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VOLATILE COMPOUNDS AND POTENTIAL BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL OF *CITRUS amblycarpa HASSK. OCHSE*

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Abstract: The utilization of volatile compounds from essential oils, one of which is citrus peels, in the food, industry, and medical fields continues to develop. This study aimed to identify volatile compounds present in the essential oils of sambal orange peels (*Citrus amblycarpa Hassk. Ochse*) and its potential biological activity. The research method begins with the isolation of volatile compounds via steam distillation techniques, followed by identification via gas chromatography–mass spectrometry (GC–MS) and literature analysis related to the biological activity of the constituent compound components. The results obtained from this study revealed the dominant volatile compounds in essential oils from sambal orange peels, namely, D-limonene (37.41%), β-pinene (22.52%), germacrene D (13.21%) and α-pinene (10.79%). On the basis of the essential oil's most significant compound components, these compounds have potential as antioxidants, antibacterial agents, anti-inflammatory agents, anticancer agents, and antifungal agents. Overall, the results of this study are expected to provide direction for further research, especially for testing the biological activity of the essential oil of sambal orange peel (*Citrus amblycarpa Hassk. Ochse*) experimentally.

Keywords: *Volatile compounds*, *essential oil*, *GC–MS*, *biological activity*, *Citrus amblycarpa Hassk. Ochse*

Abstrak: Pemanfaatan senyawa volatil dari minyak atsiri dalam dunia pangan, industri, dan pengobatan terus berkembang, salah satunya berasal dari kulit jeruk. Penelitian ini bertujuan untuk mengidentifikasi senyawa volatil yang terdapat pada minyak atsiri kulit jeruk sambal (*Citrus amblycarpa Hassk. Ochse*) dan potensi aktivitas biologisnya. Metode penelitian diawali dengan isolasi senyawa volatil menggunakan teknik destilasi uap, dilanjutkan dengan identifikasi menggunakan GC–MS dan analisis literatur terkait aktivitas biologis komponen senyawa penyusunnya. Hasil yang diperoleh dari penelitian ini adalah ditemukannya senyawa volatil yang dominan pada minyak atsiri dari kulit jeruk sambal, yaitu D-Limonene (37,41%), β-pinene (22,52%), Germacrene D (13,21%) dan α-Pinene (10,79%). Berdasarkan komponen senyawa yang paling signifikan dari minyak atsiri, senyawa-senyawa tersebut berpotensi sebagai anti oksidan, anti bakteri, anti inflamasi, anti kanker, dan anti jamur.

Secara keseluruhan, hasil penelitian ini diharapkan dapat memberikan arahan terhadap penelitian lebih lanjut, khususnya pengujian aktivitas biologis minyak atsiri kulit buah jeruk sambal (*Citrus amblycarpa Hassk. Ochse*) secara eksperimental.

Kata kunci: *Senyawa Volatile*, *minyak atsiri*, *GC–MS*, *Aktivitas Biologis*, *Citrus amblycarpa Hassk. Ochse*

INTRODUCTION

Essential oils are complex mixtures of volatile organic components such as alcohols, aldehydes, esters, ketones, phenylpropanoids, and terpenoids (Tangpao *et al.* 2022). Essential oils can be found in the leaves, flowers, branches, and even roots of aromatic plants (Chukwuma *et al.* 2023). Essential oils can be separated without heating by steam, dry distillation or suitable mechanical techniques (Kant and Kumar, 2022). Essential oils have a strong hashish odor, are generally clear in color, and are easily oxidized if contaminated with outside air, heat or exposure to light (Masyita *et al.* 2022). Essential oils were originally used in perfumes and were developed widely as flavorings and aroma enhancers in the food industry. Some essential oils with antioxidant and antimicrobial properties are often used in the cosmetic and pharmaceutical industries (Silvestre *et al.* 2019).

Citrus is a genus in the Rutaceae family distributed worldwide with more than 1300 species (Bora *et al.* 2020). Citrus essential oils are secondary metabolites composed mostly of aromatic compounds found in flowers, leaves, and fruit peels (Agarwal *et al.* 2022). The peel of citrus fruits contains more essential oil than the leaves do, so essential oil is a byproduct of the citrus beverage manufacturing industry (Mustafa, 2015). The chemical composition of citrus essential oils consists of approximately 85–99% volatile and nonvolatile compounds, ranging from 1–15% depending on the species (Jing *et al.* 2014). Volatile components found in citrus essential oils include monoterpene compounds, monoterpene hydrocarbons, monoterpene alcohol, monoterpene aldehydes, sesquiterpenes, sesquiterpene alcohol and nonvolatile components from the hydrocarbon group, sterols, fatty acids, waxes, carotenoids, coumarins, psoralen, and flavonoids (González-Mas *et al.* 2019).

Research on the biological activity of citrus essential oils continues to progress. Citrus essential oils can inhibit fungal growth; are antimicrobial, anticancer, insecticidal, and anti-inflammatory; and can overcome metabolic disorders (Bourgou *et al.* 2012; Jing *et al.* 2014; Ben Hsouna *et al.* 2017; Himed *et al.* 2019; Noshad, Alizadeh Behbahani and Nikfarjam, 2022). The biological activity of citrus essential oils is due to the volatile compounds of terpenes and terpenoids, including monoterpenes, monoterpene hydrocarbons, monoterpene alcohols, monoterpene aldehydes, sesquiterpenes, and sesquiterpene alcohols (Dhifi *et al.* 2016). Since citrus species are numerous and plants are quite abundant worldwide, the use of citrus essential oils is an opportunity to provide a substitute treatment for synthetic drugs.

Sambal orange (*Citrus amblycarpa Hassk. Ochse*) is Indonesia's endemic citrus plant (Lim, 2012). Morphologically, the sambal orange plant has a height of 140–160 cm, is a green compound leaf and fragrant unifoliate type with a length ranging from 18–33 mm, and has a small fruit. The surface of the fruit skin is rough, and if peeled, the fruit skin has three layers: a green flavedo as an outer layer, a yellowish white albedo as a middle layer and an endocarp as a layer near the contents of the fruit with a light green color (Budiarto *et al.* 2017). The sour taste of sambal orange fruit is commonly used as a flavoring in food, and dried fruit peel is commonly used as an additive in burgundy tea drinks (Dewi, 2022).

Studies on the essential oil of Citrus amblycarpa Hassk Ochse have yet to be performed. Given the potential of fruit skin, which has yet to be maximally utilized, it is necessary to study the content of volatile compounds in *Citrus amblycarpa Hassk. Ochse*. This study aims to identify the content of volatile compounds by gas chromatography–mass spectrometry (GC–MS) and literature analysis to determine the potential biological activity of the volatile compound components. The results of this study can be used as a reference or basis for further research on the biological activity of the essential oil of *Citrus amblycarpa Hassk. Ochse*.

METHOD

Materials

The peel of sambal orange (Citrus amblycarpa Hassk. Ochse) was taken in the Pontianak city area. The peels were then cleaned with distilled water and aerated for one hour. Next, the peels were blended dry to obtain a smaller size.

Isolation of essential oil

The essential oils from the peels of sambal citrus fruits were isolated via steam distillation using the equipment shown in Figure 2. The steam distillation process refers to the procedure of (Bhandari *et al.* 2021), who isolated essential oils from fresh citrus peels. In this study, 819.5 g of fresh sambal citrus fruit peels were pulverized with a blender and distilled for six hours. The essential oil obtained was then added to Na_2SO_4 at a ratio of 2:1 and allowed to stand until the water content separated from the essential oil. The results obtained were then filtered through filter paper.

Figure 1. Steam distillation used

Table 1. GC–MS parameters

Parameter Method	Value
Flow rate	1.2 mL/min
Temperature Programme	Initial: 60^0C to 140^0C at 15^0C/min (hold for 2 min); to 180^0C at 50C/min (hold for 3 min); to 250^0C at 10^0C/min (hold for 3 min)
Run time	28.333 min
Injection type	Split (20:1)
Injection Volume	1µL
Ion source	Electron Ionization, 70 eV
MS Source	230^0C maximum 250^0C
MS Quad	150^0C maximum 200^0C
Scan range	60 (*m/z*) s/d 600 (*m/z*)

Determination of the composition

The equipment used was an Agilent 8890/5977B GC–MS with an Agilent 19091S-433UI: 1429244H column (HP-5MS UI) with a nonpolar stationary phase (5%-phenyl)-methylpolysiloxane, a H_e carrier gas (99.999%), column dimensions of 30 m × 250 µm × 0.25 µm, and an MSD output. Furthermore, the sample was injected via control parameters according to Noshad et al. (2022), and the details are listed in Table 1.

RESULTS AND DISCUSSION

Essential oils from sambal orange peels

After the steam distillation process for 6 h, 15 mL of essential oil was obtained, which was clear in color and had a sharp citrus aroma (Figure 2). The density of the essential oil produced was 1.0766 g/mL, and the percentage yield was 1.906%.

a b

Figure 2. (a) Essential oil from sambal citrus peel before separation, the upper part is essential oil, the lower part is water, and (b) the volume obtained from essential oil separation.

Essential oil composition

The CG-MS results (Figure 3) revealed 12 compound peaks from the essential oil of Citrus amblycarpa Hassk. Ochse. To confirm the components of the volatile compounds and the percentage of their composition, the resulting peaks were matched for retention time (Rt) peak area (Area %), and then the type of volatile compound component was determined by comparing the value (m/z) with library data from NIST14. L (Table 2).

Table 2 shows the percentage of volatile compounds that make up the essential oil of Citrus amblycarpa Hassk. Ochse by 94.89% and nonvolatile compounds by 5.11%. According to Jing et al. (2014), the volatile constituents of essential oils from various citrus species range from 85% to 99%, including a mixture of hydrocarbon monoterpenes, sesquiterpenes, and their oxygenated derivatives, such as aldehydes, ketones, acids, alcohols, and esters. In contrast, nonvolatile constituents include long-chain hydrocarbons, fatty acids, lime, carotenoids, coumarins, psoralen, and flavonoids (Agarwal *et al.* 2022).

The volatile compound group is composed of monoterpene compounds (70.72%), monoterpene alcohol (5.59%), monoterpene hydrocarbons (3.41%), sesquiterpenes (1.96%) and sesquiterpene hydrocarbons (13.21%). The largest component of the essential oil of Citrus amblycarpa Hassk. The Ochse is D-Limonene (37.41%), β-pinene (22.52%), Germacrene D (13.21%) and α-Pinene (10.79%), where all the largest compounds include volatile compounds. D-Limonene is the largest volatile compound found in almost all essential oils from various types of citrus.

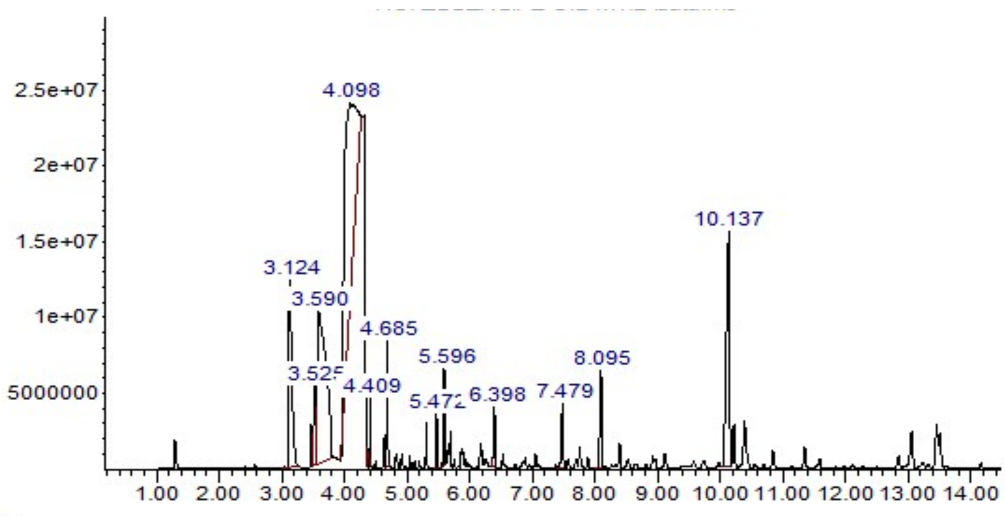

Figure 3. GC–MS Chromatogram results

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Table 2. Volatile compounds of the essential oil of Citrus amblycarpa Hassk. Ochse

Classification	Peak	Rt	Compound	Quality	% Area
Monoterpene	1	3.124	α-Pinene	95	10.79
	3	3.590	β-pinene	94	22.52
	4	4.098	D-Limonene	98	37.41
Monoterpene alcohol	6	4.685	Linalool	95	2.58
	7	5.472	Terpinen-4-ol	94	1.01
	8	5.596	L-α-Terpineol	91	2.00
Monoterpene hidrokarbon	2	3.525	Bicyclo[3.1.0]hexane, 4-methylene- 1-(1-methyl ethyl- *(Sinonim: Sabinene)*	94	3.41
Sesquiterpene	10	7.479	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methyl ethenyl)-1-(1-methyl ethyl)-, (3R-trans)- *(synonym: σ-Elemene)*	97	1.96
Sesquiterpen hidrokarbon	12	10.137	Germacrene D	98	13.21
			Total		**94.89**
Non-Volatile Compounds					
Fatty alcohol esters	5	4.409	Formic acid, octyl ester	72	0.47
	11	8.095	Butanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester,(E)- *(sinonim: Neryl (S)-2-methylbutanoate)*	91	3.14
Fatty alcohols	9	6.398	1-Decanol	91	1.50
			Total		**5.11**

Table 3. Main compounds of essential oils from various citrus species

Volatile compounds	**Percentage (%)**							
	C.a*[1]	**C.m*[2]**	**C.l*[3]**	**C.aa*[4]**	**C.s*[5]**	**C.g*[6]**	**C.h*[7]**	**C.aL*[8]**
α-Pinene	10.79	1.94	3.39	0.9	2.10	0.45	2.22	12.6
β-pinene	22.52	1.05	12.61	1.5	-	-	21.10	-
D-Limonene	37.41	72.85	43.07	90.4	60.23	97.87	25.28	0.7
Linalool	2.58	0.17	1.08	1.6	1.28	-	1.62	-
Terpinen-4-ol	1.01	-	2.85	2.85	-	-	5.06	-
L-α-Terpineol	2.00	0.32	7.20	1.7	0.40	-	2.82	1.4
Sabinene	3.41	0.28	0.06	0.4	1.13	-	14.99	-
σ-Elemene	1.96	0.08	0.15	0.15	-	-	-	-
Germacrene D	13.21	1.01	0.32	0.14	-	-	0.11	-
γ-Terpinene	-	13.44	11.48	11.48	-	-	0.15	0.1
α-Terpinolene	-	-	2.37	0.3	-	-	2.82	0.4
Geranial	-	-	1.48	1.48	-	-	-	0.9
β-Bisabolene	-	0.49	2.61	2.61	-	-	-	1.8
cis-α-Bergamotene	-	0.78	1.38	1.38	-	-	-	2.7
β-myrcene	-	1.57	1.87	2.6	5.33	0.92	1.02	1.6
Citronellal	-	-	-	-	-	-	7.63	-
α-phellandrene	-	0.42	-	-	-	-	-	48.5
p-cymene	-	0.23	-	-	-	-	-	16.5
(E,E)-α-farnesene	-	-	-	-	-	-	-	12.6
α-thujene	-	-	0.37	-	-	-	0.13	2.7

Description:
*[1]: sambal orange peel essential oil, latin: *Citrus amblycarpa Hassk. Ochse* from research results
*[2]: alemow orange, latin: *Citrus macrophylla* data from (Mohammed *et al.* 2021)
*[3]: lemon, latin: *Citrus lemon* data from (Jain and Sharma, 2017)
*[4]: Seville orange, latin: *Citrus aurantium amara* data from (Vukić, Branković and Ristić, 2023)
*[5]: sweet orange, latin: *Citrus sinensis* data from (Abdel Samad *et al.* 2023)
*[6]: pamelo oranges, latin: *Citrus grandis L.* data from (Tan *et al.* 2021)
*[7]: Kaffir lime, latin: Citrus hystrix data from (Sreepian, Sreepian and Chanthong, 2019)
*[8]: limes, latin: Citrus aurantifolia L data from (Galovičová *et al.* 2022)

Volatile compounds from the essential oil of Citrus amblycarpa Hassk. Ochse CG–MS results were then compared with those of citrus peel essential oils from several widely used Citrus species. Table 3 provides the results of the comparison. Table 3 shows that α-pinene, β-pinene and germacrene D are essential oils of Citrus amblycarpa Hassk. Ochse is more common than other Citrus varieties (except α-pinene, which is most common in Citrus aurantifolia L.). In contrast, D-Limonene is a relatively low-quality compound compared with other varieties.

Potential Biological Activity

The volatile compound D-limonene (37.41%) is the largest component of the essential oil of Citrus amblycarpa Hassk. Ochse has the potential to have a variety of biological activities. A literature review conducted by Anandakumar et al. (2021) revealed that D-Limonene has the potential to be used as an antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune-modulating, and antioxidant agent. The antifungal properties of D-Limonene are known for its ability to inhibit the growth of *C. tropicalis,* with MICs of 20 μL/mL and 40 μL/mL in PDB media (Yu *et al.* 2022). The antitumor activity of D-Limonene is known for its ability to reduce cell viability and, within 14 days, can reduce the volume of K562 xenograft tumors in C57BL/6 mice (Shah *et al.* 2018). The anticancer activity of D-Limonene is known for its chemopreventive ability through the inhibition of inflammation, oxidative stress and Ras signaling, as well as the induction of a proapoptotic state during TPA-mediated promotion of DMBA-induced skin cancer in mice (Chaudhary *et al.* 2012).

Volatile compounds α-pinene (10.79%) and β-pinene (22.52%) from the essential oils of *Citrus amblycarpa Hassk. Ochse* potentially has a wide range of biological activities. A literature review by Park et al. (2021) and Salehi et al. (2019) summarized α-pinene and β-pinene compounds as having anticoagulant, anti-inflammatory, anti-Leishmania, antimalarial, antimicrobial, antioxidant, antitumor, analgesic, and antibiotic resistance-modulating effects. The anti-inflammatory properties of α-pinene are known to suppress the mitogen-activated protein kinase (MPK) and nuclear factor-kappa B (NF-B) pathways in rat peritoneal macrophages (Kim *et al.* 2015). Anticancer activity is known from the ability of α-pinene to reduce the rate of cell proliferation in healthy neurons only at a dose of 400 mg/L and the decrease in N2a cells at doses of 100, 200 and 400 mg/L (Aydin, Türkez and Geyikoğlu, 2013). The antimicrobial properties of (+)-α-pinene and (+)-β-pinene against *Candida albicans*, with minimal inhibitory concentrations (MICs) ranging from 3,125 µg/mL to 187 µg/mL, can kill 100% of the *C. albicans* inoculum within 60 minutes (Rivas *et al.* 2012).

CONCLUSION

On the basis of the results of previous studies, it can be concluded that the volatile compounds that make up the essential oil of Citrus amblycarpa Hassk fruit peel. Ochse of 94.89%. The main components are D-limonene (37.41%), β-pinene (22.52%), germacrene D (13.21%) and α-pinene (10.79%). From these main components, the potential biological activity of Citrus amblycarpa Hassk can be predicted. Ochse includes antioxidant, antibacterial, anti-inflammatory, anticancer and antifungal effects.

In this study, only steam distillation was used to isolate essential oils, which can cause decomposition. Therefore, further research must be conducted to compare various isolation techniques, such as supercritical fluid extraction and ultrasonic and microwave extraction, to obtain more effective and efficient isolation results. However, research on the biological potential of sambal citrus peel essential oils can be used as a basis for experimentally testing their biological activity.

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