INTRODUCTION

The Citrus (Linn) family of Rutaceae, aromatic green shrubs and small tree plants has an important role in India's medicine. Scientifically this plant is known as *Aurantium maximum* Burm. Ex Rumph, *Citrus aurantium* L. Var grandi L., *Citrus decumana* L, *Citrus grandis* Osbeck & *Citrus pamplemos*. *Citrus grandis* Osbeck is a plant spread in India, China, Indonesia, US, Thailand, and others. The orange tree is about 16 to 50 feet tall. Oranges are native to Malayu and East India. Widespread in China, Japan, Philippines, Indonesia, US, and Thailand1,2. *Citrus maxima* L. or grapefruit is a plant commonly known as Papanus, spread throughout India. The bark and roots of *C. maxima* contain β-sitosterol, an acridone alkaloid. Essential oils from the leaves and fruits of *C. maxima*, which are still raw, contain limonin, nerolol, neryl acetate, and geraniol. This plant contains vitamin C as well as other citrus plants and usually used as fruit for consumption. On the other hand, this plant has been used in medicine, such as sedatives for nervous disorders, cough spasms, hemorrhagic diseases, and epilepsy. It is said to be appetizing, cardiac stimulant, and antitoxic properties4. *Citrus maxima* fruit also contains high amounts of polyphenol compounds such as hesperidin, naringin, caffeic acid, p-Coumaric acid, ferulic acid, and vanillic acid5.6.7.8.

Gas Chromatography-Mass Spectrophotometry (GC-MS) is a chemical tool widely used to analyze

Abstract

The grapefruit (*Citrus maxima* L.) is a plant known by the public as a fruit consumed with various properties. This plant's use is well known, such as antioxidants, enhancing immunity, anti-aging, and antibacterial properties. This study aimed to identify and analyze the chemical compounds contained in *C. maxima* rind. The extract was obtained by the maceration method using ethanol and ethyl acetate as solvents. The fractionation process was carried out by Column Chromatography. Observation of thin-layer chromatography profiles with UV lamps 254 and 366 nm. Analysis of chemical compound components using GC-MS and data interpretation based on the Wiley 7.0 data library. The interpretation results of the EF1 fraction are β-copaen-4-α-ol; pentadecanoic acid; hexadecanoic acid; tetradecanoic acid; dotriacontane; osthol; 2H-1-benzopyran-2-one, 7-methoxy-8-(3-methyl-2-oxobutyl); furfural; 6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin; and 6-(iodomethyl)-5-methyl-4-oxahexanolate. The chemical compounds identified in EAF2 are 1-octadecanol; decane; tetradecane; hexadecane; and 1,2-benzenedicarboxylic acid (2-ethylhexyl) ester. It can be concluded that these compounds have biological and pharmacological activities.
compounds in medicinal plants such as essential oils, fatty acids, hydrocarbons, lipids, and others. This method is simple, sensitive, and effective in separating the mixture's components⁶⁻⁷. Besides, GC-MS is a reliable tool for identifying bioactive compounds⁸. Research on C. maxima with GC-MS has previously been conducted. The results of other studies on C. maxima showed that it has antibacterial activity with essential oils, namely α-pinene, myrcene, limonene, germacrene, and β-asarone compounds⁹. Some studies also said that the C. maxima rind extract has chemical compounds such as the flavonoid group, which has several biological activities, one of which is antioxidants¹⁰⁻¹¹. In another study, it was said that the essential oil of C. maxima rind contains essential oils such α-pinene, myrcene, limonene, germacrene, β-asarone and has antimicrobial activity against the bacteria Escherichia coli and Staphylococcus aureus⁹. However, no studies have identified the chemical compound content of the ethanol and ethyl acetate fraction of C. maxima rind. Therefore, this study aims to identify the chemical compounds of the ethanol and ethyl acetate fraction of C. maxima rind based on data from the analysis using GC-MS.

MATERIALS AND METHODS

Materials
The material used in the study was C. maxima rind, which was obtained from the Bantul Regency, Yogyakarta. The determination was carried out at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada. The chemicals used are ethanol (JT-Baker), ethyl acetate (JT-Baker), and chloroform (Merck). The tools used were ovens (Memmert), analytical scales (Sartorius), Thin Layer Chromatography (TLC) plates (Merck), silica gel GF 60, capillary tubes, chambers, UV lamps (254 and 366 nm), GC/MS (Shimadzu), and a set of column chromatography equipment.

Methods

Material preparation
The C. maxima rind (Figure 1) was sorted, washed, and dried at 50°C in a drying oven for six hours¹², then the sample size was reduced using a machine to produce dry simplicia in 20 mesh to increase the touch surface area of the solvent absorption area. Furthermore, the sample was macerated using ethanol and ethyl acetate as solvents. Fractionation was carried out using column chromatography with a stationary phase of silica gel GF 60 and mobile phases of ethyl acetate, chloroform, and ethanol. The fractionation result was identified using a TLC plate to see the fractionation result profile.

Figure 1. Fruit (a) and rind (b) of C. maxima

GC-MS analysis
A sample solution of 1 μL is injected into GC-MS-QP2010 SE, which has a capillary column with a length of 30 mm, a diameter of 0.25 mm, and a thickness of 0.25 μm. Helium carrier gas at a flow rate of 1 mL/min with a split ratio of 1 : 50. The pre-programmed oven temperature was 150°C and stored isothermal for five minutes, the rate of increase was 10°C/minute, and the temperature was increased to 250°C for five minutes.

Compound identification
Interpretation of the GC-MS mass spectrum was performed using the Wiley 7.0 database. The spectrum of components compared to the Wiley 7.0 data library. The
identification of chemical compounds was confirmed based on the peak area and retention time.

RESULTS AND DISCUSSION

Maceration results obtained ethanol fraction (EF) and ethyl acetate fraction (EAF) of C. maxima rind. The fractionation results of EF were obtained four fractions with Rf value of EF1 (0.36; 0.44; 0.58; 0.74; 0.9; and 0.98), Rf value of EF2 (0.36), Rf value of EF3 (0.36), while the fractionation results of ethyl acetate extract were obtained six fractions with Rf value of EAF1 (0.47; 0.63; 0.7; and 0.8), Rf value of EAF2 (0.18; 0.28; 0.47; 0.63; and 0.72), Rf value of EAF3 (0.28; 0.47; 0.63; and 0.72), Rf value of EAF4 (0.28; 0.47; and 0.72), Rf value of EAF5 (0.28), and Rf value of EAF6 (0.28). The fractionated TLC profiles are shown in Figure 2, while Rf values for EF and EAF are shown in Tables I and II, respectively.

The selection of isolate fractions to be used in the GC-MS analysis was carried out based on the number of stains or spots that could represent all stains or spots in each fraction and the degree of separation from the TLC profile. The fraction of the isolate selected in the ethanol fraction was fraction number 1 (EF1), while the isolate selected in the ethyl acetate fraction was fraction number 2 (EAF2). The isolates EF1 and EAF2 were identified by GC-MS. The chromatogram results of the GC-MS analysis of the EF1 and EAF2 fractions of the grapefruit rind extract can be seen in Figures 3 and 4, respectively. The identification of the components of chemical compounds in EF1 and EAF2 was carried out by comparing the mass spectrum fragmentation patterns with the fragmentation patterns of the reference compounds using the Wiley 7.0 data bank.

The major chemical compounds identified by GC-MS in EF1 (Table III) were β-copaen-4-α-ol (11.66%); pentadecanoic acid (3.08%); hexadecanoic acid (5.72%); tetradecanoic acid (6.66%); dotriacontane (3.79%); osthol (12.33%); 7-methoxy-8-(2-oxo-3-methylbutyl)coumarin (32.77%); furfural (3.95%); 6-(2,3-dihydroxy-3-methylbutyl)-7-methoxycoumarin (10.18%); and 6-(iodomethyl)-5-methyl-4-oxahexanolide (9.86%). Meanwhile, the major chemical compounds identified by GC-MS in EAF2 (Table IV) were 1-octadecanol (7.75%); decane (3.97%); tetracosane (2.49%); hexacosane (3.07%), and 1,2-benzenedicarboxylic acid, (2-ethylhexyl) ester (82.71%). The results of the compound component analysis showed that the compound with the largest percentage was 7-methoxy-8-(2-oxo-3-methylbutyl) coumarin of 32.77% in the EF1 fraction, while in the EAF2 fraction the largest content was 1,2-benzenedicarboxylic acid, (2-ethylhexyl) ester equal to 82.71%.

![Figure 2. TLC profile of EF (a) and EAF (b)](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Rf value</th>
<th>EF1</th>
<th>EF2</th>
<th>EF3</th>
<th>EF4</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.36</td>
<td>0.56</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>0.44</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>0.98</td>
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Table I. Rf value of EF

<table>
<thead>
<tr>
<th>No.</th>
<th>Rf value</th>
<th>EAF1</th>
<th>EAF2</th>
<th>EAF3</th>
<th>EAF4</th>
<th>EAF5</th>
<th>EAF6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>0.18</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>0.63</td>
<td>0.28</td>
<td>0.47</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>0.47</td>
<td>0.63</td>
<td>0.72</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>0.63</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>0.72</td>
<td></td>
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</tr>
</tbody>
</table>

Table II. Rf value of EAF
Table III. The results of the GC-MS analysis of the chemical components of EF1

<table>
<thead>
<tr>
<th>No</th>
<th>Retention Time (minutes)</th>
<th>% Content</th>
<th>Molecular Weight (g/mol)</th>
<th>Molecular Formula</th>
<th>Compound</th>
<th>2D Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.82</td>
<td>11.66</td>
<td>220</td>
<td>C_{15}H_{24}O_{18}</td>
<td>β-Copaen-4-α-ol</td>
<td><img src="image" alt="β-Copaen-4-α-ol" /></td>
</tr>
<tr>
<td>2</td>
<td>12.38</td>
<td>3.08</td>
<td>270</td>
<td>C_{15}H_{24}O_{18}</td>
<td>Pentadecanoic acid</td>
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<tr>
<td>3</td>
<td>12.755</td>
<td>5.72</td>
<td>284</td>
<td>C_{16}H_{32}O_{2}</td>
<td>Hexadecanoic acid</td>
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<tr>
<td>4</td>
<td>13.105</td>
<td>6.66</td>
<td>256</td>
<td>C_{16}H_{32}O_{2}</td>
<td>Tetradecanoic acid</td>
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<tr>
<td>5</td>
<td>14.720</td>
<td>3.79</td>
<td>338</td>
<td>C_{16}H_{32}</td>
<td>Dotriacontane</td>
<td><img src="image" alt="Dotriacontane" /></td>
</tr>
<tr>
<td>No</td>
<td>Retention Time (minutes)</td>
<td>% Content</td>
<td>Molecular Weight (g/mol)</td>
<td>Molecular Formula</td>
<td>Compound</td>
<td>2D Structure</td>
</tr>
<tr>
<td>----</td>
<td>--------------------------</td>
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<td>-------------------</td>
<td>----------</td>
<td>--------------</td>
</tr>
<tr>
<td>6</td>
<td>14.793</td>
<td>12.33</td>
<td>244</td>
<td>C_{15}H_{15}O_{3}</td>
<td>Osthol</td>
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<tr>
<td>7</td>
<td>15.804</td>
<td>32.77</td>
<td>260</td>
<td>C_{15}H_{16}O_{4}</td>
<td>7-methoxy-8-(2-oxo-3-methylbutyl)coumarin</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.3</td>
<td>3.95</td>
<td>260</td>
<td>C_{15}H_{15}O_{3}</td>
<td>Furfural</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18.105</td>
<td>10.18</td>
<td>278</td>
<td>C_{15}H_{18}O_{5}</td>
<td>6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin</td>
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</tr>
<tr>
<td>10</td>
<td>18.535</td>
<td>9.86</td>
<td>270</td>
<td>C_{7}H_{11}I_{1}O_{3}</td>
<td>6-(iodomethyl)-5-methyl-4-oxahexanolide</td>
<td></td>
</tr>
</tbody>
</table>

Table IV. The results of the GC-MS analysis of the chemical components of EAF2.
From the results of GC-MS analysis, it was found that some of the compounds identified were derived from fat, sesquiterpenes, and coumarin. This plant group does have several biological activities. EF1 and EAF2 fraction metabolite content have many isolates with various biological activities such as antimicrobial, antioxidant, antifungal, and anti-inflammatory. Some of the component compounds' biological activities from the EF1 and EAF2 fractions are presented in Tables V and VI, respectively.

**Table V.** Bioactivity of compounds identified in the EF1

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Biological Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Copaen-4-a-ol</td>
<td>Antimicrobials¹³</td>
</tr>
<tr>
<td>2</td>
<td>Pentadecanoic acid</td>
<td>Antibacterial¹⁴</td>
</tr>
<tr>
<td>3</td>
<td>Hexadecanoic acid</td>
<td>Antioxidants¹⁵</td>
</tr>
<tr>
<td>4</td>
<td>Tetradecanoic acid</td>
<td>Antifungal, Antioxidant¹⁶, antitoxicant¹⁷</td>
</tr>
<tr>
<td>5</td>
<td>Dotriacontane</td>
<td>Antimicrobial¹⁸</td>
</tr>
<tr>
<td>6</td>
<td>Osthol</td>
<td>Antioxidant, anti-inflammatory¹⁹</td>
</tr>
<tr>
<td>7</td>
<td>7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin</td>
<td>Antimicrobial, thiol-peroxidase¹⁸</td>
</tr>
<tr>
<td>8</td>
<td>Furfural</td>
<td>Antityrosinase, antimicrobials²⁰</td>
</tr>
<tr>
<td>9</td>
<td>6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin</td>
<td>Antimicrobials²⁰</td>
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</tbody>
</table>

The many types of plants that exist require scientists to carry out phytochemical screening to identify the content of compounds in medicinal plants used by the public for treatment. The search for active compounds in plants is then examined to determine their biological and pharmacological activity so that they can be used to be developed as materials for new drug discovery.

**CONCLUSION**

In this study, ten compounds were found in the ethanol fraction and five compounds in the ethyl acetate fraction of C. maxima rind. Citrus maxima rind can be used as a source for developing new medicinal substances requiring clinical testing to assess their effectiveness.

**ACKNOWLEDGMENT**

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**REFERENCES**


