



Full Length Article

Comparative *In vitro* Assessment of Antioxidant, Anti-inflammatory and Antibacterial Activities of Aqueous Peel Extracts from Two Moroccan *Persea americana* Cultivars (Zutano and Fuerte)

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Abstract

Recent research shows the ability of *Persea americana* peels as a resource of metabolites of natural origin with notable biological activities. This study aimed to assess the phytochemical composition, antibacterial, antioxidant and anti-inflammatory potential of aqueous peel extracts from *P. americana* (Fuerte and Zutano) in Morocco. Qualitative phytochemical analysis was conducted to determine the main families of secondary metabolites. The total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC) were then quantified using standardized spectrophotometric methods. The free radical scavenging capacity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while the anti-inflammatory potential was assessed through prevention of denaturation of bovine serum albumin (BSA), and the disc diffusion technique was used to assess the antibacterial activity. The results showed that the peels were rich in phenolic compounds, particularly flavonoids and tannins, confirming their strong biological potential. The extracts showed significant antioxidant activity ($IC_{50} = 62.060 \pm 0.234 \mu\text{g/mL}$) for the Zutano variety, as well as a marked inhibition of protein denaturation at $500 \mu\text{g/mL}$. The extracts exhibit intense inhibitory activity with levels of 69.65% for Zutano and 65.17% for Fuerte, suggesting an interesting anti-inflammatory activity. Both varieties exhibited similar antibacterial activity against *E. coli*, while no activity was observed against the other Gram-negative bacterial strains tested, while Fuerte showed superior activity against Gram-positive bacteria. These results underscore the valorisation potential of *P. americana* peels, generally considered as agri-food waste, as a promising natural source of bioactive compounds.

Keywords: *Persea americana*; Phenolic compounds; DPPH; Anti-inflammatory activity; Antibacterial activity

Introduction

The avocado (*Persea americana* Mill.) is today one of the most appreciated fruits in the world, both for its flavor and for its nutritional richness. Originally from Central America, it is now cultivated in many tropical and subtropical regions where the climatic conditions are favorable. Belonging to the *Lauraceae* family, this fruit, often called "alligator pear" because of the appearance of its skin, is distinguished by its high content of unsaturated fatty acids, vitamins (B, C and E) and essential minerals like potassium, which gives it an important role in preventing several chronic illnesses, including metabolic and cardiovascular diseases (Dreher and Davenport 2013; Ge *et al.* 2017).

In recent years, the cultivation of *P. americana* has developed strongly in Morocco, which is now among the

main producers in Africa. The Gharb, Loukkos and Souss-Massa regions concentrate the bulk of national production, with widely marketed varieties such as Hass, Fuerte, Zutano and Bacon. This agricultural dynamic contributes to economic growth, but it is also accompanied by a significant increase in waste from consumption and fruit processing.

Indeed, the peel and the seed account for about one-third of its weight and are generally eliminated as simple residues (Rodríguez-Carpena *et al.* 2011). However, these by-products are a rich source of bioactive compounds (Soong and Barlow 2004; Kosińska *et al.* 2012).

The extracts obtained from these inedible parts have revealed remarkable biological activities, such as antibacterial, antifungal, anti-inflammatory and antioxidant effects (Tremocoldi *et al.* 2018). These properties thus bring the avocado seed and peel closer to the plant resources

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traditionally used in natural medicine, paving the way for their integration into the field of plants with therapeutic potential.

In a context where research is increasingly moving towards sustainable and environmentally friendly solutions, the recovery of agri-food waste appears to be a priority. The exploitation of *P. americana* by-products meets this double requirement: to reduce the environmental impact of waste while identifying new natural sources of molecules of interest for the pharmaceutical, cosmetic and nutraceutical sectors (FAO 2019; Boukhatem and Setzer 2020).

In this context, the present study aimed to compare the peels of two avocado cultivars, Fuerte and Zutano, grown in Morocco. Specifically, the phytochemical composition of their aqueous extracts was investigated through qualitative and quantitative analyses. In addition, their antioxidant, antibacterial, and anti-inflammatory activities, based on the inhibition of bovine serum albumin (BSA) denaturation, were evaluated. The overall objective was to highlight the potential of these agro-industrial by-products as natural sources of bioactive compounds with promising applications in various industrial sectors.

Materials and Method

Plant material

The fruits of avocado cultivars Zutano and Fuerte were harvested in November and December 2023 from an orchard located in Mnasra (34° 36' 00" N, 6° 19' 00" W), in the Rabat-Salé-Kenitra region of Morocco. The fruits were collected at commercial maturity from several trees per cultivar, ensuring a representative sample. Only healthy fruits, free of visible defects or infections, were selected.

Sample preparation

The peels of the two studied varieties were manually separated, thoroughly washed with distilled water, and subsequently air-dried in a well-ventilated, light-protected environment until a constant weight was reached (mass variation < 0.01 g over 24 h). The dried peels were then ground using an electric grinder. The resulting powder was stored at room temperature in airtight containers shielded from light and moisture until further analyses.

Preparation of extracts

Approximately 10 g of dried peel powder from each avocado cultivar (Zutano and Fuerte) was mixed with 100 mL of distilled water (1:10 w/v) and extracted by maceration under continuous stirring at room temperature (25 ± 2°C) for 72 h in the dark (Afqir *et al.* 2024). The resulting mixture was filtered using Whatman No. 1 filter paper. The filtrates were then evaporated to obtain dried crude extracts, which were subsequently stored at 4°C until further use.

Yield (%) = (weight of dry extract / weight of dry plant material) × 100 (1)

Phytochemical screening

A preliminary qualitative phytochemical screening was carried out to identify the major classes of secondary metabolites present in the aqueous peel extracts of avocado cultivars (Zutano and Fuerte), which are likely to contribute to their potential biological activities. The presence of polyphenols and tannins was determined using ferric chloride (FeCl₃) and Stiasny's reagent, respectively. Flavonoids were identified by the cyanidin reaction, steroids and triterpenes by the Liebermann-Burchard test, reducing sugars by Fehling's reagent, saponins by the foam test, and alkaloids by Dragendorff's reagent (Noorul *et al.* 2017; Mgamat *et al.* 2023).

Quantitative analysis

Total phenolics content (TPC): TPC was conducted using the Folin-Ciocalteu reagent (Oubih *et al.* 2020). The technique involved the association of the extract with the diluted Folin-Ciocalteu reagent 1/10 and Na₂CO₃ solution. Absorbance was measured at 765 nm after incubation at 45°C for 30 min and quantification was performed using a gallic acid calibration curve.

Total flavonoids content (TFC): The aluminum chloride colorimetric method illustrated by Merabet *et al.* (2025) was used to quantify the TFC. Assay mixture was analysed spectrophotometrically at 510 nm, and the total amount of flavonoids was expressed in (mg QE/g E), with reference to the calibration curve obtained from the quercetin used as standard.

Condensed tannin content (CTC): CTC was quantified using the vanillin-acid test (Haida and Kribii 2020). The reading of the absorbances was carried out by UV-VIS spectroscopy at 500 nm, after an incubation of 20 min. The results were presented as mg CE/g E.

Assessment of antioxidant activity

Using the method described (Aouji *et al.* 2023; Naji *et al.* 2025), the antioxidant activity was evaluated based on their ability to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ascorbic acid was used as standard. The radical scavenging capacity was expressed in terms of IC₅₀ values.

Anti-inflammatory activity (denaturation of BSA proteins)

The anti-inflammatory potential was examined using the BSA protein denaturation inhibition test, with slight modifications to the methods described (Williams *et al.* 2008; Saleem *et al.* 2020). The extract at concentrations ranging from 50 to 500 µg/mL was combined with a 5% (w/v) BSA solution. The mixture was heated to 57°C for three min after being incubated at 37°C for twenty min. After cooling phosphate buffer (pH 6.3) was added, the

absorbance at 660 nm was measured. Diclofenac sodium served as the positive control, while distilled water served as the negative control. Each concentration was tested in three repetitions. The percentage of inhibition (I%) was determined by the equation below:

$$I\% = [(Ac - At) / Ac] \times 100 \quad (2)$$

Where, Ac = control absorbance; At = test absorbance

Antibacterial activity

Four Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Acinetobacter baumannii*) and two Gram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) were used to test the antibacterial activity of the extracts using disc diffusion method. Sterile Whatman paper discs (6 mm in diameter), impregnated with 15 μ L of extract, were deposited in the middle of each Petri dish of agar previously inoculated. The positive controls were carried out using commercial antibiotic discs (ofloxacin 5 μ g, streptomycin 10 μ g and oxacillin 5 μ g). The diameters of the inhibition zones were measured in order to assess the extracts' efficacy, after incubation in darkness at 37°C for 24 h (Oubihiet al. 2020; Laarifi et al. 2025).

Statistical analyses

Based on three replicates (n = 3), the results are shown as mean $\pm$ standard deviation (SD). One-way ANOVA and Tukey's post hoc test were used for statistical analysis, with a significance level of $P < 0.05$. IBM SPSS Statistics (v. 26) was used for all analyses.

Results

The results represent the yield of aqueous extraction of the peels of two varieties targeted in our study (Fig. 1). The Fuerte variety provided the highest yield with $9.20 \pm 0.43\%$, followed by Zutano with $6.59 \pm 0.41\%$. A significant difference was found between the percentage of extraction yields of varieties, according to Tukey's test ($P < 0.05$), indicating that Zutano presented a lower yield than the Fuerte variety.

The phytochemical screening showed the occurrence of secondary metabolites in the aqueous extracts of the two varieties with similar intensity profiles (Table 1). These samples exhibited a strong presence of polyphenols, tannins, sterols, and terpenes, as well as reducing sugars. A moderate presence of flavonoids, alkaloids, and saponins was also observed.

Quantitative analysis showed that variety Zutano consistently exhibited higher levels of total polyphenols (64.83 ± 2.10 mg GAE/g), TFC (18.973 ± 0.789 mg QE/g), and CTC (165.513 ± 2.771 mg CE/g extract) compared to variety Fuerte (59.140 ± 0.636 mg GAE/g, 16.58 ± 0.29 mg

Table 1: Phytochemical screening of aqueous peel extracts from two *Persea americana* cultivars

Tests	Varieties	
	Fuerte	Zutano
Polyphenols	+++	+++
Tannins	+++	+++
Flavonoids	++	++
Alkaloids	++	++
Saponins	++	++
Sterols and terpenes	+++	+++
Reducing sugars	+++	+++

Note: +++ = Abundant, ++ = Moderate

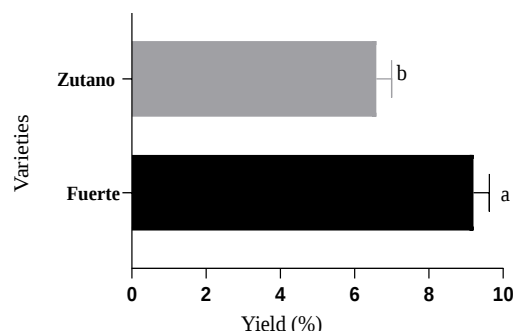


Fig. 1: Comparison of extraction yields of aqueous peel extracts from two varieties of *Persea americana*

Alphabets on the columns show significant difference between the extracts ($P < 0.05$)

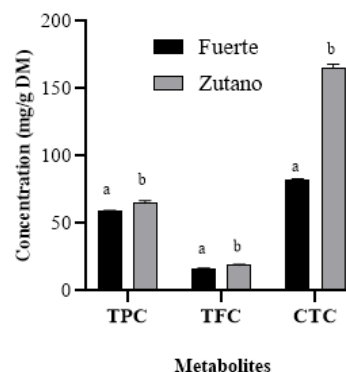


Fig. 2: Quantitative determination of total phenolic, flavonoid and condensed tannin contents in aqueous peel extracts from two *Persea americana* cultivars

Alphabets on the columns show significant difference between the extracts ($P < 0.05$)

QE/g and 82.557 ± 0.768 mg CE/g, respectively), with all differences being statistically significant ($P < 0.05$) (Fig. 2).

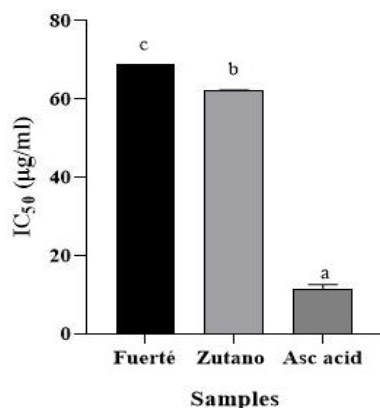
The antiradical activity was presented in μ g/mL as IC_{50} , corresponding to the extract concentration required to scavenge 50% of DPPH radicals. As shown in Fig. 3, a significant difference ($P < 0.05$) between the Zutano and Fuerte varieties (62.060μ g/mL ± 0.234 ; 68.834μ g/mL ± 0.024 respectively). Both extracts showed significantly lower antioxidant activity than ascorbic acid ($11.300 \pm 1.377 \mu$ g/mL), which was used as the reference standard due to its well-established antioxidant properties.

The effect of the *P. americana* peel extracts on the inhibition of BSA denaturation is represented in Fig. 4.

Table 2: Antibacterial activity of aqueous peel extracts from two *Persea americana* cultivars

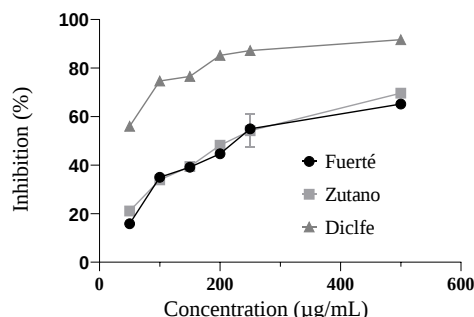
Microorganisms		Inhibition zone diameter (mm)		Antibiotics		
		Avocado Peels		Ofloxacin	Streptomycin	Oxacillin
		Fuerte	Zutano			
Gram negative	<i>E. coli</i>	6.5 ± 0.00 ^a	6.5 ± 0.00 ^a	20	12	Na
	<i>A. baumannii</i>	Na	Na	8	Na	Na
	<i>K. pneumoniae</i>	Na	Na	7	Na	Na
	<i>E. cloacae</i>	Na	Na	20	10	Na
Gram positive	<i>S. aureus</i>	6.6 ± 0.12 ^a	6.5 ± 0.00 ^a	20	10	Na
	<i>S. epidermidis</i>	6.54 ± 0.20 ^a	6.3 ± 0.1 ^a	20	14	Na

Na = Not active; Mean ± standard deviation

Note: According to Tukey's test, means within the same column sharing the same letter are not significantly different at the ($P < 0.05$)

Fig. 3: IC₅₀ values (µg/mL) for the DPPH radical scavenging activity of aqueous peel extracts from two *P. americana* cultivars. Statistical differences between varieties are denoted by distinct letter labels (a-c), with significance determined at $P < 0.05$ using Tukey's post hoc test

The two varieties showed a significant inhibitory effect, which gradually increased as a function of the tested concentrations (50, 100, 150, 250 and 500 µg/mL). At the lowest concentration tested (50 µg/mL), the Zutano variety demonstrated an inhibition of $21.09 \pm 0.24\%$, whereas the Fuerte variety showed an inhibition rate of $15.83 \pm 0.25\%$. Diclofenac sodium showed a significantly higher activity, reaching $56.01 \pm 0.12\%$. A progressive increase in concentration up to 500 µg/mL resulted in a marked inhibitory activity of the extracts, reaching $69.65 \pm 0.18\%$ for the Zutano variety and $65.17 \pm 0.45\%$ for the Fuerte variety, whereas diclofenac sodium exhibited a higher inhibition of $91.66 \pm 0.37\%$. These results demonstrate the slight superiority of the Zutano variety, although both varieties exhibited a significant inhibitory effect that increased with increasing extract concentration. However, this activity remained lower than that of Diclofenac sodium, which was employed as the positive control due to its well-established anti-inflammatory efficacy.

Concerning the antibacterial activity, the findings recorded in Table 2 indicate that the two varieties exhibit a similar activity against *E. coli*, with an inhibition zone of 6.5 mm. On the other hand, *K. pneumoniae*, *A. baumannii* and *E. cloacae*, which are also part of Gram-negative bacteria, did not exhibit any sensitivity to the varieties examined. In addition, the Fuerte variety demonstrated better efficacy on Gram-positive bacteria.


Fig. 4: Inhibitory effect of aqueous *P. americana* peel extracts on BSA protein denaturation

Discussion

The observed diversity of bioactive compounds in the two varieties of avocado peels highlights their phytochemical richness and their potential biological relevance. The phytochemical groups detected are consistent with those of Rao (2018), who reported the presence of alkaloids, polyphenols, tannins and phytosterols, as well as the absence of flavonoids in the fruits of *P. americana*. However, contrary to these observations, the peels analyzed in this work showed a moderate presence of flavonoids.

Quantification of total polyphenols showed that both varieties are rich in phenolic compounds, as reported by Tremocoldi et al. (2018), who detected a significant amount of 120.3 mg GAE/g for the Fuerte variety. However, Teixeira et al. (2025) found a higher content in the Hass variety (159.07 mg GAE/g). The TFC in our study were higher than those reported by Oliveira et al. (2022) in avocado peels analyzed (Margarida, Breda and Geada) that had respectively (1.31, 4.57 and 6.90 mg EQ/g). However, Lyu et al. (2023) showed a concentration of 3.44 mg EQ/g for the unripe Hass variety. In another similar study, Rahman et al. (2022) reported a total tannin content of 38.357 ± 0.202 mg/100 g extract for a methanol extract of avocado peel without indicating the variety, this result remains significantly lower than that of our study. Similar observations have been documented by Laarifi et al. (2025), indicating that the content of condensed tannins varied depending on the variety, with Zutano exhibiting higher levels than Fuerte. According to Chung et al. (1998) a diet enriched in condensed tannins

may provide protective effects against cancer development, attributed to their antioxidant activity.

The variations observed within the results of the quantitative analyses concerning the TPC, TFC and CTC can be attributed to the different extraction techniques used and the choice of solvent as well as the diversity of the varieties and the growing conditions, which have a direct impact on the bark, and therefore on the richness in phenolic compounds of the extracts (Ferreira and Santos 2022).

The ability of plant-derived antioxidants and their secondary metabolites to counter oxidative stress and prevent diseases has been widely recognized (Dibacto *et al.* 2021). In this sense, the antiradical capacity of the two varieties of avocado peel indicated their great potential as a natural source of bioactive compounds. These findings differ from Dibacto *et al.* (2021), who worked on the peels of several *P. americana* cultivars in Cameroon and demonstrated that the aqueous extract of the Fuerte variety has a greater antioxidant power than the Zutano variety. However, Kosińska *et al.* (2012) demonstrated that the Hass variety's peels revealed the maximal level ($IC_{50} = 0.358$ mg), against the Shepard variety in a comparative study between Hass and Shepard using methanol as extraction solvent. Moreover, a significant antioxidant activity, evaluated by the DPPH test, was reported by Rosero *et al.* (2019) for the peel extract of *P. americana* obtained by acetone/water extraction, with an IC_{50} estimated at 138.2 g/kg, without specifying the variety analyzed.

The inhibitory activity observed in our results is in agreement with that reported by Ndidiamaka *et al.* (2023), who recorded an inhibitory effect of 51.47% for methanol extracts and 54.80% for butanolic extracts of avocado peels at a concentration of 40 mg/mL. Moreover, a study carried out by Alkhalaf *et al.* (2019) concerning lipid extracts from *P. americana* fruit and seeds reported an anti-denaturation effect on BSA, exceeding 60% for the fruit extract and 70% for the seed extract at a concentration of 200 μ g/mL. These differences may be attributed to variations in experimental conditions, extraction methods, and the nature of the samples. According to our findings and previous studies, the inhibitory activity against BSA denaturation is concentration-dependent, confirming a dose-dependent effect.

Faced with the increase in bacterial resistance and the side effects linked to certain synthetic antibiotics, there is a growing interest in the discovery of new antibacterial substances of plant origin, perceived as safer alternatives for human health. In this context, the studied extracts exhibited moderate antibacterial activity against *S. aureus*, *S. epidermidis* and *E. coli*, probably linked to their richness in polyphenols and flavonoids capable of altering membrane integrity (Chen *et al.* 2017). However, no activity was shown against Gram-negative bacteria. This could be explained by the complex structure of their cell walls, limiting the penetration of bioactive compounds. These observations are partially consistent with those of Umeaku *et al.* (2018), who found that *P. americana* seed aqueous extracts were effective against *E. coli*, but ineffective against *S. aureus*. In

comparison, ofloxacin has demonstrated remarkable efficacy against all the strains tested, confirming its broad spectrum of action. However, streptomycin showed limited activity, being ineffective against certain Gram-negative bacteria, oxacillin showed no activity, which could reflect intrinsic or acquired resistance mechanisms.

Conclusion

P. americana peels constitute a rich source of bioactive compounds (polyphenols, flavonoids, saponins and alkaloids), as well as elevated levels of TPC, TFC and CTC. The assessment of antioxidant properties demonstrated a notable DPPH radical scavenging potential, likely related to the presence of various phenolic constituents. In addition, both varieties displayed a remarkable inhibitory effect on BSA protein denaturation, similar to that of diclofenac. Also, the findings indicated that the Zutano variety showed the highest values compared to the Fuerte for the tests carried out. However, Fuerte showed superior antibacterial activity compared to Zutano against Gram-positive bacteria. The aqueous extracts of peels from *P. americana* (Zutano and Fuerte) are rich in secondary metabolites and have the potential to be valued and used in several fields: medical, pharmaceutical, agri-food and cosmetics.

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Authors' Contributions

JL: Conceptualization, methodology, investigation, data curation, validation, visualization, writing, review and editing. MA: methodology, investigation, data curation, validation and review. AO: Investigation and microbiological analysis. DN and DEM: Methodology and critical revision of the manuscript. FZR: Methodology and statistical analysis. FZM: investigation and revision of the manuscript. IK: Critical review of the manuscript. YT: Conceptualization, supervision, scientific guidance, validation and review.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI Use

Generative AI tool was used in the preparation of this manuscript, including for language improvement. The AI tool used was Quillbot (Web version). The authors have reviewed and verified all AI-assisted content and take full responsibility for the content, accuracy, originality, and integrity of the manuscript.

Ethics Approval

Not applicable to this study.

References

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- ## Ethics Approval
- Not applicable to this study.
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