Evaluating the capabilities of avocado (*Persea americana* Mill.) seeds as a potential source of nutrients, nutraceutical compounds, and natural antioxidant for industrial food applications

Muhammad Khalid Saeed[1], Naseem Zahra[1], Najeeb Ullah[1], Ijaz Ahmad[1], Abid Sarwar[1], Fida Hussain[2], Tariq Aziz[3]*, Hanan M. Alharbi[4], Nawal Al-Hoshani[4], Maher S. Alwethaynani[5], Saleh A. Alsanie[6,7], Mansour Alblaji[6], Rewaa S. Jalal[8]

[1]Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, 54600 Punjab, Pakistan; [2]Department of Pharmacy, University of Swabi, 23000, Swabi, Khyber Pakhtunkhwa, Pakistan; [3]Laboratory of Animal Health, Hygiene and Food Quality, University of Ioannina Arta, 47132, Ioannina, Greece; [4]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [5]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Shaqra University, Alquwayiyah, Riyadh, Saudi Arabia; [6]Department of Basic Health Sciences, College of Applied Medical Sciences, Qassim University, Saudi Arabia; [7]Department of Clinical Nutrition, Medical City, Qassim University, Saudi Arabia; [8]Department of Biological Sciences, College of Science, University of Jeddah, 21493, Jeddah, Saudi Arabia

***Corresponding Author:** Tariq Aziz, Laboratory of Animal Health, Hygiene and Food Quality, Department of Agriculture, University of Ioannina Arta, 47132, Ioannina, Greece. Emails: tariqckd@uoi.gr; iwockd@gmail.com

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ORIGINAL ARTICLE

Abstract

Beneficial bioactive compounds, including minerals, polyphenols, phytochemicals, and natural antioxidants, are present in substantial amounts in avocado seeds (*Persea Americana* Mill.). Its seeds are one of the underutilized nonedible sections that are typically discarded as a residue. Avocado seeds have numerous biological activities, such as analgesic, amoebic, antioxidant, antibacterial, anti-arthritis, antihypertensive, fungicidal, larvicidal, and wound-healing. In this study, avocado seed powder was formulated and amounts of its nutritional fats, phytochemicals, polyphenols, flavonoids and antioxidant activity were assessed. Nutritional analysis of avocado seeds powder showed that it contains moisture (9.52±0.92%), ash (2.38±0.05%), fat (12.90±0.18%), fiber (3.70±0.12%), proteins (7.30±1.08%), carbohydrates (63.80±4.02%), energy (398.70±6.48 Kcal/100 g), sodium (1.2±0.03 mg/100 g), potassium (3.95±0.05 mg/100 g), and calcium (0.72±0.01 mg/100 g). The phytochemical analysis of aqueous and methanol extracts identified the presence of fatty acids, polyphenols, flavonoids, phytosterols, triterpenes, alkaloids, tannins, and saponins, while anthraquinones and glycosides are absent in both extracts. It contains total polyphenols of 45.50±0.94 mg/100 g gallic acid equivalent (GAE) (in aqueous extract) and 52.60±1.03 mg/100 g GAE (in methanol extract), and total flavonoids of 12.08±1.02 mg/100 g quercetin equivalent (QE) (in methanol extract) and 9.30±0.94 mg/100 g QE in aqueous extract. The antioxidant activity of its water and methanol extracts was calculated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which ranged from 20.30±1.18–74.88±3.52 in aqueous extract and 25.19±1.32–84.10±4.02 in methanol extract at a concentration 100–500 µg/mL. Significance differences (p = 0.05) in results were found in both extracts. This study demonstrates that avocado seeds (residue) could be a significant source of nutrients, bioactive compounds and natural antioxidants that enhance the value of organic waste.

Keywords: antioxidants; avocado seeds; food applications; nutraceutical compounds; nutrients

Introduction

The foundation of healthcare delivery is natural products, especially in the environments with limited resources. According to current estimates, conventional medicine is the primary method of providing healthcare to around 80% of global population. As long as efficacy and safety are guaranteed, natural products are to be encouraged in countries with limited access to conventional medicine (Emmanuel *et al.,* 2026; Nazir *et al.,* 2026; Naveed *et al.,* 2026; Emmanuel *et al.,* 2025a; Arshad *et al.,* 2025; Emmanuel *et al.,* 2025b; Aziz *et al.,* 2024). According to scientific research on medicinal plants, their biological components have a wide range of qualities and are used to treat a variety of illnesses (Kiran *et al.,* 2025; Nazir et al., 2025; Ammara *et al.,* 2023; Naveed *et al.,* 2023; Rauf *et al.,* 2023; Shah *et al.,* 2023; Odoh and Uju, 2020). Avocado is a dicotyledonous plant of the *Lauraceae* family with an olive-green skin (Figure 1) and a dense oil-rich pulp. Avocado fruit is found in both tropical and subtropical areas, where it has been used traditionally as a food and is prized for its sensory qualities because its unsaturated fatty acids. The strong nutritional content of this fruit has led to a 52% increase in global cultivation since 1999 (Kurniawan and Rahmat, 2023). Only a small percentage of the entire avocado fruit is edible, which includes its pulp, eaten as raw. Pulp is utilized as a major component of guacamole and sauce, or processed to extract oil for using in cuisine, cosmetics, and pharmaceutical preparations (David *et al.,* 2022).

Seeds of *Persea americana* make up to 13–18% of the fruit mass (Siol and Sadowska, 2023). In keeping with the idea of zero waste, proper utilization of fruit remnants lessen the issues brought on by the abundant use of fruit and the negative effects of food waste. According to Boyadzhieva *et al.* (2018), these by-products are thought to be a viable source of different bioactive chemicals, especially polyphenols such as phenolic acid (Figure 2), flavonoids (Figure 3), and alkanols, terpenoids, glycosides, various furan ring-containing derivatives, coumarins, and tannins. These ingredients have many health benefits, including as antioxidants.

Avocado seeds also contain secondary metabolites having various biological activities, such as anti-inflammation, hypoglycemic, antihypertensive, fungicide, and larvicide properties (Rojas-García *et al.,* 2022). As a result, a number of studies have examined the benefits of avocado seeds and their potential for future applications.

It is crucial to emphasize the promise of secondary metabolite substances obtained from plants and fruits that have antioxidant properties. Recent scientific investigations across the globe have unveiled the medicinal attributes of plants, subject to scrutiny because of their effective pharmacological properties, economic feasibility, and less toxicity. Compounds such as polyphenols demonstrate the ability to scavenge free radicals and enhance a balance between oxidative stress and antioxidant property (Naqbi *et al.,* 2025). Recently, phytochemical properties and antioxidant activity of medicinal fruit, *Physalis alkekengi* (wild and cultivated genotypes) and *P. peruviana* commercial species, were investigated by Yari *et al.* (2025), who demonstrated that they possessed higher values of phytochemicals. In the cited study, maximum phenolic content and antioxidant activity were observed in cultivated *Physalis* genotype. As opposed to synthetic antioxidants, natural antioxidants obtained from plants and fruits are less likely to cause unappealing effects. Hence, numerous efforts are made to investigate foster natural sources of antioxidants (Heriansyah *et al.,* 2025; Zhao *et al.,* 2025). In order to identify natural sources of antioxidants, the chemical composition of plants with antioxidant characteristics along with nutritional facts are investigated in that had not been previously studied regarding avocado seeds.

Figure 1. Fruit and seeds of avocado fruit.

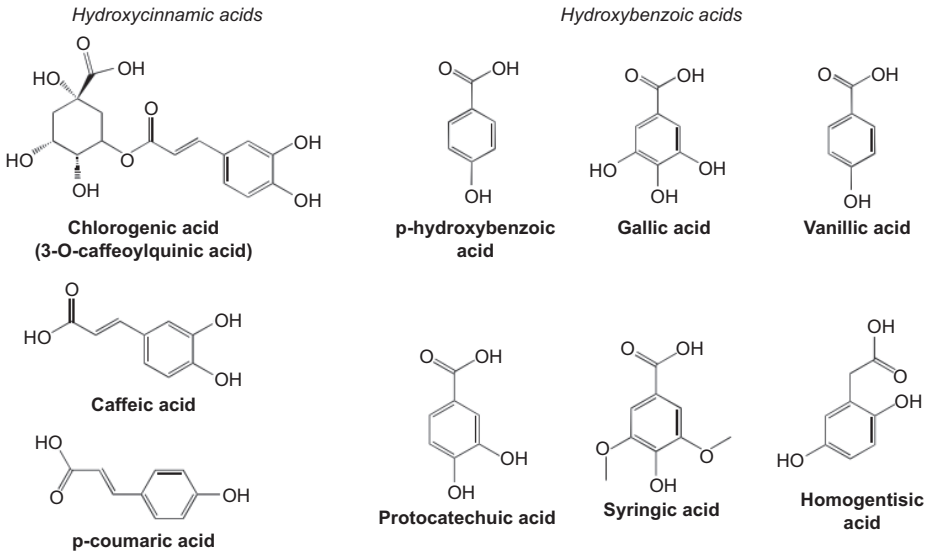

Figure 2. Major polyphenols found in avocado seeds.

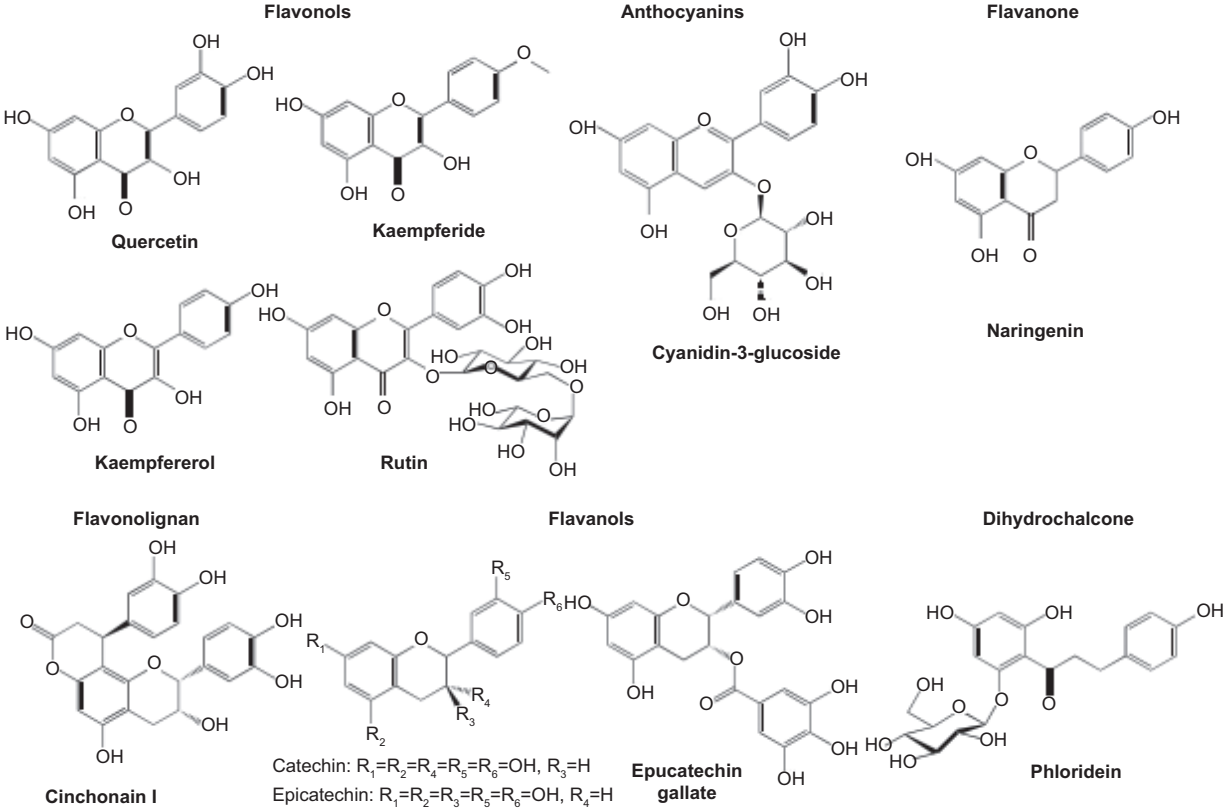

Figure 3. Major flavonoids found in avocado seeds.

The main objective of this study was to investigating nutritional facts, phytochemicals, total phenolic content, total flavonoids and free radical activity of avocado seeds extract.

Materials and Methods

Sample preparation

In 2022, the Hass variety of avocado were bought from a nearby store and cleaned with distilled water to get rid of any dirt or soil particles. The fruit was left out for ripening at room temperature (25±5°C). Then the fruit was flaked manually into seeds, peel, and pulp. Seeds' outer layers were scraped off, and slices with an average thickness of 5 mm were cut down and dried in a hot air oven at 50–60°C until completely dried (Saavedra *et al.,* 2017). Using an electronic blender (Panasonic, Malaysia), dried seeds were crunched into powder and passed through a 250-mm stainless steel sieve. The crunched sample was saved in an air-tight container at –5°C until used.

Reagents and chemicals

For analysis, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, anhydrous sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), and methanol were acquired from Sigma Chemical Co (Burlington, MA, USA). All other reagents utilized in the study were of investigative grade, and distilled water was produced using a distillation machine in laboratory.

Determination of Nutrients

Avocado seed powder's proximate composition (moisture, ash, fat, crude fiber, crude protein, and carbohydrate) and minerals (Na, K and Ca) were evaluated according to recognized protocols (Association of Official Analytical Chemists [AOAC], 2023), using a flame photometer. The seed powder was dried to a constant weight at 105°C in an oven to determine its moisture content. Hexane was used for 6 h to extract fat by using a Soxhlet device at 40–60°C. Protein was determined by the Micro–Kjeldahl method. The following equation was used for determining carbohydrates:

Carbohydrates (%) = 100 – (%moisture + %protein + %ash + %fat + %crude fiber).

By using the information of proximate analysis, samples' gross energy (expressed as Kcal/100 g) was estimated by multiplying the percentages of crude protein, crude carbohydrate, and crude fat by 4.0 and 9.0, respectively (Akinyeye *et al.,* 2011).

Phytochemical examination

In all, 1% solute with solvent water and methanol was primed. These were examined for active phytochemicals such as fatty acids, polyphenols, flavonoids, phytosterols, triterpenes, tannins, alkaloids, anthraquinones, glycosides, and saponins by using established procedures (Harborne, 1998).

Preparation of extract for phenolic and antioxidant investigation

A total of 50-mL methanol containing 2.5-g avocado seed powder was extracted using a Soxhlet apparatus; methanol was subsequently removed at 40°C using a rotatormachine. In the case of aqueous extraction, 2.5-g sample was boiled for 2 h at 60°C and then shaken for 15 min (Kosińska et al., 2012). To study antioxidants, supernatant was collected after filtration using a filter paper.

Estimation of phenols

Phenols in avocado seeds powder were dogged quantitatively by Folin–Ciocalteu reagent at 760 nm, as reported by Singleton and Rossi (1965) with minor changes (Saeed *et al.,* 2021b). The result was expressed as mg gallic acid equivalent (GAE)/100-g sample.

Total flavonoids content

Aluminum chloride was used to fortitude flavonoids in avocado seed powder. This was performed by the protocol used by Dewanto *et al.* (2002) with slight changes (Saeed *et al.,* 2013). Quercetin was used as a reference flavonoid for estimation, and the result was expressed as mg quercetin equivalent (QE)/100 g.

Antioxidant activity by DPPH assay

Using methanol and aqueous extracts of avocado seed powder, the antioxidant activity was assessed by the method used by Brand-Williams *et al.* (1995) with slight modifications (Saeed *et al.,* 2021a). DPPH, 0.004%, in methanol was prepared and 3 mL of DPPH solution was added to 100 µL of samples (concentration 100–500 µg/mL). After 30 minutes incubation the

absorbance of sample was taken at 517 nm bu using UV-spectrophotometer. As a blank, 3 mL of DPPH solution in 100 µL of methanol was used to measure antioxidant activity as follows:

$$\text{Antioxidant activity (\%)} = 1 - [A_{sample} \div A_{control}] \times 100.$$

Statistical evaluation

The result was shown as mean values and standard deviation (SD). One-way analysis of variance was used to statistically evaluate the data. Tukey's HSD test was used to check whether any changes in sample's mean values were significant at $p = 0.05$.

Results and Discussion

Nutrient information

The proximate analysis of avocado seed powder showed valuable nutrients (proteins, lipids, fiber, and carbohydrates) that might significantly improve the human diet. Seed powder contained moisture (9.52±0.92%), ash (2.38±0.05%), fat (12.90±0.18%), fiber (3.70±0.12%), proteins (7.30±1.08%), carbohydrates (63.80±4.02%), energy (398.70±6.48 Kcal/100 g), Na (1.2±0.03 mg/100 g), K (3.95±0.05 mg/100 g), and Ca (0.72±0.01 mg/100 g), as shown in Table 1.

According to this investigation, the moisture level of the seed powder was 9.52±0.92%, which was less than result of Mahawan *et al.* (2015). High moisture in flour encourages mould formation and insect infestation, which degrade food items (Abdissa *et al.,* 2020).

Ash concentration in the current study was 2.38±0.05%, which was more than the results reported by Ntuli *et al.* (2013) as between 0.29% and 1.21% for maize and wheat flour. The ash level of its powder as reported by Nwaokobia *et al.* (2018) was 0.84±0.02%, which was lower than the results reached by this study but higher than as reported by Egbuonu *et al.* (2018), which was 3.82±0.00%.

In the current study, protein and fat contents of the seed powder were 7.30±1.08% and 12.90±0.18%, respectively, and these values were less those reported by Ejiofor *et al.* (2018), who showed the protein content of 15.55±0.36% and fat content of 17.90±0.4%. These variations may be due to different varieties and regions.

Avocado seeds had a crude fiber level of 3.7±0.12%, demonstrating that it could be a rich basis of fiber. The seed powder had a high carbohydrate content of 63.8±4.02%, indicating that it could be a strong source of energy (398.7±6.48 Kcal/100 g).

Bioactive components of avocado seeds were shown to have positive impacts on diabetes alleviation. Carbohydrates and polypeptides are the main bioactive components associated with diabetes alleviation through different pathways. Carbohydrates present in avocado seeds help in proper digestion, maintain cholesterol levels, and improve liver functioning. Avocado consists of fermentable carbohydrates, such as mannans and galactans. When consumed, these fiber-rich carbohydrates help in improving beneficial microbiome in the gut and produce short-chain fatty acids, showing prebiotic effects. In general, we can say that proteins show the highest satiating effect, followed by fiber-rich carbohydrates. Fats have the least satiating effect per kilocalories among the above two, followed by simple or high glycemic index carbohydrates (Nyakangi *et al.* 2023).

The mineral analysis for sodium, potassium, and calcium was done by photometer. The findings agreed with those of Ifesan *et al.* (2015). Calcium, along with phosphorus, is a crucial component of body fluids and aids in bone formation, while sodium and potassium manipulate osmotic pressure and contribute to normal pH equilibrium, fluid balance, and nerve transmission (Oluyemi *et al.,* 2006). Minerals also help the body to use energy and build bones, teeth, blood, and other structures. Each mineral serves a particular purpose in the body and controls many bodily functions (Maitera *et al.,* 2014; Merck, 2005).

Table 1. Avocado seeds' nutrient profile and mineral composition.

Parameters	Percentage (%) values
Moisture	9.52 ± 0.92
Ash	2.38 ± 0.05
Fat	12.90 ± 0.18
Crude fiber	4.75 ± 0.12
Crude protein	7.30 ± 1.08
Carbohydrates	63.80 ± 4.02
Energy (kcal/100g)	398.70 ± 6.48
Na (mg/100g)	1.2 ± 0.03
K (mg/100g)	3.95 ± 0.05
Ca (mg/100g)	0.72 ± 0.01

Note: Values are expressed as mean ± SD and analyzed in triplicate.

Screening for phytochemicals

Qualitative phytochemical analysis is essential for determining whether a novel medicine contains any specific primary or secondary metabolites that are clinically significant and are available from plant extracts. It is vital to use appropriate chromatographic techniques to isolate important bioactive compounds from the mixture of compounds. Various phytochemical analyses of *Persea americana* seed extracts revealed the presence of fatty acids, phenols, flavonoids, phytosterols, triterpenes, tannins, alkaloids, and saponins, but no anthraquinones and glycosides (Table 2). According to Wang *et al.* (2010), avocado has a potential for extracting phytochemicals and contains significant levels of phenols, flavonoids, and proanthocyanidin. According to Figueroa *et al.* (2018) and Setyawan *et al.* (2021), phytochemicals, such as alkaloids, flavonoids, and phenolic compounds, as well as other nutrients, such as amino acids and proteins, are responsible for therapeutic properties.

Estimation of total phenols and flavonoids

Folin's reagent was used to determine polyphenols present in avocado seeds, and it was discovered that total polyphenols in water extract were 45.5±0.94 mg/100 g GAE, whereas total polyphenols in methanol extract were 52.6±1.03 mg/100 g GAE (Table 3). Significant differences in total phenolic content (TPC) and total flavonoid content (TFC) were found between two extracts by using Tukey's HSD test (p = 0.05). Our study's findings were consistent with the available literature (Santana et al., 2019; Tan et al., 2022). Secondary byproducts of plant metabolism are phenolic chemicals, possessing antioxidant properties that may offer defense against oxidation reaction that gives therapeutic benefits (Segovia et al., 2018; Sharma et al., 2022). According to Bahru et al. (2019), phenols (64%) present in avocado seeds were higher than that discovered in fruit's peel (23%) and pulp (13%).

In the current investigation, it was discovered that total flavonoids in water extract were 9.30 mg/100 g QE, whereas total flavonoids in methanol extract were 12.08 mg/100 g QE (Table 3). Owing to numerous health benefits, flavonoid compounds' secondary metabolites are the most studied bioactive substances. The TFC study of the extract was based on color change induced by the interaction of flavonoid molecules with $AlCl_3$. According to a prior study (Noorul et al., 2017), the TFC values of water extract and methanol extract of avocado seeds were 3.72 mg Rutin equivalent (RE)/100 mL and 4.96 mg RE/100 mL, respectively. These findings were comparable with the findings of the current research. According to Vinha et al. (2013), avocado seeds have higher levels of flavonoids than found fruit skin, pulp, and leaves. The findings show that avocado seeds have anti-carcinogenic, anti-inflammatory, hepatoprotective, antioxidant, anti-hyperlipidemic and anti-atherosclerotic properties. Positive correlation ($p < 0.05$) was found between total phenolics, flavonoid content, and antioxidant activity as assessed by DPPH assay, indicating that these phytochemicals provide high antioxidant activity in both extracts. This observation was in agreement with Wang et al. (2010), who reported high antioxidant activity of Hass avocado seeds extract because of high concentration of flavonoids and phenolic content.

Table 2. Screening of avocado seed powder's phytochemicals.

Phytochemicals	Tests/experiments	Aqueous extract	Methanol extract
Tannin	Ferric chloride	+	++
Alkaloids	Dragendorff's Test	+	++
Saponins	Foam test	+	+
Triterpenoids	Salkowski Test	++	+++
Phytosterols	Liebermann–Burchard Test	+	+
Glycosides	Keller-Killani Test	–	–
Flavonoids	Lead acetate test	++	+++
Anthraquinones	Potassium hydroxide test	–	–
Fatty acids	Copper sulfate	+	+
Carbohydrates	Molisch's test	++	++
Proteins	Biuret test	+	++

Note: +++: very high; ++: high; +: moderate; and –: nil.

Antioxidant activity by DPPH assay

According to Runyogote *et al.* (2021), oxidative stress is linked to early events that lead to the development of serious infectious diseases. Since oxidative stress can be readily mitigated by using antioxidants, eating foods high in these nutrients may have favorable impact on person's health. The assessed antioxidant activity of water and methanol extracts of avocado seeds are shown in Figure 4. The antioxidant activity (%inhibition) of both extracts varied from 20.30±1.18% to 74.88±3.52% in aqueous solution, while it varied from 25.19±1.32% to 84.10±4.02% in methanol at concentrations of 100–500 µg/mL. Significant difference was found in the antioxidant activity of both extracts (p = 0.05) by using Tukey's HSD test. These findings were consistent with relevant literature (Alkhalaf *et al.,* 2018; Amado *et al.,* 2019; Hue *et al.,* 2021; Saad, 2020). According to Saeed *et al.* (2023), the DPPH assay is fast, sensitive, and engaged in scavenging free radicals through an electron transfer process.

Methanol as a solvent was more effective than water for extracting antioxidant components of avocado seeds. Folasade *et al.* (2016) also stated that antioxidant properties of *Persea americana* seeds are affected by different extraction solvents. On the other hand, the antioxidant content of dried seeds was shown to be higher than those of fresh seeds (Rotta *et al.,* 2015). However, a number of other naturally occurring lipophilic antioxidants may have been degraded by drying (Guil-Guerrero, 2022). David *et al.* (2022) also determined the antioxidant activity of ethanol and aqueous extracts of avocado seed by DPPH assay and discovered that the antioxidant activity of ethanol extract (0.73±0.06 µm TE/100 g) was higher than that of aqueous extract (3.0±0.68 µm TE/100 g). However, these activity results were higher than the results of our study. This variation may be due to the use of ethanol solvent as well as regional and variety differences. Similar results were obtained by Dabas *et al.* (2019) and Weremfo *et al.* (2020). Both studies stated that methanol is the best solvent for the extraction of natural antioxidants from avocado seeds. According to the current study's findings, phenols and flavonoids are best extracted in methanol solvent because of avocado seeds' capacity to scavenge free radicals.

Table 3. Total polyphenols and total flavonoids content of Avocado seed powder.

Extracts	Avocado seed powder	
	TPC (mg GAE/g)	TFC (mg QE/g)
Water (H_2O)	45.50 ± 0.94	9.30 ± 0.94
Methanol (MeOH)	52.60 ± 1.03	12.08 ± 1.02

Note: Data are presented as mean ± SD.

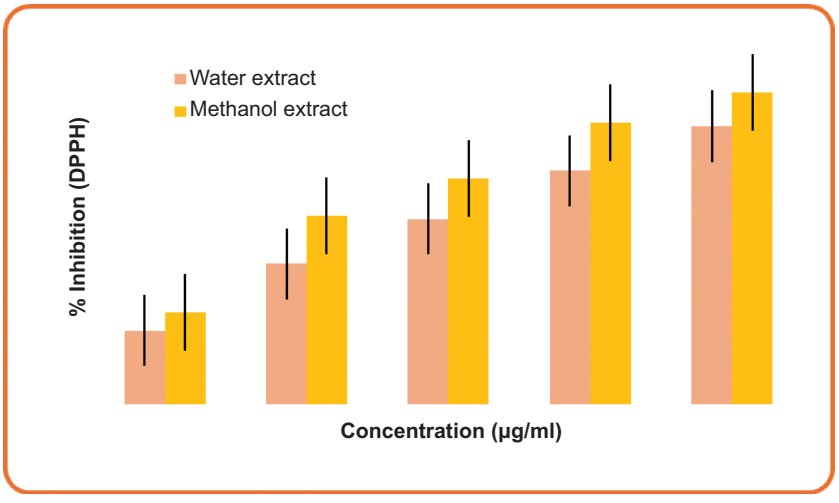

Figure 4. Proportion of inhibition (DPPH) of water and methanol extracts of avocado seeds powder.

Applications of avocado seed powder in bakery products

Following the incorporation of 0, 2, 5, and 10% avocado seeds powder into baked products (biscuits and cake), the antioxidant and sensory evaluation of formulations was done. The results showed that the amount of antioxidants in prepared products increased with the increased addition of avocado seeds powder. The bakery product (cake and biscuits) produced using 10% avocado seed powder had acceptable organoleptic properties that were comparable with the cakes with 0% avocado seed powder, making it the ideal formulation to produce acceptable cakes & biscuits and further work is in progress.

Conclusions and Future Research

Avocado seed is the by-product of direct food consumption and food processing industry which could be further utilized to develop nutraceutical products. This study discovered nutrients, phytochemicals, and antioxidants in avocado seeds' water and methanol extracts, with potential to improve health. Despite recent discoveries, little research is conducted to support the medicinal usefulness of phytochemicals present in *Persea americana* seeds to cure particular disorders. The available literature on phytochemicals derived from avocado seeds discusses their potential of being nutrients, but investigation on their medicinal attributes is scarce. Hence, further research is required regarding avocado seeds' medicinal properties and their potential application in various food products. Moreover, in future *in vitro* and *in vivo* studies must be conducted to assess the bioavailability and efficacy of avocado seeds-derived antioxidants.

Availability of Data and Material

All data produced are included in the manuscript.

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Author Contributions

Conceptualization, Muhammad Khalid Saeed; methodology, Naseem Zahra; software, Najeeb Ullah; validation, Saleh A. Alsanie; formal analysis, Ijaz Ahmad; investigation, Abid Sarwar and Fida Hussain; resources, Tariq Aziz; data curation, Maher S Alwethynani.; writing—original draft preparation, Muhammad Khalid Saeed and Abid Sarwar.; writing—review and editing, Hanan M. Alharbi and Nawal Al-Hoshani; visualization, Mansour Alblaji; supervision, Tariq Aziz and Muhammad Khalid Saeed.; project administration, Muhammad Khalid Saeed; funding acquisition, Tariq Aziz.

Conflicts of Interest

The authors declared no conflict of interest.

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