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Antibacterial Test of α-pinene compounds from Turpentine oil in Hand Sanitizer Gel

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Abstract. Research on the antibacterial test of the α-pinene compound from turpentine oil in hand sanitizer gel has been carried out. The purpose of this study was to determine the antibacterial activity of turpentine oil gel and α-pinene gel against S. aureus and E. coli. The method used for α-pinene isolation is done by fractionation distillation with pressure reduction. Analysis of α-pinene compounds from turpentine oil using FT-IR and GC-MS. The results of GC-MS analysis show that α-pinene is a terpenoid group compound. Antibacterial activity testing uses the paper disc diffusion method. Antibacterial activity test of turpentine oil and α-pinene showed inhibition of S. aureus and E. coli bacteria. Turpentine oil has the highest antibacterial activity with a inhibition zone of 13.8 mm against S. aureus and 8.83 mm against E. coli bacteria, whereas α-pinene has the highest antibacterial activity with a 2.2 mm inhibition zone against S. aureus and 2.34 mm against E. coli bacteria. Based on the results of the antibacterial gel test showed that the turpentine oil gel gave a better antibacterial effect than the α-pinene gel, so that it can be applied in the form of hand sanitizer gel.

Keywords: antibacterial; α-pinene; turpentine oil; hand sanitizer

1. Introduction

Health is an important aspect which can affect the quality of life of each individual. One effective way to maintain body health is by maintaining hand hygiene (Radji et al., 2007). Through physical contact, various kinds of viruses and bacteria attach to the hand. The most appropriate way to prevent this is to wash your hands with soap and clean running water (Wijaya, 2013). This effort is carried out because the hands can be a cause of pathogens moving from one hand to another, being a germ-carrying agent and can cause disease, therefore, as the times progress, hand washing habits have been diverted by hand sanitizer.

Turpentine oil is an example of natural ingredients that have the potential as an antiseptics. Turpentine oil is a colored (clear) liquid and has a distinctive odor derived from the distillation of tree sap which is classified as pine resin. Market demand for this oil is increasing every year because "Back to

Nature" tends to be chosen to meet the needs of the industry so that demand and the price of turpentine oil tend to increase. Turpentine oil has increased industrial demand which is used for pharmaceutical raw materials, resins, polymers, perfumes and solvents (Amini *et al.,* 2014).

Alpha-pinene compounds are the main components of turpentine oil. The higher the α-pinene content the higher level of purity and quality of turpentine oil to become better and have a high selling value (Utami *et al.,* 2011). Turpentine oil contains hydrocarbon monoterpenes such as α-pinene, β-pinene and 3-carena (Haneke, 2002; Lindmark, 2003). According to research Nohong *et al* (2000) stated that the main composition of turpentine oil is volatile compounds which are terpenoids with levels between 60-80%. Several studies relating to the use of terpenoids which have been done are inhibitors of bacterial growth with 13 mm inhibition zones against *S. aureus* and 8 mm against *E. coli* (Mimoune *et al.,* 2013). The results of other studies showed the presence of antibacterial activity of turpentine oil on bacterial growth with a 13,33 mm inhibition zone against *S. aureus* and 8,33 mm against *E. coli*. Masruri *et al* (2007) conducted a study of α-pinene antibacterial activity against bacterial growth with 8,3 mm inhibition zones against *S. aureus* and 8,9 mm against *E. coli*. From the results of this study, it is possible to use turpentine oil and α-pinene in the hand sanitizer gel dosage form. The purpose of this study was to determine the antibacterial activity of turpentine oil gel and α-pinene gel against *S.aureus* and *E.coli* bacteria.

2. Methods

In this study the tools used include a set of fractionation distillation devices, Fourier Transform Infra Red (FT-IR) PerkinElmer Frontier 10.03.06, Gas Cromatography mass spectrophotometer (GC-MS) Perkin Elmer, autoclaves, incubators, laminar air flow and vortices. The research material used was turpentine oil, anhydrous Na_2SO_4, carboxymethylcellulose, triethanolamine, propylene glycol, glycerin, aquades, nutrient agar, Mc farland 0,5 standard, *S. aureus* and *E.coli* bacteria obtained from the UPT Integrated Laboratory of Diponegoro University.

Alpha-pinene isolation was carried out by the fractionation distillation method. A total of 500 mL turpentine oil is inserted in erlenmeyer while filtered using filter paper. Anhydrous Na_2SO_4 is added to bind water in turpentine oil. The non-aqueous turpentine oil is put into a flask which is connected to a pressure reduction fractional distillation device with a temperature of 50-60^{o}C. The results of the distillation were tested with FT-IR and GC-MS.

The paper disc diffusion method will be used in the antibacterial test in this study. The testing process includes sterilization of tools and materials, preparation of nutrient agar media and preparation of bacterial suspensions. Inserted as much as 1 ounce of each *S.aureus* and *E. coli* bacteria into a test tube containing physiological saline solution then homogenized and measured turbidity according to McFarland 0.5 standard. A total of 1 mL of microbial suspension was taken using a micropipette and put into a sterile petri dish. Nutrient media that it is still liquid is poured into the petri dish and shaken to homogeneous. Disc paper that has been soaked in turpentine oil and α-pinene is placed on the surface of the NA which has been planted with bacteria. Furthermore, the media was incubated for 24 hours into an incubator with a temperature of 37°C and the inhibition zone could be observed and measured.

The hand sanitizer gel formula refers to the modified Manus *et al* (2016) study where citronella oil is the active ingredient in the formula replaced with turpentine oil and α-pinene. Making gel is done by developing CMC in hot distilled water then stirring. Added TEA drop by drop while stirring. Added by propylene glycol, turpentine oil, glycerin and aquades to the desired volume. Complete treatment of hand sanitizer gel formulations is presented in Table 1.

Table 1. Hand sanitizer gel formulation

Material	Positive control	Negative control	Turpentine oil	α-pinene
Active ingredients	Hand sanitizer of commercial		1,5 mL	1,5 mL
CMC	-	0,25 g	0,25 g	0,25 g
TEA	-	2 drops	2 drops	2 drops
Propylene glycol	-	0,5 mL	0,5 mL	0,5 mL
Glycerin	-	1 mL	1 mL	1 mL
Add aquades	-	10 mL	10 mL	10 mL

(Manus *et al*,. 2016, with modifications)

Results and Discussion

In this study, turpentine oil as a base material obtained from Perhutani Unit I Central Java and isolated by fractionation distillation. Before being isolated, turpentine oil was added to anhydrous Na_2SO_4. This addition aims to purify the desired results by binding to the remnants of water that is still mixed with turpentine oil. Turpentine oil to be used is analyzed first using GC. Turpentine oil has a component of chemical compounds in the form of α-pinene with a percentage of 86,02%, delta-3-carena 12,85% and β-pinene are 0,87%. After turpentine oil was isolated, α-pinene was analyzed using an infrared spectrophotometer (FT-IR). The α-pinene IR spectrum is presented in Figure 1.

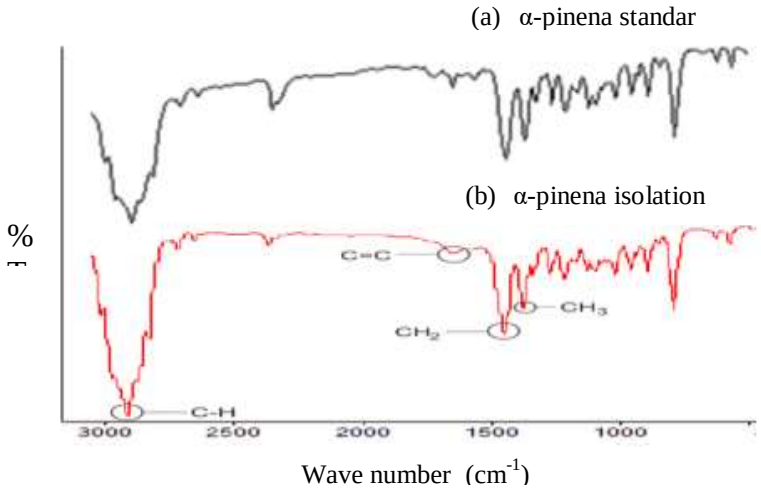

Figure 1. Spectra IR of α-pinene

Based on Figure 1 the results of isolation showed some characteristic absorption. The area of C-H absorption occurs at wave number 2920 cm^{-1}, the absorption area of C = C occurs at wave number 1651 cm^{-1}, the absorption area of CH_2 occurs at wave number 1444 cm^{-1}, and at wave number 1369 cm^{-1} absorption occurs which shows CH_3 absorption area. Based on the results of the FT-IR above, the α-pinene spectrum is the same isolation as the standard α-pinene spectrum.

Analysis of the composition content of samples from turpentine oil isolation was carried out by Gas Chromathography and Mass Spectroscopy (GC-MS) detectors. Chromatogram from the analysis of turpentine oil isolation showed 4 detected peaks, but only one peak with high abundance was analyzed by a mass spectrometer, the peak with a retention time of 3,814. Figure 2 shows the results of GC-MS α-pinene analysis.

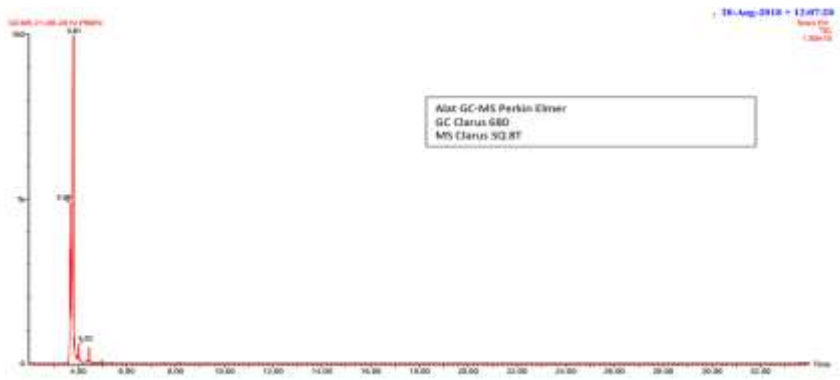

Figure 2. Chromatogram of α-pinene

The chromatogram shows the presence of several chromatogram peaks that have the highest peak. The higher the peak of a chromatogram, the greater the area of the chemical component. The largest components contained in turpentine oil from isolation are presented in Table 2.

Table 2. Results of α-pinene GC-MS analysis

Peak	Retention time (minutes)	Area (%)	Name of compound based on *library* MS
1	3,679	23,56	α-pinene
2	3,814	73,79	α-pinene
3	4,019	1,13	camphene
4	4,449	1,51	β-pinene

The results of α-pinene analysis using GC-MS appeared at two peaks allegedly due to equipment errors. Based on the MS library, it cannot distinguish between the first α-pinene and the second α-pinene. Both are $C_{10}H_{16}$. MS says the results are the same, meaning that it only moves. Retention time is 3,679 minutes and 3,814 minutes is identical. At a certain time retention GC does not accept a number of α-pinene. The first peak appears α-pinene then the second peak appears again α-pinene which means that at retention time 3,679 minutes stop first (not exit). So it does not retain a certain amount of time so it is not able to go out and the peak does not continue because it does not come out automatically down, then exit again remains detected as the same compound that came out before.

Table 3. Results of Antibacterial Activity Test

No	Sample code	Isolate	Inhibitory zone (mm)	The average inhibition zone (mm)
1	**A**	***S. aureus***	**13,5**	
2			**14,2**	**13,8**
3			**13,7**	

4		*E. coli*	8,9	
5			8,9	8,83
6			8,7	
7	F	*S. aureus*	3,5	
8			3,2	3,47
9			3,7	
10		*E. coli*	3,3	
11			3,6	3,33
12			3,1	
13	B	*S. aureus*	8,7	
14			8,7	8,83
15			8,5	
16		*E. coli*	7,5	
17			7,8	7,57
18			7,4	
19	C	*S. aureus*	2,3	
20			2,1	2,2
21			2,2	
22		*E. coli*	2,2	
23			2,4	2,37
24			2,5	
25	D	*S. aureus*	-	
26			-	-
27			-	
28		*E. coli*	-	
29			-	-
30			-	
31	E	*S. aureus*	8,2	
32			8,3	8,3
33			8,4	
34		*E. coli*	9,4	
35			8,9	9,13
36			9,1	

Information :

A: Turpentine oil
B: α-pinene
C: α-pinene gel
D: Negative control (gel base)
E: Positive control (Dettol)
F: Turpentine oil gel

Turpentine oil and α-pinene then tested for antibacterial and formulated into hand sanitizer gel. Hand sanitizer gel made with CMC base as gelling agent, TEA as alkalizing agent, propylene glycol as humectant, preservative, stabilizing agent and can dissolve oil. Addition of glycerin functions as humectants and emollients (Manus *et al.,* 2016). Antibacterial tests for 24 hours were then carried out. Antibacterial activity test results are presented in Table 3.

Based on Table 3 the results of the antibacterial test above are due to the presence of secondary metabolites contained in turpentine oil. The main chemical contents identified in the GC-MS analysis are α-pinene, β-pinene and camphene compounds. This compound is a terpenoid group compound which is thought to have antibacterial power. Terpenoids are the largest group of secondary metabolites which are seen from the number of compounds and variations in their basic structure and are the main constituents of essential oils. The mechanism of action of terpenoids as an antibacterial substance is thought to damage the cell membrane by lipophilic compounds although not fully known. In the outer membrane of the cell wall, terpenoids react with porins and then damage porins and form

strong polymer bonds, reducing the permeability of bacterial cell walls so that cell nutrients become deficient and will die or be inhibited (Haryati *et al.,* 2015; Taurina *et al.,* 2014) . Porin is the entry and exit of nutrients, if the porin is damaged, the permeability of the bacterial cell membrane is reduced. This condition will result in the death of bacterial cells (Miradiana *et al.*, 2017).

The concentration of turpentine and α-pinene oil used in this antibacterial activity test was 100% which was tested triploly. Turpentine oil antibacterial test results showed that turpentine oil before isolation had better antibacterial power than turpentine oil after isolation (α-pinene), which was evidenced by the larger diameter of the bacterial inhibition zone in *S. aureus* and *E. coli* isolates.

Based on the results of antibacterial activity tests, the growth of *S. aureus* and *E. coli* bacteria can be inhibited by turpentine oil and α-pinene, so it can be said to be antibacterial. This is consistent with research conducted by Mimoune *et al.,* (2013) and Zeynep *et al.,* (2014) which states that turpentine oil had antibacterial activity against *S. aureus* and *E. coli* bacteria. In addition, Masruri *et al.,* (2007) stated that α-pinene had the potential as an antibacterial against *S. aureus* and *E. coli* bacteria.

The results of antibacterial activity testing showed that the hand sanitizer gel which only contained a base (negative control) did not provide an inhibitory zone. The inhibition zone produced by turpentine oil gel is greater than the α-pinene gel. This is because turpentine oil has a chemical component that has the potential to be the greatest inhibitory against *S. aureus* and *E. coli*. The main components contained in turpentine oil are α-pinene, delta-3-carena and β-pinene. Although the α-pinene compound in turpentine oil that has been isolated has the largest percentage, other chemical components have a lower percentage than turpentine oil before being isolated. It is suspected that the chemical components that act as antibacterials are not only α-pinene, but other components such as 3-carena, β-pinene and camphene are also very influential. It can be estimated that the presence of other compounds which are both terpenoids, namely monoterpenoids, will improve their ability as antibacterials (Amini *et al.,* 2014). The inhibitory power of turpentine hand sanitizer gel and α-pinena hand sanitizer gel is weak because the hand sanitizer is thick so it affects the disc paper immersion (Kusumawati *et al.,* 2017).

The concentration of bacterial suspension that is opposed is one of the factors that influence the weak or not the ability of an antibacterial substance in inhibiting bacterial growth. The high concentration of cells allows it to affect antibacterial work (Ningsih *et al.,* 2013). In addition, bacterial properties which include age, type and state of bacteria are also influential. Bacterial dilution to bacterial concentrations 10^5 and 10^6 was carried out in several studies regarding antibacterial activity test. This statement is reinforced by Pelczar & Chan (2007) that if there are more microorganisms, the time needed to inhibit or kill is also increasing.

3. Conclusion

Based on the results of the study it can be concluded that turpentine oil gel and α-pinena gel can inhibit the growth of *S. aureus* and *E.coli* bacteria which are in the weak category, namely the inhibition zone ≤ 5 mm.

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