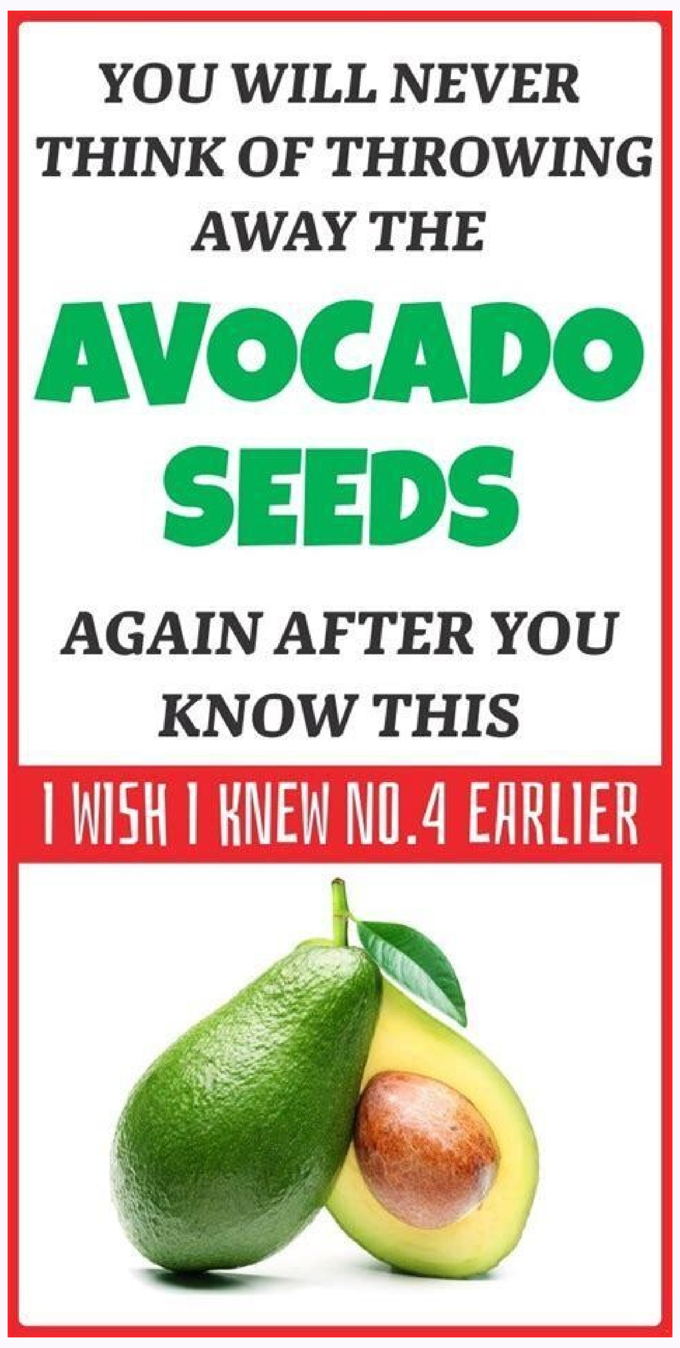
Avocado seed health benefits pdf

The processing industry discards avocado seeds, which increases production and ultimately pollutes the environment. It would be advantageous to handle these waste by-products both economically and environmentally. helabo Avocado seeds are rich in polysaccharides, proteins, lipids, vitamins, minerals, and other bioactive substances. The nutritional and phytochemical composition of avocado seeds has been well studied and discussed.Avocado-seed extracts also have many health-related bioactive properties, such as anti-hyperglycaemic, anticancer, anti-hypercholesterolemia, antioxidant, anti-inflammatory, and anti-neurogenerative effects are clearly demonstrated how these properties can be used to formulate or fortify food. The health-promoting properties of avocado seeds have been studied. These properties are attributed to various phytochemicals, such as acetogenin, catechin, epicatechin, procyanidin B1, estragole, etc. Additionally, items made from valorized avocado seeds that people can consume have been explored. The best applications of valorized by-products have been created for the pharmaceutical, functional food, and nutraceutical sectors while considering quality and safety. More clinical testing and product development research are required to prove the effectiveness of avocado seeds.Keywords: Avocado seed, Bioactive compounds, Phytochemical, Health-promoting effects, Industrial applicationAvocado (Persea americana Mill.) crop is cultivated and highly demanded internationally because of the growing demand for fruit and food products. It is a dicotyledonous plant that belongs to the flowering plant family Lauraceae, a native of Central America and Mexico. It is mainly grown in Mexico, Saint Dominic, Peru, Indonesia, Colombia, Brazil, Kenya, Venezuela, Chile, the United States, New Zealand and South Africa (FAO, 2018). Generally, avocado seeds are discarded, considering them a waste by-products of avocado processing industries. This by-product has not been used significantly, causing serious environmental pollution (Figueroa, Borrás-Linares, Lozano-Sánchez, Quirantes-Piné, & Segura-Carretero, 2018). Effective waste by-product management would benefit from an economic and environmental perspective (Araújo et al., 2020). Seeds of avocados represent a substantial percentage (13 %–17 %) of the avocado fruit and are rich in various functional and bioactive components, namely polysaccharides, proteins, lipids, minerals, and vitamins (Melgar et al., 2018, Tremocoldi et al., 2018). Avocado seeds contain many plethoras of bioactive viz., phenolics, flavonoids, and condensed tannins. These extracts have been examined for their bioactivities, such as anti-hyperglycemic (Tremocoldi et al., 2018), anti-cancer (Lara-Marquez et al., 2020), anti-inflammation (Dabas, Elias, Ziegler, & Lambert, 2019), anti-hypercholesterolemia (Uchenna, Shori, & Baba, 2017), anti-oxidant (Soledad et al., 2021), anti-microbial (Villarreal-Lara et al., 2019), and anti-neurogenerative, with numerous traditional uses as dermatological applications. They are a good natural source of biologically active ingredients for the food, pharmaceutical, and cosmetic sectors because they contain no harmful or dangerous compounds. lunovuci (Tremocoldi et al., 2018). Additionally, because of their high antioxidant potential, they prevent food oxidation, a degrading process of proteins, vitamins, carbohydrates, and lipids with reactive nitrogen and oxygen species that modifies the nutritional and sensory properties of food products (Calder & Iztapalapa, 2016). The exploring potential of seeds as a promising source of natural bioactive components can develop a novel product with added value and a safe alternative to synthetic compounds. In addition, the valorization of avocado seed residue significantly influences the environmental benefits and avocado processing industry (Saavedra et al., 2017). yopufo



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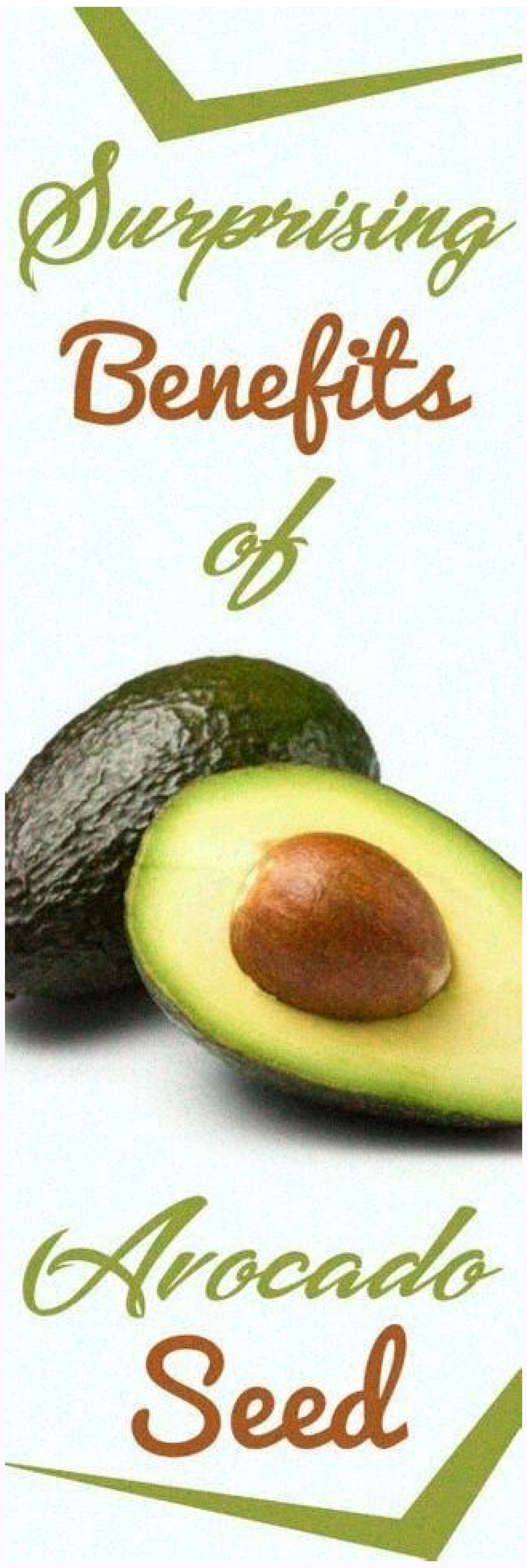
This review is an updated compilation of various aspects of avocado seed, such as nutritional composition, bioactive compounds, health-promoting biological activities, and its application in the food industry.The avocado seed is rich in various nutritional and bioactive compounds, especially proteins, starch, lipids, crude fiber, vitamins, minerals, and numerous phytochemicals. The nutritional profile of the avocado seed in several studies is summarized in Table 1.Nutritional composition of avocado seeds.GroupCompositionReferencesProximate analysisMoisture Content13.09 %Egbuonu et al., 2018Dry Matter86.91 %Crude Fibre2.87 %Ash3.82 %Sugar components (mg/g of DW)Hexose1.9Tesfay et al., 2012, Liu et al., 2002Glucose5.62Fructose12.93Sucrose7.86–18.5d-Mannuheptulose10.51–63.8Perseitol12.54–88.3Carbohydrate (%)64.9Protein%Crude protein content (AOAC, 1990 method)2.64Egbuonu et al., 2018Protein content23Ifesan & Olorunsola, 2015Protein content17.94Arukwe et al., 2012Protein content7.75Macey et al., 2015Protein content15.55Ejiofor et al., 2018Lipid's profileLong-chain fatty acids(µg/g)Tetracosanoic acid4.29Báez-Magaña et al., 2019Nervonic acid2.88Behenic acid3.63Erucic acid2.44Arachidic acid2.39Stearic acid5.06Oleic acid5.32Linoleic acid4.06Palmitic acid7.1Myristic acid2.49Fatty acid derivatives (aliphatic acetogenins)Avocatins32.28Báez-Magaña et al., 2019Polyhydroxy fatty acids24.26Pahuatins4.26Persins10.12Mineralsmg/100 gCalcium0.82Ifesan & Olorunsola, 2015Potassium4.16Phophorus0.09Zinc0.18Sodium1.41Iron0.31Arukwe et al., 2012Copper0.98Vitaminsmg/100 gVitamin A10Seed et al., 2017Thiamin0.33Riboflavin0.29Niacin0.06Ascorbic acid97.8Vitamin E0.12Among all the macromolecules found in avocado seeds, carbohydrates are said to make up a significant portion (64.9 %). Starch makes up 91.2 % of the total carbohydrates in avocado seeds (Tesfaye et al., 2018). It has been found that plant-based polysaccharide fractions contain a variety of biological activities (Bangar, Ashogbon, Lorenzo, Phimolsiripol, & Chaudhary, 2022).

The two C7 sugars, namely perseitol (88.3 mg/g) and d-mannoheptulose (63.8 mg/g), were abundant in avocado seeds (Liu, Sievert, Lu Arpaia, & Madore, 2002). The dominance of these C7 sugars in avocado seeds indicates their importance in these tissues. These sugars might have a role as transport and storage sugars in avocados. Tesfay, Bertling, and Bower (2011) concluded that the abundance of perseitol, at physiological maturity, among all sugars in the avocado cotyledons indicates their role as a C7 carbon storage compound. Liu et al.

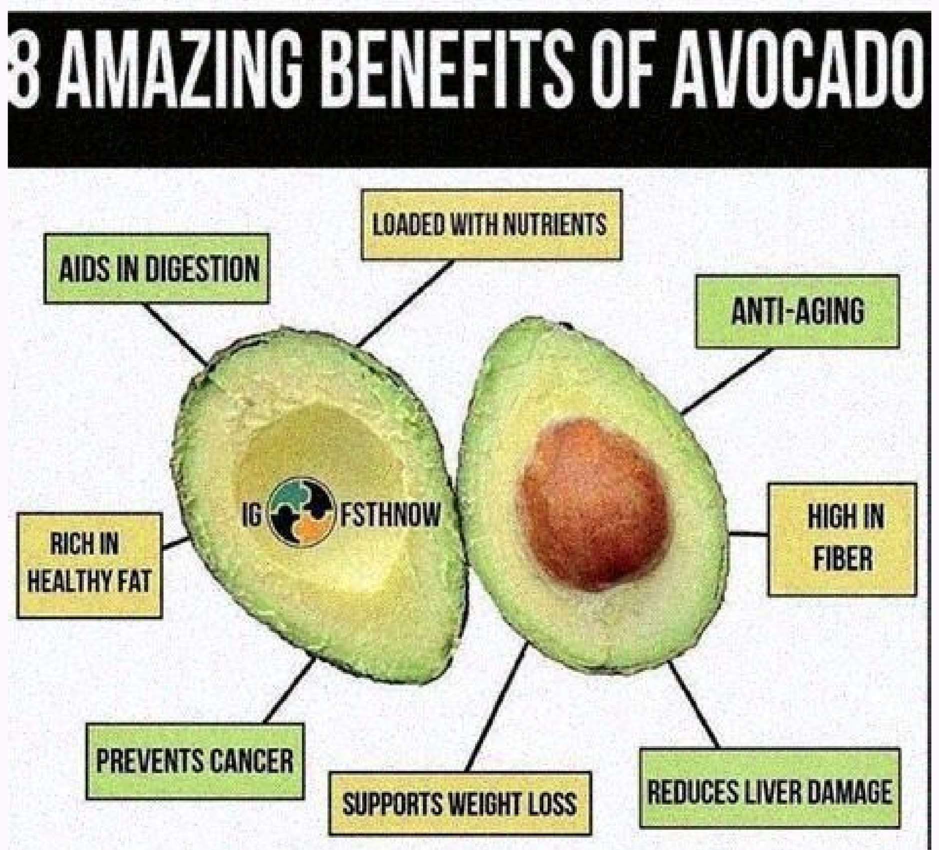
(2002) reported the carbohydrate profile of avocado seed as 246.1 (starch), 18.5 (sucrose), 1.9 (hexose), 63.8 (d-mannoheptulose), and 88.3 (perseitol) mg/g of dry weight (DW). fowa The quantity of C7 sugar found was 36.3 % of the total sugars in the avocado seed. Similarly, another study reported various sugars, including fructose (12.93), glucose (5.62), sucrose (7.86), d-mannoheptulose (10.51), and perseitol (12.54 mg/g of DW) (Tesfay, Bertling, Bower, & Lovatt, 2012) present in avocado seeds.Plant-derived lipids are mostly used for food and non-food industrial utilization. Takenaga, Matsuyama, Abe, Torii, and Itoh (2008) investigated the fatty acids and lipid profile of avocado seeds from 3 different cultivars: Bacon, Fuerte, and Hass. They reported total lipid (TL) content of1.1 %–1.6 % in avocado seeds. Further analysis of TL using thin-layer chromatography revealed the presence of neutral lipid, glycolipid (GL), and phospholipid (PL) as 77.1–80.3, 12–13.2, and 7.4–10.9 % of TL, respectively. Authors reported GL composition as 17.5–18.6 (acylsterylglucoside), 56.3–57.7 (monogalactosyl-diacylglycerol), 10.1–10.8 (sterylglucoside), 9.8–10.7 (cerebroside), 1.7–2.0 (digalactosyl-diacylglycerol) and 1.9–2.4 % (others) of total GL.

While PL composition contains 14.5–17.6 (phosphatidic acid), 30.7–31.9 (phosphatidyl ethanolamine), 10.5–13.4 (phosphatidylglycerol), 28.9–31.4 (phosphatidylcholine), 3.6–4.2 (phosphatidylinositol) and 6.3–6.9 % (others) of total PL. An investigation of the fatty acid profile of avocado seeds displayed that linoleic acid is present in the highest amount (35 %–38 %), followed by oleic acid (22 %–24 %) and palmitic acid (17 %–19 %). sarucutete Similarly, Báez-Magaña, Ochoa-Zarzosa, Alva-Murillo, Salgado-Garciglia, and López-Meza (2019) performed fatty acid profiling of the lipid-rich extract of avocado seeds by GC–MS. They reported fatty acids, including palmitic (7.1 µg/g), nervonic (2.88 µg/g), arachidic (2.39 µg/g), linoleic (4.06 µg/g), oleic (5.32 µg/g), stearic (5.06 µg/g), myristic (2.49 µg/g), erucic (3.63 µg/g) andtetracosanoic acid (4.29 µg/g), and their derivatives such as avocations (32.28 µg/g), persins (10.12 µg/g), polyhydroxy fatty acids (24.26 µg/g), and pahuatins (4.26 µg/g). yiyejetiware These results concluded that avocado seeds extract is abundant in fatty acids (particularly oleic, linoleic, and palmitic acid) and derivatives, viz., acetogenins, pahuatins, persins, avocatins, or fatty acid alcohols.Protein is a major component among various macromolecules in avocado seeds (Egbuonu, Opara, Onyeabo, & Uchenna, 2018). Proteins are large, complex molecules made of amino acids that play a key role in growth and development, cell signaling, enzyme regulation, and biocatalysts. Due to the increased need for nutritionally superior food, plant-based nutrients, especially protein, have gained attention. Thus, much emphasis has been given to finding sustainable alternative nutritionally dense food sources (Lonnie et al., 2018).

Various studies reported protein content in avocado seeds as 23 % (Ifesan & Olorunsola, 2015), 17.94 % (Arukwe et al., 2012), 15.55 % (Ejiofor, Ezeagu, Ayoola, & Umera, 2018), 7.75 % (Mahawan, Francia, Tenorio, Gomez, & Bronce, 2015), and 2.64 % (Egbuonu et al., 2018). voga Thus, the substantial amount of nutrients in avocado seeds, including carbohydrate, protein, and dietary fibers, could warrant their utilization in human supplements (Ejiofor et al., 2018). There are limited research reports available with regard to the quantified amino acids and protein in the avocado seeds; therefore, more focus is required to unearth its amino acid and protein profiles.The avocado seeds are a rich source of various minerals, namely phosphorus (P), calcium (Ca), potassium (K), iron (Fe), sodium (Na), zinc (Zn), copper (Cu), cobalt (Co), and lead (Pb), and vitamins including vitamin A, thiamine (B1), riboflavin (B2), niacin (B3), Vitamin C and vitamin E. Ifesan and Olorunsola (2015) found the concentration of various minerals, namely, P, Ca, Na, and Zn as 4.16, 0.09, 0.82, 1.41, and 0.18 mg per 100 g of the avocado seed, respectively. The minerals in avocado seeds make them a preferable choice for animal feed and human nutrition to fulfill micronutrient deficiency (Justina, Olukemi, Ajayi, & Adegoke, 2016). Egbuonu, Opara, Atasie, and Mbah (2017) observed the concentration of various vitamins as 10 (A), 0.33 (B1), 0.29 (B2), 0.06 (C), and 0.12 (E) mg per 100 g of the avocado seed. The vitamins A, C, and E in the avocado seed may improve the health of the immune system, vision, and blood vessels. jocekonifi In contrast, vitamin B displays a major role in cognitive function stimulation, nerve relaxation, and improving blood circulation.Recently, numerous research and reviews articles on the utilization of by-products of horticultural crops showed that phytochemicals and their health-promoting activities could boost their use in the preparation of innovative foods (Bangar et al., 2022, Punia and Kumar, 2021). This will improve the overall profitability of the farmers and reduce the cost of disposal of the by-products. Avocado seeds contain severalfold phenolics compared to popular antioxidant sources such as raw blueberry (Wang, Bostic, & Gu, 2010). It constitutes phenolics from five groups viz., procyanidins, catechins, flavonols, hydroxycinnamic, and hydroxybenzoic acids (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011). Further, Kosińska et al. (2012) reported 9.5 and 13.04 mg CE/g dry weight (DW) in Hass and Shephard varieties of avocado. In contrast, Soong and Barlow (2004) stated relatively high levels of 88.2 mg of GAE/g of DW. The variation in the bioactive profile is attributed to the variety, soil type, agronomic conditions, and post-harvest handling of the fruits (Kosińska et al., 2012).



The health-promoting properties of avocado seeds have been studied. These properties are attributed to various phytochemicals, such as acetogenin, catechin, epicatechin, procyanidin B1, estragole, etc. Additionally, items made from valorized avocado seeds that people can consume have been explored. The best applications of valorized by-products have been created for the pharmaceutical, functional food, and nutraceutical sectors while considering quality and safety. More clinical testing and product development research are required to prove the effectiveness of avocado seeds.Keywords: Avocado seed, Bioactive compounds, Phytochemical, Health-promoting effects, Industrial applicationAvocado (Persea americana Mill.) crop is cultivated and highly demanded internationally because of the growing demand for fruit and food products. It is a dicotyledonous plant that belongs to the flowering plant family Lauraceae, a native of Central America and Mexico. It is mainly grown in Mexico, Saint Dominic, Peru, Indonesia, Colombia, Brazil, Kenya, Venezuela, Chile, the United States, New Zealand and South Africa (FAO, 2018).



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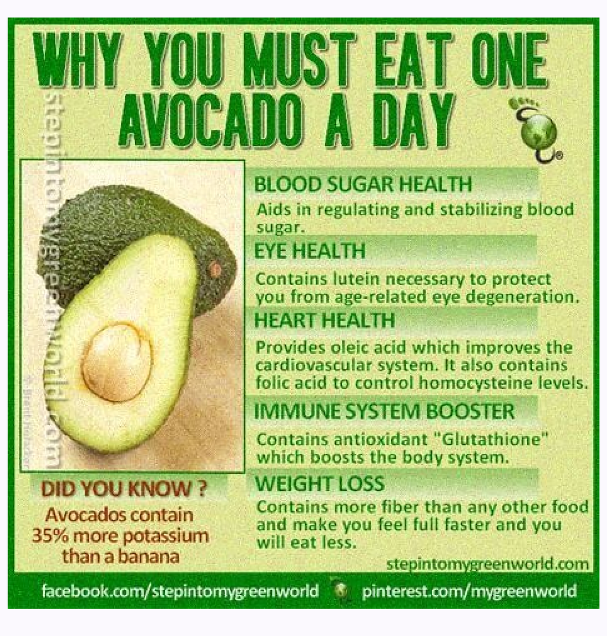

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Starch makes up 91.2 % of the total carbohydrates in avocado seeds (Tesfaye et al., 2018). It has been found that plant-based polysaccharide fractions contain a variety of biological activities (Bangar, Ashogbon, Lorenzo, Phimolsiripol, & Chaudhary, 2022). The two C7 sugars, namely perseitol (88.3 mg/g) and d-mannoheptulose (63.8 mg/g), were abundant in avocado seeds (Liu, Sievert, Lu Arpaia, & Madore, 2002). The dominance of these C7 sugars in avocado seeds indicates their importance in these tissues. These sugars might have a role as transport and storage sugars in avocados. Tesfay, Bertling, and Bower (2011) concluded that the abundance of perseitol, at physiological maturity, among all sugars in the avocado cotyledons indicates their role as a C7 carbon storage compound. Liu et al. (2002) reported the carbohydrate profile of avocado seed as 246.1 (starch), 18.5 (sucrose), 1.9 (hexose), 63.8 (d-mannoheptulose), and 88.3 (perseitol) mg/g of dry weight (DW). The quantity of C7 sugar found was 36.3 % of the total sugars in the avocado seed. Similarly, another study reported various sugars, including fructose (12.93), glucose (5.62), sucrose (7.86), d-mannoheptulose (10.51), and perseitol (12.54 mg/g of DW) (Tesfay, Bertling, Bower, & Lovatt, 2012) present in avocado seeds.Plant-derived lipids are mostly used for food and non-food industrial utilization. Takenaga, Matsuyama, Abe, Torii, and Itoh (2008) investigated the fatty acids and lipid profile of avocado seeds from 3 different cultivars: Bacon, Fuerte, and Hass. They reported total lipid (TL) content of1.1 %–1.6 % in avocado seeds. Further analysis of TL using thin-layer chromatography revealed the presence of neutral lipid, glycolipid (GL), and phospholipid (PL) as 77.1–80.3, 12–13.2, and 7.4–10.9 % of TL, respectively. Authors reported GL composition as 17.5–18.6 (acylsterylglucoside), 56.3–57.7 (monogalactosyl-diacylglycerol), 10.1–10.8 (sterylglucoside), 9.8–10.7 (cerebroside), 1.7–2.0 (digalactosyl-diacylglycerol) and 1.9–2.4 % (others) of total GL. While PL composition contains 14.5–17.6 (phosphatidic acid), 30.7–31.9 (phosphatidyl ethanolamine), 10.5–13.4 (phosphatidylglycerol), 28.9–31.4 (phosphatidylcholine), 3.6–4.2 (phosphatidylinositol) and 6.3–6.9 % (others) of total PL. An investigation of the fatty acid profile of avocado seeds displayed that linoleic acid is present in the highest amount (35 %–38 %), followed by oleic acid (22 %–24 %) and palmitic acid (17 %–19 %). Similarly, Báez-Magaña, Ochoa-Zarzosa, Alva-Murillo, Salgado-Garciglia, and López-Meza (2019) performed fatty acid profiling of the lipid-rich extract of avocado seeds by GC–MS. They reported fatty acids, including palmitic (7.1 µg/g), nervonic (2.88 µg/g), arachidic (2.39 µg/g), linoleic (4.06 µg/g), oleic (5.32 µg/g), stearic (5.06 µg/g), myristic (2.49 µg/g), erucic (3.63 µg/g) andtetracosanoic acid (4.29 µg/g), and their derivatives such as avocations (32.28 µg/g), persins (10.12 µg/g), polyhydroxy fatty acids (24.26 µg/g), and pahuatins (4.26 µg/g). These results concluded that avocado seeds extract is abundant in fatty acids (particularly oleic, linoleic, and palmitic acid) and derivatives, viz., acetogenins, pahuatins, persins, avocatins, or fatty acid alcohols.Protein is a major component among various macromolecules in avocado seeds (Egbuonu, Opara, Onyeabo, & Uchenna, 2018).

Proteins are large, complex molecules made of amino acids that play a key role in growth and development, cell signaling, enzyme regulation, and biocatalysts. Due to the increased need for nutritionally superior food, plant-based nutrients, especially protein, have gained attention. Thus, much emphasis has been given to finding sustainable alternative nutritionally dense food sources (Lonnie et al., 2018). Various studies reported protein content in avocado seeds as 23 % (Ifesan & Olorunsola, 2015), 17.94 % (Arukwe et al., 2012), 15.55 % (Ejiofor, Ezeagu, Ayoola, & Umera, 2018), 7.75 % (Mahawan, Francia, Tenorio, Gomez, & Bronce, 2015), and 2.64 % (Egbuonu et al., 2018). Thus, the substantial amount of nutrients in avocado seeds, including carbohydrate, protein, and dietary fibers, could warrant their utilization in human supplements (Ejiofor et al., 2018). There are limited research reports available with regard to the quantified amino acids and protein in the avocado seeds; therefore, more focus is required to unearth its amino acid and protein profiles.The avocado seeds are a rich source of various minerals, namely phosphorus (P), calcium (Ca), potassium (K), iron (Fe), sodium (Na), zinc (Zn), copper (Cu), cobalt (Co), and lead (Pb), and vitamins including vitamin A, thiamine (B1), riboflavin (B2), niacin (B3), Vitamin C and vitamin E. Ifesan and Olorunsola (2015) found the concentration of various minerals, namely, P, Ca, Na, and Zn as 4.16, 0.09, 0.82, 1.41, and 0.18 mg per 100 g of the avocado seed, respectively. The minerals in avocado seeds make them a preferable choice for animal feed and human nutrition to fulfill micronutrient deficiency (Justina, Olukemi, Ajayi, & Adegoke, 2016). Egbuonu, Opara, Atasie, and Mbah (2017) observed the concentration of various vitamins as 10 (A), 0.33 (B1), 0.29 (B2), 0.06 (C), and 0.12 (E) mg per 100 g of the avocado seed. The vitamins A, C, and E in the avocado seed may improve the health of the immune system, vision, and blood vessels. In contrast, vitamin B displays a major role in cognitive function stimulation, nerve relaxation, and improving blood circulation.Recently, numerous research and reviews articles on the utilization of by-products of horticultural crops showed that phytochemicals and their health-promoting activities could boost their use in the preparation of innovative foods (Bangar et al., 2022, Punia and Kumar, 2021). This will improve the overall profitability of the farmers and reduce the cost of disposal of the by-products. Avocado seeds contain severalfold phenolics compared to popular antioxidant sources such as raw blueberry (Wang, Bostic, & Gu, 2010). It constitutes phenolics from five groups viz., procyanidins, catechins, flavonols, hydroxycinnamic, and hydroxybenzoic acids (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011). Further, Kosińska et al. (2012) reported 9.5 and 13.04 mg CE/g dry weight (DW) in Hass and Shephard varieties of avocado. In contrast, Soong and Barlow (2004) stated relatively high levels of 88.2 mg of GAE/g of DW. The variation in the bioactive profile is attributed to the variety, soil type, agronomic conditions, and post-harvest handling of the fruits (Kosińska et al., 2012). Specific phenolics in avocado seeds were identified using UV spectra characteristics and retention times, and HPLC-ESI-MS was employed for the structural confirmation. Catechin/epicatechin gallate, 3-O-caffeoylquinic acid, procyanidin trimer A (II), 3-O-p-coumaroylquinic acid procyanidin trimer A (I), were found in the concentration presented in Table 2 (Kosińska et al., 2012). In another study, phenolic compounds in Hass and Fuerte variety were evaluated using chromatographic analysis. The authors identified four phenolic compounds, namely trans-5-O-caffeoyl-d-quinic acid, procyanidin B1, catechin, epicatechin, and the concentrations of the respective compounds are shown in Table 2. The volatile compounds of the seed extracts were investigated and showed esters of fatty acids and their derivatives and isoprenoid derivatives (Soledad et al., 2021). Under the terpenoid and phenylpropanoid compounds category, seven compounds were identified: estragole, isoestragole, cubebene, α-cubebene, α- germacrene α-farnesene, and caryophyllene. Another important component of the lipid fraction of avocado seeds is polyhydroxylated fatty alcohol (PHFA) derivatives.

Acetogenins (type of PHFA) originated from fatty alcohols with unsaturated aliphatic chains, commonly acylated. The concentration of total acetogenins varied between 1090 and 8330 µg/g DW in avocado seed among 22 cultivars.

Acetogenins viz., persenone A & B, AcO-avocadene contributed the maximum to the acetogenin profile of the avocado seeds, followed by persenone C, AcO-avocadenyne, persin, and persediene (Rodríguez-López, Hernández-Brenes, & de la Garza, 2015). Alkaloids, phytosterols, and tocopherols are other minor components in avocado seeds.Bioactive compounds associated with avocado seeds.SourceCompoundCultivar and concentrationReferencesTotal phenolic content–Hass: 9510 and Shephard: 13040 µg/g dwKosińska et al. (2012)Total phenolic content–Hass: 57,300 and Fuerte: 59200 µg/g dwTremocoldi et al. (2018)Phenolic compounds and its derivativesPhenolic acidsQueensland, Australia3-O-caffeoylquinic acidHass: 57.5 and Shephard: 53.5 µg/g dwKosińska et al. (2012)Queensland, Australia3-O-p- coumaroylquinic acidHass:13.6 and Shepard: 8.1 µg/g dwKosińska et al. (2012)Jaguacy Avocado Brasil Bauru, SP, Braziltrans-5-O-caffeoyl-d-quinic acidHass: 1630 and Fuerte: 5740 µg/g dwTremocoldi et al. (2018)FlavonoidsQueensland, AustraliaCatechin/ epicatechin gallateHass: 152.8 and Shephard: 105.4 µg/g dwKosińska et al. (2012)Jaguacy Avocado Brasil Bauru, SP, BrazilEpicatechinHass: 10,270 and Fuerte: 11060 µg/g dwTremocoldi et al. (2018)Jaguacy Avocado Brasil Bauru, SP, BrazilCatechinHass: 3640 and Fuerte: 8130 µg/g dwTremocoldi et al. (2018)ProcyanidinsQueensland, Australia; Level of ripening: ready-to-eat ripenessProcyanidin trimer A (I)Hass: 81.7 and Shepard: 98.9 µg/g dwKosińska et al. (2012)Queensland, Australia; Level of ripening: ready-to-eat ripenessProcyanidin trimer A (II)Hass: 89.3 and Shepard: 73 µg/g dwKosińska et al. (2012)Jaguacy Avocado Brasil Bauru, SP, BrazilProcyanidin B1Hass: 48,380 and Fuerte: 28340 µg/g dwTremocoldi et al. (2018)Polyhydroxylated fatty alcohol derivativesFundacion Sanchez Colin – CICTAMEX, Coatepec Harinas, Estado de Mexico, MexicoAcetogenins1090 to 8330 µg/g dw in 22 cultivars of avocadoRodríguez-López et al. (2015)Fundacion Sanchez Colin – CICTAMEX, Coatepec Harinas, Estado de Mexico, MexicoPersin0–300 µg/g dw in 22 cultivars of avocadoRodríguez-López et al. (2015)AlkaloidsBotanical garden in Akure metropolis, NigeriaHyoscyamine 0.6600 µg/g dwOboh et al. (2016)Atropine460 µg/g dwScopolamine240 µg/g dwNorhyoscyamine40 µg/g dwSolanidine41 µg/g dwSolanin40 µg/g dwSolasoline80 µg/g dwPhytosterolsLocal market in EgyptCampesterol–Alkhalaf et al. (2019)Stigmasterol1.11 %β-sitosterol2 %–5α-colestane49.77 µg/g dwBarrera López and Arrubla Vélez (2017)Stigmasterol19.17 µg/g dwDue to their importance in human health, the separation and identification of functional components from natural resources have become the main research focus of the food, nutraceutical, and pharmaceutical industries. This is because these components play a role in various biological and health-promoting processes in the human body. Avocado seeds are high in phytochemicals and are utilized for medicinal purposes. The bioactivities of avocado seed extracts will be discussed in the sections below. An illustration showing various bioactivities of the avocado seed extract is presented in Fig. 1.Globally, cancer has become a serious health issue, with the global cancer burden increasing to 18.1 million with 9.6 million deaths (GLOBOCAN, 2018). Cancer is characterized by the growth and multiplication of abnormal cells that invade neighboring tissues and spread outward (Zheng, Zhang, & Zeng, 2016). Synthetic anti-tumor medications have been found in clinical research to have possible therapeutic results but substantial toxicity to normal cells, posing a threat to human health. Due to its safety and immune-enhancing effect in humans, plant sources are gaining interest as anti-tumor medicines with lower toxicity. Avocado seeds and their biologically active components exhibited anti-cancer potential in human and animal cell lines, including prostate and lung cancer (Dabas et al., 2019), breast cancer (Dabas et al., 2019, Widiyastuti et al., 2018), colon cancer cells (Alkhalaf et al., 2019, Dabas et al., 2019), and hepatocellular carcinoma (Alkhalaf et al., 2019). Polyphenols from avocado seeds can inhibit human prostate cancer cells (LNCaP), breast cancer cells (MCF7), lung cancer cells (H1299), and colon cancer cells (HT29) with inhibition rates of 19, 19.1, 67.6, and 132.2 µg/mL in a dose-dependent manner (Dabas et al., 2019).The authors explained that avocado seed extracts induced G0/G1 cell cycle arrest via downregulating cyclin D1 and E2 expression in prostate cancer cells. Further, similar results were shown by Lee, Yu, Lee, and Lee (2008) in breast cancer cell lines (MDA-MB-231) by methanolic extracts of avocado seeds. Seed extracts (0.1 mg/mL) increased activation of caspase-3 and caspase-3 target protein, poly (ADP-ribose) polymerase (PARP), resulting in apoptosis. Ethanolic extracts of avocado seeds induced apoptosis in Jurkat lymphoblastic leukemia cells in an oxidative stress-dependent manner through depolarization of the mitochondrial membrane, activating protease caspase-3, and transcription factor p53, and predominancy of apoptosis-inducing factor (Bonilla-Porras, Salazar-Ospina, Jimenez-Del-Rio, Pereañez-Jimenez, & Velez-Pardo, 2014). Avocado seeds can inhibit the proliferation of immortalized HaCaT keratinocytes, which could be due to proanthocyanidins B1, proanthocyanidins B2, and A-type trimer (Ramos-Jerz, Villanueva, Jerz, Winterhalter, & Deters, 2013).Triterpenoid, an important secondary metabolite in avocado seeds, has anticancer activity (Iskandar, Novriyani, Damayanti, Afriani, Sukmawaty, Iqraini, & Razak, 2019). These secondary metabolites disrupt the membrane permeability of the mitochondrial cell wall, resulting in cell necrosis. It has been reported that triterpenoids have cytotoxic activity for lung cancer cells (A549), gastric cancer cells (SGC-7901), breast cancer cells (MCF-7), liver cancer cells (HepG2), and colon cancer cells (HCT15) (Hu et al., 2014). Further, ethanolic extract of avocado seeds triterpenoids displayed significant cytotoxic activity against Vero, human breast cancer cells (MCF-7), and human liver carcinoma cells (HepG2). In vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay displayed that triterpenoid of avocado seeds have the potential to inhibit proliferation of MCF-7 and HepG2 having the IC50 values of 62 and 12 mg/mL, respectively (Abubakar, Achmadi, & Suparto, 2017). Ethanolic extracts of phenolic compounds, alkaloids, glycosides, and saponins were reported to have a cytotoxic effect on breast cancer (T47D) cell lines with IC50 values of 107 µg/mL (Kristanty, Suriawati, & Sulistiyo, 2014).Lipidic extracts of avocado seeds were targeted for anticancer action on the HCT116 and HepG2 cancer cells. Authors described that seed lipids at a concentration of 100 µL exhibited an inhibitory percentage of 65 and 58 % in HCT116 and HepG2 cancer cell lines compared to avocado fruit lipids (Alkhalaf et al., 2019). Ethnopharmacological studies of Widiyastuti et al. (2018) reported cytotoxic and apoptosis effects of avocado seeds on MCF-7 cell lines. The authors examined cytotoxic activity by MTT assay and apoptosis by flow cytometric analysis. The cytotoxic test revealed the potent cytotoxicity of chloroform extract on MCF-7 cancer cell lines with an IC50 concentration of 94.9 µg/mL.Moreover, increased cytotoxicity with IC50 of 34.5 and 66.0 µg/mL was observed for methanol-soluble and non-soluble forms. Flow cytometry study concluded that methanolic fraction induced apoptosis by modulating sub-G1 phase arrest in MCF-7 cells. The lipidic extract of avocado seeds also has a cytotoxic effect on colorectal cancer. The avocatins and polyhydroxylated fatty alcohols in avocado seeds are associated with the possible cytotoxic reaction on Caco-2 cells (Lara-Marquez et al., 2020). These compounds induced apoptosis by activating caspases 8 and 9. Extracts can induce loss of mitochondrial membrane potential, inhibit fatty acid oxidation, and increase the superoxide anion (O_2^-) and mitochondrial reactive oxygen species (ROS). Additionally, lipidic extracts encouraged the release of cytokines IL-6, IL-8, and IL-10; but inhibited IL-1β secretion.Diabetes mellitus is a common genetic disorder caused by the impairment of insulin secretion and its deficiency. The International Diabetes Federation (IDF) reported that diabetes mellitus had reached epidemic levels worldwide. Currently, 463 million people and about 10 % (USD 760 billion) of global health expenditures are on diabetes (IDF, 2019). Chronic hyperglycemia is caused by insulin insufficiency, disturbing carbohydrate, protein, and lipid metabolism. Type 2 diabetes can be delayed and managed by altering one's lifestyle and developing good habits. Natural products with anti-diabetic properties could be a viable option to treat diabetes with minimum adverse effects (Zhao et al., 2018). Avocado seed help in treating type 2 diabetes by targeting peroxisome proliferator-activated receptor-gamma in the same way as an anti-diabetic drug (thiazolinediones) (Dabas, Shegog, Ziegler, & Lambert, 2013). Avocado seeds (2 %–8%) were added to a high-sugar diet and given to spontaneously hypertensive rats, which had an anti-diabetic and lipid-lowering impact by lowering blood glucose and cholesterol. The blood-glucose-lowering effect was attributed to bioactive compounds that assist in depositing glucose into the glycogen in the liver cells (Uchenna et al., 2017). In alloxan-induced diabetic rats, treatment of 300 or 600 mg/kg body weight avocado seed extract lowered glycemia (>70 %) and restored damage to pancreatic islet cells (Edem, Ekanem, & Ebong, 2009).Supplementation of 40 g/L of hot aqueous avocado seed extracts and glibenclamide (5 mg/kg) to alloxan-induced Wistar albino rats significantly decreased the blood glucose of diabetic rats. They observed that the reference drug glibenclamide provided the highest response (58.9 %) on day 14, equivalent to the reaction of 40 g/L avocado seed extract on day 21 (Ezejiofor, Okorie, & Orisakwe, 2013).

According to pancreas histology, the normal control rats had intact pancreatic islets and exocrine cells. Alloxan-induced diabetes rats (diabetic control rats) showed reduced islet cells and necrosis regions. Compared to the untreated alloxan-induced diabetic rats, diabetic rats treated with the 20 g/L extracts showed tiny, maintained islet cells. The studies above have revealed that avocado seeds extract may have anti-diabetic characteristics, indicating that more study is needed.Free radicals are generated due to oxidative stress and autoxidation of human lipids and lipoproteins, which are linked to diabetes, cardiovascular disease, respiratory disease, cancer, neurodegenerative and many other diseases (Punia et al., 2020, Dhull et al., 2020). An interest in using natural plant antioxidants, including polyphenols, flavonoids, and alkaloids, is increasing daily to solve these health problems (Dhull, Kaur et al., 2020; Bangar et al., 2022c).

These compounds can quench free radicals, scavenge free oxygen and chelate catalytic metals (Kaur, Dhull, Sandhu, Salar, & Purewal, 2018), which have shown promising potential in reducing oxidative stress, preventing several diseases, maintaining health, and delaying the aging process.

Avocado seed displays in vitro antioxidant potential by stabilizing peroxyl radicals and superoxide anions and DPPH and ABTS, ferric reducing power, inhibiting the β-carotene blanching and development thiobarbituric acid reactive substances (Tremocoldi et al., 2018).

Colored avocado seed extracts displayed oxygen radical absorbance capacity (ORAC) of 2012 Trolox equivalents/mg, and electron paramagnetic resonance spectroscopy assay observed radical scavenging potential of seed extracts with EC50 of 42.1 µg/mL (Dabas et al., 2019). A dose of 0.75 % avocado seed extracts causes an 80 % delay in oxidation as measured by oxidation induction time (Segovia, Hidalgo, Villasante, Ramis, & Almajano, 2018).Aqueous extracts of avocado seeds exhibit antioxidant potential and can prevent radical-induced oxidative damage (Oboh et al., 2016). The authors induced rat brains with Fe^{2+} and sodium nitroprusside (SNP) solutions. They observed an increase in thiobarbituric reactive species (TBARS) level resulting in oxidative damage caused by free radicals by Fe^{2+} and SNP. Furthermore, avocado seed extract reported decreased TBARS levels in Fe^{2+} and SNP-induced lipid peroxidation due to the synergic effect of phenolic components and saponins of seeds. In avocado seeds, phenolic components and procyanidins (catechin and epicatechin) contribute 38 % antioxidant activities of whole avocado fruit (Wang et al., 2010).

Ethanolic extracts of Hass and Fuerte avocado seeds possessed many phenolic compounds (Tremocoldi et al., 2018). The reported antioxidant potential of 1175.1 and 1881.4 µmol Fe^{2+}/g for Hass and Fuerte peel extracts. They reported that epicatechin and catechin in seeds could stabilize peroxyl radicals (ROO.) and superoxide anions (O2.). Interestingly, catechin had 1.3, 2.5, and 1.6 folds better-stabilizing activity to stabilize ROO., O2., and hypochlorous reactive species than epicatechin. Lyophilized avocado seed power was added in oil in water emulsion and beef meat burger to evaluate the delay in oxidation (Gómez, Sánchez, Iradi, Azman, & Almajano, 2014). They observed oxidation inhibition of 30 % (pure extracts) and 60 % (extract + egg albumin) in emulsion and 90 % inhibition of TBAR substances in meat burgers. The authors suggested that avocado seeds could be used in meat to increase shelf life.The volatile or lipophilic chemical profile of avocado seeds indicates their potential application as an antioxidant additive (Soledad et al., 2021). Acetone and ethanolic extracts of avocado seeds total phenolic content of 30.80 and 30.25 GAE/100 g, respectively, and DPPH inhibition of 212.75 and 183.75 mg Trolox/100 g, respectively.

Also, acetone extract of avocado seeds exhibited a higher power reduction of 56.35 ascorbic acid equivalents (AAE)/100 g than ethanol extract (45.05 g AAE/100 g). Therefore, it is suggested that avocado seeds have potential application as an antioxidant additive in food products.Alzheimer's disease (AD) is a brain disorder characterized by the gradual degeneration of nerve cells, which leads to deficits in cognitive ability (Oboh et al., 2016). A cholinergic hypothesis states that acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) catalyze the breakdown of acetylcholine into choline and acetate groups. Several studies indicate that 40–90 % of AD patients had reduced AChE activity and increased BChE activity (Brimijoin, 1983). Reducing acetylcholine levels may inhibit brain transmission in Alzheimer's patients (Ahmed, Ghalib, Sasikala, & Ahmed, 2013). Therefore, restoring acetylcholine by inhibiting AChE and BChE with phytoconstituents from plants is the modern method for treating Alzheimer's disease and neurodegenerative illnesses. Avocado seed aqueous extracts inhibited the AChE and BChE enzymes with IC50 values of 27.93 and 30.08 mg/mL, respectively (Oboh et al., 2016). Inhibition of both enzymes by phenolic compounds such as caffeic acid in avocado seeds inhibits the breakdown of butyrylcholine and acetylcholine in the brain neurons, resulting in increased levels of these neurotransmitters at the synaptic clefts. This ultimately improves the communication between the nerve cells and helps to manage neurodegenerative ailments such as AD. Various bioactivities of the avocado seed extracts are presented in Table 3.Health-related bioactive properties of extracted compounds from avocado seeds.RegionExtraction method/solvent usedCompounds/componentModeIn vitro/In vivoTarget mechanismKey findingsReferencesAnticancer activityMexicoLipidic extractAvocatins, polyhydroxylated fatty alcohols and saturated long-chain fatty acidsColon cancer cell line Caco-2In vitro(+) Caspases 8(+) Caspases 9(+) Cytokines IL-6(+) IL-8(+) IL-10(-) IL-1β-Lara-Marquez et al. (2020)USAMethanolPolyphenols–In vitro(-) Cyclin D1(-) Cyclin E2(+) Caspase 3(+) PARPAvocado seed extracts could be a functional source of anticancer compoundsDabas et al. (2019)Saudi ArabiaMethanol/chloroformLipidsHepG2 and HCT11 cancer cell linesIn vitro–-Alkhalaf et al. (2019)VietnamEthanol–-In vitro–-Vo and Le (2019)IndonesiaChloroform and methanol–-(+) Apoptosis–Widiyastuti et al. (2018)IndonesiaEthanolTriterpenoid–In vitro–Avocado seed triterpenoids exhibit cytotoxic activity with low IC50 valueAbubakar et al. (2017)ColombiaEthanol–-In vitro(+) Transcription factor p53(+) Protease caspase-3(+) AIFAvocado seeds function as a pro-apoptotic componentBonilla-Porras et al. (2014)JakartaEthanolPhenolic compounds, alkaloids, saponinsT-47D breast cancer cell lineIn vitro–-Kristanty et al. (2014)GermanyMethanolProanthocyanidins B1,Proanthocyanidins B2 and A-type trimer–––-Ramos-Jerz et al. (2013)KoreaMethanolPolyphenols–In vitro(+)Caspase 3(+) PARP(+) apoptosis–Lee et al. (2008)Antidiabetic activityBrazilEthanolPhenolic compounds–In vitro–To stabilize peroxyl radicals (ROO.), superoxide anions (O2.) and hypochlorous reactive species.Tremocoldi et al. (2018)Saudi Arabiaseed-supplemented dietAnti-hyperglycemia and Anti-hypercholesteremiaIn vitro–Avocado seeds extract Improved carbohydrate and lipid metabolism.Uchenna et al. (2017)NigeriaHot water–-In vivo–Seeds have anti-diabetic and protective effects on some rat tissues such as the pancreas, kidneys, and liver.Ezejiofor et al. (2013)Antioxidant activityMexicoAcetone/ethanolLipophilic compoundsAntioxidant potntialIn vitro(+) Phenolic compounds(+) DPPH inhibition(+) Reducing powerAvocado seeds have potential application as antioxidant additive.Soledad et al. (2021)SpainMethanol;Ethanol/waterCatechinepicatechinRadical scavenging activityIn vivo(-) oxidationAvocado seed extracts are effective as a natural antioxidantSegovia et al. (2018)NigeriaAqueous extractsPhenolic compounds and alkaloidsIn vivo–Oboh et al.(2015)Anti-neurogenerative activityNigeriaAqueous extractsPhenolic compounds and alkaloidsIn vivo(-) AChE(-) BChEAvocado seed extracts may serve as a cheap therapeutic drug for preventing/treating AD.Oboh et al. (2015)Anti-inflammation activityUSAMethanolPolyphenolsRAW264.7 cellsIn vitro(-) IL-6(-) TNF-α(-) IL-1β(-) NOAvocado seeds exhibited anti-inflammatory compounds which could be as functional food ingredients.Dabas et al. (2019)Saudi ArabiaMethanol/chloroformLipidsIn vitro–Alkhalaf et al. (2019)BrajilEthanolPhenolic compoundsRAW264.7 cellsIn vitro(-) TNFα(+) Nitric oxideTremocoldi et al. (2018)IndonesiaInfusion and methanolKristanti et al. (2017)Antimicrobial activityMexicoAcetone/ethanolLipophilic compoundsStaphylococcu aureus and Salmonella enterica serovar typhimuriumIn vitro(-) gram positive bacteria(-) gram negative bacteriaMaximum microbial reductions of 4 and 1.8 log cycles for Staphylococcus aureus and Salmonella enterica serovar TyphimuriumSoledad et al. (2021)USADichloromethaneAcetogeninsIn vitro(-) gram positive bacteriaVillarreal-Lara et al. (2019)MexicoHeptane:Methanoland Heptane: ethanol:methanol:waterAcetogeninsClostridium sporogenes PA 3679In vitro(-) C. sporogenesAvocado seeds as a potential source of functional compounds with anti-inflammatory activityPacheco et al. (2017)MexicoHeptane:Methanoland Heptane: ethanol:methanol:waterAcetogeninListeria monocytogenes (ATCC 35152)In vitro(-) Listeria monocytogenesAn anti-listeral agentSalinas-Salazar et al. (2017)The inflammatory response is a defense response of an individual against invaders. It involves various chemical mediators capable of triggering vascular changes, such as plasma protein extravasation and defense cell recruitment. (Tremocoldi et al., 2018). Many immune cells involved in the inflammatory response, such as neutrophils, macrophages, and phagocytes, secrete inflammatory mediators such as nitric oxide (NO), interleukins, and TNF-, as well as inflammatory proteins such as nitric oxide synthase (NOS) and cyclooxygenase (COX)-2 in response to exogenous stimulation (Hyung, Ahn, Kim, Kim, & Je, 2016). Excessive inflammatory mediator release due to inflammatory responses has been linked to atherosclerosis, diabetes, tumor growth, immunological illness, and other inflammatory diseases. A novel glycosylated benzotropolone-containing polyphenol was identified in colored avocado seed extracts (Hatzakis, Mazzola, Shegog, Ziegler, & Lambert, 2019).

Many studies have reported the anti-inflammatory effect of benzotropolone-containing natural products. At the dose of 6 µg/mL, avocado seeds extract reduced the secretions of IL-1β and tumor necrosis factor (TNF)-α in lipopolysaccharide-stimulated RAW264.7 cells. Avocado seed extracts at a concentration of above 5 µg/mL reduced NO production resulting in reduced inducible nitric oxide synthase (iNOS) expressions. Similar findings were reported by (Tremocoldi et al., 2018) for avocado seed extracts of Hass and Fuerta cultivars, which can inhibit TNFα and produce NO in lipopolysaccharides-stimulated RAW 264.7 macrophage culture (Tremocoldi et al., 2018). Kristanti, Simanjuntak, Dewi, Tianri, and Hendra (2017) demonstrated the anti-inflammatory activities of infusion (0.67 g/kg BW) and methanolic (3.33 g/kg BW) extract of avocado seed in carrageenan-induced paw edema in mice.

They observed a decrease in area under curve values and percentage inhibition of inflammation results in the decreased thickness of paw edema on the test animals' paws.Numerous studies have been conducted to find natural alternatives to the synthetic antimicrobial used in food, medicine, and pharmaceuticals. These efforts have been made for consumers' concerns regarding the safety of synthetic chemical products. Many scientists claimed the potentiality of avocado seed to control human food-borne pathogenic bacteria and spoilage microbes. Leite et al., 2009, Idris et al., 2009 concluded that avocado seeds organic extracts inhibited Candida spp., Cryptococcus neoformans, and Malassezia pachydermatis and bacteria including S. aureus, S. pyogenes, C. ulcerans, C. albicans, E. coli, and S. typhi. Further, methanolic and chloroform extract of avocado seed exhibited antifungal potential against Cryptococcus neoformans with IC50 value of less than 8 µg/mL and 8.211 µg/mL, and petroleum ether extracts exhibited inhibition activity against S. aureus, IC50 8.7 µg/mL (Falodun et al., 2014). Jiménez-Arellanes, Luna-Herrera, Ruiz-Nicolás, Cornejo-Garrido, Tapia, and Yépez-Mulia (2013) also observed anti-parasital activity of seeds for E. histolytica, and G. lamblia.Avocado seeds contain fatty acid derivatives with antimicrobial potential called acetogenins. The first report on the antilisteral potential of avocado acetogenins was conducted by Salinas-Salazar et al. (2017).

They identified AcO-avocadene, persediene, persenone C, persenone A, persin, and persenone B in avocado seeds and considered persenone C, persenone A, and AcO-avocadenyne as the most powerful acetogenin.

The acetogenin extracts showed a minimum inhibitory concentration of 7.8 mg/L and a bactericidal activity due to an enhancement in membrane permeability resulting in cell lysis. Further, they added that antilisteral activity is a combined result of the trans-enone feature and the number of unsaturated molecules in the aliphatic chain.In another study, acetogenins (AcO-avocadene, AcO-avocadenyne, persediene, persenone A, persenone B, persenone C, and others), naturally occurring lipidic molecules of avocado seeds were evaluated to control growth and endospore germination of Clostridium sporogenes PA 3679 (ATCC 7955)in carrot puree under high hydrostatic pressure (HHP) (300–600 MPa), time (3–6 min), temperature (25–120 °C) and salt (1 %–3%). The authors reported that AcO-avocadene exhibited the highest antimicrobial activity, whereas the extract was resistant to high temperature, HHP, and salt, with greater stability at pH ≥ 7.0. However, acetogenins were reduced by 63 and 32 % at 25 and 4 °C for 42 days. Among acetogenins, persediene was the most stable, followed bypersenones andAcO-avocadene with an aliphatic chain, a keto group, or trans-enone in C-4 allow hydrogen donation to a carbon atom and inhibit oxidation (Pacheco et al., 2017).Villarreal-Lara et al. (2019) conducted a study to evaluate the anti-microbial spectrum of avocado seeds acetogenins. They added purified acetogenins meat inoculated with Listeria monocytogenes and then stored at 20 and 4 °C. They exposed eight gram-positive bacteria to Nisaplin® and Mirenat® (food preservatives) for comparative analysis.

The authors concluded that the inhibition zone of avocado seeds acetogenins was two-four times higher than Nisaplin® and Mirenat® for gram + ve bacteria, except for Staphylococcus aureus. Additionally, after storage at 4 °C for 72 d, acetogenins inhibited L. monocytogenes completely. These suggested avocado seeds as a good source of functional compounds with anti-microbial potential.Chemical profiling of volatile compounds indicated that avocado seed contains sesquiterpenoids, poly, and unsaturated fatty acid esters (Soledad et al., 2021).

They reported that the fatty acids of avocado seeds display antimicrobial activities. The fatty acid has double bonding in the cis-configuration. They alter its functionality by disordering the phospholipid chain, resulting in fluidity, disorganization, and disintegration of the cell membrane resulting in leakage of intracellular content and cell death. A high concentration of 2000 mg/L exhibited maximum microbial reductions of 4 and 1.8 log cycles for Staphylococcus aureus and Salmonella enterica serovar typhimurium, respectively (Soledad et al., 2021). They explained that minimum microbial reduction for S. typhimurium could be due to the composition and cell wall structure. Gram-negative bacteria exhibit lipidic bilayer providing more protection against antimicrobial components (Beristain-Bauza et al., 2019).The industrial processing of avocados generates various by-products such as peel and seed, in which seed is a major waste product that accounts for about 13 % to 17 % of avocados. The avocado seed is ideal for valorization because it includes various nutritious components with numerous potential industrial uses.

Its seed powder and flour have many specific nutrient contents, which encourage scientists to work on the utilization of seed in various food products, i.e., Instant soup and beverages (Alissa, Hung, Hou, Lim, & Ciou, 2020), antioxidant-rich tea (Araujo et al., 2018); antibacterial agent in meat products (Villarreal Lara et al., 2019), antioxidant in sunflower oil (Segovia et al., 2018), used as a preservative (Pachego et al., 2017), and bakery products (Rivera Gonzalez et al., 2019). Further development and commercialization of these research efforts by the food industry will provide an opportunity for a raw material source that is still underutilized and generally treated as waste.The avocado seed contains various classes of nutritional components (carbohydrate, protein, olefinic and acetylenic bond containing fatty acids, fiber, and minerals) as summarized in section 2 and other natural products such as phytosterols, triterpenes, dimmers of flavonols, and oligomeric pro-anthocyanidins (discussed in section 3) which can be explored in designing of different functional foods to stimulate growth and metabolism (Permal, Chang, Seale, Hamid, & Kam, 2020). The up-to-date food applications of avocado seeds (flour and extract) have been summarized in Fig. 2. When included in the diets, avocado seed flour showed dose-dependent partial effects on the feeding and growth performance

of rats (Uchenna et al., 2017). The cholesterol levels were lowered; high blood glucose was suppressed, especially after adding sucrose to the diet.
The liver glycogen storage of rats improved after avocado seed infusion in their diets. Therefore, avocado seed flour can modulate lipid and carbohydrate metabolism and improve the glycogen storage ability of the liver and can be utilized in the diets of people with hyperglycemia and/or hypercholesterolemia. Due to the seeds' dietary and crude fibre, antioxidants, and phenolic content, Pahua-Ramos et al. (2012) also discovered minimal toxicity, hypocholesterolemia, and low LDL cholesterol in hypercholesterolemic model mice. The avocado seed powder-supplemented diet of culled female quail improved its kidney and liver functions, meat quality, tenderness, protein, and fat content, while cooking losses were reduced (Tugiyanti, Iriyanti, & Apriyanto, 2019).A vegetable extract prepared from avocado and soybean fruit and seed oil (mainly in the ratio of 1:2) is known as avocado and soybean unsaponifiables (ASU). It has anti-inflammatory effects attributed to many phytosterols and isoflavones, which suggests its possible role in the prevention of osteoarticular, autoimmune, and meningeal disorders (Eser et al., 2011). ASU as a pure extract or mixed with other plant extracts (e.g., Uncaria tormentosa and Zingiber officinalis) is available as food supplements in many countries (Ghasemian et al., 2016, Salehi et al., 2020). The seed extract can be used as a nutraceutical due to its antioxidant, anti-inflammatory, and antibacterial potential, having more powerful effects than avocado bulb extracts (Alkhalaf et al., 2019). In an indomethacin-induced ulcer study in mice, the ethyl acetate fraction of avocado seed extract was found effective in decreasing the level of oxidized products and increased superoxide dismutase enzyme activity, thus mitigated the oxidative stress and also prevented the increase in the ulcer and lesions (Athaydes et al., 2019). The extract is rich in substances such as flavonoids, epicatechin, catechin, caffeoylquinic acid, phenylpropanoids, and tannins, proving it a valuable nutraceutical that can be used a safe, effective, and cheap alternative to conventional treatments to prevent or treat gastric ulcers. However, further studies and clinical assays are required to develop more scientific information and one formulation for sustainable utilization (Athaydes et al., 2019).To improve the nutritional quality and antioxidant profile of different seeds, solid substrate fermentation has been focused on these days (Dhull et al., 2020, Dhull et al., 2021). Solid substrate fermentation of avocado seeds using fungi such as Aspergillus niger resulted in the secretion of bound phenolic compounds and increased antioxidant capacity. Several factors such as low cost, fast growth rate of microbes, easy downstream of phenolic/fermentation compounds and eco-friendly nature have proven fermentation as an efficient process for the production of polyphenolic compounds. Meanwhile, different enzymes (protease, amylase, lipase, phytase etc.) produced by fermenting microbes convert complex carbohydrates, proteins and lipids into easily digestible components with an appealing taste and texture (Dhull et al., 2020, Dhull et al., 2021). Also, these enzyme significantly reduce different anti-nutritional factors, including tannins, phytic acid, and protease inhibitors (Soetan & Oyewole, 2009), and help to improve the absorption and bioavailability of certain minerals present in seeds. This suggested an opportunity to increase the value of processing avocado waste and develop new products to avoid processing waste (Yepes-Betancur et al., 2021). A seed powder prepared by spray drying a mixture of avocado seed extract, maltodextrin, and water showed good yield (24.46 %–35.47 %), water activity (0.27 %–0.34 %), solubility (55.50 %–79.67 %), and color values (Alissa et al., 2020). This powder can be used in different food such as instant soups and beverage products, simultaneously adding value to the waste product.The growing trend towards no or minimal use of synthetic additives forced the food industries to use natural additives and discover new antimicrobial molecules (Tiwari et al., 2009; Negi, 2012). Avocado seed extract is a rich source of acetogenins which have strong antimicrobial, antifungal, and insecticidal properties (Pacheco et al., 2017; Salinas- Salazar et al., 2017; Villarreal-Lara et al., 2019, Salazar-López et al., 2020).
The seeds are almost 1.6 times richer source of acetogenins than the pulp, showing a good waste management solution for the avocado processing industry (Salinas- Salazar et al., 2017). The extract inhibited Listeria monocytogenes completely and showed antibacterial activities against several Gram-positive bacteria, including Bacillus subtilis, Staphylococcus aureus, Clostridium perfringens, C. sporogenes, and Alicyclobacillus acidocaldarius (Villarreal-Lara et al., 2019). The acetogenins from avocado seeds were characterized for the anticlostridial activity, stability and effectiveness under different food processing conditions in a model food system (Pacheco et al., 2017).
The extract bioactivity showed resistance to different food processing conditions such as HPP (300 MPa –600 MPa, 3–6 min, 25 °C), high temperature (≤120 °C), and salt concentration (≤3 % w/v), the extract had good resistance while showed higher stability at pH ≤ 7.0. Additionally, after exposure to HHP treatment and pH 9.5, an increase in the potency against endospores was observed, suggesting a positive effect on the stability or structure of particular acetogenins. However, the initial quantity of acetogenins was gradually decreased in HHP processed model food system (carrot puree) during storage at different temperatures. The antioxidant activity conferred by hydrogen donation to surrounding carbon atoms could be due to a keto or trans-enone group at C-4 in the aliphatic chain of the acetogenins. This suggested the potential of avocado seeds as natural food preservatives, but further investigation regarding the effectiveness of acetogenins against different microorganisms, its stability in more complex food systems, effect on sensory attributes, and human consumption safety evaluation is needed.
In combination with nisin (an antimicrobial peptide), the seed extract acted synergistically in its microbial response, providing a novel combination to decrease nisin use at the industrial level, reducing cost, and promoting the utilization of natural resources compounds (Calderón-Oliver et al., 2016).In lipids and protein-rich foods, lipid oxidation and protein carbonylation lead to nutritional loss, off-flavors, loss of essential amino acids, reduced digestibility of myofibrillar protein, and degradation of their texture and other quality traits (Shahidi & Zhong, 2010). Avocado seed extracts are an interesting natural source of rich phenolic compounds with strong antioxidants and antimicrobial properties. Its addition to the meat system would enhance nutritional and sensory properties by effectively inhibiting the oxidation of protein and lipids. In raw pork patties, avocado seed extracts reduced oxidative reactions and color deterioration during storage through protein carbonyl formation and TBARS reduction (Rodríguez-Carpena et al., 2011, Rodríguez-Carpena et al., 2011).In emulsion-based foods, lipids are present in dispersed colloidal particles (O/W emulsions) and stabilized by surface-active compounds, including proteins, polysaccharides, gums which act at the interfacial regions (Decker et al., 2017). As the molecules at the interface come in contact with many pro-oxidants (enzymes, metals, photosensitizers, etc.), the oxidative and colloidal stability of emulsions highly depends on the composition of the interface (Yi et al., 2019). In the O/W emulsion, lipid oxidation results in the development of off-flavors, and shortening of shelf-life, which causes rejection by consumers and influences food safety by forming toxic reaction products. The phenolic-rich avocado seed extract significantly affect the colloidal stability of O/W emulsions, depending on the emulsifier used to prepare the emulsion/nanoemulsion (Velderrain-Rodríguez, Salvia-Trujillo, González-Aguilar, & Martín-Belloso, 2021). Also, the lipid oxidative stability of emulsions and nanoemulsions was enhanced as the phenolic components in the extract retarded the oxidation process. It decreased the formation of secondary lipid oxidation products during storage. The extract from avocado seeds also inhibited oxidation of about 30 % in pure form and about 60 % in combination with egg albumin. In comparison, TBARS formation was reduced by 90 % in meat burgers (Gómez et al., 2014), suggesting its possible use as an antioxidant in foods. The oxidation was also delayed in sunflower oil with added avocado seed extracts (Segovia et al., 2018) which suggested its use in reducing synthetic surfactants and additives.Avocado seed processed into flour have good yield (46.3 %), protein (6.7 %), fat (3.4 %), ash (2.7 %), and dietary fibre (45.53 %) contents which can be an alternative source of nutrients for the preparation of bread, cakes, and cookies (Rivera-González et al., 2019). The flour has good water and oil absorption capacity and solubility (2.4 %, 2.16 %, 11.2 %, respectively) related to different macro and micronutrients present in the seeds and their strong intermolecular interactions. Still, additional compounds such as gum, pectin, alginates can be added to avocado flour to alter its properties for better exploration. Avocado seeds are a rich source of dietary fiber which has many health benefits such as hypoglycemia, hypocholesterolemia (Hu & Yu, 2013), cardioprotective and prebiotic (Slavin, 2013), early satiety (Kristensen & Jensen, 2011), and excretion and retention of bile juices (Kristensen et al., 2012). The avocado seeds flour processing residue has useful technological properties, including good water and oil absorption properties, suggesting its use as an important ingredient to improve softness, freshness, and viscosity in bakery products and juiciness in meat products (Barbosa-Martín, Chel-Guerrero, González-Mondragón, & Betancur-Ancona, 2016). The seed also contains fatty acids esters, and unsaturated fatty acids, which are beneficial for human health; therefore, suggesting the use of these solvent-extracted compounds in developing functional foods (Soledad et al., 2021). Natural anti-diabetic and flavonoid-rich substances from avocado seeds can regulate blood sugar levels in many ways (Brahmachari, 2011), which can be explored in the preparation of alternative snacks such as biscuits used for diabetes management (Mursyid & Kadir, 2020).The avocado seeds can also be utilized as alternatives/non-conventional starch sources (Rivera-González et al., 2019). Its starch fraction (27.3 % yield) have low total dietary fibers (7.32 %) (Rivera-González et al., 2019), which can be useful as a thickener, emulsifier, and gelling agent in different food applications (Dhull et al., 2022, Chandak et al., 2022, Chandak et al., 2022). The avocado starch contains 15–16 % amylose, with a gelation range at 56–74 °C having good water absorption capacity (22–24 g of water/g of starch), solubility (19 %–20 %), swelling power (28 g–30 g of water/g of starch), and a maximum viscosity (380 BU–390 BU) which makes it an ideal ingredient for gelling and thickening, pharmaceuticals and biodegradable packaging materials for foods (Chel-Guerrero, Barbosa-Martín, Martínez-Antonio, González-Mondragón, & Betancur- Ancona, 2016). Further, treatment like microwave-assisted extraction of avocado seed starch has been reported improving the extraction yield and induce some changes in the structure and properties of starch, for example, the production of small size starch chain and improving the starch solubility (Araújo et al., 2020). These changes can improve the starch functionality and provide new biotechnological applications, such as formulation of nanoparticles and preparation of alginates with bioactivity (Araújo et al., 2020). Starch has a huge world of utilization, which can be further extended by many modifications such as oxidation of avocado seed starch with sodium hypochlorite solutions (Lacerda et al., 2014) and heat moisture treatment (Lacerda et al., 2015). These modification changes various physicochemical properties of starches, such as average roughness, gelatinization enthalpy, pasting properties, degree of relative crystallinity, making them more suitable for several food industry applications (Punia et al., 2019, Punia et al., 2020, Dhull et al., 2020c). Ahrafai et al. (2020) prepared bioplastic sheets with optimal properties and cost by mixing potato starch (47 g), mango starch (7.6 g), and avocado starch (35.3 g), opening more ways for utilization of avocado seeds.Recently, avocado seeds demonstrated their utilization as a bio platform for producing relatively new nanomaterials, i.e., carbon dots (CD) with polyfunctional surfaces and different physico-chemical properties (Monje et al., 2021). Apart from its several applications in other fields, the CD can be effectively used as a Pickering emulsion (i.e., an emulsion stabilized by solid surfactant) stabilizer (Zhai et al., 2018) due to its high dispersibility in water. The CD can be used as a solid surfactant to avoid the adverse effect of soluble surfactants, affecting human health and the environment due to their mutagenic, toxic and carcinogenic properties (Chevalier & Bolzinger, 2013).
Further, using a nanomaterial like CD with all dimensions less than 10 nm would help prepare emulsion with very small droplet sizes (Zhai et al., 2018), which may find its utilization in food drug delivery, and cosmetics. Copper nanoparticles ranging from 42 to 90 nm synthesized using a green route with avocado seed extract were found stable and reproducible with excellent antioxidant and antimicrobial properties against the plant pathogens (A. niger, A. fumigatus, F. oxysporum) (Rajeshkumar & Rinitha, 2018). These bio-medically important nanoparticles can be utilized in drug delivery, nutraceuticals, and other food and pharma applications.Apart from this, distinct photoluminescent properties and singlet oxygen photosensitizing capacity of the CD is of interest in wastewater treatment and catalysis (Abd Rani et al., 2020, Monje et al., 2021). Similarly, different avocado seed based adsorbents find several applications in wastewater treatment by removing basic dyes (Elizalde-González, Mattusch, Peláez-Cid, & Wennrich, 2007), phenol (Rodrigues, da Silva, Alvarez-Mendes, dos Reis Coutinho, & Thim, 2011), ammonium, and p-cresol (Zhu et al., 2016, Zhu et al., 2018), fluoride (Salomón-Negrete, Reynel-Avila, Mendoza-Castillo, Bonilla-Petriciolet, & Duran-Valle, 2018), organic pollutants including pharmaceuticals and phenols (Leite et al., 2018), methylene blue dye (Dhaoudi et al., 2020), anticancer drug (Della-Flora et al., 2020), and heavy metals (Boeykens et al., 2019, Dhaouadi et al., 2021, Díaz-Muñoz et al., 2016).Moreover, a yellow-orange-colored pigment extracted from avocado seeds (Dabas et al., 2011, Hatzakis et al., 2019) can also be a potential food ingredient (Arlene, Prima, Utama, & Anggraini, 2015), pharma and cosmetic industries but after its further safety assessment studies. Eighteen patents related to avocado have been reported by Araújo, Rodríguez-Jasso, Ruiz, Pintado, and Aguilar (2018). The majority (i.e., ten) of that is related to the food industry, such as using avocado seed as a tea ingredient or a drink and as a substance to prepare culture media. Besides food, nutraceutical, pharma, and feed applications, avocado seeds may be important for personal care.
Avocado seed extract flavonoids and secondary metabolite, such as catechin, can inhibit the process of melanogenesis and have skin lightening potential by inhibiting the tyrosinase activity (Laksmiani, Sanjaya, & Leliqia, 2020). Also, four patents related to cosmetic, including one for an avocado facial cleanser, stands out for avocado-related products (Araújo et al., 2018, Salazar-López et al., 2020). This should demonstrate the diverse and effective use of avocado seeds, reducing contamination by not ending up as waste and generating nutritional and health benefits and economic gains. Avocado is widely grown and consumed fruit crop in tropical and subtropical regions while exported to the rest of the world because of its delicious taste, rich nutrient composition, and several health-promoting bioactivities in the human system. However, its seeds are generally considered as agricultural and food processing waste. The present review article has discussed that the seed remnants generated from the avocado fruit processing industries also exhibit several important constituents such as proteins, polyphenolic compounds, unsaturated fatty acids, antimicrobials and polysaccharides with promising biological and functional properties. The in vitro and in vivo studies on animal models along human cell lines using avocado seed extracts have proved its health-promoting properties like a strong antioxidant, anti-microbial, anticancer, anti-obesity, anti-inflammation, anti-diabetes and anti-neurodegenerative agent. Based on the nutritional and health/bioactivities of avocado seeds, the present review also summarized their applications in the development of functional food for cancer and diabetic patients. Several experiments have been performed to validate the safety of avocado seed consumption; however, reports on its mechanism of action and metabolism in humans are limited.
It is evident that a multidisciplinary research approach has encouraged the utilization of avocado seed residue as a healthy ingredient in the food industry. Recently, the application of avocado seed in functional food or food ingredients has gained much attention from many researchers. However, the understanding is still unearthed with respect to the molecular mechanism of bioactivities of avocado seed extracts. As the food industry looks to become more sustainable, repurpose of waste generated during processing into value-added products is essential. An in-depth investigation on the safety and pharmacological activities of specific compounds from avocado seed extract for pharma and food application needs to be proved. Also, there needs to be further research into valorization approaches as a cost-effective raw material.Abd Rani U., Ng L.Y., Ng C.Y., Mahmoudi E. A review of carbon quantum dots and their applications in wastewater treatment. Advances In Colloid and Interface Science. 2020;278 [PubMed] [Google Scholar]Abubakar A.N.F., Achmadi S.S., Suparto I.H. Triterpenoid of avocado (Persea americana) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. Asian Pacific Journal of Tropical Biomedicine. 2017;7(5):397–400. [Google Scholar]Ahmed F., Ghalib R.M., Sasikala P., Ahmed K.M. Cholinesterase inhibitors from botanicals. Pharmacognosy Reviews. 2013;7(14):121.
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2018;9:63–73. [Google Scholar] Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain Thrombosis Research Center, Medical Technology School, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, Universidad de Talca, Talca 3460000, Chile Authors to whom correspondence should be addressed. Antioxidants 2022, 11(6), 1049; Received: 28 April 2022 / Revised: 18 May 2022 / Accepted: 23 May 2022 / Published: 25 May 2022 : Avocado seed and peel are the main by-products from avocado industrialisation, and account for nearly 30% of fruit weight.
Although they are usually discarded, their high phenolic content has been deeply associated with several nutritional and functional benefits. Thus, for a comprehensive analytical evaluation of both semi-industrial extracts, various steps have been developed: tentative characterisation and quantification of the phenolic composition using HPLC-ESI-qTOF-MS, determination of TPC and antioxidant activity by Folin–Ciocalteu, FRAP, TEAC and ORAC methods, evaluation of scavenging capacity against different ROS and measurement of the enzymatic inhibitory potential against potentially harmful enzymes. Finally, their bioactive potential was tested in a human platelet model where antiaggregatory activity was measured. Hence, 48 different compounds were identified, where flavonoids and procyanidins were the most representative groups. The higher TPC was found in avocado peel extract (190 ± 3 mg/g), which showed more antioxidant power and more capacity to decrease ROS generation than seed extract (60 ± 2 mg/g). In addition, both extracts showed enzymatic inhibition, especially against hyaluronidase, xanthine oxidase and acetylcholinesterase. Lastly, avocado peel was proven to inhibit platelet aggregation with significant results at 1, 0.75 and 0.5 mg/mL, where the extract showed reducing effects on agonists' expression such as p-selectin or GPIIb/IIIa complex. These results demonstrate that both semi-industrial extracts—above all, avocado peel—have an interesting potential to be exploited as a natural by-product with antioxidant properties with multiple applications for the prevention of different pathologies. The avocado (Persea americana Mill.) is an important Central American fruit belonging to the Lauraceae family, produced mainly in tropical and subtropical areas, although it is currently cultivated throughout the world. Approximately, about six million tons of avocado are thought to be produced annually around the world [1], being the tropical fruit with the greatest production growth in recent years.An average avocado fruit is composed of pulp (among 65–73%), peel (among 11–15%) and seed (among 16–20%) [2]. Recent studies have demonstrated that avocado fruit possesses high nutritional quality. The pulp is recognised for its high levels of vitamins, minerals, proteins and fibers, as well as high concentrations of unsaturated fatty acids and bioactive compounds such as carotenoids, hydroxybenzoic and hydroxycinnamic acids, procyanidins, condensed tannins and flavonoids, especially flavonols [3]. Nevertheless, peels and seeds have been also identified as interesting matrices due to their high content of bioactive compounds such as carotenoids, tocopherols and especially phenolic compounds [3]. Their main phenolics ever identified are derivatives of chlorogenic acid (caffeoylquinic and coumaroylquinic acids) and flavonoids (catechins, quercetin glycosides and procyanidins) [4]. Former studies have also demonstrated that avocado seed and peel show even higher amounts of phenolics than the pulp [5].Because of this, avocado by-product phenolic compounds have already been associated with a host of health-related benefits: antioxidant, anti-inflammatory, anticarcinogenic, antiaging, antiaggregatory, antibacterial and antifungal activity, among others [6]. Due to antioxidant and anti-inflammatory activity, related to their ability to eliminate free radicals [7], these by-products are known to promote therapeutic effects against some human degenerative diseases associated with the presence of reactive oxygen species (ROS) and oxidative stress, including prevention and protection against neurodegeneration, cardiovascular and gastrointestinal failures and cancer development [1,8,9], or even against skin-aging-related issues, encouraging photoprotection from harmful UV sun rays, increasing of the wound-healing process and mitigation of skin hyperpigmentation [10,11,12].Despite their proven bioactivity, these nonedible parts are commonly discarded. Annually, at least 1.6 million tons of avocado seeds and peels are estimated to be thrown away globally [1], turning them into a remarkable source of environmental contamination and provoking a huge wastage of nutritional value. Instead, these residues could be a low-cost onset to obtain a wide variety of phenolic acids and flavonoids with a high functional potential for the formulation of foods, nutraceuticals or cosmetic products [13].In this context, and being aware of high economic value of avocado production, a comprehensive in vitro evaluation of the semi-industrial extract of P. americana Mill. by-products was carried out with the aim of highlighting the potential of seeds and peels as phytochemical sources, and their possible revalorisation from a preindustrial-scaling point of view. For this purpose, a tentative analytical characterisation/quantification of the phenolic composition was carried out, followed by an in-depth in vitro study evaluating parameters such as total phenolic content, antioxidant potential and free-radical scavenging activity, as well as the affinity of its phenolics with several enzymes involved in physiological phenomena by determining their inhibitory concentration (IC50). Finally, an evaluation of platelet antiaggregatory activity was performed to determine the anticoagulation capacity of avocado seed and peel, and thereby prove their cardiovascular benefits.
In the present work, for the first time, a comprehensive enzymatic study was carried out on the main avocado by-products on a preindustrial scale, as well as their possible therapeutic applications (platelet aggregation), thus addressing their potential for the development of future nutraceuticals.For extractions and solutions, ultrapure water was obtained with a Milli-Q system Millipore (Bedford, MA, USA) and absolute ethanol was purchased from VWR chemicals (Radnor, PA, USA).The following reagents were provided from the indicated suppliers: Sodium carbonate, acetic acid, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), sodium hydroxide and hydrochloridic acid were purchased from Fluka (Honeywell, NC, USA).
Absolute ethanol and sulfuric acid were purchased from Riedel-de-Haën (Honeywell, NC, USA). Sodium hypochlorite solution EMPLURA was purchased from Merck (Darmstadt, Germany). NOC-5 was purchased from Chemcruz (Santa Cruz Biotech., Dallas, TX, USA). Gallic acid, Folin reagent, ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonate)), potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium acetate, ferric chloride, heptahydrate ferrous sulphate, fluorescein, AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride), sodium phosphate monobasic and dibasic, DHR (dihydrorhodamine), DMF (dimethylformamide), potassium dihydrogen phosphate anhydrous, NADH (β-nicotinamide adenine dinucleotide), NBT (nitrotetrazolium blue chloride), PMS (phenazine methosulfate), DAF-2 (diaminofluorescein diacetate) tyrosinase inhibitor screening kit (colorimetric), Tris (tri(hydroxymethyl)aminomethane), acetylthiocholine iodide, 5.5-dithiobis-(2-nitrobenzoic acid), acetylcholinesterase from Electrophorus, Cayman's xanthine oxidase fluorometric assay kit, neutrophil elastase colorimetric drug discovery kit, sodium chloride, hyaluronic acid, hyaluronidase from sheep testes, tricine, 1-10 phenantroline, collagenase from Clostridium histolyticum, FALGPA (N-[3-(2-furyl)acryloyl]-L-leucyl-glycyl-L-prolyl-L-alanine), adenosine diphosphate (ADP), thrombin receptor activating peptide 6 (TRAP-6), sodium citrate 3.2% and phosphate buffer solution (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Collagen was purchased from Havertown, PA, USA.Fresh dark avocado fruits of the variety 'Hass' were donated by the commercial group La Caña, Miguel García Sánchez e Hijos, S.A. (Motril, Spain) to NATAC Biotech. S.L. (Cáceres, Spain) in order to obtain a preindustrial extract from avocado by-products. Complete avocado seeds and peels were manually separated and cleaned under continuous flow of tap water. Then, a 3-cycle solid–liquid extraction (maceration) at 50–70 °C (seeds and peels, respectively) using a hydroalcoholic mixture (EtOH 60%-peels and 70%-seeds) was carried out. Ethanol/water is considered as a favourable solvent in the extraction of polar substances such as phenolic compounds, which does not have toxic effects on humans and is environmentally friendly (GRAS solvent) [7]. Each cycle was performed on 20 kg of seeds or peels mixed with 200 L of extractant over 2 h. Subsequently to decantation, microfiltration and liquid-extract concentration, a biconical rotary vacuum dryer was used to obtain the final dry extracts at 50–60 °C (seeds and peels, respectively), with a certain amount of silicon dioxide to promote peels (4%) and seeds (10%) drying. Once well-dried, both extracts were ground and sieved, turning them into samples of an average size of 2 mm. The extraction efficiency from extracts obtained were 15.7 ± 0.9 g dry extract per 100 g of raw material for peel, and 14.6 ± 1.2 g dry extract per 100 g of raw material. The material was stored at room temperature and protected from light until their analysis.Avocado seed and peel extracts at 5000 mg/L were analysed using high-performance liquid chromatography (HPLC), specifically an ACQUITY UPLC H-Class System (Waters, Milford, MA, USA) coupled to an electrospray-quadrupole time-of-flight mass spectrometer (ESI-qTOF-MS, Synapt G2, Waters Corp., Milford, MA, USA) working in negative-ion mode over a range from 50 to 1200 m/z. The separation was performed in a ACQUITY UPLC BEH Shield RP18 Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm at a flow rate of 0.7 mL/min using volume injection of 10 µL.The mobile phases were (A) acidified water with 1% of acetic acid (v/v), and (B) acetonitrile. The following multistep linear gradient was used in order to achieve efficient separation: 0.0 min [A:B 99/1], 2.33 min [A:B 99/1], 4.37 min [A:B 93/7], 8.11 min [A:B 86/14], 12.19 min [A:B 76/24], 15.99 min [A:B 60/40], 18.31 min [A:B 2/98], 21.03 min [A:B 2/98], 22.39 min [A:B 99/1] and 25.0 [A:B 99/1]. To acquire mass spectrum, two parallel scan functions were performed, switching among them rapidly. Of both scans, one was operated at low collision energy in the gas cell (4 eV) and the other at an elevated collision energy (MSE energy linear ramp: from 20 to 60 eV). Leu-enkephalin was injected for mass calibration continuously. Other parameters were as follows: source temperature 100 °C; scan duration 0.1 s; resolution 20,000 FWHM; desolvation temperature 500 °C; desolvation gas flow 700 L/h; capillary voltage 2.2 kV; cone voltage 30 V; cone gas flow 50 L/h.Finally, data obtained were processed and visualised using MZmine 2.53 open-source software and Sirius 4.4.29. In addition, by contrasting information provided by the software with the literature available for both avocado and other species belonging to Lauraceae family, the compounds' characterisation was achieved. Literature search for published spectral information was carried out by using SciFinder®.The phenolic compounds identified in the extracts were tentatively quantified using calibration curves of the respective reference compounds, all of them obtained with a good linearity ($R2 > 0.99$) by plotting the standard concentration as a function of the peak area obtained from HPLC-ESI-qTOF-MS analyses [14]. For this purpose, a pattern mix was prepared as from stock solutions (500 mg/L) diluted to concentrations of 0.488–31.25 mg/L (catechin, procyanidin B, verbascoside, myricetin-3-glucoside, quercetin, quercetin glucoside and quinic acid). In the case that reference compounds were not relatable enough, the quantification of some compounds was performed using structurally related substances, provided that the phenolic compound standard had an aglycon moiety similar to those present in the test sample. Table 1 summarises the analytical parameters for the different phenolic compounds present in avocado extracts.All undermentioned assays performed were adapted to a 96-well polystyrene microplate, and the absorbance measurement was carried out on a Synergy H1 Monochromator-Based Multi-Mode Micro plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA).(a)Total Phenolic Compound Content Assessment by Folin–Ciocalteu MethodTotal phenolic content was determined following Folin–Ciocalteu (F-C) method, reported by Gurrea-Cadiz et al., with some modifications, and using different proportions of by-product extract (100, 200 and 500 mg/L) [15]. Total phenol content was expressed as microgram of gallic acid equivalent (GAE) per milligram of dry extract (DE) (µg GAE/mg DE). Measurements were made in triplicate.(b)Ferric Reducing Antioxidant Power (FRAP)This method, carried out by Al-Duais et al., with some modifications, is used to measure the reducing antioxidant power of the different proportions of avocado wastes extracts (100, 200 and 500 mg/L) in comparison with a calibration curve constructed with ferrous sulfate ($FeSO4·7H2O$). Results were expressed as µmol of iron equivalent per DE (µM Fe (II)/g). Measurements were made in triplicate.(c)Trolox Equivalent Antioxidant Capacity (TEAC)TEAC was in vitro measured as the reducing activity of extracts at different concentrations (100, 200 and 500 mg/L) against ABTS * +, a way of calculating antioxidant capacity based on ability to scavenge that radical. As Re et al. reported, Trolox was used as the standard at concentrations from 0.5 to 15 µM [16]. The results were expressed as mmol Trolox equivalent per grams of DE.

Measurements were made in triplicate.(d)Oxygen Radical Absorbance Capacity (ORAC)To assay the capacity of the extracts to scavenge peroxyl radicals, a validated ORAC method by Huang et al., was carried out [17]. ORAC values were calculated using a regression equation between the Trolox concentration and the area under the fluorescence decay curve. The results are expressed as μmol Trolox equivalents per grams of DE. Measurements were made in triplicate.All free-radical assays were performed according to Gomes et al. and Pinto et al. [18,19].(a)Scavenging Ability of Superoxide Anion Radical (O2 *)Superoxide anions were generated by a nonenzymatic PMS-NADH system, and the scavenging activity was evaluated using a colorimetric methodology in the microplate reader based on the reduction of NBT into a purple-coloured diformazan as result of the reaction with superoxide anions at 560 nm. The sample concentration providing 50% inhibition (IC50) was achieved by interpolating this inhibition percentage against extract concentrations.(b)Scavenging Ability of Nitric-Oxide Radical (NO *)Nitric-oxide anions were generated by the presence of NOC-5, and 4,5-diaminofluorescein (DAF-2) was used as the fluorescence probe applied [18,19].An incubation at 37 °C was needed, and a fluorescence measure at 485–528 nm for excitation emission was performed. Results are expressed as IC50 values obtained as aforementioned.(c)Scavenging Ability of Hypochlorous Acid (HOCl)The method was based on the fluorescent HOCl-induced oxidation of DHR to rhodamine [18,19]. Results are expressed as the inhibition, in IC50, of this oxidation of DHR induced by HOCl.(a)Inhibition of Acetylcholinesterase (AChE)AChE inhibitory activity was measured by using a photometric colour-based assay described by Ellman et al., with certain modifications [20]. The reaction starts with acetylthiocholine (ATCI) acting as the substrate and being cleaved by AChE to form thiocholine, which in turn reacts with DTNB to give the yellow 5-thio-2-nitrobenzoate anion. The enzyme activity was measured by following the rate of production of thiocholine, thus the increase in yellow colour produced.The ratio of colour production was measured every minute at 405 nm. Tests were carried out in triplicate, and the IC50 was calculated using different avocado-extract concentrations.(b)The test was carried out utilizing the "Tyrosinase Inhibitor Screening Kit (Colorimetric)" (Sigma-Aldrich, USA). Tyrosinase catalyses the oxidation of tyrosine, producing a chromophore that can be detected at 510 nm. Thus, stablishing an inhibition control using kojic acid and an enzyme control using only tyrosinase, every avocado sample tyrosinase inhibition activity could be measured by calculating chromophores production.Briefly, solvents were added to the microplate according to its role (sample, inhibitor or enzyme control). Later, enzyme and substrate solution were added into each well and the measurement took place at 510 nm. Tests were performed in triplicate, and the IC50 was calculated using different avocado-extract concentrations.(c)Inhibition of Xanthine Oxidase (XO)Avocado by-products' XO inhibitory activity was measured using the kit "Cayman's Xanthine Oxidase Fluorometric Assay Kit" (Cayman Chem. Ann Arbor, MI, USA). The method is based on the production of a highly fluorescent compound named resorufin as of oxidation of hypoxanthine by XO releasing H_2O_2.

[illegible]

The nitric-oxide radical (NO•) is widely created by living organisms: endothelial cells, macrophages, neurons, etc. [69]. It is involved in modulation and regulation of certain physiological processes, reacting with singlet oxygen to produce, through intermediates, stable products of nitrate and nitrite related to blood and 'haemo' phenomena [70]. However, nitric oxide is also regarded as an important mediator of acute and chronic inflammation, easily reacting with superoxide anion to form potent oxidizing molecules that provoke cellular damage. Thus, an excess concentration is linked with corporal detriment, displaying multiple cytotoxic effects that would trigger a host of different diseases such as inflammation, cancer or atherosclerosis, among others [67,71]. Hypochlorous acid (HOCl) is generated in neutrophils by reactions of chlorides with hydrogen peroxide. Its endogenous production constitutes an important defence mechanism against microorganisms [7]; nevertheless, it also promotes haemolysis on erythrocytes, being associated with several pathological processes such as atherosclerosis [58,72].Table 3 shows the results of avocado by-product extracts obtained after evaluation of the radical-scavenging capacity of three average endogenous reactive species whose corporal excessive concentration develops serious physiological damage. Generally, avocado peel resulted in the best ROS scavenger extract, with almost the lowest IC50 values, although avocado seed exhibited more hypochlorous-acid-scavenging capacity than peel (2.3 mg/L vs 7.1 mg/L, respectively).

Table 4 also shows positive controls tested to compare to our results: for radical neutralisation, gallic acid (GA) and epicatechin (EPI) were chosen as positive scavengers. Avocado-peel extract showed worse results for $O_2^{•−}$ scavenging (380 ± 69 mg/L vs.
50 ± 3 mg/L, GA, and 70 ± 5 mg/L, EPI) and almost the same results for NO• scavenging (1.90 ± 0.09 mg/L vs. 1.4 ± 0.3 mg/L, GA, and 0.87 ± 0.02 mg/L, EPI). Avocado seed showed better results than GA for HOCl scavenging (2.3 ± 0.1 mg/L vs. 3.8 ± 0.3 mg/L, respectively), which brings interest to the extract. Except for $O_2^{•−}$, avocado peel exerted a significantly high antiradical activity, with similar magnitude to those showed by GA and EPI.Plant phenolics can act as antioxidant agents by different mechanisms. The first one, radical scavenging, was formerly mentioned and evaluated, highlighting avocado peel's ability to neutralise free-radical species. Another antioxidative mechanism, metal chelation, is also closely related to this matter [73]. Catechol-moiety flavonoids and phenolic acids are also efficient at chelation of transition metals, which have the role of natural enhancers of ROS formation in living organisms [68]. Andjelkovic et al. observed that hydroxycinnamic acids (chlorogenic and caffeic acids) performed the best complex formation thanks to their ortho-diphenol chelating domain [73].Taking together metal chelation activity and the formerly mentioned radical scavenging ability, the antioxidant activity of avocado peel and seed is totally proven. The peel is pointed out as a significant better source of antioxidant substances than avocado seed, based on reported values and a wide-ranging diversity of phenolic compounds qualitatively determined by HPLC-ESI-qTOF-MS. However, both are practical and feasible options in food, pharmacological and cosmetic industries [1].After reviewing reported literature, almost nothing was found about testing the radical-scavenging ability of 'Hass' avocado by-products' extracts, specially about hypochlorous acid. Tremocoldi et al. addressed this issue and obtained, from Hass seed to peel, 52 and 70 mg/L for superoxide radical, and 5.2 and 6.7 mg/L for hypochlorous acid. In comparison, although our avocado extracts displayed somewhat less superoxide radical-scavenging capacity than reported, our peel extract exhibited nearly half the seed value. On the other hand, both hypochlorous-acid-scavenging studies bore some similarity [7].

Other studies did exhibit lower results than ours, such as Alagbaoso et al., with antioxidant activity against superoxide anion values between 1500 and 3400 mg/L for avocado seed, or Kamaraj et al., which reported nitric-scavenging activity of 79.05 mg/L and superoxide-scavenging activity of 103.05 mg/L for avocado peel [66,69]. However, the variety of fruit was not specified in any of the latter two studies, and since no direct comparison could be made among the different species, it is difficult to assess with the results reported. Concerning the differences among experiments, it could be thought that the composition of by-products is a determining factor in the total scavenging ability of the extract.

Therefore, observing the identification of phenolic compounds, quercetin is highlighted for being located only in avocado peel, and for a remarkable bioactivity against species such as $O_2^{•−}$ and NO• [74,75].

The higher presence of quercetin in avocado peel probably promotes its ability for blocking radical species, making its IC50 lower than the seed one.Regarding other tropical species, Limonia acidissima L. showed a range from 60 to 125 mg/L as IC50 of nitric-oxide-scavenging radical activity [76]. The stem bark of mango showed an IC50 scavenging activity against HOCl of 400 mg/L [77]. In nontropical fruit by-products, shells from Castanea sativa were tested for $O_2^{•−}$ and HOCl scavenging capacity, showing 49.42 ± 0.41% at 500 mg/L, and 50% at 1.57 ± 0.10 mg/L, respectively [19]. In conclusion, avocado extracts (especially peel) show excellent properties at scavenging radical species in comparison to other fruit species.ROS can be originated by intrinsic or extrinsic factors, with first referring to oxidative/nitrosative stress and altered metabolism, and the second referring to long exposures to exogenous harmful agents, e.g., UV radiation. In this sense, the excess of reactive species leads to many detrimental conditions for human body, in which the activation of different enzymes, such as acetylcholinesterase, tyrosinase, xanthine oxidase, elastase, hyaluronidase and collagenase is closely linked [21].Acetylcholinesterase (AChE) enzyme is responsible for acetylcholine (ACh) regulation, a significantly important compound involved in nerve-impulse transmission between cells (cholinergic synapses).

In its presence, AChE rapidly breaks down ACh into choline and acetate, thus promoting neurological disorders related to cholinergic transmission deficit: Alzheimer's disease, senile dementia, ataxia and myasthenia gravis.

Tyrosinase enzyme is responsible for the physiological synthesis of melanin, the production of which in human skin is known as a primordial defence mechanism against UV radiation. However, overproduction and unrestricted accumulation could lead to the formation of epidermal pigmentation, considered the first sign to skin aging and some deleterious disorders related: melasma, age spots, flecks, ephelides and sites of actinic damage. Xanthine oxidase (XO) enzyme is a dehydrogenase responsible for catalysing hypoxanthine to xanthine, and subsequently to uric-acid oxidation.

However, under oxidative-stress conditions, XO is transformed in an oxidase, responsible for dangerous superoxide-radical production and causing many pathological diseases, such as gout, hyperuricemia, hepatitis, carcinogenesis and aging. Elastase, hyaluronidase (HYALase) and collagenase enzymes are responsible for, respectively, elastin, hyaluronic acid (HYAL) and collagen regulation, the main substances in the extracellular matrix (ECM) and closely related in order to maintain its structural organisation, structure integrity and elasticity. These enzymes, under ROS overproduction, promote skin-aging phenomena through fibre-network depletion, leading to the loss of skin elasticity, decrease in holding-water capacity and consequent skin disorders such as skin sagging, wrinkle formation and eczemas, among others [78,79,80,81,82].Therefore, the coherent blocking of these key enzymes would improve their beneficial state on human health. This effect can be induced by plant phenolics with strong antioxidant power.

Table 3 shows results obtained from the enzyme-inhibition assessment.According to their IC50 or IC30 values, the peel exhibited a higher degree of inhibition of XO and elastase, while the seed showed it for AChE and HYALase, in spite of the fact that it showed the lowest degree of all inhibition values for elastase, and no inhibitory effect at all on tyrosinase. Several assays previously carried out stated that hydroxyl groups from flavonoids and phenolic compounds could interact with the backbone or with some functional group of enzymes; or that interactions between the enzyme and the antioxidant result in conformational changes that finally lead to unfunctional enzymes. Enzyme inhibitory capacity is directly related to the existence of certain phenols and flavonoids such as epicatechin, catechin or quercetin [82,83,84].

Hence, the inhibitory activity of avocado by-products extracts could be explained again by their phenolic high concentrations. For example, quercetin activity against collagenase has been reported before, and being aware that only avocado peel harbours quercetin and derivatives, it could explain its lower IC50 [85]. The power this flavonol exerts against tyrosinase and elastase activity has also been noted [86]. Nevertheless, the slight anti-elastase potential from both extracts may be due to the attachment between main flavonoids with sugars to positions 3 or 7, which seems to downregulate the anti-elastase activity of the aglycone [87]. In the case of anti-acetylcholinesterase activity, previous reports highlight avocado leaf extract as interesting neuroprotector solutions; a quantification was performed and the levels of chlorogenic acid stood out, which may be the reason why both our extracts showed cholinergic potential [88].Comparing our results with controls from Table 4, some interpretations can be made: positive controls from tyrosinase, elastase and AChE exerted higher inhibitory activity than both avocado extracts. Drugs as positive controls, such as physostigmine, offer extremely specific activity against certain targets, so the comparison among it and extracts could be confusing. Nevertheless, the remaining enzymes were more inhibited by avocado seed and/or peel than the controls: in the case of collagenase, knowing that 4500 ppm of phenanthroline were needed to inhibit 83 ± 2%, avocado seed and peel needed 104 and 81 ppm, respectively, to inhibit a 50%. It could be thought that avocado extracts exert higher inhibitory activity than the control. In the case of HYAL, once the IC50 value was known, the final order was GA = EPI < AP < AS; both extracts highlighted as better HYALase inhibitors than positive controls. Similarly, in the case of XOD, the order was EPI < AS < AP, also offering higher activity than the chosen control. These studies confirm the possibilities that both extracts offer depending on the target.Concerning the industrial potential of these extracts to regulate enzyme production, the quite limited number of studies published in literature is noteworthy. In the case of AChE, Oboh et al. only reported avocado (unknown variety) leaf and seed extract values, 33.72 and 27.93 mg/mL, respectively, against our seed value of 0.0583 mg/mL (IC50) [48]. Tyrosinase was also found in literature only performed for avocado seed (unknown variety), offering an IC50 of 93.02 mg/L, oppositely to our nonexistent result [12]; other inhibition values were also reported by Fawole et al., from seven cultivars of pomegranate peel, in ranges from 3.66 ± 0.11 to 98.66 ± 0.12 mg/L (IC50) [89]. XOD inhibition assay was only found on Persea leaves, with a resulting IC50 of 63.39 mg/L [90]. In the case of elastase and HYALase, no literature was found concerning avocado or its by-products. Samejima et al. performed the anti-elastase and anti-HYALase assay on guava leaf extract, another tropical fruit, with IC50 results between 17.7 and 42.5 mg/L for elastase; and between 377 and 875.4 mg/L for hyaluronidase [91]. Finally, regarding anticollagenase activity, Figueroa et al. reported an inhibition of 43.7% at 150 mg/L of avocado 'Hass' peel, less active in comparison to our result (50% at 81 mg/L) [35]. Mangiferin obtained from Mangifera indica leaves, peels and barks reported inhibition results against elastase (IC50 58.9 ± 3.9 mg/L) and collagenase (IC50 107.09 ± 3.19 mg/L) [92]. Comparisons highlight avocado peel for its use inhibiting AChE, collagenase, HYALase and XO.Cardiovascular diseases (CVDs) are considered as the leading cause of death worldwide, with ischemic heart disease and stroke being the two main causes of death, as pointed out by the World Health Organization [93]. In addition, these pathologies have been deeply associated with an increase in platelet function. Platelets are small anucleated blood cells responsible for maintaining a balance between activatory and inhibitory signalling pathways for haemostasis and thrombosis phenomena. Nevertheless, excessive platelet activation is a decisive factor enhancer of CVDs disorders such as hypertension, diabetes and atherosclerosis, among others [94].The platelet activation process, composed of different cellular signalling pathways, allows the involvement of a host of different substances and species, such as platelet agonists or ROS.

They act as second messengers by stimulating the arachidonic-acid metabolism and phospholipase C pathway. Excessive production enhances oxidative stress, which would regulate several components of thrombosis, including platelet activation. All this cellular stress displays a critical role in CVDs [95]. Agonists such as collagen, von Willerbrand factor (VWF), adenosine diphosphate (ADP), thrombin or thromboxane 2 (TXA2), are platelet-activation stimulators. They are responsible for inducing signalling cascades that result in conformation changes in αIIb3 integrin, creating an activated complex with improved affinity to fibrinogen and enhanced adhesive properties [96].In this sense, it is necessary to search for new strategies to modulate platelet activity. The consumption of fruits and vegetables with high phenolic-compound content has formerly been profoundly reported because of their important role exerting platelet antiaggregatory activity [27,97]. This effect on platelet function seems to be possible at different levels: due to structure-dependent interferences (number of hydroxyl groups, C4 carbonyl substituted, C3 hydroxylated and a B ring with catechol moiety); inhibition of ROS production, reducing oxidative burst, modulating certain pathways or blocking agonistic substances [94]. Accordingly, platelet antiaggregatory assessments could be performed through several mechanisms; in this study, the measure was carried out, in the first place, using the inhibition of ADP, collagen and TRAP-6 technique in order to choose the most significant powerful extract capable of inhibiting the action of these different thrombus agonists. Subsequently, the inhibition of ADP, collagen or TRAP-6-stimulated P-selectin secretion and GP IIb/IIIa activation were also evaluated, and both events related to platelet activation and adhesion: the first one is a cohesive molecule released during the platelet activation and promoted the thrombus formation, and the latter is an integrin complex whose main function is the reception of different agonists that regulate the activation of platelets [98,99].In the first place, the antiplatelet activity of avocado seed and peel extracts was evaluated by turbidimetry at 1 mg/mL. Platelet aggregation was stimulated with ADP (4 μM), TRAP-6 (10 μM) and collagen (1 μg/mL), as it is shown in Table 5. It was observed that avocado peel and seed extracts inhibited agonist-stimulated platelet aggregation in study.Avocado-peel extract showed greater antiplatelet potential than avocado-seed extract against the evaluated agonists, TRAP-6, ADP and collagen. The antiplatelet activity of avocado-peel extract was higher when platelet aggregation was stimulated with ADP and collagen: 78 ± 2% and 55 ± 2%, respectively. This effect was less marked compared to TRAP-6, 42 ± 1. Meanwhile, the avocado-seed extract only showed significant inhibition of platelet aggregation against collagen, 45 ± 2%. Considering the positive control adenosine (10μM), avocado peel stands out as a great antiaggregatory extract, especially when promoted by ADP and collagen.The extracts that showed higher antiplatelet potential were selected, with a percentage of platelet inhibition above 50%. The concentration-dependent antiplatelet activity of avocado-peel extract induced by ADP and collagen was also studied.In this sense, the obtained results (Figure 1) show that the antiplatelet activity of avocado-peel extract is concentration-dependent, being able to inhibit collagen-stimulated platelet aggregation up to 0.5 mg/mL compared to the control. Nevertheless, when platelet aggregation was induced with ADP, the antiplatelet potential remained significant at higher concentrations, 1 mg/mL and 0.75 mg/mL, while at the lowest concentration the activity decreased markedly. Actually, avocado peel showed higher anticoagulant activity than adenosine, even at 0.75 mg/mL.On the other hand, other studies were conducted to determine the effect of avocado-peel extract on platelet activation, p-selectin expression and activation of GP IIb/IIIa (Figure 2). Platelet activation was stimulated with the agonists ADP and collagen, since in these conditions relevant antiplatelet effects of avocado peel extract were observed. When platelets were stimulated with ADP, only at 1 mg/mL p-selectin expression was inhibited.

However, when platelet activation was stimulated with collagen, the expression of p-selectin was significantly reduced to a concentration of 0.25 mg/mL with respect to the activated condition (maximum expression of p-selectin). Comparing to positive control, the extract works better in a collagen-agonist model at concentrations from 0.5 to 1 mg/mL.Finally, the evaluation of the activation of GP IIb/IIIa showed that the avocado-peel extract reduced the platelet activation induced by ADP and collagen (activated state), with this effect being more powerful when activated with collagen. Activation of GP IIb/IIIa decreased at higher concentrations of avocado extract, 0.75 and 1 mg/mL. These results suggest that avocado-peel extract inhibits platelet activation by reducing P-selectin secretion and GP IIb/IIIa activation.The use of HPLC-ESI-qTOF-MS enabled the identification of 48 different compounds in avocado seed and peel semi-industrial extracts—some of them never identified before in the avocado by-products matrix—and the tentative quantification of 38 phenolic compounds from different families. Glycosylated flavonols, hydroxycinnamic acids and procyanidins are the most representative groups in both samples, mostly in avocado peel.Avocado-peel extract showed quite greater antioxidant effects than avocado seed, in terms of TPC, capacity to donate electrons (FRAP and TEAC) and transfer hydrogen atoms (ORAC), and ability to scavenge reactive species (·O2 and NO).

In addition, results of enzyme assay revealed that compounds from avocado peel were effective inhibitors for every enzyme tested, especially for hyaluronidase, xanthine oxidase and acetylcholinesterase. Regarding avocado-seed extract, it also shown significant phenolic content, antioxidant capacity and enzyme inhibition (HOCl, hyaluronidase, xanthine oxidase).Moreover, platelet antiaggregatory effect has been proven to be performed by avocado-peel extracts, which means a reduction in thrombus formation and other cardiovascular benefits. The mechanism through this effect is exerted must be studied more profoundly, but it seems that phenolic compounds and other bioactives from avocado peel are able to reduce the effect of agonists, inhibit adhesive molecules and inactivate protein complexes closely involved in platelet aggregation. Therefore, due to differences among phytochemical compositions and the biological potential, avocado by-products can be considered as an excellent source of antioxidants that could be employed for therapeutical needs.Regarding the extraction procedure, although emergent extraction techniques such as ultrasound or microwave-assisted extractions are very popular for the obtainment of phytochemicals through environmentally friendly procedures, these methods have been used almost exclusively on a laboratory scale. This study aims to provide new information about how avocado wastes could be treated from a preindustrial scale-up point of view for those industries that generate many tons of waste per year and want to invest on revalorisation. The main disadvantage of emergent techniques for industry is that they are very expensive and require rather high temperatures (> 130 °C) for their application, which are major limitations for industrial equipment and make these methodologies not very feasible to an industrial scale-up.Consequently, in order to ease industrial application, SLE was chosen as the extraction method. As a future perspective, knowing the high number of greener extraction techniques than SLE that currently exist, research and studies must focus on applying them in a practical way for industrial scale-up, thus reducing operation time and pollutant emission without a disproportionate rise in costs. The following supporting information can be downloaded at: Figure S1: (a) Best peak chromatogram of avocado seed performed by HPLC-ESI-qTOF-MS. The main peaks are numbered as they appear on the table. (b) Best peak chromatogram of avocado peel performed by HPLC-ESI-qTOF-MS. The main peaks are numbered as they appear on the table. (c) Enlarged areas of the avocado-peel chromatogram.Conceptualisation, M.d.l.L.C.-G., A.S.-C. and I.P.; methodology, M.d.l.L.C.-G., A.R.-G., M.d.C.V.-A., E.F. and L.R.; validation, M.d.l.L.C.-G. and I.P.; formal analysis, M.d.l.L.C.-G., A.R.-G., M.d.C.V.-A., E.F. and L.R.; investigation, A.R.-G., M.d.C.V.-A., E.F. and L.R.; resources, A.S.-C. and I.P.; data curation, M.d.l.L.C.-G., A.R.-G. and I.P.; writing—original draft preparation, A.R.-G., E.F. and L.R.; writing—review and editing, M.d.l.L.C.-G., A.R.-G. and D.A.-R.; visualisation, A.S.-C. and I.P.; supervision, M.d.l.L.C.-G., D.A.-R. and I.P.; project administration, A.S.-C., D.A.-R. and I.P.; funding acquisition, A.S.-C., D.A.-R. and I.P. All authors have read and agreed to the published version of the manuscript.This research received no external funding.The study was conducted in accordance with the Declaration of Helsinki and approved by the Scientific Ethics Committee of the University of Talca (protocol no. 19/2018).Informed consent was obtained from all subjects involved in the study.The work was supported by the project P18-TP-3589 (Regional Ministry of Economy, Knowledge, Enterprise and Universities of Andalusia). The author A.R.-G.
would like to thank the project P18-TP-3589, University of Granada and AGR274 group for the contract (265). The author M.d.C.V.-A. would like to thank the Spanish Ministry of Science, Innovation, and Universities for the grant FPU19/01146. The author M.d.l.L.C.-G. would like to thank the Regional Ministry of Economy, Knowledge, Enterprise and Universities of Andalusia for the contract for Young Researchers (PAIDI) at the University of Granada.The authors declare no conflict of interest.Salazar-López, N.J.; Domínguez-Avila, J.A.; Yahia, E.M.; Belmonte-Herrera, B.H.; Wall-Medrano, A.; Montalvo-González, E.; González-Aguilar, G.A. Avocado fruit and by-products as potential sources of bioactive compounds. Food Res. Int. 2020, 138, 109774. [Google Scholar] [CrossRef] [PubMed]Calderón-Oliver, M.; Escalona-Buendía, H.B.; Medina-Campos, O.N.; Pedraza-Chaverri, J.; Pedroza-Islas, R.; Ponce-Alquicira, E. Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts. LWT Food Sci. Technol. 2016, 65, 46–52. [Google Scholar] [CrossRef]Bhuyan, D.J.; Alsherbiny, M.A.; Perera, S.; Low, M.; Basu, A.; Devi, O.A.; Barooah, M.S.; Li, C.G.; Papoutsis, K. The odyssey of bioactive compounds in Avocado (Persea Americana) and their health benefits. Antioxidants 2019, 8, 426. [Google Scholar] [CrossRef] [PubMed][Green Version]Velderrain-Rodríguez, G.R.; Quero, J.; Osada, J.; Martín-Belloso, O.; Rodríguez-Yoldi, M.J. Phenolic-rich extracts from avocado fruit residues as functional food ingredients with antioxidant and antiproliferative properties. Biomolecules 2021, 11, 977. [Google Scholar] [CrossRef]Rosero, J.C.; Cruz, S.; Osorio, C.; Hurtado, N. Analysis of Phenolic Composition of Byproducts (Seeds and Peels) of Avocado (Persea americana Mill.) Cultivated in Colombia.
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Chem. 2020, 192, 112187. [Google Scholar] [CrossRef]Olas, B. A review of in vitro studies of the anti-platelet potential of citrus fruit flavonoids. Food Chem. Toxicol. 2021, 150, 112090. [Google Scholar] [CrossRef] Figure 1. Study of platelet aggregation of avocado peel and seed extract induced by collagen and ADP. The PRP was previously incubated with vehicle or avocado extract (0.1, 0.25, 0.50, 0.75 and 1 mg/mL). After 3 minutes of incubation at 37 °C, it was stimulated with the agonist to initiate platelet aggregation for 6 minutes. The negative control is in the absence of the extracts. Bar graph indicates maximum aggregation expressed as a percentage (mean ± SEM; n = 6). Differences between groups were analysed by ANOVA using Dunnet's post hoc test. *** p < 0.001 and ** p < 0.01, denote statistically significant differences compared to the vehicle; ns: nonstatistical difference with respect to the vehicle (PBS). Figure 1.
Study of platelet aggregation of avocado peel and seed extract induced by collagen and ADP. The PRP was previously incubated with vehicle or avocado extract (0.1, 0.25, 0.50, 0.75 and 1 mg/mL).
After 3 minutes of incubation at 37 °C, it was stimulated with the agonist to initiate platelet aggregation for 6 minutes. The negative control is in the absence of the extracts.
Bar graph indicates maximum aggregation expressed as a percentage (mean ± SEM; n = 6). Differences between groups were analysed by ANOVA using Dunnet's post hoc test. *** p < 0.001 and ** p < 0.01, denote statistically significant differences compared to the vehicle; ns: nonstatistical difference with respect to the vehicle (PBS). Figure 2.
Effect of avocado-peel extract on the expression of platelet-activation markers. (A) Effect on P-selectin expression; (B) Effect on PAC-1 expression. Platelets were stimulated with ADP or Collagen. Platelets were identified as a CD61 + population. Statistical analysis was performed by ANOVA (Dunnet's test). * p < 0.05, ** p < 0.01 and *** p < 0.001 vs. Vehicle (PBS) vs. activated control (agonist) (n = 5). Figure 2. Effect of avocado-peel extract on the expression of platelet-activation markers. (A) Effect on P-selectin expression; (B) Effect on PAC-1 expression. Platelets were stimulated with ADP or Collagen. Platelets were identified as a CD61 + population. Statistical analysis was performed by ANOVA (Dunnet's test). * p < 0.05, ** p < 0.01 and *** p < 0.001 vs. Vehicle (PBS) vs. activated control (agonist) (n = 5). Table 1. Quantification data of identified phenolic compounds from avocado seed and peel. Table 1. Quantification data of identified phenolic compounds from avocado seed and peel. StandardLOD(μg/mL)LOQ(μg/mL)Calibration Range (mg/L)Calibration EquationsR2Quinic acid (1)0.040.14(0.977–7.813)y = 1099.56 x − 21.480.999Quinic acid (2)0.040.14(3.906–31.25)y = 2155.60 x − 5059.590.99Procyanidin B1 (1)0.370.95(0.977–3.906)y = 336.61 x − 39.080.999Procyanidin B1 (2)0.370.95(3.906–15.625)y = 857.10 x − 1913.350.998Catechin0.461.43(1.953–31.25)y = 857.50 x − 748.370.999Quercetin0.080.19(0.488–31.25)y = 3177.80 x − 2495.070.997Quercetin glucoside0.090.29(0.488–31.25)y = 2820.85 x + 688.340.993Myrecetin-3-glucoside0.180.48(0.488–15.625)y = 1289.08 + 737.990.995Verbascoside0.090.29(0.488–15.625)y = 2199.92 x − 213.060.998 Table 2. Identification and quantification of phytochemical compounds in avocado seed and peel extracts with ethanol/water by HPLC-ESI-qTOF-MS. Table 2. Identification and quantification of phytochemical compounds in avocado seed and peel extracts with ethanol/water by HPLC-ESI-qTOF-MS. PeakRT (min)[M-H]−Mol. FormulaCompoundContent (mg/g DE)Seed 10.46343.0352C14H16O10Galloylquinic acid4.0 ± 0.120.62211.0805C7H16O7PerseitolNQ30.68191.0546C7H12O6Quinic acid3.3 ± 0.440.78191.0539C6H8O7Citric acid1.8 ± 0.354.28597.2170C28H38O14Picraquassioside CNQ64.41351.0695C16H16O9Chlorogenoquinone isomer 1NQ75.38443.1907C21H32O10PenstemideNQ86.05351.0705C16H16O9Chlorogenoquinone isomer 2NQ96.38387.1643-UnknownNQ108.66441.1741C21H30O10Hydroxyabscisic acid glucosideNQ118.81863.1824C45H36O18Procyanidin A trimer isomer 1NQ129.87863.1804C45H36O18Procyanidin A trimer isomer 22.5 ± 0.31310.12863.1821C45H36O18Procyanidin A trimer isomer 32.7 ± 0.51411.29472.1606-UnknownNQ1512.11461.2371-UnknownNQ1615.07329.2321C18H34O5Trihydroxyoctadecenoic acidNQ1716.01329.2330C18H34O5Trihydroxyoctadecenoic acidNQ1817.68315.2522C14H20O8Hydroxy salidrosideNQTotal phenolic amount14 ± 1Peel 10.46343.0360C14H16O10Galloylquinic acid3.1 ± 0.120.67191.0544C7H12O6Quinic acid5.7 ± 0.730.78191.0542C6H8O7Citric acid2.0 ± 0.340.83545.0979C14H20O7Trigalacturonic acidNQ55.37443.1907C21H32O10PenstemideNQ66.02351.0711C16H16O9Chlorogenoquinone isomer 1NQ76.02173.0445C21H32O10Shikimic acidNQ86.27351.0717C16H16O9Chlorogenoquinone isomer 2NQ96.80289.0704C15H14O6(Epi)catechin7 ± 2107.19577.4579C30H26O12Procyanidin B dimerNQ117.89865.1994C45H38O18Procyanidin B trimer isomer 12.1 ± 0.2128.221153.2635C60H50O24Procyanidin B tetramer isomer 11.31 ± 0.10138.55865.1959C45H38O18Procyanidin B trimer isomer 2NQ148.64441.1741C21H30O10Hydroxyabscisic acid glucosideNQ158.85863,1796C45H36O13Procyanidin A trimer2.8 ± 0.3168.971153.2576C60H50O24Procyanidin B tetramer isomer 22.1 ± 0.2179.11521.2003C26H34O11Isolariciresinol glucid derivativeNQ189.23625.1390C27H30O17Quercetin diglucoside isomer 13.9 ± 0.3199.33625.1389C27H30O17Quercetin diglucoside isomer 20.7 ± 0.1209.57565.2265C28H38O12Quercetin derivative isomer 11.48 ± 0.09219.81595.1292C26H28O16Quercetin arabinosyl glucoside isomer 13.4 ± 0.3229.90595.1311C26H28O16Quercetin arabinosyl glucoside isomer 20.6 ± 0.22310.00609.1468C27H30O16Quercetin rutinoside isomer 11.79 ± 0.092410.03505.2083C23H22O13Quercetin acetylglucoside1.10 ± 0.072510.08575.1190C30H24O12Procyanidin A dimer isomer 12.2 ± 0.22610.33575.1185C30H24O12Procyanidin A dimer isomer 22.0 ± 0.22710.36595.1302C26H28O16Quercetin arabinosyl glucoside isomer 30.53 ± 0.072810.49463.0859C21H20O12Quercetin glucoside isomer 11.5 ± 0.12910.62463.0849C21H20O12Quercetin glucoside isomer 20.55 ± 0.073010.73579.1335C26H28O16Luteolin pentosyl hexoside2.1 ± 0.23110.93565.1187C28H38O12Quercetin derivative isomer 20.52 ± 0.023211.00299.0178C15H8O7NorwedelactoneNQ3311.08609.1468C27H30O16Quercetin rutinoside isomer 29.6 ± 0.73411.18447.0891C21H20O11Quercetin rhamnoside isomer 10.52 ± 0.053511.32433.0750C20H18O11Quercetin arabinosideEvaluation of platelet-aggregation inhibition of avocado seed and peel against thrombus-formation agonists TRAP-6, ADP and collagen. Table 5. Evaluation of platelet-aggregation inhibition of avocado seed and peel against thrombus-formation agonists TRAP-6, ADP and collagen.
ExtractsTRAP-6 (10 μM)ADP (4 μM)Collagen (1 μg/mL)PA (%)Inh. (%)PA (%)Inh. (%)PA (%)Inh. (%)AS85 ± 1 ns089 ± 1 ns3 ± 140 ± 6 ***45 ± 2AP45 ± 1 ***42 ± 120 ± 2 ***78 ± 232 ± 6 ***55 ± 2Ctrl (−)88 ± 1094 ± 1082 ± 30Ctrl (+)22 ± 379 ± 227 ± 469 ± 427 ± 159 ± 2
Rojas-García, A.; Fuentes, E.; Cádiz-Gurrea, M.d.l.L.; Rodriguez, L.; Villegas-Aguilar, M.d.C.; Palomo, I.; Arráez-Román, D.; Segura-Carretero, A. Biological Evaluation of Avocado Residues as a Potential Source of Bioactive Compounds. Antioxidants 2022, 11, 1049. AMA Style Rojas-García A, Fuentes E, Cádiz-Gurrea MdlL, Rodriguez L, Villegas-Aguilar MdC, Palomo I, Arráez-Román D, Segura-Carretero A. Biological Evaluation of Avocado Residues as a Potential Source of Bioactive Compounds. Antioxidants. 2022; 11(6):1049. Chicago/Turabian Style Rojas-García, Alejandro, Eduardo Fuentes, María de la Luz Cádiz-Gurrea, Lyanne Rodriguez, María del Carmen Villegas-Aguilar, Iván Palomo, David Arráez-Román, and Antonio Segura-Carretero. 2022. "Biological Evaluation of Avocado Residues as a Potential Source of Bioactive Compounds" Antioxidants 11, no. 6: 1049. For more information on the journal statistics, click here.