Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2017.55.61

Research Article
Antioxidant Activities and Phenolics Contents of Avocado (*Persea americana* Mill.) Peel *in vitro*

[1]Irma Antasionasti, [1]Sugeng Riyanto and [1,2]Abdul Rohman

[1]Faculty of Pharmacy, Gadjah Mada University, 55281 Yogyakarta, Indonesia
[2]Research Center of Halal Products, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Abstract

Background: Synthetic-based antioxidants are suspected to cause toxic or mutagenic effects toward human health, therefore, the demand for natural antioxidants increased. One of the natural antioxidant sources is Avocado Peel (AP). **Objective:** The objective of this study is to determine antioxidant activities of the extract, fractions and isolates from AP using *in vitro* method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging, reducing power of iron (III) as well as total phenolic and flavonoid contents. **Materials and Methods:** Ethyl acetate fraction of AP is fractionated using vacuum liquid chromatography and gravitation column chromatography methods. To identify the chemical structure of the active compound of antioxidants in AP, FTIR spectroscopy and gas chromatography-mass spectrometry (GC-MS) are used. **Results:** Among extracts evaluated, methanol extract has the strongest antioxidant activity. Further fractionation of methanolic extract showed that fraction 8 is the most active fraction with IC_{50} values of 4.221±0.137 mg mL^{-1} (DPPH assay) and 0.855±0.013 mg mL^{-1} (ABTS assay), with reducing power of iron (III) of 723.067±18.849 mg ascorbic acid g^{-1} fraction. Phenolics contents contributed 37, 40.78 and 47.45%, compared with the contribution of flavonoids compounds of 26.71, 19.25 and 34.62% for DPPH radical scavenging, ABTS radical scavenging and reducing power of iron (III) to iron (II), respectively. Isolation of the fraction 8 of methanol extract indicated the presence of 1,2,4-trihidroksiheptadek-12,16-diyne and isolation of the ethyl acetate extract indicated the presence of 1,2,4-trihidroksiheptadek-16-yne-18-ene. **Conclusion:** The AP is potential to be used as food supplement due to its antioxidant activities.

Key words: Avocado peel, ABTS, DPPH, reducing power, antioxidant compounds

Received: January 05, 2017 Accepted: March 03, 2017 Published: March 15, 2017

Citation: Irma Antasionasti, Sugeng Riyanto and Abdul Rohman, 2017. Antioxidant activities and phenolics contents of avocado (*Persea americana* Mill.) peel *in vitro*. Res. J. Med. Plants, 11: 55-61.

Corresponding Author: Abdul Rohman, Faculty of Pharmacy, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The existence of free radicals such as superoxide (O_2^{-}), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) in large quantities is a problem for human health, because unpaired electrons in free radicals are the highly reactive species which cause cell damage[1]. The free radicals can cause several degenerative diseases such as atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer and neurodegenerative[2,3]. The most effective way to neutralize and reduce free radical is the use of antioxidants[4].

Antioxidants are group of compounds capable of delaying, reducing or inhibiting the oxidation reactions of macromolecules including lipids, nucleic acids and proteins[5]. Antioxidants can be obtained synthetically such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) or naturally. Fruits and vegetables are rich sources of natural antioxidants. Synthetic-based antioxidants like BHA and BHT are suspected to cause toxic or mutagenic effects[6], therefore, the demand for natural-based antioxidants increased due to the safety related issues of synthetic antioxidants[7-9]. One of the interesting natural antioxidant sources to be developed are parts of plants which are not consumed (underutilized part) such rambutan peel[10,11]. In this study, the potential of Avocado Peel (AP) as natural antioxidant is explored to get added value of AP.

Avocado (*Persea americana* Mill.) fruit has been widely known as functional fruit due to its bioactive compounds having the beneficial effects to human health, including vitamin C, vitamin E, carotenoids and phenolics compounds[12]. Avocado fruit is consumed fresh and this implies that avocados can produce large amount of peel and seeds which are considered as wastes, which are nuisance during food processing[13]. Therefore, some phytochemicals have been isolated from seed and peel which provide good sources of antioxidants[14], capable of preventing inflammatory diseases[15].

Several studies have been carried out to evaluate antioxidant activities of avocado fruit and its part. Calderon-Oliver *et al.*[16] have evaluated antioxidant activity of avocado peel extract using radical scavenging assay and Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP). Antioxidant activities of seed and peel are also evaluated by Daiuto *et al.*[17]. Using literature review, there is no reports regarding the determination of antioxidant activities of extracts and fractions of AP. In this study, the antioxidant activities of methanolic extracts of AP *in vitro* are evaluated. Furthermore, the chemical compounds responsible for these activities were also identified.

MATERIALS AND METHODS

Avocado fruit is obtained from Ampelgading Hamlet, Bandungan, Semarang Indonesia and is authenticated in the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (UGM), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, rutin, gallic acid and quercetin were purchased from Sigma (Aldrich, USA). Silica gel F_{254}, Folin-Ciocalteu and other reagents and solvents were purchased from E. Merck (Darmstat, Germany).

Preparation of extracts and fractionation: Avocado Peel (AP) is separated from the pulp and is subjected sun drying. The dried powder (4.8 kg) was macerated using petroleum ether, ethyl acetate and methanol to get its corresponding extracts under room temperature and the eluent was replaced every 24 h. The extraction process was reapeated 3 times. Each extract was evaporated using vacuum rotary evaporator at 60°C. The methanolic and ethyl acetate extracts of AP was fractionated using vacuum column chromatography with stationary phase of silica gel and eluents were delivered in gradient manner in the order n-hexane: $CHCl_3$ (100:0-0:100), $CHCl_3$:EtOAc (100:0-0:100), EtOAc:MeOH (100:0-0:100). Each eluate (100 mL) obtained was subsequently evaporated and subjected to TLC profiling using silica gel F_{254} with the mobile phase of chloroform:methanol (9:1 v/v). The eluates with similar TLC profiles were then pooled and were designated as "fraction". Each fraction was further used for DPPH radical scavenging test, ABTS radical scavenging test, reducing power of iron (III) and determination of total phenolics and flavonoid contents.

Determination of DPPH free radical scavenging activity: The scavenging activity of DPPH radical of samples was performed based on Kikuzaki *et al.*[18]. In this assay, a 50 µL test solutions (extract or fraction solutions with different levels) was added with 1.0 mL of 0.4 mM methanolic-DPPH and added with methanol in volumetric flask 5.0 mL. The mixture was shaken vigorously using vortex for 1 min and is allowed to stand for 20 min at 25°C in dark room. The absorbance of solution was measured using spectrophotometer at 517 nm using methanol as blank. The radical scavenging activity was calculated as:

$$\text{Radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is absorbance of control (DPPH radical without the addition of test solution), A_s is sample absorbance (absorbance of DPPH radical after the addition of test solutions).

Determination of ABTS radical scavenging activity: The activity of ABTS radical scavenging was measured according to Re *et al.*[19] with slight modification. Briefly, ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium per sulfate and the mixture was allowed to stand for 12-16 h in at room temperature. Prior analysis, the solution was diluted with methanol, so that the absorbance value of 0.700±0.02 was obtained at 734 nm. The samples were subsequently added into 3 mL of ABTS solution and were mixed. The mixture was left at room temperature for 6 min and absorbance was read at 734 nm. The radical scavenging activity was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is absorbance of control (ABTS radical without the addition of test solution), A_s is sample absorbance (absorbance of ABTS radical after the addition of test solutions).

Determination of reducing power: The reducing power of samples were determined according to Hinneburg *et al.*[20]. The extracts or fractions (1.0 mL) with different concentrations were mixed with 2.5 mL of phosphate buffer (200 mM; pH 6.6) and 2.5 mL of potassium ferricyanide 1%. The mixture was incubated at 50°C for 20 min. The mixture was added with 2.5 mL of 10% TCA and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. The absorbance increase of the reaction mixture was interpreted as increase in reducing activity of the evaluated samples. Ascorbic acid was used as standard for the calibration curve. Reducing power of the extracts and fractions were expressed as milligram ascorbic acid equivalents per gram of sample (mg g^{-1}).

Determination of total phenolic content: Folin-Ciocalteu (FC) reagent was used for analysis of Total Phenolics Content (TPC) according to Chun *et al.*[21]. In a 10 mL volumetric flask, the aliquot (0.2 mL) of the samples in methanol (1.0 mg mL^{-1}) was mixed with 0.4 mL FC reagent. The solution was allowed to stand at 25°C for 5-8 min before addition of 4.0 mL Na_2CO_3 7.0% and made to 10.0 mL with bidistilled water. The mixture was allowed to stand for 2 h before its absorbance was measured at 725 nm. The TPC was expressed as milligram Gallic Acid Equivalents (GAE) per gram sample (mg g^{-1}).

Determination of total flavonoid content: The total flavonoid contents were measured by colorimetric assay according to Zou *et al.*[22]. The aliquot (0.1 mL) of samples in methanol was added into 10 mL volumetric flask containing 4 mL distilled water. At zero time, 0.3 mL $NaNO_2$ 5% was added to the flask. After 5 min, 0.3 mL $AlCl_3$ 10% was added. At 6 min, 2 mL NaOH 1 M was added to the mixture. Immediately, the mixture was diluted to volume with the addition of 2.4 mL distilled water and was thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus a blank containing all reagents except the samples. Total flavonoid content of the extracts and fractions were expressed as milligram Rutin Equivalents (RE) per gram of sample (mg g^{-1}).

Identification of isolates: To identify the chemical structures of isolate(s) having the active antioxidants in avocado peel, FTIR spectroscopy and gas chromatography-mass spectrometry (GC-MS) were used.

Statistical analysis: All data were presented as Means±Standard Deviation (SD) for at least three replications for each samples. Statistical analysis was performed based on Mann Whitney U at confidence level 95% using SPSS (SPSS Corporation, Chicago, IL) version 16.0 for windows. Linear regression to correlate between total phenolics as well as total flavonoid with antioxidant activities was carried using Excel 2010.

RESULTS AND DISCUSSION

Several methods are used for evaluation of antioxidant activities *in vitro*, namely radical scavengers, lipid peroxidation inhibition, chelating agent, reducing power and synergist. Avocado Peel (AP) has been reported to have antioxidant activities using radical scavenging and reducing power[23]. In the present study, the radical scavenging activity was measured using DPPH and ABTS radicals. The DPPH method is based of reduction of colour (purple) from DPPH solution at wavelength 517 nm[24] to the presence of radical scavengers from the evaluated samples. The ABTS radical is green in color and when it is reduced by antiradicals, the color changed into blue with decreased absorbance at 732 nm. The antioxidant activity using DPPH and ABTS radicals was expressed with of IC_{50} values, a value of extracts/fractions capable of reducing 50% absorbance of initial DPPH and ABTS solution. At the lower IC_{50} value, the antiradical activity was more.

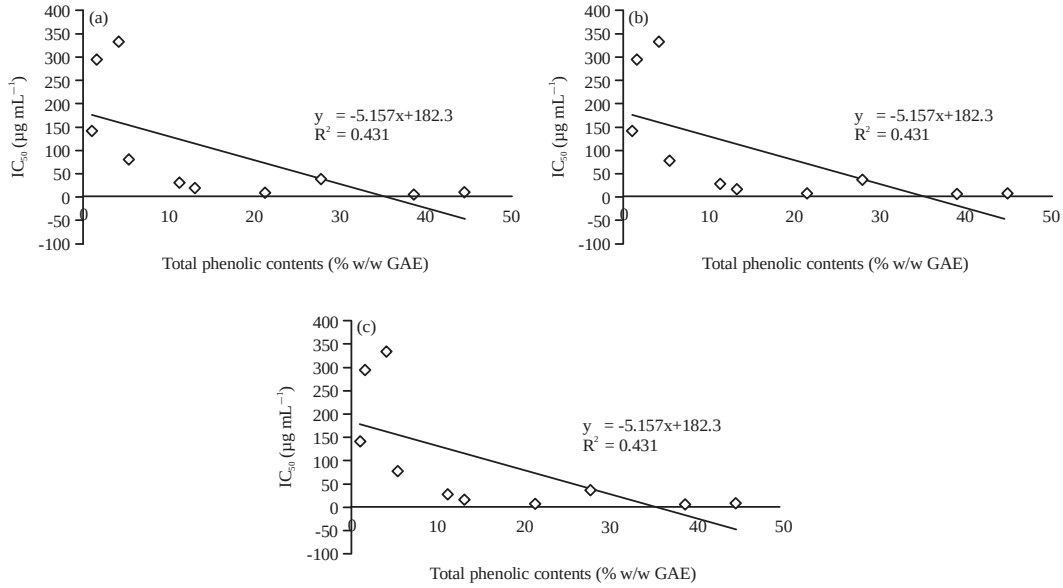

Fig. 1(a-c): Relationship between radical scavenging activity of (a) DPPH, (b) Radical scavenging activity of ABTS and (c) Reducing power with total phenolic contents of extracts and fractions of methanolic extract of avocado peel

Table 1: Antioxidant activities of extracts and fractions of avocado peel as determined using radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical as well as reducing power

Extracts/fractions	DPPH-IC_{50} ±SD (µg mL^{-1})	ABTS-IC_{50}±SD (µg mL^{-1})	Reducing power (mg g^{-1} sample)
Methanol	9.467±0.045	1.122±0.008	742.863±3.487
Ethyl acetate	18.387±0.022	5.487±0.216	179.748±3.057
Petroleum ether	78.331±0.210	156.696±4.758	63.976±0.569
1	No observed	No observed	No observed
2	293.586±2.949	198.075±2.785	66.034±5.222
3	141.213±1.275	136.302±2.356	116.052±2.920
4	332.919±2.657	55.806±4.913	287.484±5.757
5	36.520±0.447	33.074±1.772	153.271±7.882
6	30.565±1.311	17.882±1.180	737.784±15.341
7	8.637±0.095	10.611±0.230	723.067±18.849
8	4.221±0.137	0.855±0.013	774.710±26.284
9	8.894±0.094	3.373±0.026	

In reducing power method, the colorless of Fe^{3+} complex is reduced to blue Fe^{2+} complex with antioxidant[25] which can be monitored at 700 nm. The increased absorbance value at wavelength 700 nm showed increased reducing power of samples. The results of the determination of antioxidant activities of extract and fractions of AP using DPPH assay, ABTS assay and reducing power are presented in Table 1. Among extracts evaluated, methanol extract has the highest antiradical activities against both DPPH and ABTS assays with IC_{50} values of 9.467±0.045 and 1.122±0.008 mg mL^{-1}, respectively. The methanol extract also revealed the highest reducing power, accounting of 742.863±3.487 mg ascorbic acid g^{-1} of extract. Therefore, methanol extract is further fractionated to obtain more active fraction. The highest antioxidant activity in methanol extract is due to the high levels of phytochemicals having reducing properties such as phenolics, flavonoids and vitamin C.

The fractionation of the methanol extract resulted 9 fractions, in which fraction 8 has the highest antiradical activities toward DPPH assay (IC_{50} = 4.221±0.137 mg mL^{-1}), ABTS assay (IC_{50} = 0.855±0.013 mg mL^{-1}) and reducing power accounting of 723.067±18.849 mg ascorbic acid g^{-1} fractions. The fraction 9 has the highest reduction power (744.710±26.284 mg ascorbic acid g^{-1} fractions) than fraction 8. However, based on Mann Whitney test, the reducing power of fraction 8 and 9 were not significantly different ($p>0.05$). There were no significant differences in DPPH and ABTS assays between vitamin C with methanol extract and fraction 8 ($p>0.05$).

Figure 1 and 2 revealed the relationship between radical scavenging activity of DPPH, radical scavenging activity of

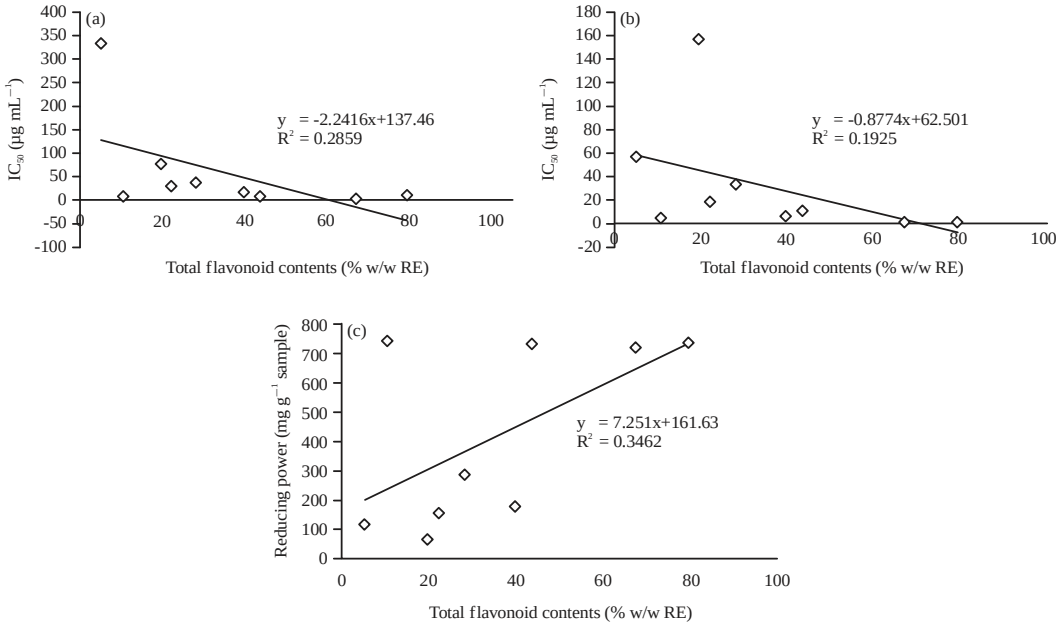

Fig. 2(a-c): Relationship between radical scavenging activity of (a) DPPH, (b) Radical scavenging activity of ABTS and (c) Reducing power with total flavonoid contents of extracts and fractions of methanolic extract of avocado peel

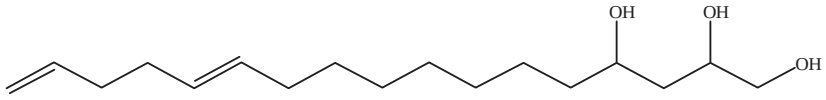

Fig. 3: Chemical structure of 1,2,4-trihidroksiheptadek-12,16-diyne, isolated from methanol extract of avocado peel

ABTS and reducing power with total phenolic contents (Fig. 1) and with flavonoid contents (Fig. 2) of extracts and fractions of methanolic extract of AP. Based on coefficient determination (R^2) value, phenolics compounds are more contributing toward antioxidant activities than flavonoid contents as indicated by R^2 value of phenolics than flavonoid. Based on R^2 values, phenolics contents contributed to DPPH radical scavenging, ABTS radical scavenging and reducing power iron (III) to iron (II), each with 43.10, 40.78 and 47.45%, respectively, compared to the contribution of flavonoids compounds accounting of 28.59, 19.25 and 34.62%. This is not surprising because flavonoids are also group of phenolics.

The methanolic and ethyl acetate extracts are further subjected to isolation to obtain an active isolate. Isolate 1 from methanolic extract is purified by recrystallization to obtain brown powder. To ensure the purity of the isolates, the melting point (56-58°C) and TLC test with 3 different mobile phases, namely ethyl acetate (Rf 0.67), ethyl acetate: methanol (1:1 v/v; Rf 0.75) and methanol (Rf 0.90) were used. The FTIR spectrum of isolate 1 revealed sharp absorption band with strong intensity at wavenumber 3305 cm^{-1} indicating the presence of hydroxyl group (OH). The absorption bands at wavenumber 2920 and 2850 cm^{-1} with a strong intensity indicated stretching vibration group $-CH_2$ aliphatic, reinforced by bending vibration of C-H at wavenumber 1471 and 1372 cm^{-1}. The FTIR spectrum of isolate 1 showed absorption at wavenumber 1610 cm^{-1} indicating the stretching vibration of C=C[26]. The GC-MS chromatogram showed that isolates are not pure, because they appear several peaks, where the dominant peak appeared at retention time of 26.650 min. At this retention time, isolate had fragmentation patterns that are similar to the results reported by Adikaram *et al.*[27] with the molecular ion M^+ at m/z 285. The fragmentation pattern is: 237 $[M-CH_2OH-H_2O]^+$ (2), 209 $[M-CH_2CHOHCH_2OH]^+$ (4), 193 (4), 149 (4), 135 (7), 123 (3), 109 (15), 87 (100), 81 (38), 69 (38), 67, 55 (59), 41 (38). It can be interpreted that isolate 1 has a molecular weight of 284. Isolate 1 is a mixture of compounds including long-chain aliphatic compounds, especially 1,2,4-trihidroksiheptadek-12,16-diyne (Fig. 3).

Isolate 2 is TLC pure with melting point of 58-60°C. The FTIR spectrum of isolate 2 revealed sharp absorption band

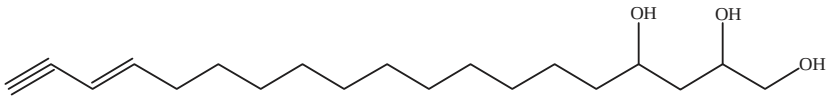

Fig. 4: Chemical structure of 1,2,4-trihidroksiheptadek-16-yne-18-ene, isolated from ethyl acetate extract of avocado peel

with strong intensity at 3311 cm^{-1} indicating the presence of hydroxyl group (OH). Peaks at wavenumbers of 2921 and 2851 cm^{-1} were observed which indicated stretching vibration aliphatic group -CH_2 and were confirmed by presence of peak at 1472 cm^{-1} due to CH_2 bending vibration. Isolates 2 also showed FTIR absorption peak at wavenumber 2361 cm^{-1} indicating C≡C group[26]. The GC-MS chromatograms showed that isolate 2 have molecular ion M^+ at m/z 311 with fragmentation patterns 285 (1), 255 $[M\text{-}CH_2OH]^+$ (2), 237 $[M\text{-}CH_2OH\text{-}H_2O]^+$ (3), 211 $[M\text{-}CH_2CHOHCH_2OH]^+$ (5), 193 (3), 149 (2), 123 (5),109 (16), 87 (100), 69 (52), 41 (38). Based on these spectra, isolates 2 had the formula $C_{19}H_{34}O_3$ identified as 1,2,4-trihidroksiheptadek-16-yne-18-ene (Fig. 4). This chemical structure is in agreement with that reported by Rodriguez-Sanchez *et al*.[28] and Adikaram *et al*.[27].

CONCLUSION

Among extracts and fractions of avocado peel evaluated, methanol extract and its fraction (fraction 8) has the highest antioxidant activities, total phenolics contents and total flavonoid contents. The isolate 1, tentatively identified as 1,2,4-trihidroksiheptadek-12,16-diyne is obtained during isolation of fraction 8 from methanolic extract of avocado peel. Furthermore, an isolate obtained from ethyl acetate extract contained 1,2,4-trihidroksiheptadek-16-yne-18-ene.

SIGNIFICANT STATEMENT

- The consumption of fresh avocado consumed fresh and this implies that avocados can produce large amount of peel and seeds
- We have evaluated the antioxidant activities of avocado peel
- The subfractions of ethyl acetate fraction of avocado peel revealed strong antioxidant activities
- Avocado peel is potential to be developed as functional food

ACKNOWLEDGMENT

The first author thanks to the Indonesia Endowment Fund for Education, Ministry of the Finance Republic of Indonesia (LPDP) for the graduate scholarship, to the Faculty of Pharmacy Gadjah Mada University for facilitating the study.

REFERENCES

1. Carocho, M. and I.C.F.R. Ferreira, 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol., 51: 15-25.
2. Aktumsek, A., G. Zengin, G.O. Guler, Y.S. Cakmak and A. Duran, 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food Chem. Toxicol., 55: 290-296.
3. Young, I.S. and J.V. Woodside, 2001. Antioxidants in health and disease. J. Clin. Pathol., 54: 176-186.
4. Arina, N.B. and A. Rohman, 2013. The phenolic contents and antiradical activity of Indonesian *Phyllanthus urinaria* L. Int. Food Res. J., 20: 1119-1124.
5. Di Carlo, G., N. Mascolo, A.A. Izzo and F. Capasso, 1999. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sci., 65: 337-353.
6. Alnajar, Z.A.A., M.A. Abdulla, H.M. Ali, M.A. Alshawsh and A.H.A. Hadi, 2012. Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of *Melastoma malabathricum*. Molecules, 17: 3547-3559.
7. Valentao, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.L. Bastos, 2002. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. J. Agric. Food Chem., 50: 4989-4993.
8. Tachakittirungrod, S., S. Okonogi and S. Chowwanapoonpohn, 2007. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chem., 103: 381-388.
9. He, N., Z. Wang, C. Yang, Y. Lu and D. Sun *et al*., 2009. Isolation and identification of polyphenolic compounds in *Longan pericarp*. Sep. Purif. Technol., 70: 219-224.
10. Rohman, A., S. Riyanto, Mistriyani, Shuhaira and A.E. Nugroho, 2016. Antiradical activities of rambutan peel: Study from two cultivars. Res. J. Phytochem., 11: 42-47.
11. Permatasari, L. and A. Rohman, 2016. 2,2'-diphenil-1-picrylhydrazil (DPPH) radical scavenging activity of extracts and fractions of rambutan (*Nephelium lappaceum* L.) peel. Res. J. Phytochem., 10: 75-80.

12. Mfonobong, A., U. Ismail, I.H. Mairo, Z. Maimuna and D. Fatima, 2013. Hypolipidemic and antioxidant effects of petroleum ether and methanolic fractions of *Persea americana* Mill seeds in Wistar rats fed a high-fat high-cholesterol diet. Int. J. Med. Pharmaceut. Sci., 3: 1-10.
13. Saavedra, J., A. Cordova, R. Navarro, P. Diaz-Calderon and C. Fuentealba *et al*., 2017. Industrial avocado waste: Functional compounds preservation by convective drying process. J. Food Eng., 198: 81-90.
14. Ayala-Zavala, J.F., V. Vega-Vega, C. Rosas-Dominguez, H. Palafox-Carlos and J.A. Villa-Rodriguez *et al*., 2011. Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Res. Int., 44: 1866-1874.
15. Dabas, D., R.M. Shegog, G.R. Ziegler and J.D. Lambert, 2013. Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. Curr. Pharmaceut. Des., 19: 6133-6140.
16. Calderon-Oliver, M., H.B. Escalona-Buendia, O.N. Medina-Campos, J. Pedraza-Chaverri, R. Pedroza-Islas and E. Ponce-Alquicira, 2016. Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts. LWT-Food Sci. Technol., 65: 46-52.
17. Daiuto, E.R., M.A. Tremocoldi, S.M. de Alencar, R.L. Vieites and P.H. Minarelli, 2014. Chemical composition and antioxidant activity of the pulp, peel and by products of avocado 'Hass. Revista Brasileira Fruticultura, 36: 417-424.
18. Kikuzaki, H., M. Hisamoto, K. Hirose, K. Akiyama and H. Taniguchi, 2002. Antioxidant properties of ferulic acid and its related compounds. J. Agric. Food Chem., 50: 2161-2168.
19. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26: 1231-1237.
20. Hinneburg, I., H.J.D. Dorman and R. Hiltunen, 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem., 97: 122-129.
21. Chun, O.K., D.O. Kim and C.Y. Lee, 2003. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem., 51: 8067-8072.
22. Zou, Y., Y. Lu and D. Wei, 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. J. Agric. Food Chem., 52: 5032-5039.
23. Kosinska, A., M. Karamac, I. Estrella, T. Hernandez, B. Bartolome and G.A. Dykes, 2012. Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. J. Agric. Food Chem., 60: 4613-4619.
24. Floegel, A., D.O. Kim, S.J. Chung, S.I. Koo and O.K. Chun, 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Comp. Anal., 24: 1043-1048.
25. Biskup, I., I. Golonka, A. Gamian and Z. Sroka, 2013. Antioxidant activity of selected phenols estimated by ABTS and FRAP methods. Postepy Higieny Medycyny Doswiadczalnej, 67: 958-963, (In Polish).
26. Silverstein, R.M., F.X. Webster and D.J. Kiemle, 2005. Spectrometric Identification of Organic Compounds. 7th Edn., John Wiley and Sons, New York, USA., ISBN: 9780471393627, Pages: 502.
27. Adikaram, N.K.B., D.F. Ewing, A.M. Karunaratne and E.M.K. Wijeratne, 1992. Antifungal compounds from immature avocado fruit peel. Phytochemistry, 31: 93-96.
28. Rodriguez-Sanchez, D.G., A. Pacheco, M.I. Garcia-Cruz, J.A. Gutierrez-Uribe, J.A. Benavides-Lozano and C. Hernandez-Brenes, 2013. Isolation and structure elucidation of avocado seed (*Persea americana*) lipid derivatives that inhibit *Clostridium sporogenes* endospore germination. J. Agric. Food Chem., 61: 7403-7411.