# *Illicium verum* Essential Oil as Antibacterial Agent

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Abstract: This aim of this research was to determine the main compounds, physico-chemical properties and the potential of Illcium *verum* essential oils as antibacterial agent. Essential oil was obtained by steam distillation of dried sample method. The yield of distillation of 500 grams of Illcium *verum* dry sample was 1.8% with physico-chemical properties of specific gravity 0.978 gr / mL and index of refraction 1.3284. The results of volatile oil characterization using GC-MS showed that the main compounds contained were anetols (97.03%). The anti-bacterial test results for gram-positive *Staphylococcus aureus* (S.*aureus*) and negative *Escherichia coli* (E.*coli*) bacteria indicate that Illcium *verum* essential oil has the potential as an antibacterial.



## 1 INTRODUCTION

*Illicium verum* called star anise is an aromatherapy plant containing essential oils (Figure 1). *Illicium verum* is usually used for spices. Europe in the 17th century introduced *Illicium verum* as a spice. The *Illicium verum* plant originated in southern China and North Vietnam, then grew exclusively in South China, Indonesia and Japan. Essential oils are obtained using the steam distillation method (Morton, 2004).

Figure 1: *Illicium verum.*

Vietnam produces more than 2000 tons of star anise seeds per year. Around 1600 tons of seeds were exported to Cuba, China and Soviet Taiwan. In addition, 200-250 tons of essential oil were sent to France and Czechoslovakia (de Beer, 1993). In 2002 the Ministry of Health of the People's Republic was very useful for food and medicine (Morton, 2004).

From this explanation, it shows that this plant is very good as a basic product for commercial products.

This article informs the main components, the physical properties of the essential oils of *Illicium verum*, and also informs that the essential oils of Illcium verum have the potential as antibacterial. Antibacterial tests were tested on gram positive (S. aureus) and negative (E. coli) bacteria. S. aureus is one of the microbial pathogens which is often positive in food (Octaviantris, 2007). Food sources of S. aureus contamination that cause epidemics are pork, bread products, beef, turkey, chicken and eggs. The Gram negative bacteria used are *E. coli* bacteria, usually these bacteria are often found in food, sewage disposal, human digestive tracts, household appliances. Selection of *E. coli* and *S. aureus* bacteria in bacterial tests because their growth is very fast and easy to handle (Octaviantaris, 2007).



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# 2 MATERIALS AND METHODS

## 2.1 Materials

The material used by *Illicium verum* obtained from the Pancing market, Medan, North Sumatra, aquades. The tools used are distillation stahl, scales, picnometers, refractometers, glassware and Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu-Qp-505).

## 2.2 Methods

The *Illicium verum* was dried gradually (± 500g each distillation) and put into Dean-Stark distillator. The sample and distillate water ratio was 1: 2, then the sample was evaporated for 6 hours at a temperature of 100-105ºC. Some amount of anhydrous $Na_2SO_4$ was added to obtained essential oils. The produced essential oils were analyzed for yield, specific gravity, refractive index (SNI 06-2385-2006), and characterization of the main chemical compounds in *Illicium verum* using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu-Qp- 505). Then, the obtained essential oil was tested against S. *aureus* and E. *coli* to measure its activity as antibacterial agent.

# 3. RESULTS AND DISCUSSIONS

## 3.1 Yield Percentage and Physical Properties of Atsiri Oil

The yield percentage of the isolated essential oils from 500 g of dried sample *Illicium verum* was 1.8% v/w. The physical properties of *Illicium verum* essential oil was yellowish and has a distinctive odor (Figure 2). In the previous research that conducted by Gholivand et al. (2009) reported that the dried *Illicium verum* contained 8 to 12% of essential oil. The physicochemical properties of essential oils that produced in this study has specific gravity of 0.978 gr / mL and index of refraction of 1.3284.

Figure 2: Essential oil of *Illicium verum.*

## 3.2 Chemical Compounds of *Illicium verum* Essential Oil

The main compounds in essential oils are terpenoids, which consist of monoterpenes with the range of boiling points 140-180°C, and sesquiterpene with boiling points above 200°C (Harborne 1987). The compound that presence in *Illicium verum* essential oil was characterized using GC-MS (Figure 3). The GC-MS results of the *Illicium verum* essential oils showed that the main components were anethol (97.03%) and estragola (1.58%). This is in according with the result of Dwivedy et al. (2018) that the main components in *Illicium verum* are anetol (89.12%) and estragola (4.86%).

This distinguish was caused by the differences in the harvest age, storage, variety, and geographical location (Sivasothy et al. 2011). Bermawie et al., (2008) also stated that the differences in the results of the amount of substances formed in plants can be influenced by several factors including environmental and geographical factors.

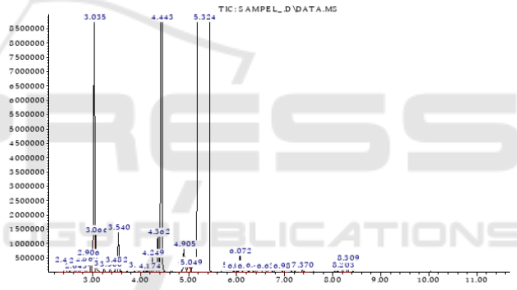


Figure 3: Chromatogram of essential oils.

The structure of the dominant compounds in the essential oils of *Illicium verum* is presented in Figure 4.

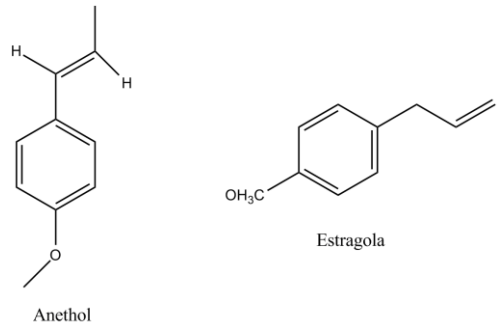


Figure 4: Structure of anethol and estragola.

Anethol is a compound that responsible for the distinctive aroma of *Illicium verum* essential oil. Mass chromatogram based on the Wiley data base

(Figure 5) shows that anetol has an SI value: 97 with molecular weight 148 (Figure 6) corresponding to the molecular weight of anethol ($C_{10}H_{12}O$). The base peak m/z 148 is the typical base peak for anethol, because anethol is stabilized by resonance. Fraction with m/z 117 is produced by the release of H and O = $CH_2$ radicals.

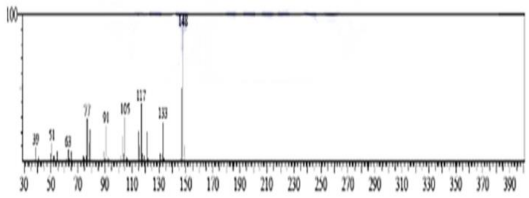

Figure 5: Anetol chromatogram based on the Wiley database.

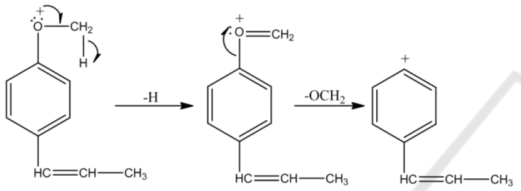


Figure 6: Proposed Fragmentation Pattern of Anethol Compounds (Kusumaningsih, 2004).

### 3.3 Antibacterial Activities of *Illicium verum* Essential Oil

The antibacterial activity of *Illicium verum* againts *E. coli* and *S. aureus* was performed using disc paper method. The diameter of the inhibition zone is an indication of the sensitivity of the testing bacteria, with the greater the inhibition zone, the antibacterial activity has better antibacterial activity (Figure 7).

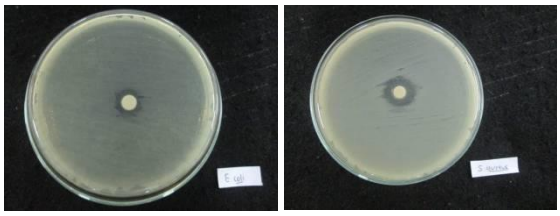

Figure 7: Zone of Inhibition againt (a) *E. coli* and (b) *S. Aureus.*

*E. coli* is one of the bacteria that used in this study and classified as gram negative bacteria. Gram negative bacteria have better resistance to antimicrobial compounds compared to gram-positive bacteria, according to Zuhud's opinion (Zuhud, 2011).

The inhibitory categories are grouped into three based on inhibition diameter, i.e. (i) 0-3 mm is weak category, (ii) 3-6 mm is moderate category, and (iii) >6 mm is strong category. A material is said to have strong antibacterial activity if the diameter is greater or equal to 6 mm (Suciati et al., 2012).

The inhibitory value of *Illicium verum* essential oil against *E. coli* and *S. aureus* respectively 0.8 mm & 1.45 mm. This shows that essential oils of *Illicium verum* against *E. coli* and *S. aureus* have the potential as antibacterial .

## 4 CONCLUSIONS

The yield percentage of 500 grams of dried sample Illcium *verum* is 1.8%. The physicochemical properties of *Illicium verum* essensial oils, i.e. specific gravity of 0.978 gr / mL and index of refraction 1.3284. The main compounds that contained in Illcium *verum* essential oils are anetol (97.03%) and Estragol (1.58%). *Illicium verum* essential oils against *E. coli* and *S. aureus* have the potential as antibacterial.

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