPPH reacts with other compounds that donate protons. The addition of protons to the DPPH radical structure will cause a reduction in the formation of nonradical DPPH. The optimum DPPH wavelength obtained was 517 nm. This wavelength was used to measure the absorbance of EDR sample. The absorbance measurement results of various concentrations of quercetin compound are presented in Table 4.

Table 4. Absorbance measurements results of quercetin compound

Concentration (ppm)	Absorbance
10	0.902
20	0.673
30	0.523
40	0.296
50	0.213

The absorbance data above are converted into % inhibition of DPPH using equation (1). The data in Table 4 are graphed as % inhibition of DPPH against the concentration of quercetin compound. The graph of% DPPH inhibition against quercetin concentration is presented in Figure 2.

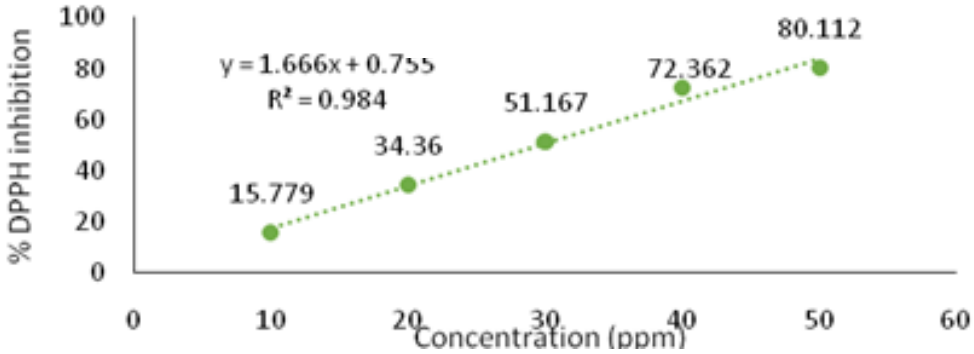

Figure 2. Graph of % DPPH inhibition against quercetin concentration

Table 5. Absorbance measurements results of EDR sample

Concentration (ppm)	Absorbance
50	0.914
100	0.796
150	0.616
200	0.496
250	0.392

Figure 2 shows a linear regression equation of the relationship between % inhibition of DPPH to the concentration of quercetin is obtained with y = 1.6667x + 0.7556 and R^2 = 0.9847. Based on calculation, the IC_{50} comparison of quercetin is 29.546 ppm. The results of absorbance measurement of various concentrations of the EDR sample are presented in Table 5. From Table 5 above, a graph of % inhibition of DPPH against EDR concentration is created. The graph of the relationship between % inhibition of DPPH and EDR sample concentration is presented in Figure 3.

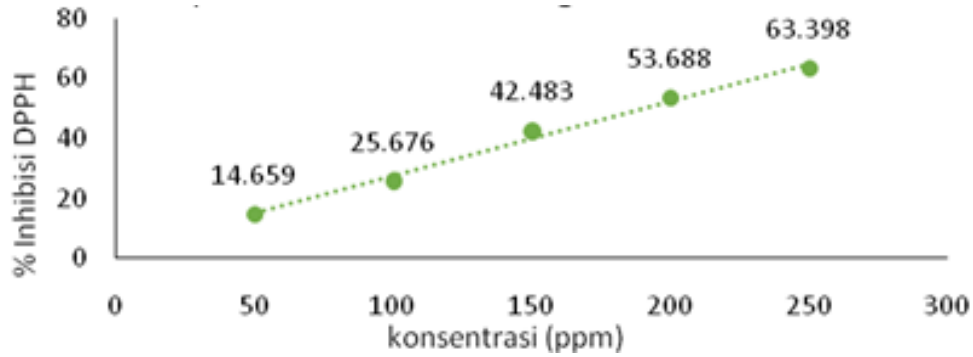

Figure 3. Graph of % DPPH inhibition against EDR concentration

Calculation of IC_{50} from the EDR sample is obtained from the linear equation y = 0.251x + 2.33338 with R^2 = 0.9915. The calculation obtained IC_{50} value of ethanol extraction product of 189.905 ppm. EDR sample shows relatively good antioxidant activity, because the price of IC_{50}<200 ppm (16). However, its antioxidant activity is much lower when compared to the quercetin which has an IC_{50} value of 29.546 ppm. This is because quercetin is a pure compound and has been shown to be an active

antioxidant compound, while EDR (ethanol extraction product of distillation residue from citronella leaves) still contains various secondary metabolites. The antioxidant activity in the EDR sample is produced by the presence of secondary metabolites which are still present in the distillation residues of fragrant lemongrass leaves, including phenolic and polyphenol groups which are known to have antioxidant activity [11]. From the results of the antioxidant activity test of the EDR sample, even though it cannot be classified as a strong antioxidant, the ethanol extraction product of the distillation residue of citronella leaves is still prospective as a natural antioxidant.

4. Conclusion

Ethanol extraction product of distillation residue of fragrant citronella leaves contain flavonoids, tannins, quinones, phenolics, and steroids. Its total phenol content amounts to 45.955 mg gallic acid equivalent/g EDR. EDR has the potential to be an antioxidant with an IC_{50} value of 189.905 ppm.

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