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Terpenoid Compounds of Lime (*Citrus Aurantifolia*) Peel Extract Using Gas Chromatography Mass Spectrometry (GCMS) Method

Mirnawati[1*], Nur Mu'min[1], Muhammad Yunus[2]

Abstract. Terpenoid compounds known as essential oils can be used as flavor enhancers and medicinal sources. Essential oils in the *citrus* genus are species of *citrus aurantifolia* containing terpenoid compounds, it's used as antibiotics and antiseptics. *Citrus aurantifolia* (Rutaceae) is a fruit type most often consumed by the public. This study aimed to determine the terpenoid compounds contained in lime peel extract (*C. aurantifolia*). This study used the extraction method for 3 x 24 hours and then continued by using a sonicator. The viscous extract was then analyzed by using a GCMS method. This study obtained that the *β*-pinene compound for the retention time was 7.92, and the percentage of the area was 34.81%. While for the D-limonene compound for the retention time was 6.62, and the percentage of the area was 20.15%.

Introduction

Terpenoid compounds are volatile secondary metabolites. It is known as essential oils that can be used as flavor enhancers and medicinal sources (Lata *et al.,* 2000). Essential oils are used to give taste and aroma to foods, beverages, perfumes, and cosmetics (Hegarty et al., 2001).

Essential oils are reported to have acted as anticancer (Ballistreti et al., 2019), antimicrobial, antioxidant (Ladaniya, 2008), anti-inflammatory, hypolipidemic, anthropoid, and hepatoprotective (Al-Snafi & Thuwaini, 2017). In addition to essential oils, the genus *Citrus* contains polyphenolic compounds (Fattore et al., 2016), flavanone glycosides, and poly methoxy flavones (Lu et al., 2015., Rafiq et al., 2018., Chen et al., 2017., Delgado et al., 2019). One of the species contained in the *Citrus* genus is the *Citrus aurantifolia* species. *Citrus aurantifolia* (Rutaceae) is one of the most commonly consumed types of fruit by the public. Lime is rich in vitamins, minerals, and essential fiber that are useful for the growth and development of the body.

C. aurantifolia thrives in tropical climates and has a height of about 150-350 cm and thin-skinned fruit and white flowers. This plant has a salt content of 10% and can thrive on soil with a slope of about 30° (Rukmana, 2003). The use of lime (*C. aurantifolia*) is still only a flavor enhancer in food. It is necessary to innovate either as an antibacterial or an antiseptic to increase its economic value. In this study, samples of lime peel (C. aurantifolia) were macerated using an organic solvent. The extract obtained was then analyzed using GCMS to determine that the lime peel extract (*C. aurantifolia*) contains terpene compounds. This study aimed to determine the terpenoid compounds contained in samples of lime (*C. aurantifolia*).

Experimental

Material and Methods

Instrumentation

In this study, the equipment used were: evaporator, glassware, incubator, colony counter, autoclave, laminar flow, oven, water bath shaker, analytical balance, and refrigerator.

Materials

The materials used were samples of lime (*Citrus aurantifolia*), *Bacillus cereus* bacteria, *Escherichia coli* bacteria, physiological sodium chloride (NaCl), solid media (NA), liquid media (NB), *n*-hexane, and ethanol (C_2H_5OH).

[1]*Department of Chemistry, Faculty of Engineering, Technology University of Sulawesi, Jl. Talasalapang No. 51, Makassar, Indonesia;* ***Email: muhsakhizaidan@gmail.com***
[2]*Department of Physics, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Gorontalo, Indonesia*

Procedure

Sample Extraction of Lime (*C. aurantifolia*)

Weigh the dried lime sample of *C. aurantifolia* as much as 500 g. The sample is put into a container and then soaked in ethanol for 3 x 24 hours. The immersion results were then sonicated for 30 minutes with a frequency of 20 Hz. The sample was evaporated using a rotary vacuum evaporator at a temperature of 40 ^{o}C to obtain a thick extract of 100 g.

Solid and Liquid Media Production

The tools used in this study were sterilized in an oven at 170 ^{o}C for ± 1 hour (dry sterilization), the media was sterilized in an autoclave at 121 ^{o}C for 15 minutes (wet sterilization). Weigh 2.5 g of NA, and it dissolves in 100 mL of warm H_2O. Then, all ingredients are put in an Erlenmeyer and sterilized in an autoclave for 15 minutes at 121 ^{o}C and 1 atm pressure.

Weigh 0.8 g of NB into a beaker, then dissolve in 100 mL of warm aquadest. They are sterilized in an autoclave at 121 ^{o}C for 15 minutes and a pressure of 1 atm.

Rejuvenation and Production of Bacteria

The test bacteria used consisted of Staphylococus aureus. Bacterial rejuvenation was carried out by taking 1 oshe of pure culture and then transferred it to a petri dish containing NA, incubated for 24 hours at 37 ^{o}C.

The rejuvenating bacterial culture was taken 1 oshe on NA media. Then suspend into NB media. Incubate for 24 hours at 37 ^{o}C. Bacterial growth is characterized by the presence of turbidity in the suspended media.

Compound Analysis using Mass Spectroscopy Gas Chromatography/GCMS

The ethanolic extract of *C. aurantophilia* was added with 1 mL of ethyl acetate solvent. Take 1 µL to be injected into the GCMS. Identify the top of the graph and match it with a reference in the GCMS program.

Result and Discussion

Phytochemical Test

In this study, the extract used was lime extract (*C. aurantifolia*). *C. aurantifolia* contains secondary metabolites with different polarities. One way to take these compounds is the maceration method using ethanol as a solvent.

The moving process using a sonicator has been carried out to speed up the compound withdrawal process. The purpose of moving is to make collisions between particles that can increase the binding and breakdown of cells so that the bioactive components can come out of the tissue and dissolve in the solvent. The pressure difference between the outside and inside the cell causes cell walls and membranes to break down. So that secondary metabolites contained in the cytoplasm would dissolve into organic solvents.

The resulting viscous extract was then subjected to a phytochemical test using a solution of glacial acetic acid reacted with sulfuric acid to obtain a colour change from blue to purple. The purple colour indicates that the ethanol extract of lime (*C. aurantifolia*) peel contains terpene compounds.

Figure 1. Phytochemical test results of lime (*C. aurantifolia*) peel extract

Terpenoid compounds with low molecular weight are volatile and are commonly found as components of essential oils. The mechanism of action of terpenoid compounds involves the breakdown of membranes by lipophilic components. In addition, terpenoid compounds have the primary target, and it is the cytoplasmic membrane refers to its hydrophobic nature.

Analysis of Compound Content of Lime (*C. aurantifolia*) Peel

Chromatogram of the ethanolic extract of lime (*C. aurantifolia*) peel involved 98 peaks. However, there were two peaks whose abundance was relatively high analyzed, namely at retention times of 7.92 and 6.62, with the percentage of the area (abundance) of 34.81% and 20.15%, respectively (Figure 2).

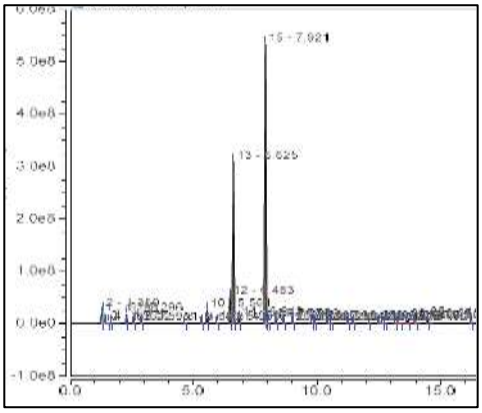

Figure 2. Results of GCMS analysis of lime (C. aurantifolia) peel ethanol extract.

Retention time indicates the length of time for a compound to move through the column to the detector. A compound has a different retention time depending on its boiling point. A compound that boils at a temperature higher than the column temperature will spend all of its time condensing as a liquid at the beginning of the column. In other words, using a high temperature, the compound will pass through the column faster, but the separation is not good. If a compound passes through the column in a short time, there will be no distance between the peaks in the chromatogram. Vice versa, the lower the column temperature, the better the separation will be obtained. But it will take a long time to get the compound due to condensation at the beginning of the column (Hendayana, 2006).

Based on the search results in the GCMS Library, β-pinene and D-limonene compounds were obtained (Figure 3).

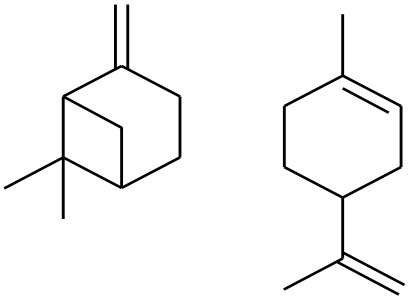

Figure 3. Compound structure of β-pinene and D-limonene.

Compounds of β-pinene and D-limonene are compounds with 10 carbons, and they belong to the monoterpene group. These compounds are reported to have antibacterial, antiseptic and anticancer activities.

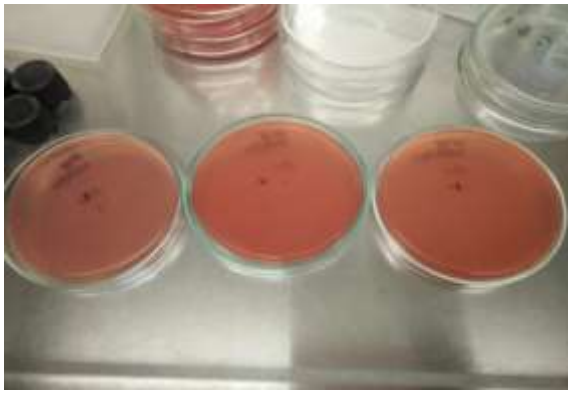

Figure 4. Bacterial test results *Staphylococcus aureus.*

Antibacterial Activity

The purpose of antibacterial activity test of lime peel extract was to determine the ability of secondary metabolite compounds contained in lime peel to kill bacteria. In this research, lime extract tested using *Staphylococcus aureus* bacteria. Bacterial test results *Staphylococcus aureus* shown in the Figure 4.

In this study, bacterial calculation result *Staphylococcus aureus* is <10 colonies/g. SNI standard contains a minimum of bacteria as antibacterial of 1,26 colonies/g. This indicates that lime peel extract (*Citrus aurantifolia*) has the potential to be used as an antibacteral agent (Hamidi, 2020). The smaller the measurement results obtained, the stronger the bioactive compounds in inhibiting bacterial growth (Ridho, 2018).

Conclusion

Based on research that has been done, the secondary metabolites compounds in the ethanolic extract of lime peel (C. aurantifolia) are β-pinene and D-limonene, which are included in the terpenoid group.In this study, Bacterial calculation result staphylococcus aureus <10 colonies/g. SNI standard contains a minimum of bacteria as antibacterial of 1,26 colonies/g. This indicates that lime peel extract (*Citrus aurantifolia*) has potential to be used as anti bacterial agent.

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