

Antibacterial and Antioxidant Activities of *Persea Americana* (Mill) Lauraceae Kernel Extracts

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Abstract

Nowadays, a large number of aromatic and medicinal plants possess highly significant bioproperties, with numerous applications in medicine, pharmacy, cosmetology, and agriculture. This work aimed to conduct a phytochemical screening and assay of phytomarkers with biopharmaceutical potential and to assess the antioxidant and antibacterial activity of *Persea americana* Mill. kernel extracts in vitro.

P. americana plant samples were collected in May 2017 in Kinshasa. Three standard strains were selected namely *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 9027 for microbiological assays. The phytomarker assay was used to detect total polyphenols, and the antibacterial activity was determined using the solid diffusion method on the Mueller Hinton medium to establish the minimal inhibitory concentration. Antioxidant activity was evaluated by the DPPH, ABTS, and Phosphomolybdate techniques. The findings showed that *P. americana* kernels contain secondary metabolites such as flavonoids, polyphenols, alkaloids, tannins, and triterpenoids, which may confer interesting biological activities. Quantitative phytomarker analysis of *P. americana* extracts showed a concentration of 69.70 ± 3.07 mg/QE of total polyphenols and 27.53 ± 1.15 mg/QE of flavonoids. All the extracts tested had low antibacterial activity against the three strains tested. However, the methanolic extract showed better antioxidant activity. In view of the IC₅₀ values of our various fractions, it is clear that *P. americana* kernels possess interesting antioxidant properties.

Keywords: Antibacterial, Antioxidant, *Persea Americana*, Kernel

INTRODUCTION

Traditional medicine has been utilized since ancient times to alleviate human illnesses. Their pharmacological activities are due to the existence of several natural compounds known as secondary metabolites. These chemicals are present in several organs and occasionally in distinct plant cells [1-3]. Nowadays, the rising adventure of microbial resistance to different compounds like antibiotics and the toxicity of synthetic antioxidants have led researchers to find an alternative to the plant world, specifically plants of medicinal and food relevance, in search of effective natural molecules devoid of any adverse effects [4, 5]. Numerous studies have highlighted the importance of secondary metabolites with effective biological activities like polyphenols and many others [5-8]. Furthermore, oxidative stress defined as a profound imbalance between pro-oxidants and antioxidants leads to irreversible cellular damage. The univalent reduction of oxygen results in the formation of activated oxygen species (AOS), like free radicals, hydrogen peroxide, etc [9, 10]. It seems that all these species are potentially toxic to the organism [11].

Recently, attention has focused on herbs as sources of antioxidants, which can be used to protect against the effects of oxidative stress [12-14]. In fact, Africa is endowed with rich plant biodiversity, especially in the central part where is located The Democratic Republic of the Congo (DRC). This latter has a huge biological richness (fauna or flora),

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unfortunately, it is not sufficiently monitored scientifically. Some of these plants are traditionally used by the population. These plant species represent a large reservoir of secondary metabolites. Among this arsenal of medicinal plants is the *Persea* genus, which is widely distributed, especially in the sub-tropical region, and the most known and consumed species is *P. americana*. This plant is widely used to treat several diseases and has been the subject of several studies to determine its chemical composition and biological properties. The current research aimed to assess the pharmacological properties of *P. americana* Mill. kernel extracts, with a focus on antioxidant and antibacterial activities.

MATERIALS AND METHODS

Biological Material

Plant samples were collected in Kinshasa-East in May 2017.

Conditioning of Plant Material

In this study we used *P. americana* kernels once dried under the conditions used in traditional pharmacopeia (five days at 450 °C in ovens), the plant part is ground or pulverized to obtain a fine powder.

Microbiological Material

The three bacterial strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 9027), which were 24 hours old, were provided by the Microbiology Laboratory of the Faculty of Pharmaceutical Sciences at the University of Kinshasa.

Methods

Preparation of Plant Material

The harvested plant parts were oven-dried (approx. 45°C) for five days in the laboratory, then ground to obtain the powder. The powder obtained was macerated for 24 hours followed by a filtration using Whatmann n°1. The filtrate was used for chemical screening and the extractions for biological testing.

Phytochemical Screening

The phytochemical screening was carried out as reported by Inkoto *et al.* [15], Bongo *et al.* [16] and Mbadiko *et al.* [17].

Extraction with Increasing Polarity

Fifty grams of powder (50g) were macerated in 500 mL of increasing polarity solvents (n-Hexane, Dichloromethane, and Methanol; 1:10, w/v) for 48 hours. After filtration, a rotary evaporator was used to concentrate the filtrates by evaporating the solvent to dryness using an oven at +40°C for 48 hours. The concentrated filtrates were used for further analyses.

Yield of Crude Extract

The raw extract yield is defined as the ratio between the mass of dry extract obtained and the mass of plant material processed. This yield is calculated using the following Eq. 1:

$$R(\%) = \frac{n}{N} \times 100 \quad (1)$$

Where: R(%): yield in en %

n: extract mass after solvent evaporation

N: Vegetal material mass used for extraction [18, 19].

Phytomarker Assay

Quantitative analysis samples were created by dissolving 10 mg of extract in 50 mL of methanol solvent. In the preparation of the Folin-Calcolteu reagent, the total polyphenol content and flavonoids were determined as per the protocol described by Dibacto *et al.* [20].

Biological Studies

Evaluation of Antibacterial Activity

The antibacterial efficacy of various extracts is evaluated using the solid-state diffusion method [4]. Dissolve 20 mg of the extract in 250µL of DMSO, then adjust the total volume to 5 mL with Mueller Hinton culture media for testing. The micro-dilution assay is performed in sterile, round-bottomed 96-well polystyrene microplates. Essentially, 100 µL of culture media is added to each well. Using a micropipette, 200 µL of each extract to be tested (1000 µg/ml) is placed in the wells respectively. 100 µL of each extract stock solution is used for creating serial dilutions.

To prepare the bacterial suspension, three isolated colonies from the test strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027) are placed in 2 mL of saline solution for each strain. The mixture is then incubated for 24 hours to achieve a 0.5 McFarland standard. Bacterial suspensions are introduced onto Petri dishes with Mueller Hinton media using a swabbing method. Wells are created in the Mueller Hinton agar in the Petri dishes using a Pasteur pipette. Subsequently, 100µL of varying quantities of each extract are distributed into individual wells. The positive control is placed in a separate well. The cultures are cultured at 37°C for 24 hours after diffusion, and the inhibitory halos around each well are then measured.

Evaluation of Antioxidant Activity

- **ABTS & DPPH**

The assessment of the antioxidant activity was performed as per the protocol described by Bongo *et al.* [4] and Mbadiko *et al.* [17].

- **Phosphomolybdate Test**

This test was performed following Tomovska and Vilasaku [21].

Preparation of Ammonium Molybdate Reagent

100 mL of distilled water was acidified with 3.27 mL of strong sulfuric acid to prepare the ammonium molybdate reagent. Subsequently, 436.6 mg of ammonium molybdate and 436.8 mg of sodium phosphate were dissolved, and the

solution was diluted to 300 mL with water that had been distilled.

Contacting the Sample with the Reagent

In test tubes, 10 mg of *P. americana* methanolic extract (1000-25.5µg/mL) was mixed with 2 mL of ammonium molybdate reagent. After shaking, the tubes were capped with absorbent cotton, and incubated in a water bath at 95°C for 1h30min. Absorbance was measured with a UV-visible spectrophotometer at 695nm. Methanol was used as the negative control, while vitamin C (100-62.5) served as the positive control.

The relative antioxidant activity is calculated according to the following Eq. 2:

$$AAR = \frac{\text{Abs. sample}}{\text{Abs. Vit C}} \times 100 \quad (2)$$

Where Abs. sample: sample absorbance

Abs. Vit C: absorbance in vitamin C equivalents/g extract

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical content of the extracts is presented in the **Table 1**.

Table 1. Phytochemical screening of *P. americana* kernel

Compounds	Used part (kernel)
1. Aqueous phase	
Polyphenols	+
Flavonoids	+
Tannins	+
Anthocyanins	+
Leuco-anthocyanins	+
Alkaloids	+
Saponins	-
Bound quinones	+
2. Organic phase	
Steroids and Triterpenoids	+
Free quinones	+

(Legend: + presence, - : absence).

From the table above, *P. americana* Mill. kernels are rich in secondary metabolites, like flavonoids, steroids, polyphenols, tannins, anthocyanins, bound quinones, alkaloids, leuco-anthocyanins, triterpenoids, and free quinones, but saponins are absent. These results corroborate those published by Kosińska *et al.* [22]. In the literature, it has been shown that *P. americana* leaves contain quercetol, catechin, epicatechin, cyanidin, procyanidin, terpenoids, catechic tannins, responsible for the anti-diarrheal effect, and essential oil (containing varying amounts of estragole, methylchavicol, α -pinene, and other terpenes) [23, 24]. The fruit pulp contains

sesquiterpenes, hydroxy tryptamine, vitamins A and E, carotenoids, and carbohydrates (glucose, fructose, perseitol, mannose, heptulose). The various classes of chemical constituents are monoterpene [25], sesquiterpene [26], triterpenoids [27], flavonoids [28], alkaloids [29], steroids [30, 31], carotenoids [32]. Much of the work carried out on the fruits of this plant has resulted in the discovery of molecules with interesting properties. An antifungal compound against the pathogen *Colletotrichum gloeosporioides* was discovered in 2000. It is (E, Z, Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene and was isolated from avocado idioblasts [33].

Corral-Aguayo *et al.* [34], demonstrated that avocado is endowed with antioxidant properties. This is due to total soluble phenols, vitamin C, β -carotene, and total carotenoids. The aqueous extract is 95 times more antioxidant than the lipophilic one, these are phenols (24.2mg/100g FM) and vitamin C (58%) that are mostly responsible for avocado's antioxidant activity. Ramos [35], showed that the methanolic extract of the kernel, followed by fractionation, enabled the separation and identification of three compounds of chlorogenic acids and their isomers, quinic acid, salidroside, pro-antocyanidins B1 and B1. *In vitro* tests of these compounds have shown them to have stimulatory inhibitory effects on human keratinocytes and fibroblasts. Other bioactive phytochemicals have been found to improve hypercholesterolemia, inflammation, diabetes, and hypertension. In addition, insecticidal, fungal, and antimicrobial effects were once again demonstrated [36-39].

Extraction Yield with Solvents of Increasing Polarity

The extraction yield of metabolites is presented in **Figure 1** below.

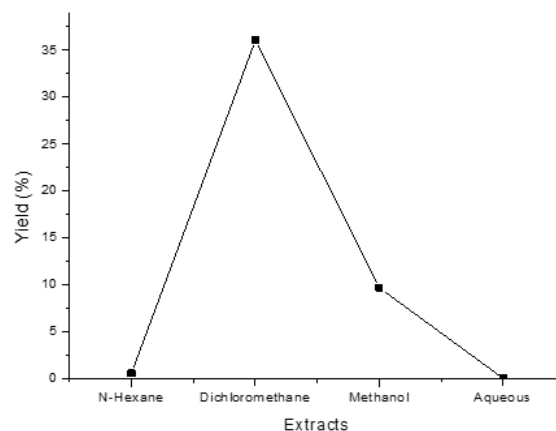


Figure 1. Extraction yield of different metabolites using different solvents

The figure shows that for the increasing polarity extraction of our samples, starting from 25g of *P. americana* Mill. kernel

powder, the yield obtained for the n-hexane fraction is 0.6%. For the dichloromethane fraction, the yield was 36.08%, and for the methanol fraction, the yield was 9.68%. The aqueous fraction gave a yield of 0.04%.

Antibacterial Activity of *P. Americana* Extracts

The antibacterial activity of the extracts is presented in Table 2.

Table 2. Antibacterial activity of *P. americana* kernel

Fractions	MIC ($\mu\text{g/mL}$)							M/C ($\mu\text{g/mL}$)
	2000	1000	500	250	125	62.5	31.25	
<i>Escherichia coli</i> ATCC 8739								
n-Hexane	+	+	+	+	+	+	+	>2000
Dichloro	+	+	+	+	+	+	+	>2000
Methanol	+	+	+	+	+	+	+	>2000
Aqueous extract	+	+	+	+	+	+	+	>2000
<i>Staphylococcus aureus</i> ATCC25923								
n-Hexane	+	+	+	+	+	+	+	>2000
Dichloro	+	+	+	+	+	+	+	>2000
Méthanol	+	+	+	+	+	+	+	>2000
Aqueous extract	+	+	+	+	+	+	+	>2000
<i>Pseudomonas aeruginosa</i> ATCC 9027								
n-Hexane	+	+	+	+	+	+	+	>2000
Dichloro	+	+	+	+	+	+	+	>2000
Méthanol	+	+	+	+	+	+	+	>2000
Aqueous extract	+	+	+	+	+	+	+	>2000

(Legend: +: bacterial growth; -: growth inhibition; ATCC: American Type Cell Collection, MIC minimum inhibitory concentration)

The table shows that all the bacterial strains tested are less sensitive to *P. americana* Mill. kernel extracts (MIC greater than or equal to 2000 $\mu\text{g/mL}$). This antibacterial activity is, however, low, and may be due to the phenolic and other compounds present in the extracts tested. *S. aureus* is a gram-positive bacteria with a thick cell wall that bioactive chemicals from *P. americana* Mill. would target pharmacologically. In contrast, *E. coli* has an outer membrane that hinders the penetration of chemical compounds into the cell [40, 41].

Phyto-Marker Assay in the Kernels of *P. Americana* Mill

In order to assess the content of secondary metabolites in our sample, we assayed total polyphenols and flavonoids (Table 3).

Table 3. Chemical composition of *P. americana* MILL. extracts in phyto-markers

Extracts	Secondary Metabolites	
	Total Polyphenols ($\mu\text{g GAE/g}$)	Flavonoids ($\mu\text{g QE/g}$) (% ratio)
<i>P. americana</i> Mill.	69.70 \pm 3.07	27.53 \pm 1.15 (39.49)

(GAE: gallic acid equivalent; QE: quercetin equivalent; Ratio: ratio of flavonoids to total polyphenols).

This table shows that *P. americana* Mill. extract has a high polyphenol content. However, it should be noted that the metabolites sought in this plant pass easily into polar solvents (methanol). Furthermore, this plant is rich in flavonoids (ratio equal to 0.39), and it is reported that flavonoids can therefore be selected as more physico-chemically stable phyto-markers, making them more suitable for further phytochemical study. In the literature, flavonoids have been shown to have anti-sickling activity [42], and the antioxidant activity is also attributed to polyphenols [43].

Antioxidant Activity

The antioxidant activity of *P. americana* kernel of different fractions and the phosphomolybdate reagent is presented in Table 4.

Table 4. Antioxidant activity of *P. americana* kernel extract

Extracts	IC ₅₀ ($\mu\text{g/mL}$)	
	ABTS	DPPH
Methanol fraction	12.22 \pm 2.67	11.94 \pm 4.45
Aqueous extract	83.95 \pm 10.67	1104.08 \pm 10.95
Vitamine C	2.94 \pm 0.27	1.52 \pm 0.19

Antioxidant activity with phosphomolybdate reagent

Concentration ($\mu\text{g/mL}$)	Antioxidant activity (%)	Equivalent in vitamin C (mg/g extract)
1000 $\mu\text{g/mL}$	63.65 \pm 0.49	636.5 \pm 4.90
500 $\mu\text{g/mL}$	61.32 \pm 0.45	613.2 \pm 4.50
250 $\mu\text{g/mL}$	56.06 \pm 0.00	560.6 \pm 0.00
125 $\mu\text{g/mL}$	21.05 \pm 0.00	210.5 \pm 0.00
62.5 $\mu\text{g/ml}$	15.55 \pm 0.00	155.5 \pm 00
31.25 $\mu\text{g/ml}$	7.35 \pm 0.00	73.5 \pm 0.00

It is observed that the methanol fraction of *P. americana* Mill. kernels showed IC₅₀ values of less than 100 $\mu\text{g/mL}$ in the ABTS and DPPH tests, while the aqueous fraction displayed an IC₅₀ value of less than 100 $\mu\text{g/mL}$ in the ABTS test. The radical-inhibiting capacities of different extracts varied significantly in each type of test. In addition, the inhibitory

concentration 50 (IC₅₀) value obtained in the DPPH test with the methanol fraction showed greater activity than the aqueous fraction against ABTS. The variation in activity can be attributed to the different reaction mechanisms. The ABTS reagent reacts with hydrophilic and lipophilic molecules, whereas the DPPH° reagent exclusively reacts with hydrophilic chemicals [44]. Our fractions exhibited lower activity compared to Vitamin C used as a positive control. However, the observed activity is noteworthy when compared to other plant species [45]. There is therefore evidence that polyphenols found in foods or medicinal plants are capable of modulating oxidative stress [46].

On the other side, the antioxidant activity of *P. americana* Mill. Kernels with the phosphomolybdate reagent varies according to extract concentration, ranging from 1000µg/mL to 31.25µg/mL. It should be noted that the activity of the extract of this plant tested is greater at higher concentrations, as we can observe. In the literature, it has been reported that the antioxidant activity of plants used in traditional pharmacopeia is attributed to phenolic compounds [47, 48]. The presence of these constituents with this property in this plant could justify its use by the population in traditional medicine [49].

CONCLUSION

This work aimed to conduct a phytochemical screening and evaluate the *in vitro* antioxidant and antibacterial properties of *P. americana* Mill. kernel extracts. All the extracts tested had low antibacterial activity against the three strains tested. However, the methanolic extract showed better antioxidant activity. In view of the IC₅₀ values of our various fractions, it is clear that *P. americana* kernels possess interesting antioxidant properties.

The findings show that this species commonly used in traditional medicine appears to be biologically active, and ethnobotanical studies could therefore be an interesting biological approach that opens up new prospects for the discovery of new drugs for the treatment of ailments caused by oxidative stress. It would therefore be interesting to extend the range of antioxidant and antibacterial tests of the different fractions by chromatography, as well as the isolation and characterization of active compounds in a view to identifying the molecules responsible for these biological activities.

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REFERENCES

- Ngbolua KN, Bongo GN, Inkoto CL, Ashande CM, Lufuluabo GL, Mukiza J, et al. A mini-review on the phytochemistry and pharmacology of the medicinal plant species *Persea americana* Mill. (Lauraceae). *Disc Phytomed.* 2019;6(3):102-11.
- Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytoactive compounds as therapeutic agents for human diseases: A review. *Food Sci Nutr.* 2023;11(6):2500-29. doi:10.1002/fsn3.3308
- Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms.* 2021;9(10):2041. doi:10.3390/microorganisms9102041
- Bongo G, Inkoto C, Masengo C, Tshiana C, Lengbiye E, Kapepula M, et al. Antisickling, antioxidant and antibacterial activities of *Aframomum albobolaleum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. f.) Skeels. *Am J Lab Med.* 2017;2(4):52-9. doi:10.11648/j.ajlm.20170204.13
- Bongo GN, Tuntufye HN, Malakalinga J, Ngbolua KN, Pambu AL, Tshiana C, et al. Anti-mycobacterial activity in Middlebrook 7H10 agar of selected Congolese medicinal plants. *Biosc Bioeng.* 2018;4(4):68-77.
- Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, et al. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *Biomed Res Int.* 2022;2022:5445291. doi:10.1155/2022/5445291
- Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, et al. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon.* 2020;6(11):e05609. doi:10.1016/j.heliyon
- Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: Current approaches and prospects. *Nucleus (Calcutta).* 2022;65(3):399-411. doi:10.1007/s13237-022-00405-3
- Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: Harms and benefits for human health. *Oxid Med Cell Longev.* 2017;2017:8416763. doi:10.1155/2017/8416763
- Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, et al. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Front Chem.* 2023;11:1158198. doi:10.3389/fchem.2023.1158198
- Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, et al. Oxidative stress in human pathology and aging: Molecular mechanisms and perspectives. *Cells.* 2022;11(3):552. doi:10.3390/cells11030552
- Chandran R, Abraham H. Identifying plant-based natural medicine against oxidative stress and neurodegenerative disorders. *Oxid Med Cell Longev.* 2020;2020:8648742. doi:10.1155/2020/8648742
- Talib WH, Al-Ataby IA, Mahmud AI, Jawarneh S, Al Kury LT, Al-Yasari IH. The impact of herbal infusion consumption on oxidative stress and cancer: The good, the bad, the misunderstood. *Molecules.* 2020;25(18):4207. doi:10.3390/molecules25184207
- Salehi B, Azzini E, Zucca P, Maria Varoni E, V. Anil Kumar N, Dini L, et al. Plant-derived bioactives and oxidative stress-related disorders: A key trend towards healthy aging and longevity promotion. *Appl Sci.* 2020;10(3):947. doi:10.3390/app10030947
- Inkoto CL, Bongo GN, Kapepula PM, Masengo CA, Gbolo BZ, Tshiana C, et al. Microscopic features and chromatographic fingerprints of selected Congolese medicinal plants: *Aframomum albobolaleum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook.f.) Skeels. *Emerg Life Sci Res.* 2017;4(1):1-10.
- Bongo G, Tuntufye H, Ngbolua KN, Malakalinga J, Tshiana C, Pambu A, et al. Comparative anti-mycobacterial activity on lowenstein-jensen slants of selected medicinal plants used in the congolese pharmacopeia. *J Dis Med Plants.* 2017;3(5):88-96. doi:10.11648/j.jdmp.20170305.12
- Mbadiko CM, Bongo GN, Ngbolua K, Kasongo SN, Masunda A, Ngombe NK, et al. Phytochemical study and evaluation of the antioxidant activity of aqueous and ethanolic extracts of *Hua gabonii*. *J Adv Pharm Res.* 2024;8(1):1-13. doi:10.21608/aprh.2024.225955.1230
- Monagas M, Brendler T, Brinckmann J, Dentali S, Gafner S, Giancaspro G, et al. Understanding plant to extract ratios in botanical extracts. *Front Pharmacol.* 2022;13:981978. doi:10.3389/fphar.2022.981978
- Cao-Ngoc P, Leclercq L, Rossi JC, Hertzog J, Tixier AS, Chemat F, et al. Water-based extraction of bioactive principles from blackcurrant

- leaves and chrysanthellum americanum: A comparative study. *Foods*. 2020;9(10):1478. doi:10.3390/foods9101478
20. Dibacto REK, Tchuenta BRT, Ngedjo MW, Tientcheu YMT, Nyobe EC, Edoun FLE, et al. Total polyphenol and flavonoid content and antioxidant capacity of some varieties of *Persea americana* peels consumed in cameroon. *Sci World J*. 2021;2021:8882594. doi:10.1155/2021/8882594
 21. Tomovska J, Vllasaku I. Phosphomolybdate test method for antioxidant activity in extracts of animal feed. III. Balkan Agriculture Congress, Edirne, Turkey; 2021:1-10.
 22. Kosińska A, Karamać M, Estrella I, Hernández T, Bartolomé B, Dykes GA. Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *J Agric Food Chem*. 2012;60(18):4613-9. doi:10.1021/jf300090p
 23. Neumann N, Honke M, Povydysh M, Guenther S, Schulze C. Evaluating tannins and flavonoids from traditionally used medicinal plants with biofilm inhibitory effects against MRGN *E. coli*. *Molecules*. 2022;27(7):2284. doi:10.3390/molecules27072284
 24. Fraga-Corral M, Otero P, Cassani L, Echave J, Garcia-Oliveira P, Carpena M, et al. Traditional applications of tannin rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods*. 2021;10(2):251. doi:10.3390/foods10020251
 25. Pino JA, Rosado A, Agüero J. Volatile components of avocado (*Persea americana* Mill.) fruits. *J Essent Oil Res*. 2000;12(3):377-8. doi:10.1080/10412905.2000.9699539
 26. Scora RW, Scora PE. Leaf oils of two new avocado varieties endemic to Costa Rica. *J Essent Oil Res*. 1997;10(6):705-7. doi:10.1080/10412905.1998.9701016
 27. Werman MJ, Mokady S, Neeman I. Partial isolation and characterization of a new natural inhibitor of lysyl oxidase from avocado seed oil. *J Agri Food Chem*. 1990;38(12):2164-8. doi:10.1021/jf00102a013
 28. De Almeida AP, Miranda MM, Simoni IC, Wigg MD, Lagrota MH, Costa SS. Flavonol monoglycosides isolated from the antiviral fractions of *Persea americana* (Lauraceae) leaf infusion. *Phytother Res*. 1998;12(8):562-7.
 29. Nagaraj M, Sandhya V, Supriya G, Manju R, Kumari P, Bole S, et al. Antioxidant and antibacterial activity of avocado (*Persea gratissima* Gaertner.) seed extract. *World Appl Sci J*. 2010;9(6):695-8.
 30. King-Loeza Y, Ciprian-Macias DA, Cardador-Martinez A, Martindel-Campo ST, Castaneda-Saucedo MC, Ramirez-Anaya JDP. Functional composition of avocado (*Persea americana* Mill. Var Hass) pulp, extra virgin oil, and residues is affected by fruit commercial classification. *J Agric Food Res*. 2023;12:100573.
 31. Flores M, Saravia C, Vergara CE, Avila F, Valdés H, Ortiz-Viedma J. Avocado oil: Characteristics, properties, and applications. *Molecules*. 2019;24(11):2172. doi:10.3390/molecules24112172
 32. Gross J, Gabai M, Lifshitz A, Sklarz B. Structures of some carotenoids from the pulp of *Persea americana*. *Phytochemistry*. 1974;13(9):1917-21. doi:10.1016/0031-9422(74)85115-0
 33. Domergue F, Helms GL, Prusky D, Browse J. Antifungal compounds from idioblast cells isolated from avocado fruits. *Phytochem*. 2000;54(2):183-9. doi:10.1016/s0031-9422(00)00055-8
 34. Corral-Aguayo RD, Yahia EM, Carrillo-Lopez A, González-Aguilar G. Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. *J Agri Food Chem*. 2008;56(22):10498-504. doi:10.1021/jf801983r
 35. Ramos-Jerz Mdel R, Villanueva S, Jerz G, Winterhalter P, Deters AM. *Persea americana* Mill. Seed: Fractionation, characterization, and effects on human keratinocytes and fibroblasts. *Evid Based Complement Alternat Med*. 2013;2013:391247. doi:10.1155/2013/391247
 36. Raj R, Shams R, Pandey VK, Dash KK, Singh P, Bashir O. Barley phytochemicals and health promoting benefits: A comprehensive review. *J Agri Food Res*. 2023;14:100677. doi:10.1016/j.jafr.2023.100677
 37. Zhao XX, Lin FJ, Li H, Li HB, Wu DT, Geng F, et al. Recent advances in bioactive compounds, health functions, and safety concerns of onion (*Allium cepa* L.). *Front Nutr*. 2021;8:669805. doi:10.3389/fnut.2021.669805
 38. Bahrapour N, Mirzababaei A, Hosseininasab D, Abaj F, Clark CCT, Mirzaei K. High intake of dietary phytochemical index may be related to reducing risk of diabetic nephropathy: A case-control study. *BMC Nutr*. 2023;9(1):14. doi:10.1186/s40795-023-00676-2
 39. Arabshomali A, Bazzazzadehgan S, Mahdi F, Shariat-Madar Z. Potential benefits of antioxidant phytochemicals in type 2 diabetes. *Molecules*. 2023;28(20):7209. doi:10.3390/molecules28207209
 40. Nikolic P, Mudgil P. The cell wall, cell membrane and virulence factors of *Staphylococcus aureus* and their role in antibiotic resistance. *Microorganisms*. 2023;11(2):259. doi:10.3390/microorganisms11020259
 41. Royani A, Hanafi M, Lotulung PDN, Julistiono H, Dinoto A, Manaf A. Analysis of the antibacterial activity and the total phenolic and flavonoid contents of the moringa oleifera leaf extract as an antimicrobial agent against *Pseudomonas aeruginosa*. *Scientifica (Cairo)*. 2023;2023:5782063. doi:10.1155/2023/5782063
 42. Souleymane HD, Djibo AK, Seyni SH, Zakaria O, Botezatu AV, Dinica RM, et al. Phytochemical characterization and in vitro evaluation of the anti-sickle cell activity of aqueous and ethanolic extracts of two medicinal plants from niger: *Flueggea virosa* (Roxb. ex Willd.) royle and *Kigelia africana* (Lam.) benth. *Plants*. 2023;12(20):3522. doi:10.3390/plants12203522
 43. Stagos D. Antioxidant activity of polyphenolic plant extracts. *Antioxidants (Basel)*. 2020;9(1):19. doi:10.3390/antiox9010019
 44. Nwachukwu ID, Sarteshnizi RA, Udenigwe CC, Aluko RE. A concise review of current in vitro chemical and cell-based antioxidant assay methods. *Molecules*. 2021;26(16):4865. doi:10.3390/molecules26164865
 45. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *monothea buxifolia* fruit. *Osong Public Health Res Perspect*. 2013;4(5):246-54. doi:10.1016/j.phrp.2013.09.003
 46. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. *Front Pharmacol*. 2022;13:806470. doi:10.3389/fphar.2022.806470
 47. Teterovska R, Sile I, Paulausks A, Kovalcuka L, Koka R, Mauriņa B, et al. The antioxidant activity of wild-growing plants containing phenolic compounds in latvia. *Plants (Basel)*. 2023;12(24):4108. doi:10.3390/plants12244108
 48. Yu M, Gouvinhas I, Rocha J, Barros AIRNA. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Sci Rep*. 2021;11(1):10041. doi:10.1038/s41598-021-89437-4
 49. Agidew MG. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bull Natl Res Cent*. 2022;46(1):87. doi:10.1186/s42269-022-00770-8