Formulation of Hand Sanitizer Gel of A-Pinene Isolated from Turpentine Oil and its Antibacterial Activity

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Abstract. Research on the antibacterial assay of the α-pinene and turpentine oil in hand sanitizer gel formulation has been carried out. Antibacterial activity assay using the disc diffusion method showed that turpentine oil has the highest antibacterial activity with a inhibition zone of 13.8 mm against Staphylococcus aureus and 8.83 mm against Escherichia coli, 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.

1. Introduction

Health is an important aspect which can affect the quality of life. One effective way to preserve health is by maintaining hand hygiene [1]. Through physical contact, various kinds of viruses and bacteria attach to the hand. The most appropriate way to prevent this is to properly washing hands with soap and clean running water [2]. This effort is carried out because the hands can be germ-carrying agent and cause disease. Over time, hand washing habits have been diverted by hand sanitizer because it is more practical and easier to use. Turpentine oil is an example of natural ingredients that have the potential as an antiseptics. Turpentine oil is a clear colored 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 due to its useful purpose for pharmaceutical raw materials, resins, polymers, perfumes and solvents [3]. Alpha-pinene is a natural terpene and is the main components of turpentine oil [4]. The higher the α-pinene content, the higher level of purity and quality of turpentine. Beside α-pinene, turpentine oil contains monoterpene hydrocarbons such as β-pinene, camphene [5] and 3-carene [6], [7].

Several studies revealed that terpenoids from turpentine oil can inhibit bacterial growth with 13 mm inhibition zones against *S. aureus* and 8 mm against *E. coli* [8]. Masruri conducted a study of antibacterial activity of α-pinene against bacterial growth with 8.3 mm inhibition zones against *S. aureus* and 8.9 mm against *E. coli* [9]. Based on these researches, we performed formulation of turpentine oil and α-pinene in the gel hand sanitizer 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*

2. Materials and Methods

2.1. Research instruments

The instruments used in this study include set of fractional distillation devices, Fourier Transform Infra Red (FT-IR) PerkinElmer Frontier 10.03.06, Gas Chromatography-Mass Spectrophotometer (GC-MS) Perkin Elmer, autoclaves, incubators, laminar air flow and vortex mixer.

2.2. Materials

The material used include turpentine oil, anhydrous Na_2SO_4, carboxymethylcellulose (CMC), triethanolamine (TEA), propylene glycol, glycerin, distilled water, nutrient agar, McFarland 0,5 standard, bacteria cultures of *S. aureus* and *E. coli* which obtained from the Integrated Laboratory of Universitas Diponegoro, Semarang, Indonesia.

2.3. Alpha-pinene isolation

Turpentine oil was obtained from Indonesian State Forest Company Unit I Central Java, and isolated by fractional distillation. The anhydrous Na_2SO_4 was added to purify the desired results by binding to the remnants of water that is still mixed with turpentine oil. Alpha-pinene was isolated using the fractional distillation method with reduced pressure. A total of 500 mL turpentine oil is inserted in flask and 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 fractional distillation device with a temperature of 50-60^{o}C. The results of the distillation were investigated using FT-IR and GC-MS.

2.4. Antibacterial activity assay

The disc diffusion method was used in the antibacterial assay. The testing procedures includes sterilization of tools and materials, preparation of nutrient agar media and preparation of bacterial suspensions. One ounce of each *S. aureus* and *E. coli* bacteria was added into a test tube containing physiological saline solution then homogenized and measured its 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. Liquid nutrient media that was poured into the petri dish and shaken homogeneously. Paper disc that has been soaked in turpentine oil and α-pinene was placed on the surface of the nutrient agar which has been planted with bacteria. Next, 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.

2.5. Hand sanitizer gel formulation

The hand sanitizer gel formula refers to the modified formula by Manus [10]. The production of gel was carried out by adding CMC in hot distilled water then stirred. TEA was added drop by drop while stirring, then propylene glycol, turpentine oil, glycerin and distilled water was added to the desired volume. Commercial hand sanitizer (Dettol Brand) was used as positive control. The gel hand sanitizer formulations is presented in Table 1.

Table 1. Hand sanitizer gel formulations

Component	Negative control	Turpentine oil gel	α-pinene gel
Active ingredients	-	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
Distilled water ad	10 mL	10 mL	10 mL

3. Result and discussion

The analysis of chemical components of turpentine oil was performed using Gas Chromatography. The chemical components including α-pinene with a percentage of 86.02%, delta-3-carene of 12.85% and β-pinene of 0.87%. The α-pinene was analyzed using an Infrared Spectrophotometer (FT-IR). The α-pinene IR spectra is presented in Figure 1.

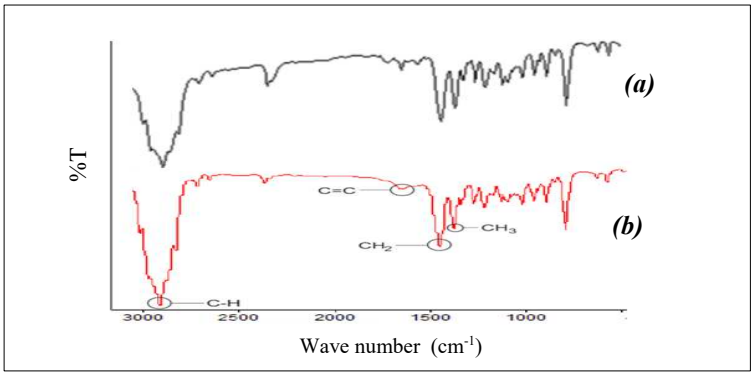

Figure 1. IR Spectra of α-pinene (a: standard α-pinene, b: isolated α-pinene)

Figure 1 showed the results of characteristic absorptions of α-pinene which was isolated previously. The area of C-H absorption, C = C, and CH_2 was occurred at wave number 2920 cm^{-1}, 1651 cm^{-1}, and 1444 cm^{-1}, respectively. Wave number 1369 cm^{-1} indicated the CH_3 absorption. Based on the results of the FT-IR above, the isolated α-pinene spectrum was the same as the standard α-pinene spectrum. Analysis of the composition of turpentine oil isolation was carried out by Gas Chromatography and Mass Spectroscopy (GC-MS) detectors. Chromatogram from the turpentine oil analysis showed four detected peaks, but only one peak with high abundance was analyzed by a mass spectrometer, that is the peak with a retention time of 3.814 min. Figure 2 shows the results of α-pinene GC-MS analysis.

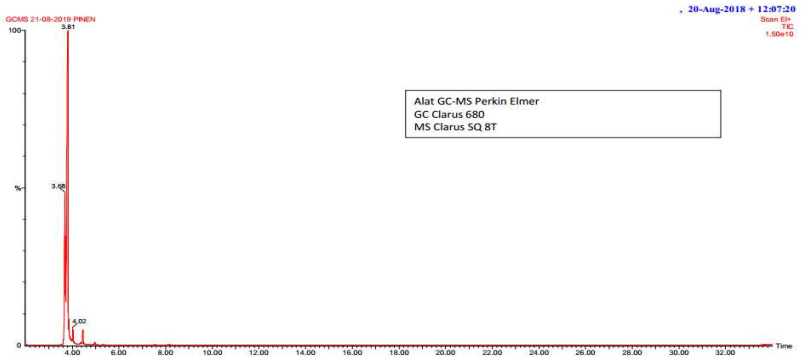

Figure 2. Chromatogram of α-pinene

The chromatogram showed the presence of several high peak. The higher the peak of a chromatogram, the greater the area (concentration) of the chemical component. The largest components contained in turpentine oil from isolation are presented in Table 2.

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

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 α-pinene analysis using GC-MS showed two peaks allegedly due to equipment errors. Based on the MS library, it could not be distinguished between the first α-pinene and the second α-pinene, because it was both possessed molecular formulas of $C_{10}H_{16}$. Turpentine oil and α-pinene then tested for antibacterial activity and formulated into gel hand sanitizer. The formulation of gel hand sanitizer was carried ot by using CMC base as gelling agent, TEA as alkalizing agent, propylene glycol as

humectant, preservative, and stabilizing agent. Addition of glycerin functioned as humectants and emollients [11]. Antibacterial activity test results are presented in Table 3.

Table 3. Results of antibacterial activity test

No	Sample code	Bacteria	Inhibitory zone (mm)	The average of inhibitory 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	B	*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	C	*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	D	*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	E	*S. aureus*	-	
26			-	-
27			-	
28		*E. coli*	-	
29			-	-
30			-	
31	F	*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	

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

The results above indicated that turpentine oil and turpentine oil gel exhibited antibacterial activity due to the presence of secondary metabolites. The main chemical contents identified in the GC-MS analysis were terpenoid compounds, namely α-pinene, β-pinene, and camphene. Terpenoids are the largest group of secondary metabolites which has large 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 by disrupting the cellular membrane [12], [13]. 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 Porin is the entry and exit site 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 [14].

The concentration of turpentine and α-pinene oil used in this antibacterial activity test was 100% which was tested triplicates. Antibacterial activity assay showed that turpentine oil had better antibacterial activity than α-pinene, which was proven by the larger diameter of the bacterial inhibition zone in *S. aureus* and *E. coli*. This is consistent with research conducted by Mimoune and Zeynep which stated that turpentine oil had antibacterial activity against *S. aureus* and *E. coli* [8], [15].

The results of antibacterial activity assay showed that the negative control did not exhibited bacteria inhibitory zone. The inhibition zone produced by turpentine oil gel is greater than the α-pinene gel. This is due to the the presence of the other secondary metabolites in turpentine oil which has the antibacterial potency against *S. aureus* and *E. coli*, such as delta-3-carene, camphene, and β-pinene. It can be estimated that the presence of other compounds can improve the antibacterial activity [16], [17]. The inhibitory activity of gel hand sanitizer gel of turpentine oil and α-pinene was considered weak (≤ 5 mm) because the gel has thick consistency, hence it could affect the disc paper immersion.

The concentration of bacterial suspension that is opposed is one of the factors that influence whether the samples showed inhibition of bacterial growth. The high concentration of cells allows it to affect antibacterial activity [11]. In addition, bacterial properties which include age, type and state of bacteria are also influential. Bacterial dilution to reach concentrations of 10^5 and 10^6 was carried out in several studies regarding antibacterial activity test. This statement is reinforced by Pelczar & Chan that if there are more microorganisms, the more time needed to inhibit or kill [18]

4. Conclusion

Based on the results of the study, it can be concluded that turpentine oil and α-pinena in hand sanitizer gel formulations exhibit antibacterial activity against *S. aureus* and *E. coli*.

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