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Flavonoids from Limau Peel (*Citrus amblycarpa* (Hassk.) Ochse) and Their Antioxidant Activity

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ABSTRACT. *Citrus amblycarpa* (Hassk.) Ochse is an endemic Indonesian plant from West Java. This study aims to determine the chemical structure of the flavonoid compounds of *C. amblycarpa* peel ethanol extract and their antioxidant activity. Three flavonoid compounds, namely 5-hydroxy-3',4',6,7,8-pentamethoxyflavone (**1**), 8-hydroxy-3,3',4',5,6,7-hexamethoxyflavone (**2**), and 3',4',5,6,7,8-hexamethoxyflavone (**3**), were isolated for the first time from the ethanol extract of *C. amblycarpa* peel. Their chemical structure was determined by spectroscopic methods (MS, IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT) and compared with previous reported spectral data. Furthermore, these compounds were evaluated for their antioxidant activity using the DPPH method. The results showed that 8-hydroxy-3,3',4',5,6,7-hexamethoxyflavone (**2**) has the highest antioxidant activity with IC_{50} value of 121.09 ± 0.24 ppm.

Keywords: Antioxidant activity, *Citrus amblycarpa*, flavonoid, limau

INTRODUCTION

The *Citrus* genus is a member of the Rutaceae family, consisting of 1300 species, and widely distributed in the tropical and subtropical regions (Anwar et al., 2008). *C. amblycarpa* is a species of the *Citrus* genus originating from West Java, Indonesia, and the fruit is often used as a spice in cooking, while it is usually not used and ends up as waste (Budiarto et al., 2017). Furthermore, its peels have benefits in the food, pharmaceutical, and cosmetic industries (Mahato et al., 2018).

A study on the biological activity of *C. amblycarpa* showed many interesting activities, including *Aedes aegypti* larvicidal (Ishak et al., 2019), anti-diabetic (Tambunan et al., 2020), analgesic (Maharani et al., 2020), antibacterial (Mulyani et al., 2009), and antioxidant (Stevenie et al., 2019). The chemical analysis of its peels led to the isolation of various compounds, including flavonoids, alkaloids, phenolics (Fahrurroji & Riza, 2020), tannins, triterpenoids, quinones, steroids, vitamins C and A, coumarins, rosmarinic acid derivatives, and essential oils (Pedana et al., 2017). Furthermore, the flavonoid is a source of promising antioxidant compounds (Panche et al., 2016). It was reported that antioxidant flavonoids such as quercetagetin (Yang et al., 2011), quercetin, rutin (Kim et al., 2013), and kaempferol (Hayat et al., 2010), with IC_{50} of 7.89 μM (Yang et al., 2011), 4.36 ± 0.10 μM, 6.36 ± 0.12 μM, and 16.09 ± 0.10 μM (Zhu et al., 2017), respectively, were isolated from *Citrus* genus. However, report on the isolation of antioxidant flavonoids from *C. amblycarpa* has not been conducted. This study analyzed the isolation of flavonoid compounds from the fruit peel of *C. amblycarpa* alongside their antioxidant activity. The compounds were isolated and characterized using various chromatographic and spectroscopic (MS, IR, ^{13}C-NMR, and ^{1}H-NMR) techniques. Additionally, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method measured the antioxidant activity.

EXPERIMENTAL SECTION

General Experiment Procedure

IR spectra was recorded using Thermo Scientific Nicolet Summit FTIR (Thermo Fisher Scientific, Madison, WI, USA). High-resolution of mass spectra (HR-TOFMS) were determined on a Waters Xevo Q-TOF direct probe/MS system, utilizing ESI mode and micro channel plates MCPs detector (Milford, MA, USA). NMR was recorded with ^{1}H and ^{13}C-NMR JEOL 500 MHz and 125 MHz using TMS as the internal standard. Vacuum liquid chromatographic (VLC) separation and column chromatography were performed on silica gel 60 GF_{254} and 70-230 mesh, Merck, respectively. TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), and detection was performed by spraying $AlCl_3$ in ethanol, then heated and analyzed under UV light at λ 254 and 367 nm.

Plant Material

The fruit peels of C. *amblycarpa* were collected in January 2021 from Bandung, West Java. The plant was identified by the staff of the Taxonomy Laboratory, Department of Biology, Faculty Mathematics, and Natural Sciences, Universitas Padjadjaran, Jatinangor, Sumedang, West Java, Indonesia.

Extraction and Flavonoid Test

The fruit of C. *amblycarpa* (5 kg) was peeled, dried, and ground into powder (327.83 g), then extracted by maceration using ethanol for 3*x*24 hours to obtain a concentrated ethanolic extract (122.92 g). Afterward, the resulting product was tested for flavonoids by dissolving 0.1 g of sample in 10 mL of alcohol and dividing it into four test tubes. The first tube was used as a control, while the second, third, and fourth were added with NaOH, concentrated Mg-HCl powder, and concentrated H_2SO_4, respectively.

Separation and Purification of Flavonoid Compounds

The ethanol extract (24 g) was separated by vacuum liquid column chromatography using *n*-hexane-EtOAc-MeOH gradient 10% elution to generate 21 fractions (**1-21**). The 6th and 7th fractions were combined into **A**, while the 8th, 9th, 10th, and 11th were combined into **B**. Fraction **A** (1.13 g) was separated by column chromatography using an *n*-hexane-EtOAc solvent eluted gradient of 5%, resulting in two positive flavonoid fractions (**A8** and **A9**). The **A8** fraction (90.5 mg) was separated using column chromatography with *n*-hexane:chloroform:acetone 6:3:1 to obtain nineteen fractions (**A8a-A8s**). The **A8d-A8h** fractions were purified using *n*-hexane:MTC:acetone 7:2:1 to produce compound **1** (17.5 mg). Fraction **A9** (57 mg) was separated using the *n*-hexane:MTC:acetone 7:2:1 solvent to produce compound **2** (9.2 mg). Fraction **B** (0.70 g) was separated using an *n*-hexane-EtOAc-MeOH solvent with 5% gradient elution, resulting in four flavonoid-positive fractions (Fraction **B14-B17**), which were then separated using *n*-hexane:MTC:acetone 7:2:1 to produce compound **3** (23.1 mg).

Antioxidant Activity by DPPH Assay

The sample of 0.4 mg was dissolved in 4 mL MeOH to obtain 1000 ppm of standard sample. Concentration variations of 10, 50,100, and 200 ppm were produced, and each of them was measured with the absorbance value by mixing the sample with 0.5 mL of DPPH (1 mM in methanol). The mixture was shaken and allowed to stand at room temperature for 30 minutes. Furthermore, the resulting absorption was measured at a wavelength of 515 nm. Finally, the percentage of sample inhibition was calculated based on the difference in the absorbance value between the blank (DPPH) and the sample.

RESULTS AND DISCUSSION

The flavonoid test on the ethanol extract peel of C. *amblycarpa* showed that adding NaOH, Mg-HCl, and H_2SO_4 solution changed the colors to yellow, green, and yellow, respectively. When a solution of NaOH and H_2SO_4 is added, the color changes to green, brown, and yellow, which indicates that flavonoids are present (Machado et al., 2013). A positive result for flavonoids is obtained when the solution turns yellow, orange, red, or green after adding Mg-HCl (Depkes RI, 1979).

Figure 1 shows that the ethanol extract of C. *amblycarpa* peel was separated and purified by VLC and column chromatography to produce compounds **1**, **2**, and **3**. Compound **1** was obtained as a light yellow needle, which was soluble in methylene chloride, ethyl acetate, and acetone. It showed a yellow stain after being sprayed with $AlCl_3$, stained ethanol, and heated. Furthermore, compound **1** fluoresces at λ 254 and 365 nm under UV light. The molecular formula was $C_{20}H_{20}O_8$ based on the HR-TOFMS spectrum showing $[M+H]^+$ *m/z* 389.1434 (calculated *m/z* 389.3706), with eleven degrees of unsaturation. The IR spectrum of compound **1** showed the absorption peaks for the OH group (3080 cm^{-1}), aromatic C-H stretch (2976 cm^{-1}), aliphatic C-H stretch (2836 cm^{-1}), C=O stretch (1646 cm^{-1}), C=C stretch (1514 cm^{-1}), C-H bending (1368 cm^{-1}), C-O stretch (1269 cm^{-1}), and C-H out-of-plane bend (841 cm^{-1}). The OH group generally appears in the 3570–3200 cm^{-1} area (Nandiyanto et al., 2019), but in compound **1**, it appears at 3080 cm^{-1}. This is due to the strong intramolecular hydrogen bond involving a characteristic feature of the OH group located at the C-5 position, which forms a chelate with the carbonyl in flavonoids (Machado et al., 2013; Shiono et al., 2013).

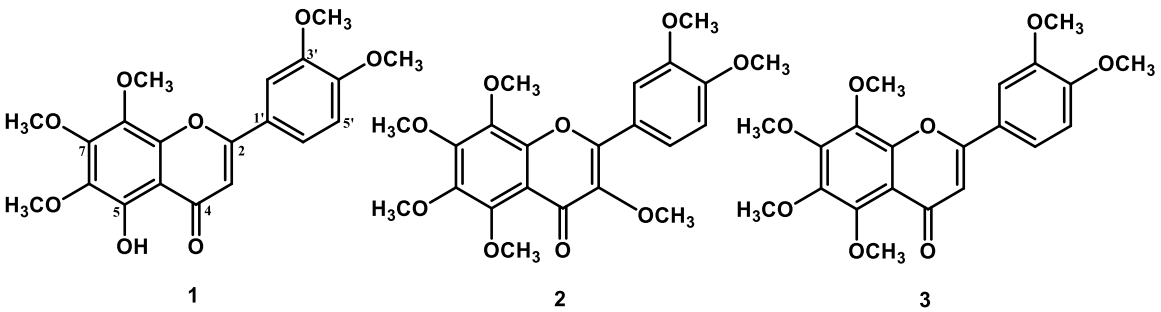

Figure 1. Chemical structures of compounds **1–3**

The ^{13}C-NMR and DEPT 135° showed twenty carbon atoms consisting of five methoxy carbons at δ_C 55.2, 55.4, 60.2, 61.1, and 61.5 ppm, four methine carbons at δ_C 103.5 (C-3), 109.2 (C-5'), 111.7 (C-2'), and 120.1 ppm (C-6'), two quaternary carbons at δ_C 106.7 (C-10), 123.4 ppm (C-1'), five oxygenated quaternary carbons (C-OCH_3), at δ_C 133.2 (C-8), 136.5 (C-6), 147.0 (C-3'), 153.0 (C-4') and 153.1 ppm (C-7), two oxygenated quaternary carbons (C-O) at δ_C 146.5 (C-9) and 164.2 ppm (C-2), one oxygenated quaternary carbon (C-OH) at δ_C 149.8 ppm (C-5), and one quaternary carbon which is a carbonyl at δ_C 183.0 ppm (C-4). The ^{13}C-NMR data shows characteristics that are in accordance with flavonoid compounds. According to (Markham et al., 1982), flavonoids showed at least 15 carbon and carbonyl signals in the 170-210 ppm area. Compound **1** is a flavonoid with five methoxys and one substituted hydroxyl. Based on the molecular formula and NMR data in **Table 1**, eleven degrees of unsaturation were identified and described as eight pairs of carbon sp^2 and tricyclic flavonoids. The ^{1}H NMR spectrum showed five singlet signals indicating methoxy resonating at δ_H 3.90 (3H, s), 3.94 (3H, s), 3.97 (3H, s), 3.96 (3H, s), and 4.05 ppm (3H, s), one singlet signal indicating the presence of methine which resonates at δ_H 6.78 ppm (1H, s, H-3), two doublet signals indicating the presence of methine which resonates at 7.15 (1H, d, J = 8.5 Hz, H-5') and 7.61 ppm (1H, d, J = 2.5 Hz, H-2'), one double doublet signal indicating the presence of methine which resonates at 7.72 ppm (1H, dd, J = 8.5, 2.5 Hz, H-6'). The signal confirms the proton in ring B, and there is one singlet signal showing the presence of a hydroxyl group that forms a chelate with the carbonyl at δ_H 12.7 ppm. Furthermore, the ^{1}H NMR data strengthens the notion that compound **1** is a flavonoid with a hydroxyl group substituted at the C-5 position, forming a chelate with the carbonyl. The data is in accordance with (Li et al., 2006), which state that the structure of compound **1** was determined as 5-hydroxy-3',4',6,7,8-pentamethoxyflavone. This compound was isolated previously from *C. sinensi* and for the first time in this species.

Compound **2** was obtained as a yellow needle, which was soluble in methylene chloride, ethyl acetate, and acetone. In TLC, it showed a yellow stain after being sprayed with $AlCl_3$, stained in ethanol, and heated. Furthermore, compound **2** fluoresces at λ 254 and 365 nm under UV light. The molecular formula was $C_{21}H_{22}O_9$ based on the HR-TOFMS spectrum showing $[M+H]^+$ *m/z* 419.1354 (calculated *m/z* 419.3960), with eleven degrees of unsaturation. The IR spectrum of compound **2** showed the absorption peaks for the OH group (3295 cm^{-1}), aromatic C-H stretch (2933 cm^{-1}), aliphatic C-H stretch (2837 cm^{-1}), C=O stretch (1593 cm^{-1}), C=C stretch (1513 cm^{-1}), C-H bending (1407 cm^{-1}), C-O stretch (1265 cm^{-1}), and C-H out-of-plane bend (867 cm^{-1}). Additionally, the IR spectrum data shows characteristics in accordance with the typical spectrum of flavonoid compounds. According to (Noh et al., 2017), the IR spectrum of flavonoids shows the presence of C=O stretch (1630-1665 cm^{-1}), C-O stretch (1000 -1300 cm^{-1}), aromatic C-H curve in the fingerprint area (600-980 cm^{-1}), and the C=C stretch (1600-1500 cm^{-1}) (Awouafack et al., 2017; Sianturi et al., 2016). The ^{13}C-NMR and DEPT 135° data in **Table 1** showed twenty-one carbon atoms consisting of six methoxy carbons at δ_C 55.2, 55.2, 61.2, 61.6, 61.4, and 61.1 ppm, three methine carbons at δ_C 110.3 (C-2'), 111.5 (C-5'), and 121.1 ppm (C-6'), two quaternary carbons at δ_C 112.0 (C-10) and 123.9 ppm (C-1'), six oxygenated quaternary carbons (C-OCH_3) at δ_C 137.6 (C-8), 143.7 (C-6), 147.6 (C-5), 149.2 (C-3'), 150.9 (C-4'), and 151.7 ppm (C-7), two oxygenated quaternary carbons (C-O) at δ_C 142.5 (C-2) and 146.9 ppm (C-9), one oxygenated quaternary carbon (C-OH) at δ_C 138.0 ppm (C-3), and one quaternary carbon which is a carbonyl at δ_C 172.5 ppm (C-4).

Based on ^{13}C-NMR data, compound **2** has the same characteristics as flavonoids stated in ^{13}C-NMR compound **1**. The ^{1}H-NMR spectrum showed signals at δ_H 7.86 (1H, d, J =2.0 Hz, H-2'), 7.14 (1H, d, J =9.0 Hz, H-5'), and 7.92 (1H, dd, J = 2.0, 9.0 Hz, H-6') corresponding to the protons of ring B and methoxy groups at δ_H 3.89 (3H, s), 3.86 (3H, s), 4.03 (3H, s), 3.87 (3H, s), 3.88 (3H, s), 4.06 ppm (3H, s). According to Johann et al., (2007), despite the presence of the OH group in the IR spectrum, there was no proton signal of the OH group due to the action of the solvent (3295 cm^{-1}). Based on data, the structure of compound **2** was determined as 8-hydroxy-3,3',4',5,6,7-hexamethoxyflavone. This compound was isolated previously from *C. aurantifolia* (Johann et al., 2007) and for the first time in this species.

Compound **3** was obtained as a yellow needle, which was soluble in methylene chloride, ethyl acetate, and acetone. In TLC, it showed a yellow stain after being sprayed with $AlCl_3$, stained in ethanol, and heated. Furthermore, compound **3** fluoresces at λ 254 and 365 nm under UV light. The molecular formula was $C_{21}H_{22}O_8$ based on the HR-TOFMS spectrum showing $[M+H]^+$ *m/z* 403.1311 (calculated *m/z* 403.3970), with eleven degrees of unsaturation. The IR spectrum of compound **3** showed absorption peaks for the aromatic C-H stretch (2946 cm^{-1}), aliphatic C-H stretch (2843 cm^{-1}), C=O stretch (1644 cm^{-1}), C=C stretch (1519 cm^{-1}), C-H bending (1369 cm^{-1}), C-O stretch (1271 cm^{-1}), and C-H out-of-plane bend (847 cm^{-1}). Additionally, the IR spectrum data shows characteristics in accordance with the typical spectrum of flavonoids identical to the discussion of compound **2.** The ^{13}C-NMR and DEPT 135° data in **Table 1** shows twenty-one carbon atoms consisting of six methoxy

carbons (-OCH_3) at δ_C 55.3, 55.3, 61.0, 61.1, 61.4, and 61.5 ppm, four methine carbons at δ_C 106.3 (C-3), 108.9 (C-2'), 111.7 (C-5') and 119.4 ppm (C-6'), two quaternary carbons at δ_C 114.9 (C-10), and 123.8 ppm (C-1'), six oxygenated quaternary carbons (C-OCH_3), at δ_C 138.3 (C-8), 144.2 (C-6), 148.2 (C-5), 149.7 (C-3'), 151.4 (C-7) and 152.4 ppm (C-4'), two quaternary carbons bonded to the ether functional group (C-O) at δ_C 147.7 (C-9) and 160.7 ppm (C-2), and one quaternary carbon which is carbonyl (C=O) at δ_C 175.8 ppm (C-4). Based on ^{13}C-NMR data, compound **3** has the same characteristics as flavonoids as in the discussion of ^{13}C-NMR compound **1**. The ^{1}H NMR spectrum showed six singlet signals indicating resonance of methoxy at δ_H 3.82 (3H, s), 3.86 (3H, s), 3.89 (3H, s), 3.93 (3H, s), 4.01 (3H, s), and 4.04 ppm (3H, s), one singlet signal indicating the presence of methine resonated at δ_H 6.60 ppm (1H, s, H-3), two doublet signals indicating the presence of methine resonate at 7.12 (1H, d, J = 8.0 Hz, H-5') and 7.56 ppm (1H, d, J = 2.5 Hz, H-2'), and one double doublet signal indicating the presence of methine (-CH-) which resonates at 7.65 ppm (1H, dd, J = 2.5, 8.0 Hz, H-6'). According to (Wang et al., 2007), the signal confirms the proton in ring B. Based on data, the structure of compound **3** was determined as 3',4',5,6,7,8-hexamethoxyflavone. This compound was isolated previously from *C. hassaku* Hort. ex Tanaka (Machida & Osawa, 1989), setoka (Nakanishi et al., 2019), and *Pericarpium citri* reticulatae viride (Wang et al., 2007) and for the first time in this species.

Table 2 shows the results of compounds **1-3**, which were tested for antioxidant activity by the DPPH method. Compound **2** has stronger antioxidant activity than **1** and **3**. This is because it has an OH group at C-8 which contributes to electron transfer for radical scavenging at DPPH. There is also an OH group at the C-5 position of compound **1**. However, its antioxidant activity is lower than compound **2**, with an OH group at C-8. This is because the OH at C-5 does not play a role in antioxidant activity (Herath et al., 2008). Compound **3** also has weaker antioxidant activity because it has no OH group. The presence of the OH group indicates more positive roles than methoxy groups. These results are consistent with previous reports, which showed that multiple OH groups on the molecule have a substantial antioxidant activity, and methoxy groups generate unfavorable steric effects and increased lipophilicity and membrane partitioning (Jeong et al., 2007).

Table 1. NMR data for compounds **1-3** (acetone-d_6, 500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position Carbon	**1**		**2**		**3**	
	δ_H [(ΣH, mult., J(Hz)]	δ_C	δ_H [(ΣH, mult., J(Hz)]	δ_C	δ_H [(ΣH, mult., J(Hz)]	δ_C
2	-	164.2	-	142.5	-	160.7
3	6.78 (1H, s)	103.5	-	138.0	6.60 (1H, s)	106.3
4	-	183.0	-	172.5	-	175.8
5	-	149.8	-	147.6	-	148.2
6	-	156.5	-	143.7	-	144.2
7	-	153.1	-	151.7	-	151.4
8	-	133.2	-	137.6	-	138.3
9	-	146.5	-	146.9	-	147.7
10	-	106.7	-	112.0	-	114.9
1'	-	123.4	-	123.9	-	123.8
2'	7.61 (1H, d, 2.5)	111.7	7.86 (1H, d, 2.0)	110.3	7.56 (1H, d, 2.5)	108.9
3'	-	147.0	-	149.2	-	149.7
4'	-	153.0	-	150.9	-	152.4
5'	7.15 (1H, d, 8.5)	109.2	7.14 (1H, d, 9.0)	111.5	7.12 (1H, d, 8.0)	111.7
6'	7.72 (1H, dd, 8.5, 2.5)	120.1	7.92 (1H, dd, 2.0, 9.0)	121.1	7.65 (1H, dd, 2.5, 8.0)	119.4
-OCH_3						
3'	3.97 (3H, s)	55.4	3.89 (3H, s)	55.2	3.93 (3H, s)	55.3
4'	3.94 (3H, s)	55.2	3.86 (3H, s)	55.2	3.86 (3H, s)	55.3
3	-	-	4.03 (3H, s)	61.2	-	-
5	-	-	3.87 (3H, s)	61.6	4.04 (3H, s)	61.5
6	3.90 (3H, s)	60.2	3.88 (3H, s)	61.4	3.82 (3H, s)	61.4
7	3.96 (3H, s)	61.5	4.06 (3H, s)	61.1	3.89 (3H, s)	61.0
8	4.05 (3H, s)	61.1	-	-	4.01 (3H, s)	61.1
5-OH	12.75 (1H, s)	-	-	-	-	-

Table 2. Antioxidant activity compound **1-3**

Compounds	IC_{50} (ppm)
1	269.16 ± 0.51
2	121.09 ± 0.24
3	308.34 ± 0.62
Quercetin*	3.77 ± 0.01

*positive control

The antioxidant activity of flavonoids is generally associated with three chemical features, namely an *ortho*-dihydroxy structure in the B-ring, the presence of 2,3 double bonds in the C-ring, and the presence of a 4-oxo function in the C-ring (Leonarduzzi et al., 2009). Furthermore, the OH group at position 3 of the C ring correlates with antioxidant properties (Amić & Lučić, 2010).

CONCLUSIONS

This study concluded that three polymethoxylated flavones, namely 5-hydroxy-3',4',6,7,8-pentamethoxy flavone (**1**), 8-hydroxy-3,3',4',5,6,7-hexamethoxyflavone (**2**) and 3′,4′,5,6,7,8-hexamethoxyflavone (**3**) was isolated from the ethanol extract of C. *amblycarpa* peel for the first time. Therefore, compounds **1, 2, 3** showed antioxidant activity with IC_{50} values of 269.16 ± 0.51, 121.09 ± 0.24, and 308.34 ± 0.62 ppm, respectively.

REFERENCES

Amić, D., & Lučić, B. (2010). Reliability of bond dissociation enthalpy calculated by the PM6 method and experimental TEAC values in antiradical QSAR of flavonoids. *Bioorganic and Medicinal Chemistry*, *18*(1), 28–35. https://doi.org/10.1016/j.bmc.2009.11.015

Anwar, F., Naseer, R., Bhanger, M. I., Ashraf, S., Talpur, F. N., & Aladedunye, F. A. (2008). Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *JAOCS, Journal of the American Oil Chemists' Society*, *85*(4), 321–330. https://doi.org/10.1007/s11746-008-1204-3

Awouafack, M. D., Tane, P., & Morita, H. (2017). Isolation and structure characterization of flavonoids. *Flavonoids - From Biosynthesis to Human Health*. 46-58. https://doi.org/10.5772/67881

Budiarto, R., Poerwanto, R., Santosa, E., & Efendi, D. (2017). The potentials of limau (*Citrus amblycarpa* Hassk. Ochse) as a functional food and ornamental mini tree based on metabolomic and morphological approaches. *Journal of Tropical Crop Science*, *4*(2), 49–57. https://doi.org/10.29244/jtcs.4.2.49-57

Depkes RI (1979). Farmakope Indonesia, Edisi III. *Departemen Kesehatan Republik Indonesia, Jakarta*.

Fahrurroji, A., & Riza, H. (2020). Karakterisasi ekstrak etanol buah *Citrus amblycarpa* (L), *Citrus aurantifolia* (S.), dan *Citrus sinensis* (O.). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *7*(2), 100. https://doi.org/10.20473/jfiki.v7i22020.100-113

Hayat, K., Zhang, X., Chen, H., Xia, S., Jia, C., & Zhong, F. (2010). Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity. *Separation and Purification Technology*, *73*(3), 371–376. https://doi.org/10.1016/j.seppur.2010.04.026

Herath, W., Mikell, J. R., Hale, A. L., Ferreira, D., & Khan, I. A. (2008). Microbial metabolism part 9.1 Structure and antioxidant significance of the metabolites of 5,7-dihydroxyflavone (chrysin), and 5- and 6-hydroxyflavones. *Chemical and Pharmaceutical Bulletin*, *56*(4), 418–422. https://doi.org/10.1248/cpb.56.418

Ishak, N. I., Kasman, K., & Chandra, C. (2019). Efektivitas ekstrak kulit buah limau kuit (*Citrus amblycarpa*) sebagai larvasida *Aedes aegypti* Instar III. *Media Kesehatan Masyarakat Indonesia*, *15*(3), 302. https://doi.org/10.30597/mkmi.v15i3.6533

Jeong, J. M., Choi, C. H., Kang, S. K., Lee, I. H., Lee, J. Y., & Jung, H. (2007). Antioxidant and chemosensitizing effects of flavonoids with hydroxy and/or methoxy groups and structure-activity relationship. *Journal of Pharmacy & Pharmaceutical Sciences : A Publication of the Canadian Society for Pharmaceutical Sciences, Société Canadienne Des Sciences Pharmaceutiques*, *10*(4), 537–546. https://doi.org/10.18433/j3kw2z

Johann, S., Smânia, A., Pizzolatti, M. G., Schripsema, J., Braz-Filho, R., & Branco, A. (2007). Complete ^{1}H and ^{13}C NMR assignments and antifungal activity of two 8-hydroxy flavonoids in mixture. *Anais Da Academia Brasileira de Ciencias*, *79*(2), 215–222. https://doi.org/10.1590/S0001-37652007000200004

Kim, S. H., Hur, H. J., Yang, H. J., Kim, H. J., Kim, M. J., Park, J. H., Sung, M. J., Kim, M. S., Kwon, D. Y., & Hwang, J. T. (2013). *Citrus junos* Tanaka peel extract exerts antidiabetic effects via AMPK and PPAR- γ both *in vitro* and *in vivo* in mice fed a high-fat diet. *Evidence-Based Complementary and Alternative Medicine*. Vol 2013. https://doi.org/10.1155/2013/921012

Leonarduzzi, G., Testa, G., Sottero, B., Gamba, P., & Poli, G. (2009). Design and development of nanovehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids. *Current Medicinal Chemistry*, *17*(1), 74–95. https://doi.org/10.2174/092986710789957760

Li, S., Lo, C. Y., & Ho, C. T. (2006). Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (*Citrus sinensis*) peel. *Journal of Agricultural and Food Chemistry*, *54*(12), 4176–4185. https://doi.org/10.1021/jf060234n

Maharani, R. A. I. K., Cahyaningsih, N. K., Abimanyu, M. D., Astuti, K.W. (2020). Kulit buah jeruk limau (*Citrus amblycarpa* (Hassk.) Osche) sebagai analgesik. *Jurnal Kimia*. *14*(1), 24-29. doi: https://doi.org/10.24843/JCHEM.2020.v14.i01.p05

Machado, N. F. L., Batista De Carvalho, L. A. E., Otero, J. C., & Marques, M. P. M. (2013). A conformational study of hydroxylated isoflavones by vibrational spectroscopy coupled with dft calculations. *Vibrational Spectroscopy*, *68*, 257–265. https://doi.org/10.1016/j.vibspec.2013.08.010

Machida, K., & Osawa, K. (1989). On the flavonoid constituents from the peels of *Citrus hassaku* Hort: Ex Tanaka. *Chemical and Pharmaceutical Bulletin*, *37*(4), 1092–1094. https://doi.org/10.1248/cpb.37.1092

Mahato, N., Sharma, K., Sinha, M., & Cho, M. H. (2018). Citrus waste derived nutra-pharmaceuticals for health benefits: current trends and future perspectives. *Journal of Functional Foods*, *40*, 307–316. https://doi.org/10.1016/j.jff.2017.11.015

Markham, K. R., Chari, V. M., & Mabry, T. J. (1982). The Flavonoids: Advances in Research. *London: Chapman and Hall*, 19–134.

Mulyani, S., Susilowati, Hutabarat, M. M. (2009). Analisis GC-MS dan daya anti bakteri minyak atsiri *Citrus amblycarpa* (Hassk) Ochse. *Majalah Farmasi Indonesia*, *20*(3), 127–132.

Nakanishi, M., Hino, M., Yoshimura, M., Amakura, Y., & Nomoto, H. (2019). Identification of sinensetin and nobiletin as major antitrypanosomal factors in a citrus cultivar. *Experimental Parasitology*, *200*, 24–29. https://doi.org/10.1016/j.exppara.2019.03.008

Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret ftir spectroscope of organic material. *Indonesian Journal of Science and Technology*, *4*(1), 97–118. https://doi.org/10.17509/ijost.v4i1.15806

Noh, C. H. C., Azmin, N. F. M., & Amid, A. (2017). Principal component analysis application on flavonoids characterization. *Advances in Science, Technology and Engineering Systems*, *2*(3), 435–440. https://doi.org/10.25046/aj020356

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, 1-15. doi: https://doi.org/10.1017/jns.2016.41

Pedana, F., Fadhlillah, F. M., Lestari, W., Garut, F. M., & No, J. J. (2017). Aktivitas antioksidan minyak jeruk sambal (*Citrus amblycarpa*) ditiga daerah di Jawa Barat dengan metode carotenoid bleaching. *Jurnal Ilmiah Farmako Bahari*, *8*(1), 1–4. doi: http://dx.doi.org/10.52434/jfb.v8i1.625

Shiono, Y., Sasaki, T., Shibuya, F., Yasuda, Y., Koseki, T., & Supratman, U. (2013). Isolation of a phomoxanthone a derivative, a new metabolite of tetrahydroxanthone, from a *Phomopsis sp*. isolated from the mangrove, *Rhizhopora mucronata*. *Natural Product Communications*, *8*(12), 1735–1737. https://doi.org/10.1177/1934578x1300801220

Sianturi, J., Harneti, D., Darwati, Mayanti, T., Supratman, U., & Awang, K. (2016). A new (–)-5′,6-dimethoxyisolariciresinol-(3″,4″-dimethoxy)-3α-O-β-D-glucopyranoside from the bark of *Aglaia eximia* (Meliaceae). *Natural Product Research. 30(19): 2204–2208*. https://doi.org/10.1080/14786419.2016.1160233

Stevenie, Girsang, E., Nasution, A. N., & Lister, I. N. E. (2019). Comparison activities of peel and extract of lime (*Citrus amblycarpa*) as antioxidant and antielastase. *American Scientific Research Journal for Engineering (ASRJETS)*, *57*(1), 77–84.

Tambunan, G. C. A., Dutt, A., Nadhifa, S., Amelia, F., & Girsang, E. (2020). The *in vitro* anti-diabetic activity of lime peels (*Citrus amblycarpa* (Hassk.) Ochse). *Journal of Health Sciences*, *13*(01), 26–33. https://doi.org/10.33086/jhs.v13i01.1437

Wang, D., Wang, J., Huang, X., Tu, Y., & Ni, K. (2007). Identification of polymethoxylated flavones from green tangerine peel (*Pericarpium Citri Reticulatae Viride*) by chromatographic and spectroscopic techniques. *Journal of Pharmaceutical and Biomedical Analysis*, *44*(1), 63–69. https://doi.org/10.1016/j.jpba.2007.01.048

Yang, X., Kang, S. M., Jeon, B. T., Kim, Y. D., Ha, J. H., Kim, Y. T., & Jeon, Y. J. (2011). Isolation and identification of an antioxidant flavonoid compound from citrus-processing by-product. *Journal of the Science of Food and Agriculture*, *91*(10), 1925–1927. https://doi.org/10.1002/jsfa.4402

Zhu, L., Chen, J., Tan, J., Liu, X., & Wang, B. (2017). Flavonoids from *Agrimonia pilosa* Ledeb: free

radical scavenging and dna oxidative damage protection activities and analysis of bioactivity-structure relationship based on molecular and electronic structures. *Molecules*, *22*(3), 195. https://doi.org/10.3390/molecules22030195.