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Antioxidant Activity of Avocado Seed Extract: A Systematic Review of Extraction Methods

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ABSTRACT

The extraction method, solvent type, concentration, and various other factors highly influence the extraction of antioxidants from avocado seeds. This study explores the impact of these variables on the yield and bioactivity of bioactive compounds, with a particular focus on the antioxidant capacity. Ethanol extraction, especially at specific concentrations, has demonstrated the highest effectiveness in isolating compounds with significant antioxidant properties, as evidenced by low IC50 values from multiple studies. However, the efficiency of the extraction method alone does not suffice for selecting the optimal process. The choice of extraction method must be aligned with the research objectives, the specific phytochemical class targeted for isolation, and the intended application of the final product. This review highlights the importance of balancing extraction methods, solvent characteristics, and process conditions to maximize antioxidant yield while maintaining high bioactivity. Furthermore, it discusses emerging techniques such as microwave-assisted extraction, ultra-sound, and fermentation processes that offer sustainable and efficient alternatives for obtaining bioactive compounds from avocado seeds. Ultimately, this work provides insights into the optimization of extraction protocols for industrial and health-related applications, contributing to the sustainable utilization of avocado seed waste.

Keywords: Bioactive Compounds; Avocado Seed; Antioxidants; Phytochemicals

Abbreviations: IC50: Half Maximal Inhibitory Concentration (50%) ;UAE: Ultrasound-assisted Extraction; PHWE: Pressurized Hot Water Extraction; MAE: Microwave-assisted Extraction; EAE: Enzyme-Assisted Extraction; PLE: Pressurized Liquid Extraction; SFE: Supercritical Fluid Extraction; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; TAC: Total Antioxidant Capacity; ORAC: Oxygen Radical Absorbance Capacity; TE: Trolox Equivalent; TPC: Total Phenolic Content; DPPH: 2,2-difenil-1-picril-hidrazila (2,2-Diphenyl-1-picrylhydrazyl); ABTS: Ácido 2,2'-azino-bis(3-etilbenzotiazolina-6-sulfônico) (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); FRAP: Ferric Reducing Antioxidant Power; CEAC: Vitamin C Equivalent Antioxidant Capacity; TAA: Total Antioxidant Activity; TFC: Total Flavonoid Content; AA: Antioxidant Activity

Introduction

The food industry generates numerous by-products and residues during the manufacturing of goods, leading to significant environmental impacts due to the high organic load associated with these wastes and the costs related to their handling, storage, and transportation [1,2]. Consequently, alternative approaches for utilizing these residues are currently being explored, guided by knowledge of their composition [1]. According to Setyawan, et al. [3], industry and research could potentially benefit from the use of fruit seeds. The global avocado industry has seen remarkable growth, with production reaching approximately 8.98 million tonnes in 2022, an increase from 8.57 million tons in the previous year [4,5]. This growth trend is expected to continue, with projections indicating that global production volume could reach 12 million tonnes by 2030, which would be three times more than a decade ago [4,5]. Mexico remains the largest producer, contributing about 33% of global production, followed by other significant producers in Central and South America [4-6]. In Brazil, avocado production (Persea americana Mill.) increased from 197,000 tons in 2016 to 301,000 tons in 2021 [7]. The peel and seed are predominantly discarded; however, these residues are rich in bioactive

substances with antibacterial and antioxidant properties [2]. As a result, investigations on this fruit's characteristics have increased due to its high seed content, with the main goal being the bioprospecting of substances with scientific and commercial value [2]. Setyawan, et al. [3] indicated that avocado seeds have more phenolic compounds and antioxidant activity (AA) than pulp and peel. Tannins, flavonoids, phenolics, saponins, oxalates, phytates, and alkaloids are the main phytochemicals found in avocado seeds [3]. More specifically, avocado seeds are rich in:

1. Phenolic compounds, including hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids.
2. Flavonoids such as catechin and epicatechin are known for their antioxidant and anti-inflammatory properties.
3. Tannins and saponins, which exhibit anti-microbial and anti-inflammatory effects.
4. Alkaloids are associated with various health benefits, including neuroprotective effects.
5. Unsaturated fatty acids, including oleic and linoleic acids, are beneficial for cardiovascular health [3,8,9].

The diversity of chemical compound classes in avocado seeds has attracted interest in clinical research. Many of these compounds have demonstrated various beneficial activities, including anti-inflammatory, anti-allergic, antibiotic, antiviral, and anticancer properties, as reported in scientific literature [10]. Furthermore, the phytochemical compounds present in avocado seeds may also be associated with other activities, such as antioxidant effects (free radical scavenging), transition metal chelation (primarily through complexation with highly oxidative metal ions such as Fe^{2+} and Cu^{+}), and mitigating oxidative stress in biological systems [10].

Avocado seed extracts have potential applications beyond health and nutrition. They show promise in the development of functional foods, nutraceuticals, pharmaceuticals, and cosmetics [11,12]. Additionally, avocado seeds can be valorized to produce activated carbon, which is useful for environmental applications such as water purification [11,12]. Despite the growing body of research on avocado seed extracts, significant gaps in knowledge remain [10]. One of the key issues is the need for optimization and standardization of extraction methods, as various techniques can yield differing results in terms of efficiency and the stability of bioactive compounds [9]. Additionally, comprehensive identification and characterization of these compounds are still lacking, which hinders a deeper understanding of their specific health benefits and potential applications in food and cosmetics [6,8,9]. Stability challenges also need to be addressed, particularly given the sensitivity of antioxidant capacity to changes in pH, which can impact the application of these compounds in diverse food environments [10]. Therefore, there is a pressing need for the exploration of advanced and sustainable extraction techniques that can efficiently isolate valuable compounds from avocado seeds [10]. Finally, further extensive research is required to explore the full range of applications for the phytochemical compounds found in avocado seeds, including their potential uses in pharmaceuticals and nutraceuticals [11,12]. Therefore, using resources that are frequently discarded as waste-like avocado seeds-becomes sustainable and profitable when aimed at extracting components for use in industry and scientific study [9]. However, the concentration and variability of these bioactive compounds are directly influenced by the extraction method employed This investigation seeks to address the existing knowledge gaps by conducting a systematic review of the literature on extraction methods for avocado seeds. The primary objective of this study is to provide a comprehensive analysis and comparison of various extraction techniques, such as ultrasound-assisted extraction (UAE), pressurized hot water extraction (PHWE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and traditional solvent extraction methods. In addition, the study aims to assess the efficiency of these methods in terms of both yield and the preservation of bioactive compounds. Another key focus is to identify the most promising ex-traction strategies for future research that aims at bioprospecting new chemical compounds from avocado seeds. Furthermore, the study will offer recommendations for optimizing extraction processes, ultimately improving the utilization of avocado seed by-products across various industries [13-15].

Materials and Methods

This study was conducted as a systematic review, adhering to the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Titles and abstracts were comprehensively and unbiasedly screened, after which two independent authors selected studies that met the predefined inclusion criteria. The selected articles comprised peer-reviewed research conducted on human subjects and encompassed various study designs (Figure 1 provides an overview of the research selection process). The review protocol has been publicly available on the Open Science Framework and is registered under DOI 10.17605/OSF.IO/DQC67. A literature search was conducted on the SCOPUS database, a widely recognized multidisciplinary repository of peer-reviewed scientific literature, including journal articles, books, and conference proceedings. The search covered publications from January 2014 to September 2024. To refine and broaden the search strategy, a combination of the following key terms was employed: "Avocado seed", "Extraction", and "Antioxidants". These keywords were required to appear in the title, abstract, or keyword section of the retrieved studies.

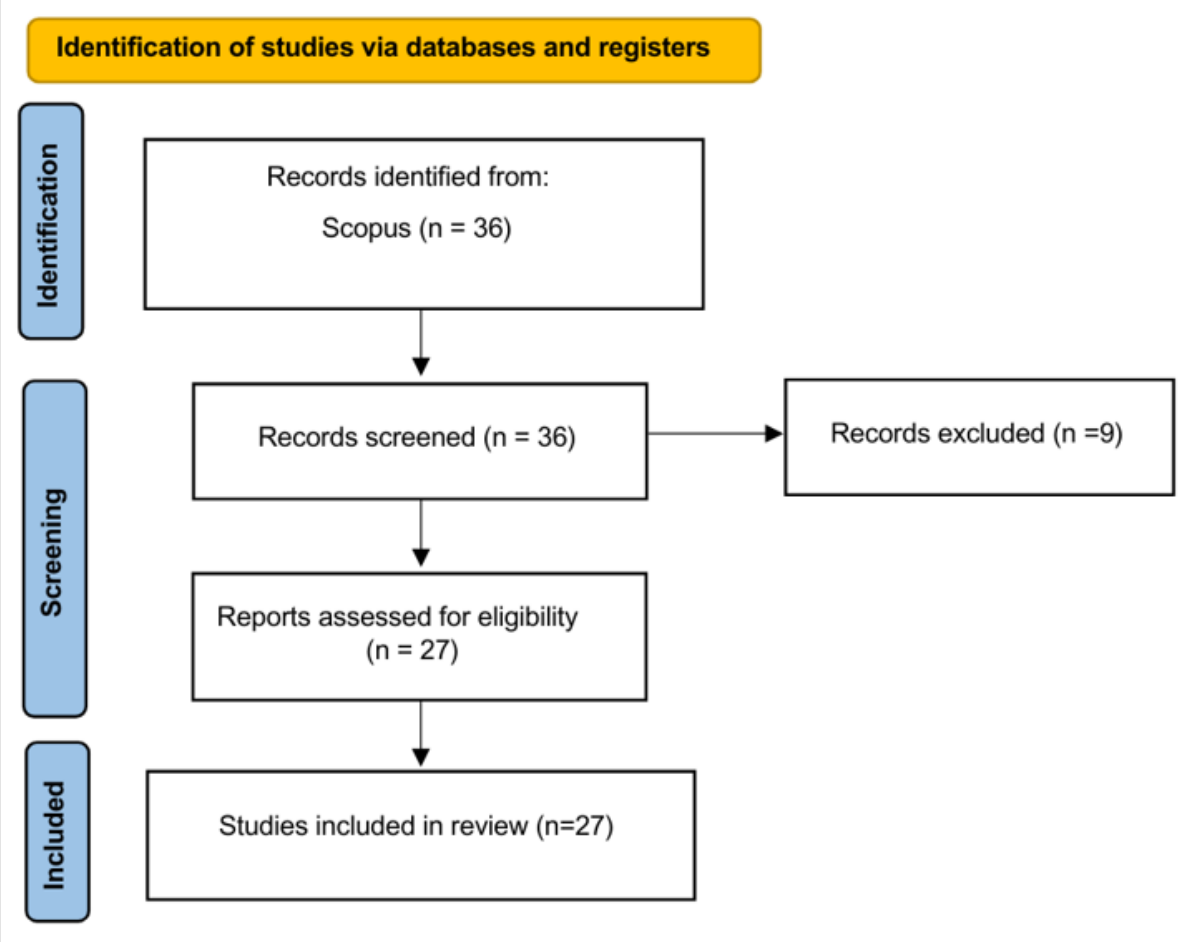

Figure 1: PRISMA flow diagram illustrates the inclusion and exclusion criteria applied to peer-reviewed studies in the present systematic review.

Only peer-reviewed publications in English were considered. The inclusion criteria were as follows:

a) Articles published between 2014 and 2024;

b) Studies explicitly addressing all three keywords in combination.

c) Research that included at least one assay for antioxidant capacity.

Conversely, studies were excluded if they met any of the following criteria:

a) Articles mentioning only one of the specified keywords;

b) Review articles;

c) Studies that assessed only total phenolic content without performing a direct antioxidant capacity assay.

Inclusion Criteria

The inclusion criteria were as follows:

a) Papers from the last 10 years were included;

b) The researchers should address at least one of the phenolic quantification tests or antioxidant capacity tests (TPC, DPPH, ABTS, or FRAP);

c) Only studies published in the English language were taken into consideration.

All included articles were thoroughly reviewed and analyzed.

Exclusion Criteria

We excluded the following types of experimental papers: (a) After reading the title, abstract, or full text, it did not address the investigated methodology.

Results and Discuss

The literature search and selection process were conducted in March and April 2024. Initially, 36 articles related to the topic were identified. Following an abstract review, the number of studies eligible for inclusion in the systematic review was reduced to 27 (Figure 1). Table 1 provides a detailed summary of the key findings, highlighting the main results identified across the selected studies, including information on the extraction methods, antioxidant capacity, and the bioactive compounds analyzed [16-42].

Table 1: Systematic Review of major Extraction Methods for Antioxidants.

Author	Extraction method	Antioxidant evaluation method	Results
Miramontes-Corona, et al. [16]	Maceration	FRAP	Antioxidant activity were higher for Hass (1329.48±6.89 µmol Fe^{+2}/g dry seed) as compared to Criollo (1276.18 ± 6.4 µmol Fe^{+2}/g dry seed).
Hassan, et al. [17]	Microwave-assisted	DPPH	The extracted and modified variants are then analyzed for their antioxidant properties against oxidative stress by checking their scavenging activity against DPPH (58.20% scavenging).
Bastos, et al. [18]	Soxhlet	DPPH	Avocado extract (91,4%) presented an antioxidant activity (AA%) that was 3.5% more effective than Quercetin (87,7%).
Del Castillo-Llamosas, et al. [19]	Microwave-assisted autohydrolysis	TAC, ABTS, FRAP and DPPH	This work demonstrates that microwave-assisted autohydrolysis is a promising technology for obtaining antioxidant compounds in avocado seeds. Regarding ABTS, the values ranged from 16.52–88.23 mg TE/g of AS and DPPH reached values from 7.31 mg TE/g of AS
Munthe, et al. [20]	Reflux extraction	DPPH	Extract ethanol 70% of P. americana seeds obtained from the maceration and reflux method has strong antioxidant activity (77,298 µg/mL and 98,626 µg/mL, respectively)
Razola-Díaz, et al. [21]	Ultrasound-assisted extraction	ABTS, FRAP and DPPH	The results showed that applying the sonotrode extraction method could increase antioxidant activity by 62–76% compared to ultrasound bath technology (values ranged from 0.31-12.29 µg TE/g d.w. for DPPH, 1.17–23.12 mg TE/g d.w. for ABTS, and 1.05–14.79 mg TE/g d.w. for FRAP technique
Garcia-Vallejo, et al. [22]	Ultrasound-assisted extraction	ABTS and DPPH	The highest antioxidant activity was obtained at 30% w/w ethanol, 20min, 40°C, and 1:10 solid-to-liquid ratio for the seed (736.22µmol Trolox/100g dry seed)
Razola-Díaz, et al. [23]	Bacterian fermented	DPPH and FRAP	Avocado seeds fermented by *Lactiplantibacillus plantarum* CECT 9567 showed the highest antioxidant activity measured by both DPPH and FRAP assays (6294.67 ± 19.44 and 6846.91 ± 2.13 µg TE/g d.w., respectively).
ONG, et al. [24]	Pressurized Hot Water Extraction	ABTS, CAEC and DPPH	Results demonstrated that avocado seed extracts have antioxidant activity (IC50 11-20 mg/mL) and inhibited oxidative stress-induced metabolomics changes in endothelial cells
DO, et al. [25]	Thermostated water bath shaker	DPPH	The extract TN-1 showed the highest total phenolic compounds value of 104.30 mg GAE/g DW, followed by PD-1 (97.24) and PD-2 (95.25) with no significant difference ($p > 0.05$).
David, et al. [26]	Aqueous, Ethanolic, and Supercritical fluid extracts	ABTS, FRAP and ORAC	The aqueous extracts had a greater capacity to trap the hydroxyl radical (52.23 µmol TE/ g and 51.47 µmol TE/ g for DS-H2O and FS-H_2O, respectively) than the ethanolic extracts (10.62 µmol TE/ g and 14.75 µmol TE/ g for DS-EtOH and FS-EtOH, respectively)
Peccin, et al. [27]	Maceration	DPPH	The ethanolic extracts showed greater antioxidant activity (IC50 = 0.013 mg/mL and 0.018 mg/mL) at lower extraction temperatures, 4°C and 25 °C, respectively.
Tan, et al. [28]	Ultrasound-assisted extraction	DPPH and FRAP	Ultrasound-assisted extraction for 60 mins resulted in the highest antioxidant activity (ethanolic avocado seed extract - 158.77 mg TE/g)
HUE, et al. [29]	Maceration, Percolation, Hot extraction and Soxhlet	DPPH and FRAP	For instance, sample 4B (Soxhlet method) exhibited the highest DPPH inhibitory activity (IC50 = 19.24±1.23 µg/mL)
SHI, et al. [30]	Soxhlet	ABTS and DPPH	The seed extracts showed higher antioxidant activity, the Soxhlet ripe seed extract had the highest DPPH and ABTS scavenging capacity (70 ± 0.12%; 78 ± 0.56%)
Yepes-Betancur, et al. [31]	Solid-state fermentation	ABTS and DPPH	The ability of the fungus to degrade compounds present in the avocado seed was decisive in enhancing the antioxidant capacity. ABTS and DPPH were 108.47 mg catechin/g, 1361.57 mg TE/g and 19.17 mg TE/g
YEO, et al. [32]	Maceration, Heating and Ultrasonication	DPPH	The extraction of avocado seeds and seed husks with 100% ethanol by maceration showed highest antioxidant activities and lowest IC50 values compared to 80% ethanol extract.

Ibarra-Buenavista, et al. [33]	Methanolic extracts	ABTS and DPPH	The use of instant controlled pressure drop (DIC) method allowed increasing three times the antioxidant capacity of seed extracts.
Paramos, et al. [34]	Soxleht Extraction and Supercritical fluid	ABTS and DPPH	The highest Antioxidant Activity measured by DPPH assay in extracts from ME seeds using the SE + EtOH was 65750 ± 1720 µmol TEAC/100 g
Araújo, et al. [35]	Micro-wave Extraction	ABTS, DPPH and ORAC	The optimized extracts obtained with acetone and ethanol showed a high antioxidant activity measured by DPPH (266.56 ± 2.76 and 221.69 ± 20.12mg ET/g extract), ABTS (607.28 ± 4.71 and 516.34 ± 11.81mg ET/g extract) and ORAC (475.55 ± 47.82 and 495.25 ± 14.52mg ET/g extract)
Weremfo; Adulley, et al. [36]	Micro-wave Extraction	ABTS and DPPH	Optimal conditions for the simultaneous extraction of phenolic compounds and antioxidant activity were ethanol 58.3% (v/v), microwave power of 400W, and extraction time of 4.8min (experimental values obtained ranged from 22.93-79.76% DPPH inhibition, and 11.21-80.32% ABTS inhibition)
VO, et al. [37]	Ethanol extract, Hexane fraction, Dichloromethane fraction, Ethyl acetate fraction, and Distilled water fraction	ABTS and DPPH	The solvents with moderate polarity such as dichloromethane (IC50 48.0 ± 3.4 µg/mL) and ethyl acetate (88.0 ± 2.8 µg/mL) were suitable for extracting potential DPPH and ABTS+.
Segovia, et al. [38]	Centrifuged	ABTS and ORAC	Radical scavenging methods have values between 1310–263 µmol TE/g for ORAC and ABTS, respectively.
Figueroa, et al. [39]	Accelerated solvent extraction	ABTS, DPPH, AAPH and TROLOX	Concerning the DPPH assay, the concentration of avocado extracts required to decrease by 50% (IC50) absorbance were 10.4 and 15 µg extract/ml for seed coat and seed, respectively.
Boyadzhieva, etal. [40]	Thermostated water bath shaker	DPPH	The method of antioxidant extraction from avocado waste (seeds) is determined as follows: 30% ethanol, 70ºC, solvent-to-solid material ratio 8, process duration 60 min obtain 1250 mg DPPH/g DE
Segovia; Corral--Pérez, et al. [41]	Ultrasound-assisted extraction	ORAC	The experimental results showed that temperature and ultrasound power had a significant influence on the extraction of polyphenols from avocado seeds. Increasing temperature and ultrasound power resulted in extracts with higher polyphenol content and antioxidant capacity.

Antioxidant Capacity Tests

The most regularly used assays for antioxidant capacity were DPPH (22 papers) and ABTS (14 studies), which are considered standard analyses and extensively studied in literature [30]. The ABTS and DPPH antioxidant capacity tests are widely accepted and recognized for their reliability in measuring the antioxidant activity of compounds, foods, and plant extracts [43,44]. Both the ABTS and DPPH assays operate effectively in both hydrophilic and lipophilic environments, providing a wide spectrum of results and applications across diverse samples, such as foods, extracts, and pharmaceutical products [43,44]. The high reproducibility and widespread use of these tests in literature can be attributed to their simplicity, speed, and suitability for use with common laboratory equipment. A comprehensive database is also provided, which is necessary for comparative research [43,44]. However, while these methods are widely used and accepted, they have some limitations [45].

Both DPPH and ABTS assays primarily measure the hydrogen-donating abilities of antioxidants, which do not necessarily reflect the full spectrum of antioxidant mechanisms present in complex biological systems [45,46]. For instance, these tests may not accurately capture the antioxidant capacity of compounds that function via electron transfer mechanisms or those that interact with specific cellular targets [45]. Additionally, the DPPH test is highly sensitive to pH and solvent effects, which can lead to variability in results depending on the matrix of the sample [45,46]. The ABTS test, although versatile, can suffer from interference in the presence of high concentrations of reducing agents or other compounds in the matrix that may skew the results [46]. These limitations underline the need for complementary testing methods to capture the full scope of antioxidant activity and to validate the results obtained from DPPH and ABTS assays [45,46].

Extraction Methods

The most used extraction methods were maceration (5 articles), ultra-sound-assisted extraction (4 articles), and microwave-assisted extraction (4 articles), accounting for 48% of the studies analyzed. The extraction method is a critical factor in determining the concentration and chemical characterization of natural extracts, as different methods yield varying concentrations and types of phytochemicals [3]. The extraction process determines the concentration of alkaloids, flavonoids, phenols, and tannins in the extracts, which directly influences their biological and antioxidant activities [3]. The techniques used for extracting bioactive components are crucial for de-fining the compound profile of the extract, optimizing extraction efficiency, and maximizing the bioactivity of the compound's present [47]. It is essential to note that the extraction method does not only impact on the yield of bioactive compounds but also their biological quality. For example, micro-wave-assisted extraction (MAE) and ultrasound-as-

sisted extraction (UAE) are often favored for their ability to reduce extraction time and solvent usage. However, they can sometimes induce thermal degradation of sensitive compounds, such as flavonoids and polyphenols, leading to a reduction in their biological activity. Conversely, maceration, although a slower process, is less likely to cause degradation, but it may result in lower yields due to its inefficient use of solvents and extraction time. The choice of solvent also plays a critical role-polar solvents such as ethanol or methanol may be more effective for extracting phenolic compounds, while non-polar solvents may be better suited for extracting lipophilic compounds like essential oils and terpenes [3,48].

Moreover, different extraction methods can lead to variations in the phytochemical profiles of the extracts, which directly influence their antioxidant and biological activities [47]. For example, a study comparing maceration and UAE found that UAE produced extracts with higher levels of bioactive compounds but also higher levels of degradation byproducts due to the generation of heat during the process [3]. These differences highlight the importance of selecting an extraction method that aligns with the specific goals of the research [13]. If the primary goal is to maximize the antioxidant potential of an extract, methods that balance yield and preservation of bioactivity should be prioritized [13]. Therefore, the selection of an extraction method should not only consider the quantity of compounds extracted but also the quality-how well the method preserves the bioactive potential of the phytochemicals [41,47]. This distinction is crucial for ensuring that the extract retains its biological efficacy in applications ranging from pharmaceutical formulations to functional foods [41,47]. In this sense, the choice of extraction method significantly impacts the scientific interpretation of bioactivity results, as different methods may lead to different biological outcomes due to alterations in the chemical composition of the extract [13,48].

Extraction Methods: Evaluation of IC50 Values: Antioxidant capacity assays are crucial for evaluating the antioxidant effects of natural extracts and, more importantly, for assessing the efficiency of different extraction methods [43,44]. The IC50 value refers to the concentration of an antioxidant required to inhibit 50% of the free radical activity in each system [29,32]. In this context, the study by Hue, et al. [29] effectively demonstrated the analysis of yield and antioxidant capacity of 70% ethanolic extracts obtained via four distinct ex-traction methods (Maceration, Percolation, Hot Extraction, and Soxhlet). The study concluded that the Soxhlet method is the most effective strategy for extracting antioxidants from avocado seeds [29]. IC50 value for the Soxhlet method using 70% ethanol (at a temperature of 70–80°C) was 9.24 µg/mL, the lowest reported among all other studies and extraction methods analyzed [29]. The IC50 values for the other methods were as follows: Maceration 42.98 µg/mL, Hot Extraction 43.44 µg/mL, and Percolation 67.48 ± 0.98 µg/mL [29]. Subsequently, the best IC50 values for DPPH were reported by Peccin and colleagues [27], with values ranging from 13 to 18 µg/mL for the maceration extraction process. Similar results were observed by Ong , et al. [14], with IC50 values ranging from 11–20 µg/mL. Another investigation, by Yeo, , et al. [32], reported slightly higher IC50 values of 23.0 µg/mL for the maceration technique.

Study by Ong, et al. [14] elucidated the efficiency of Pressurized Hot Water Extraction (PHWE), presenting one of the best IC50 values for DPPH. This technique, which uses a green solvent (pressurized hot water), is employed to extract compounds from plant materials [14]. Munthe, et al. [20] obtained some of the least effective IC50 values for DPPH (Maceration method: IC50 = 77.298 µg/mL and Reflux method: IC50 = 98.626 µg/mL), suggesting that these methods are less effective compared to others analyzed in this review. Similarly, Vo, et al. [37] reported lower antioxidant activity with extraction using ethyl acetate (88.0 ± 2.8 µg/mL). However, it is important to note that IC50 values can be influenced by several experimental conditions, such as solvent type, temperature, extraction time, and the chemical composition of the plant material. Variations in these parameters can lead to discrepancies in the reported antioxidant capacity across studies. Furthermore, the IC50 value alone does not fully capture the bioactivity of an extract, as it is a measure of radical scavenging ability, but does not account for other mechanisms of antioxidant activity, such as metal chelation or enzymatic interactions. It would be beneficial to include a broader range of assays (e.g., FRAP, ORAC) to provide a more comprehensive understanding of the antioxidant potential of the extracts.

Extraction Methods: Comparison Between ABTS and DPPH

Analyzing the DPPH inhibition percentage, the Soxhlet method still prevailed as the most efficient Bastos, et al. [18] found more significant values for DPPH inhibition, with 91.4%, followed by 86% reported by Shi, et al. [30] using Soxhlet extraction for antioxidant compounds. The differences in methodology could have been significant, such as the solvent used (Ethanol and Methanol), extraction time (2 hours versus 6-8 hours), and temperature (70°C and 64°C, respectively). Bastos, et al. [18] may have highlighted one of the best Soxhlet extraction strategies, using ethanol as a solvent and a shorter extraction time. The seed pre-treatment could also have been crucial for the final antioxidant activity result, with Bastos and colleagues [18] using dried seeds at 60°C for 96 hours compared to Shi, et al. [30], who used freeze-drying at −80°C for 4 days. Another methodological difference to consider is the solid-to-liquid ratio, with the solvents used in the following proportions: 1:10 and 1:18 [18] and [30], respectively). Paramos, et al. [34] also re-ported greater antioxidant efficiency for the Soxhlet extract compared to the Super-critical CO2 fluid extract. This finding highlights the effectiveness of the Soxhlet method, which may offer superior extraction of bioactive compounds with antioxidant properties when compared to alternative extraction techniques such as supercritical fluid extraction. The use of Soxhlet extraction is well documented for producing crude extracts from various plants, as seen

in the studies by Oliveira, et al. [49,50], which also reported the best extraction results using this method. Soxhlet extraction has proven to be an efficient and reliable technique for obtaining high yields of bioactive compounds from plant materials, making it a preferred choice in many phytochemical investigations.

Among the studies that analyzed Ultrasound extraction, Razola-Díaz, et al. [21] reported better DPPH values than Tan, et al. [28], with values of 0.31–12.29 µg TE/g and 0.158 µg TE/g, respectively. The use of a sonotrode (high-intensity ultrasound) resulted in significant increases in antioxidant activity of the extracts compared to the control, showing a 70% increase in DPPH, a 76% increase in ABTS, and a 70% increase in FRAP in their study [21]. High-intensity ultrasound was much more effective than the ultrasound bath method in enhancing the extraction of antioxidant compounds [21]. The difference between the methods highlights the importance of ultrasound intensity in maximizing antioxidant extraction, suggesting that the use of a sonotrode is preferable for obtaining higher quantities of bioactive compounds [21,28]. The use of sonotrode likely improves the release of bioactive compounds by promoting greater cavitation, which disrupts plant cells, thereby re-leasing more soluble compounds [21]. The microwave extraction method showed differences in DPPH % values between studies, with Weremfo, Adulley, and Adarkwah-Yiadom [36] reporting 79.76% and Hassan, et al. [17] reporting 58.2%. The experimental study by Weremfo, Adulley, and Adarkwah-Yiadom [36] involved ethanol extractions with different concentrations (via Microwave-assisted extraction), determining the "optimal conditions for the simultaneous extraction of maximum phenolic compounds (TPC and TFC) and antioxidant activity (DPPH and ABTS) from avocado seeds were ethanol concentration of 58.3%, microwave power of 400 W, and extraction time of 4.8 min" ([36] pp. 9). The methodology likely played a key role in the discrepancy of results, as Hassan, et al. [17] used a microwave-assisted heating process with only water (heating treatment up to 150°C and a treatment time of 30 minutes). The comparison of extraction methods based on ABTS and DPPH assays highlightsthe variability in antioxidant activity depending on the technique and conditions employed. Soxhlet extraction, particularly with ethanol as a solvent, generally provided the most efficient results across various studies, showcasing higher antioxidant capacities. Similarly, ultrasound-assisted extraction, particularly when using a sonotrode, demonstrated substantial improvements in antioxidant activity, likely due to enhanced cavitation and cell rupture. Microwave-assisted extraction also exhibited promising results, although variations in solvent choice, temperature, and time influenced the outcomes. Overall, selecting the appropriate extraction method should consider the desired compound profile, efficiency, and the final application, ensuring that both high yield and optimal antioxidant activity are achieved.

Solvents, Concentrations, Temperature, and Extraction Time

The choice of solvent is crucial in extraction procedures since each material has a unique solubility in various solvents, which directly affects the extraction effectiveness [51]. As a result, a more thorough and effective product can be produced by carefully choosing the solvent to optimize the yield of target compounds while reducing the ex-traction of undesirable contaminants [51]. Furthermore, the concentration, temperature, and extraction time are also key factors [35]. The solvent concentration affects the dissolution capacity of the components, while the temperature can accelerate the solubility of compounds [51]. Controlling the extraction time is crucial to avoid excessive extraction of unwanted compounds [35]. The solvent concentration affects the dissolution capacity of the components, while temperature can accelerate the solubility of compounds and help overcome the energy barriers associated with extraction [50,51]. Controlling extraction time is crucial to prevent excessive extraction of unwanted compounds and to ensure that the extraction reaches its optimal point [50,51]. All these factors need to be balanced to achieve effective extraction, ensuring the quality and efficiency of the process and resulting in a final product of higher purity [51]. The analysis of the influence of temperature, solvent, and extraction time on chemical compounds in fruits has become an area of growing interest in scientific literature, particularly concerning the optimization of processes aimed at maximizing yield and effectiveness in extracting bioactive compounds [19,35,47]. The authors emphasize that optimizing methodologies not only reduces extraction time but also ensures high extraction rates, which is crucial for obtaining target phytochemicals [35,47]. The authors emphasize that optimizing methodologies not only reduces ex-traction time but also ensures high extraction rates, which is crucial for obtaining tar-get phytochemicals. However, the interplay of these variables-temperature, solvent, and time-requires a careful balance to prevent degradation of the target compounds [19,35,47].

The investigation by Guerra, Garcia, and da Silva [52] presents a literature report that can be extrapolated to our study. By analyzing the optimization of phenolic com-pound extraction from mango peel, it was revealed that the solvent polarity and temperature are determining factors for extraction, with an increase in temperature favoring the solubility of phenolic compounds [52]. Furthermore, the interaction between temperature and time demonstrated a positive relationship in the extraction process, reinforcing the connection between sample heating and the disruption of plant tissue, which facilitates the release of desired compounds [52]. Del Castillo-Llamosas, et al. [52] achieved 16.52 mg TE/g of AS at 150°C and 88.2 mg TE/g of AS at 260°C, determining those higher temperatures in aqueous solvent extraction led to more efficient extraction of antioxidant compounds.

These results were superior to those obtained by Ultrasound-assisted extraction [21], Micro-wave Extraction [35], and Solid-state fermentation [31]. A difference in ABTS values is observed when comparing the microwave-assisted extraction by Del Castillo-Llamosas, et al. [19] and the centrifugation extraction by Segovia [41]. The centrifugation (50% ethanol) showed more efficient results, with 65.97 mg TE/g of seed, compared to Segovia [38], who obtained 30.97 mg TE/g using methanol. Peccin and colleague also highlighted that the ethanolic extract exhibited greater antioxidant capacity (maceration extraction). Yeo, Lee, et al. [32] report that the cold maceration method with 100% ethanol exhibited the best antioxidant activity. When analyzing three ethanol concentrations in maceration, the IC50 value of the 80% ethanol extract was 58.7 µg/mL, while the IC50 value of the 100% ethanol extract was 23.0 µg/mL [32]. Razola-díaz, et al. [21] compared antioxidant assays (DPPH, ABTS, and FRAP) under different extraction conditions, using sonotrode (high-intensity ultra-sound) and bath ultrasound. The lowest antioxidant activity for DPPH, ABTS, and FRAP was obtained under extraction conditions with 100% ethanol, 25 minutes, and 20% amplitude [21]. The lower efficiency of this protocol is likely due to the limitation in extracting bioactive compounds that dissolve better in solutions with lower alcohol proportions, which are more amphiphilic, as reported by other studies in this review [22,36,38,42]. In their research, the optimal extraction conditions for avocado seeds were determined to be a 55:45 (v/v) ethanol/water mixture, 30 minutes, and 90% amplitude [21]. Under these conditions, antioxidant activity was maximized, suggesting that an appropriate ethanol/water mixture enhances the efficient extraction of both hydrophilic and lipophilic antioxidant compounds. The ethanol proportion was quite similar to that reported by Gómez et al. [42,36,22], indicating one of the best strategies for extracting antioxidants from avocado seeds using Thermostat water bath shaker, Microwave, and Ultrasound Extraction.

Gómez, et al. [42] discuss the importance of determining the ideal sol-vent concentration and temperature to achieve the best yield and antioxidant capacity. It was found that the optimal ethanol concentrations ranged from 10% to 60% in the extraction method using a Thermostat water bath shaker [42]. Consequently, the optimized extraction conditions were identified as 56% ethanol, 63°C, and 23 minutes of extraction, which was considered the ideal condition for maximizing antioxidant activity. Another interesting study to compare the importance of extraction methodology is by Araújo ,et al. [35], who elucidated that variations in temperature and extraction time affect the content of phenolic compounds and antioxidant activity in extracts from dried avocado seeds. Within the range of 60-90°C, both antioxidant activity and phenolic concentration remain stable, regardless of the extraction time variation [35]. These data indicate that phenolic compounds are extracted similarly within this temperature range, with no significant loss of antioxidant activity, as evidenced by authors [19,40,42]. Ong and colleagues [14] also evaluated antioxidant activity at different temperatures for PHWE, with extracts obtained at 60 °C and 80 °C showing significantly lower IC50 values for DPPH inhibition when compared to the temperatures of 100 °C and 120 °C. Another important finding by Araújo, et al. [35] regarding the correlation between phenolic concentration and antioxidant capacity was that at 75°C, the reduction in phenolic content did not affect the antioxidant activity in microwave-assisted extraction. At this temperature, the highest antioxidant activity was reported (134.15 mg TE/g of dry seed). Although the phenolic compound content was lower, the remaining compounds may have higher antioxidant potency and synergistic effects, or other non-phenolic antioxidants may contribute more significantly to the total antioxidant activity [35].

The study by Wolff, et al. [53], which investigated the extraction of antioxidant compounds in yerba mate (Ilex paraguariensis), highlighted the need for a minimum extraction time to achieve a balance between the concentrations of extracted compounds and the solvent. The determination of the optimal extraction time is essential to avoid the risk of degradation of the compounds due to prolonged extraction [53]. Their research found that polar organic solvents, such as methanol and ethanol, are more effective in extracting polyphenols, supporting the discussion on the importance of solvent selection [53]. The studies analyzed highlight the importance of temperature, solvent type, and extraction time in optimizing the processes for extracting chemical compounds from fruits, contributing to the development of more efficient and sustainable methodologies in the extraction of bioactive compounds. The optimization of extraction processes is crucial for maximizing the yield and bioactivity of target compounds. By carefully adjusting these factors, it is possible to enhance the extraction process, achieving higher yields while maintaining or even improving the antioxidant capacity of the extracts. The studies reviewed indicate that a combination of polar solvents, moderate temperatures, and carefully controlled extraction times can lead to more sustainable and effective methodologies, ensuring that the extracted compounds retain their desired bioactivity for various applications.

Other Relevant Factors

Shi and colleagues [30] highlighted an important point regarding the need to analyze different plant structures and the appropriate maturation stage to optimize the extraction of antioxidants. In their study, which evaluated extracts from mature and immature avocado seeds and peels, the mature seed extract exhibited the highest DPPH and ABTS sequestration capacities (values of 86±0.21% and 91±0.06%, respectively). Paramos, et al. [34] also investigated the relationship between the maturation stage and antioxidant concentration, finding higher antioxidant activity in mature seeds. The pretreatment of the seed must also be analyzed to determine the quality and quantity of the extracted compounds, with various techniques employed to facilitate the rupture of plant structures and cell walls [47]. However, high temperatures (>100 °C) during this process can negatively impact the yield of oil extraction [47]. The drying of the seed before extraction can also affect the result, as evidenced by David, et al. [26]. In their

study, the drying process of avocado seeds reduced the amount of antioxidant compounds and, consequently, the antioxidant capacity of the extracts [26]. The relationship between drying temperature and extraction process, ensuring it does not negatively impact antioxidant capacity, was also mentioned in previous studies reviewed, such as those by Ong ,et al. [14,38,40,42].

An interesting pre-processing method was suggested by Del Castillo-Llamosas, et al. [19], autohydrolysis, a process that uses water to hydrolyze hemicellulose in lignocellulosic biomass. Microwave-assisted autohydrolysis stood out as a promising alternative; however, its results were still inferior to those reported by Araújo, et al. [35]. Further investigations could be conducted to evaluate the efficiency of this pretreatment in combination with other extraction methods and protocols. A new strategy for extracting antioxidant compounds was reported by Razo-la-Díaz, et al. [23], who used lactic acid bacteria to enhance the extraction of bioactive compounds. They reported a higher antioxidant capacity compared to non-fermented seeds in antioxidant capacity tests. Among the different strains tested, Lactiplantibacillus plantarum CECT 9567 showed the highest antioxidant activity, measured by both DPPH and FRAP assays, with values of 6294.67 ± 19.44 and 6846.91 ± 2.13 µg TE/g d.w. (TROLOX), respectively. However, their results were inferior when compared to the TROLOX values of microwave-assisted extraction (266.56 ± 2.7 mg TE/g [35]) and solid-state fermentation (19.17 mg TE/g [31]). According to all the previously discussed viewpoints, using natural products-like avocado seeds-that are frequently thrown away become sustainable and commercially feasible when they are used to extract compounds for use in industry or health-related research [9]. The selection of extraction techniques and methodology should be based on the nature of the target compounds and the objective final application. Processes that preserve the integrity of bioactive compounds and utilize sustainable methods are preferred to ensure high-quality products and biological efficiency. In addition to the efficiency of the extraction method, it is essential to consider the chemical nature of the phytochemicals to be isolated, as each chemical compound has specific characteristics of polarity, stability, and solubility, which directly influence its extraction. Therefore, the choice of solvent and method should be strategically adjusted to maximize the ex-traction of the desired phytochemicals. The destination of the extracts -whether for food formulations, cosmetics, or pharmaceuticals- may require different levels of purity, toxicity, or concentration of specific phytochemicals.

Conclusions

The extraction of antioxidants from avocado seeds can vary significantly based on the extraction method, solvent used, and its concentration. The method of extraction directly influences the yield of phytochemicals, which are closely linked to antioxidant activity. Ethanol extraction, particularly at specific concentrations, has proven to be the most effective in isolating compounds with high antioxidant capacity, as demonstrated by the low IC50 values reported in various studies. However, the efficiency of each method should not be the only criterion for selection. The research objectives, the specific class of phytochemicals targeted for isolation, and the intended application of the extract are essential factors in determining the optimal extraction method. Therefore, the final decision must balance the extraction method, the desired phytochemical class, and the anticipated antioxidant activity. Author Contributions: All authors have made substantial contributions to the work and agree to be accountable for all aspects of it. All authors have approved the final version of the manuscript to be published and agree to be personally accountable for their own contributions. They also ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and documented.

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Conflicts of Interest

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