Phytochemicals properties of avocado seed: A review

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Abstract. Persea americana Mill. seed or avocado seed contains phytochemical components such as flavonoids, tannins, saponins, phenolic, and alkaloid. The phenolic compounds of avocado seed are mainly catechin, hydroxybenzoic acid, caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, and triterpenoid glycosides. This review also discusses various extraction methods using an organic solvent, inorganic solvent, and a mixture of solvents. The analysis methods to measure the chemical properties of extracted phytochemicals from avocado seed are discussed. The functional properties of the phytochemicals obtained from the seed are presented.

1. Introduction
Avocado is a nutritious fruit native to Central and South America [1, 2]. Genus Persea and Family Lauraceae belong to Persea americana Mill. or avocado. It has several varieties, and the most popular one is the Hass variety [3]. The fruit characteristic depends on the cultivar, storage conditions, ripeness, and edaphoclimatic conditions [4].

The avocado industrial processing unit generates large amounts of peels and seeds [2]. The seeds are underutilized as a non-edible part of the fruit and discarded as wastes [5]. Untreated waste can potentially increase the number of insects and rodents [1]. The avocado waste (seed and leaves) has a range of useful chemicals. Thus, these seeds and leaves may potentially be extracted and produced valuable income for the avocado industries. The avocado seed comprises 13–18% of the whole fruit's size and is one source of phytochemicals. The seed and leaves have higher total phenolic content and antioxidant capacities than those of its pulp and peels [6]. The leaves have more phenolic content than avocado seed [7].

This paper reviewed the phytochemical properties of avocado seed derived from its industrial waste. The information on avocado's phytochemical properties can be used for further derivative product development, particularly in the phytopharmaceutical industry.

2. Phytochemical Components
Bioactive compounds are chemicals separated from plants. The components of avocado seed are protein, sugar, starch, fat, and water. The bioactive compounds found in avocado are phenolics, flavonoids, carotenoids, vitamin C, and vitamin E [4]. Table 1 presents the composition of phytochemicals in the avocado seed. Phytochemicals present in avocado seed include flavonoids, tannins, saponins, phenolics, antioxidant capacity, oxalates, phytates, and alkaloids [8].

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Table 1. Composition of phytochemicals in avocado seed [9].

| Phytochemicals | (mg/100g) |
|----------------|-----------|
| Flavonoids     | 20.33±0.01|
| Tannins        | 0.76±0.17 |
| Saponins       | 0.52±0.42 |
| Oxalates       | 4.40±0.30 |
| Phytates       | 0.44±0.01 |
| Alkaloids      | 5.40±0.00 |

The polyphenols are one of the bioactive compounds distributed in all parts of avocado plants [10]. Table 2 lists the polyphenols in the avocado seed. The other polyphenol compounds found in avocado seeds are 3-O-caffeoylquinic acid, 3-O-p-coumaroylquinic acid, procyanidin trimer A(I), procyanidin trimer A(II), catechin, or epicatechin gallate [11]. Other research stated that avocado seeds have tannins, saponins, and flavonoids, which are parts of polyphenols [12].

Table 2. The identified polyphenols in avocado seed [10].

| Compounds                                         | Content (mg/100g) |
|---------------------------------------------------|-------------------|
| Catechin                                          | 24.3-2,000        |
| Caffeic acid                                      | 13.7-22.5         |
| Chlorogenic acid                                  | 0.0516-1,953      |
| (Epi)catechin                                     | 1.106-2,906       |
| Ferulic acid                                      | 0.09-1.2          |
| Kaempferol                                        | 10.74             |
| Kaempferide                                       | 10.74             |
| Procyanidins                                      | 152-5,560         |
| Rutin                                             | 0.22              |
| Trans-5-O-caffeoyl-D-quinic acid                  | 163-574           |
| Vanillic acid                                     | 286               |

Different varieties of avocado produce different content of phenolic compounds [13]. The phenolic compound profile of avocado seed can be classified into alkanols, terpenoid glycosides, fural ring-containing derivatives, flavonoids, and coumarin [14]. The type of alkanols is 4-acetoxy-1,2-dihydroxy heptadec-16-ene, 4-acetoxy-1,2-dihydroxy heptadec-16-yne, and 1,2,4-trihydroxy heptadec-16-yne. Part of terpenoid glycosides is (1'S, 6'R)-8'-hydroxy abscisic acid β-D-glc and (1'R, 3'R, 5'R, 8'S)-epidihydrophaseic acid- β-D-glc. 14 2-(pentadecyl)furan, 16 2-(heptadecyl)furan, 2-(12-tridecyl)furan, 2-(12-tridecyl)furan and dimethyl scadinonate as furan ring-containing derivative. Astragal, luteolin, apigenin, luteolin-7-O-D-glc, quercetin-3-O-D-diglc, quercitri, isoquercitrin, afzelin, quercetin-3-O-D-ara, (+)-catechin and (-)-epicatechin are part of flavonoids and scopoletin as coumarin compound [13].
3. Extraction Methods
The extraction method to obtain bioactive compounds from avocado seed varies. The freeze-drying method is commonly used for avocado seed's pre-treatment. The avocado seed powder was extracted using freeze-drier with 80% methanol in a 1:8 ratio of solid to solvent, heated at 60℃, and shook for 15 minutes [13]. The solvent was then evaporated using rotavapor at 40℃. The aqueous residue was lyophilized to protect oils from oxidation in water emulsions [13]. The freeze-drying method was done by grounding avocado seed into powder. 0.5 g of the seed powder was extracted in 10 ml solvent (70 v acetone, 29.7 v water, and 0.3 v acetic acid) [6]. During the extraction process, there was a mixing process using a vortex mixer for 30 s and two sonication processes for 5 min with an interlude for 20 min at room temperature. The extract was then centrifuged for 10 min [6].

Solvant extraction is another method that can be used to separate bioactive compounds from the avocado seed. Gomez et al. (2014) extracted a lyophilized avocado seed powder using ethanol, centrifuged at 2500 rpm for 10 minutes. The powder was macerated in ethanol at a ratio of 1:10 (w/v), then was evaporated at 50℃ using a rotary evaporator [15]. Another ethanol extraction method was performed by Egbuonu et al. (2018). In short, the avocado seed was sun-dried and milled, and then the powder was extracted with cold maceration using 90% ethanol in ratio 1:2 solid to solvent. Afterwards, the extract was filtered using filter paper. The filtrate was concentrated at 60℃ using a water bath and dried at 50℃ with an oven [16].

Organic solvent, such as hexane, is also used for the extraction. Ogbuagu et al. (2020) used sun-dried avocado seed and grounded the seed into a powder then extracted them using Soxhlet with n-Hexane as a solvent in ratio 1:25 (w/v) [16]. The avocado seed powder was wrapped in filter paper and placed in a thimble. The oil was obtained by evaporating the solvent at 50-60℃ [17]. Adaramola et al. (2016) performed a hexane-based extraction by oven-dried avocado seed for 48 hours at 50℃ then pulverized [5]. A 100 g sample was extracted for 8 hours using Soxhlet and n-Hexane as the solvent. The seed oil was recovered using a vacuum rotary evaporator at 40℃ [5].

Extraction using water as a solvent was also performed. A 50 g avocado seed and 500 ml water are placed in a water bath at 96℃ for 30 min. Then the extract filtered using flannel cloth [18]. Mixtures of solvents were also used for avocado seed extraction. Rivai et al. (2019) macerated a 50 g avocado seed powder using a mixture of hexane, acetone, and ethanol at a ratio of 1:1:1 [18]. The extract was then evaporated using a rotary evaporator at 50℃ to obtain the filtrate [18].

Those various extraction methods' filtrate showed different quantities of alkaloids, flavonoids, phenol, and tannin. Fatty acids are the chemical compounds in the extraction of hexane. Fatty acids are also found in acetone extract with other chemical compounds like phenols, tannins, and flavonoids. In the ethanol extraction, the extracted chemical compounds are phenols, tannins, alkaloids, and flavonoids. In water extraction, the chemical compounds found are carbohydrates, tannins, and phenols. 0.435% of total alkaloids were only found in ethanol extraction, while 0.1068% and 0.1084% of total flavonoids were found in acetone extract and ethanol extraction, respectively. Total phenol for extraction using acetone was 0.0476%, 0.0309% in the ethanol extraction and 0.0494% in water extraction. 0.1989%, 0.2044%, and 0.1804% of total tannins were found in acetone extract, ethanol extract, and water extract, respectively [18]. The levels of phytochemicals in the avocado seed are based on the growth condition, variety of avocado, and the age of the avocado plant [11].

4. Phytochemical Analysis of Avocado Seed
The total phenolic content of avocado seed extract was determined by diluting Folin-Ciocalteu reagent and 15% sodium carbonate. Then measured the absorbance at 765 nm using a microplate reader [6]. In short, a 0.25 ml methanolic solution extract, 0.25 ml Folin-Ciocalteu reagent diluted with distilled water (1:1), 0.5 ml sodium carbonate are mixed, and 4 ml water was added. The mixture was incubated at room temperature for 30 minutes, then the absorbance at 725 nm is measured using a spectrophotometer [13].

The presence of saponins, tannins, and flavonoids in avocado seed was tested using 0.1% ferric chloride added with a few drops in filtered, boiled water of avocado seed [12]. Indication of tannins
showed by blue-black or brownish-green colour. Indication of saponins showed by emulsion in the solution of 10 ml filtrate water, 5 ml distilled water, and three drops of olive oil. While the indication of flavonoids showed by yellow colouration in the mixture of 5 ml, dilute ammonia solution, 5 ml filtrate, and 1 ml sulphuric acid.

The antioxidant properties on extracted avocado seed oil contained flavonoid content, phenolic content, and free radicals. Assays in flavonoid content are measured using the Aluminum trichloride spectrophotometric method and quercetin as a standard. Methanol, distilled water, 5% NaNO2 solution, 10% AlCl3, and NaOH are used in this assay. The phenolic content was evaluated using the reduction of the Folin-Ciocalteu reagent method and gallic acid as a standard. The phenolic content assays' solution is methanol, distilled water, 10 ml of 7% sodium carbonate. The free radical scavenging was assayed using the spectrophotometry method and gallic acid as a standard. Methanol, 2,2-diphenyl-2-picrylhydrazyl, or DPPH, and distilled water are used to assay free radical scavenging. Reducing power on avocado seed oil was evaluated using spectrophotometry and gallic acid as standard. Solutions of phosphate buffer, 1% potassium ferricyanide, 10% trichloroacetic acid, 1% ferric chloride, and distilled water are applied to evaluate the reducing power [5].

5. Functional Properties
The high nutritional value in avocado seed, especially phenolics, was reported to have various functional properties such as antioxidant, antimicrobial, and analgesic. Glutathione has a role as an antioxidant to protect cells through oxygen and free radical harm. The methanolic extract has been found as an agent to prevent and treat bacterial infections caused by pathogenic bacteria such as E. coli and S. pyogenes [8]. Polyphenols, as natural oxidants, have some beneficial properties to human health. It can prevent lipid oxidation and reduce the risk of inflammatory diseases [19].

Henry et al. (2015) claimed that saponins have hypertensive activity and cardiac depressant because cholesterols are bound with saponins and form insoluble complexes. Saponins in the human body can affect the immune system, help against cancer, and lower cholesterol levels. The characteristics of saponins are stable in aqueous solutions forming soap-like foams and diverse in a group of compounds. At the same time, flavonoids have beneficial effects on body health as antiviral, antitumor, anti-inflammatory, antioxidant, and antiplatelet. Tannins are known as polyphenols, which have high molecular weight and water-soluble. It also can precipitate proteins. The benefits of tannins in the human body are reducing the respiratory problem, improving appetite, and lowering blood pressure [12].

Yasir et al. (2010) argued that the potential activity of avocado seed due to the rich source of nutrients, such as vasorelaxant activity, analgesic, and anti-inflammatory activity, hypotensive activity, anticonvulsant activity, antiviral activity, wound healing activity, antiulcer effect, antihepatotoxic activity, antioxidant activity, hypoglycemic activity, and reducing body weight [14]. The avocado seed is mentioned in research as a medical agent such as anticancer, anti-inflammatory, antidiabetic, antihypertensive, cholesterol reducer, dermatological effects, antimicrobial, insecticidal, and even as a colourant [11].

Recent research of terpenoid in avocado seed extract has been investigated for an anticancer component [22]. The isolation of the terpenoid compound used ethanol extract and in vitro cytotoxic test using MTT assay. It showed the IC50 value is 62µg/mL as a cytotoxic activity under a safe concentration limit of 100µg/mL for ethanol extract on normal cells. Anticancer activity against human breast MCF-7 (Michigan Cancer Foundation-7) cancer cells enhanced by using a purified extract of terpenoid from avocado seed extract. Thus, isolated triterpenoid can be further developed for
chemotherapeutic agents to inhibit tumour and cancer cell growth [20]. Extraction of avocado seed using chloroform and further partition with soluble methanol fractions (FLM) and non-soluble methanol fractions (FTLM) showed the IC50 was 94.87, 34.52, and 66.03 µg/mL, respectively. More than 50% of MCF-7 cells were killed at 30µg/mL concentration and caused cell shrinkage than FTLM within 48h. Both FLM and FTLM avocado seed extract induced apoptosis in MCF-7 cells [21].

6. Future Research
Phytochemicals in avocado seed extract are the potential for increasing health. Despite the recent findings, there is a limited study to prove the beneficial effect of phytochemicals found in avocado seed to treat specific diseases. The available literature on phytochemical derived from avocado seed discusses the potential, but investigation on medicine production is scarce. Hence, further research of avocado seed as medicine is potential.

7. Conclusions
Different varieties of avocado produce different content of phenolic compounds. The natural products contained in avocado seeds are phytosterols, fatty acids, triterpenes, abscisic acid, furanoic acids, polyphenols and proanthocyanidins. Several methods like water extraction solvent extraction using ethanol, methanol, acetone, and organic solvent such n-Hexane give different levels of phytochemicals. Those phytochemicals are beneficial due to rich sources of nutrients, such as vasorelaxant activity, analgesic and anti-inflammatory activity, hypotensive activity, anticonvulsant activity, antiviral activity, wound healing activity, antiulcer effect, antihepatotoxic activity, antioxidant activity, hypoglycemic activity, and reducing body weight.

References
[1] Ifesan B O T, Olorunsola B O and Ifesan B T 2015 Nutritional composition and acceptability of candy from avocado seed (Persea americana) Int. J. Agric Innovations Res. 3 1732-1735
[2] Segovia F J, Hidalgo G I, Villasante J, Ramis X and Almajano M P 2018 Avocado seed: A comparative study of antioxidant content and capacity in protecting oil models from oxidation Molecules. 23 10 2421
[3] Orhevba B A and Jinadu A O 2011 Determination of physico-chemical properties and nutritional contents of avocado pear (Persea americana M.) Acad. res. Int. 1 3 372
[4] Vinha A F, Moreira J and Barreira S V 2013 Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (Persea americana Mill.) J. Agric. Sci. 5 12 10
[5] Adaramola B, Onigbinde A and Shokunbi O 2016 Physiochemical properties and antioxidant potential of Persea americana seed oil Chem. Int. 2 3 168-17
[6] Wang W, Bostic T R and Gu L 2010 Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars Food Chem. 122 4 1193-1198
[7] Oboh G, Odubanjo V O, Bello F, Ademosun A O, Oyeleye S I, Nwanna E E and Ademiluyi A O, 2016 Aqueous extracts of avocado pear (Persea americana Mill.) leaves and seeds exhibit anti-cholinesterases and antioxidant activities in vitro J Basic Clin Physiol Pharmacol. 27 2 131-140
[8] Bahru T B, Tadele Z H and Ajebe E G 2019 A review on avocado seed: functionality, composition, antioxidant and antimicrobial properties Int. J. Chem. Sci. 27 2 1-10
[9] Oluwaniyi O O, Nwosu F O and Okeye C M 2017 Comparative Study of the Constituents of the Fruits Pulps and Seeds of Canarium ovatum, Persea americana and Dacryodes edulis J. Chem. 13 2 113-125
[10] Jimenez P, Garcia P, Quitral V, Vasquez K, Parra-Ruiz C, Reyes-Farias M, Garcia-Diaz D F, Robert P, Encina C and Soto-Covasich J 2020 Pulp, leaf, peel and seed of avocado fruit: a review of bioactive compounds and healthy benefits Food Rev. Int. 1-37
[11] Dabas D, M Shegog R, R Ziegler G and D Lambert J 2013 Avocado (Persea americana) seed as
a source of bioactive phytochemicals Curr. Pharm. Des. 19 34 6133-6140
[12] Henry L N, Mtaita U Y and Kimaro C C 2015 Nutritional efficacy of avocado seeds Global J. Food Sci. Technol. 3 5 192-196
[13] Kosinska A, Karamac M, Estrella I, Hernandez T, Bartolome B and Dykes G A 2012 Phenolic compound profiles and antioxidant capacity of Persea americana Mill. peels and seeds of two varieties J. Agric. Food Chem. 60 18 4613–4619
[14] Yasir M, Das S and Kharya M D 2010 The phytochemical and pharmacological profile of Persea americana Mill Pharmacogn Rev. 4 7 77
[15] Gómez F S, Sánchez S P, Iradi M G G, Azman N A M and Almajano M P, 2014 Avocado seeds: extraction optimization and possible use as antioxidant in food Antioxidants. 3 2 439-454
[16] Egbuonu A C, Opara I C, Onyeabo C and Uchenna N O 2018 Proximate, functional, antinutrient and antimicrobial properties of avocado pear (Persea americana) Seeds J. Nutr Health Food Eng. 8 1 00260
[17] Ogbuagu A S M and Okoye C I 2020 Physico-chemical characterization of Avocado (Persea americana Mill.) oil from tree indonesian avocado cultivars Prog. Chem. Biochem. Res. 3 1 39-45
[18] Rivai H, Putri Y T and Rusdi R 2019 qualitative and quantitative analysis of the chemical content of hexane, acetone, ethanol and water extract from avocado seeds (Persea americana Mill.) Sch. Int. J. Tradit. Complement. Med. 1 1 25-31
[19] Segovia F J, Corral-Pérez J J and Almajano M P 2016 Avocado seed: modeling extraction of bioactive compounds Ind. Crops Prod. 85 213-220
[20] Abubakar A N F, Achmadi S S and Suparto I H 2017 Triterpenoid of avocado (Persea americana) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells Asian Pac. J. Trop. Biomed. 7 5 397-400
[21] Widiyastuti Y, Pratiwi R, Riyanto S and Wahyuono S 2018 Cytotoxic activity and apoptosis induction of avocado Persea americana Mill. seed extract on MCF-7 cancer cell line Ind. J. Biotech. 23 2 61-67