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***FORMULATION AND ANTIBACTERIAL TEST OF STAR ANISE  
EXTRACT (ILLICIUM VERUM HOOK.F) AS A HAND SANITIZER***

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***Abstract***

*The hand becomes one of the body parts that are easily exposed to microorganisms because of their high mobility. Hand sanitizer is a liquid that can be used to kill microbes. Hands become one of the body parts that are easily exposed to microorganisms because of their high mobility. Hand sanitizer is a liquid that can be used to kill microorganisms. Most hand sanitizers contain alcohol and triclosan, which can be bad for the skin if used in excessive amounts. It is necessary to substitute alcohol and triclosan as an antibacterial, one of which is star anise (Illicium verum Hook.f). Star anise contains flavonoids and tannins, which can function as antibacterial and safe for health. The objective of this study was to make a hand sanitizer formulation from star anise and test its antibacterial strength. The method used was a laboratory experiment with stages; Star anise extraction and antibacterial test. The results of the hand sanitizer formulation of the biggest star anise extract in inhibiting are 10% star anise extract. Inhibitory zone of 10% star anise extract at  $6.2 \pm 0.76$  mm and included in the medium category.*

**Keywords:** Antibacterial hand sanitizer, star anise

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## INTRODUCTION

The hand is one of the organs that have high mobility. Hand has a high content of microbes, where per cm<sup>2</sup> contains 1000 CFU microbes (Harsa et al., 2016). High mobility or movement causes hands to have exposure to pathogenic microorganisms, which potentially hands become vectors of diseases and infections. In addition to keeping sanitation, hand washing is the most practical way to prevent infection or the spread of pathogenic microbes. Demands of the times that require practicality then created materials that can inhibit contamination, disease, and spread of microbial pathogens by using a liquid antiseptics (Wijaya, 2013).

The utilization of antiseptics in the form of gel or spray has become a lifestyle. The use of antiseptic hand gel use in the dosage form is to be dropped into the palm, then spread all the surface of the hand (Sari & Isadiartuti, 2006). The antiseptic known as hand sanitizer was chosen because it is more effective, efficient, and practical than using soap. Hand sanitizer can kill germs or pathogens because it contains alcohol (ethanol, propanol, isopropanol) at a concentration of ± 60-80% and phenols (chlorhexidine, triclosan) (Aminah et al., 2018) and can eliminate germs in less than 30 seconds. Alcohol (Rini & Nugraheni, 2018) and

Grace et al. (2015) hand sanitizer with 99% alcohol (Grace et al., 2015) can kill bacteria within seconds after usage.

The chemical compound in hand sanitizer can denature and agglutinate the proteins in microbes. Alcohol as a main ingredient of hand sanitizer has bactericidal. Triclosan has inhibitory trait on bacterial growth, as well as antifungal and antiviral trait, and it is not corrosive (Aminah et al., 2018). The problem in the utilization of hand sanitizer is excessive consumption can cause irritation and a burning sensation on the skin. Also, the strong alcohol scent is less preferred. It is necessary a solution to substitute alcohol with a compound that has an antibacterial trait that has a pleasant aroma and does not cause irritation.

Anise (*Illicium verum* Hook.f) is a spice used as seasoning and herbs. Anise has a distinctive taste and intense aroma. In Indonesia, known as anise, fennel China, is used as a spice that has a very similar flavor to fennel, obtained from the star-shaped pericarp (Hilmarni, 2019). Chemical contents of anise are saponins, tannins, flavonoids, Orthidine E, Adriadyiolide, 8-Decene-4,6-diyne-1,2,10-triol, hrysogedone A, Egomaketone, and Lepalone (Winarsih et al., 2018) and essential oils (Nurmalasari & Sofian, 2012). The chemical compound can

inhibit the growth of bacteria (Ave et al., 2018).

With the presence of these compounds, apart from being seasoning, anise also can be used as a mosquito repellent (Eva et al., 2019). Also, it is the potential to be used as a hand sanitizer. The objectives of this study are to formulate and antibacterial test of anise extract used as a hand sanitizer.

## **MATERIALS AND METHODS**

This study was experimental laboratory research conducted at the Laboratory of Microbiology FKIK UKSW. Materials used were anise extract, witch hazel-original (Thayers), alcohol (Merck), nutrient agar (Merck), nitrogen gas, and distilled water. Tools used include; oven (Memmert), incubator (memert), autoclave (all American), rotary vacuum evaporator (Dlabs), stirrer (IKA), a micropipette (Eppendorf) and centrifuged (Hettich). The stages of research were star anise extraction, hand sanitizer formulations, and anti-microbial test.

### *Star Anise Extraction*

A total of 20 grams of anise washed and dried for 6 hours in an oven. They were once dried then crushed using a mortar and filtered using 60 mesh sieves.

Sawdust mace then dissolved in alcohol in the ratio 1:5 in the Erlenmeyer, then macerated using a magnetic stirrer and stirred for 24 hours at room temperature. Once macerated, then filtered and centrifuged at a speed of 40,000 rpm for 10 minutes. The supernatant results of centrifugation then evaporated with a rotary vacuum evaporator with a temperature of 50°C to stay 1/4 volume. Anise extract was then concentrated with nitrogen gas.

### *Formulations Hand Sanitizer*

Anise extract was mixed with witch hazel with a concentration of 1, 2.5, 5, 7.5, and 10% in the test tube. Homogenization was done by vortex for 1 minute. The formulation was then prepared for the antibacterial test.

### *Anti-Bacterial Test - Well*

The media of the antibacterial test used was Nutrient Agar (6.6 g) and was dissolved in 300 ml of distilled water in the Erlenmeyer. The solvent is then sterilized using an autoclave at a temperature of 121°C for 15 minutes at a pressure of 2 atm. After that, the Agar solvent was then poured into a sterile petri dish and allowed to harden. After hardening, the surface was perforated by

sanitary glass pipes, and the surface was smeared with a suspension of bacteria. The formula of hand sanitizer was inserted into the wellbore as much as 100 µl using a micropipette. Incubation was performed for 48 hours in an incubator at a temperature of 31°C. The bacterial inhibition zone was calculated based on the width of the diameter. It was used the witch hazel and commercial hand sanitizer as a control.

## RESULTS AND DISCUSSION

Anise has bioactive compounds that can be dissolved by using alcohol. The solvent will be got and used as a mixture

of hand sanitizer with various concentrations. One of the indicators of hand sanitizer can inhibit the growth of bacteria based on antibacterial tests. The bacterial sensitivity test is a way to find and get a natural product that has potential as an antibacterial ingredient and can inhibit the growth of or kill bacteria (Haryati et al., 2017).

The research results can be seen in Table 1. The table indicates that the inhibition zone difference of each treatment (1 – 10%). There is a significant difference in each treatment, where the higher the concentration, the more wide the inhibitory zone.

Table 1. The measurement results inhibition zone (mm) of various formulations of hand sanitizer

Treatment	The mean inhibitory zone (mm)	Category Davis and Stout (1971)
1%	0.2 ± 0.45	Weak
2.5%	1.1 ± 0.225	Weak
5%	1.9 ± 0.225	Weak
7.5%	2.4 ± 0.42	Weak
10%	6.2 ± 0.76	moderate
Witch Hazel	4.6 ± 1.34	Weak
Control	4.2 ± 0.45	Weak

Note 10 – 20 mm (strong) 5 – 10 mm (medium) and a diameter of <5 mm (weak) Davis and Stout (1971) in (Rahmawati & Sudjarwo, 2011).

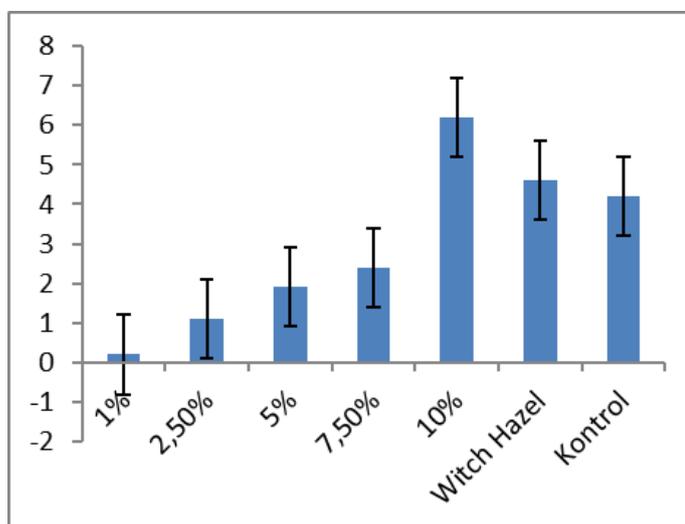
In this study, only 10% concentration of anise that has inhibitory effects in the medium category, which is 6.2 ± 0.76 mm, while concentrations under 10% are categorized as weak inhibition.

### *Zone of Hand Sanitizer Inhibition*

Witch hazel is one of the cosmetic ingredients derived from extracts of flowers *Hamamelis Virginiana* from family Hamadelidaceae. *Hamamelis virginiana* flower extract contains several bioactive and volatile compounds. Distinctive aroma and character that does not cause toxicity

(toxic) and irritation of the skin make this flower used as cosmetic ingredients. In *Hamamelis virginiana* flowers, some antibacterial agents are tannin concentration of 10000 - 70000 ppm (Engel et al., 1998). Therefore, the flower extracts can be used as necessary materials as hand sanitizer, and the results of this study have inhibitory zone  $4.6 \pm 1.34$  mm and included in the low category.

Witch hazel can be used as an alternative to alcohol and triclosan hand sanitizer. Alcohol and triclosan have the potential to cause skin irritation at the use of excessive and aroma are less preferred. Therefore, witch hazel can be combined with star anise extract. The combination of witch hazel and anise extract is expected to provide benefits as an antibacterial as well as giving a good aroma.



**Image 1.** Chart comparison of inhibition zone.

Figure 1 is an inhibitory zone comparison of anise extract mixed with witch hazel for hand sanitizer. The higher concentration of star anise extract mixed with witch hazel, the greater the inhibitory zone. This is supported by Ave et al (2018) that used anise extract with a concentration of 20% has inhibitory zone of  $5.76 \pm 0.23$  mm, 40% ( $7.03 \pm 0.05$  mm), 60% ( $7.13 \pm 0.55$  mm), 80% ( $8.36 \pm 0.11$

mm) and 100% ( $10.36 \pm 1.58$  mm). However, witch hazel, without the addition of anise extract, has a smaller inhibitory zone than witch hazel, which was added. The only possibility is tannins that work as an antibacterial compound.

Mixing witch hazel with star anise extract provides additional inhibition zone becomes wider and aroma produced is typical anise. Hand sanitizer in the market

with alcohol-70% as an essential ingredient gives an inhibition zone of  $4.2 \pm 0.45$  mm does not vary much with the inhibition zone of witch hazel. From these results, it can be said anise extract is added to the witch hazel have a higher area of inhibition than with commercial hand sanitizer. Therefore, the star anise extract has potential as an essential ingredient of hand sanitizer.

#### *Anise Antibacterial*

Star anise is widely used in some areas in Indonesia as a condiment for spicy cuisine, for instance, Aceh curry, Rendang Minang, Javanese cuisines, and Bali cuisines. Fruits of anise contain essential oils (anethole 85-90%), resins, fats, tannins, pectin, terpenes, limonene, estradiol, safrole, timokuinon, flavonoids, glucosides, and saponins. Flavonoid compounds are kind of flavones, flavonols, flavononol, tannins, saponins, alkaloid are potential as an antibacterial. Thus, the bioactive compounds that exist in anise can be used as an antibacterial. Rosary & Duniaji, (2018) tested the antibacterial anise extract activity with a concentration of 20% to *Staphylococcus aureus* bacteria has an inhibition zone of  $5.76 \pm 0.23$  mm and is in the medium category. In this study, the highest power resistor is at a concentration of 10%, which is 6.2 mm and falls into the medium category.

Anise extract has antibacterial compounds such as flavonoids and tannins. Flavonoids have traits as a disinfectant. Flavonoids work by denaturing the proteins in microbes, by forming a complex compound with extracellular proteins. The formed complexes disturb the integrity of the bacterial cell membrane by denaturalizing bacterial cell protein and damaging cell membrane irreparably (Rizkita, 2017), Tannins also have antibacterial power. Tannins will react with the microbial cell membrane. Tannins will damage the polypeptides in microbial cell walls so that forming cell walls become less perfect. The broken cell wall can cause bacteria to be lysed, for their osmotic pressure and can cause death.

#### **CONCLUSION**

From this study, it can be concluded that the greatest hand sanitizer formulation of anise extract in inhibiting microorganisms is at a concentration of 10% of anise extract with the addition of witch hazel. Inhibition zone of 10% star anise extract is at  $6.2 \pm 0.76$  mm and falls into the medium category.

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