

Phytochemical composition, antioxidant activity and antibacterial efficacy of methanolic extracts of by-products of three varieties of *Persea americana* Mill. grown in Morocco

Jihane Laarifi^{1*}, Marouane Aouji¹, Asmaa Oubih¹, Douae El Merabet¹, Fatima-Zahra Rhebbar¹, Fatima-Zahra Mekaoui¹, Doha Naji¹, Youness Taboz¹

¹ Laboratory of Natural Resources and Sustainable Development, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco

* Corresponding author's e-mail: Jihane.laarifi@uit.ac.ma

ABSTRACT

This study aims to study the phyto-chemical composition of the methanolic extracts of the fruit by-products (seeds and peels) of three varieties of *Persea americana*, in order to evaluate their antioxidant and antibacterial activities. The contents of total polyphenols, flavonoids and condensed tannins were determined respectively by the Folin-Ciocalteu methods of the aluminum chloride reagent and vanillin. The antibacterial activity of the extracts was assessed using the diffusion technique on discs, and the antioxidant capacity was assessed using the free radical scavenging method DPPH. The results of phytochemical screening showed a richness in secondary metabolites on the seeds and peels of all the varieties studied, in particular polyphenols, free tannins, alkaloids, proteins, and saponosides. Regarding the quantitative analysis, the seeds of the Hass variety showed high TPC contents (108.026 ± 0.71 mg of GAE/g) in TFC (44.23 ± 0.91 mg of QE/g) and Tannins (357.59 mg of CE/g), for the peels, Zutano showed the highest TPC values (88.51 ± 0.36 mg of GAE/g) in TFC (39.856 mg of QE/g) and in tannins (216 ± 2.03 mg of CE/g), while Fuerte has moderate concentrations in both parts for all dosages. In terms of antibacterial activity, a marked effectiveness has been found against Gram-positive bacteria, and only against *E. coli* among Gram-negative ones. For antioxidant activity, the Zutano variety showed the best antioxidant activity ($IC_{50} = 21.268$ μ g/ml for the seed and $IC_{50} = 33.182$ μ g/ml for the peels). According to these results, methanolic extracts from seeds and peels represent a potential source of bioactive compounds that must be valued for use in different fields.

Keywords: *Persea americana*, bioactive compounds, antioxidant activity, antibacterial activity, Morocco.

INTRODUCTION

Persea americana Mill is a tropical fruit that is high in nutrients. It is a member of the *Lauraceae* family, and to the genus *Persea*, which has three species: *Persea schiedeana*, *Persea parvifolia* and *Persea americana*. Due to varying regional, climatic, genetic, and evolutionary circumstances, the latter exhibits a number of phenotypic variations (Shafer et al., 2015). Today, the avocado is recognized and cultivated throughout the world, mainly for the consumption of its fruits due to its nutritional as well as cosmetic benefits. In Morocco, its cultivation has experienced a remarkable boom in recent years, especially along

the coast between Larache and Rabat (Nasri et al., 2022), with an abundance of Hass, Zutano, Fuerte and Bacon varieties.

The avocado tree is considered in traditional medicine as a plant with a variety of therapeutic effects, in particular as a hypotensive and hypoglycemic agent, as well as for the treatment of cardiovascular diseases and ulcers (Anita et al., 2005; Nayak et al., 2008; Anaka et al., 2009; Kosińska et al., 2012). Additionally, its analgesic and anti-inflammatory qualities are acknowledged. (Adeyemi et al., 2002). Moreover, the fruit by-products (peel, seed) represent a high content of bioactive compounds, but their valorization is still insufficient.

Indeed, the seeds have shown significant antibacterial activity (Rodríguez et al., 2011; Ekom et al., 2022) against several pathogens, especially *Pseudomonas species*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Escherichia coli*. Moreover, they also have various pharmacological activities, such as anti-inflammatory, anti-cancer, antioxidant and analgesic actions (Alkhalaf et al., 2019) as well as anti-diabetic activity (Alhassan et al., 2012). However, the peels have also demonstrated properties such as antioxidant and antibacterial activities (Melgar et al., 2018). We opted for the analysis of the phytochemical profile, in particular the preliminary screening as well as quantify the total polyphenols, flavonoids and tannins, and also for the evaluation of the antioxidant and antibacterial activities of the methanolic extracts of peels and seeds of three varieties (Fuerté, Hass, Zutano) grown in Morocco. This approach aims to enhance these by-products as a natural source of bioactive agents, with a view to developing new ingredients for pharmaceutical, cosmetic or agri-food use.

MATERIALS AND METHODS

Plant material

The ripe fruits of three varieties of *Persea americana* (Hass, Fuerte and Zutano) were harvested between October and November 2023 in the Rabat-Salé-Kenitra region, more precisely in Mnasra, located 15 km from Kenitra and known for its humid climate (Latitude: 34° 46' 0" North, 5° 31' 0" West). The pulp was separated in order to recover the peels and the seeds. The latter were carefully cleaned with distilled water, then cut into pieces using stainless steel knives. They were allowed to dry at ambient temperature and in the dark until a constant weight was obtained. Once dried, the seeds and the peels were crushed separately to obtain fine powders, which were kept in storage at +4 °C.

Preparation of extracts

Initially, an amount of 10 g was subjected to maceration in 100 ml of methanol for 48 hours, under cold and dark conditions. The macerates obtained were filtered using Whatman paper (8 µm). The extracts were then kept in flasks at

4 °C after being concentrated using a rotary evaporator under vacuum at 40 °C. The following formula is used to calculate the extraction yield.

$$EY (\%) = (M_{ex} / M_0) \times 100 \quad (1)$$

where: *EY* – stands for extraction yield (%),
M_{ex} for extract mass (g), *M₀* – for test sample mass (g).

Qualitative analysis

The phytochemical screening of the main secondary metabolites was carried out on vegetable powders using specific tests based on staining and precipitation reactions. The polyphenols and the tannins were determined by the FeCl₃ reaction and Stiasny's reagent (Alilou et al., 2014), the flavonoids by the Cyanidine reaction (Daoudi et al., 2016), the sterols and terpenes by Liebermann-Burchard test, the reducing sugars by Fehling's reagent, the saponosides by the foam test, the proteins by the Biuret reaction. (Bekro et al., 2007) and the alkaloids by the Dragendorff reagent, (Iqbal et al., 2015).

Quantitative analysis

Total polyphenol content – the Folin-Ciocalteu methodology was used to quantify the total polyphenols in the methanolic extracts of the seeds and peels of each variety under study (Nounah et al., 2019). After mixing 2.5 ml of diluted Folin-Ciocalteu reagent (1:10 in distilled water) with 0.5 ml of sample solution, 4 ml of 7.5%, w/v Na₂CO₃ was added. A UV-Vis spectrophotometer was used to detect the absorbance at 765 nm following a 30-minute incubation period at 45 °C in a water bath, using a white sample as a reference. Gallic acid's calibration curve was performed with varying concentrations (Mg GAE/g DM) under identical circumstances.

Total flavonoid content – the following method was used to quantify the total flavonoid content (Elbouzidi et al., 2023). 1.25 ml of distilled water and 0.25 ml of extract solution were placed in a test tube. The mixture was then kept for five minutes after the addition of 0.075 ml of a 5% NaNO₂ sodium nitrite solution. Following this incubation, 0.5 ml of 1 M sodium hydroxide NaOH and 0.15 ml of 10% aluminum chloride are added six minutes later. After then, 0.275 cc of distilled water was added to dilute the mixture. At 510 nm, the mixture's absorbance was promptly measured

using a standard curve made using quercetin. In milligrams of quercetin equivalent (EQ) per gram of dry matter, the flavonoid concentration was reported (mg EQ/g DM).

Condensed tannin content – the acidified vanillin technique, which was modified by (Haida et al., 2020), was used to quantify the condensed tannins. Three milliliters of a 4% vanillin-methanol solution and 1.5 milliliters of hydrochloric acid were combined with 500 microliters of the extract solution. After letting the mixture sit for fifteen minutes, the absorbance at 500 nm was measured. The catechin equivalent (CE) per gram of dry matter (mg CE/g DM) was used to express the results.

Antioxidant activity

According to the procedure outlined by Aouji et al. (2023), the antioxidant activity was assessed using the trapping technique of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), which is based on the antioxidants' capacity to neutralize the free radical DPPH. This was accomplished by dissolving 500 µL of extract solutions in methanol at various concentrations, and then adding 2500 µL of DPPH solution (0.2 mM) to each concentration. The mixture was stirred and then allowed to sit at room temperature in the dark for half an hour. At 517 nm, the sample's absorbance was measured. A negative control (2500 µL of the DPPH solution and 500 µL of methanol) was made in similar conditions, while the methanol, serving as a blank control, was processed simultaneously with the extract to be tested. A method based on absorbance was used to determine the percentage of antioxidant activity.

$$\% \text{ Inhibition} = \frac{(\text{Abs control} - \text{Abs test})}{\text{Abs control}} \times 100 \quad (2)$$

Antimicrobial activity

Studied microorganisms

Six bacterial strains were chosen due to their high level of antibiotic resistance in order to assess the antibacterial activity of the methanolic extracts under study. They include four Gram-negative bacteria: *Escherichia coli* (EC), *Acinetobacter baumannii* (Acineto), *Klebsiella pneumoniae* (Kleb), and *Enterobacter cloacae* (Entero), and two Gram-positive bacteria: *Staphylococcus*

aureus (SA) and *Staphylococcus epidermidis* (SE). Nutrient agar was used to cultivate these strains.

Disc broadcasting

According to Oubihi et al. (2020) and Afqir et al. (2024), the disc diffusion method on Mueller-Hinton medium was used to assess the antibacterial activity of the extracts. A microbial suspension with an optical density equivalent to 1 McFarland was prepared, then seeded by flooding onto the surface of Petri dishes containing an agar medium. The methanolic extracts were dissolved in DMSO (dimethyl sulfoxide). The use of DMSO has been justified by its wide adoption in the scientific community. This solvent does not exhibit significant antibacterial activity (Gachkar et al., 2007). Whatman absorbent paper discs No. 1, with a diameter of 6 mm, were sterilized and then soaked with 15 µL of each extract. These discs were then placed on the surface of the inoculated agar in the center of each box. Reference antibiotics were used as positive controls, in particular amoxicillin (25 µg) AML₂₅ and spiramycin (100 µg) SP₁₀₀. For a whole day, every dish was incubated at 37 °C. The diameter of the inhibitory zones surrounding each disc, measured in millimeters, was used to assess the antibacterial activity.

Statistical analysis

Following three iterations (n = 3), the experimental data were presented as a mean ± standard deviation (SD). To assess the differences, a one-way analysis of variance (ANOVA) was conducted. When there were notable differences, the Duncan test's post-hoc test was used to compare the means at the 5% significance level (α = 0.05). The SPSS program (IBM SPSS version 26) was used for all statistical analyses.

RESULTS AND DISCUSSION

Extract yield

The results of the yields are presented in Figure 1. The three varieties of *P. americana* show that the average yields of the extracts vary between 9.01% and 15.34% for the peels, between 7.08% and 13.63% for the seeds. The Fuerté and Zutano varieties showed the highest values, while the Hass variety showed the lowest values in both fruit parts.

Qualitative analysis

The results of the phytochemical screening tests (Table 1) revealed the presence of the various secondary metabolites with a high intensity in the two studied parts of these three varieties, these are polyphenols, free tannins and reducing sugars, and a moderate intensity of proteins in all varieties. In addition, alkaloids and saponosides were detected with a high intensity for the three varieties at the seed level and a moderate intensity at the bark level, while for sterols and terpenes they present a high intensity at the peels level and a moderate intensity for the seeds. As far as gallic tannins and flavanols are concerned, an absence of these products has been observed, these results confirm those found by Idriss et al. (2009). On the other hand, Munthe et al. (2023) confirmed that the ethanol extract of avocado seeds included flavonoids, tannins, saponins, and alkaloids. Previous research on the methanolic extract of *P. americana* leaves by Ajayi et al. (2017) found that there were no steroids present and that bioactive compounds such tannins, flavonoids, saponins, alkaloids, and terpenoids were present.

Content of total polyphenols, flavonoids and condensed tannins

The results of the TPC, TFC and TTC are illustrated in Table 2. In our study, we note that all the samples analyzed were relatively rich in polyphenols. The PTC contents of the seeds varied between 81.233 ± 0.71 and 108.026 ± 0.71 mg

of GAE/g DW, with the abundance of the Hass variety, while those of the peels ranged from 75.23 ± 0.44 to 85.46 ± 0.26 mg of GAE/gDW with a maximum concentration observed in Zutano. Whereas, the Fuerte variety has moderate TPC contents in both parts (seeds and peels) compared to other varieties.

Several studies, including one by Wang et al. (2010), have reported high concentrations of phenolic compounds in avocado seeds and skins. These findings support our own research into the Hass variety, which revealed TPC concentrations of 51.5 mg GAE/g in the seeds and 12.6 mg GAE/g in the peels. These results highlight a greater accumulation of phenolic compounds in the seeds, followed by the peels. According to Daiuto et al. In 2014, the Hass variety has concentrations of 63.5 mg GAE/g DM in the peels and 57.3 mg GAE/g DM in the seeds, from a hydroalcoholic extract (ethanol /water), these results differ from those of our study. This difference could be related to the type of solvent used for the extraction, although this cannot be stated with certainty.

Rodriguez et al. (2011) analyzed the TPC of avocado by-products, specifically the Fuerte and Hass varieties, and demonstrated that the Fuerte variety has higher values than the Hass variety, both in seeds and in peels. In addition, they found that the peels contain higher concentrations than the seeds in the acetone and methanol-based extracts, which diverges from our study.

The total flavonoid measurements of the extracts varied depending on the part analyzed and

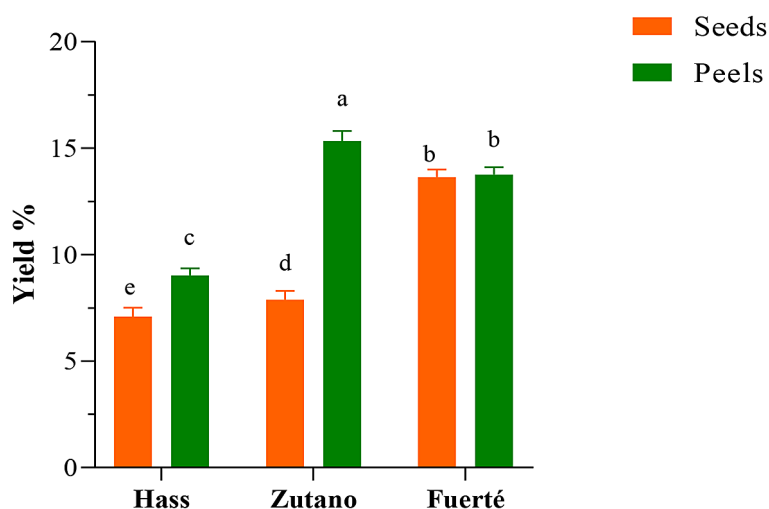


Figure 1. Yields of methanolic extract from the seeds and the peels of three varieties of *P. americana*. a, b, c, d, e shows a significant difference ($a > b > c > d > e$; $p < 0.05$)

Table 1: Phytochemical screening of the three varieties of seeds and peels of *P. americana*

Tests		Parts					
		Seeds			Peels		
		Hass	Zutano	Fuerte	Hass	Zutano	Fuerte
Polyphénols		+++	+++	+++	+++	+++	+++
Tanins	Tanins cathéchiques	+++	++	++	+++	+++	+++
	Tanins galliques	-	-	-	-	-	-
Flavonoïdes	Flavones	+	-	-	+	+	+
	Flavanones	++	++	++	-	-	-
	Flavonols, Flavanonols	-	-	-	-	-	-
Alcaloïdes		+++	+++	+++	++	++	++
Saponosides		+++	+++	+++	++	++	++
Protéïnes		++	++	++	++	++	++
Sucres réducteur		+++	+++	+++	+++	+++	+++
Stérols et terpènes		++	++	++	+++	+++	+++

Note: +++: Strongly positive test, ++: Moderately positive test, +: weakly positive test, -: negative test.

the varieties. For seeds, the results indicate that the Hass variety reached the highest level with 44.23 mg EQ/g DM, followed by Zutano with 40.65 mg EQ/g DM and Fuerte with 34.58 mg EQ/g DM. Regarding the peels, the Zutano variety has the highest value with 39.856 mg EQ/g DM, followed by Hass with 37.27 mg EQ/g DM and then Fuerte with 32.99 mg EQ/g DM (Table 2).

These results are consistent with those of Vinha et al., (2013), where they found that the Hass variety grown in the Faro region, in the south of Portugal, has contents of 47.9 mg/100 g and 44.3 mg/100 g in fresh matter, respectively for seeds and peels. Moreover, another study conducted by Ge et al., (2017) on the seed of the two varieties of avocado revealed flavonoid concentrations of 1636.25 mg/100g and 936.60 mg/100 g of fresh matter.

The condensed tannin contents showed higher values in the seeds, with 357.59 mg/CE g DW for the Hass variety, compared to the peels where 216 mg/CE g DW were noted for Zutano (Table 2). However, Ge et al., (2017) reported concentrations of 2.02 ± 0.04 and 2.45 ± 0.09 mg/100 g in fresh seeds of the two varieties grown in China. Moreover, Lyu et al., (2023) reported a higher tannin content in the peel of the ripe fruit of the Hass variety grown in Australia, reaching 148.98 mg CE /g, compared to other samples analyzed such as the peels of the Hass avocados and the seed and the peel of the other varieties (Reed, Wurtz). These results remain lower than those obtained in the present study.

Antioxidant activity

The analysis of the antioxidant activity of the extracts of the by-products of *Persea americana* revealed significant differences depending on the part and the varieties examined (Figure 2). The Zutano variety is distinguished by a notable antioxidant activity of the seeds with an IC_{50} of 21.268 ± 4.23 μ g/ml, followed by the Hass variety which displays an IC_{50} of 25.33 ± 0.415 μ g/ml and finally the Fuerte variety with 27.876 ± 0.434 μ g/ml. However, the peels showed a low free radical scavenging activity compared to the seeds, with IC_{50} values of 33.182 ± 1.521 μ g/ml, 37.872 ± 0.640 μ g/ml, 44.607 ± 0.746 μ g/ml, respectively for Zutano, Hass and Fuerte. Ascorbic acid showed a much lower IC_{50} of 11.30 ± 1.38 μ g/ml, thus highlighting its exceptional effectiveness as a standard antioxidant.

The results showed a considerable correlation between the antioxidant capacity and the total amount of phenolic compounds in the seeds and peels. This correlation has been widely observed in other similar studies (Wang, 2010, Shan, et al., 2005). According to Vinha et al., (2013) the seeds demonstrated a higher antioxidant activity than the peels, which supports our results. Indeed, the seed contains a higher concentration of flavonoids and phenolic compounds, while the peel is more abundant in carotenoids.

However, Lyu et al., (2023), stated that Hass peels have a higher antioxidant activity than seeds, which contradicts our findings, several factors could explain this difference, including the

Table 2. Total polyphenol, total flavonoid and condensed tannin content

Assays	Avocado seeds			Avocado peels		
	Hass	Zutano	Fuerte	Hass	Zutano	Fuerte
TPC	108.02±0.71 ^a	88.51±0.36 ^b	81.23±0.71 ^d	78.88±1.07 ^e	85.46±0.26 ^c	75.23±0.44 ^f
TFC	44.23±0.91 ^a	40.65±0.91 ^b	34.58±0.75 ^d	37.27±0.91 ^c	39.856±0.78 ^b	32.99±0.89 ^e
TTC	357.59±1.33 ^a	338.52±2.03 ^b	330.09±3.34 ^c	88.32±2.03 ^e	216.00±2.03 ^d	86.99±2.03 ^e

Note: Different letters indicate significant statistical differences ($p < 0.05$).

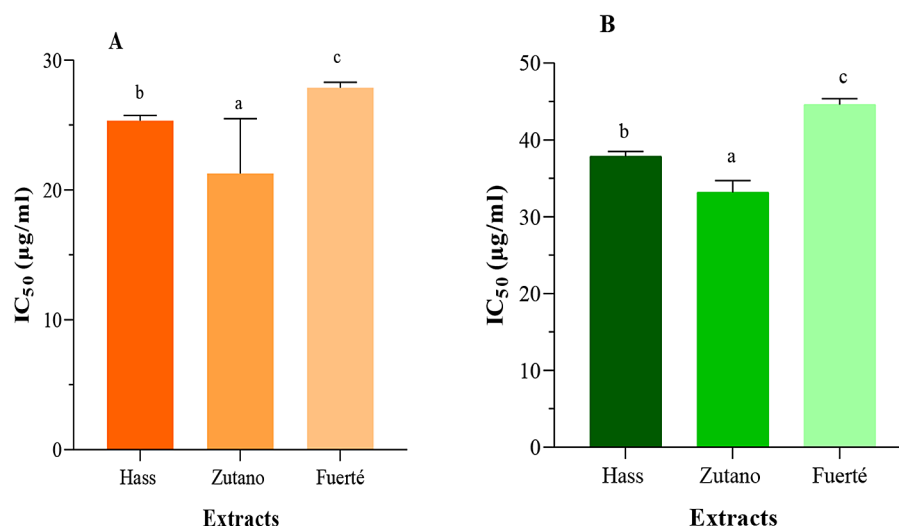


Figure 2. Inhibitory concentration (IC_{50}) expressed in $\mu\text{g/ml}$ of extracts of *P. americana*. A: Seeds; B: Peels
Duncan's post hoc test revealed significant differences between the seed extracts and peel extracts ($a > b > c$, $p < 0.05$)

choice of extraction solvent and the condition of the sample used (fresh or dried). On the other hand, a study by Kupnik et al. (2023) which analyzed the seeds extracted by different methods and using three different solvents revealed that the highest antioxidant activity was observed in the extract obtained by Soxhlet extraction, compared to other extraction techniques.

Antibacterial activity

The antibacterial activity of *Persea americana* seeds and peels extracts was evaluated by the disc diffusion technique, by observing the inhibition zones created around the examined extracts. This approach is part of the current context of research for new therapeutic alternatives, motivated by the alarming rise in antibiotic resistance and by the side effects associated with their use. The results of the antibacterial activity are indicated in the (Table 3). The seed extracts vary according to the variety and the bacterial strain, it is found that the Zutano variety has shown maximum efficacy against *S. aureus*, with a diameter

reaching 9.16 ± 0.76 mm followed by Hass and Fuerte, displaying respectively a diameter of 8.66 ± 0.57 mm and 7.33 ± 0.57 mm. For *S. epidermidis*, the Hass variety has shown a diameter of inhibition zone of 7.83 ± 0.28 mm and the Zutano and Fuerte varieties have shown a slightly worse efficiency, displaying respectively a diameter of 7.5 ± 0.5 mm and 6.66 ± 0.57 mm. On the other hand, the peel extracts of the Hass, Fuerte and Zutano varieties showed inhibition zones of 8.83 ± 0.76 mm, 8.16 ± 0.76 mm and 7.83 ± 0.28 mm respectively against *S. aureus*, similarly the Hass variety demonstrated a significant inhibitory action against *Staphylococcus epidermidis* with an inhibition zone diameter of 9.33 ± 0.57 mm against 7.5 ± 0.5 mm for Fuerte and 7.16 ± 0.28 mm for Zutano which show less effective antibacterial activity. For Gram-negative bacterial strains such as *Kleb. pneumonia*, *Acineto. baumannii* and *Entero. cloacae*, the tested extracts showed lower efficiencies, we find the most effective inhibition zone reaching 6.83 ± 0.28 mm for the seeds of Hass and Fuerte against *Acinetobacter baumannii*. For the *E. coli* bacterium, the seed extracts

Table 3. Antibacterial activity of methanolic extracts of *P. americana* seeds and peels and antibiotics by the disk diffusion method

Microorganisms		Inhibition zone diameter (mm)*							
		Avocado seeds			Avocado peels			AML ₂₅	SP ₁₀₀
		F	Z	H	F	Z	H		
Gram-positive	<i>Staphylococcus aureus</i>	7.33±0.57 ^b	9.16±0.76 ^a	8.66±0.57 ^a	8.16±0.76 ^a	7.83±0.28 ^a	8.83±0.76 ^a	0	17
	<i>Staphylococcus epidermidis</i>	6.66±0.57 ^b	7.5±0.5 ^{a b}	7.83±0.28 ^a	7.5±0.5 ^b	7.16±0.28 ^b	9.33±0.57 ^a	0	17
Gram-negative	<i>Escherichia coli</i>	8.16±1.25 ^a	8.00±0.5 ^a	7.86±0.23 ^a	7.16±0.28 ^a	7.66±0.57 ^a	8.16±0.76 ^a	0	10
	<i>Klebsiella pneumonia</i>	0.00±0.00	6.13±0.11 ^a	6.20±0.17 ^a	0.00±0.00	0.00±0.00	0.00±0.00	0	0
	<i>Acinetobacter baumannii</i>	6.83±1.04 ^a	6.10±0.17 ^a	6.83±0.28 ^a	6.16±0.28 ^a	6.33±0.57 ^a	6.10±0.17 ^a	0	0
	<i>Enterobacter cloacae</i>	0.00±0.00	6.13±0.11	0.00±0.00	0.00±0.00	6.16±0.28	0.00±0.00	0	8

Note: * The disc diameter (6 mm) is included in the zone of inhibition measurement, AML₂₅: amoxicillin (25µg), SP₁₀₀: spiramycin (100 µg); a, b shows a significant difference (a > b; p < 0.05).

showed inhibition zones varying from 7.86 ± 0.23 to 8.16 ± 1.25 mm and for the peels from 7.16 ± 0.28 to 8.16 ± 0.76 mm. On the other hand, the antibiotics tested, namely amoxicillin and spiramycin, showed contrasting results. Spiramycin has shown inhibitory effects against *S. aureus* (SA), *S. epidermidis*, *E. coli* and *Entero. cloacae* with diameters of inhibition zones estimated at 17 mm, 10 mm and 8 mm respectively. Unlike amoxicillin which has not proven effective against the same targeted bacteria.

The extracts of our study showed a notable inhibitory power vis-à-vis Gram-positive bacteria, while the majority of Gram-negative bacteria were resistant to it, with the exception of *E. coli*. It should be emphasized that the increase in the resistance of Gram-negative strains can be attributed to the existence of an additional outer cell membrane, which serves as a barrier against the infiltration of active compounds (Burt and Reinders, 2003). These observations highlight the richness of the extracts in polyphenols and flavonoids, renowned for their potential to alter the structure of bacterial membranes. Their action consists in deteriorating the integrity of the cell wall, targeting specific membrane receptors, as well as lipid membranes and ion channels, which leads to irreparable damage and effectively slows down the multiplication of bacteria (Chen et al., 2017). These findings are in agreement with those reported by Nasri et al., (2022) who demonstrated that the essential oils of the leaves of *P. americana* exhibit antibacterial activity against Gram-positive strains such as *S. epidermidis* and *S. aureus*.

CONCLUSION

Avocado residues, including peels and seeds, represent a crucial resource of secondary metabolites, such as flavonoids, tannins and polyphenols. These substances not only exhibit strong antioxidant activity, but also recognized antibacterial properties. For the recent study the varieties of *P. americana* studied showed interesting contents of phenolic compounds either for the seeds or for the peels with an abundance of the variety Hass, on the other hand Zutano showed an antiradical capacity more than the other varieties (Hass, Fuerte), in addition all the varieties showed efficacy against Gram-positive bacteria and *E. coli* (Gram-negative).

Valorizing these avocado by-products would provide a means of lowering agri-food residues considerably and investigating their uses in the pharmaceutical, cosmetic, and agri-food industries.

REFERENCES

1. Adeyemi, O. O., Okpo, S. O., Ogunti, O. O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia*, 73(5), 375–380. [https://doi.org/10.1016/S0367-326X\(02\)00118-1](https://doi.org/10.1016/S0367-326X(02)00118-1)
2. Afqir, H., Belmalha, S., Farihi, A., Elbouzidi, A., Bouhrim, M., Elrherabi, A.,..., Ouhssine, M. (2024). Comparative Analysis of Phenolic and Flavonoid content, Antioxidant, Antibacterial Activities, and Functional Groups of Chemicals from *Hypericum perforatum* L., and *Papaver*

- Rhoeas L. Flower Extracts. *Ecological Engineering & Environmental Technology*, 25. <https://doi.org/10.12912/27197050/175801>
3. Ajayi, O. E., Awala, S. I., Olalekan, O. T., Alabi, O. A. (2017). Evaluation of antimicrobial potency and phytochemical screening of *Persea americana* leaf extracts against selected bacterial and fungal isolates of clinical importance. *Microbiology Research Journal International*, 20(1), 1–11. <https://doi.org/10.9734/MRJI/2017/24508>
4. Alhassan, A. J., Sule, M. S., Atiku, M. K., Wudil, A. M., Abubakar, H., Mohammed, S. A. (2012). Effects of aqueous avocado pear (*Persea americana*) seed extract on alloxan induced diabetes rats. *Greener Journal of Medical Sciences*, 2(1), 5–11. <https://doi.org/10.15580/GJMS.2012.1.GJMS1202>
5. Alilou, H., Bencharki, B., Hassani, L. I., Barka, N. (2014). Screening phytochimique et identification spectroscopique des flavonoïdes d'*Asteriscusgraeolens* subsp. *odorus*. *Afrique Science: Revue Internationale des Sciences et Technologie*, 10(3).
6. Alkhalaf, M. I., Alansari, W. S., Ibrahim, E. A., ELhalwagy, M. E. (2019). Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract. *Journal of King Saud University-Science*, 31(4), 1358–1362. <https://doi.org/10.1016/j.jksus.2018.10.010>
7. Anaka, O. N., Ozolua, R. I., Okpo, S. O. (2009). Effect of the aqueous seed extract of *Persea americana* Mill (Lauraceae) on the blood pressure of Sprague-Dawley rats. *African Journal of Pharmacy and Pharmacology*, 3(10), 485–490. <https://doi.org/10.5897/AJPP.9000120>
8. Antia, B. S., Okokon, J. E., Okon, P. A. (2005). Hypoglycemic activity of aqueous leaf extract of *Persea americana* Mill. *Indian journal of pharmacology*, 37(5), 325–326. <http://doi.org/10.4103/0253-7613.16858>
9. Aouji, M., Imtara, H., Rkhaila, A., Bouhaddioui, B., Alahdab, A., Parvez, M. K.,..., Bengueddour, R. (2023). Nutritional composition, fatty acids profile, mineral content, antioxidant activity and acute toxicity of the flesh of *Helix aspersa* Müller. *Molecules*, 28(17), 6323. <https://doi.org/10.3390/molecules28176323>
10. Bekro, Y. A., Mamyrbekova, J. A., Boua, B. B., Bi, F. T., Ehile, E. E. (2007). Étude ethnobotanique et screening phytochimique de *Caesalpinia benthiana* (Baill.) Herend. et Zarucchi (Caesalpinaceae). *Sciences & nature*, 4(2), 217–225. <http://doi.org/10.4314/scinat.v4i2.42146>
11. Burt, S. A., Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. *Letters in applied microbiology*, 36(3), 162–167. <https://doi.org/10.1046/j.1472-765X.2003.01285.x>
12. Chen, M., Zhao, Z., Meng, H., Yu, S. (2017). The antibiotic activity and mechanisms of sugar beet (*Beta vulgaris*) molasses polyphenols against selected food-borne pathogens. *LWT-Food Science and Technology*, 82, 354–360. <https://doi.org/10.1016/j.lwt.2017.04.063>
13. Daoudi, A., Hrouk, H., Belaidi, R., Slimani, I., Ibbijben, J., Nassiri, L. (2016). Valorization of *Ruta montana* and *Ruta chalepensis*: Ethnobotanical study, phytochemical screening and antibacterial activity Valorisation de *Ruta montana* et *Ruta chalepensis*: Etude ethnobotanique, Screening phytochimique et pouvoir antibactérien. *Journal of Materials and Environmental Science*, 7(3), 926–935.
14. Ekom, S. E., Kuete, V. (2022). Methanol extract from the seeds of *Persea americana* displays antibacterial and wound healing activities in rat model. *Journal of Ethnopharmacology*, 282, 114573. <https://doi.org/10.1016/j.jep.2021.114573>
15. Elbouzidi, A., Taibi, M., Ouassou, H., Ouahhoud, S., Ou-Yahia, D., Loukili, E. H.,..., Addi, M. (2023). Exploring the multi-faceted potential of carob (*Ceratonia siliqua* var. *Rahma*) leaves from Morocco: A comprehensive analysis of polyphenols profile, antimicrobial activity, cytotoxicity against breast cancer cell lines, and genotoxicity. *Pharmaceuticals*, 16(6), 840. <https://doi.org/10.3390/ph16060840>
16. Ferreira da Vinha, A., Moreira, J., Barreira, S. (2013). Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (*Persea americana* Mill.). *Journal of Agricultural Science*, 5(12), 100–109. <https://doi.org/10.5539/jas.v5n12p100>
17. Gachkar, L., Yadegari, D., Rezaei, M. B., Taghizadeh, M., Astaneh, S. A., Rasooli, I. (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *Food chemistry*, 102(3), 898–904. <https://doi.org/10.1016/j.foodchem.2006.06.035>
18. Ge, Y., Si, X., Cao, J., Zhou, Z., Wang, W., Ma, W. (2017). Morphological characteristics, nutritional quality, and bioactive constituents in fruits of two avocado (*Persea americana*) varieties from Hainan province. *China. J. Agric. Sci*, 9(2), 8–17. <https://doi.org/10.5539/jas.v9n2p8>
19. Haida, S., Kribii, A. (2020). Chemical composition, phenolic content and antioxidant capacity of *Haloxylon scoparium* extracts. *South African Journal of Botany*, 131, 151–160. <https://doi.org/10.1016/j.sajb.2020.01.037>
20. Huang, B., He, J., Ban, X., Zeng, H., Yao, X., Wang, Y. (2011). Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. *Meat science*, 87(1), 46–53. <https://doi.org/10.1016/j.meatsci.2010.09.001>

21. Idris, S., Ndukwe, G., Gimba, C. (2009). Preliminary phytochemical screening and antimicrobial activity of seed extracts of *Persea americana* (avocado pear). *Bayero Journal of Pure and Applied Sciences*, 2(1), 173–176. <https://doi.org/10.4314/bajopas.v2i1.58538>
22. Iqbal, E., Salim, K. A., Lim, L. B. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniotalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science*, 27(3), 224–232. <https://doi.org/10.1016/j.jksus.2015.02.003>
23. Kosińska, A., Karamac, M., Estrella, I., Hernández, T., Bartolomé, B., Dykes, G. A. (2012). Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *Journal of agricultural and food chemistry*, 60(18), 4613–4619. <https://doi.org/10.1021/jf300090p>
24. Kupnik, K., Primožič, M., Kokol, V., Knez, Ž., Leitgeb, M. (2023). Enzymatic, antioxidant, and antimicrobial activities of bioactive compounds from avocado (*Persea americana* Mill.) seeds. *Plants*, 12(5), 1201. <https://doi.org/10.3390/plants12051201>
25. Lyu, X., Agar, O. T., Barrow, C. J., Dunshea, F. R., Suleria, H. A. (2023). Phenolic compounds profiling and their antioxidant capacity in the peel, pulp, and seed of Australian grown avocado. *Antioxidants*, 12(1), 185. <https://doi.org/10.3390/antiox12010185>
26. Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M., Rodriguez-Lopez, A. D.,..., Ferreira, I. C. (2018). Bioactive characterization of *Persea americana* Mill. by-products: A rich source of inherent antioxidants. *Industrial Crops and Products*, 111, 212–218. <https://doi.org/10.1016/j.indcrop.2017.10.024>
27. Munthe, S. W. N., Riskianto, R., Juvi, D., Novia, J. (2023). Antioxidant, total phenolic, and total flavonoid of 70% ethanol extract of avocado seeds (*Persea americana* Mill.). *Pharmacognosy Journal*, 15(4). <https://doi.org/10.5530/pj.2023.15.126>
28. Nasri, C., Halabi, Y., Aghzaf, S., Nounah, I., Brunel, M., Oubihi, A.,..., Tabyaoui, M. (2022). Seven *Persea americana* varieties essential oils comparison: Chemical composition, toxicity, antibacterial, and antioxidant activities. *Biocatalysis and Agricultural Biotechnology*, 44, 102468. <https://doi.org/10.1016/j.bcab.2022.102468>
29. Nayak, B. S., Raju, S. S., Chalapathi Rao, A. V. (2008). Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *Journal of wound care*, 17(3), 123–125. <https://doi.org/10.12968/jowc.2008.17.3.28670>
30. Nounah, I., Hajib, A., Oubihi, A., Hicham, H. A. R. H. A. R., Gharby, S., Kartah, B. E.,..., Bougrin, K. (2019). Phytochemical screening and biological activity of leaves and stems extract of *hammada scoparia*. *Moroccan Journal of Chemistry*, 7(1), J-Chem. <https://doi.org/10.48317/IMIST.PRSM/morjchem-v7i1.14218>
31. Oubihi, A., Ouryemchi, I., Nounah, I., Tarfaoui, K., Harhar, H., Ouhssine, M., Guessous, Z. (2020). Chemical composition, antibacterial and antifungal activities of *Thymus leptobotrys* Murb essential oil. *Advances in Traditional Medicine*, 20, 673–679. <https://doi.org/10.1007/s13596-020-00488-w>
32. Rodríguez-Carpena, J. G., Morcuende, D., Andrade, M. J., Kylli, P., Estévez, M. (2011). Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of agricultural and food chemistry*, 59(10), 5625–5635. <https://doi.org/10.1021/jf1048832>
33. Wang, W., Bostic, T. R., Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food chemistry*, 122(4), 1193–1198. <https://doi.org/10.1016/j.foodchem.2010.03.114>
34. Wang, M., Zheng, Y., Khuong, T., Lovatt, C. J. (2016). Developmental differences in antioxidant compounds and systems in normal and small-phenotype fruit of ‘Hass’ avocado (*Persea americana* Mill.). *Scientia Horticulturae*, 206, 15–23. <https://doi.org/10.1016/j.scienta.2016.04.029>