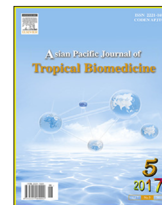
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Original article http://dx.doi.org/10.1016/j.apjtb.2017.01.010

Triterpenoid of avocado (*Persea americana*) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells

CrossMark

Andi Nur Fitriani Abubakar[1], Suminar Setiati Achmadi[1*], Irma Herawati Suparto[1,2]

[1]*Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia*

[2]*Laboratory of Microbiology & Immunology, Primate Research Center, Bogor Agricultural University, Bogor 16151, Indonesia*

ARTICLE INFO

Article history:
Received 1 Aug 2016
Accepted 7 Sep 2016
Available online 6 Jan 2017

Keywords:
Persea americana
Triterpenoid
MCF-7
HepG2
Cancer cells

ABSTRACT

Objective: To determine the structure of triterpenoid isolated from avocado seeds and the cytotoxic effect on MCF-7 and HepG2 cells.

Methods: The powder sample was macerated with ethanol, followed with separation of the extract by column chromatography. The target compound was monitored on thin layer chromatography plate and reagent Lieberman–Buchard. The isolated compound was characterized by spectral analysis, mainly ultraviolet, infrared, and liquid chromatography-mass spectroscopy and their spectroscopic data with those reported in literature were compared. *In vitro* cytotoxic activity was investigated against Vero, MCF-7, and HepG2 cell lines using MTT assay.

Results: A triterpenoid compound was isolated from ethanol extract. The extracts, fraction (F_3), and the isolated compound showed a significant cytotoxic activity against all investigated cell lines. MTT assay showed that the triterpenoid isolate inhibited cell proliferation of MCF-7 and HepG2 cell line with the IC_{50} values of 62 µg/mL and 12 µg/mL, respectively, and was safe to normal cells.

Conclusions: The results of the present study reveal that triterpenoid from avocado seeds have the potential for further development as anticancer agents.

1. Introduction

Avocado plant (*Persea americana*) is one member of Lauraceae family that grows in many tropical and subtropical regions such as Indonesia. Almost all parts of this plant have been used in traditional medicine by the local communities. One interesting part of the avocado crop is the seed, which is usually wasted. The seed is reported to contain secondary metabolites that belong to the class of alkaloids, triterpenoids, tannins, flavonoids, saponins [1], and polyphenols [2], which generally has a pharmacological effect. The ethanol extract of the seed shows antioxidant activity *in vitro* [3] and cytotoxic activity on breast cancer cells T47D [4]. Other studies also report that the seed extract is able to reduce the bacteria proliferation of *Mycobacterium tuberculosis* [5], *Proteus mirabilis*, and *Aerobacter aerogenes* [6]. The antioxidant activity, cytotoxic and antibacterial of the secondary metabolites indicate that the avocado seed is potential as an anticancer agent.

An important secondary metabolite in avocado seed is triterpenoid, which is widely reported to have anticancer activity. However, the previous studies were focused on the crude seed extract and limited phytochemical tests, and none on its bioactive compounds. Given the potential of triterpenoids and the benefits of avocado seed, there should be further investigation of this bioactive compounds derived from avocado seeds toward effective and safe anticancer agent. The purpose of this study was to determine chemical structure of the triterpenoid isolated from avocado seeds and the cytotoxic effect on MCF-7 and HepG2 cells. The results of this study are expected to contribute to the development of avocado seed as anticancer agents and a strong basis of the efficacy of the ethanol extract.

*Corresponding author: Suminar Setiati Achmadi, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia.

Tel/Fax: +62 8161857886

E-mail: ssachmadi@yahoo.com

Foundation Project: Supported by Ministry of Finance of Indonesia through Education Fund Management Institution (LPDP) under a contract number PRJ-541/LPDP.3/2016.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

2221-1691/Copyright © 2017 Hainan Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Materials and methods

2.1. Plant material

Avocado seeds were collected from Malino, Gowa, South Sulawesi Province, Indonesia in July, 2015. The plant material was identified in Herbarium Bogoriense, Department of Botany Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia. The seed sample was sorted, washed, dried, and ground to 60 mesh-size.

2.2. Isolation of triterpenoid

The samples (2.5 kg) were macerated in ethanol (five times replacement of solvent, every 24 h) at room temperature. After filtration and removal of the solvent under vacuum, 327 g of crude extract was obtained. The crude extract (174 g) was partitioned using *n*-hexane to eliminate lipid constituents. Three grams of the ethanol extract was fractionated by column chromatography on silica gel Merck 60 (0.063–0.200 mm) with *n*-hexane: ethyl acetate (3:7) mixture and isocratic elution system. The eluate was collected in 10 mL vials. The success of separation was monitored using thin layer chromatography (TLC). The eluates producing similar pattern of the same stain were combined into one faction. The existence of triterpenoids in each fraction was identified by spraying Lieberman–Burchard reagent on the TLC plates and followed by heating. The fractions indicating positive test fraction and in the form of solids were washed with *n*-hexane.

2.3. TLC of the isolated triterpenoid

Analysis was performed on TLC silica gel 60 F_{254} (Merck, Darmstadt, Germany, 20 cm × 20 cm, 1 mm thickness) plates. The freshly prepared plates were dried at room temperature, thereafter these were kept at 100 °C for 30 min to activate and then cooled at room temperature. Chromatographic development was performed using *n*-hexane: ethyl acetate (5:5, v/v) as mobile phase. After development, the TLC plates were dried. To visualize the spots, the plates were sprayed with a Lieberman–Burchard reagent (acetic anhydride – conc. H_2SO_4) and heated for 10 min at 110 °C and observed under UV light (365 nm) [7].

2.4. Characterization of the isolated triterpenoid

Triterpenoids isolates were characterized by UV PharmaSpec 1700 (Shimadzu, Kyoto, Japan) in methanol, Fourier transform infrared spectrophotometer (FTIR) Bruker Tensor 37 using KBr plates, and liquid chromatography mass spectrometry Waters Acquity.

2.5. In vitro cytotoxicity assay

2.5.1. Cell cultures

Cytotoxicity assay was performed on Vero cell line (ATCC CCL-81), human breast cancer cell line MCF-7 (ATCC HTB-22), and human liver cancer cell line HepG2 (ATCC HB-8065) from collection of the Primate Study Center, Bogor Agriculture University, Indonesia. Vero and HepG2 cells line were cultured in Dulbecco's modified eagle medium media, while MCF-7 cells line in RPMI media with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 μg/mL) from Gibco (Carlsbad, CA, USA). Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air and sub-cultured when they were 50% confluent (24 h).

2.5.2. MTT assay

The cytotoxic properties of the samples (extract, fraction, and isolated compounds) were assessed using MTT assay [8]. The cell suspension was dispensed into a 96-well microplate at 100 mL/well containing 5 000 cells/well and incubated in humidified atmosphere with 5% CO_2 at 37 °C for 24 h, and then treated with the compounds at various concentrations. After 48 h of treatment, 10 μL MTT solution was added to each well, and further incubated for 4 h. Living cells upon reaction with MTT will form formazan blue. The formazan formed was dissolved in 96% ethanol. The cytotoxic effects of the samples were measured as the absorbance of each well at 595 nm on ELISA microplate reader (Bio-Rad imark 11421). All assays were performed using samples in three replicates. The percentage inhibition of cells line was calculated by the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The sample concentration which inhibited 50% cell line (IC_{50}) was determined using the relationship curve between the concentration of sample (*x*) and the percentage inhibition (*y*).

3. Results

3.1. Chemical properties of the isolated triterpenoid

The yield of ethanol extract was 13.09% (w/w). Fractionation over silica gel column of the ethanol extract from the avocado seeds yielded eight main fractions (F_1, F_2, F_3, F_4, F_5, F_6, F_7 and F_8). Out of these eight fractions, only one fraction (F_3) showed a single spot indicating triterpenoids characteristics. Repeated purification of F_3 gave 124 mg of white solid. Identification on TLC with Lieberman–Burchard reagent produced a green spot under UV light (365 nm).

The UV spectra showed a maximum absorption at wavelength of 217 nm (Figure 1). The FTIR spectrum showed absorptions at 3 428 cm^{-1} (OH), 2 920 cm^{-1} (C−H aliphatic), 2 851 cm^{-1} (C−H aliphatic), 1 710 cm^{-1} (C═O), 1 641 cm^{-1} (C═C), 1 468 cm^{-1} (C−H in CH_2), and 1 382 cm^{-1} (C−H in CH_3) (Figure 2). The molecular weight of the isolate was analyzed by liquid chromatography mass spectrometry.

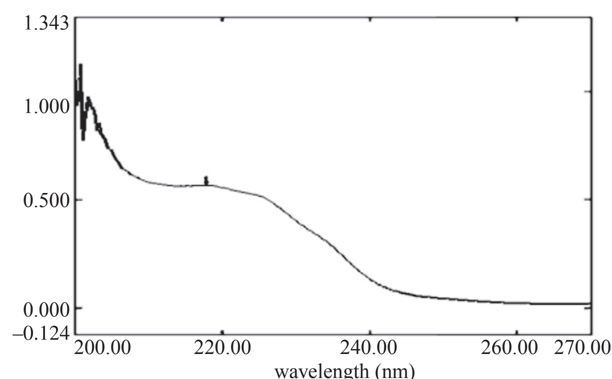


Figure 1. UV spectrum of the isolated triterpenoids in methanol.

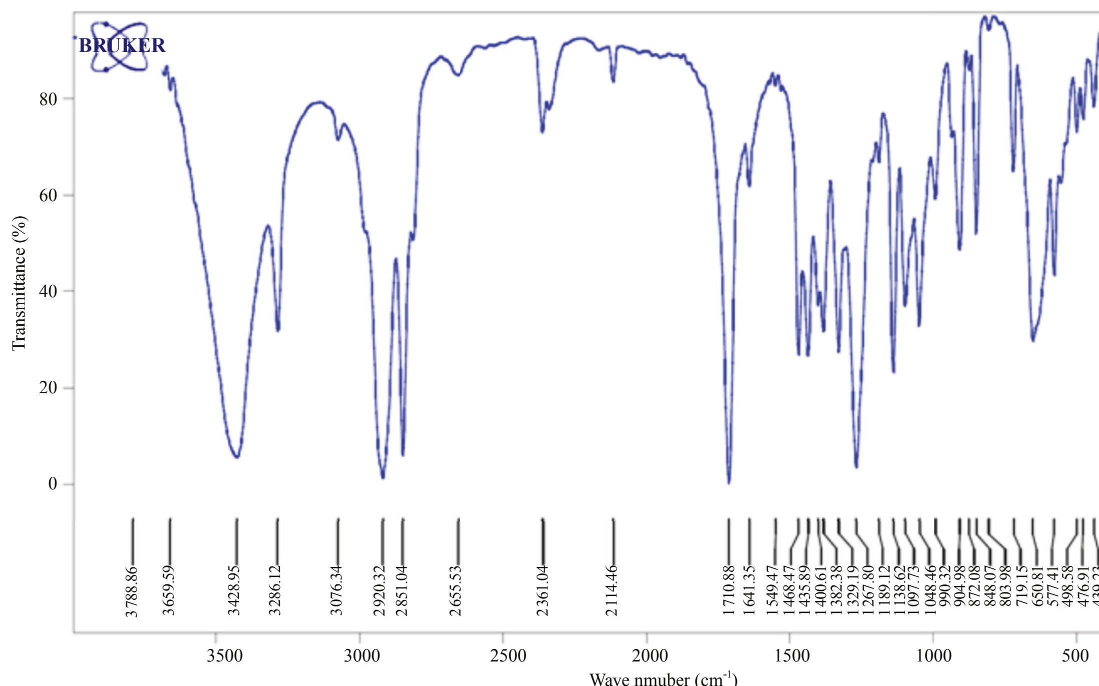


Figure 2. FTIR spectrum of the isolated triterpenoid.

Separation using liquid chromatography showed a single peak with a high intensity with a retention time at 19.00 min. This particular peak was further analyzed using mass spectrometry, showing a peak of mass 506 $[M+H]^+$ (Figure 3).

3.2. Cytotoxicity activity

Three samples [ethanol extract, fraction (F_3), and the isolate] in this study were challenged against Vero cells and MCF-7 cells. Various concentrations of each sample to Vero cells were 200.0, 150.0, 100.0, 50.0, 25.0, and 12.5 µg/mL. The toxicity test of the three samples that showed safe concentration limit for ethanol extract on normal cells was 100 µg/mL, whereas that of the F_3 and the isolates were 12.5 µg/mL (Figure 4). Higher concentrations may produce inhibition value above 50% to normal cells. Samples with safe concentration

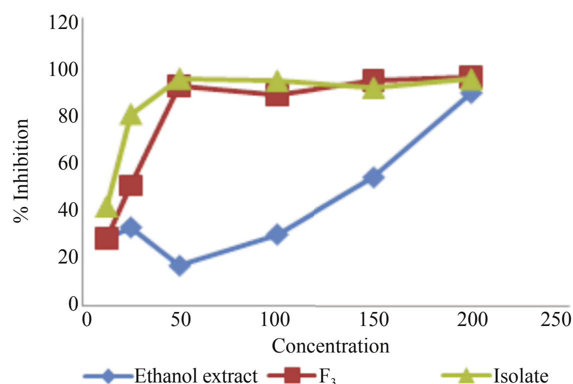


Figure 4. Cytotoxic effects of avocado seed samples on Vero (normal African green monkey kidney cells).

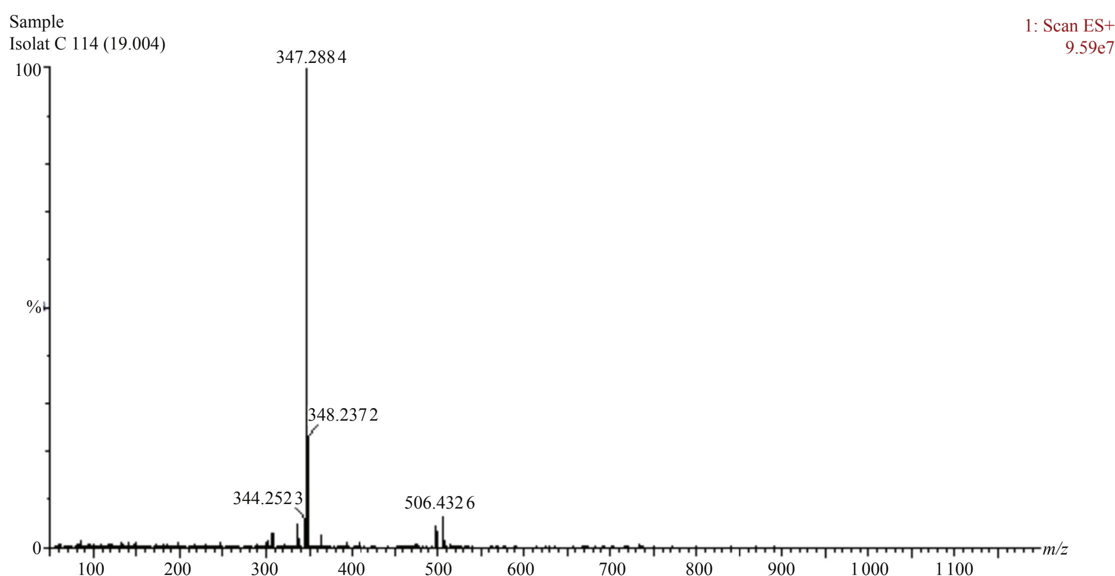


Figure 3. The mass spectrum of the isolate with a retention time of 19.00 min.

toward normal cells were further tested against breast cancer cells MCF-7. The result of the ethanol extract, fraction (F_3), and the isolate against MCF-7 cells with IC_{50} values were 99.74 μg/mL, 80.05 μg/mL and 62.43 μg/mL, respectively. Cytotoxic test of the isolate against other cancer cells, in this case was the HepG2 cells, showing IC_{50} value of 12.03 μg/mL. Therefore *in vitro* cytotoxicity test of the isolate showed its significant anticancer activity against MCF-7 and HepG2 cell line.

4. Discussion

4.1. Phytochemical constituents

We report in this work the isolation and characterization of triterpenoid compound of the ethanol extract obtained from the avocado seeds. Identification isolated on TLC plates with Lieberman–Burchard reagent produced a green spot under UV light (365 nm), indicating the existence of triterpenoids [9]. Absorption peaks the isolated compound in the UV spectrum is typical for triterpenoids which has a chromophore form double bonds (C=C) that is unconjugated [10]. Most triterpenoids have no strong UV absorption due to the lack of conjugated functional groups [11]. The FTIR spectrum showed absorptions at 1 468 cm^{-1} and 1 382 cm^{-1} indicating the gem dimethyl groups as typical compounds of triterpenoids [12]. IR spectrum showed characteristic absorption bands for the carboxylate group (1 710 cm^{-1}) and unsaturation (1 641 cm^{-1}). In this study, we report for the first time a triterpenoid compound from avocado seed with a molecular weight of 505 g/mol. Other studies found acetogenin compound with a molecular weight of 353 g/mol [13].

4.2. Cytotoxic effects

The extract, a particular fraction, and the isolated compounds were evaluated for cytotoxic activity. The Vero cells were used to evaluate the toxicity to normal cells to determine the safe level of administration of the sample as a medicine from some natural materials. Higher concentrations that inhibit above 50% of the normal cell growth should not be used for testing against cancer cells [14]. The cytotoxic result of the ethanol extract, F_3 fraction, and the isolate against MCF-7 cells showed anticancer activity. The IC_{50} values of less than 100 μg/mL indicated the potential sample as chemoprevention agent [15]. Based on the IC_{50} values, the three samples increase cytotoxic activity against cancer cells MCF-7, meaning that the purified extract enhances the activity as anticancer against MCF-7 cells. Thus, the triterpenoids isolates are more likely to be developed as a new chemotherapeutic agent for inhibiting tumor and cancer cell growth. The *in vitro* cytotoxicity of the isolated compounds showed its significant anticancer activity against HepG2 cells. This is in accordance with the standard of the National Cancer Institute (NCI, USA), stating that the effectiveness of bioactive components to fight cancer cells must be lower than 30 μg/mL. With a very low IC_{50} value, the isolated triterpenoid is again confirming its potential to become an alternative material to inhibit liver cancer cells. We conclude that a triterpenoid, derived from ethanol extract of avocado seed, demonstrates cytotoxic activity with a very low IC_{50} value, thus is potential to become an alternative material to inhibit liver cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to the Ministry of Finance of Indonesia through Education Fund Management Institution (LPDP) for financial assistance under a contract number PRJ-541/LPDP.3/2016.

References

[1] Torres RC, Garbo AG, Walde RZM. Lavicidal activity of *Persea americana* Mill. against *Aedes aegypti*. *Asian Pac J Trop Med* 2014; **7**(Suppl 1): S167-70.
[2] Kosińska A, Karamać M, Estrella I, Hernández T, Bartolomé B, Dykes GA. Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *J Agric Food Chem* 2012; **60**: 4613-9.
[3] Soong YY, Barlow PJ. Antioxidant activity and phenolic content of selected fruit seeds. *J Food Chem* 2004; **88**(3): 411-7.
[4] Kristanty RE, Suriawati J, Sulistiyo J. Cytotoxic activity of avocado seeds extracts (*Persea americana* Mill.). *Int Res J Pharm* 2014; **5**(7): 557-9.
[5] Jiménez-Arellanes A, Luna-Herrera J, Ruiz-Nicolás R, Cornejo-Garrido J, Tapia A, Yépez-Mulia L. Antiprotozoal and antimycobacterial activities of *Persea americana* seeds. *BMC Complement Altern Med* 2013; **13**: 109.
[6] Dewi S, Sulistyowati. [Use of avocado (*Persea americana* Mill.) seed extract as antibacterial for *Proteus mirabilis* and *Aerobacter aerogenes*]. *Stigma* 2013; **6**(2): 31-4. Indonesian.
[7] Nikam PH, Kareparamban JA, Jadhav AP, Kadam VJ. Development and validation of high-performance thin layer chromatographic method for ursolic acid in *Malus domestica* peel. *Indian J Pharm Sci* 2013; **75**(4): 486-9.
[8] Thakur AN, Thakur NL, Indap MM, Pandit RA, Datar VV, Müller WE. Antiangiogenic, antimicrobial, and cytotoxic potential of sponge-associated bacteria. *Mar Biotechnol (NY)* 2005; **7**: 245-52.
[9] Khlif I, Jellali K, Michel T, Halabalaki M, Skaltsounis AL, Allouche N. Characteristics, phytochemical analysis and biological activities of extracts from Tunisian chetoui *Olea europea* variety. *J Chem* 2015; **2015**: 418731.
[10] Zetra Y, Prasetya P. [Isolation of α-amyrin compound from *Beilschmiedia roxburghianan* (medang) plant and its bioactivity]. *Akta Kim* 2007; **3**: 27-32. Indonesian.
[11] Xu R, Ye Y, Zhao W. *Introduction to natural products chemistry*. Boca Raton: CRC Press; 2011, p. 138.
[12] Mathias L, Vieira JC, Filho RB, Filho ER. A new pentacyclic triterpenoid isolated from *Myroxylon balsamum*. *J Braz Chem Soc* 2000; **11**(2): 195-8.
[13] Rodríguez-Sánchez DG, Pacheco A, García-Cruz MI, Gutiérrez-Uribe JA, Benavides-Lozano JA, Hernández-Brenes C. Isolation and structure elucidation of avocado seed (*Persea americana*) lipid derivatives that inhibit *Clostridium sporogenes* endospore germination. *J Agric Food Chem* 2013; **61**(30): 7403-11.
[14] Senthilraja P, Kathiresan K. *In vitro* cytotoxicity MTT assay in Vero, HepG2, and MCF-7 cell lines study of Marine Yeast. *J Appl Pharm Sci* 2015; **5**(3): 80-4.
[15] Meiyanto E, Susidarti RA, Handayani S, Rahmi F. [Ethanolic extract of *Areca catechu* L. seeds inhibit proliferation and induce apoptosis on MCF-7 cells]. *Indones J Pharm* 2008; **19**(1): 12-8. Indonesian.