AGRINIKA
Jurnal Agroteknologi dan Agribisnis
p - ISSN 2579 - 3659
e - ISSN 2721 - 2807
http://ojs.unik-kediri.ac.id/index.php/agrinika/index

Antioxidant Activity of Non-Volatile Lime (*Citrus aurantifolia* Swingle) Extract

Nandang Permadi[1], Euis Julaeha[2], Yudi Rosandi[3], Mohamad Nurzaman[1*]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang 45363, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang 45363, Indonesia
[3]Department of Geophysics, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang 45363, Indonesia

*Correspondence: m.nurzaman@unpad.ac.id

Diterima 24 Juli 2021/ Direvisi 07 September 2021/ Disetujui 20 September 2021

ABSTRACT

The paper reports the antioxidant activity of a non-volatile fraction of lime processing by-products from the lime syrup home industry. The activity was measured by spectrophotometry to obtain the 50% inhibition concentration (IC_{50}) using the 2,2-*diphenyl-1-picrylhydrazyl* (DPPH) method. The sample was extracted by the maceration method with *n*-hexane, ethyl-acetate, and ethyl-ethanol. The IC_{50} values of 681 ppm, 458 ppm, and 2,775 ppm were *n*-hexane, ethyl-acetate, and ethyl-ethanol. The experiments concluded that the ethyl-acetate extract provides the best inhibition value to scavenge free-radicals DPPH. The HPLC and mass spectroscopy were measured to find out the content and group of active compounds. The significant compounds consisted of naringin, poncirin, or neoponcirin, which are known as antioxidant substances. The result shows the potential application of the lime by-products, its volatile fraction, and the non-volatile fraction, which is the production residue of lime peel. This work can be applied as an alternative to zero-waste lime production, which may benefit the industry and the environment.

***Keywords*:** Antioxidant; *Citrus aurantifolia*; DPPH; Zero-waste industry

INTRODUCTION

Citrus aurantifolia, a member of the Rutaceae family, has been commonly used for food and health products. In the conventional industry, the main product is obtained from lime syrup. The peel is considered as waste and usually trashed without further processing. Some research reports that the peel is rich in substances that can be extracted further to obtain various active compounds for food, medicine, and health applications (Loizzo *et al.,* 2012; Zhuo Zou *et al*., 2016). The biological function of lime peel is originated from the phytochemical content. Among the biological activities of the peel are antioxidant, anti-inflammation, anti-bacterial, anti-viral and anti-fungi, and anti-diabetic substances (Safdar *et al.,* 2017; Samira Lagha-Benamrouche & Madania, 2013; Shofinita, Feng, & Langrish, 2015).

Citrus fruits are the primary source of ascorbate acid, containing many bioactive substances, such as coumarin, carotenoid, limonoid, and flavonoid, especially polymethoxy-flavone and flavanone. Many species of citrus own a

broad spectrum of biological activities. According to the literature, the plant can also be applied for traditional medical treatment of digestion system disorder. It is also reported that the polyphenols substances can obtain potent free-radical scavenging (Loizzo *et al*., 2012) due to the ability to act as hydrogen, electron, and chelate transition metals donor.

In this work, the antioxidant activity of the *Citrus aurantifolia* extract was observed. The antioxidant substance has been used for food products additive, which can protect from product quality decrease through the oxidative-degradation. Although the synthetic antioxidant is commonly used, the natural substance is preferable to avoid side effects, such as liver function disturbance and carcinogenic diseases (Chen *et al*., 2017). Hence, the isolation and characterization of natural antioxidant substances are demanding. The solvent extraction method, which is commonly used in preparing plant extract, was used in this work. The method is widely applied, efficient, and relatively easy to operate. The most used solvents are hot water, methanol, ethanol, acetone, and ethyl-acetate (Chen *et al.,* 2017; Samira Lagha-Benamrouche & Madania, 2013). In contrast, solid-liquid maceration is the most used conventional extraction method to obtain bioactive compounds of plants.

This paper reports the result of the non-volatile extraction process of the C. *aurantifolia*, after separating volatile fraction (essential oil) by hydro-distillation technique. The residue was further extracted by the maceration method using n-hexane, ethyl-acetate, and ethanol solvents, respectively. The antioxidant activity of each produced extract was evaluated using the *2,2-diphenyl-1-picrylhydrazyl (DPPH)* method. The polyphenols profile of the most active extract was studied using HPLC and Mass-spectroscopy instruments.

MATERIALS AND METHODS

Materials and characterization

The *C. aurantifolia* peel was obtained from the residue of the lime home industry in Kuningan, West Java. The laboratory standard Sthal distillation equipment was used to extract the volatile fraction. HPLC apparatus with RF-18 column and photodiode array detector (PDA) was used to analyze the chemical content of the sample. The mass spectroscopy apparatus uses TOP MS ES^+. The used organic solvents are n-hexane, ethyl-acetate, and ethanol, obtained from the local chemical supply.

Preparation of *C. aurantifolia* peel extract

The peel is shredded approximately 2 cm wide, weighed, and put in the distillation flask, soaked in distilled water. Then the distillation process is operated for 3 hours to remove the volatile fraction. After this process, the residue of citrus peels was taken and blended with n-hexane solvent. The first maceration procedure for three days. The same procedure is performed sequentially using different solvents, ethyl-acetate and 80% ethanol, as the second and third maceration processes. The extracts are collected daily, and the same type of solvent is refilled to the flask. The resulted extracts from this three-stage

procedure are collected for further measurements.

Antioxidant activity test

The antioxidant activity was tested using the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) method, a very reactive free-radical chromogen. The substance absorbed visible light at 517 nm, detected by spectroscopy apparatus. The concentration of the tested substance is varied according to the ability to scavenge DPPH free radicals, which is shown by the color change from purple to yellow. The method is widely used due to its easiness, measurement speed, and sensitivity.

The amount of 0.1 mL n-hexane, ethyl-acetate, and ethanol extracts with around 100 to 4,000 ppm were added in 2 mL DPPH (0.21 mM in 95% ethanol). The mix was shaken and kept for 60 minutes in a dark room and ambient temperature. A spectrometer measured the light absorbance at 517 nm. The DPPH inhibition, %I, is calculated by (Maemulyati & A. R. Prihadi, 2016; Safdar *et al.,* 2017; Samira Lagha-Benamrouche & Madania, 2013),

$$\%I = \frac{A_c - A_S}{A_c} \times 100 \quad \text{..... (1)}$$

where A_c and A_s is the absorbance control and sample, respectively. The IC_{50} value is measure by,

$$IC_{50} = (50 - b)/a \quad \text{..... (2)}$$

a and b were taken as the line fitting function parameter from the plotted measurement data of %I for each extract.

RESULTS AND DISCUSSION

Extraction yield

The yield of the extraction process can be seen in Table 1. Using the maceration process was obtained 0.3% to 2.2% yield from the total peel weight, and it strongly depends on the used solvent

Table 1. The yield of extracts. The initial peel weight is 400 g

Solvent	Extract weight (g)	%Yield
n-hexane	1.20	0.30
ethyl-acetate	2.97	0.74
80% ethanol	8.80	2.20

The extraction sequence is arranged in such a way depending on the solvent polarity, n-hexane as a non-polar, ethyl-acetate as a semi-polar, and ethanol as a polar solvent. The ethanol gave an enormous yield value due to its polarity, which indicates that polar components dominate the crude extract.

Antioxidant test

The inhibition value was evaluated using the DPPH method, which measured the ability of the substance to scavenge free radicals. The DPPH captures antioxidant substances through reaction, which changes the substance into pale yellowish colored *diphenylhydrazine*. The reaction is accompanied by the decrease of light absorption at 517 nm wavelength. The decrease is due to the disappearance of paramagnetic resonance signals induced by the electron dynamics of the free radicals. Hence, the decrease is

proportional to the number of free radicals stabilized by free radicals. The phenomenon is observed using the spectrophotometer. In order to quantize the activities, the IC50 is defined, which stands for the amount of concentration of the substance that can inhibit 50% of oxidation reaction by DPPH. Figure 1 shows the inhibition value (in percentage) as a function of substance concentration.

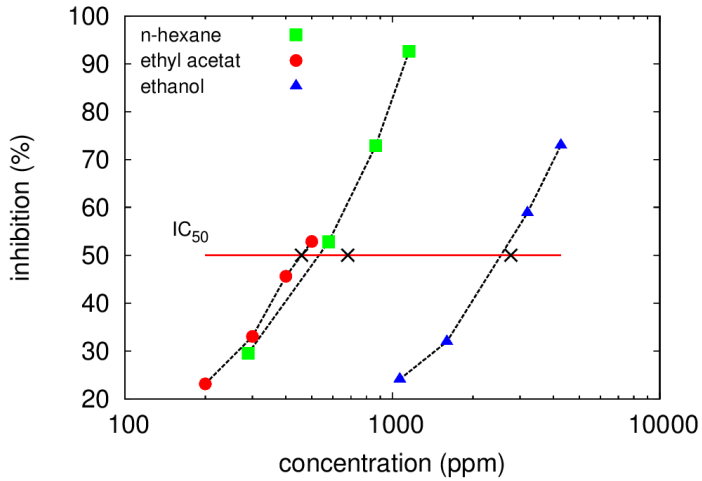


Figure 1. The correlation between the concentration and the inhibition percentage for *n*-hexane, ethyl-acetate, and ethanol extract of lime peel. The red horizontal line is the 50% inhibition value (IC_{50}). The black cross symbols denote the values from line regression approximation. The dashed lines are to guide the eye

The plot shows a positive correlation between the concentration of the n-Hexane extract with the inhibition percentage. The correlation demonstrates the effectiveness of the inhibition mechanism induced by the number of polyphenols contained in the extract. The result agrees with the data previously reported in (Samira Lagha-Benamrouche & Madania, 2013). The obtained IC50 number extracted by n-hexane, ethyl-acetate, and 80% ethanol is 681.2 ppm, 457.6 ppm, dan 2,775.4 ppm, respectively. The result shows that the ethyl-acetate gives the lowest value. Hence, the extract is the most active compared to other extracts.

The results of HPLC and mass spectroscopy measurement

The most active extract (ethyl-acetate) is to be analysed further by HPLC and mass spectroscopy. Figure 2 shows the chromatogram profile taken from the HPLC experiment.

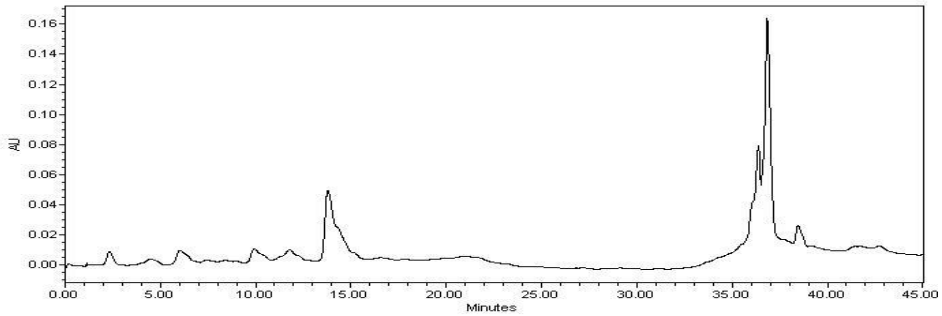
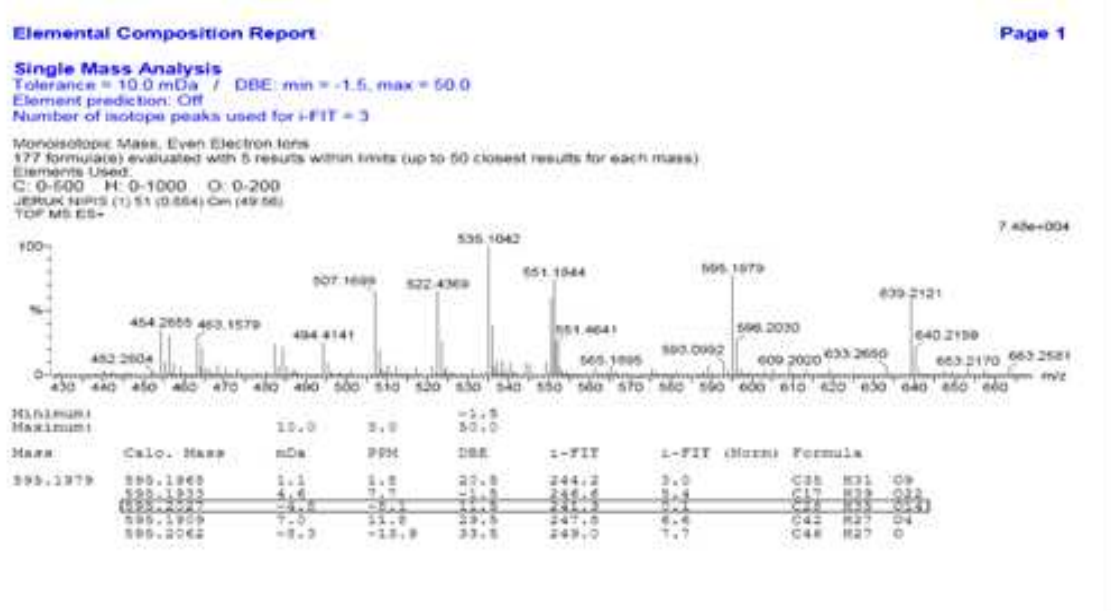
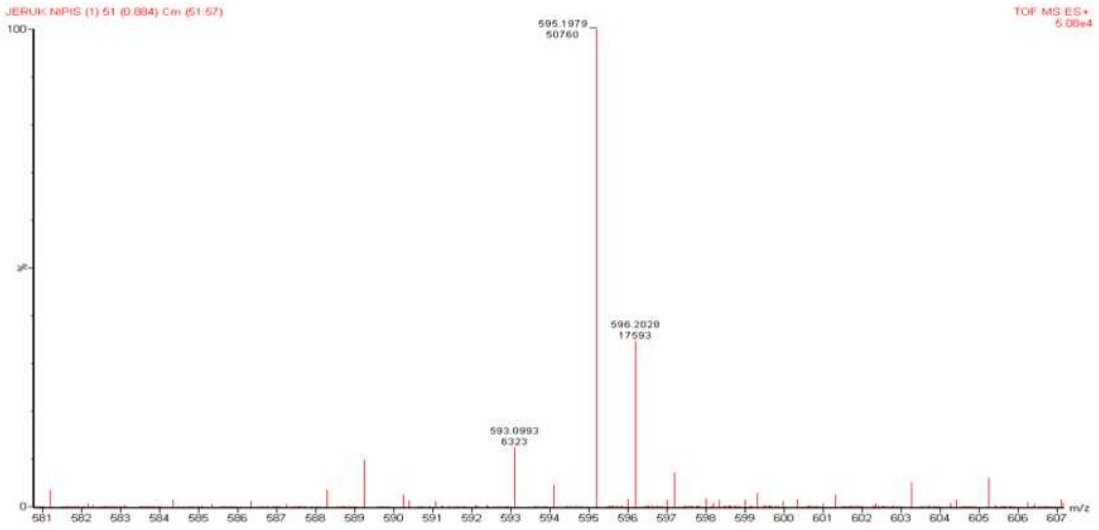


Figure 2. The chromatogram of ethyl-acetate extract of the lime peel with RP-18 PDA detector.

Figures 3 and 4 show the mass spectrum obtained from the experiment. The Beynon table shows two dominant masses, 594 amu and 580 amu, corresponding to $C_{28}H_{34}O_{14}$ and $C_{27}H_{32}O_{14}$ molecular structures.



(a)



(b)

Figure 3. (a) The mass spectrum of the main chemical component of the ethyl-acetate extract. (b) mass spectrum of the $C_{28}H_{34}O_{14}$ molecule.

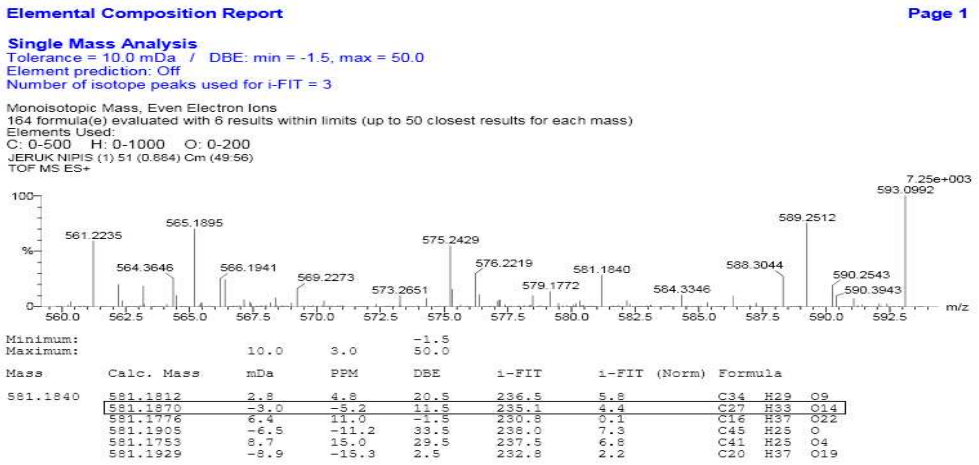


Figure 4. The mass spectrum of the main chemical component of the ethyl-acetate extract indicates the $C_{27}H_{32}O_{14}$ molecule.

According to the phytochemistry tracing of the citrus family, this molecular formulae leads to the poncirin or neoponcirin, and naringin compounds, respectively. These compounds are members of the flavonoid group, which are the effective antioxidants (Chen *et al.,* 2017; Safdar *et al.,* 2017; Xia, Fanga, Zhaob, Jiaob, & Zhoua, 2014).

CONCLUSION

From this research, an ethyl-acetate extract is the most active antioxidant extract, having 457.6 ppm IC_{50}. Compared to the value of *n*-hexane and ethanol extract, this value is superior. Hence this paper concludes that ethyl-acetate is the preferable solvent to extract *C. aurantifolia* peels. The main components for antioxidant activity, poncirin or neoponcirin and naringin, are identified in the resulted extract. The result confirms that the lime peel has the potential to be used as an antioxidant product.

ACKNOWLEDGEMENT

The Ministry of Research and Technology/National, Research and Innovation Agency, supports this work, which has provided research funding through the Master Thesis Research Grant scheme in 2020, contract number: 1827/UN6.3.1/LT/2020.

REFERENCES

Chen, X. M., Tait, A. R., & Kitts, D. D. (2017). Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food Chemistry*, *218*, 15–21. http://doi.org/10.1016/j.foodchem.2016.09.016

Loizzo, M. R., Tundis, R., Bonesi, M., Menichini, F., De Luca, D., Colica, C., & Menichini, F. (2012). Evaluation of *Citrus aurantifolia* peels and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. *Journal of the Science of Food and Agriculture*, *92*(15), 2960–2967. http://doi.org/10.1002/jsfa.5708

Maemulyati, A., & A. R. Prihadi. (2016). Chemical composition of essential oil and hexane extract and antioxidant activity of various extracts of *Acmella uliginosa* (Sw.)

Cass flowers from Indonesia. *Agriculture and Natural Resources*, *50*(4), 264–269.

Safdar, M. N., Kausar, T., Jabbar, S., Mumtaz, A., Ahad, K., & Saddozai, A. A. (2017). Extraction and quantification of polyphenols from kinnow (*Citrus reticulate* L.) peel using ultrasound and maceration techniques. *Journal of Food and Drug Analysis*, *25*(3), 488–500. http://doi.org/10.1016/j.jfda.2016.07.010

Samira Lagha-Benamrouche, & Madania, K. (2013). Phenolic contents and antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Algeria: Peels and leaves. *Industrial Crops and Products*, *50*, 723–730.

Shofinita, D., Feng, S., & Langrish, T. A. G. (2015). Comparing yields from the extraction of different citrus peels and spray drying of the extract. *Advanced Powder Technology*, *26*(6), 1633–1638.

Xia, W., Fanga, B. B., Zhaob, Q., Jiaob, B., & Zhoua, Z. (2014). Flavonoid composition and antioxidant activities of local Chinese pummelo (*Citrus grandis* Osbeck.) varieties. *Food Chemistry*, *162*, 230–238.

Zhuo Zou, Wanpeng Xi, Yan Hu, Chao Nie, Z. Z. (2016). Antioxidant activity of Citrus fruits. *Food Chemistry*, *196*, 885–896.