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Research Article

GC-MS Analysis of Bioactive Compounds in Ethanol and Ethyl Acetate Fraction of Grapefruit (*Citrus maxima* L.) Rind

La Ode Muhammad Andi Zulbayu [1,2]

Endang Lukitaningsih [2*]

Rumiyati [2]

[1]Department of Pharmacy, Universitas Mandala Waluya, Kendari, Southeast Sulawesi, Indonesia

[2]Department of Pharmacy, Universitas Gadjah Mada, Sleman, Special Region of Yogyakarta, Indonesia

*email: lukitaningsih_end@ugm.ac.id

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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 were 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-oxahexanolide. The chemical compounds identified in EAF2 were 1-octadecanol, decane, tetracosane, hexacosane, and 1,2-benzenedicarboxylic acid (2-ethylhexyl) ester. It can be concluded that these compounds have biological and pharmacological activities.

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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 Thailand[1,2]. *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[3]. 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 properties[4]. *Citrus maxima* fruit also contains high amounts of polyphenol compounds such as hesperidin, naringin, caffeic acid, p-Coumaric acid, ferulic acid, and vanillic acid[3,5].

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 components[6,7]. Besides, GC-MS is a reliable tool for identifying bioactive compounds[8].

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 α-pinene, myrcene, limonene, germacrene, and β-asarone compounds[9]. Some studies also said that the *C. maxima* rind extract has chemical compounds such as the flavonoid group, which has several biological activities, including antioxidants[10,11]. 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 aureus*[9]. 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 hours[12]; 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 µL 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 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 were shown in **Figure 2**, while Rf values for EF and EAF were shown in **Tables I** and **II**, respectively.

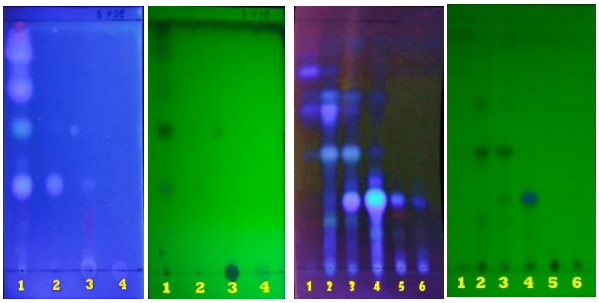


Figure 2. TLC profile of EF (a) and EAF (b)

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	0.9	-	-	-
6	0.98	-	-	-

Table II. Rf value of EAF

No.	Rf value					
	EAF1	EAF2	EAF3	EAF4	EAF5	EAF6
1	0.47	0.18	0.28	0.28	0.28	0.28
2	0.63	0.28	0.47	0.47	-	-
3	0.7	0.47	0.63	0.72	-	-
4	0.8	0.63	0.72	-	-	-
5	-	0.72	-	-	-	-

The selection of isolate fractions to be used in the GC-MS analysis was carried out based on the number of stains or spots representing 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 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-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%.

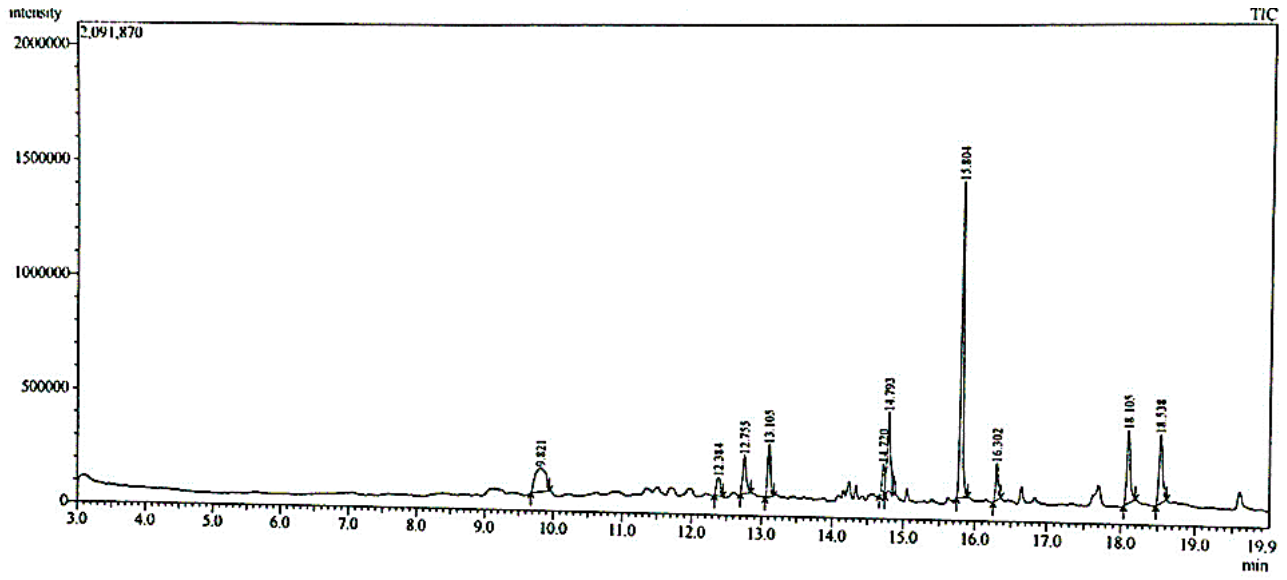


Figure 3. GC-MS chromatogram of EF1

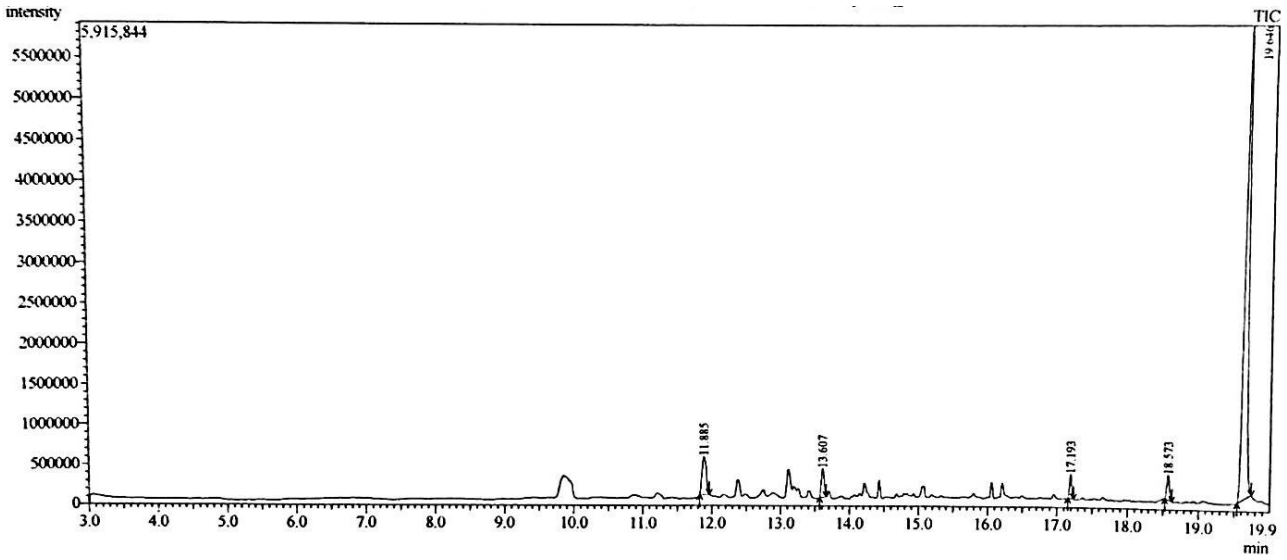
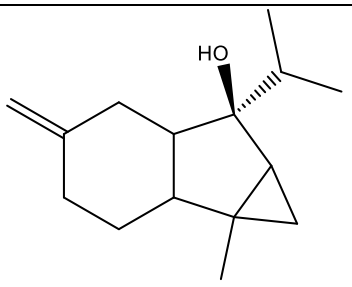
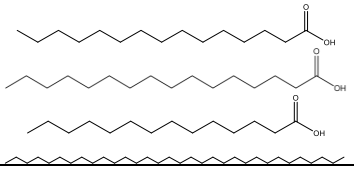


Figure 4. GC-MS chromatogram of EAF2

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	$C_{15}H_{24}O$	β-Copaen-4-α-ol	
2	12.38	3.08	270	$C_{17}H_{34}O_2$	Pentadecanoic acid	
3	12.755	5.72	284	$C_{18}H_{32}O_2$	Hexadecanoic acid	
4	13.105	6.66	256	$C_{16}H_{32}O_2$	Tetradecanoic acid	
5	14.720	3.79	338	$C_{32}H_{66}$	Dotriacontane	

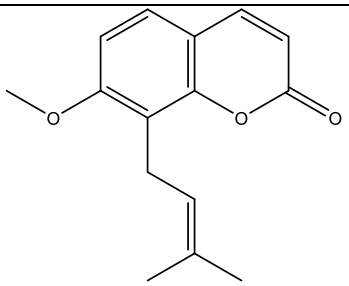
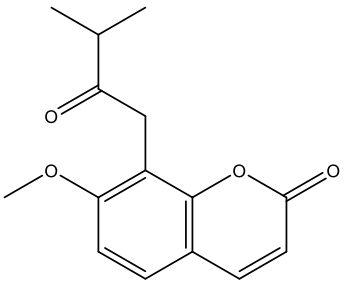
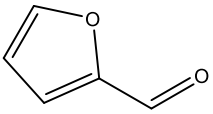
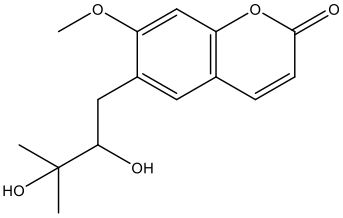
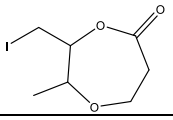
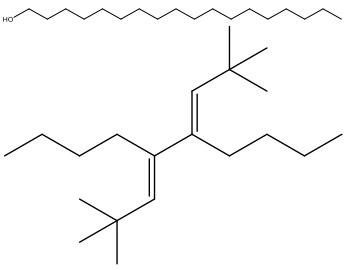
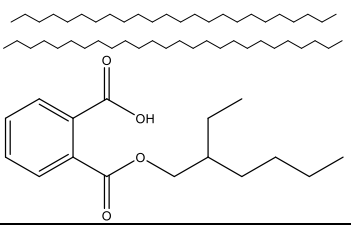
No	Retention Time (minutes)	% Content	Molecular Weight (g/mol)	Molecular Formula	Compound	2D Structure
6	14.793	12.33	244	$C_{15}H_{16}O_3$	Osthol	
7	15.804	32.77	260	$C_{15}H_{16}O_4$	7-methoxy-8-(2-oxo-3-methylbutyl)coumarin	
8	16.3	3.95	260	$C_{11}H_{10}O_3$	Furfural	
9	18.105	10.18	278	$C_{15}H_{18}O_5$	6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin	
10	18.535	9.86	270	$C_7H_{11}IO_3$	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	$C_{18}H_{38}O$	1-Octadecanol	
2	13.607	3.97	278	$C_{20}H_{38}$	Decane, 5,6-bis(2,2-dimethylpropylidene)-	
3	17.193	2.49	338	$C_{24}H_{50}$	Tetracosane	
4	18.573	3.07	366	$C_{26}H_{54}$	Hexacosane	
5	19.646	82.71	390	$C_{24}H_{38}O_4$	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	β-Copaen-4-α-ol	Antimicrobials[13]
2	Pentadecanoic acid	Antibacterial[14]
3	Hexadecanoic acid	Antioxidants[15]
4	Tetradecanoic acid	Antifungal, Antioxidant[14]
5	Dotriacontane	Antimicrobial[16], antioxidant[17]
6	Osthol	Antioxidant, anti-inflammatory[18]
7	7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin	Antioxidants[10]
8	Furfural	Antityrosinase, antimicrobial[19]
9	6-(2,3-Dihydroxy-3-methylbutyl)-7-methoxycoumarin	Antimicrobials[20]

Table VI. Bioactivity of compounds identified in the EAF2

No.	Compound	Biological Activity
1	1-Octadecanol	Antibacterial, antifungal, anti-larval[21]
2	Tetracosane	Antioxidants[22]
3	Hexacosane	Antimicrobial[23]
4	1,2-Benzenedicarboxylic acid, (2-ethylhexyl) ester	Antimicrobial, Antifouling[21]

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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