In-Vitro Antioxidant Activity of Avocado Fruit Oil in Erbil, Kurdistan Region

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Abstract: Many human diseases are linked to the accumulation of free radicals. Antioxidants can scavenge free radicals, lessening their impact. As a result, searching for naturally occurring antioxidants in plants is critical. This research aimed to investigate avocado's antioxidant and free radical scavenging properties (*Persea Americana* Mill). Several standard methods for determining antioxidant and free radical scavenging activity were applied, including a spectrophotometer. Avocado fruit oil (AFO) has the highest overall antioxidant capacity, followed by Avocado seed oil (ASO). Based on ABTS and hydroxyl radical scavenging activity, IC_{50} values of 62.99 ug/ml and 50.68 ug/ml, respectively. According to these results, the AFO had a more active radical scavenging system than the ASO. AFO showed the strongest inhibitory activity in the lipid peroxidation inhibition experiment, with an IC_{50} of 318.8 ug/ml; however, ASO's lipid peroxidation is 315.2 ug/ml, which is extremely similar to the IC_{50} of value for AFO. The nitric oxide radical scavenging activity and reduced glutathione assays were also in the AFO>ASO order. A positive correlation ($p<0.001$) was found between free radical scavenging efficiency and lipid peroxidation inhibition activities. Avocado fruit oil contains natural antioxidants and can serve as a free radical scavenger or inhibitor. As a result, Avocado may be a beneficial oil-based treatment for various illnesses caused by free radicals.

Keywords: *Persea Americana*, Free Radicals, ABTS, Antioxidant, Glutathione, ABTS

1. Introduction

Because native plants are only found in certain areas and are only known by the native community, current discovery on their antioxidant qualities is scarce. As a result, evaluating such antioxidant activity remains an intriguing and valuable endeavour, especially when looking for potential natural antioxidant sources for food supplements and naturopathic remedies (Buenrostro and López-Munguia, 1986, Arancibia et. al., 2017). Antioxidants are substances that deliver electrons to damaged cells to prevent and stabilize free radical damage. Free radicals are also converted by antioxidants into waste by products that the body eliminates. Antioxidant-rich fruits and vegetables have been shown to reduce the risk of free radical-related illnesses (Flores et. al., 2019). These health benefits are attributed to phytochemicals such as carotenoids, polyphenols, β-Sitosterol Sitoestanol and vitamins E and C (Wang et.al., 2002, Costagli et.al., 2015, Santana et. al., 2015).

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Although phenolic compounds are present in both edible and non-edible herbs, grains, fruits, vegetables, oils, spices, and other plant parts (Forero-Doria et al., 2017), they are most frequently found in fruits.

Oxidative stress is a key driver in the initiation and progression of diabetes, cancer, inflammatory disorders, and neurodegenerative illnesses, among other ailments (Flores et. al., 2014). Free oxygen and nitrogen species are unstable molecules found in the environment (exogenous) and mechanisms that, in a normal physiologic state, ensure a consistent balance of prooxidants and antioxidants, supporting health (Flores et. al., 2014). Catalase, glutathione peroxidase, and superoxide dismutase are enzyme-based antioxidants, while bilirubin, uric acid, and lactoferrin are non-enzymatic antioxidants. The natural antioxidant mechanisms, on the other hand, are overworked during illness, resulting in more than free radicals, which produce oxidative stress-related damage to cellular machinery, which has been associated to a variety of diseases (Dos Santos et al., 2014; Martnez-Padilla et al., 2018)

Avocados (*Persea americana* Mill.) are members of the Lauraceae family. In the tropical and subtropical parts of the world, avocado is one of the most important crops. Morocco's northwest coast, between Rabat and Tangier, is where the majority of the nation's agricultural output is grown. Due to its high nutritional value, it is typically eaten in the form of fresh fruit. Avocados have a sizable global market and are utilized in food processing, cosmetics, and edible oils. This fruit's pulp is renowned for having a high lipid content that is on equal with olive oil. Fatty acids and minerals including iron, magnesium, phosphorus, and potassium comprise the majority of the composition. Avocado oil has the highest concentration of fatty acids of any vegetable oil. Oleic acid is reported to have immunomodulatory properties that affect a variety of physiological activities. According to certain research, it may help with “cancer, autoimmune, and inflammatory diseases, as well as wound healing” (Sales-Campos et. al., 2013).

A necessary fatty acid for human health is linolenic acid. Cardioprotective, anticancer, neuroprotective, and anti-osteoporotic effects have been established. Furthermore, these fats are said to be beneficial to one's health because they help to raise HDL cholesterol levels (Lunn J and Theobald H., 2006). This study aims to know the secondary strong fatty acid composition and antioxidant & free radical scavenging characteristics of avocado oil. According to ethnobotanical and ethnopharmacological studies on avocados, a variety of illnesses may be treated using these plants.

2. Material and Methods

2.1 Plant Material Collection and Identification

In the Kurdistan region, avocado fruit varieties such as Ettinger, Fuerte, Hass, and Reed were accessible. Skin color ranges from bright green to dark green. When Hass is fully ripe, it turns purplish-black (Ojewole et. al., 2007). Each type of avocado is distinguished by its form, color, skin, and size when it comes to the morphological traits that characterize many different varieties. The Hass and Reed variants are oval and spherical, whereas the Ettinger and Fuerte types are often pear-shaped. Avocados are classified into four varieties, each with its own skin structure. Some species have smooth skin (Ettinger), whereas Fuerte and Hass have grainy or granular skin. The sample was taken on January 2019. The avocado pulp and seed were separated and dried overnight in an oven at 45°C. Following drying, the dried fruit material is converted into a powder in a blender and kept in bags.

2.2 Preparation of the Extract

For 8 hours, a Soxhlet extractor was used with 250 mL of n-hexane and 50 g of powdered, dried avocado pulp. A rotary evaporator operating at 50 °C and low pressure was used to extract this solvent. The extracted oils were then put in sample vials of brown color and refrigerated.

2.3 Chemicals

Trichloroacetic acid (TCA), Tris-HCl buffer, Thiobarbituric acid (TBA), malondialdehyde, Phosphate buffer, Ellman's reagent, precipitating reagent, Standard glutathione were obtained from Himedia laboratories, Mumbai. All the other reagents used were of analytical grade.

2.4 Qualitative Phytochemical Screening

Standard phytochemical screening protocols were used to conduct qualitative assays for different phytochemical constituents found in the Avocado fruits and seeds. Visual assessment of the color or foaming was used to determine the presence or absence of phytochemical constituents.

2.5 Standardization of Avocados Oil

Physical constants that affect avocado oils are occasionally taken into consideration when calculating their oil content. The Indian Pharmacopoeia, 1996, A.O.A.C. 2000, analyses the acid value, saponification, esters value, iodine value, peroxide value, weight per millilitre, refractive index, and viscosity. The components in avocado fruits and oil can be identified and detected using all of these physical characteristics.

2.6 In-vitro Antioxidant Activities of the Avocados Oil

2.6.1 Experimental Animals

Male albino Wistar rats (weighing 160–180 g) were kept in the departmental animal house one week prior to the experiment at 22 ± 2°C, 30 to 40 % of relative humidity, and a 12-hour light/dark cycle. The animals were fed rodent diet and had unlimited access to water. A rat was chosen at random and fasted overnight before being sacrificed via cervical dislocation. The liver was excised and homogenized in 0.15 M KCl after being rinsed in ice-cold saline. After that, the homogenate was employed for anti-lipid peroxidation and reduced glutathione assay.

2.6.2 ABTS Radical Decolorization Assay

Antioxidant scavenging activity for the ABTS radical cation was evaluated. On the day before the assay, a stock solution of ABTS radical cation was made by mixing 5.0 ml of 7.0 mM ABTS with 1.0 ml of 14.7 mM ammonium persulphate and storing it in the dark at room temperature. The ABTS radical cation stock solution was diluted with water to absorb around 0.7 O. D at 734 nm. The decolorization experiment began with 2.0 ml of diluted ABTS solution mixed with various proportions of Avocado. The total volume of all the tubes was increased to 2.5 ml using distilled water. 2.0 mL ABTS was combined with 0.5 mL pure water as a control. After 30 minutes, the absorbance was measured at 734 nm. The antioxidant activity was indicated by a decrease in absorbance (Re et. al., 1999).

2.6.3 Lipid Peroxidation Inhibitory Assay

The degree of lipid peroxidation was calculated using the traditional method by estimating the thiobarbituric acid reactive compounds (Ohkawa, et. al., 2002). The liver homogenate was mixed with various amounts of the formulation developed in this section (0.5 ml). Lipid peroxidation was initiated by mixing 100 microliters of 15mM FeSO4 solution with 100 microliters of water and incubating for 30 minutes at 37oC. After 30 minutes, 1.0 mL of 10% TCA was added, and the mixture was centrifuged. After 10 minutes, 1.0 mL thiobarbituric acid was added to the supernatant. After boiling the tubes for 20 minutes, the pink color developed was measured at 535 nm.

2.6.4 Reduced Glutathione Assay

0.3 ml of the serum or 0.5 ml of homogenate was mixed thoroughly with 3.0 ml of precipitating reagent and allowed to stand for 5 minutes and centrifuged. A set of standards were taken and made upto 1.0 mL with distilled water. 1.0 ml of supernatant along with 1.0 ml blank containing distilled water was taken. To all the tubes 2.0 ml of 0.3 M disodium hydrogen phosphate and 0.5 ml of DTNB reagent were added. The colour developed was read at 412 nm. Reduced glutathione levels were expressed as µM of GSH/gm of protein (Tripathi and M Sharma, 1998).

2.6.5 Nitric Oxide Radical Scavenging assay

The activity of nitric oxide radical scavenging was evaluated spectrophotometrically (Govindarajan, et. al., 2002, Uthirapathy S., 2021). In phosphate buffer (pH 7.4, 0.1 M), 1.0 ml of sodium nitroprusside (5 mM) was combined with various doses of the formulation (pH 7.4, 0.1 M). The tubes were then incubated for two hours at 25°C. 1.5 ml of the reaction mixture was withdrawn at the end of the second hour and diluted with 1.5 ml of Greiss reagent. The absorption wavelength of the chromophore generated by nitrite diazotization with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride is 546 nm. All compounds were present in the control tube except the AFO and ASO.

% Inhibition was calculated by using this formula,

% Inhibition = (Control – Test) / Control) × 100

The IC_{50} value was defined as the concentration of studies Avocado oil that exhibits 50 % activity, and this was calculated by nonlinear regression mode of statistics.

3. Result and Discussion

Avocado oil is getting more popular, and researchers are trying to figure out what the primary and minor components are. It is therefore necessary to consider the many kinds and portions of the fruit in order to fully understand the nutritional and functional properties of this oil. Indriyani et al., (2016) stated that the several varieties of avocado seed oil comprised roughly 8.47% oil, with the unsaponifiable matter accounting for 76.9%. “Ergosterol, 5-cholestane, and stigmasterol were the phytosterols that were quantified in a higher proportion".

Due to their remarkable antioxidant activity, extractable polyphenols, which are abundant in avocado seeds, have gained popularity. It was shown that raising the temperature and ultrasonic power improved the polyphenol content and antioxidant capacity (Wang et. al., 2010). The unsaponifiable portion of the sample had weak antioxidant activity, which the DPPH technique demonstrated was

indicated by the accumulation of polyphenols and steroids. The quality indicators, such as specific gravity, viscosity, iodine value, acid value, peroxide value, and saponification value were comparable to those for extra virgin olive oil (A.O.C. 2000).

The lipid content of the pulp and seed of the Fuerte variety grown in North eastern Brazil differs significantly when the oil composition of the pulp and seed is compared. Gas chromatography revealed that seed oil had a wider range of fatty acids than pulp oil. Furthermore, “the pulp's fatty acid profile was substantially higher in monounsaturated fatty acids than that of the seed” (Dubois et. al., 2007). Seed oil, on the other hand, is higher in “polyunsaturated fatty acids than pulp oil” (Bora et. al., 2001, Martinez-Nieto et. al., 1994).

The active phyto-constituents discovered through qualitative phytochemical investigations, such as alkaloids, glycosides, phytosterol, tannins, and proteins, are identified using chemical assays. A preliminary phytochemical analysis of Avocado oil was performed by Khandelwal (Khandelwal, 2003) and Kokate (Kokate, 2005). The presence of secondary metabolites is presented in Table 1. The Avocados oils were standardized by Indian Pharmacopoeia methods such as weigh per ml, acid value, iodine value, ester value, saponification value, peroxide value viscosity and refractive index as shown in Table 3. Three successive readings were collected, and then mean values and standard deviation were calculated statistically. They claim that the quality of avocado oil is determined by the fruit's quality and ripeness, as well as the extraction procedure in terms of temperature, solvents, and storage (Uthirapathy, et. al., 2021; Thenmozh et. al., 2021). While the avocado fruit has been extensively researched, little is known about avocado oil and its possible health benefits. As a result, it's critical to consider the many kinds and portions of the fruit in order to fully comprehend the nutritional and functional features of this oil (Indian Pharmacopoeia, 1996, A.O.A.C. 2000).

Figures 1 and 2 shows that both AFO and ASO may scavenge the ABTS radical cation. Avocado fruit and oil both increase in concentration as concentration increases. When AFO and ASO are compared, the IC50 value demonstrates that ASO can scavenge ABTS at a lower concentration. Avocado also contains unsaponifiable substances including “beta-sitosterol, vitamins, carotenoids, tocopherols, and phenolic compounds, all of which have antioxidant and anti-inflammatory properties” (Martinez-Nieto et al., 1994; Kosiska et al., 2012). Avocados are also known for possessing alkanols, a fat-soluble chemical that inhibits cancer cells (Zhang et. al., 2013). As a result, the avocado pulp contains hundreds of phytochemicals that may help to prevent cancer. Studies have shown that avocado pulp can inhibit the growth of a variety of cancer cells (Zhang et. al., 2013).

Figures 3 and 4 indicates that both AFO and ASO can scavenge nitric oxide radicals, increasing with concentration. The IC50 values of fruits and oils are practically comparable in this scenario and no much difference is observed in the IC50 value of both of them. Similarly, in in vitro lipid peroxidation assay (Figure 5 and 6) and inhibition of glutathione oxidation (Figure 7 and 8), both the AFO and ASO exhibit good efficacy in in-vitro lipid peroxidation assays and glutathione oxidation inhibition. The IC50 value of AFO shows that it is more effective than ASO in inhibiting glutathione oxidation (Table 3). These data imply that both oils have antioxidant activity in vitro. ABTS radical cation scavenging is a generally accepted paradigm for in-vitro antioxidant activity (Ahamad and Uthirapathy, 2021). It has also been discovered that as concentration increases, so does the activity.

Table 1: Qualitative phytoconstituents of AFO and ASO

S.No.	Phytoconstituents	Avocado fruit and seed	
		AFO	ASO
1.	Alkaloids (Dragendroff's Reagent, Mayers reagent, Wagner's reagents)	+++	++
2.	Phytosterols (Libermann Burchard Test)	+++	++
3.	Glycosides (Libermann Burchard Test, Legals and Borntrager test))	++	++
4.	Phenolic compounds and Tannins (5% Fecl soln, and 1% gelating containing 10 % NaCl and 10 % lead acetate in bromine soln	+++	++
5.	Flavonoids (Lead subacetate test, Shinoda's test, Antimony pentachloride test.	+++	++
6.	Oils and Fatty Acids (0.5N KOH)	++++	+++
7.	Volatile oil (Hydrodistillation method)	++++	++
8.	Carbohydrates (Molisch's Test, Fehling's, Barfoed's and Benedict's reagents)	++	++
9.	Proteins and Aminoacids (Biuret test, Millon's and Ninhydrin test)	++	+
10	Saponins (Frothing test)	++	+
11.	Terpenoids (Salkowsky test)	++	++

Key: ++ - low concentration +++ - High concentration, - absence

Table 2: Avocados (AFO and ASO) Oil Standardizations (Indian Pharmacopeia, 1996a).

S. No.	Avocados Oil	Description	Wt/ml At 20^0C	Acid Value	Saponification Value	Iodine Value	Ester Value	Peroxide Value	Viscosity AT RT	Refractive index
1.	AFO	Greenish Yellow colour Characteristic-odour	0.9099 gm/ml	0.5528	154.603	147.7899	157.0497	5.1747	1697.2370 cps	1.4210
2.	ASO	Brownish Yellow colour Characteristic-Odour	0.9526 gm/ml	0.6384	211.023	181.1298	210.3844	5.5222	362.32 cps	1.4020

Fig 1Effect of AFO on ABTS Radical scavenging assay

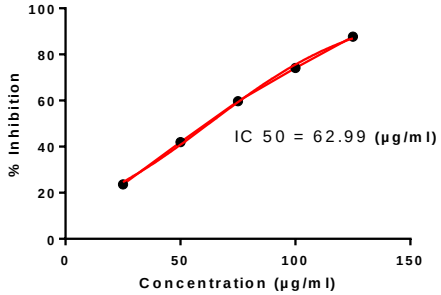


Fig 2 Effect of ASO on ABTS Radical scavenging assay

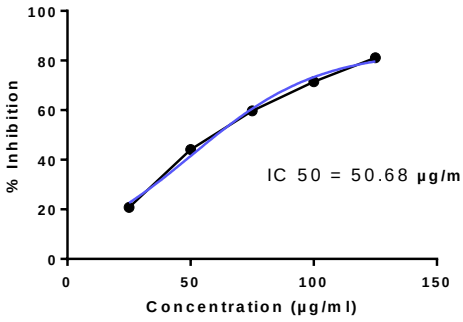


Fig 3 Effect of AFO on Nitric oxide radical scavenging assay

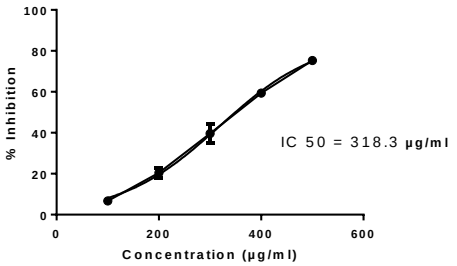


Fig 4 Effect of ASO on Nitric oxide rdical scavenging assay

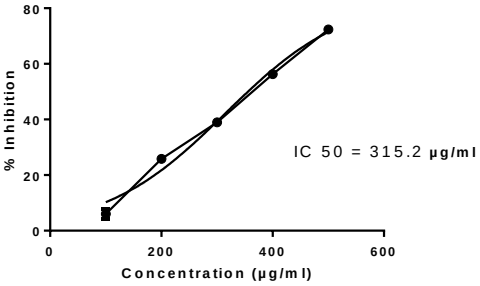


Fig 5 Effect of AFO on Anti lipid peroxidation assay

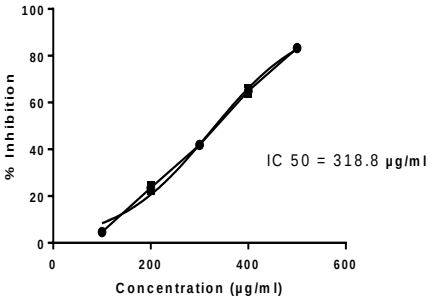
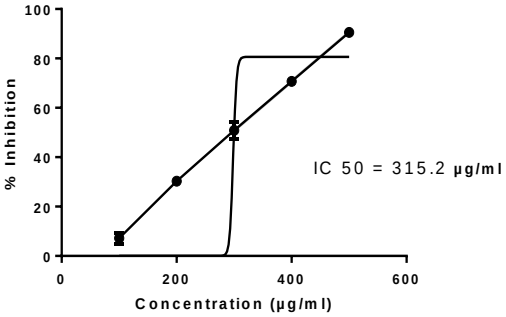


Fig 6 Effect of ASO on Anti lipid peroxidation assay



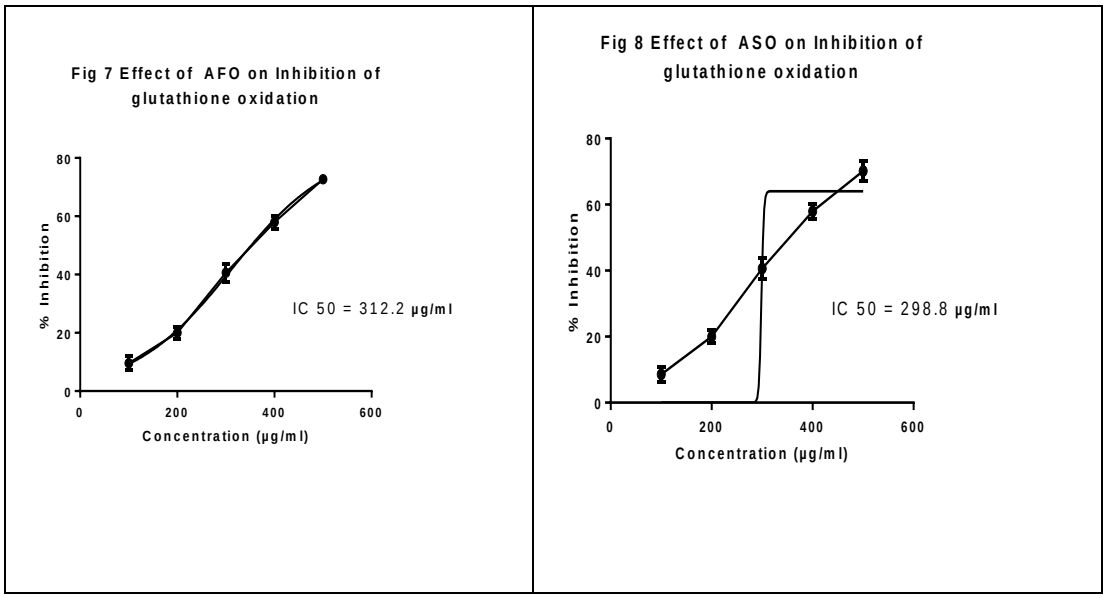


Figure 1-8: Effect of Avocados (AFO and ASO)- in vitro studies.

Table 3: IC_{50} value of Avocados oil in different in-vitro antioxidant activities.

S. No	Different Antioxidant Methods	IC_{50}	
		AFO	ASO
1	ABTS decolorization	62.99	50.68
2	NO radical scavenging	318.3	315.2
3	Anti-lipid peroxidation	318.8	315.2
4	Inhibition of glutathione oxidation	312.2	298.8

4. Conclusion

These experiences will help demands for a more significant advancement in research on the contamination and adulteration of avocado oil as well as a more thorough investigation of the potential biological consequences of the numerous components found in the oil, whether in humans or animals. According to the results, AFO has higher in-vitro antioxidant activity than ASO. The metabolic enzymes found in avocado fruit and seed oil may function in a number of different ways. The action might be different in the in-vivo environment. As a result, avocados' antioxidant activity in vivo has to be further studied.

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