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Avocado (*Persea americana*) Seed as a Source of Bioactive Phytochemicals

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Abstract: The pulp of avocado (*Persea americana*, Lauraceae) has been reported to have beneficial cardiovascular health effects. Avocado oil is used for dermatological applications and its unsaponifiable portion is reported to have beneficial effects against osteoarthritis. Although the seed represents a considerable percentage of the total fruit, scientific research on the phytochemistry and biological effects of avocado seeds is in the nascent stages,. Currently, the seed represents an under-utilized resource and a waste issue for avocado processors. There is ethno-pharmacological information on the use of seeds for the treatment of health-related conditions, especially in South American countries where avocados are endemic and currently grown on a large scale. Current research has shown that avocado seeds may improve hypercholesterolemia, and be useful in the treatment of hypertension, inflammatory conditions and diabetes. Seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities. The avocado seeds and rich in phenolic compounds, and these may play a role in the putative health effects. Historically, extracts of avocado seeds were also used as ink for writing and research in our laboratory has explored the potential colorant properties of a polyphenol oxidase-produced colored avocado seed extract. Here, we review the currently-available data on the bioactivity and other functional properties of avocado seeds. We discuss the strength of the available data, the putative active compounds, and potential directions for future studies.

Keywords: Avocado, *Persea americana*, phenolic compounds, human health, seed.

1. INTRODUCTION

The avocado (*Persea americana* Mill., Lauraceae) is a large drupe and has the highest oil content of all fruits, with the possible exception of the olive. The avocado's status as a food varies with the region where it is consumed and the degree of familiarity with which it is regarded by the local populace. The fruit is a traditional dietary staple in Guatemala and nearby countries. It is also an ingredient of widely consumed, elaborately prepared traditional foods in Mexico such as guacamole, which is composed of pureed avocado pulp and other vegetables [1]. The archaeological record reveals them to be one of the oldest food plants of Mexico (8000 BCE) [2]. Avocados are endemic to the tropical regions of the New World but are now cultivated around the world.

The avocado is a member of the commercially important plant family, Lauraceae, and has eight well-defined geographical ecotypes, of which three (so called horticultural races) comprise the commercial avocado crop. These horticultural races are the Mexican (*P. americana* var. *drymifolia*), the West Indian (*P. americana* var. *americana*) and the Guatemalan (*P. nubigena* var. *nubigena* and *P. nubigena* var. *guatemalensis*) [2]. Current commercial varieties are hybrids of these races. For example the Hass variety belongs to the Guatemalan-Mexican hybrid group [3]. Other common varieties in commerce include Bacon, Feurte, Gwen, Lamb Hass, Pinkerton, Reed and Zutano [4]. The Hass variety is commonly grown in Mexico and California and is one of the major varieties in commerce [5]. The fruits of the Hass variety are pear to ovoid shaped and the skin is tough, leathery and dark brown or black when ripe. It also contains a smaller seed than most other common varieties [6]. Total U.S. avocado production during the 2007/08 season was 170,000 tons: total world production in 2007/08 was 1.4 million tons with the largest producer being Mexico [1]. The European Union and North America are the largest importers of avocados in the world [3].

To date, most chemical and bioactivity studies have focused on the pulp (mesocarp) of the avocado. Avocado pulp contains up to 30% oil which is rich in monounsaturated fatty acids. These fatty acids have been proposed to have beneficial effects on cardiovascular disease risk factors [7, 8]. The pulp also contains several bioactive phytochemicals including carotenoids (lutein, zeaxanthin, α-carotene, cryptoxanthin), B vitamins, vitamins C and E, D-mannoheptulose, β - sitosterol, and persenone A and B. These constituents have demonstrated antifungal, antitumor and antioxidant activities [9]. Avocado pulp has been found to have *in vitro* growth inhibitory effects against cancer. Lutein, other carotenoids, and tocopherols are proposed as the active compounds [10]. Avocado pulp is also well known for its beneficial skin properties. The unsaponifiable fraction of avocado pulp in combination with soybean oil unsaponifiable is used for treatment of osteoarthritis, and new research shows its anti-carcinogenic and anti-inflammatory effects [4, 9, 11-13].

In the present review, we will discuss the current literature regarding the chemical composition, ethnopharmacological data, and modern scientific data regarding the beneficial biological effects of avocado seeds. We will review and critique available studies and attempt to provide insight into potential directions for future studies. It is our hope that this review will stimulate further discussion and new research efforts to understand the potential usefulness of avocado seeds with regard to human health.

2. AVOCADO SEEDS

The avocado seed represents up to 16% of the total weight of the fruit, has a rich phytochemical profile and a long history of ethnobotanical use [14, 15]. In spite of this, the avocado seed is largely considered a waste product and is an under-utilized resource. Modern scientific research into the potential bioactivities of avocado seeds remains in the early stages. Current knowledge on the biological activities of avocado seed extracts is summarized in Table 1.

2.1. Ethnobotanical and Historical use of Avocado Seed

Ethnopharmacological studies of the Aztec and Mayan cultures showed the use of decoctions of avocado seeds for the treatment of mycotic and parasitic infections [16]. Seed preparations have also been reported for use against diabetes and gastrointestinal irregularity. The seed is considered to have anti-anemic and diuretic effects

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Table 1. Summary of Current Findings Regarding the Biological Effects of Avocado Seed Extracts.

Effect	Model	Extract/dose	Main Effect	Reference
Anticancer	MDA-MB-231 breast cancer cells	Methanolic extract at concentrations greater than 100 μg/mL	Induction of apoptosis measured by increased caspase-3,caspase-7 and poly (ADP ribose) polymerase (PARP) cleavage.	Lee *et al.* 2008
Anti-inflammatory	Secretary PLA_2	Lipidic polyols	Inhibit activity of PLA_2.	Etoxioni 2003
	Keratinocytes *in vitro*	PFA isolated from pulp and seed	Decreased generation of pro-inflammatory mediators IL-6, PGE_2, and cyclobutane pyrimidine dimers after exposure to UVB radiation.	Rosenblat *et al.* 2010
Antidiabetic	Alloxan-induced diabetic rats	450 and 900 mg/kg bw seed extract treatment for 14 d	Reduced blood glucose levels by 47-55%. Histological study suggested restorative effect on pancreatic islets.	Edem 2009
	Alloxan-induced diabetic rats, non-diabetic rats	300 and 600 mg/kg bw aqueous seed extract for 21 d	Reduced plasma glucose concentrations by 73% and 78% respectively in diabetic rats. Plasma glucose concentrations of non-diabetic rats were reduced by 35-39%.	Edem, Ekanem, and Ebong 2009
Antihypertensive	NaCl induced hypertensive rats	200-700 mg/kg bw aqueous seed extract for 4 wk	Reduced blood pressure at all doses; reduced plasma TG, TC, and LDL at doses at 500 mg/kg bw and above.	Imafidon and Amaechina 2010
	NaCl hypertensive rats	200-700 mg/kg bw aqueous seed extract for 5 wk	Dose-dependently reduced blood pressure, cholesterol, glucose, urea an dsodium levels.	Kate and Lucky 2009
	Acetylcholine-induced hypertensive rats	260 mg/kg aqueous seed extract for 10 d	39.3-43.5% reduction in mean arterial pressure compared to baseline; heart rate reduced by 9.4-19.8%	Anaka, Ozolua, and Okpo 2009
Hypocholesterolemic	Mice	125, 250, and 500 mg ASF/kg bw dried avocado seed "flour" for 6 d	Significant reduction in TC and LDL-C.	Pahua-Ramos *et al.* 2012
	Cholesterol induced hyper-cholesterolemic mice	50-300 mg/kg bw methanolic seed extract for 10 d	Dose-dependent reduction in TC, TG, LDL-C, and VLDL-C	Asaolu 2010
	Rabbits	100 and 200 mg/kg bw aqueous seed extract for 2 mos	Dose-dependent reduction in TC, TG, LDL, and total lipids in the plasma	Nwaoguikpe and Braide 2011
Dermatological uses	Keratinocytes *in vitro*	Up to 5 μg/mL PFA derived from hexane seed extract	Increased cell viability; reduction in number of sunburned cells after irradiation; enhanced DNA repair	Rosenblat *et al.* 2010
	Keratinocytes *in vitro*	10 μg/mL water-ethyl acetate seed extract and HSCCC fraction of the seed	Proliferation and cell viability	Ramos-Jerz, Villanueva, and Deters 2007
	Human subjects	5-10% unsaponifiable fraction of avocado seed lipids applied to skin for 2-6 wk	Reduction of stretch marks, keratosis, redness, hypopigmentation, hyperpigmentation and ridging; increased skin moisture retention and skin elasticity; increased skin thickness.	Moy 1999
Dermatological uses	*In vitro* rat skins and tibias of 17 day old chick embryos	Unsaponifiable component of avocado seed oil	Inhibited action of lysyl oxidase, which can lead to wrinkles and lack of skin elasticity	Werman, Mokady, and Neeman 1990

(Table 1) Contd.....

Effect	Model	Extract/dose	Main Effect	Reference
	Hairless mice	3% natural or synthetic isolated linoleic furan from seed extract	Enhanced metabolic activity in dermal and epidermal tissues leading to increased elasticity, protein content, DNA content, skin thickness, fibroblast population, collagen synthesis, and glucose metabolism.	Counts and Huber 1996
	Human subjects	Avocado seed soaked in mineral oil for 4 wk then applied to the scalp	Prevents frying and falling of hair	Ruiseco 1989
Antimicrobial	Select gram positive and gram negative bacteria *in vitro*	125-250 μg/mL ethanolic seed extract	Antimicrobial effects shown against *Salmonella enteritidis, Citrobacter freudii, Pseudomonas aeruginosa,* and *Enterobacter aerogenes*	Raymond, Chia and Dykes 2010
	Candida spp, *Cryptococcus neoformans,* and *Malassezia pachydermatis in vitro*	Methanolic seed extract at 0.125-0.625 mg/mL, 0.08-0.156 mg/mL, and 0.312-0.625 mg/mL, respectively	Fungicidal effects	Leite *et al.* 2009
	S. aureus and *B. subtilis in vitro*	Crude terpenoid and alkaloid fractions from solvent seed extraction	Growth inhibition	Nagaraj *et al.* 2010
Insecticidal	*Aede aegypti*	16.7 mg/mL hexane seed extract; 8.9 mg/mL methanol extract	LC_{50} against *Aedes aegypti* larvae (yellow fever vector)	Leite *et al.* 2009
	Trypanosoma cruzi in vitro	>500 μg/mL methanolic extract	Moderate activity against mobilization of epimastigotes and trypomastigotes of *Tryanosoma cruzi*	Abe *et al.* 2005
Toxicity	Rats	2-10 g/kg aqueous extract administered as single dose	Showed no signs of toxicity in two wk following gavage	Ozolua *et al.* 2009
	Rats	2.5 g/kg bw for 28 d	No gross or hematological changes observed	Michaelakis *et al.* 2009
	Mice	1767 mg/kg bw	Determined to be LD_{50}	Ramos *et al.* 2012

[17]. A piece of the seed or the decoction put into a tooth cavity is reported to relieve toothache. A powder prepared from avocado seed is believed to cure dandruff and the seed oil is sometimes applied to skin eruptions [6]. Topical application of avocado seed paste has been used for treatment of arthritis. In Nigeria, it is mixed with soups and puddings because it is considered to be effective for management of hypertension [18]. The seed was also used to treat snakebite. Crushed seed has been used as a contraceptive and as an abortive agent [17]. In African tradition, the ground seeds were consumed to treat whitlows and dysentery [19]. Historically, colored exudate from avocado seeds was used as indelible ink by the *Conquistadors* in the 1500s [6].

2.2. Chemical Components of Avocado Seeds

The proximate composition of the seeds of two varieties of avocado, Hass and Feurte, was determined as follows: moisture content, 54.1% and 50.2%; ash content, 1.2% and 1.3%; protein, 2.4% and 2.5%; sugars, 3.5% and 2.2%; starch, 27.5% and 29.6 %; and fat 0.8 % and 1.0%, respectively [20]. Seeds of both varieties had lower fat content than the corresponding pulp.

The lipid and fatty acid composition of the pulp and seed of the Feurte, Bacon, and Hass varieties were investigated and the total lipid content of the seeds has been determined as 1.1, 1.6 and 1.1 %, respectively. The majority of lipids in the pulp were neutral lipids (95 – 99%) with a smaller amount of glycolipids (2.5 – 3.2%) and phospholipids (0.7 – 2.1%). By contrast in the seed, the percentage of neutral lipids was (77 – 80%) and the percentage of the other two fractions – glycolipids (12 – 13%) and phospholipids (7.4 – 10.9%) was higher. The neutral lipids were largely monoacylglycerols, diacylglycerols, free sterols and free fatty acids with a lesser amount of triacylglycerols in the neutral lipids of seeds than pulp [21].

Phytochemical studies on avocado seeds have identified various classes of natural products including phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins (PAC)s and other polyphenols (Fig. 1) [4, 22] . The levels of these compounds in the seeds vary with the variety of avocado, conditions of growth and stage of maturity. Measured levels are also influenced by the method of extraction employed in the experiment.

Soong and Barlow (2004) determined the phenolic compound levels in the seed and pulp as 88.2 and 1.3 mg/g gallic acid equivalent (GAE) [23]. Wang and others (2010) determined the phenolics for the seed of Hass variety as 51.6 mg/g GAE. By comparison, the phenolic compound content for the pulp was 4.9 mg/g GAE. The content of PACs in the Hass variety was found to be 47.7 mg/g. Phenolic content was found to be 137 μg/mg tannic acid in seed

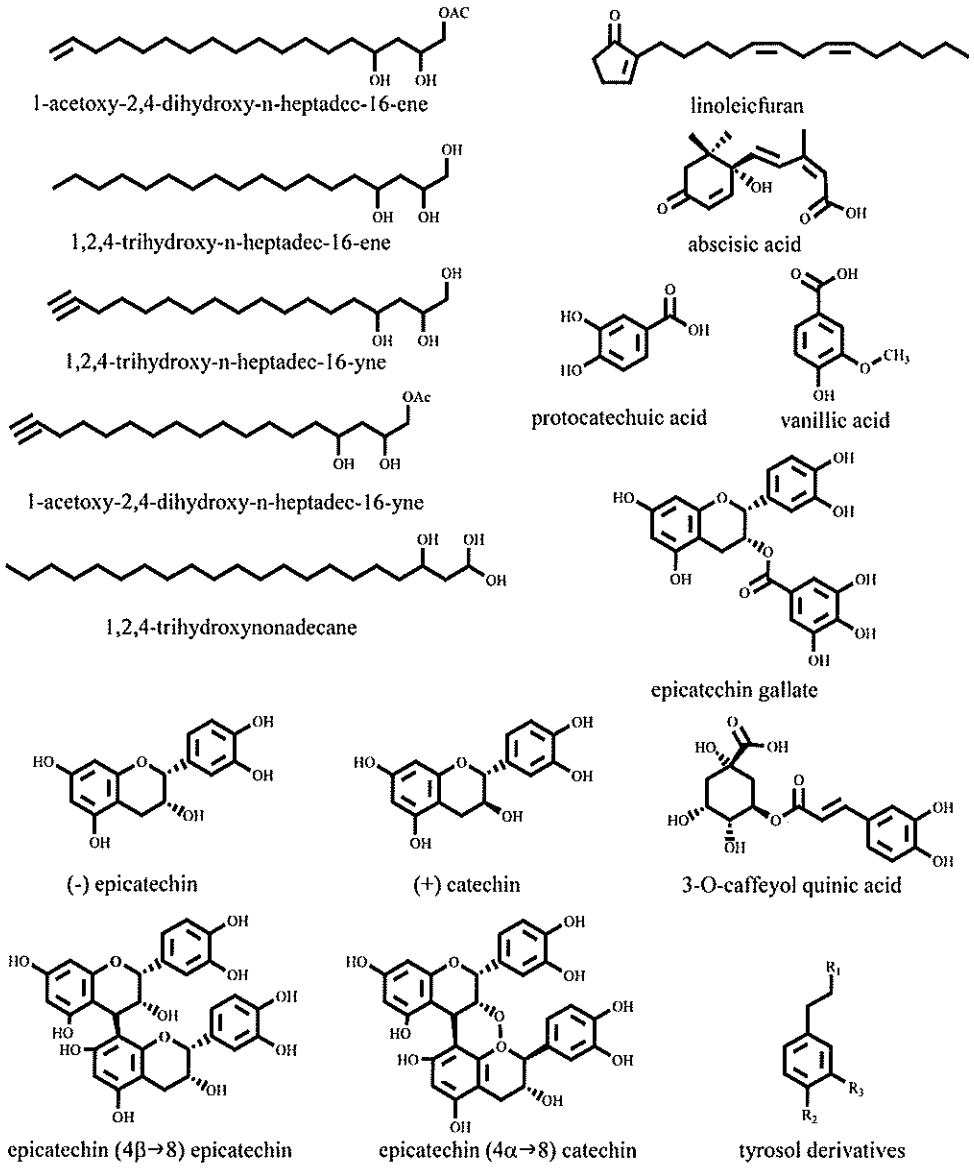

Fig. (1). Potential biologically-active phytochemicals in avocado (*Persea Americana* Mill) seeds.

[24]. Total phenolic content of ethyl acetate, acetone and methanolic extracts of seed were evaluated with the acetone extract containing the highest concentration of phenolic compounds (6082 mg GAE/g) followed by methanol and ethyl acetate extracts [25].

Catechin, epicatechin, and A- and B-type PACs (degree of polymerization = 2 – 6) and leucoanthocyanidins (3, 4-flavandiols) were identified as the major phenolic compounds in avocado seeds [23, 26, 27]. Epicatechin (4β→8)-catechin, epicatechin (4β→8) epicatechin, and the A-type trimers, A_2 –(+)-catechin and A_2-(-)-epicatechin have been reported in seed (Fig. 1) [28]. 3-O-caffeyol quinic acid (57.5 μg/g DW), 3-*O*-coumaroyl quinic acid (13.6 μg/g DW), procyanidin trimer A (170 μg/g DW), catechin/epicatechin gallate (152 μg/g DW) were isolated from the methanol extracts of Hass avocado seeds (Fig. 1) [29]. Pahua-Ramos *et al.* (2012) have reported that protocatechuic acid, kaempferide and vanillic acid are also present in the avocado seed (Fig. 1) [30].

2.3. Antioxidant Activity of Avocado Seeds

Avocado seeds have been found to have higher antioxidant activity than the pulp and this activity has been attributed to the high content of the phenolic compounds. The antioxidant capacity of avocado seeds has been studied mainly using electron transfer-based assays. Soong and Barlow (2004) found that an ethanol: water (1:1, v/v) extract of the avocado seed contained 1160 μmol/g and 1484 μmol/g ascorbic acid equivalent antioxidant capacity (AEAC) in the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) cation radical scavenging assay and the ferric reducing antioxidant power assay, respectively. These values were 55 and 155 times greater than that of the pulp, respectively.

The antioxidant activity of the ethyl acetate, acetone and methanol extracts of the avocado seed were compared using ABTS radical scavenging assay: the acetone extract had the highest antioxidant activity (158.3 mmol Trolox equivalent (TE)/g fresh weight) [25]. The radical scavenging capacity of 20 μg/mL of avocado seed methanolic extract using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was comparable to 15 μM Trolox. Nagaraj and others (2010) prepared alkaloid and terpenoid-rich extracts of avocado seeds and determined radical scavenging activity using the DPPH assay [31]. The terpenoid extract had higher antioxidant activity (4.9 μg/mL AEAC) than the alkaloid fraction (3.4 μg/mL AEAC).

The antioxidant activity of the seed extract has also been evaluated using the oxygen radical absorbance capacity assay, a hydrogen atom transfer-based reaction. The seed extract was found to contain 428.8 TE/g, whereas the pulp contained only 11.6 TE/g [32]. Total antioxidant levels were higher in the peel and seed tissues than in the pulp. The antioxidant activity of seeds was due to the presence of ascorbic acid and phenolic compounds, whereas D-mannoheptulose was found to be responsible for the antioxidant activity of the pulp [33].

2.4. Functional Properties of Avocado Seed

Despite its historical use as a medicinal agent and current advances in scientific research, the biological activities of avocado seeds remain underexplored. Avocado seed has been studied for anticancer, antidiabetic, cholesterol-lowering, antimicrobial, and hypotensive activity, as well as for its dermatological effects. Recently the potential of avocado seeds as a source of natural colorants was explored by the authors of this review [34].

2.4.1. Anticancer Effects

To date, only one paper has reported the anticancer effects of extracts of the avocado seed and peel. Treatment of MDA-MB-231 human breast cancer cells with the methanolic extract of avocado seed (100 μg/mL) led to induction of apoptosis as measured by increased caspase-3, caspase-7 and poly (ADP ribose) polymerase cleavage. DNA laddering was also observed. The peel extract exerted similar effects but was more potent. This observed differences in anticancer effects correlated with higher total phenolic compound levels and higher total flavonoid levels in the peel than in the seed [24]. Further studies are required to determine the spectrum of anticancer activity of the avocado seed methanolic extract, the active compounds, and whether anticancer activity is observed *in vivo*.

2.4.2. Anti-inflammatory Effects

Arachidonic acid is the precursor to a large family of compounds called eicosanoids which include the cyclo-oxygenase-derived prostaglandins and lipoxygenase-derived leukotrienes. Eicosanoids play important physiologic and pathologic functions including mediation of inflammation. The enzyme Phospholipase A (PLA_2) releases arachidonic acid from membrane phospholipids from the *sn*-2 position and thus plays an important role in inflammation [35]. Lipidic polyols purified from avocado seeds were found to inhibit activity of secretory PLA_2. The active compounds were identified as 1-acetoxy-2,4-dihydroxy-n-heptadec-16-ene (olefin A), 1,2,4-trihydroxy-n-heptadec-16-ene (olefin B), 1-acetoxy-2,4-dihydroxy-n-heptadec-16-yne (acetylene A) and 1,2,4-trihydroxy-n-heptadec-16-yne (acetylene B) (Fig. 1). Compared to the control, almost complete inhibition was observed after treatment with 12.2 mM of olefin A, 7.5 mM of acetylene A, 0.17 mM olefin B and 0.08 mM of acetylene B [36]. Although these findings are interesting, they must be interpreted with care since the effective concentrations are quite high.

Treatment of keratinocytes (1 μg/mL for 60 min) with polyhydroxylated fatty alcohols (PFA) isolated from seed and pulp prior to exposure to ultra violet (UV) B radiation resulted in decreased generation of pro-inflammatory mediators including interleukin (IL)-6 and prostaglandin E2 (PGE_2), as well as reduced formation of cyclobutane pyrimidine dimers. In human skin explants, treatment with PFA significantly reduced UV-induced cellular damage. The major constituents were identified as olefin A and acetylene B (Fig. 1) [37].

2.4.3. Antidiabetic Effects

Avocado seed extracts have been shown in laboratory models to reduce blood glucose and ameliorate diabetes. Treatment of alloxan-induced diabetic rats with an ethanolic seed extract (450 or 900 mg/kg bw) for 14 d reduced blood glucose levels by 47 – 55 %. Histological examination of the pancreas of treated rats suggested that the extract protected pancreatic islet cells [38]. Edem *et al.*, found that treatment with an aqueous seed extract (300 or 600 mg/kg bw) for 21 d reduced plasma glucose concentrations by 73 and 78 % respectively in alloxan-induced diabetic rats [39]. The glucose levels of non-diabetic rats were also reduced by 35 – 39% suggesting that the effects are not specific to the disease model. The authors hypothesized that the hypoglycemic effects are due to the presence of insulin-mimetic substances in the extract which stimulates production of insulin by β cells or enhances glucose utilization. This mechanism remains to be further studied.

2.4.4. Antihypertensive Effects

Avocado seed extract is used traditionally in Nigeria for the treatment of hypertension [40]. An aqueous seed extract from Fuerte avocados (200 - 700 mg/kg bw) significantly reduced the blood pressure in NaCl-induced hypertensive rats after 4 wk of treatment. A reduction in plasma triglycerides (TG), total serum cholesterol (TC) and serum low density lipoprotein-associated cholesterol (LDL-C) levels was also observed at doses of 500 mg/kg bw and higher [40]. In a similar study 200 – 700 mg/ kg bw aqueous avocado seed extract dose-dependently reduced blood pressure, cholesterol, glucose, urea and sodium levels in hypertensive rats after 5 wk [41]. Acute treatment of acetylcholine-induced hypertensive rats with aqueous seed extract (260 mg/kg bw) for 10 d led to a reduction in mean arterial pressure (39 – 44% reduction) and heart rate (9.4-19.8% reduction) [42].

2.4.5. Cholesterol-lowering Effects

Recent research has suggested that avocado seeds can reduce cholesterol in laboratory animal models. Avocado seed flour (125, 250 and 500 mg/kg bw) significantly reduced TC and LDL-C in mice after 6 d [30]. In another study, a dose-dependent reduction in TC, TG, LDL-C and very low density lipoprotein-associated cholesterol in hypercholesterolemic rats after treatment with a methanolic extract of avocado seed (50 – 300 mg/kg bw) for 10 d [43]. Nwaoguikpe and Braide (2011) observed similar effects in rabbits after treatment with an aqueous seed extract (100 and 200 mg/kg bw) for 2 mos [44].

2.4.6. Dermatological Effects

Components of avocado seeds may promote the growth of keratinocytes. Pretreatment of keratinocytes with avocado seed-derived PFAs improved cell viability following UVB irradiation compared to vehicle-treated controls [37]. The treatment also reduced the number of sunburned cells in human skin explants, enhanced DNA repair, and reduced the secretion of pro-inflammatory mediators IL-6 and PGE_2. Proliferation and cell viability of keratinocytes were also enhanced by treatment with10 μg/mL of a water-ethyl acetate extract of the avocado seed [45].

Avocado seed unsaponifiable fatty acids (UFA) have patented for use in a cosmetic base to improve skin quality in human subjects based of several different studies. In the first study, after six wk topical treatment, 28 of 40 volunteers showed at least mild or moderate improvement in stretch marks and keratoses. In a second study, treatment for 4 wk with UFA improved stretch marks compared to controls. A third study observed that redness, hypopigmentation, hyperpigmentation and ridging were reduced significantly as a result of treatment. A fourth study observed a 33% increase in skin moisture retention and an 8% increase in skin elasticity occurred compared to the control after 2 wk of UFA application. Addition of metal chelators improved the UFA effects on the damaged skin [46].

The unsaponifiable component of avocado seed oil was shown to inhibit the action of lysyl oxidase. This enzyme initiates cross-links in collagen and the overabundance of these cross-links results in wrinkles and a lack of skin elasticity [47].

Hairless mice treated for 14 d with 3% natural or synthetic isolated linoleic acid-acid conjugated furan enhanced metabolic activ-

ity in the skin, caused an increase in elasticity, protein content, DNA content and an increased epidermal thickness in the treatment group. Compounds with a similar structure, having at least 9 carbons units attached to number 2 position of furan ring were also considered effective [48]. The compounds have been the subject of two patents [48, 49].

2.4.7. Antimicrobial Activity

The antimicrobial activity of an ethanolic avocado seed extract was tested in select gram positive and gram negative bacteria. The extract was found to be effective against *Salmonella enteriditis*, *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* (minimum inhibitory concentration (MIC) = 125 to 250 μg/mL) [50]. The antimicrobial activity of crude terpenoid fractions and crude alkaloid fractions obtained using solvent extraction was assessed and both the fractions were found to inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis* [31].

Fungicidal effects of a methanolic avocado seed extract were observed against *Candida* spp, *Cryptococcus neoformans* and *Malassezia pachydermatis* with MIC = 0.125 to 0.625 mg/mL, 0.08 to 0.156 mg/mL and 0.312 to 0.625 mg/mL [22].

These results are interesting, but it is unclear how these extracts will perform *in vivo*. It is also unclear what phytochemicals account for the anti-microbial and anti-fungal activity. Further studies are needed in these areas.

2.4.8. Insecticidal Effect

There is a pressing need for safe and effective new insecticides to deal with the spread of insects representing agricultural pests or disease vectors [51]. Leite *et al.* (2009) tested the larvicidal activity and *in vitro* insecticidal potential of hexane and methanol extracts of avocado seeds and found that the median inhibitory concentration against *Aedes aegypti* larvae (Yellow Fever Vector) was16.7 mg/mL and 8.9 mg/mL for hexane and methanol extracts, respectively. Phytochemical analysis revealed the presence of 1,2,4-trihydroxy decane and β-sitosterol in the hexane fraction and flavonoids, tannins, alkaloids and triterpenes in the methanol extract: though the presence of these compounds was not directly related to the insecticidal effects [22]. A methanol extract of the avocado seed showed moderate activity against the mobilization of epimastigotes and trypomastigotes of *Trypanosoma cruzi in vitro* (MIC > 500 μg/mL). A series of 1,2,4-trihydroxy heptadecane derivatives and 1,2,4-trihydroxy nonadecane derivatives were isolated as active compounds (Fig. 1) [52].

2.4.9. Colorant Effects

Historical reports indicate that the Spanish *Conquistadors* used a colored exudate from avocado seeds as an indelible ink [6]. Studies in our laboratory have reported the development of a stable orange pigment when avocado seeds are crushed in air [34]. This development of color was dependent on the action of the enzyme polyphenol oxidase indicating that the resulting pigment is a polyphenolic compound. Further studies are needed to determine the identity of the compounds responsible for the orange color, and their colorant characteristics in various systems.

2.4.10. Safety of Avocado Seed Extract

Crucial to its use in various applications including as a natural colorant and as a potential medicinal agent is the safety of an avocado seed extract. Contrary to popularly held opinions, the fact that avocado seed extracts are derived from natural sources implies nothing about their inherent safety. To date, there have been a limited number of studies on this topic. Oral administration of a single bolus dose of an aqueous extract of avocado seed (2 – 10 g/kg bw) was found to result in no significant toxic effect in rats within two subsequent weeks of observation. Sub-chronic administration of the same extract (2.5 g/kg bw) for 28 d also caused significant gross toxicological or hematological changes [18, 51]. In another study, the median lethal dose of dried avocado seed in mice was determine as 1767 mg/kg bw [30]. These doses are quite high and suggest the overall safety of the aqueous seed extract. These data, however, remain preliminary and further studies are needed to more carefully determine the maximum tolerated dose of this extract. Additional studies are needed to determine the safe dose range for avocado seed extracts prepared with other solvents, since these are likely to concentrate different compounds.

3. CONCLUSION

Avocado seeds represent 12 – 16% of the total weight of the fruit, and currently is regarded a waste product. Phytochemical studies to date have indicated that the avocado seed is rich in a number of classes of secondary metabolites including polyphenols, fatty acids, and alkaloids. Emerging laboratory studies have indicated a number of potential health beneficial effects for avocado seed extracts including anticancer, cholesterol-lowering, antimicrobial, and anti-inflammatory effects. Although the currently-available data is promising, for most indications it remains very preliminary. Further studies are needed to identify putative active compounds, determine the spectrum of biological activity and whether activities observed *in vitro* can be translated to the *in vivo* situation. In addition, the safety of various extracts of avocado seeds must be assessed in order to more fully estimate the usefulness of this resource. In conclusion, there is a growing body of data indicating potential health beneficial effects of avocado seed extracts. Additional research will reveal if these promising results translate into clinically- or nutritionally-useful agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

ABBREVIATIONS

ABTS	=	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
AEAC	=	Ascorbic acid equivalent antioxidant capacity
BW	=	Body weight
DW	=	Dry weight
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
GAE	=	Gallic acid equivalent
IL	=	Interleukin
LDL-C	=	Low density lipoprotein-associated cholesterol
MIC	=	Minimum inhibitory concentration
PAC	=	Proanthocyanidin
PFA	=	Polyhydroxylated fatty alcohol
PGE_2	=	Prostaglandin E_2
TC	=	Total cholesterol
TE	=	Trolox equivalent
TG	=	Triglycerides
UFA	=	Unsaponafiable fatty acids
UV	=	ultraviolet

REFERENCES

[1] Knight RJ. History, Distribution and Uses. In: Whiley AW, Schaffer B, Wolstenholme BN, eds., The avocado: botany, production, and uses. CABI Pub.: Wallingford; New York 2002; pp. 1-12.

[2] Heiser CB. Origins of Some Cultivated New World Plants. Ann Rev Ecol Sys 1979; 10: 309-26.

[3] Chanderbali AS, Albert VA, Ashworth VETM, *et al.* Persea americana (avocado): bringing ancient flowers to fruit in the genomics era. BioEssays 2008; 30: 386-96.

[4] Ding H, Chin Y-W, Kinghorn AD, D'Ambrosio SM. Chemopreventive characteristics of avocado fruit. Sem Cancer Biol 2007; 17: 386-94.

[5] USDA. Avocado situation and outlook for selected countries. In: World Horticultural Trade & U.S. Export Opportunities 2006.

[6] Morton JF, Dowling CF. Avocado. In: Fruits of Warm Climate.: Winterville, N.C. 1987; pp. 91-102.

[7] Colette C, Percheron C, Pares-Herbute N, *et al.* Exchanging carbohydrates for monounsaturated fats in energy-restricted diets: Effects on metabolic profile and other cardiovascular risk factors. Int J Obesity 2003; 27: 648-56.

[8] Pieterse Z, Jerling JC, Oosthuizen W, *et al.* Substitution of high monounsaturated fatty acid avocado for mixed dietary fats during an energy-restricted diet: Effects on weight loss, serum lipids, fibrinogen, and vascular function. Nutrition 2005; 21: 67-75.

[9] Lu QY, Arteaga JR, Zhang Q, Huerta S, Go VLW, Heber D. Inhibition of prostate cancer cell growth by an avocado extract: role of lipid-soluble bioactive substances. J Nutr Biochem 2005; 16: 23-30.

[10] Lu QY, Zhang Y, Wang Y, *et al.* California Hass Avocado: Profiling of Carotenoids, Tocopherol, Fatty Acid, and Fat Content during Maturation and from Different Growing Areas. J Ag Food Chem 2009; 57: 10408-13.

[11] Altinel L, Saritas ZK, Kose KC, Pamuk K, Aksoy Y, Serteser M. Treatment with unsaponifiable extracts of avocado and soybean increases TGF-beta1 and TGF-beta2 levels in canine joint fluid. The Tohoku J Exp Med 2007; 211: 181-6.

[12] Kawcak CE, Frisbie DD, McIlwraith CW, Werpy NM, Park RD. Evaluation of avocado and soybean unsaponifiable extracts for treatment of horses with experimentally induced osteoarthritis. Am J Vet Res 2007; 68: 598-604.

[13] Boileau C MP, J., Caron J, Msika P, Guillou GB, Baudouin C, Pelletier JP. Protective effects of total fraction of avocado/soybean unsaponifiables on the structural changes in experimental dog osteoarthritis: inhibition of nitric oxide synthase and matrix metalloproteinase-13 Arthritis Res Ther 2009; 11: 1-9.

[14] Ramos-Jerz MDR. Phytochemical analysis of avocado seeds (*Persea americana* Mill., c.v. Hass). Cuvillier: Göttingen 2007.

[15] Oleata JA, Schwartz, M. and Contreras, S. Use of Hass avocado (Persea americana Mill.) seed as a processed product. In: VI World Avocado Congress (Actas VI Congreso Mundial del Aguacate): Chile 2007; pp. 1-8.

[16] Kunow MA. Plant Catalog: Appendix A. In: Kunow MA, ed., Maya medicine : traditional healing in Yucatan. University of New Mexico Press: Albuquerque 2003; pp. 129.

[17] Duke JA, Vásquez R. Amazonian ethnobotanical dictionary. CRC Press: Boca Raton, Fla. 1994.

[18] Ozolua RI, Anaka ON, Okpo SO, Idogun SE. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. African Journal of Traditional and Complementary Alternative Medicines 2009; 6: 573-8.

[19] Hatfield G. Encyclopedia of folk medicine : old world and new world traditions. ABC-CLIO: Santa Barbara, CA. 2004.

[20] Weatherby LS, Sorber DG. Chemical Composition of Avocado Seed. Ind Eng Chem 1931; 23: 1421-3.

[21] Takenaga F, Matsuyama K, Abe S, Torii Y, Itoh S. Lipid and fatty acid composition of mesocarp and seed of avocado fruits harvested at northern range in Jpn J Oleo Sci 2008; 57: 591-7.

[22] Leite JJG, Brito ÉHS, Cordeiro RA, *et al.* Chemical composition, toxicity and larvicidal and antifungal activities of Persea americana (avocado) seed extracts. Revista da Sociedade Brasileira de Medicina Tropical 2009; 42: 110-3.

[23] Soong YY, Barlow PJ. Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 2004; 88: 411-7.

[24] Lee SG, Yu MH, Lee SP, Lee IS. Antioxidant Activities and Induction of Apoptosis by Methanol Extracts from Avocado. J Korean Soc Food Sci Nutr 2008; 37: 269-75.

[25] Villa-Rodríguez JA, Molina-Corral FJ, Ayala-Zavala JF, Olivas GI, González-Aguilar GA. Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado. Food Res Int 2011; 44: 1231-7.

[26] Prabha TN, Patwardhan MV. Polyphenols of avocado (*Persea americana*) and their endogenous oxidation. J Food Sci Technol 1980; 17: 215-7.

[27] Rodriguez-Carpena JG, Morcuende D, Andrade MJ, Kylli P, Estevez M. Avocado (Persea americana Mill.) Phenolics, *In vitro* Antioxidant and Antimicrobial Activities, and Inhibition of Lipid and Protein Oxidation in Porcine Patties. J Ag Food Chem 2011; 59: 5625-35.

[28] Ramos-Jerz MDR. Phytochemical analysis of avocado seeds (*Persea americana* Mill., c.v. Hass). In: Cuvillier: Göttingen 2007.

[29] Kosinska A, Karamac M, Estrella I, Hernandez T, Bartolome B, Dykes GA. Phenolic compound profiles and antioxidant capacity of Persea americana Mill. peels and seeds of two varieties. J Agric Food Chem 2012; 60: 4613-9.

[30] Pahua-Ramos M, Ortiz-Moreno A, Chamorro-Cevallos G, *et al.* Hypolipidemic Effect of Avocado (*Persea americana* Mill) Seed in a Hypercholesterolemic Mouse Model. Plant Foods Human Nutr 2012; 67: 10-6.

[31] Nagaraj M, Sandhya V, Supriya G, *et al.* Antioxidant and antibacterial activity of avocado (*Persea gratissima* Gaertner) seed extract. World Appl Sci J 2010; 9: 695-8.

[32] Wang W, Bostic TR, Gu L. Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. Food Chem 2010; 122: 1193-8.

[33] Bertling I, Tesfay SZ, Bower JP. Antioxidants in 'Hass' Avocado. In: South African Avocado Grower's Association 2007; pp. 17-9.

[34] Dabas D, Elias RJ, Lambert JD, Ziegler GR. A colored avocado seed extract as a potential natural colorant. J Food Sci 2011; 76: C1335-41.

[35] Sudhir K. Lipoprotein-Associated Phospholipase A2, a Novel Inflammatory Biomarker and Independent Risk Predictor for Cardiovascular Disease. J Clin Endocrinol Metab 2005; 90: 3100-5.

[36] Etozioni S. Lipidic polyols from plants as a natural source for phospholipase A2 inhibitors. In: Biotechnology. Technion Israel Institute of Technology: Haifa 2003.

[37] Rosenblat G, Meretski S, Segal J, *et al.* Polyhydroxylated fatty alcohols derived from avocado suppress inflammatory response and provide non-sunscreen protection against UV-induced damage in skin cells. Arch Dermatol Res 2010: 1-8.

[38] Edem DO. Hypoglycemic effects of ethnaolic extracts of alligator pear seed (*Persea Americana* Mill) in rats. European J Sci Res 2009; 33: 669-78.

[39] Edem D, Ekanem I, Ebong P. Effect of aqueous extracts of alligator pear seed (*Persea americana* mill) on blood glucose and histopathology of pancreas in alloxan-induced diabetic rats. Pakistan J Pharm Sc 2009; 22: 272-6.

[40] Imafidon KE, Amaechina FC. Effects of Aqueous Seed Extract of Persea americana Mill. (Avocado) on Blood Pressure and Lipid Profile in Hypertensive Rats. Adv Biol Res 2010; 4: 116-21.

[41] Kate IE, Lucky OO. Biochemical evaluation of the tradomedicinal uses of the seeds of *Persea americana* Mill.,(Family Lauraceae). World J Med Sci 2009; 4: 143-6.

[42] Anaka ON, Ozolua RI, Okpo SO. Effect of the aqueous seed extract of *Persea americana* Mill (Lauraceae) on the blood pressure of sprague-dawley rats. African J Pharm Pharmacol 2009; 3: 485-90.

[43] Asaolu M, Asaolu, Samuel S, Oyeyemi S O and Aluko, BT. Hypolipemic effects of methanolic extract of *Persea americana* seeds in hypercholestrolemic rats J Med Medical Sci 2010; 1: 126-28.

[44] Nwaoguikpe R, Braide W. The effect of aqueous seed extract of *Persea americana* (avocado pear) on serum lipid and cholesterol levels in rabbits African J Pharm Pharmacol Res 2011; 1: 26-9.

[45] Ramos-Jerz MDR, Villanueva S, Deters AM. Influence of avocado seed fractions (*Persea americana* Mill.) obtained by HSCCC on human skin keratinocytes and fibroblasts: Differences of effects in regard of tested cell types. In: 55th International Congress and Annual Meeting of the Society for Medicinal Plant Research: Graz, Austria 2007.

[46] Moy LS. Cosmetic formulation and method for amerlioration of skin keratoses and striae distensae. US Patent 5759555 1999.

[47] Werman MJ, Mokady S, Neeman I. Partial isolation and characterization of a new natural inhibitor of lysyl oxidase from avocado seed oil. J Ag Food Chem 1990; 38: 2164-8.

[48] Counts DF, Huber R. Lipidic furans and retinol palmitate compositions useful for skin therapeutics. US Patent 5514709 1996.

[49] Ruiseco MG. Oil based scalp treatment composition. US Patent 4849214 1989.

[50] Raymond Chia TW, Dykes GA. Antimicrobial activity of crude epicarp and seed extracts from mature avocado fruit (Persea americana) of three cultivars. Pharm. Biol. Pharmaceutical Biology 2010; 48: 753-6.

[51] Michaelakis A, Strongilos A, Bouzas E, Koliopoulos G, Couladouros E. Larvicidal activity of naturally occurring naphthoquinones and derivatives against the West Nile virus vector (*Culex pipiens*). Parasitol Res 2009; 104: 657-62.

[52] Abe F, Nagafuji S, Okawa M, *et al.* Trypanocidal constituents in plants- evaluation of some mexican plants for their trypanocidal activity and active constituents in the seeds of *Persea americana*. Biol Pharm Bull 2005; 28: 1314-7.

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