Study of the water hyacinth extract concentration to the characteristics of gel hand sanitizer

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Abstract. Hand hygiene is one of the most important things in the spread of bacteria and virus. The spread of this organism can be stopped by cleaning hands either by washing or sanitizing. Water hyacinth extract contain high flavonoid and tannin which can be used as an active agent in hand sanitizer. Three different extract concentration were examined which are 8, 10 and 12%. Organoleptic test were conducted to examine the shape, colour and scent of the hand sanitizer. Biological test were performed to find the effectiveness of the hand sanitizer against the bacteria. The results showed that hand sanitizer product with 8% water hyacinth extract have better potential in killing the bacteria compared to the other concentration.

1. Introduction
Indonesia is known as a rich archipelago with abundant natural resources [1]. It provides a great opportunity for Indonesia to optimize its utilization, especially in the health sector. Public health can become a measure to attribute the prosperity and welfare of a country. In fact there are still many people suffering from illness. One of the diseases that is often suffered is diarrhea [2,3]. The number of sufferers in Semarang itself is quite high, which is more than 30,000 cases. This is due to hand contamination that can be a vector for the transmission of microorganisms[4]. One of solution to prevent the microorganism transmission through human-human interactions, WHO suggested the use of alcohol based hand sanitizer [5].

Water hyacinth is a plant with a very high growth rate. Based on the data from the Department of Animal Farm and Fisheries in Semarang, Indonesia, the growth rate of water hyacinth reaches 7.1% per year with a growth interval of 37.6 times in 1 year [6]. Water hyacinth has various side effects, such as creating anoxic conditions in the lake, thereby increasing the level of toxicity and disease [7] damage to the ecosystem, increase the mosquito populations [8,9], threats to the function and biodiversity of aquatic ecosystems and fisheries [8], disruptions in irrigation systems, increased sedimentation [10] and cause increased water loss through evapotranspiration [11].

Instead of its negative effects, water hyacinth leaves extract contains flavonoids, alkaloids, tannins, phenols, which have biological activities such as antiviral, antifungal agents and antibacterial agents [12]. The presence of flavonoids and tannins in water hyacinth indicates that water hyacinth has the potential to be used as an antiseptic hand sanitizer gel (hand sanitizer) with low costs and abundant raw material.
The production of hand sanitizer is an interesting topic during this COVID-19 era. This paper will focus on the potential utilization of water hyacinth leaves extract as a natural antiseptic in the form of gel hand sanitizer. The characteristics of hand sanitizer will be evaluated such as the organoleptic, pH, homogeneity and antiseptic test.

2. Materials and Methods

2.1. Materials
In this research, water hyacinth leaves were obtained from Rawa Pening, Ambarawa, Indonesia. The chemicals used were Triethanolamine (TEA), carbopol 940, ethanol 70%, methylparaben, gliserin, aquadest, triclosan and nutrient agar were obtained from Indrasari, Semarang, Indonesia.

2.2. Preparation of Water Hyacinth Leaves
Water hyacinth leaves (Eichhornia crassipes) were cleaned by washing with clean water and was then weighed as much as ± 4 kg. The cleaned of water hyacinth leaves were then dried under the sun for 4 days; the water hyacinth leaves were considered dried when it became fragile (easily crushed by hands). Dried water hyacinth leaves were then mashed up using a blender became a dry powder. The dry powders were then weighed.

2.3. Water Hyacinth Leaves Extraction
100 grams of water hyacinth powder was weighed. 800 mL of Ethanol 70% was then poured into the aqua bottle and then the water hyacinth powder was then soaked for 24 hours in the ethanol solution and closed by using black plastic and stored in a dark place to avoid sunlight. The mixture was then filtered using a cloth and inserted into the glass bottles. The screening result in the glass bottle was then placed in the incubator with temperature of 80 °C for 6 hours. The water hyacinth extracts were then left to dry to remove the remaining solvent.

2.4. Production of Gel Hand Sanitizer
1 gram of Carbopol 940 was weighed and poured into the porcelain mortar. 20 mL of aquadest was then added ant the solution was then stirred rapidly in the mortar until the gel mass was formed. After that, 1.25 gram of TEA (Tri-Ethanolamine) was then added in a mortar and continuously stirred until the solution become homogeneous. After the solution become homogeneous, 0.1 grams of methyl parabens was weighed then dissolved in a 5 mL of aquadest, the solution was then inserted into the mortar. Next, 5 grams of glycerin was then added, solution was then stirred continuously. Then, 2 grams of triclosan was added. After that, water hyacinth extract then added with different concentration (8% w/v, 10% w/v and 12% w/v) into 18 mL of aquadest and then mixed with the solution in the mortar until homogeneous and crushed until gel was formed. The Gel was then stored into the bottle.

2.5. Characteristic Test of Gel Hand Sanitizer
Once the gel hand sanitizer was prepared, several characteristic tests were performed such as the organoleptic test, pH test, homogeneity and antiseptic test.

3. Results and Discussion

3.1. Organoleptic Test
Organoleptic test is a test that is often performed as a quality control of a dosage. The research uses 10 untrained panelists who are required to assess the smell, color, gel form, easily applied and do not contain the gel granules hand sanitizer through the provided questionnaire sheet. Every panelist get 4 types of gel hand sanitizer which is base gel and water hyacinth leaves extract gel, so panelists can feel...
the difference of the four types of gel directly. The results of organoleptic test of a gel hand sanitizer (*Eichhornia Crassipes*) can be seen in Table 1.

| Sample | Shape | Colour | Smell | Total | Average |
|--------|-------|--------|-------|-------|---------|
| 1      | 60    | 60     | 60    | 180   | 60      |
| 2      | 47    | 57     | 50    | 154   | 51.3    |
| 3      | 43    | 50     | 49    | 142   | 47.3    |
| 4      | 41    | 41     | 42    | 124   | 41.3    |

Notes: Sample 1 = gel Base; Sample 2 = water hyacinth concentration 8% w/v; Sample 3 = Concentration 10%; Sample 4 = Concentration 12%.

Based on Table 1, sample 2 gave the most preferable organoleptic test among the sample of water hyacinth leaves extract gel hand sanitizer. This sample has a characteristic of yellow color, easily applied to the skin and does not cause a sense of heat on the skin. This gel also does not have coarse grain and does not feel sticky on the skin, a little smell of green tea to cover the water hyacinth aroma also preferable.

### 3.2. pH Test

In this test, 2 grams of samples were weighed and then 20 mL of aquaest was added, and stirred. Once the solution is homogenized, pH measurement was done by dipping the calibrated pH meter in to the solutions. The pH of samples is presented in Table 2.

| Sample | pH   |
|--------|------|
| 1      | 3.37 |
| 2      | 3.22 |
| 3      | 5.61 |
| 4      | 3.17 |

Form Table 2, it can be seen that Sample 3 have the lowest pH which is close to neutral condition while the remaining samples have a pH value around 3. Based on the Indonesian National Standard No 06-2588, the range of pH value for hand sanitizer should be between 4.5-6.5[13]. According to Kulthanan et al. [14], a very low pH condition can cause skin irritation, while in very high pH conditions it can cause a scaly condition.

### 3.3. Homogeneity Test

Homogeneity tests were performed by plating each sample on different glass or any other suitable transparent material. The samples should show a homogeneous arrangement and invisible presence of coarse grain [15]. Results of homogeneity test can be seen in Table 3.

| Sample | Homogeneity          |
|--------|----------------------|
| 1      | Homogeneous, No Coarse Grain |
| 2      | Homogeneous, No Coarse Grain |
| 3      | Homogeneous, No Coarse Grain |
| 4      | Homogeneous, No Coarse Grain |
All water hyacinth gel hand sanitizer indicates the absence of coarse grain on the dosage, meaning that all the ingredients of the gel are well blended, so it can be said that they have good homogeneities.

3.4. Antiseptic Test
The antiseptic test on the sample of water hyacinth gel hand sanitizer was done by using the Total Plate Count (TPC) before and after using the hand sanitizer. TPC method does not identify the type of bacteria, but only counts the total number of bacterial colonies. The advantage of this technique is that the growing microbes can spread evenly on the media. The TPC results of bacteria can be seen in Table 4.

| Sample | 1st Examination (CFU/mL) | 2nd Examination (CFU/mL) | Average (CFU/mL) |
|--------|--------------------------|--------------------------|------------------|
| 1      | 8 x 10^6                 | 5 x 10^6                 | 6.5 x 10^6       |
| 2      | 4 x 10^6                 | 4 x 10^6                 | 4 x 10^6         |
| 3      | 10 x 10^6                | 17 x 10^6                | 13.5 x 10^6      |
| 4      | 32 x 10^6                | 47 x 10^6                | 39.5 x 10^6      |

Table 4. The results of TPC test on the water hyacinth leaves extract gel hand sanitizer

Calculation of the number of colonies is using the plate count method (Total Plate Counts). The principle of the plate count method is when the surviving cell of the body is grown in the medium, the body's cells will multiply and form a colony that can be seen directly and calculated visually without the use of a microscope [16]. Calculations were done by using the number of bacterial colony calculation tool (Colony Counter). The number of colonies that can be calculated as required is 30-300 colonies [17].

Table 4 shows that the antiseptic test result obtained the average number of colonies on the I, II, III, and IV. Replication of the positive control gel obtained 6.5 x 10^6 colonies, while negative control results are obtained on average the largest number with 39.5 x 10^6 colonies. The result of a gel antiseptic test with an extract of 8% obtained the lowest yield, which 4 x 10^6 compared to a gel with 10% extract is obtained an average number of colonies amounting to 13.5 x 10^6. This suggests that the concentration of water hyacinth leaf extract is 8% more effective than other variables.

4. Conclusion
Water hyacinth leaves extract has been successfully become an active agent in the production of gel hand sanitizer. All samples have been analyzed its physical properties including organoleptic, homogeneity, pH and antiseptic test. From the antiseptic test against bacteria, gel hand sanitizer with the concentration of 8% water hyacinth leaves extract gave the best performance with only 4 x 10^6 CFU/mL.

Acknowledgements
This research is supported by Research Grant from Universitas Negeri Semarang with grant No. 023.17.2.677507/2020. Authors also would like to acknowledge La Nina Veren and Juliawan Arif Permana for the contribution in this research.

References
[1] Herdiansyah H, Soepandji B S, Seda F S S E and Dewi O 2014 Conflict Management of Renewable Natural Resources in the Border of Indonesia-Malaysia: Sustainable Environmental Approach Procedia Environ. Sci. 20 444–50
[2] Mulyani N S, Prasetyo D, Karyana I P G, Sukardi W, Damayanti W, Anggraini D, Palupi-Baroto R, Nirwati H, Wahab A, Mulyadi A W E, Nakamura T and Soenarto Y 2018 Diarrhea among hospitalized children under five: A call for inclusion of rotavirus vaccine to the national immunization program in Indonesia Vaccine 36 7826–31
[3] Nuraida L 2015 A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods *Food Sci. Hum. Wellness* 4 47–55
[4] Dinas Kesehatan Kota Semarang 2018 *Profil Kesehatan Kota Semarang 2018* (Semarang)
[5] Mahmood A, Eqan M, Pervez S, Alghamdi H A, Tabinda A B, Yasar A, Brindhadevi K and Pugazhendhi A 2020 COVID-19 and frequent use of hand sanitizers; human health and environmental hazards by exposure pathways *Sci. Total Environ.* 742 140561
[6] Ilmiawan D F 2015 *Analisis Dinamik Model Predator-Prey Pada Populasi Eceng Gondok Dengan Adanya Ikan Grass Carp dan Pemanenan* (Universitas Negeri Semarang)
[7] Sasaqi D, Pranoto P and Setyono P 2019 Estimation of Water Losses Through Evapotranspiration of Water Hyacinth (Eichhornia crassipes) *Caraka Tani J. Sustain. Agric.* 34 86
[8] Sanmuga Priya E and Senthamil Selvan P 2017 Water hyacinth (Eichhornia crassipes) – An efficient and economic adsorbent for textile effluent treatment – A review *Arab. J. Chem.* 10 S3548–58
[9] Sindhu R, Binod P, Pandey A, Madhavan A, Alphonsa J A, Vivek N, Gnansounou E, Castro E and Faraco V 2017 Water hyacinth a potential source for value addition: An overview. *Bioresour. Technol.* 230 152–62
[10] Bordoloi S, Yamsani S, Garg A, Sreedee S and Borah S 2015 Study on the efficacy of harmful weed species Eichornia crassipes for soil reinforcement *Ecol. Eng.* 85 218–22
[11] Villamagna A M and Murphy B R 2010 Ecological and socio-economic impacts of invasive water hyacinth (Eichhornia crassipes): a review *Freshw. Biol.* 55 282–98
[12] Wijaya J I 2013 Formulation of Hand Sanitizer Gel Formulation with Triclosan 1.5% and 2% Active Ingredients. University of Surabaya Student Scientific Journal 2 1–14
[13] Indonesia S N 1992 *Detergen Sintetik Cair Pembersih Tangan* 06-2588-1992 (Jakarta)
[14] Kulthanan K, Maneeprasopchote P, Varothai S and Nuchkull P 2014 The pH of antiseptic cleansers *Asia Pac. Allergy* 4 32–6
[15] Indonesia K K R 2013 *Farmakope Indonesia* (Jakarta: Kementrian Kesehatan Republik Indonesia)
[16] Martins W F, Longhi D A, de Aragão G M F, Melero B, Rovira J and Diez A M 2020 A mathematical modeling approach to the quantification of lactic acid bacteria in vacuum-packaged samples of cooked meat: Combining the TaqMan-based quantitative PCR method with the plate-count method *Int. J. Food Microbiol.* 318 108466
[17] Zhu G, Yan B, Xing M and Tian C 2018 Automated counting of bacterial colonies on agar plates based on images captured at near-infrared light *J. Microbiol. Methods* 153 66–73