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INTRODUCTION The Citrus (Linn) family of Rutaceae, aromatic green shrubs and small tree plants have an important role in India's medicine. Scientifically this plant is known as Aurantium maximum Burm. Ex Rumph, Citrus aurantium L. Var grandis 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 ß-sitosterol, an acridone alkaloid.

Essential oils from the leaves and fruits of C. maxima, which are still raw, contain limonin, nerolol, neryl acetate, and geraniol3. This plant contains vitamin C as well as other citrus plants and is 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 acid3,5.

Gas Chromatography-Mass Spectrophotometry (GC-MS) is a chemical tool widely used to analyze 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. Other studies on C. maxima showed that it has antibacterial activity with essential oils, namely a-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, including antioxidants10,11. In another study, it was said that the essential oil of C. maxima rind contains essential oils such a-pinene, myrcene, limonene, germacrene, \(\beta\)-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 were 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. / a b Figure 1. Fruit (a) and rind (b) of C. maxima GC-MS analysis A sample solution of 1  $\mu$ L was injected into GC-MS-QP2010 SE, which had a capillary column with a length of 30 mm, a diameter of 0.25 mm, and a thickness of 0.25  $\mu$ m. 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 were shown in Figure 2, while Rf values for EF and EAF were shown in Tables I and II, respectively. a b Figure 2.

TLC profile of EF (a) and EAF (b) Table I. Rf value of EF No. \_Rf value \_ \_ \_EF1 \_EF2 \_EF3 \_EF4 \_ \_1 \_0.36 \_0.36 \_0.36 \_- \_2 \_0.44 \_- \_- \_ \_3 \_0.58 \_- \_- \_- \_4 \_0.74 \_- \_- \_5

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 could 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-a-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 3. GC-MS chromatogram of EF1 / Figure 4. GC-MS chromatogram of EAF2 Table III. The results of the GC-MS analysis of the chemical components of EF1 No \_Retention Time (minutes) \_% Content \_Molecular Weight (g/mol) \_Molecular Formula \_Compound \_2D Structure \_ \_1 \_9.82 \_11.66 \_220 \_C15H24O \_B-Copaen-4-a-ol \_/ \_ \_2 \_12.38 \_3.08 \_270 \_C17H34O2 \_Pentadecanoic acid \_/ \_ \_3 \_12.755 \_5.72 \_284 \_C18H32O2 \_Hexadecanoic acid \_/ \_ \_4 \_13.105 \_6.66 \_256 \_C16H32O2 \_Tetradecanoic acid \_/ \_ \_5 \_14.720 \_3.79 \_338 \_C32H66 \_Dotriacontane \_/ \_ \_6 \_14.793 \_12.33 \_244 \_C15H16O3 \_Osthol \_/ \_ \_7 \_15.804 \_32.77 \_260 \_C15H16O4 \_7-methoxy-8-(2-oxo-3-methylbutyl)coumarin \_/ \_ \_8 \_16.3 \_3.95 \_260 \_C11H10O3 \_Furfural \_/ \_ \_9 \_18.105 \_10.18 \_278 \_C15H18O5 \_6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin \_/ \_ \_10 \_18.535 \_9.86 \_270 \_C7H11IO3 \_6-(iodomethyl)-5-methyl-4-oxahexanolide \_/ \_ \_ Table IV.

The results of the GC-MS analysis of the chemical components of EAF2 No \_Retention Time (minutes) \_% Content \_Molecular Weight (g/mol) \_Molecular Formula \_Compound \_2D Structure \_ \_1 \_11.885 \_7.75 \_270 \_C18H38O \_1-Octadecanol \_/ \_ \_2 \_13.607 \_3.97 \_278 \_C20H38 \_Decane, 5,6-bis(2,2-dimethylpropylidene)- \_/ \_ \_3 \_17.193 \_2.49 \_338 \_C24H50 \_Tetracosane \_/ \_ \_4 \_18.573 \_3.07 \_366 \_C26H54 \_Hexacosane \_/ \_ \_5 \_19.646 \_82.71 \_390 \_C24H38O4 \_1,2-benzenedicarboxylic acid, (2-ethylhexyl) ester \_/ \_

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 had several biological activities. EF1 and EAF2 fraction metabolite content had 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. Table V. Bioactivity of compounds identified in the EF1 No. \_Compound \_Biological Activity \_ \_1 \_\textit{B}-Copaen-4-a-ol \_Antimicrobials13 \_ \_2 \_Pentadecanoic acid \_Antibacterial14 \_ \_3 \_Hexadecanoic acid \_Antioxidants15 \_ \_4 \_Tetradecanoic acid \_Antifungal, Antioxidant14 \_ \_5 \_Dotriacontane \_Antimicrobial16, antioxidant17 \_ \_6 \_Osthol \_Antioxidant, anti-inflammatory18 \_ \_7 \_7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin \_Antioxidants10 \_ \_8 \_Furfural \_Antityrosinase, antimicrobial19 \_ \_9 \_6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin \_Antimicrobials20 \_ \_ Table VI.

Bioactivity of compounds identified in the EAF2 No. \_Compound \_Biological Activity \_ \_1 \_1-Octadecanol \_Antibacterial, antifungal, anti-larval21 \_ \_2 \_Tetracosane \_Antioxidants22 \_ \_3 \_Hexacosane \_Antimicrobial23 \_ \_4 \_1,2-Benzenedicarboxylic acid, (2-ethylhexyl) ester \_Antimicrobial, Antifouling21 \_ \_ 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 was then examined to determine their biological and pharmacological activity so that they could 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 could be used as a source for developing new medicinal substances requiring clinical testing to assess their effectiveness.

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