

Contribution to Phytotherapy Valorization of the Seed of Avocado, *Persea Americana*

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Abstract: Plants represent an immense storehouse of biomolecules endowed with diverse biological properties. This study was carried out to analyze the phytochemical composition and evaluate the free radical scavenging and antioxidant activities of *Persea americana* seed. The dried avocado pit powder was first delipidated with hexane and the residue was successively extracted by maceration with ethanol-water mixture (50% -50%: v/v), and ethanol. Qualitative phytochemical analyses were carried out on the two extracts; and quantitative assays, to assess total polyphenol, flavonoid and condensed tannin contents. The free radical scavenging and antioxidant activities of the extracts were assessed. The results had revealed the presence of polyphenols, alkaloids, flavonoids, saponosides, anthocyanins, anthraquinones, steroids, cardiac glycosides, and condensed tannins in both extracts. In addition, total polyphenol, flavonoid and condensed tannin contents were: 70.427±0.113 mg GAEq/g DE and 194.986±2 mg GAEq/g DE; 19.667±1.732 mg QtEq/g DE and 23.111±0.509 mg QtEq/g DE; then 65.134±1.672 mg CEq/g DE and 203.616±4.931 mg CEq/g DE, respectively in the hydroethanolic and ethanolic extracts. The hydroethanolic extract showed a free radical scavenging activity evaluated as 13.84 ± 0.01%, whereas that of the ethanolic extract was 34.41 ± 4.70%; while measurement of antioxidant activity provided values of 24.30 ± 0.63 mg AAEq/g DE and 42.35 ± 1.20 mg AAEq /g DE, respectively. In conclusion, ethanolic extract showed better free radical scavenging and antioxidant activities, with higher contents of bioactive compounds. These results justified the common use of avocado seeds in phytotherapy in Togo for treatment of various pathologies.

Keywords: *Persea Americana*, Seed, Phytochemical Composition, Antioxidant and Antiradical Activities

1. Introduction

According to the WHO observation, almost 65-80% of people living in developing countries are still treating themselves with herbal medicines, due to lack of access to modern medicine and, above all, poverty [1]. Furthermore, despite the considerable advances made in the chemical

synthesis of molecules with therapeutic properties, more than 25% of drugs prescribed in rich countries derive directly or indirectly from medicinal plants [2, 3].

Plants are an important resource for researchers involved in the formulation of phytomedicines, as well as in the manufacture of therapeutic products used in modern medicine. In pharmaceutical and organic chemistry

laboratories, the investigation and identification of new plant sources for the isolation and characterization of new active ingredients for drug formulation are therefore a major interest for researchers.

Throughout history, plant organs, particularly fruit seeds from certain agricultural products, have been used as waste. Unfortunately, the degradation of these wastes remains a serious threat to the environment [4]. Yet these wastes contain natural substances known as secondary metabolites, which are endowed with various biological activities [5]. This is the case, for example, with the seeds of *Persea americana*, commonly known as avocado, a medium-sized tree that can reach a height of twenty meters. This tree, originally from Tropical America [6], is one of the agricultural plants whose fruit use generates plant residues considered as waste less value, with a rate of around 26% and mostly represented by its seed [7].

In fact, the biological diversity of living beings is proportional to the problems and threats that humans face, especially in terms of health, due to the proliferation of pathogenic microbial agents [8], probably resulting from the insalubrities that surround them. According to current observations, the human species is constantly facing various forms of epidemics. The most recent example is the Corona virus 2019 pandemic.

Among the microbial diseases endangering the human species are fungal infections, known as mycoses [9]. Nowadays, due to the resistance of pathogenic microorganisms to pharmaceutical products, phytotherapy is a promising alternative in the therapy of diseases of various origins [10]. One of the major advantages of phytotherapy is that it prevents pathogenic microorganisms from developing resistance phenomenon. As a result, the health and well-being of patients is enhanced or improved. So far, the use of plant extracts and phytomedicines is increasingly attracting the attention of researchers due to their: availability, cost-effectiveness, efficacy, proven specificity, biodegradability, and low toxicity to various ecosystems [11]. Thus, many works have been conducted to show that many species possess antifungal and antibacterial properties [12-14].

Previous studies have shown that *Persea americana* seed is a potentially rich source of polyphenols and contains a large variety of different classes of phytochemical compounds. Indeed, it had been noted that the seed has a higher polyphenol content and greater antioxidant activity than the pulp [15-17]. This is why some believe that *Persea americana* seed could play a vital role in the treatment of heart disease and some cancers in which develop inflammatory processes [18]. Recently, it has been reported that the Aztecs and Mayans used avocado seed decoctions for the treatment of mycotic and parasitic infections, diabetes, inflammation and gastrointestinal problems [19].

In Togo, after consumption of avocado pulp, large quantities of *Persea americana* seeds are often discarded in the nature. However, the degradation of these seeds can be detrimental to the survival of the human species, as it is a source of proliferation of pathogenic microorganisms. In

order to fight this phenomenon, it is therefore useful to valorize avocado seed by giving added value to all those involved in the chain of production and sale of *Persea americana* fruit.

The current study was undertaken to analyze the phytochemical composition and evaluate antioxidant and free radical scavenging activities of avocado seed extracts.

2. Materials and Methods

2.1. Plant Material

In the current work, avocado seeds were used as plant material. The seeds were collected in Lomé from avocado sellers. The fruits were harvested in Plateaux Region, the major area for avocado cultivation in Togo. They were stripped of their seed coats and the recovered seeds were washed, then cut into small pieces by using a stainless steel knife, and dried at laboratory room temperature (30°C) for at least two weeks before being ground. The powder obtained was placed in a sealed bottle and stored in the laboratory for future use.

2.2. Solid-Liquid Extraction of the Seed

The extraction method used in this study was maceration, using three different solvents: hexane (100%), ethanol (95% vol.) and an ethanol-water mixture (50%-50%: v/v).

A mass of 1 kg of dry avocado seed powder was weighed and first delipidated by maceration for 48 hours in 1.25 L of hexane. The recovered pomace was then dried for 24 hours. After drying, 314 g of dry pomace were dissolved and left to macerate in 1.0 L of ethanol for 72 hours. A further 314 g of dry pomace were also dissolved and macerated in 1.0 L of hydroethanolic mixture (50%-50%: v/v).

The extract solutions obtained, were first gravimetrically filtered on Wattman paper N°1 before being concentrated using a Büchi rotary evaporator system.

The yield (Y_{ext} : %) of each type of extraction was calculated through *Formula 1*.

$$Y_{\text{ext}}(\%) = \left(\frac{M_{\text{ext}}}{M_{\text{pom}}} \right) \times 100 \quad (1)$$

With: M_{ext} = mass of extract;

M_{pom} = mass of pomace.

2.3. Qualitative Phytochemical Analyzes of *Persea Americana* Seed

To reveal the presence of secondary metabolites in our ethanolic and hydroethanolic extracts, the following protocols described were followed. Adopting the procedure of [20], alkaloids were characterized by Dragendorff's reagent and aqueous HCl solution (10%); polyphenols and tannins by aqueous FeCl_3 solution.

Flavonoids were highlighted by soda solution (10%); anthraquinones, by aqueous ammonia solution (10%) with reference to the method described by Rajbhar *et al.* (2015)

[21], while saponins were characterized according to the method described by Daira et al. (2016) [22]. With chloroform and concentrated sulfuric acid, cardiac glycosides were characterized by applying the method of Berran et al. (2015) [23]. Finally, soluble starch was revealed using successively concentrated sulfuric acid and aqueous potassium hydroxide solution (10%) according to the method used by Berran et al. (2015) [23].

2.4. Quantitative Phytochemical Analyses of *Persea Americana* Seed

Quantitative phytochemical analyses focused on the determination of phytochemical constituents in our extracts, such as: fat, total polyphenols, total flavonoids and total condensed tannins.

2.4.1. Determination of Fat Content in Avocado Seed

The fat content (Fat: %) of avocado seeds was determined using *Formula 2*.

$$F_{at}\% = \left(\frac{M_1}{M_0}\right) \times 100 \quad (2)$$

With: M_0 = mass of avocado seed dry powder used for delipidation and M_1 = mass of fat recovered by delipidation of avocado seed dry powder using hexane maceration.

2.4.2. Total Polyphenol Determination

Total polyphenol quantification was performed by applying the method similar to the one described by Suman Joshi et al. (2017) [24]. This method stated that an aqueous sodium carbonate solution (6%: w/v) had to be freshly prepared. To 500 μ L of each extract, 500 μ L of Folin Ciocalteu reagent (10%: v/v) were added and the resulting mixture was vortexed. After a 5 min rest, 500 μ L of sodium carbonate solution (6% w/v) was added to the previous mixture. The new mixture obtained was left to stand for 30 minutes at laboratory room temperature and protected from light.

The optical density of the various samples prepared was read at a wavelength of 725 nm against a blank using a METASH UV-5200PC spectrophotometer. A calibration curve (Figure 1) was established under the same conditions, using gallic acid as standard for a range of concentrations from 0 to 300 μ g/mL. The total polyphenol content of the samples analyzed was expressed in milligrams of gallic acid equivalent (mg GAEq) per gram (g) of dry extract (DE), i.e. mg GAEq/g DE.

2.4.3. Quantitation of Total Flavonoids

The colorimetric method based on the use of aluminum trichloride ($AlCl_3$) as a reagent by adopting the slightly modified experimental protocol described by Mahmoudi et al. (2013) [25] allowed us the quantification of the total flavonoid content in the ethanolic and hydroethanolic extracts. To 200 μ L of solution of each extract, with a concentration of 500 μ g. mL^{-1} , were added 1600 μ L of distilled water, 120 μ L of aqueous sodium nitrite solution ($NaNO_2$: 5%), then 80 μ L of aqueous aluminum trichloride hexahydrate solution ($AlCl_3 \cdot 6H_2O$: 10%). After vortexing, the final mixture was incubated

at 30°C in the dark for 5 minutes.

The absorbance of the final solution obtained was read on a METASH UV-5200PC spectrophotometer against a blank, at a wavelength of 510 nm. Tests were carried out in triplicate. Quercetin was used as a reference to establish the calibration curve for a concentration range from 0 to 700 μ g/mL (Figure 2) for the calculation of total flavonoid content. The total flavonoid content of the samples was expressed in milligrams (mg) of quercetin (Qt) equivalent (Eq) per gram (g) of dry extract (DE), i.e. mg QtEq/g DE.

2.4.4. Quantitation of Total Condensed Tannins

Total condensed tannins were assayed in the extracts table.

μ L of each extract, 2000 μ L of vanillin solution (4%) in methanol and 750 μ L of an aqueous hydrochloric acid solution (37%) were added.

The resulting mixture was shaken vigorously before being incubated at 30°C for 30 minutes. The absorbance of the final solution obtained was measured at a wavelength of 500 nm against a blank consisting of a 4% solution of vanillin in methanol and HCl (37%). Triplicates were run for each unknown sample. Catechin was used to establish the calibration curve (Figure 3) for the measurement of total condensed tannin content, expressed as milligram (mg) catechin (C) equivalent (Eq) per gram (g) dry extract (DE), i.e. mg CEq/g DE.

2.5. Biological Activity Evaluation

The two biological activities tested on ethanolic and hydroethanolic extracts from avocado seeds were: anti-free radical activity and antioxidant activity.

2.5.1. Evaluation of the Anti-Free Radical Activity of the Seed Extracts

The antiradical activity was determined using the experimental protocol previously described by Bouchenak et al. (2020) [27]. According to this experimental protocol, to 100 μ L of solution of each extract, 3 mL of the methanolic solution of DPPH \cdot (0.4%) were added. After incubation for 10 min at room temperature (30°C) and in the dark, the absorbance of the solution obtained was measured at a wavelength of 517 nm against a blank prepared using the methanolic solution of DPPH \cdot (100 μ L of methanol in 3 mL of DPPH \cdot solution).

Three assays were carried out for each extract and the average of the three values was taken into consideration. The gallic acid solution, prepared under the same conditions as the ethanolic and hydroethanolic extracts, was used as a positive control. The calibration curve for gallic acid (Figure 4) was plotted with concentrations ranging from 0 to 80 μ g. mL^{-1} . The results obtained were expressed as a function of the percentage absorbance inhibition (%A) of the DPPH \cdot radical, obtained by calculation using *Formula 3*.

$$\%A = \frac{Abs(DPPH\cdot) - Abs(Ext)}{Abs(DPPH\cdot)} \times 100 \quad (3)$$

With: Abs = Absorbance and Ext = Extract.

2.5.2. Evaluation of the Antioxidant Activity of the Seed Extracts

The antioxidant activity of the extracts was measured using a protocol described by Bouchenak *et al.* (2020) [27]. The FRAP reagent solution was obtained by mixing: 17 mL (10mM) of TPTZ reagent (53.4mg of powder dissolved in 17 mL of hydrochloric acid HCl (40 mM)); 170 mL of acetate buffer solution (pH = 3.6; 0.2 M: 320 mg sodium acetate introduced into 200 mL acetic acid) and 17 mL ferric chloride FeCl₃ solution (20 mM: 8.1 mg FeCl₃ introduced into 17 mL distilled water).

The absorbance reading on the spectrophotometer was taken at a wavelength of 593 nm after mixing 100 µL of extract solution with 3000 µL of FRAP reagent solution. Ascorbic acid was used as a reference over a range of 0.000 to 0.200 mg/mL to establish the calibration curve (Figure 5). Results were expressed as milligram (mg) of ascorbic acid (AA) equivalents (Eq) per gram (g) of dry extract (DE), i.e. mg AAEq /g DE.

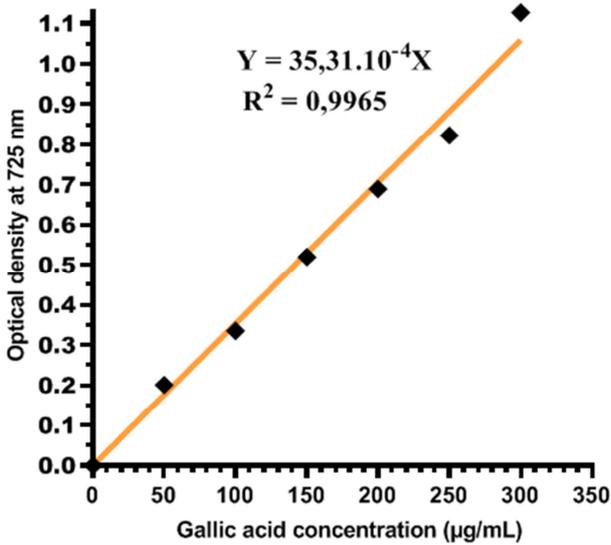


Figure 1. Gallic acid calibration curve for total polyphenol quantification.

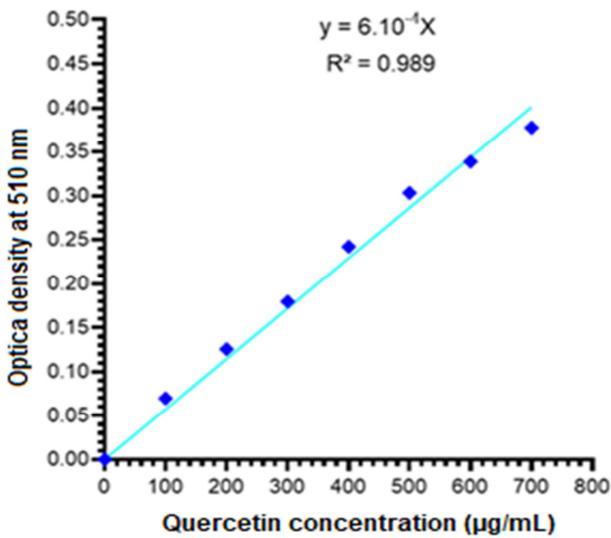


Figure 2. Quercetin calibration curve for total flavonoids analysis.

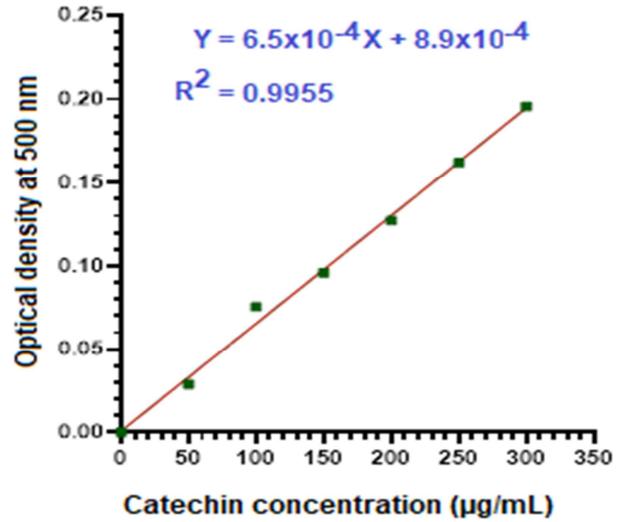


Figure 3. Catechin calibration curve for the analysis of total condensed tannins.

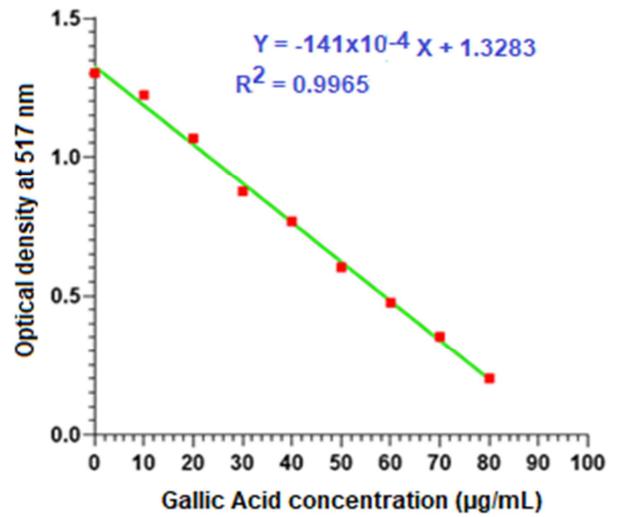


Figure 4. Gallic Acid calibration curve for assessing the free radical scavenging activity of the extracts.

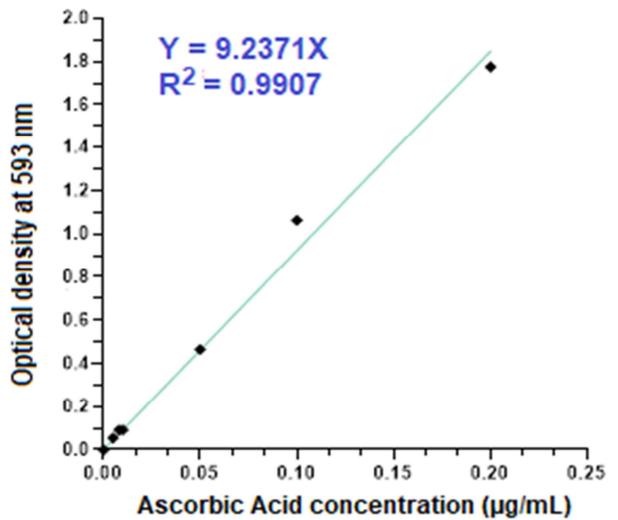


Figure 5. Ascorbic Acid calibration curve for assessing the antioxidant activity of the extracts.

3. Results

3.1. Yields of *Persea Americana* Seed Extracts

In table 1 were shown the fat extraction yield and those of the ethanolic and hydroethanolic extracts of the avocado seed.

Table 1. Extraction yields of fat, ethanolic and hydroethanolic extracts of *Avocado* seed.

Extract types	Yields (%)
Fat content	0.58 ± 0.19
Hydroethanolic extract (H-E/Ext)	10.57 ± 1.84
Ethanolic extract (E/Ext)	4.06 ± 0.96

3.2. Results of Qualitative Phytochemical Analyses of *Persea Americana* Seed

The results of analyses of the qualitative phytochemical composition of the *Persea americana* seed were reported in Table 2.

Table 2. Results of qualitative phytochemical analyses of *Persea americana* seed.

Phytoconstituents tested	Hydroethanol extract	Ethanolic extract
Alkaloids	+	+
Polyphenols	+	+
Flavonoids	+	+
Condensed tannins	+	+
Saponins	+	+
Anthocyanins	+	+
Steroids	+	+
Anthraquinones	+	+
Soluble starch	+	+
Cardiac glycosides	+	+

Legend: + = Effective presence of the phytoconstituent in the seed.

3.3. Results of Quantitative Phytochemical Analyses of *Persea Americana* Seed

Figures 6, 7 and 8 illustrated the comparisons of the contents of total polyphenols, total flavonoids and total condensed tannins in the ethanolic and hydroethanolic extracts of *Persea americana* seed.

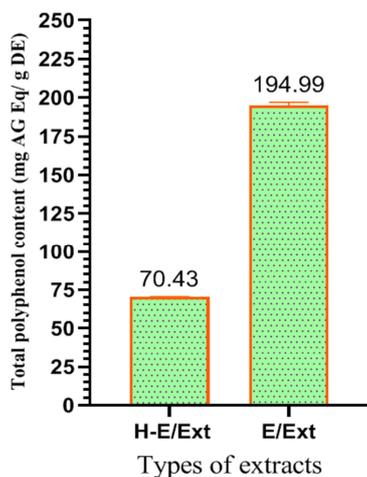


Figure 6. Comparison of total polyphenol content in extracts.

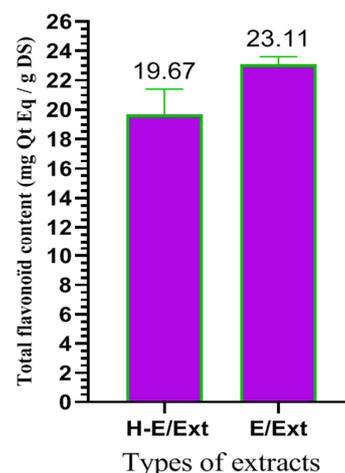


Figure 7. Comparison of total flavonoid content in extracts.

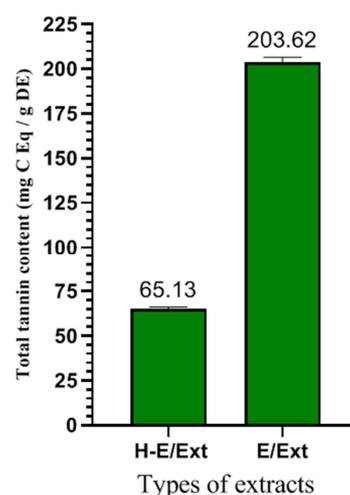


Figure 8. Comparison of total condensed tannin content in extracts.

3.4. Results of Anti-Free Radical and Antioxidant Activity Evaluation of Seed Extracts

The histograms in Figures 9 and 10 showed the comparison of the reduction rates of DPPH[•] radical and Fe³⁺ ion, followed by the comparisons of anti-free radical and antioxidant activity evaluation of *Persea americana* seed extracts.

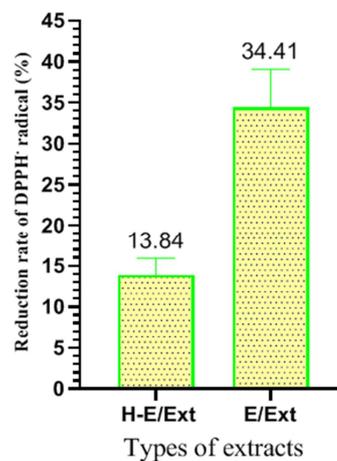
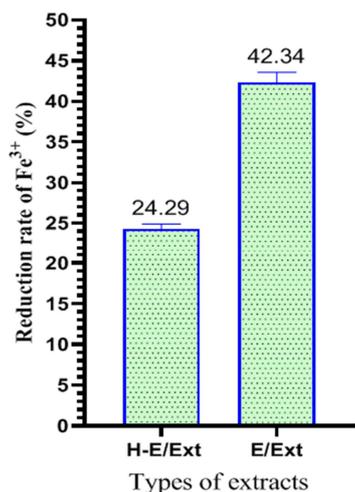


Figure 9. Comparison of DPPH- radical reduction rates by extracts.



Legend: H-E/Ext = hydroethanolic extract; and E/Ext = ethanolic extract

Figure 10. Comparison of Fe³⁺ ion reduction rates by extracts.

4. Discussion

4.1. Yields of *Persea Americana* Seed Extracts

The results presented in Table 1 indicated that the fat content in *Persea americana* seed was almost insignificant ($0.58 \pm 0.19\%$). This yield was much lower than that reported by Adaramola *et al.* (2016) [28], which was $8.10 \pm 0.07\%$. Indeed, the fat extraction method applied in this work was delipidation by maceration with n-hexane, whereas that adopted by Adaramola *et al.* (2016) [28] was the Soxhlet extraction method with n-hexane.

In the current study, the yield of the hydroethanolic extract was significantly better when it compared to the one of ethanolic extract, i.e. 10.57% versus 4.06% respectively. The yield obtained for hydroethanolic extraction (50%-50%: v/v) was very low (10.57%) compared to that reported by Hamani & Boudaoud (2018) [29] which was about 23.2% for hydroethanolic extraction (20%-80%: v/v). This difference could be linked to the polarity of the used solvents, which was not identical for the two studies, and also to the varieties of the used raw materials. However, this yield was similar to that obtained by the same authors for the aqueous extraction of *Persea americana* seed, which was 10.5% .

However, it was recognized that extraction efficiency depends not only on the chemical nature of the phytochemicals to be extracted, but also on the extraction method used, the contact surface of the sample with the solvent, the type of solvent used and the presence of interfering substances [27]. In addition, it was remarked that the extraction yield becomes better when the size of the particles to be extracted is small.

4.2. Phytochemical Constituents Identified Qualitatively in the Seed

The results of the qualitative phytochemical tests (Table 2) revealed the presence of various phytoconstituents such as: alkaloids, polyphenols, flavonoids, tannins, saponins,

anthocyanins, steroids, anthraquinones, soluble starch and cardiac glycosides in the ethanolic and hydroethanolic extracts. These results were almost the same as those obtained by Mamadou *et al.* (2016) [30] and Alagbaoso *et al.* (2017) [17] during their work on ethanolic and aqueous extracts of unripe and ripe *Persea americana* seed.

4.3. Phytoconstituents Quantified in Ethanolic and Hydroethanolic Extracts

Total polyphenol contents were equal to 70.427 ± 0.113 mg GAEq/g DE and 194.986 ± 2.000 mg GAEq/g DE, for the hydroethanolic and ethanolic extracts respectively (Figure 6). Analysis of these results showed that the ethanolic extract was richer in total polyphenolic compounds than the hydroethanolic extract. These results were in agreement with the work carried out by Bourgou *et al.* (2016) [31] who reported that the most suitable solvent for extracting polyphenols is ethanol. However, in their studies, Athaydes *et al.* (2019) [32] had obtained a total polyphenol content of 366.79 ± 5.05 mg GAEq/g DE, which is significantly higher than that obtained in the current study. The disparity between the results could probably be due to the different extraction methods used in the compared studies.

The total flavonoid contents of the hydroethanolic and ethanolic extracts were about 19.667 ± 1.732 mg QtEq/g DE and 23.111 ± 0.509 mg QtEq/g DE, respectively (Figure 7). The ethanolic extract had a slightly higher total flavonoid content than the hydroethanolic extract. In their study, Athaydes *et al.* (2019) [32] obtained a total flavonoid content equal to 28.09 ± 0.64 mg QtEq/g DE, which is slightly higher than those obtained in the current study.

The ethanolic extract showed a total condensed tannin content, i.e. 203.616 ± 4.931 mg CEq/g DE, which was significantly higher than that of the hydroethanolic extract evaluated at 65.134 ± 1.672 mg CEq/g DE (Figure 8). However, for the same species studied, Athaydes *et al.* (2019) [32] had found a higher total tannin content (314.64 ± 2.53 mg CEq/g DE) for ethanolic extract than that obtained in the current work. The extraction method should be the main factor behind this difference.

The use of *Persea americana* seed extracts in formulation of phytomedicines would therefore be great interest for primary healthcare, because the seeds are a very rich source of tannins, which are biomolecules endowed with multiple therapeutic virtues thanks to their specific intrinsic properties. Indeed, tannins are capable of toning the skin and restoring [33]. They are also involved in normalizing blood flow and play a venous protective role [34]. Furthermore, in the industrial sector in particular, they are involved in the manufacture of varnishes and paints [35].

4.4. Anti-Free Radical Activity of the Seed Extracts

The DPPH- radical reduction rates were about $13.840 \pm 2.111\%$ and $34.411 \pm 4.700\%$, respectively for the hydroethanolic and ethanolic extracts of *Persea americana* seed (Figure 9), corresponding to antiradical activities equivalent to 496.398

$\pm 48.000 \mu\text{mol GAEq/g DE}$ and $410.611 \pm 19.623 \mu\text{mol GAEq/g DE}$, respectively for the hydroethanolic and ethanolic extracts.

According to this study, the ethanolic extract had shown the best antiradical activity due to its high total polyphenol content, since Lubis et al. (2017) [36] found that polyphenols, particularly flavonoids and tannins, are biomolecules with the ability to stabilize the DPPH[•] radical via the labile H⁺ protons transfer mechanism.

The results obtained in this work were comparable to those obtained in previous studies. Addressing the same subject, Ferreira da Vinha et al. (2013) [37] had obtained a percentage reduction of the DPPH[•] radical of $90.270\% \pm 0.100\%$ for the aqueous extract by infusion at $90 \pm 5^\circ\text{C}$ with the core powder roasted at 180°C , while for the core dried at laboratory room temperature, they had obtained a percentage reduction of $95.66\% \pm 0.57\%$.

On the other hand, in their investigation, Segovia et al (2018) [38] had obtained antiradical activities equivalent to $410.700 \pm 35.000 \mu\text{mol TE/g DE}$ and $464.900 \pm 32,700 \mu\text{mol TE/g DE}$, respectively for two Hass and Fuerte varieties of *Persea americana*. Rodríguez-Carpena et al. (2011) [39] had also reported free radical scavenging activities of $66.240 \pm 24.840 \mu\text{mol TE/g DM}$ and $94.270 \pm 30.720 \mu\text{mol TE/g DM}$ for the hydroethanolic extract (30% -70%: v/v), then $130.260 \pm 36.800 \mu\text{mol TE/g DM}$ and $167.500 \pm 42.080 \mu\text{mol TE/g DM}$ for the hydroketone extract (30%-70%: v/v), respectively for the Hass and Fuerte varieties of *Persea americana*.

4.5. Antioxidant Activity of Seed Extracts

The reduction rates of Fe³⁺ ions were evaluated at $42.345 \pm 1.193\%$ and $24.295 \pm 0.625\%$, respectively for the ethanolic and hydroethanolic extracts of *Persea americana* seed (Figure 10), corresponding to antioxidant activities equivalent to $801.136 \pm 34.090 \mu\text{mol AAEq /g DE}$ and $460.227 \pm 11.364 \mu\text{mol AAEq /g DE}$ for the ethanolic and hydroethanolic extracts, respectively. Comparatively, it is ethanolic extract that showed also the highest antioxidant activity in the current study. However, in their work, Rodríguez-Carpena et al. (2011) [39] had obtained an estimated antioxidant activity of $2114.4 \mu\text{mol Fe}^{3+}/\text{g DE}$, while Flores et al. (2019) [40] had rather found $2086.33 \mu\text{mol Fe}^{3+}/100\text{g DE}$ as antioxidant activity for their ethanolic extract.

5. Conclusion

In this study, the qualitative and quantitative phytochemical characterization and evaluation of the free radical scavenging, and antioxidant activities of *Persea americana* seed were carried out.

The results had shown qualitatively that *Persea americana* seed from togolese flora had contained bioactive phytochemicals, in particular: alkaloids, polyphenols, flavonoids, tannins, saponins, anthocyanins, steroids, anthraquinones, soluble starch and cardiac glycosides.

Regarding the results obtained in the current study, it would be interesting to exploit this seed as an alternative resource of natural origin for the formulation of products with therapeutic properties. Indeed, the results of this study had shown that the seed was rich in nutritional substances that could be beneficial to the cardiovascular system thanks to the antioxidant and anti-radical properties, and that could also help the immune system to defend itself. In addition, the presence of polyphenols, flavonoids and tannins could confer various biological activities on the seed.

Considering the composition of this seed which is so rich in polyphenol, it would be interesting to evaluate its antifungal activity on various strains of fungi.

Competing Interests

Authors have declared that no competing interests exist about this project publication.

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