Antioxidant and antimicrobial activity of whole seed extracts of *Persea americana Mill*

Sooad Al-Daihan, Abeer M Aldbass, Latifah M Alotebi & Ramesa Shafi Bhat

**College of Science, Biochemistry Department, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia**

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**ABSTRACT**

*Persea americana Mill* used in traditional medicine for the treatment of various ailments and has antibacterial, antifungal, anti-viral and wound-healing properties. In the present study, antioxidant and antibacterial activity of different extracts of *Persea americana Mill* were investigated. Antioxidant potential of plant extracts was evaluated by means of total phenolic, total flavonoids content and DPPH radical scavenging activity. The highest phenolic and flavonoid content was observed in methanol extract while the lowest was achieved in aqueous extract. At concentration of 500 µg/mL, DPPH radical scavenging activity was found to be highest in methanol extract (70%) and lowest in aqueous extract (51%). Antibacterial activity of different extracts was evaluated by using the disk diffusion method. Highest antibacterial activity was observed with methanol extract against *S. pyogenes*, while minimum activity was observed with aqueous extract against *E.coli*.

**Introduction**

Human health is mostly affected by free radicals which can cause severe diseases like cancer and cardiovascular diseases by cell degeneration. Free radicals are produced during normal body function and also can be acquired from the environment. Oxygen radicals can cause damage to biomolecules (lipids, proteins, and DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, postischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging, and other degenerative diseases in humans[1]. Although our body contain many enzymatic and nonenzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage but it may not be adequate for continuous oxidative stress [2]. An imbalance between ROS and the inherent antioxidant capacity of the body, directed the use of dietary and medicinal supplements particularly during the disease attack. Studies on herbal plants, vegetables, and fruits have indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and proanthocyanidins. The antioxidant contents of medicinal plants may contribute to the protection they offer from disease. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders[3].

*Persea americana Mill* is a flowering plant belongs to the family Lauraceae. The English name is avocado, and it is widely cultivated in tropical and subtropical regions[4]. The fruits are loaded with nutrients, such as vitamin E, potassium, magnesium, vitamin B and K and monounsaturated fatty acids[5] (Navel et al., 2002). The plant is used in the management of hypertension [6] while the carotenoid content of the edible fruit pulp may play a significant role in cancer reduction[7]. The seeds of *P. Americana* are used in the treatment of diarrhea, dysentery, toothache and skin infections[8]. Apart from its use as food the avocado is traditionally utilized for various medicinal purposes including as hypotensive, hypoglycemic and anti-viral, and is applied for the treatment of ulcers and cardiovascular diseases[9]. In view of the medicinal uses of *Persea americana Mill* described above, the antibacterial activity of the extracts of plant together with their radical scavenging activities were studied. Phenolic and flavonoid content of the extracts were also determined.

**Material and methods**

**Seeds collection and extraction**

The commercially available *Persea americana* var. Wurtz were collected Riyadh. Uniform seeds of fruit were selected, rinsed with water and Distilled Water (DW) and dried under a chemical hood. The dried material was ground into a powder using mortar and pestle and passed through a sieve of 0.3mm mesh size. The powder obtained was extracted with different solvents like methanol, ethanol, ethyl acetate, and water for 48
The antimicrobial assay was performed by paper disc method with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

**Determination of Total Phenolic Content**

The TPC of the extracts of *Persea americana* was measured by the Folin-Ciocalteu method described with some modifications[10]. Briefly, an aliquot of 0.5mL of sample solution (with appropriate dilution to obtain absorbance in the range of the prepared calibration curve) was mixed with 1.0mL Folin-Ciocalteu reagent (10 times dilution before use) and allowed to react at 30°C for 5min in the dark. Then 2.0mL of saturated Na2CO3 solution was added and the mixture was allowed to stand for 1h before the absorbance of the reaction mixture was read at 747 nm. A calibration curve, using gallic acid with a concentration range of 0.01–0.10 mg/mL, was prepared. The TPC of the samples was standardized against gallic acid and expressed as mg gallic acid equivalent (GAE) per gram of sample on a dry weight basis.

**Total flavonoids estimation**

Aluminium chloride colorimetric technique was used for flavonoids estimation [11]. Each extract (0.5 mL of 1:10 g mL-1) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was plotted by preparing the quercetin solutions at concentrations 12.5 to 100 g mL-1 in methanol.

**DPPH Radical Scavenging Activity**

DPPH method was carried out according to the method modified by Kim et al.[12]. An aliquot of the radical formed from DPPH was left to react with 50,100–500 μg/mL of the extract for 30min. The absorbance was read at 517 nm. Ascorbic acid was used as the standard (10mg/10 mL). The percentage of radical inhibition was calculated by the following formula:

% inhibition = [1 – \((Ae/A0)\)] × 100, (1)

where A0 is the absorbance without sample, and Ae is absorbance with sample.

**Antimicrobial activity**

The antimicrobial assay was performed by paper disc diffusion method[13]. For all the bacterial strains, overnight cultures grown in broth were adjusted to an inoculum size of approximately 106 CFU/ml for inoculation of the agar plates. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates and plates were allowed to dry for 5 min. Sterile filter paper discs (6 mm in diameter) impregnated with different test extracts (1 mg/disc) were then placed on the surface of seeded agar plate. The plates were then incubated at 37°C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

**Statistical analysis**

The values are expressed as mean ± standard deviation(SD). The results were evaluated by using the SPSS (version12.0) and Origin 6 softwares.

**Results and discussion**

**Total Phenolic and total flavonoids content**

Phenolic compounds in plants are powerful free radical scavengers that can inhibit lipid peroxidation by neutralizing peroxyl radicals generated during the oxidation of lipids[14]. The TPC of the different extracts of *Persea americana Mill* was assayed by the Folin-Ciocalteu method using gallic acid as standard. It was found that the TPC of different extracts was in the descending order of methanol > propanol > ethanol > acetone > Aqueous extract (Table 1). The highest TPC of 2.7mg GAE/g was obtained in methanol, whereas the lowest TPC of 1.2mg GAE/g was achieved in aqueous extract. It is well known that plants contain many phenolic compounds which contain a hydroxyl group on an aromatic ring. These phenolic compounds interrupt chain oxidation reactions by donation of a hydrogen atom or chelating metals. So they act as reducing agents and antioxidants[15]. Total flavonoids content of aqueous extract of *Persea americana Mill* was expressed as mg quercitine equivalents/g of extract. Samples were analyzed in triplicate. Table 1 represents the data for flavonoid content of all test extracts of *Persea americana Mill*. Plants possess high amounts of polyphenols and flavonoids and potent antioxidant activity leading therefore to various defensive and disease fighting properties. Phenolic compounds are plants secondary metabolites considered as very important plant constituents due to the presence of one or more hydroxyl groups on their aromatic ring. Those phenolic compounds being non harmful to human’s health, there is an increase of the use of plants with high phenolics amount in the food industry aiming to improve the quality of foods[16].

| Plant        | Total phenol(mg GAE/g) | Flavonoids(mg quercitine /g) |
|--------------|------------------------|------------------------------|
| Methanol     | 2.7                    | 0.51                         |
| Propanol     | 2.6                    | 0.47                         |
| Ethanol      | 2.2                    | 0.46                         |
| Acetone      | 1.4                    | 0.44                         |
| Aqueous      | 1.2                    | 0.32                         |

| Table 1: Concentration of total phenol and flavonoids in different extracts of *Persea americana Mill* |
DPPH radical scavenging activity

DPPH free radical scavenging activities of the different extracts of *Persea americana* Mill are shown in Figure 1. For each sample, six concentrations (50, 100–500 μg/mL) of the plant extract were tested. All tested extracts showed a DPPH scavenging effect in a concentration-dependent manner. Methanol extract exhibited considerably higher DPPH radical scavenging activity than other extracts, and the lowest DPPH radical scavenging rate was found in aqueous extract. The free radical scavenging activities of different extracts decreased in the order of methanol > acetone > propanol > ethanol > aqueous extract. DPPH radical scavenging assay is one of the most commonly used methods to evaluate the radical scavenging activity of antioxidants because of its quickness, reliability, and reproducibility. This method depends on the reduction of the purple DPPH by accepting an electron or hydrogen radical to become a stable diamagnetic molecule with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the antioxidant compounds or extracts in terms of hydrogen-donating ability [17,18]

![Fig 1: DPPH radical scavenging activity of various extracts of *Persea americana* Mill](image)

Antibacterial activity

Antibacterial activities of the different extracts of *Persea americana* Mill against the tested organisms are shown in Table 2. Almost all the extracts tested showed antibacterial activity; however, the extracts differ in their activities against the micro-organisms tested. Highest antibacterial activity was observed with methanol extract against *S. pyogenes*, while minimum activity was observed with aqueous extract against *E. coli*. Some extracts displayed a potent antibacterial activity indicating that this seeds could be a good source for the antibacterial to combat bacterial infections. However, the larger inhibition zone was found against the Gram-positive bacteria when compared against the Gram-negative bacteria. Our results are in agreement with several previous findings demonstrating greater activity of the plant extracts towards Gram-positive bacteria [19]

| Test extract  | *S. aureus.* | *B. subtilis* | *S. pyogenes* | *E. coli.* | *P. aeruginosa* |
|---------------|--------------|-------------|--------------|------------|----------------|
| Methanol      | 15±33        | -           | 21±98        | 13±44      | 12±80          |
| Propanol      | 13±41        | -           | 20±00        | 15±87      | 17±70          |
| Ethanol       | 17±76        | -           | 20±65        | 18±12      | 15±66          |
| Acetone       | 13±88        | 10±00       | 13±23        | 14±65      | 17±55          |
| Aqueous       | 10±61        | -           | -            | 9±88       | 10±60          |
| Kanamycin     | 26.5 ±0.33   | 25±00       | 28 ±0.57     | 20 ±0.33   | 25 ±0.10       |

Table 2: Antibacterial activity of various extracts of *Persea americana* Mill against bacterial species tested by disc diffusion assay

Values are mean inhibition zone (mm) ± S.D of three replicates
Conclusion
Plants under study contain active antioxidants and antibacterial compounds that could be further isolated and possibly be exploited for pharmaceutical use.

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