

EFFECT OF EXTRACTION TEMPERATURE ON POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF BOOTH AVOCADO SEEDS (*Persea americana*)

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Abstract

This study examined how extraction temperature influences the total phenolic content and free radical scavenging activity of avocado seed extract. Avocado seed extracts were obtained using a reflux Soxhlet method with 70% ethanol as the solvent at temperatures ranging from 30 to 70 °C. The highest extraction yield ($34.67 \pm 1.06\%$) was obtained at 50 °C. A significant increase in total phenolic content ($P < 0.05$) was observed as the extraction temperature increased from 30°C to 50°C. However, further increases in temperature (≥ 60 °C) resulted in a reduction in total phenolic content. The total phenolic content of the 70% ethanol extract of avocado seeds obtained at 50°C was 129.29 ± 5.92 mg GAE/kg DW. Extracts obtained at lower temperatures exhibited higher free radical scavenging activity than those obtained at higher temperatures, with 50°C identified as the optimal extraction temperature, yielding the lowest half maximal inhibitory concentration ($IC_{50} = 30.23 \pm 2.15$ mg/mL).

Keywords: antioxidant activity, Avocado seed, booth avocado, DPPH assay, ethanol extraction, extraction temperature, total phenolic content.

1. Introduction

Avocado is a widely consumed fruit in Vietnam, with various cultivars such as Booth, Tu Quy, and wax avocado. It is highly regarded for its nutritional value and potential health benefits. However, the seed and peel are discarded as inedible parts, accounting for approximately 25-30% of the total fruit weight. (Domínguez et al., 2014). The disposal of avocado seeds and peels may contribute to organic waste accumulation and environmental management challenges (Mora-Sandí et al., 2021). Thus, the valorization of avocado seed waste emerges as an effective and sustainable approach. Rich in bioactive compounds, avocado seeds can be transformed into value-added products, thereby reducing environmental impacts, lowering emissions, and promoting circular production systems. At present, avocado seeds are used only in small quantities and on a limited basis for nutritional and health applications. As a result, the potential of avocado seeds has not yet been fully exploited, though avocado trees are widely cultivated nationwide.

Avocado seed has a high oleic acid content and contains significant levels of acid, iodine, and peroxide values, as well as unsaponifiable matter (Charles, Dadmohammadi, & Abbaspourrad, 2022). Notably, it is rich in phenolic compounds and tocopherols, which underpin its extensive utilization across the food, cosmeceutical, and pharmaceutical industries. As a functional food ingredient, avocado oil offers health benefits beyond basic nutrition, including enhanced health status and reduced disease risk.

Numerous extraction strategies have been proposed for the recovery of bioactive compounds from plant-based materials, including conventional solvent extraction, ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, and other emerging green extraction technologies. Despite the availability of these advanced techniques, conventional solvent extraction remains the most widely used approach due to its simplicity, reproducibility, and scalability for industrial applications.

In this study, ethanol was used as the solvent in Soxhlet extraction to ensure safety and environmental friendliness. The phenolic compounds were analyzed, and antioxidant activity was evaluated using the DPPH radical assay.

2. Literature review

The valorization of avocado waste has emerged as a promising strategy not only to mitigate environmental issues associated with organic waste accumulation but also to exploit its rich bioactive profile, particularly phenolic compounds with antioxidant, antimicrobial, and anti-inflammatory activities. Previous studies have consistently demonstrated that avocado by-products, especially seeds and peels, are richer in phenolic compounds than the pulp. For instance, Xiaoyan Lyu et al. reported that seeds and peels of Hass, Reed, and Wurtz avocados exhibited significantly higher total phenolic content (TPC), with seed values ranging from approximately 26–45 mg GAE/g, along with strong DPPH radical scavenging activity, while the pulp showed negligible antioxidant capacity (Lyu et al., 2023). Similarly, Wang et al. investigated several avocado cultivars, including Booth 7 and Booth 8, using an acetone/water/acetic acid extraction system, and confirmed that seeds possessed the highest phenolic content and antioxidant activity, followed by peels and pulp (Wang, Bostic, & Gu, 2010).

In addition to cultivar differences, extraction techniques and solvent systems have been shown to significantly influence phytochemical recovery. Ultrasound-assisted extraction (UAE), for example, enhances the extraction efficiency of bioactive compounds, with TPC values reported in the range of 31.90–41.62 mg GAE/100 g depending on sonication time (Tan et al., 2022). Moreover, solvent fractionation using organic solvents such as hexane, dichloromethane, ethyl acetate, and ethanol has revealed that certain fractions exhibit strong antioxidant activity, with dichloromethane extracts showing DPPH ($IC_{50} = 48.0 \mu\text{g/mL}$) (Vo & Le, 2019). Overall, the TPC of avocado residues varies widely, typically ranging from 45 to 180 mg GAE/g dry weight, depending on cultivar, extraction method, and processing conditions. Despite these findings, most studies have focused on commonly cultivated varieties such as Hass, while limited attention has been given to Booth avocado, particularly under optimized, food-grade extraction conditions. In addition, although various organic solvents have been employed, there remains a need to evaluate environmentally friendly, safe solvent systems suitable for practical applications. Therefore, this study aims to investigate the phenolic content and antioxidant activity of Booth avocado seed extracts using 70% ethanol as a green and effective extraction solvent.

3. Research Methods

3.1. Materials

Booth avocado fruits were obtained from Thu Dau Mot Market, Ho Chi Minh City, Vietnam. Booth avocados were acquired from Thu Dau Mot Market in Ho Chi Minh City,

Vietnam, between October and November 2025. These fruits were sourced from local suppliers, confirming that they were grown in southern Vietnam. A total of 30 avocados were selected for this study. Only whole fruits that showed no visible defects, mechanical damage, or signs of fungal infection were included. The ripeness of the avocados was evaluated based on their external color and firmness, and only those that were moderately ripe were selected to ensure uniformity. The Booth cultivar was identified by its distinguishing characteristics, such as larger size, thick green skin, round to slightly oval shape, and firmer flesh when compared to other avocado varieties. Once collected, the fruits were transported to the laboratory and kept at room temperature for no more than 48 hours before processing. To prepare the seeds, they were manually extracted from the fruit, washed with distilled water to eliminate any remaining pulp, and then air-dried. The initial moisture content of the seeds was measured at 65.42% using the gravimetric method.

3.2. Research chemicals

- Na_2CO_3 (China, 96%), HCl (China, 36%), urea (China, 99%), ethanol (Vietnam, 99.5%), Folin–Ciocalteu’s phenol reagent (2N, India), 1,1-diphenyl-2-picrylhydrazyl (DPPH, USA, 99.9%), ascorbic acid (VWR Chemicals, 99.9%).

- Spectrophotometer (Jasco J770), rotary evaporator (Neyela-1110), Soxhlet (Iwaki), analytical balance (Onelab)

3.3. Methods

3.3.1. Extracts preparation

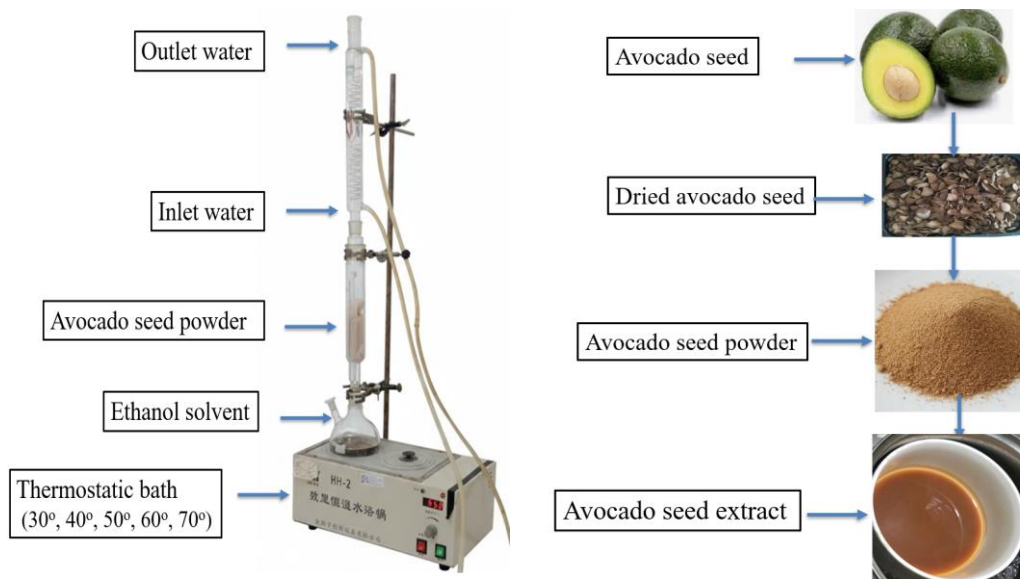


Figure 1. Schematic diagram of avocado seed extraction

The avocado was washed, the seeds were manually removed, and any damaged seeds were discarded. The seeds were washed again to remove adhering impurities, sun-dried, ground into powder, and sieved to obtain a uniform particle size. 5 g of the seed powder were weighed into a filter bag and placed in a Soxhlet extractor containing 250 mL of ethanol as the extraction solvent. The extraction was carried out at 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C using a thermostatically controlled water bath for 6 h (Fig. 1). After

filtration, the combined extracts were evaporated to dryness under reduced pressure at 50 °C using a rotary evaporator. The dried residues were collected in amber glass bottles and stored at 4 °C before further analyses (Rodríguez-Carpena et al., 2011).

3.3.2. Measurement of total phenolic content (TPC)

Total phenolic content was measured using the Folin–Ciocalteu colorimetric assay, which relies on the reduction of the reagent by phenolic compounds. Antioxidant constituents cause the reagent to change from pale yellow to an intense blue, with color intensity proportional to phenolic concentration (Singleton & Rossi, 1965). Before analysis, 0.10 g of the dried extract was dissolved in 70% (v/v) ethanol and diluted to a final volume of 100 mL with distilled water. An aliquot of 0.1 mL of the extract sample or standard solution was pipetted into a test tube, followed by the addition of 0.50 mL of Folin–Ciocalteu reagent (10%, v/v), and mixed thoroughly. Subsequently, 0.40 mL of 10% (w/v) Na₂CO₃ solution and 1.50 mL of distilled water were added. The mixture was shaken well and incubated at room temperature in the dark for 2 h. The reaction mixture changed in color from yellow to dark blue, after which the absorbance was measured at 765 nm. The total phenolic content was calculated based on a gallic acid standard curve (mg GAE/kg DW), with all experiments performed in triplicate and statistical significance set at $P < 0.05$.

3.3.3. Determination of antioxidant activity based on DPPH radical scavenging capacity

DPPH is a stable free radical commonly used to screen the antioxidant activity of test compounds. Antioxidant activity is evaluated by the reduction in DPPH's purple color, quantified as absorbance at 517 nm. (Brand-Williams, Cuvelier, & Berset, 1995).

Preparation of DPPH reagent (0.60 mM): 5.92 mg of DPPH was accurately weighed, dissolved in absolute ethanol, and transferred to a 25 mL volumetric flask. The volume was adjusted to the mark with absolute ethanol.

Preparation of test samples: The sample solution with an initial concentration of 1000 µg/mL was serially diluted to obtain concentrations of 100, 50, 25, 12.50, and 6.25 µg/mL. Vitamin C was used as a positive control and prepared in absolute ethanol at concentrations of 50, 25, 12.50, 6.25, and 3.13 µg/mL—absolute ethanol served as the negative control.

First, 0.50 mL of the sample solution was added to a test tube, followed by 3.00 mL of absolute ethanol. Then, 0.50 mL of DPPH solution (0.60 mM) was added. The mixture was thoroughly shaken and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer (J770). The DPPH radical-scavenging activity and IC₅₀ values (µg/mL) were calculated to assess the relationship between scavenging activity and sample concentration. The experiment was performed in triplicate, and the results were analyzed at a significance level of $P < 0.05$.

4. Results and Discussion

4.1. Extraction yield (%) of avocado seed extract

Avocado seed powder was extracted by Soxhlet reflux with 70% ethanol at temperatures ranging from 30 to 70°C. As illustrated in Fig. 2, the maximum extraction yield ($34.67 \pm 1.06\%$) was achieved at 50°C. Extraction temperature significantly influences both the

efficiency of the process and the stability of bioactive compounds. Increasing the temperature enhances solvent penetration, solubility, and mass transfer, thereby facilitating the release of target compounds from the plant matrix. Nevertheless, excessive temperatures (60–70°C) may induce oxidative degradation of thermolabile constituents, such as vitamin C and polyphenols, thereby diminishing antioxidant activity. Conversely, lower extraction temperatures limit extraction efficiency due to reduced solubility, slower diffusion kinetics, and restricted mass transfer between the solvent and solid matrix (Ma et al., 2022).

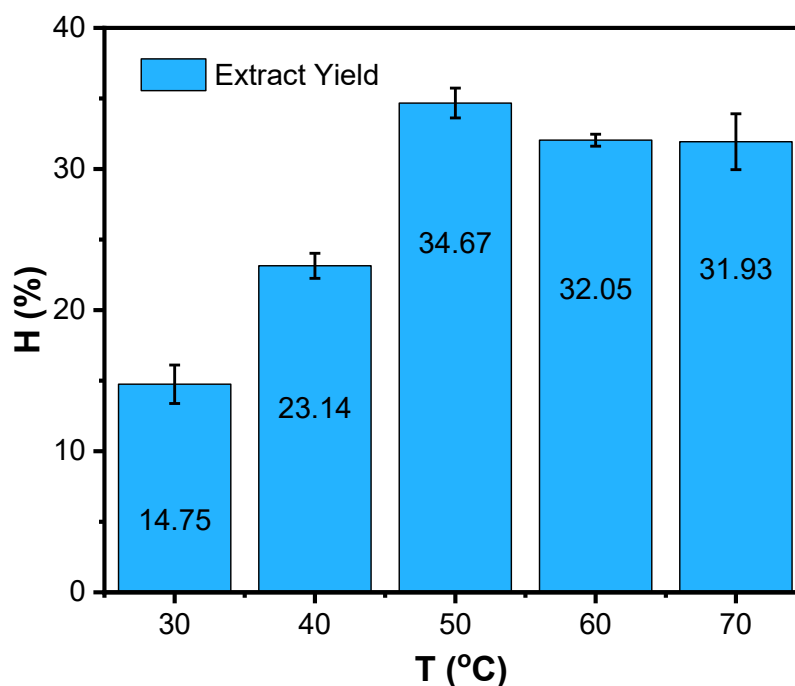


Figure 2. Effect of extraction temperature (30 - 70°C) on the extraction yield of avocado seed powder obtained by Soxhlet extraction using 70% ethanol

4.2. TPC of the extracts

TPC of the extracts was calculated using the linear regression equation derived from the gallic acid equivalent (GAE) calibration curve. As illustrated in Fig. 3, TPC increased with increasing extraction temperature from 30 to 50 °C, reaching a peak of 129.29 ± 5.92 mg GAE/kg DW extract at 50 °C. In contrast, increasing the temperature further to 60 and 70 °C led to a slight decline in TPC. These results suggest that optimal extraction of phenolics occurs at moderate temperatures, whereas elevated temperatures can result in their thermal decomposition. Error bars represent standard deviations of triplicate measurements. This result is consistent with previous findings indicating that phenolic compounds are more efficiently extracted at moderate temperatures (40–60 °C), while higher temperatures (above 70 °C) may cause thermal degradation of phenolic acids (Antony & Farid, 2022). The TPC of Booth avocado seeds was higher than that reported for Slimcado seed (19.20 mg GAE/kg), Hass seed (51.60 mg GAE/kg), Tonnage seed (33.10 mg GAE/kg), Loretta seed (31.15 mg GAE/kg), and Simmonds seed (40.20 mg GAE/kg) (Rosero et al., 2019).

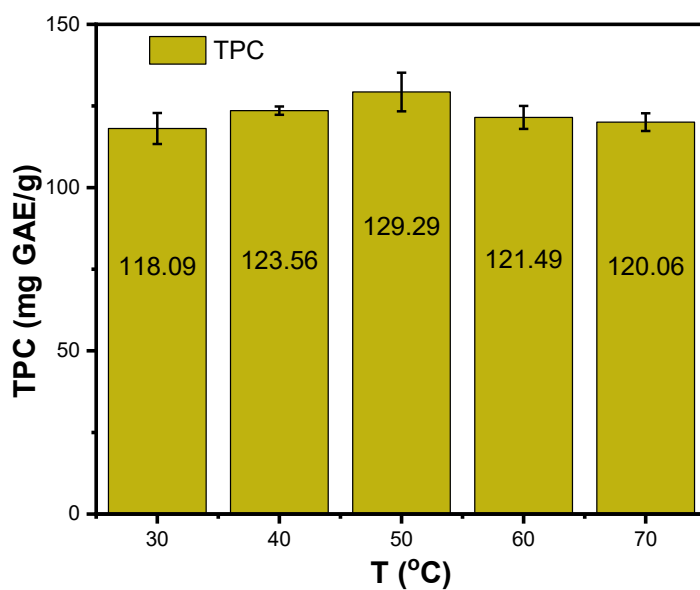


Figure 3. Variation of total phenolic content in avocado seed extracts as a function of extraction temperature

4.3. Antioxidant activity of the extracts (DPPH assay)

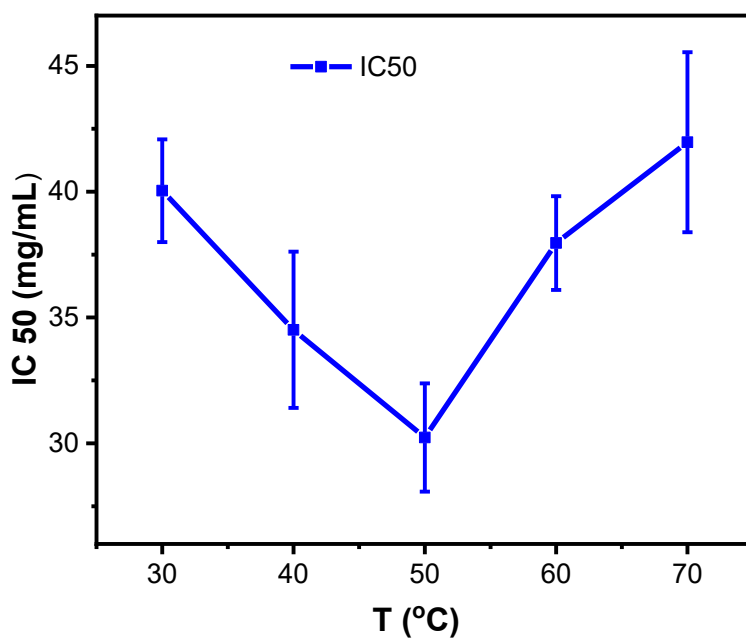


Figure 4. Variation of antioxidant activity content in avocado seed extracts as a function of extraction temperature

The antioxidant potential of avocado seed extracts, determined using the DPPH assay, is presented as IC₅₀ values (Fig. 4). The extracts exhibited good antioxidant activity, with

the highest activity observed at 50 °C ($IC_{50} = 30.23 \pm 2.15 \mu\text{g/mL}$). This sample exhibited stronger antioxidant activity than *Persea americana* Mill., as indicated by its lower IC_{50} value (77.29 mg/mL) (Munthe et al., 2023). Increasing the extraction temperature from 30 to 50°C enhanced antioxidant activity (lowered IC_{50}), whereas further increasing it to 70°C decreased activity (increased IC_{50}), suggesting that excessive heat may degrade temperature-sensitive antioxidants. Extraction temperature influences polyphenol stability, as chemical and enzymatic degradation at elevated temperatures can decrease total phenolic content and reduce antioxidant capacity (Akowuah, Mariam, & Chin, 2009).

All figures and tables in this article are the results of the authors' own research.

5. Conclusion

The experimental results showed that the extraction yield, polyphenol content, and antioxidant activity were significantly influenced by the extraction temperature during Soxhlet extraction using ethanol as the solvent. Avocado seeds exhibited relatively high polyphenolic content and antioxidant activity compared with those reported in previous studies. The extracts also exhibited pronounced DPPH radical scavenging activity. These results provide a scientific rationale for the further isolation of bioactive compounds and their potential application in targeted products.

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