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Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract

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ABSTRACT

Avocado (*Persea americana*) seeds represent under-utilised resources and waste issues in avocado processing. This study was produced to compare the lipid contents of the avocado bulb and seed. The study also evaluated the anti-oxidant, anti-inflammatory and anti-cancer potentiality of each extract. Oleic acid was the predominant unsaturated fatty acid in a chloroform/methanol extract of *P. americana* fruit and seed. The seed extract was richer with sterol compounds than the fruit extract. The extracts exhibited anti-inflammatory and anti-cancer activities against cell line of colon cancer (HCT116) and cell line of liver cancer (HePG2) in a dose-dependent manner. It also exhibited powerful scavenging of free radical by using- DPPH and ABTS. IC_{50} of seed extract against the aforementioned cancer cell lines was more or less near the values of a reference drug (sorafinib). In conclusion, *P. americana* seed extract has more powerful effects than avocado bulb extract. The seeds should not be neglected.

1. Introduction

Persea americana, avocado or alligator pear, has excellent nutritional and medical applications. It contains high amount of fats, proteins, fibres as well as vitamins and minerals such as (C, E, K, B1, B2, B6, B9) also, phosphorus, sodium, magnesium, potassium, iron and zinc (Orhevba and Jinadu, 2011; Oluwole et al., 2013; Maitera et al., 2014). In addition, it possesses sum claiming edible oil and it is a low sugar content in fruit that makes *P. americana* an essential recommended part in the diet of diabetic people. *P. americana* seed considered the under-utilised parts of the fruit non-edible, that are usually neglected remains. Abuse of this neglected part of fruits is in developing pattern that might demonstrate a chance to be precise gainful in the future. Using the remaining parts in fruits diminished the number of wastes and explore new active compounds in these parts especially anti-oxidants (Vinha et al., 2013; Mensah and Golomeke, 2015; Mensah et al., 2015). Consumable foods grown from the ground mash holds up to 33% oil rich with monounsaturated fatty acids (Ortiz et al., 2004).

Phytochemical compounds separated from *P. americana* may affect cell cycle arrest, retard development and activate apoptosis in some cancer cell lines (D'Ambrosio et al., 2011). The bioactive compounds separated from *P. americana* have a role in scavenging very active radicals. Essential anti-oxidants extracted from *P. americana* pith are the oxygenated carotenoids. Also, *P. americana* possess persenones A and B vitamins which prevent inflammation and carcinogenesis. Following to the American Dietetic Association (ADA), *P. americana* can be considered as an effective food due to its high nutritional value that has a valuable role in human health (Thomson et al., 1999).

The goal of this study was to isolate lipids of *P. americana*, identify sterols and fatty acids and assess their anti-oxidant, anti-inflammatory activities as well as anti-cancer potentiality against cell line of colon cancer (HCT116) and cell line of liver cancer (HePG2).

2. Materials and methods

2.1. Isolation of plant parts

P. americana fruits purchased from local market in Egypt. Fruits were peeled (FP), the seed was isolated from the fruit and the kernel was obtained by removing the seed cover (SK).

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2.2. Separation and identification of lipid content

Lipids of *P. americana* were separated by the method of Roughan and Bartt (1968).

2.3. Chemical composition of lipid

2.3.1. Analysis of the fatty acids composition by GC

Separation of lipid of (FP) and (SK) were methylated according to the method described by Luddy et al. (1960). Analysis of Fatty acid methyl ester using gas chromatography was occurred (Perkin Elmer Auto system XL) gas chromatography equipped with a flame ionization detector and fused silica capillary column (DB-5 (American) 60 m × 0.32 mm, i.e.) with a thick film (0.25/25 μm). Temperature program of column was initially 150 °C then gradually increased at rate of 3 °C/min up to 250 °C. Temperature of the injector and detector were 230 and 250 °C respectively. Helium used as carrier gas (1 ml/min).

2.3.2. Separation and identification of the hydrocarbon lipid

Lipid extract of the two parts of *P. americana* were identified using GC/MS, under certain conditions: Hewlett Packard HP 6890 prepared with methyl siloxane HP1 capillary column. Utilizing ionization detector (FID) and a gas carrier nitrogen. Flow rates of nitrogen, hydrogen and gas carrier were 30, 30 and 300 ml/min, respectively. The temperature of the oven was 70–280 °C. Sterols and hydrocarbon contents were detected and specified by comparing with standard matters injected under the same conditions.

2.4. Biological activity

2.4.1. Determination of free radical scavenging by ABTS and DPPH

Antioxidant activity of the lipid extract of *Persea americana* was measured using ABTS by method of Arnao et al. (2001) and DPPH free radical-scavenging were assayed by method of Ye et al. (2008)

2.4.2. The activity of total antioxidant of lipid extract of Persea americana

The total antioxidant assays were determined by Prieto et al. (1999).

2.4.3. Anticancer activity

Potential antitumor activity of *Persea americana* lipid extracts were tested using MTT (Mossman, 1983; Takeuchi et al., 1991).

2.4.4. Anti-inflammatory activity using serum bovine albumin

Anti-inflammatory of (FP) and (SK) of *Persea americana* were tested using the method of Rahman et al. (2015).

2.5. Data analysis

Data were analyzed statistically by ANOVA analysis of variance for three replicates and data were illustrated as mean ± SE at $p < 0.01$.

3. Results

3.1. Total lipid and fatty acid contents of the fruit and seed of P. americana

Table 1 represented the percentage of total lipid content in the chloroform/methanol extracts of the edible (fruit) and non-edible (seed) parts of *P. americana*. The results showed that the edible (fruit) part of *P. americana* is rich, with a higher percentage of lipid than the seed. The fatty acid compositions of isolated lipids were investigated by gas chromatographic analysis. Several fatty acids were present, as shown in Table 2. The fruit was rich with oleic acid (C18, 75.16%) and palmitic acid (C16, 14.72%); however, α-linoleic acid and linoleic acid represent 3.57% and 3.37% of the fatty acids, respectively. The seed of *P. americana* was fruitful, with a larger proportion of minor fatty acids than the fruits, as depicted in Table 2. The seed extract contained linoleic (39.43%), oleic (25.30%), palmitic (20.05%), palmitoleic (3.66%), stearic (3.36%) and α- linoleic acid (2.62%) acids.

3.2. Identification of hydrocarbon and sterols of fruits and seeds of P. americana

Predominant components of parts of *P. americana* were hydrocarbon and sterols. Other types of sterols campesterol, stigmasterol and sitosterol derivatives were the smallest components in the fruit than that seed of *P. americana* (Table 3).

3.3. Anti-oxidant activities of the lipid extracts of the fruit and seed of P. americana (scavenging activity by DPPH and capacity of total anti-oxidants)

The ATBS and DPPH inhibitory activity of the lipid extracts of the seed and fruit of *P. americana* is depicted in Figs. 1A and 1B. The lipid extract of seed showed a more pronounced inhibitory effect than that of the fruit, in a dose-dependent manner. It demonstrated strong, significant inhibitory activities at the highest concentration 200 μg/ml (69.73% and 36.64%, respectively for ATBS and DPPH) as compared with their relative standards, Trolox and BHT, at the same concentration (122.30 and 113.87%, respectively). Moreover, the total anti-oxidant capacity of the lipids of *P. americana* seed and fruit are expressed in Fig. 1C. The seed extract recorded higher significant activity than that of the fruit. Seed extracts demonstrated inhibition percentages depend on a dose pattern. The highest suppression percent of total anti-oxidant capacity occurred at the dose 300 μg/ml (435.28%), as compared with that of the standard (Vitamin C, 230.14%) at the same inhibitor concentration.

3.4. Anti-inflammatory activity of lipid extract of fruits and seeds of P. americana

Anti-inflammatory activity of isolated lipids from the fruit and seed of *P. americana,* shown in Fig. 2, revealed that both parts have significant potentiality as anti-inflammatory depends on a dose level (the activity increases as the dose increases), as compared with a standard. Isolated lipids from seed produced more pronounced effect than the isolated lipids from the fruit.

3.5. Anti-cancer activity of lipid extract of the fruit and seed of P. americana against cancer HepG2 and HCT11 cancer cell lines

The gradual, but marked inhibition of hepatocellular carcinoma and colon cancer cell lines is depicted in Figs. 3 and 4. These results show significant inhibition by the lipid extracts of both seed and fruit for cell line of hepatocellular carcinoma (HepG2) and cell line of colon cancer (HCT116), compared with the reference drug sora-

Table 1
Total lipids content of *Persea americana*.

	Total lipids %	
Persea americana	Seed	Fruit
chloroform/methanol extract	0.85	30.86

Table 2
Percentage of Fatty acids composition of fruit lipid peel and seed *Persea americana.*

Fatty acid	Common name	Fruit lipid extract	Seed lipid extract
$C_{14:0}$	Myristic acid	0.31	1.28
$C_{14:1}$	Myristoleic	0.27	1.22
$C_{16:0}$	Palmitic acid	14.72	20.05
$C_{16:1}$	Palmitoleic acid	0.19	3.66
$C_{18:0}$	Stearic acid	0.87	3.36
$C_{18:1}$	Oleic acid	75.16	25.30
$C_{18:(2\ ω\ 6)}$	Linoleic acid	3.37	39.43
$C_{18:(3\ ω\ 3)}$	α-Linolenic acid	3.57	2.62
$C_{20:0}$	Eicosadienoic acid	0.15	1.86
$C_{20:1}$	Eicosatrienoic acid	0.19	1.22

Table 3
Hydrocarbon and Sterol composition of fruit lipid peel and seed *Persea americana.*

Hydrocarbon &Sterols	Fruit lipid extract	Seed lipid extract
Octadecane (C_{18})	–	10.98
Nonadecane (C_{19})	36	10.4
Eicosane (C_{20})	–	8.55
Heneicosane (C_{21})	–	6.33
Docosane (C_{22})	62.88	30.79
Tricosane (C_{23})	0.8	10.99
Tetracosane (C_{24})	0.02	6.42
Pentacosane (C_{25})	0.01	0.56
Hexacosane (C_{26})	0.01	6.47
Heptacosane (C_{27})	0.01	2.66
Squalene	0.03	0.88
Octacosane (C_{28})	0.02	0.57
Nonacosane (C_{29})	0.02	0.68
Tricocontane (30)	0.02	0.51
Cholesterol	0.03	0.1
Campasterol	0.04	–
Stigmastero	0.02	1.11
β-Sitostero	0.1	2
Total hydrocarbon	99.82	96.79
Total sterols	0.18	3.21

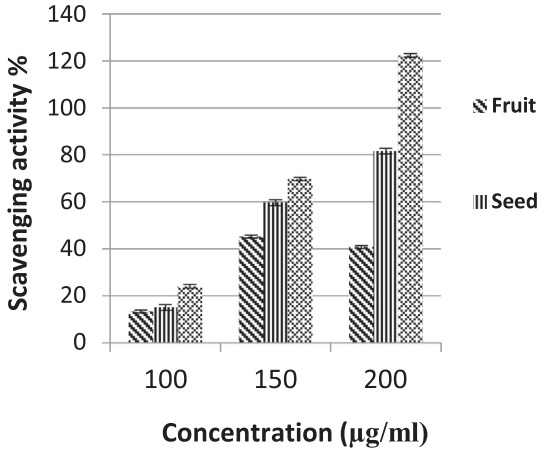

Fig. 1A. ATBS.

finib. Inhibitory effects of the seed lipids were highly significant in both cell lines, as compared with the other groups.

3.6. IC_{50} values of the lipid extracts of the fruit and seed of P. americana against HepG2 and HCT116 cancer cell lines.

IC_{50} values of extracts of the fruit and seed of *P. americana* against HepG2 and HCT116 cancer cell lines are presented in Figs. 5 and 6. The data revealed that the IC_{50} value of the extract of seeds was less than the IC_{50} value of the extract of fruit against both HepG2 and HCT116 cell lines, as compared with that of the reference drug.

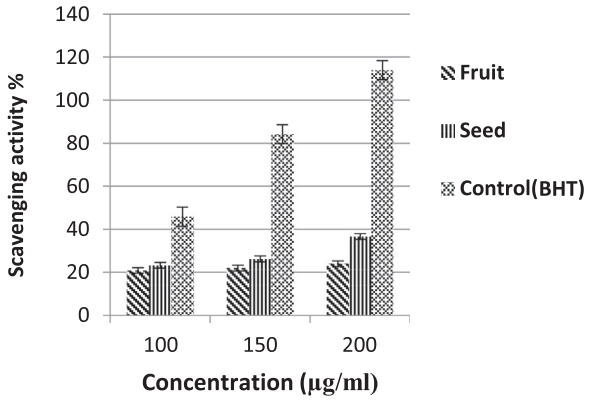

Fig. 1B. DPPH.

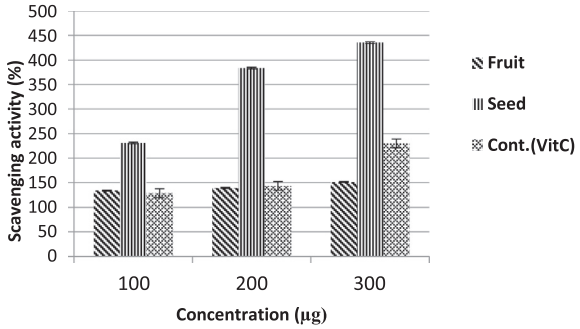

Fig. 1C. Total Anti-oxidant Capacity.

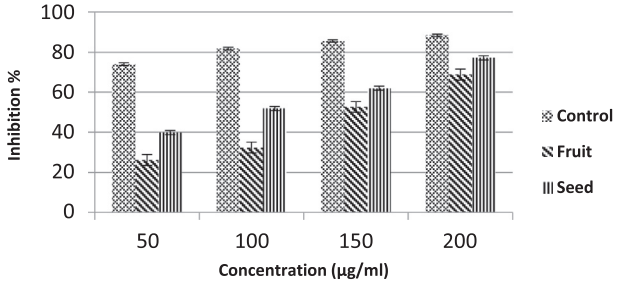

Fig. 2. Anti-inflamatory activity of *Persea americana* using bovin serum Albumin.

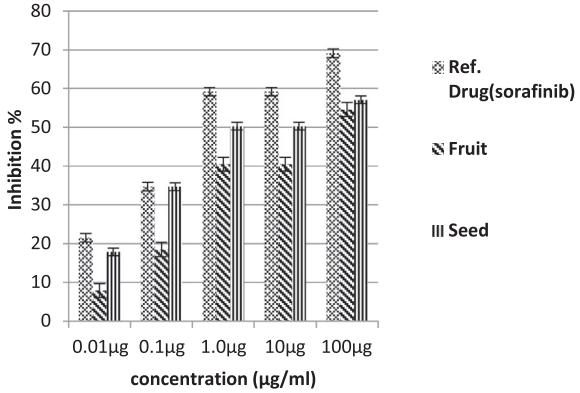

Fig. 3. Antitumer (HepG2).

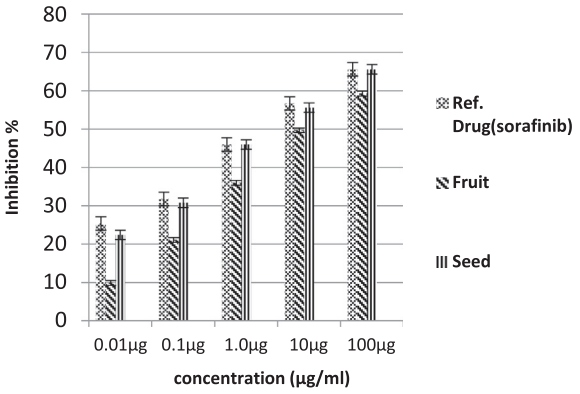

Fig. 4. Antitumer (HCT116).

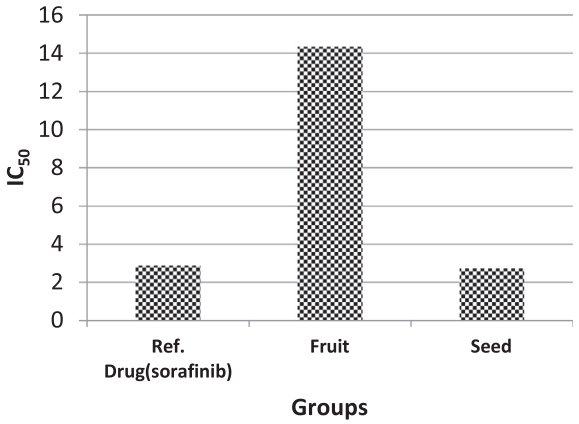

Fig. 6. IC_{50} of *Persea americana* lipid against human (HCT116).

4. Discussion

P. americana, known as avocado alternately gator pear, is a standout amongst those fruits have phenomenal dietary as well as medicinal qualities (Orhevba and Jinadu, 2011). Avocado seeds represent more than 16% of the total weight of the fruit. The seeds are rich with phytochemical compounds (Ding et al., 2007). This work was carried out to compare the fruit and seed of *P. americana*, by isolating the lipids content in each, identifying the hydrocarbon, sterols and fatty acids, and assessing the potentiality of anti-oxidant, anti-cancer and anti-inflammatory activities of each. Results demonstrated that total lipid content of fruit was larger than that of seed. These findings run parallel to those of Bwade et al. (2013); plants rich with oil in their fruits or seed considered a good industrial source of oil. Referring to the FAO, it was recorded by Akinoso and Raji (2011) that seeds contain more than 17% as seed oils. The major important properties of the avocado fruit are lipid components. Avocado fruits are rich with about five to six fatty acids in significant amounts. Those acids may be saturated fatty acids palmitic acid (16:0) and stearic acid (18: 0), the monounsaturated fatty acids oleic acid (18:1) and palmitoleic acid (16:1) and the polyunsaturated fatty acids linoleic acid (18:2) and linolenic acid (18:3). 60% of the total lipids is Oleic acid that was the most abundant monounsaturated fatty acid at both sites. Avocados have been reported to contain a monounsaturated fatty acid (MUFA)-rich fruit oil with 71% MUFA, 13% polyunsaturated fatty acids (PUFA), and 16% saturated fatty acids (SFA) (USDA and HHS, 2010a; Jakobsen et al., 2009). The aforementioned information confirms our finding that the total lipid content of the edible (fruit) and non-edible (seed) parts of *P. americana*, obtained using chloroform/methanol extraction, are rich with saturated fatty acids and oleic acid. Oleic acid is the major fatty acid in both parts. The chief component of the plant cell membrane is Phytosterols which are important like cholesterol in animal cell membranes. Higher concentration of plant sterols was detected in nuts and seeds of fatty foods (Hicks and Moreau, 2001). The chemical structure of phytosterols resembles that of cholesterol; but differ in the length of the side chain, that explains the ability of phytosterols to decrease cholesterol (Hicks and Moreau, 2001). Both the fruit and seed of *P. americana* are rich with other sterols. Stigmast-5-en-3-ol is abundant in both parts with the 59.55%. Other types of campesterol, stigmasterol and sitosterol derivatives are present in both parts, with different percentages present in the fruit and seed. These results are confirmed by Law (2000), who reported that β-sitosterol group is about 80% of sterols in the seed and fruit of *P. americana* present in un-saponifiable matter. Furthermore, Campesterol, stigmasterol, and cholesterol are the detected sterols. Lipid extracts of the seed and fruit of *P. americana* have significant ATBS and DPPH inhibitory activity. Moreover, the total anti-oxidant capacity of the extracted seed lipid was more pronounced than in fruit extract in a dose-dependent relationship. These findings are inconsistent with those recorded by Gómez et al. (2014), who found that the water and ethanol extracts of avocado seed have high anti-oxidant and inhibitory activity, and the seed extract reported a significantly greater effect than the fruit extract. Furthermore, the present work declared anti-inflammatory activity of seed chloroform extract of *P. americana* is pronounced than that of fruit. And it is also dose-dependent. It is well-known that oxidative stress play important roles in endothelial dysfunction, lung disease, gastrointestinal dysfunction, and atherosclerosis, and inflammatory symptoms are involved in these all disorders (Rozner and Garti, 2006). The anti-inflammatory compound of the avocado extract was observed in mice with carrageenan - induced oedema, as it reduced swelling after administration of 10 g/kg of extract (Adeyemi et al., 2002).

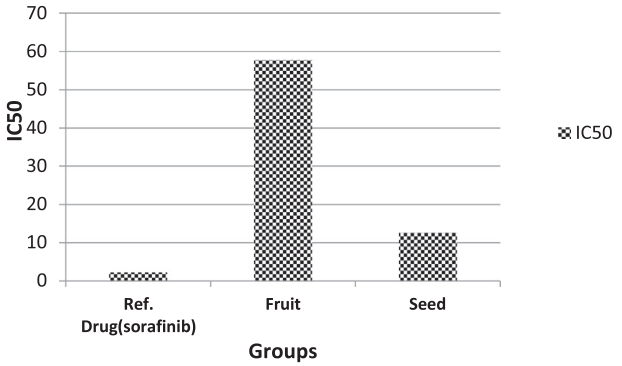

Fig. 5. IC_{50} of *Persea americana* lipid extract against HePG2.

Water extract of avocado exhibited small LC_{50} values 13.3 µg/ml in HepG-2 and 22 µg/ml in HT-29 cell lines, respectively. So, avocado considered a very favourable source of drugs treat cancer as it is effective in treating liver and colon cancers (Barakat et al., 2013). These findings run partially within our results, where the lipids extract of the fruit and seed of *P. americana* demonstrated significant inhibition of hepatocellular carcinoma HepG2 and colon cancer HCT116 cells versus the reference drug sorafinib. The inhibitory effects of the seed lipids were highly significant in both cell lines, as compared with the fruit lipids. Moreover, our results revealed that the IC_{50} value of the seed extract was less than that of the fruit extract against the HepG2 and HCT116 cell lines, as compared with the reference drug. Phytochemicals extracted from *P. americana* have a mazing effect in cell cycle arrest, growth inhi-

bition of some cancer cells through stimulation of apoptosis (D'Ambrosio et al., 2011). *P. americana* has been used in curing tumours in ethno medicine which offering a new therapeutic target may have a good impact on human (Paul et al., 2011).

5. Conclusion

A lipid extract of the seed of *P. americana* exhibited remarkably significant anti-oxidant, anti-inflammatory and anti-cancer effects, as compared with a lipid extract of the fruit. This may be refered to the high percentages of hydrocarbon, sterols and unsaturated fatty acids. These promising outcomes point to values of the waste parts of the plants may be behind their medicinal values in phytomedicine.

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