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UTILIZATION OF CITRONELLA HYDROSOL AS A DISINFECTANT PRODUCT WITH THE ADDITION OF PATCHOULI OIL AS A FIXATIVE COMPOUNDS

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ABSTRACT. Hydrosol is a byproduct of the distillation process of citronella oil, which serves as an aromatic water with antibacterial activity. Citronella hydrosol has the potential to be used as a disinfectant because it can reduce the excessive use of alcohol, and producing an environmentally friendly disinfectant. This research aims to determine the best formulation in the production of disinfectant from citronella hydrosol with the addition of patchouli oil as fixative compound, based on total microbial, antibacterial activity, and organoleptic test. The methods used are 1) descriptive for total microbial and antibacterial activity, and 2) quantitative for the organoleptic tests with analysis of variance (ANOVA) and follow-up tests if significant difference are found. The research show that the best formulation is F2 (40% hydrosol, 20% distilled water, 30% alcohol antiseptic, 9% patchouli oil, and 1% surfactant). Formulation F2 has a Total Plate Count (TPC) value in the range of 0.3 x 10^4 CFU/mL – 0.9 x 10^4 CFU/mL and an inhibition zone for antibacterial activity of 20 mm for *Escherichia coli* and 15.8 mm for *Staphylococcus aureus*, classified as strong. These results show that the disinfectant is capable of reducing total microorganism growth. The organoleptic test of formulation F2 showed thr results for color, aroma, and overall acceptance of 5.76, 4.84, and 5.16, respectively, with a neutral to like moderately.

INTRODUCTION

Citronella hydrosol is a by-product of the distillation of citronella plants that has many benefits. Hydrosols contain a small amount of essential oils. Although hydrosols are by-products of distillation, they also have the potential to be developed into products with added value (Almeida *et al*., 2024). Hydrosol is also referred to as a byproduct of distillation, with a pH lower than that of essential oils (Aswandi and Kholibrina, 2021). Hydrosols are very liquid, the odor depends on the aromatic plants, contains few essential oils, and has a pH between 3.5 and 6.5 (Rao, 2013). Citronella hydrosol can be used as a disinfectant because it has antibacterial activity (D'Amato *et al*., 2018). This explanation is reinforced by Shen *et al.* (2017), who reported that the hydrosol from plants containing volatile compounds has medium to strong antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

The need for hygiene and sanitation products, especially disinfectants, has become an integral part of modern society's lifestyle. The increased awareness of the importance of maintaining a hygienic environment to prevent the spread of infectious diseases has been the main driver of the high demand for disinfectant products in the market. Global phenomena such as the COVID-19 pandemic have further strengthened this awareness and made disinfectants essential products in households, public facilities, and industries.

Disinfectants are chemical compounds that prevent the growth of, and kill some or all microorganisms in direct contact with the disinfectant. The microorganisms in question are diverse, including bacteria that often attach to the surface of inanimate objects such as furniture, work tools, and medical equipment. However, the use of chemical compounds can have harmful effects on health and the environment if used frequently. Disinfectants have varying effectiveness against different types of microorganisms. Bacteria, viruses, fungi, and spores may respond differently to disinfection methods. This can also be influenced by the contact time of the disinfectant (Bhargava *et al*., 2024).

The use of essential oils as disinfectants can help eradicate microorganisms. This can potentially be developed to reduce the use of chemical compounds (Benaziz *et al*., 2022), and essential oils can be mixed into disinfectant materials or serve as a substitute for disinfectants (Kamali *et al*., 2024). Therefore, the author attempted to utilize the by-product of the distillation of citronella essential oil, namely hydrosol, as the main ingredient for making disinfectants, as an alternative to produce more environmentally friendly products. The antibacterial activity of essential oils can be used as a disinfectant that can kill or inhibit bacterial growth. Places such as hospitals, health centers, and other public areas are vulnerable to contamination.

Citronella hydrosol disinfectant was produced using several formulations to determine the best one. Patchouli oil as a fixative compounds are added to improve the quality of citronella hydrosol disinfectant. Fixative compounds function as a binder for other aromatic compounds so that they can maintain and increase aroma spread. Based on the results of research conducted by Nurjanah *et al*. (2019), patchouli oil also increased antibacterial activity, namely the diameter of the inhibitory zone in several bacteria, namely *Propioni bacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Micrococcus luteus*, with a range of 13 – 31.22 mm.

The utilization of hydrosol as a by-product of citronella plant distillation will increase the efficiency of citronella essential oil production. Utilizing by-products in a production process is also helpful for increasing the income obtained from the sale of by-products. However, studies on the combination of citronella hydrosol and patchouli oil in disinfectant formulations remain limited. Based on the explanation above, this research aims to find the best formulation for making disinfectants from citronella hydrosols by adding patchouli oil as a fixative compound, based on total microbial, antibacterial activity, and organoleptic tests.

MATERIALS AND METHODS

Materials

The materials used in this study were citronella hydrosol from "*CV Asliko Nusantara*," distilled water, alcohol antiseptic 70% from Brataco, patchouli oil from West Sumatera farmers, Tween 80 from Merck, Mueller Hinton Agar media, Nutrient Agar media, *Staphylococcus aureus*, and *Escherichia coli* bacterial cultures.

The tools used in this study were disinfectant bottles, glass beakers, analytical balances, colony counters, autoclaves, spatulas, measuring cups, hotplates, magnetic stirrers, sterile disc paper, microliter pipettes (µL), and Petri dishes.

Research Procedure

Production of Citronella Hydrosol Disinfectant

The first stage of this study is the process of producing surface disinfectant products based on citronella hydrosol. Citronella hydrosol was used as a substitute for antiseptic alcohol. The formulation used refers to the research of Benaziz *et al*. (2022), but has been modified to adjust to the preliminary research the researcher has conducted. The process of producing citronella hydrosol disinfectant is designed in five formulations, as shown in Table 1. Based on that formulation, all components were mixed and homogenized using a hot plate stirrer at a temperature of 40ºC and a speed of 800 rpm for 30 min. The disinfectant was then placed in an antiseptic bottle.

The methods used in this study were as follows: 1) descriptive for total microbial and antibacterial activity analysis, with a control of a surface without the application of disinfectant as a comparison for each formulation, and 2) quantitative for the organoleptic tests with analysis of variance (ANOVA) and follow-up tests if significant differences were found.

Total Microbial Testing

The formulations used in this method were F1, F2, F3, F4, and F5, with the treatment being the surface of inanimate objects sprayed with disinfectant for 30, 60, 90, 120 min, and the control treatment being without any disinfectant spray at all. The calculation of bacterial colonies in this study was based on Rosmania and Yanti (2020). The bacterial suspension was then

diluted from 10^{-1} to 10^{-4}. Dilution is carried out to reduce the number of microbes that grow, making it easier to calculate. In the following process, 1 mL of the bacterial suspension was taken using a micropipette, placed into the first dilution tube 10^{-1} containing 9 mL of physiological Sodium Chloride solution, and then homogenized using a vortex mixer. The same step was performed until the fourth tube was diluted. The following process was used: 1000 µL was taken using a micropipette at the last two dilutions and placed in a sterile Petri dish. Next, use the pour plate method to put the Nutrient Agar (NA) medium at 40°C. The medium was homogenized by rotating the dish slowly in a figure-eight motion and allowing it to sit for approximately 15-20 minutes. In the final stage, the Petri dish was wrapped in paper covering the entire surface of the cup and then placed in an incubator at

37°C for a minimum of 1 × 24 h and a maximum of 2 × 24 h.

Antibacterial Activity

The method used in this research refers to Shatnawi *et al*. (2021), but it has been modified. Antibacterial activity of citronella hydrosols using the disk diffusion method. Antibacterial testing was performed on *Escherichia coli* and *Staphylococcus aureus*. Muller Hinton Agar (MHA) was used as the medium. The first stage was the sterilization of the media. The sterilized media was then placed in a 25 mL Petri dish. The process then continued with the preparation of the test bacterial suspension.

The next step was the suspension of the test bacteria, which was inoculated on 0.1 mL of MHA medium, flattened with a hockey stick, and left to dry. Next, soak the sterile disc paper in the citronella hydrosol for 15 minutes. The sterile disc paper was aseptically placed on the surface of the media. The last process is the observation of the clear zone in the area around the disc paper.

Sensory Analysis

This study will use the hedonic test to analyze the sensory analysis, with assessment parameters for Color, Odor and Overall Acceptance. This study will
The hedonic test was used for the sensory analysis. This hedonic test also develops a product and makes decisions about which products will be further processed to be marketed to the public. The hedonic test was carried out using a scale of 1-7, namely, 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = like very much (Setyaningsih *et al*., 2014).

The panelists used in this study were 25 untrained panelists. The panelists were randomly selected in terms of age, gender, and profession so that the data obtained sufficiently represented consumers who would use this disinfectant product. A descriptive presentation in the form of a diagram was used to display the data from the sensory analysis.

Statistical Analysis

The experimental design was a Completely Randomized Design for organoleptic tests, with five formulations and 25 replications, resulting in 125 observation data. The data were analyzed using ANOVA and followed by Duncan's Multiple Range Test (DMRT) if significant differences were found. The methematical model of the Completely Randomized Design can be seen in the equation below.

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Description:

Y_{ij}	=	observation value from the i-th and the j-th replication
μ	=	overall mean
τ_i	=	effect of the i-th treatment
ε_{ij}	=	experimental error from the i-th treatment and the j-th replication

RESULTS AND DISCUSSIONS

Total Microbial Testing of Citronella Hydrosol Disinfectant

Hydrosol, a by-product of citronella essential oil distillation, can be used as a mixture in disinfectant-producing materials. In this case, by-products can provide added value by becoming new products that can be produced after the main product is produced.

Disinfectants are chemical-based products commonly used to reduce, inhibit, and kill microorganisms that adhere to the surfaces of inanimate objects. On average, inanimate objects often infested by microorganisms are objects close to our daily lives, such as household furniture, door handles, and other objects in direct contact with human hands. Total microbial testing in this study used standard methods, namely Total Plate Count (TPC), which counts the number of microorganisms that live on certain media (Houchmandzadeh and Ballet, 2023).

Table 1. Formulations of disinfectant (%)

Component	F1	F2	F3	F4	F5
Citronella Hydrosol	35	40	45	50	55
Distilled Water	20	20	20	20	20
Alcohol Antiseptic 70%	35	30	25	20	15
Patchouli Oil	9.5	9	8.5	8	7.5
Tween 80	0.5	1	1.5	2	2.5

The results of bacterial colony calculations based on the time of exposure to the disinfectant are graphically presented in Figure 1.

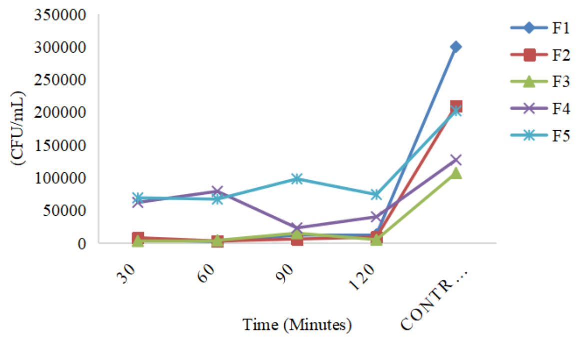

Figure 1. Graphics of CFU/mL for all samples of citronella hydrosol disinfectant

The results show that each disinfectant formulation can reduce contamination compared to the control. Disinfectant was sprayed onto the surface of inanimate objects, and the number of colonies growing was observed after predetermined times of 30, 60, 90, and 120 min. The control in this study was the surface of an inanimate object without exposure to disinfectant. The average number of colonies grown on each formulation was calculated. The lowest number of growing colonies was in the F2 formulation in the range of 0.3×10^4 CFU/mL – 0.9×10^4 CFU/mL, and the highest was in the F5 formulation in the range of 6.7×10^4 CFU/mL – 9.8×10^4 CFU/mL.

Formulation F2 consisted of 40% citronella hydrosol, 20% distilled water, 30% antiseptic alcohol 70%, 9% patchouli oil, and 1% Tween 80. Based on the study by Alajlan *et al.* (2022), alcohol-based disinfectants or mixtures of alcohol with other antimicrobial ingredients reduced the growth of various types of bacteria. Formulation F2 has 30% of antiseptic alcohol with the addition of 40% hydrosol and 9% patchouli oil. The addition of 9% patchouli oil also contributes to reducing the number of growing microbes, which is reinforced by the study of Ahmad *et al.* (2024), where patchouli oil had a total coloni growth value of 0.1×10^3 CFU/mL.

Sukamdi *et al.* (2021) prepared a hand sanitizer by adding lemongrass essential oil and compared it with commercial products. Sukamdi *et al.* (2021) found that the total microbes were 144×10^7 CFU/mL for commercial hand sanitizer products and 167×10^7 CFU/mL for lemongrass hand sanitizer. These results show that the disinfectant from citronella hydrosol in this study has a better ability to inhibit bacterial growth than similar commercial products such as hand sanitizers.

Bayhan *et al.* (2020) observed colony growth on hands after applying hydrosol from *Rosa damascene*. The results showed that the number of colonies that grew significantly decreased after the hydrosol was applied to the hands. Sengun *et al.* (2020) also reported that essential oils have high potential to be the main ingredient in disinfectants. Sengun *et al.* (2020) used a mixture of oregano oil, cinnamon, and cloves; this essential oil mixture was able to reduce the total mesophilic aerobic bacteria by 2 × 27 log CFU per 25 cm^2 and *Escherichia coli* by 4 × 60 log CFU per 25 cm^2 below the detection limit. Research on the use of hydrosols as a mixing ingredient for antiseptic alcohol in the production of surface disinfectants has not been widely conducted; therefore, the comparative references used are generally similar sanitation products. Based on the explanation above, it shows that disinfectant from hydrosol has a very good potential as disinfectant product.

The potential of citronella hydrosol as an active ingredient in disinfectants is a promising breakthrough. Its utilization not only offers a solution to reduce essential oil production waste but also adds value to the entire production process. By transforming discarded by-products into functional, safe, and sustainable disinfectant products, this research can have a positive economic impact on producers and an ecological impact on the environment.

Antibacterial Activity

Antibacterial activity refers to the extent to which a material can inhibit the growth of bacteria, prevent the formation of colonies, and even kill microorganisms. Hydrosol has antibacterial properties that depend on the aromatic intensity of the bioactive components (Aćimović *et al.,* 2020). Based on the research of Shafie *et al.* (2022), the antibacterial activity of hydrosols is influenced by the active compounds contained in the hydrosol. These active compounds certainly adjust to the active compounds of essential oils still found in hydrosols.

The results of this study show that the largest diameter of zone inhibition for *Escherichia coli* and *Staphylococcus aureus* is in the F2 formulation, which is 20 mm and 15.8 mm, respectively. The negative control used is sterile distilled water and the positive control is a commercial disinfectant. The results obtained were greater than those of Shafie *et al.* (2022), who measured the diameter of the zone of inhibition of the hydrosol of several plants. Some examples of the

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results of the research of Shafie *et al.* (2022), hydrosols from arecanut and coconut are 6.7-14.2 mm for *Escherichia coli*; and hydrosols from bay leaf, black cumin, rosemary, sage, and thyme, which are 4.32-6.07 mm for *Salmonella Typhimurium* and *Escherichia coli*.

This indicates that each hydrosol has a different ability to inhibit bacterial growth, depending on the active compounds present in each plant. The antibacterial activity of the citronella hydrosols is shown in Table 2.

Based on references by Astriani and Iqbal (2024) and research results by Rahayuningsih *et al*. (2021), the inhibition zone is divided into four categories, namely the inhibition zone > 20 mm in the very strong category, 11-20 mm in the strong category, 5-10 mm in the medium category, and < 5 mm in the weak category. One of the results of antibacterial activity in this research is shown in Figure 2.

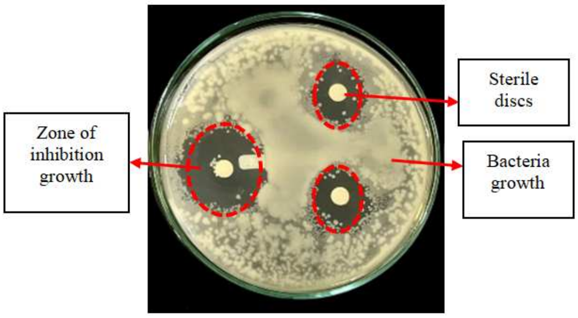

Figure 2. Sample of antibacterial activity

Disinfectants of citronella hydrosol can also be used in similar products, such as hand sanitizer and bed linen spray. Rastuti *et al*. (2024) tested the antibacterial activity of hand sanitizers made from citronella essential oil fractions and obtained 17.69-29.84 mm results against *Escherichia coli*. The research results of Bouftila *et al*. (2024) stated that essential oils, including lemongrass and citronella oil, have strong antibacterial activity. These results are in line with this research, that citronella essential oil has the ability to inhibit bacterial growth. So, as a by-product, citronella hydrosol also has the same ability but with lower power. For this reason, patchouli oil was added as an amplifier for the disinfectant produced in this research.

Essential oils, specifically their bioactive components, exhibit antibacterial activity. As seen in the study by Khwaza *et al*. (2025), the bioactive components include monoterpenes: carvacrol, menthol, and thymol, and phenylpropanoids: cinnamaldehyde and eugenol.

The results indicate that the bioactive components of these essential oils have good antibacterial activity and lower toxicity levels. Based on their good antibacterial properties, essential oils and hydrosols can be used as preservatives for various products (Hay *et al*., 2018).

The bioactive components in several essential oil plants, such as cinnamaldehyde, eugenol, 1,8-cineol, limonene, carvacrol, and thymol, have large inhibition zones against several bacteria, including *Escherichia coli* (ATCC 25922), *Fusobacterium necrophorum* (ATCC 25286), *Trueperella pyogenes* (ATCC 19411), and *Staphylococcus aureus* (ATCC 29213) (Paiano *et al*., 2020). Therefore, the bioactive components found in essential oils and hydrosols have the potential for products related to antibacterials. Hydrosols also function as antioxidants, making them useful in skincare products (Jakubczyk *et al*., 2021). This should be considered in future research on utilizing the combination of antibacterial and antioxidant properties of hydrosols to produce skincare products.

Table 2. Antibacterial activity of citronella hydrosols disinfectant

Formulation	*Escherichia coli*	Diameter of zones of inhibition (mm)		Category
		Category	*Staphylococcus aureus*	
F1	18	Strong	9.8	Medium
F2	20	Strong	15.8	Strong
F3	19	Strong	9.4	Medium
F4	17	Strong	9.2	Medium
F5	19	Strong	10	Medium
Control (-)	0	-	0	-
Control (+)	22.7	Very Strong	17.7	Strong

Organoleptic

Organoleptic tests were performed using hedonic tests. The observations included color, aroma, and overall acceptability. The panelists used were 25 untrained panelists. Thehedonic tests for products containing essential oils include parameters of color and odor (Ma'arif *et al*., 2023). In research that produces applied products, finding fundamental consumer segmentation is much more important than simply determining which products are preferred over others, as competition among products in the consumer market has become increasingly crowded and competitive. Hedonic testing can also help determine which products will be developed and marketed in the future (Lim, 2011).

The organoleptic results showed that the color and overall acceptability parameters were the highest values of 5.76 and 5.16, respectively, for the F2 formulation. These results indicate that a scale above 5 is interpreted as a "like slightly" rating. The odor parameter with the highest value is 4.92 in the F3 formulation. A rating above four is defined as "neither like nor dislike." Based on the results of the statistical analysis of the hedonic test in this study, it did not show significantly different results; therefore, Duncan's further test was not carried out. The organoleptic results of this study are shown in Figure 3.

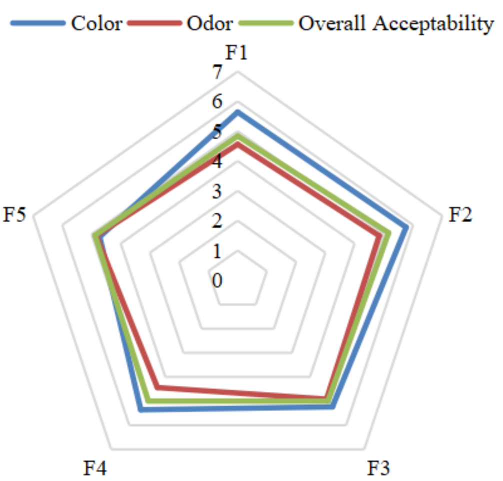

Figure 3. Spider web of organoleptic test of disinfectant

Color and odor are organoleptic properties, and the composition and amount of aromatic compounds can determine the specific odor of essential oils. The odor of essential oils is described as sweet, woody, spicy, balsamic, floral, and fresh (Abdalbasit, 2016). Because hydrosols contain fewer bioactive components than essential oils, their aroma is less intense than that of essential oils (Roanisca *et al*., 2024).

The distillation time of the essential oil also affects the aroma of the hydrosol. A longer distillation time produces a hydrosol with a rich and lasting fragrance; however, there are aromas in addition to the original scent of the plant (Wan *et al.,* 2024). The odor produced by this citronella hydrosol disinfectant product combines grassy, woody, and slightly spicy aromas. The addition of patchouli oil also caused this aroma combination.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Disinfectants from citronella hydrosol have the potential to reduce antiseptic alcohol content in disinfectant formulations, thereby reducing microbial growth and increasing antibacterial activity. Based on the results of this study, the best formulation was F2, which had a composition of 40% hydrosol, 20% distilled water, 30% alcohol, 9% patchouli oil, and 1% surfactant. Total microbial testing using the Total Plate Count (TPC) method showed that the F2 formulation with exposure times of 30, 60, 90, and 120 min had values in the range of 0.3×10^4 CFU/mL – 0.9×10^4 CFU/mL. The antibacterial activity of the F2 formulation against E. coli and S. aureus showed inhibition zones of 20 mm and 15.8 mm, respectively, in the strong category. The results of organoleptic tests on color, odor, and overall acceptability parameters showed that the F2 formulation was 5.76, 4.84, and 5.16, respectively, with a neutral to moderately like rating.

Recommendations

Subsequent research is expected to conduct an analysis of the Physical and Chemical Stability. Testing the stability of products under various storage conditions, such as temperature and light, over a certain period of time. Changes such as color, odor, pH, and sedimentation were observed. This is a crucial aspect to be carried out in determining the shelf life of the product.

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