

UV-Vis, FTIR and antioxidant study of *Persea Americana* (Avocado) leaf and fruit: A comparison

UV-Vis, FTIR y estudio antioxidante de *Persea Americana* hoja y fruto (Avocado): Una comparación

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Recibido: 21-11-2015. Aceptado: 15-03-2016

Abstract. Avocado (*Persea americana* Miller), a well-known nutritional tree crop grown in many parts of the world. The present study compares the UV-Vis and Fourier transform infrared spectroscopy (FTIR) spectrum of Avocado leaf and fruit (peel, pulp, and oil) cultivated in Ecuador; and further its antioxidant activity is evaluated against 1,1-diphenyl-2-picrylhydrazyl (DPPH•). The UV-Vis and FTIR study revealed predominant amount of flavonoids. Among the Avocado leaf and fruit studied, the DPPH• free radical scavenging assay for Avocado leaf had the highest antioxidant activity ranging from 84.46 % to 80.12% with values from 32.60-32.73 μg gallic acid equivalents per mL. It showed that the order of antioxidant activity in Avocado is leaf > peel > oil > pulp. The antioxidant activity had a positive correlation with total flavonoid content and these plant extracts (specially Avocado leaf) are useful for future antioxidant products.

Keywords. antioxidant, FTIR, *persea americana* fruit, phytochemicals, UV-visible.

Resumen. El aguacate (*Persea americana* Miller) es una conocida fruta arbórea con un alto contenido nutricional que crece en varias partes del mundo. El presente estudio compara los espectros del UV-Vis y del espectrómetro infrarrojo con transformada de Fourier (FTIR) de la fruta y de la hoja de aguacate (cáscara, pulpa y aceite) cultivado en Ecuador y posteriormente evalúa su actividad antioxidante empleando el 1,1-difenil-2-picrilhidrazil (DPPH•). El estudio de los espectros UV-Vis y FTIR reveló que el aguacate tiene predominantemente flavonoides. Entre la hoja y el fruto del aguacate, se comprobó mediante el ensayo DPPH• (captura de radicales libres), que la hoja tuvo una mayor actividad antioxidante, la que oscila entre 84,46% y 80,12%, con valores de 32.60-32.73 μg equivalentes de ácido gálico por mL. Se demostró que el orden de la actividad antioxidante de los extractos es: hoja de aguacate > cáscara > aceite > pulpa. La actividad antioxidante tuvo una correlación positiva con el contenido total de flavonoides y estos extractos de plantas (especialmente de las hojas del aguacate) son útiles para el desarrollo de futuros productos antioxidantes.

Palabras claves. antioxidante, fitoquímico, FTIR, *persea americana* fruta, UV-visible.

Forma sugerida de citación: B. Kumar, y L. Cumbal (2016), "UV-Vis, FTIR and antioxidant study of *Persea Americana* (Avocado) leaf and fruit: A comparison", Revista de la Facultad de Ciencias Químicas, N°. 14, Enero - Abril, pp.13-20, ISSN: 1390 - 1869.

1. Introduction

The *Persea americana* Miller or Avocado is an economically valuable tree crop, indigenous to tropical America and belongs to the Lauraceae plant family. This evergreen tree is native to the Central American region and bears fruit that ripens when fallen. It is appreciated worldwide because of its special organoleptic characteristics and nutritional value [1]. From a nutritional point of view, a fruit produces extremely high oil content, which is the main component of its dry weight, containing a high content of unsaturated fatty acids (55-65%, oleic acid), vitamin E, vitamin C, vitamin B6, β -carotene, and potassium [2]. It is recommended for hypercholesterolemia, hypertension, gastritis, anemia, exhaustion, and gastroduodenal ulcer [3].

The leaf extract of Avocado has been studied chemically and the presence of several constituents has already been established. These include triterpene glycosides, coumarins, saponins, alkaloids, tannins, reducing sugars and flavonoids [4]. The leaves have been reported as an effective antitussive and antidiabetic, and for relief of arthritis pain, by traditional medicine practitioners of the Ibibio tribe in Southern Nigeria. Analgesic and antiinflammatory properties of the leaves have been reported [5].

Avocado oil is very similar to olive oil and highly digestible. This, together with its low sugar content and high energy value, makes it an ideal food source for diabetics [6]. Edible oils such as avocado, Sacha inchi and olive oils contain high levels of oleic acid, a stable omega-9 monounsaturated fatty acid that is good for health. An epidemiological study in the Mediterranean region, where the diet includes a large quantity of monounsaturated fatty acids (MUFAs), shows a low incidence of atherosclerotic cardiovascular disease [7]. It is reported that an avocado-enriched diet can reduce total and low density lipoprotein (LDL) cholesterol levels whilst increasing high density lipoprotein (HDL) cholesterol levels which lowers the risk of atherosclerotic cardiovascular disease [8]. The avocado fruit contains vitamins E and C, carotenoids and sterols that possess antioxidant and radical scavenging activities [9], these being deficient in people living in the United States of America [10]. Antioxidants are considered important nutraceuticals on account of many health benefits. In general, antioxidants are the chemical substances that neutralize reactive oxygen species (ROS) and used for treating various human diseases related to muscle, lungs, heart, kidney, brain and helps to control the aging process. Antioxidants effectively function in the human body by inhibiting or delaying the formation of free radicals and lipid peroxidation that are mainly responsible for many human diseases and the aging process [11], [12]. Fruits and vegetables based natural compounds have been accounted for a wide range of biological properties such as antioxidant, anti-inflammatory and antimicrobial activities [13–15]. The presence of different phytochemicals such as polyphenolic compounds, ascorbic acid, tocopherols, carotenoids, and their combined activities result in the total antioxidant activity of a plant. However, polyphenolic compounds from plants appear to have the greatest antioxidant potential and could be the most beneficial antioxidants [16].

The requirement of a standard assay is very important in order to compare the results of different laboratories and validation of the conclusions. 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge. Scavenging of DPPH• radical is the basis of the popular DPPH• antioxidant assay [17, 18].

With these evidences, the present study highlights the UV-vis, Fourier transform infrared spectroscopy (FTIR) and antioxidant studies of Avocado leaf, peel, pulp and oil cultivated in the Andean region of Ecuador. To the best of our knowledge, no one has been reported the comparison of aqueous extract of leaf, pulp, peel and oil of Avocado and further characterized by various analytical instruments and assess their antioxidant activity.

2. Methods

2.1 Chemicals

Gallic acid (97.5-102.5%) and 1, 1-diphenyl-2- picrylhydrazyl (DPPH•, >99.5%) were purchased from Sigma Aldrich, USA. Milli-Q water was used in all experiments.

2.2 Plant Material

In this experiment, the leaves and fruits of avocado were collected in June 2015 from the residential colony Playa Chica 1; avocado oil was purchased from the Sangolqui, market near Universidad de las Fuerzas Armadas ESPE, Ecuador. The extraction procedure was carried out by adding 5 gm of avocado leaves, peel and pulp to 50 mL water and the mixture was heated for 90 mins at 60-65°C. Further, the mixture was filtered by using Whatman No. 1 paper and stored at 4°C for future studies. The avocado oil mixture is prepared by adding a mixture of avocado oil (50 µL) and acetone (950 µL) to 9 mL of water to in 25 mL round bottom flask under constant stirring at 25 °C.

2.3 Evaluation of antioxidant activity

The antioxidant activity of the leaves and fruits of avocado extracts; and avocado oil were measured by using DPPH• as a free radical model and a method adapted from [19] with slight modification. An aliquot (200- 40 µL) or control and (1800 -1960 µL) of H₂O was mixed with 2.0 mL of 0.2 mM DPPH• in 96% ethanol. The mixture was vortexed vigorously and allowed to stand at 22-25°C for 30 mins in the dark. Absorbance of the mixture was measured spectrophotometrically at λ_{max} 517 nm, and the free radical scavenging activity was evaluated using Eq. (1):

$$\text{Scavenging effect(\%)} = [1 - \{\text{absorbance of sample} / \text{absorbance of control}\}] * 100$$

Equation (1)

The scavenging percentage of all samples were plotted. The final result was expressed as % of DPPH• free radical scavenging activity (µL).

2.4 Characterization of Avocado fruit and leaf extracts

UVvis spectra was measured using a Thermo Spectronic, GENESYSTM 8, England spectrophotometer. FTIR spectra were recorded on a Perkin Elmer (Spectrum two) spectrophotometer to determine the functional groups present in Avocado fruit and leaves.

3. Results

3.1 Visual and UV-vis study

The schematic diagram for the extraction of Avocado (a) leaf, (b) peel, (c) pulp extract, and (d) oil in water is shown in Figure 1. It clearly showed that the color of Avocado leaf and peel extract are more darker/ yellowish than the pulp extract and oil. It is due to the abundance of polyphenolics/flavonoids [20]. The UV-Vis analysis is a reliable method to predict the initial phytoconstituents in plant material. Figure 2 shows the UV-Vis absorption spectrum of Avocado (a) leaf, (b) peel, and (c) pulp extract in water, indicating that all of these compounds display maximum absorption in the vicinities of 260-270 nm and 320-360 nm, which are attributed to the presence of coumarins, saponins, alkaloids, tannins, reducing sugars, catechins, epicatechins, flavonoids and polyphenolic (catechins, hydroxyl benzoicacids, hydroxyl cinnamic acids) [4, 21, 22]. Whereas (Figure 2-d) oil doesnt shows and desired peak in the

range of 260-360 nm. Interestingly, the avocado leaf and peel had significantly higher amounts of flavanoids than the pulp and the oil [21]. It is seen from the Figure 2 that the polyphenolics/flavonoids contents in Avocado increases in the following order: pulp < oil < peel < leaf. These results can be explained by the appearance of UV-Vis absorption band in between 250-350 nm.

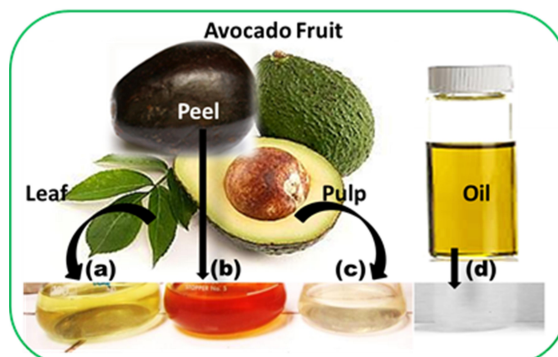


Figure 1. Schematic diagram for extraction of Avocado (a) leaf, (b) peel, (c) pulp extract, and (d) oil in water.

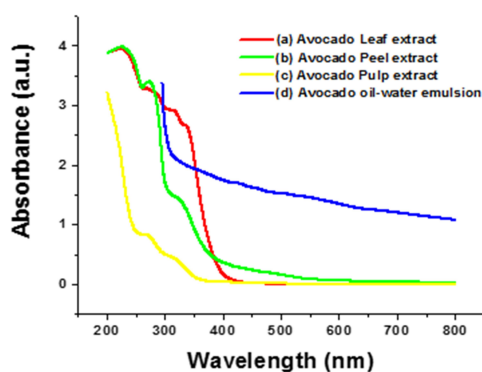


Figure 2. UV-Vis spectrum of Avocado (a) leaf, (b) peel, (c) pulp extract, and (d) oil in water.

3.2 FTIR study

FTIR technique was applied to determine the functional groups present in the Avocado leaf, peel, pulp and oil. Assignments of FTIR peaks and bands to functional groups are given in Table 1. Figure 3 shows an example of the FTIR spectra of Avocado leaf, peel, pulp and oil from the region $4000-650\text{ cm}^{-1}$. As observed in Figure 3, the asymmetric ($2916-2924\text{ cm}^{-1}$) and symmetric ($2849-2853\text{ cm}^{-1}$) CH/CH_2 stretching vibrations of all samples were similar to each other, except O-H stretch regions ($3300-3284\text{ cm}^{-1}$) was absent in oil. The strong peak at 1743 cm^{-1} (Figure 3(d)) arises from $\text{C}=\text{O}$ stretching vibrations of aldehydes and ketones from triglycerides and polyphenols [23]. Whereas weak peaks at 1731 cm^{-1} and 1737 cm^{-1} supports the low abundance of triglycerides. The presence of strong peaks at $1671-1611\text{ cm}^{-1}$, possibly due to the stretching $\text{cis C}=\text{C}/\text{C}=\text{O}$ carbonyl. The absorbance bands range at $1463-1415\text{ cm}^{-1}$, $1371-1377\text{ cm}^{-1}$ and 1315 cm^{-1} arise from CH_2 and CH_3 scissoring vibration, while those at $1159-1165\text{ cm}^{-1}$, 1117 cm^{-1} , and $1032-1096\text{ cm}^{-1}$ are associated with the $\text{C}-\text{O}$ stretching vibrations of ester. The small peak at $718-721\text{ cm}^{-1}$ corresponds to the $-\text{CH}_2$ rocking and out of plane vibration of *cis*-disubstituted olefins [23]. The appearance of peak at $3300-3284\text{ cm}^{-1}$ and $1671-1611\text{ cm}^{-1}$ supports the existence of polyphenols/flavonoids whereas, strong peak at 1743

cm^{-1} supports the presence of esters (monounsaturated and polyunsaturated acyl groups).

Table 1. FTIR peaks of Avocado (a) leaf, (b) peel, (c) pulp, and (d) oil.

Avocado Leaf, (cm^{-1} , %T)	Avocado Peel (cm^{-1} , %T)	Avocado Pulp (cm^{-1} , %T)	Avocado Oil (cm^{-1} , %T)	Functional Group
3300.9, 90.8	3276.3, 93.8	3284.7, 55.1	-	-OH, -NH ₂
2916.5, 60.9	2918.6, 80.9	2924.3, 79.9	2922.2, 58.0	-CH, -CH ₂ asymmetric stretching
2849.0, 66.3	2850.3, 84.3	2851.3, 82.5	2853.1, 68.1	-CH, CH ₂ symmetric stretching
1731.6, 84.1	1737.7, 91.0	-	1743.5, 55.4	C=O Stretching
1611.4, 84.8	1671.9, 91.2	1635.2, 67.4	-	-cis C=C stretching/ C=O carbonyl
1462.9, 81.7	-	1415.2, 78.4	1463.8, 83.5	CH ₂ & CH ₃ bending
1375.4, 85.7	-	1371.1, 77.9, 1316.9, 77.1	1377.3, 89.9	CH ₂ & CH ₃ scissoring
-	-	1245.0, 76.5	-	C-O stretching
1165.6, 77.4	1159.1, 87.9	-	1160.2, 68.2	CH ₂ bending
-	-	-	1117.8, 80.3	C-O stretching
1045.3, 73.3	1097.0, 87.6	1032.3, 47.1	1096.2, 81.0	C-O-C stretching
718.8, 72.1	719.67, 87.1	-	721.98, 81.0	-CH ₂ rocking

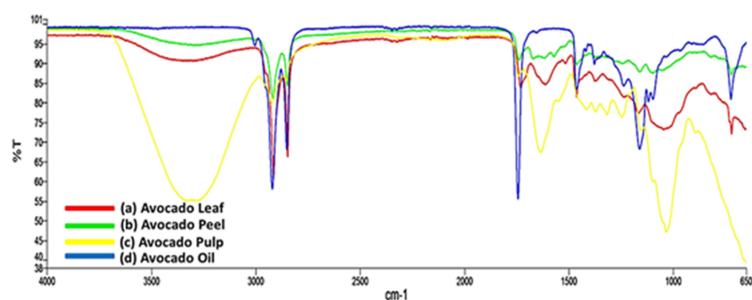


Figure 3. FTIR spectrum of Avocado (a) leaf, (b) peel, (c) pulp, and (d) oil.

3.3 Antioxidant activity

The plant extracts or their secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries related to oxidative stress and free radicals. Free radicals, mainly includes ROS such as, hydroxyl radicals, peroxy radicals, super oxide radicals, hydrogen peroxide, singlet oxygen, and various lipid peroxides [24]. The purple color DPPH• radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule with yellow in the presence of an antioxidant compound which is indicative of its radical scavenging potential [25]. The avocado fruit and leaf contain a wide variety of phytochemicals that function as antioxidants. The DPPH• radical scavenging activity of the avocado leaf, peel, pulp and oil were found to be significantly different, as shown in Figure 4 and Table 2. For the avocado peel extract (aq), DPPH• radical scavenging activity varied in the range of 80.60-75.18% (32.94-30.61 GAE, $\mu\text{g}/\text{mL}$). The aqueous extract of avocado leaf (84.46-80.12%) showed significantly higher DPPH• radical scavenging activity equivalent of 32.60-32.73 μg of gallic acid equivalents per mL of solution. Whereas, aqueous avocado pulp extract (22.53-6.51%) and oil (28.36-22.30%) showed the lowest DPPH• radical scavenging activity equivalent of 7.96-1.07 μg GAE/mL and 10.47-7.86 μg GAE/mL. The results obtained in the present study showed that, the antioxidant activity of avocado leaf was relatively high with respect to the fruit due to the presence of the higher content of polyphenolic/flavonoids in leaf.

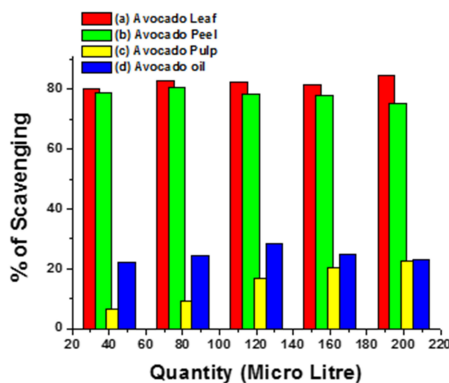


Figure 4. Antioxidant activity of Avocado (a) leaf, (b) peel, (c) pulp, and (d) oil against DPPH•.

Table 2. Comparative chart of antioxidant activity of different avocado material with respect to gallic acid equivalent (GAE).

Avocado Material	Avocado Leaf	Avocado Peel	Avocado Pulp	Avocado Oil
Scavenging (%)	84.46-80.12	80.60-75.18	22.53-6.51	28.36-22.30
GAE ($\mu\text{g}/\text{mL}$)	32.60-32.73	32.94-30.61	7.96-1.07	10.47-7.86

4. Conclusions

From the study, it can be concluded that the avocado leaf has a greater antioxidant activity compared to fruit (peel, pulp and oil) due to the occurrence of more phenolic compounds/flavanoids. It showed enhanced antioxidant activity in the range of 84.46-80.12% with values from 32.60-32.73 $\mu\text{g}/\text{mL}$ gallic acid equivalents against DPPH•. Hence, we recommend the use of Avocado leaf as natural antioxidant and health-beneficial products.

Acknowledgments

This scientific work has been funded by the Prometeo Project of the National Secretariat of Higher Education, Science, Technology and Innovation (SENESCYT), Ecuador.

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