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ANTIOXIDANT POTENTIAL OF *PERSEA AMERICANA* MILL. SEED EXTRACTS *IN VITRO*: THE ROLE OF BIOLOGICALLY ACTIVE COMPOUNDS

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Abstract. This article presents the results of a comprehensive study on the chemical composition and antioxidant activity of aqueous – ethanolic extracts obtained from *Persea americana* Mill. (avocado) seeds using extractants of different concentrations (40 % and 70 %) and raw materials in different states (fresh and dry). Thin-layer chromatography confirmed the presence of ascorbic acid as well as phenolic compounds in all extracts, including gallic acid and quercetin. The total phenolic content was determined spectrophotometrically using the Folin – Ciocalteu method, while the total flavonoid content was measured by the aluminum chloride colorimetric assay based on a linear regression equation derived from the quercetin calibration curve (expressed in QE). Quantitative analysis showed that the total phenolic content ranged from 1.204 to 1.861 mg/g, flavonoids – from 2.663 to 3.395 mg/g, and amino acids (determined by the ninhydrin reaction) – from 0.054 to 0.140 % in terms of alanine equivalent. The highest phenolic content was found in the 40 % extract from dry seeds, whereas flavonoids were more efficiently extracted with 40 % ethanol from fresh raw materials. Modeling of lipid peroxidation and protein oxidative modification processes *in vitro* revealed pronounced antioxidant activity in all extracts, particularly in the 40 % extracts from dry seeds, which reduced the levels of thiobarbituric acid-reactive substances by 44.3 % and protein carbonyl groups by 73.1 % ($p \leq 0.001$). These findings indicate the high antioxidant potential of *Persea americana* seeds, suggesting their promise as a natural source of antioxidants for pharmaceutical, cosmetic, and food applications.

Keywords: *Persea americana*, aqueous – ethanolic extracts, phenolic compounds, flavonoids, amino acids, antioxidant activity, spectrophotometry, thin-layer chromatography (TLC).

1. Introduction

Plant extracts are valuable natural sources of biologically active compounds, particularly antioxidants, which play a crucial role in maintaining redox balance and protecting cells from oxidative damage. The presence of phenolic acids, flavonoids, and other secondary metabolites determines their high therapeutic potential. These natural antioxidants contribute to the prevention of various oxidative stress-related disorders and are therefore widely studied for pharmaceutical, cosmetic, food applications, *etc.*[1, 2]

Persea americana Mill., better known as avocado, represents a valuable object of interdisciplinary research in the field of biotechnology due to its exceptional biochemical composition and wide spectrum of potential applications. As a member of the laurel family (*Lauraceae*), this tropical fruit tree originates from Central America but is now widely cultivated in many parts of the world. Growing public interest in healthy and functional nutrition has further increased the demand for avocado and its derivatives.

In contrast to the widely consumed pulp, avocado seeds are a rich source of numerous bioactive compounds that largely determine their antioxidant activity, including the ability to neutralize free radicals, chelate metal ions, and inhibit oxidative processes. Data from the scientific literature confirm the presence of lipids, vitamins, phytosterols, tannins, phenolic acids (gallic, chlorogenic, catechin, epicatechin, procyanidins), and flavonoids (quercetin, kaempferol) in avocado fruits. Phenolic compounds, flavonoids, coumarins, tannins, and other phytochemicals are well known to exhibit strong antioxidant properties.[3, 4] Both the pulp and seeds, being rich in dietary fiber, contribute to their popularity in the food, pharmaceutical, and cosmetic industries.[5–7]

Modern biotechnological studies have shown that extracts from *Persea americana* seeds exhibit not only pronounced antioxidant activity but also anti-inflammatory and antimicrobial properties. Furthermore, these extracts may serve as effective additives in food preservation, helping to extend shelf life and prevent enzymatic browning.[8, 9]

According to current research, the high potential of avocado seeds in modulating inflammatory processes is mainly attributed to flavonoids such as quercetin, which are also found in various fruits and vegetables. Studies have also demonstrated the antioxidant and anticancer properties of *Persea americana* seed extracts. According to Dayı *et al.*, the extract inhibited the expression of cyclin-dependent kinases 1 and E2 in the prostate cancer cell line (LNCaP) and exhibited antiproliferative effects by inducing cell cycle arrest at the G0/G1 phase.[10] Avocado seed extracts have also been shown to suppress the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α in stimulated macrophages. In addition, studies indicate that these extracts exhibit cytotoxic activity against colon (HCT116) and liver (HepG2) cancer cells, likely due to their ability to induce apoptosis. This suggests promising prospects for the use of avocado seed extracts in cancer prevention.[11]

Therefore, the chemical composition and antioxidant components of avocado seeds are of considerable interest to researchers and manufacturers in the food and cosmetic industries. Although natural avocado oil is relatively expensive to produce, it is rich in nutrients and has multiple potential applications, ranging from salad preparation to cosmeceutical and soap production.

The biotechnological investigation of *Persea americana* seed components opens new opportunities for developing innovative solutions in medicine, nutraceuticals, and bioengineering, using plant-derived raw materials with high potential for targeted applications. Previous studies assessing the antioxidant activity of avocado seeds have primarily relied on chemical assays, including the DPPH radical-scavenging method.[11] In this context, the present study aims to analyze the content of bioactive compounds in aqueous – ethanolic extracts of *Persea americana* seeds and to evaluate their antioxidant activity using Fe^{2+}-initiated Fenton reaction processes in rat hepatocytes under *in vitro* conditions, as well as to consider their potential practical application as natural antioxidants.

2. Experimental

2.1. Materials

The study was carried out using aqueous – ethanolic extracts (40 % and 70 %) prepared from both fresh and dried seeds of *Persea americana*. Identification and evaluation of bioactive constituents were performed employing standard reagents and reference solutions in compliance with the requirements of the State Pharmacopoeia of Ukraine.[12]

Plant material was ground using an LZMK-1 laboratory mill. Spectrophotometric measurements were conducted with a ULab 108UV instrument equipped with 10 mm optical cells, and sample clarification was achieved using an OPn-8 UKhL 4.2 centrifuge. Accurate weighing of plant samples and reference compounds was performed on a Radwag AS 220.R2 analytical balance (readability 0.0001 g).

2.2. Methods

The experimental approach included classical qualitative chemical assays, thin-layer chromatography (TLC), and biochemical evaluation of antioxidant properties in rat hepatocytes under *in vitro* free radical-induced oxidative conditions.

Results are presented as mean values with standard error (M ± SE), based on 3 or 5 independent measurements. Statistical analysis was conducted using one-way ANOVA with Tukey's multiple comparison test. A probability level of $p < 0.05$ was considered statistically significant.[13]

2.2.1. Preparation of Extracts

Fresh *Persea americana* seeds were mechanically crushed and air-dried at approximately 20 °C for 10 days until constant mass was achieved.

For extraction, 5.0 g of ground fresh or dried seed material was transferred to a flask and mixed with either 40 % or 70 % aqueous ethanol at a solid-to-solvent ratio of 1:20. Maceration proceeded for 10 days at room temperature in the absence of light. The obtained extracts were filtered and subsequently centrifuged at 6000 rpm for 10 min to remove suspended particles.[14, 15]

2.2.2. Determination of Bioactive Compounds in *Persea americana* Seed Extracts by Qualitative Reactions

Preliminary phytochemical screening was performed using established qualitative reactions to detect biologically active compounds responsible for the pharmacological potential of the extracts.[12, 14–17]

2.2.2.1. Phenolic Compounds

Phenolic constituents were detected by characteristic color reactions:

10 % $FeCl_3$ solution produced blue, green, or violet coloration.

10 % *NaOH* yielded yellow to reddish coloration.

Combined *$FeCl_3$ and $K_3[Fe(CN)_6]$* generated dark blue or green tones.

Vanillin in concentrated HCl produced a red-pink color indicative of phenolic structures.

2.2.2.2. Flavonoids

Flavonoids were identified using the following assays:

2 % *$AlCl_3$* in ethanol resulted in yellow fluorescence, intensified under UV light.

$Pb(CH_3COO)_2$ formed a pale yellow precipitate.

Treatment with 10 % *NaOH* caused yellow coloration.

Cyanidin and Shinoda reactions produced characteristic red or purple hues confirming flavonoid presence.

2.2.3. Thin-Layer Chromatographic Analysis

The phytochemical profile of aqueous – ethanolic seed extracts was further examined by TLC on Silufol UV-254 plates. Aliquots (2 μL) of purified extracts and reference standards were applied 1.5 cm above the lower plate edge (5×10 cm format).

Chromatographic development was performed in glass chambers using solvent systems selected according to the targeted compound class. Separated constituents were identified based on R_f values and specific coloration patterns before and after treatment with appropriate visualization reagents.[18]

2.2.3.1. Ascorbic Acid

Ananlysis was carried out in *n*-butanol – acetic acid – water (4:1:1, upper phase). Spots were visualized under UV light (254 nm), where ascorbic acid appeared as a white zone due to its absorption of UV radiation. A 1 % $FeCl_3$ solution was used as the visualization reagent.

2.2.3.2. Phenolic Compounds (Gallic Acid)

Toluene – acetone – formic acid (6:6:1) was used as the mobile phase. Under UV illumination (254 nm), phenolics appeared as grayish-green zones on a white background or as blue and red zones on a yellow background. A 1 % $FeCl_3$ solution was used as the visualization reagent.

2.2.3.3. Flavonoids (Quercetin)

Chromatography was performed in ethyl acetate – formic acid – water (8:1:1). The visualization reagent was a mixture of *p*-anisaldehyde and H_2SO_4.

2.2.4. Quantitative Spectrophotometric Analysis of *Persea americana* Seed Extracts

2.2.4.1. Total Phenolic Content

Total phenolics were quantified using a modified Folin – Ciocalteu assay. A diluted sample (0.1 mL, 1:10) was mixed with Folin – Ciocalteu reagent (0.1 mL), distilled water (1.5 mL), and 20 % sodium carbonate (0.3 mL). After 150 minutes of incubation in darkness, absorbance was recorded at 760 nm.

Results were calculated from a calibration curve constructed with gallic acid and expressed as gallic acid equivalents (GAE). All determinations were performed in triplicate.[19, 20]

2.2.4.2. Total Flavonoid Content

Flavonoids were determined *via* aluminum chloride complex formation. An extract aliquot (0.2 mL) was mixed with ethanol (0.8 mL) and sequentially treated with 5 % $NaNO_2$ (0.06 mL), 10 % $AlCl_3$ (0.06 mL), and 0.1 M NaOH (0.4 mL) under controlled timing conditions. Then, 0.480 mL of ethanol was added. After 5 minutes of incubation in darkness, absorbance was measured at 510 nm.

Quantification was based on a quercetin calibration curve, and results were expressed as quercetin equivalents (QE). All determinations were performed in triplicate.[20,21]

2.2.4.3. Total Amino Acid Content

Amino acids were quantified using the ninhydrin (2,2-dihydroxyindane-1,3-dione) reaction. An aliquot of the *Persea americana* extract (2 mL) was reacted with 0.2 % ninhydrin in ethanol (2 mL) and incubated at 60 °C for 15 minutes. After cooling and dilution to 5 mL with distilled water, absorbance was measured at 570 nm. Alanine was used as the reference compound, and concentrations were calculated by comparing sample absorbance with that of the standard solution:

$$C = \frac{A_x \cdot m_0}{A_0 \cdot V}, \quad (1),$$

where A_x is the sample absorbance, A_0 is the standard alanine solution absorbance, m_0 is the standard alanine sample mass (mg), and V is the volume of the extract and standard alanine solution used for analysis (mL).

All determinations were performed in triplicate.[14, 15, 22, 23]

2.2.5. Evaluation of Antioxidant Activity *in Vitro* in Rat Liver Model

2.2.5.1. Preparation of Rat Liver Homogenate

Rat liver tissue (0.5 g) was homogenized in potassium phosphate buffer (5 mL). Extract samples (0.3 mL) were added to homogenate aliquot samples (0.3 mL), while controls received solvent only.

Oxidative stress was induced by sequential addition of 2.8 % $FeSO_4$ (0.3 mL) and, 10 minutes later, 4 % H_2O_2 (0.3 mL). After 2 h incubation, the reaction was stopped using 40 % trichloroacetic acid (1.2 mL), followed by centrifugation for 10 minutes at 5000 rpm.

Lipid peroxidation was assessed in the supernatant, and protein oxidative modification was evaluated in precipitated proteins.[24]

2.2.5.2. Thiobarbituric Acid Reactive Substances (TBARS) Assay

Lipid peroxidation was assessed by quantifying TBARS. Malondialdehyde formation was quantified *via* reaction with thiobarbituric acid under acidic, high-temperature conditions. The resulting colored trimethine product (λ = 532 nm) was extracted into butanol and measured spectrophotometrically as described in our earlier work.[14] Protein content was evaluated by the Lowry method.[25] The concentration of TBARS was assessed from the formula:

$$[\boldsymbol{TBARS}] = \frac{E \cdot V_1 \cdot V_2}{\varepsilon \cdot V \cdot C}\ \mu mol/mg\ \text{protein}, \qquad (2)$$

where E is the test sample absorbance, ε is the millimolar extinction coefficient (ε = 156 $cm^2/\mu mol$), V_1 is the butanol volume (mL), V_2 is the sample volume (mL), V is the supernatant volume (mL), and C is the protein concentration in the supernatant (mg/mL).

2.2.5.3. Protein Carbonyl Determination

The degree of protein oxidative modifications was assessed by measuring carbonyl groups (CG) on amino acid side chains using reaction with DNPH (2,4-dinitrophenylhydrazin). Following incubation and washing steps, precipitated proteins were dissolved in aqueous urea and absorbance was recorded at 370 nm as described in our earlier work.[14] The content of protein CG was assessed from the formula:

$$[\boldsymbol{CG}] = \frac{\Delta D \cdot V_{samples}}{E_{370} \cdot C}\ nmol\ /\ mg\ protein, \qquad (3)$$

where $\Delta D = D_{test} - D_{control}$ is the difference in absorbance between test and control samples; $V_{samples}$ is sample volume (3 mL); E_{370} is the molar extinction coefficient of DNPH (22,000 $M^{-1}cm^{-1}$); and C is total protein concentration, mg/mL.

3. Results and Discussion

The results of the qualitative analysis showed that the 40 % and 70 % aqueous – ethanolic extracts of *Persea americana* seeds are rich in phytoconstituents with therapeutic potential (Table 1).

Table 1. Qualitative analysis of aqueous – ethanolic (40 % and 70 %) seed extracts of *Persea americana*

Group of bioactive compounds / Qualitative reaction	Characteristic color / precipitate	1A 70 %	1B 40 %	2A 70 %	2B 40 %
Phenolic compounds					
reaction with $FeCl_3$ (10 %)	dark blue-green	+	±	+	±
reaction with NaOH	change from yellow to red	+	+	+	+
reaction with $FeCl_3$ + $K_3[Fe(CN)_6]$	dark blue / green	+	+	+	+
reaction with vanillin in conc. HCl	red-pink	+	+	+	+
Flavonoids					
$AlCl_3$ (in alcohol)	yellow fluorescence, enhanced under UV light	+	+	+	+
reaction with $Pb(CH_3COO)_2$	light yellow precipitate	+	±	+	+
reaction with NaOH	yellow	+	+	+	+
cyanidin reaction	purple-red	+	+	±	±
Shinoda reaction (Mg + HCl)	red-pink	+	+	+	+

1A – 70 % ethanol, fresh seed; 1B – 40 % ethanol, fresh seed; 2A – 70 % ethanol, dry seed; 2B – 40 % ethanol, dry seed; + is a positive qualitative reaction and appearance of characteristic color; ± is a weak color of qualitative reaction.

Table 1 summarizes the results of qualitative reactions used to identify the main groups of bioactive compounds. For phenolic compounds, several characteristic reactions were performed: the addition of 10 % $FeCl_3$ solution produced a dark blue-green color in the 70 % extracts and a weakly positive (±) reaction in the 40 %

extracts; treatment with NaOH yielded a color change from yellow to red; the combination of $FeCl_3$ and $K_3[Fe(CN)_6]$ resulted in a dark blue or green coloration in all extracts; and the reaction with vanillin in concentrated HCl caused a red-pink color, confirming the presence of phenolic compounds.

For flavonoids, the results were also positive. The addition of $AlCl_3$ (alcoholic solution) produced yellow fluorescence that intensified under UV light; reaction with lead (II) acetate formed a light yellow precipitate; treatment with NaOH yielded a yellow color; the cyanidin reaction gave a purple-red coloration; and the Shinoda test (Mg + HCl) produced a pink-red color, indicating the presence of flavones and flavonols.

Overall, phenolic compounds and flavonoids were detected in all extracts, but the reactions were more intense in the 70 % extracts, which can be attributed to the higher solubility of these compounds in solvents with greater ethanol content.

Identification of bioactive compounds in the 40 % and 70 % aqueous – ethanolic extracts of *Persea americana* seeds was also performed using TLC according to standard methodological approaches. Reference standards were used, and fluorescence, spot color, and R_f values were visually evaluated. The results are presented in Table 2.

Table 2. TLC results of aqueous – ethanolic seed extracts of *Persea americana*

Aqueous – ethanolic extracts	Identified compound, R_f		
	Gallic acid	*Quercetin*	*Ascorbic acid*
1A 70 %	0.54	0.65	0.97
1B 40 %	0.52	0.68	0.99
2A 70 %	0.55	0.67	0.96
2B 40 %	0.50	0.67	0.98

1A – 70 % ethanol, fresh seed; 1B – 40 % ethanol, fresh seed; 2A – 70 % ethanol, dry seed; 2B – 40 % ethanol, dry seed; R_f is the retardation factor, average values are given.

Thin-layer chromatography performed in the solvent system toluene – acetone – formic acid (6:6:1), followed by visualization under UV light and treatment with a 1 % ferric chloride ($FeCl_3$) solution, revealed characteristic grayish-green spots with R_f values ranging from 0.50 to 0.55 (Table 2), corresponding to gallic acid. The detection of gallic acid confirms the antioxidant potential of the studied extracts.

To identify flavonoids, particularly quercetin, the ethyl acetate – formic acid – water (8:1:1) solvent system was used, followed by treatment with a mixture of *p*-anisaldehyde and H_2SO_4. Flavonoid compounds were detected in all samples, with R_f values corresponding to those of quercetin, confirming its presence.

TLC results also verified the presence of ascorbic acid in the aqueous – ethanolic extracts of *Persea americana* seeds, regardless of ethanol concentration, indicating the consistent occurrence of this potent antioxidant in the studied raw material.

The total phenolic content in the aqueous – ethanolic extracts of *Persea americana* seeds was quantitatively determined spectrophotometrically as gallic acid equivalent, using the calibration equation $y = 2.1899x$, where x is the optical density and y is the total phenolic content (mg/g) (Table 3).

Table 3. Total phenolic content expressed as gallic acid equivalent in aqueous – ethanolic seed extracts of *Persea americana*

Aqueous – ethanolic extracts	Mean values of the optical density	Total phenolic content, mg/g ($\bar{x} \pm \Delta\bar{x}$, $n = 3$)
1A 70 %	0.55	1.204 ± 0.010
1B 40 %	0.68	1.489 ± 0.010
2A 70 %	0.69	1.511 ± 0.002
2B 40 %	0.85	1.861 ± 0.003

1A – 70 % ethanol, fresh seed; 1B – 40 % ethanol, fresh seed; 2A – 70 % ethanol, dry seed; 2B – 40 % ethanol, dry seed

As shown by the obtained data, phenolic compounds were detected in all samples; however, their content depended on both the concentration of the extractant and the preliminary treatment of the raw material. The highest phenolic content was observed in the 40 % aqueous – ethanolic extract obtained from dry seeds (sample 2B), amounting to 1.861 mg/g expressed as gallic acid equivalent.

The lowest value was recorded for sample 1A (70 % ethanol, fresh seeds) – 1.204 mg/g, indicating lower extraction efficiency of phenolic compounds when using more concentrated ethanol and fresh raw material.

Overall, it was found that 40 % ethanol solutions are more effective for extracting phenolic compounds from *Persea americana* seeds, while dry raw material provides a higher yield of these substances compared to fresh seeds.

The total flavonoid content in the aqueous – ethanolic extracts of *Persea americana* seeds was quantitatively determined spectrophotometrically as quercetin equivalent, using the calibration equation $y = 7.657x$, where x is the optical density, and y is the total flavonoid content (mg/g). Table 4 presents the results of the determination of total flavonoids in the aqueous –ethanolic extracts of *Persea americana* seeds, expressed as quercetin equivalent. The extraction was carried out using 40 % and 70 % ethanol and both fresh and dry raw materials.

The results indicate that flavonoids were present in all samples, though their content varied depending on the

ethanol concentration and the physical state of the seeds. The highest flavonoid content was recorded in the 40 % aqueous – ethanolic extract from fresh seeds (sample 1B), reaching 3.395 mg/g expressed as quercetin equivalent. This suggests that a lower ethanol concentration promotes more efficient extraction of polar compounds such as flavonoids. The lowest content was observed in the 70 % aqueous – ethanolic extract from fresh seeds (sample 1A) – 2.663 mg/g.

Table 4. Total flavonoid content expressed as quercetin equivalent in aqueous – ethanolic seed extracts of *Persea americana*

Aqueous – ethanolic extracts	Mean value of the optical density	Total flavonoid content, mg/g ($\bar{x} \pm \Delta\bar{x}$, $n = 3$)
1A 70 %	0.40	2.663 ± 0.003
1B 40 %	0.51	3.395 ± 0.010
2A 70 %	0.47	3.129 ± 0.010
2B 40 %	0.42	2.796 ± 0.020

1A – 70 % ethanol, fresh seed; 1B – 40 % ethanol, fresh seed; 2A – 70 % ethanol, dry seed; 2B – 40 % ethanol, dry seed

In summary, 40 % aqueous – ethanolic extracts generally contained higher levels of flavonoids compared with 70 % extracts, and fresh seeds were characterized by slightly higher flavonoid content than dry ones.

The amino acid content in the 40 % and 70 % aqueous – ethanolic extracts of fresh and dry *Persea americana* seeds was determined using the ninhydrin method. The results are presented in Table 5. The average optical density of the alanine – ninhydrin standard complex was $A_0 = 0.6002$. Initial experimental parameters were as follows: extract volume $V = 2$ mL; alanine mass in the standard $m_0 = 0.2572$ mg.

The obtained data show that all examined extracts contained free amino acids; however, their content varied significantly depending on the extractant concentration and the physical state of the raw material. The highest relative amino acid content was observed in the 70 % aqueous – ethanolic extract from fresh seeds (sample 1A), amounting to 0.142 ± 0.001 mg/mL.

Table 5. Total amino acid content expressed as alanine equivalent in aqueous – ethanolic seed extracts of *Persea americana*

Aqueous – ethanolic extracts	Mean value of the optical density	Total amino acid content, mg/mL ($\bar{x} \pm \Delta\bar{x}$, $n = 3$)
1A 70 %	0.667	0.142 ± 0.001
1B 40 %	0.254	0.054 ± 0.001
2A 70 %	0.391	0.084 ± 0.001
2B 40 %	0.367	0.079 ± 0.001

1A – 70 % ethanol, fresh seed; 1B – 40 % ethanol, fresh seed; 2A – 70 % ethanol, dry seed; 2B – 40 % ethanol, dry seed

The results of lipid peroxidation and protein oxidative modification assays (Figs. 1 and 2) indicate that both the 40 % and 70 % aqueous – ethanolic extracts obtained from fresh and dried *Persea americana* seeds possess pronounced antioxidant properties.

A significant and reliable ($p \leq 0.001$) decrease in the content of thiobarbituric acid reactive substances (TBARS) and protein carbonyl groups was observed under the action of the 40 % extracts compared with the control.

As shown in Fig. 1, relative to the control, the 40 % aqueous – ethanolic extracts reduced TBARS levels by 29.2 % for fresh seeds ($p \leq 0.01$) and by 44.3 % for dry seeds ($p \leq 0.001$). Similarly, the 70 % aqueous – ethanolic extracts decreased TBARS by 15.0 % for fresh seeds and by 34.8 % for dry seeds. These findings indicate a decrease in the level of lipid peroxidation in all studied extracts of *Persea americana*, with the most pronounced reliability observed for the 40 % extracts ($p \leq 0.001$). Moreover, the extracts prepared from dry seeds exhibited significantly lower lipid peroxidation compared to those obtained from fresh seeds.

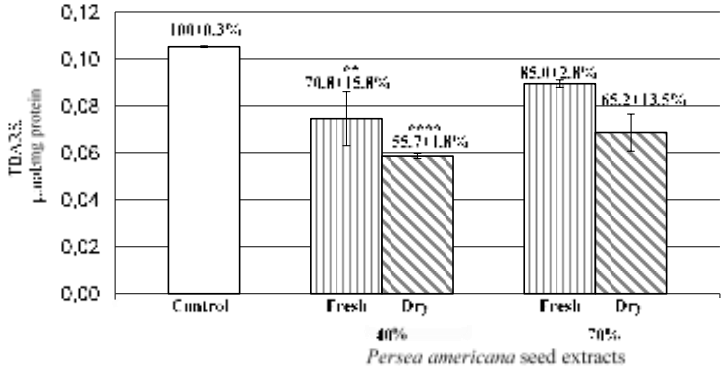

Fig. 1. TBARS content in rat liver homogenate under the action of aqueous-ethanolic *Persea americana* seed extracts (** – $p \leq 0.01$; **** – $p \leq 0.001$; M ± SE; $n = 5$)

An even greater antioxidant effect of the extracts was observed in the inhibition of protein oxidative modification (Fig. 2). As in the previous case, the content of protein carbonyl groups was significantly lower following treatment with the aqueous – ethanolic extracts from dry seeds. Specifically, compared to the control, the 40 % extracts reduced protein carbonyl group levels by 46.9 % for fresh seeds ($p \leq 0.05$) and by 73.1 % for dry seeds ($p \leq 0.005$); for the 70 % extracts, the reduction was 37.3 % and 49.9 %, respectively ($p \leq 0.005$).

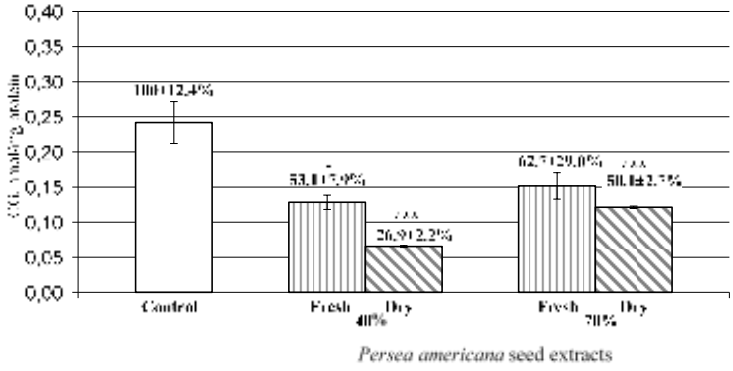

Fig. 2. Content of protein carbonyl groups in rat liver homogenate under the action of aqueous-ethanolic *Persea americana* seed extracts (* – $p \leq 0.05$; *** – $p \leq 0.005$; M ± SE; $n = 5$)

Overall, analysis of the obtained results demonstrated a statistically significant decrease in TBARS and protein carbonyl group levels for all *Persea americana* seed extracts compared to the control, confirming a reduction in the intensity of free radical processes in lipids and proteins.

The antioxidant activity assays thus showed that *Persea americana* seed extracts possess a strong capacity to neutralize free radicals, underscoring the considerable potential of avocado seeds as natural antioxidants. The observed correlations between total phenolic content and antioxidant activity emphasize the key role of phenolic compounds in determining the overall antioxidant potential of the extracts. Although these findings are promising, further studies are required to comprehensively characterize the therapeutic potential of *Persea americana* seed extracts.

4. Conclusions

1. The conducted studies revealed the presence of phenolic compounds, flavonoids, and free amino acids in all aqueous – ethanolic extracts of *Persea americana* seeds.

2. Dried seed material was found to be more efficient for the extraction of free amino acids, whereas fresh samples exhibited comparatively lower amino acid content.

3. The highest total content of bioactive compounds was observed in the 40 % aqueous – ethanolic extract of dried seeds, supporting its suitability for further pharmacological and biotechnological investigation.

4. Based on two oxidative stress indicators, both the 40 % and 70 % aqueous – ethanolic extracts of *Persea americana* seeds (fresh and dried) demonstrated pronounced antioxidant activity in rat hepatocytes under conditions of *in vitro* free-radical oxidation. The extracts effectively reduced the formation of free radicals in both lipids and proteins. The strongest antioxidant effect was observed for extracts prepared from dried seeds using 40 % ethanol as the solvent. Therefore, drying the biological material prior to extraction is recommended, as it results in greater inhibition of lipid peroxidation and protein oxidative modifications.

5. A strong correlation was established between total phenolic content and antioxidant activity, confirming the pivotal role of polyphenolic compounds in protecting against oxidative stress.

6. The obtained results highlight the high antioxidant potential of *Persea americana* seeds, indicating their promise as a natural source of antioxidants for pharmaceutical, cosmetic, and food industry applications.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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АНТИОКСИДАНТНИЙ ПОТЕНЦІАЛ ЕКСТРАКТІВ НАСІННЯ *PERSEA AMERICANA* MILL. *IN VITRO*: РОЛЬ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Анотація. *У роботі здійснено комплексне дослідження хімічного складу й антиоксидантної активності водно-етанольних екстрактів насіння Persea americana Mill. (авокадо) із використанням екстрагентів різної концентрації (40 % і 70 %) та сировини різного стану (свіжої та сухої). Методами тонкошарової хроматографії встановлено наявність у всіх екстрактах аскорбінової кислоти, а також фенольних сполук, зокрема галової кислоти і кверцетину. Загальний вміст фенолів оцінювали спектрофотометрично за методом Фоліна – Чокальтеу, а загальний вміст флавоноїдів визначали колориметричним аналізом із хлоридом алюмінію на основі рівняння лінійної регресії, отриманого з калібрувальної кривої кверцетину (вираженого у перерахунку на кверцетин). Кількісний аналіз показав, що сумарний вміст фенольних сполук коливався у межах 1,204–1,861 мг/г, флавоноїдів – 2,663–3,395 мг/г, а амінокислот (за нінгідриновою реакцією) в межах 0,054–0,140 % у перерахунку на аланін. Найвищий вміст фенолів виявлено в 40 % екстракті із сухого насіння, тоді як флавоноїди краще екстрагувалися 40 % етанолом зі свіжої сировини. За результатами моделювання процесів перекисного окиснення ліпідів і окисної модифікації білків in vitro виявлено виражену антиоксидантну активність усіх екстрактів, зокрема 40 % екстрактів із сухої сировини, які знижували рівень ТБК-активних продуктів на 44,3 % і карбонільних груп протеїнів – на 73,1 % (p≤0,001). Отримані результати свідчать про високий антиоксидантний потенціал насіння Persea americana, що може бути перспективним джерелом природних антиоксидантів для фармацевтичної, косметичної та харчової промисловості.*

Ключові слова: *Persea americana, водно-етанольні екстракти, фенольні сполуки, флавоноїди, амінокислоти, антиоксидантна активність, спектрофотометрія, тонкошарова хроматографія (ТШХ).*