

ORIGINAL RESEARCH

Phenolic Content and Antioxidant Activity of Young and Mature Mango (*Mangifera indica*) and Avocado (*Persea americana*) Leaves Extracts

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Abstract

Polyphenols are groups of secondary metabolites in plants, known with their various biological activities, including their ability to act as antioxidants. Due to the side effects of the use of synthetic antioxidants on human health, the search for natural less toxic compounds has significantly increased. This study was carried out to evaluate the phenolic content and antioxidant activity of young and mature avocado (*Persea americana*) and mango leaves (*Mangifera indica*). Different extracts were prepared by maceration in methanol, ethanol, cold and hot water. The phenolic content of the extracts was determined using the Folic-Ciocalteu Method. A total of three antioxidant tests were done on the extracts; the 2, 2-diphenyl-1-picrylhydrazyl test (DPPH test), the Ferric reducing antioxidant power and the Hydroxyl radical scavenging activity. Results of these investigation showed that the mature mango leaves and young avocado leaves exhibited the highest phenolic and flavonoid contents. They were also very efficient as antioxidants in reducing the ferric ions and in scavenging the DPPH and hydroxyl radicals.

Practical Applications

Matured mango leaves and young avocado leaves are rich in polyphenols and have good antioxidant activity. They can be used as natural sources of antioxidants for the reduction of the damages caused by free radicals and reactive oxygen species in the body and some foods such as oils and foods containing lipids that easily oxidize.

Keywords: Phenolic content, Antioxidant activity, *Persea americana*, *Mangifera indica*.

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1. Introduction

Reactive Oxygen Species (ROS) and free radicals are molecules that are naturally produced in living organisms from metabolic reactions (Rahman & Adcock, 2006). They play some beneficial role in the body as they contribute in the destruction of microorganisms or pathogens. However, when they are produced in excess they lead to oxidative stress (Arts & Hollman, 2005). It is well known nowadays that oxidative stress is the number one killer in the world as it has been proven to be implicated in several degenerative disorders such as mutagenesis, cardiovascular diseases, carcinogenesis, Parkinson's disease (Zanera & Sankar, 2009). Oxidative stress does not only affect living organisms, is also detrimental to the food industry by promoting oxidation reactions of oils, fats, and food containing lipids leading to the reduction of their nutritional value and organoleptic properties (Djikeng *et al.*, 2017).

Several biological molecules have been proven capable of preventing or delaying oxidative stress in living organisms and foods. Those molecules have the capacity to donate their hydrogen atom for the stabilization of free radicals or Reactive Oxygen Species. They are generally called antioxidants (Djikeng *et al.*, 2017). Two different types of antioxidants exist, synthetic and natural antioxidants. However, because of the side effects of synthetic antioxidants on human health, the search for natural sources of antioxidants that can be used to delay oxidative stress in living organisms and prolong the shelf-life of foods has been deeply investigated (Womeni *et al.*, 2016). In plants, the natural antioxidants are generally represented by phenolic compounds such as phenolic acids, flavonoids,

anthocyanin, tannins, carotenoids etc (Habermann *et al.*, 2016). *Mangifera indica* belongs to the family Anacardiaceae. It is commonly known as mango, which is an important tree with several medicinal properties. Mangiferin is its bioactive principle which has been proven to have good antioxidant activity (Shah *et al.*, 2010). Additionally, extracts from its leaves and stem bark have been found to be good anti-malarial, anti-fungal, anti-microbial, anti-inflammatory, anti-cancer etc (Williamson, 2002; Ayoola *et al.*, 2008; Kanwal *et al.*, 2010). *Persea americana* belongs to the Lauraceae family commonly known as avocado. Its leaves have been used in the treatment of several disorders such as hypertension, diarrhea etc. It has also been proven to possess anti-inflammatory and good anti-oxidant activity (Adeyemi *et al.*, 2002).

Though many studies have been reported on the phenolic content and antioxidant activity of *Persea americana* and *Mangifera indica* leaves, stem, fruits, flower and seeds, very few have been done on the evaluation of the phenolic content and antioxidant activity of their young and mature leaves, especially with the use of different extraction solvents and conditions. In one study, Habermann *et al.* (2016) showed that the antioxidant activity and phenolic content of aqueous and ethyl acetate extract of young leaves of *Blepharocalyx salicifolius* were higher than those of the mature leaves. Similar observations were also made by Murugan (Murugan & Velayudhan, 2016) with the leaves of *Tectona grandis*. It would be important to have an idea of the phenolic and antioxidant activity of young and mature *Mangifera indica* and *Persea americana* leaves. The state of maturity of

Mangifera indica and *Persea americana* leaves might impact on their phenolic content and antioxidant activity. The objective of this study was therefore to evaluate the phenolic content and antioxidant activity of the methanolic, ethanolic, aqueous and infusion extracts of young and mature *Mangifera indica* and *Persea americana* leaves.

2. Material and methods

2.1. Material

Young and mature leaves of Mango (*Mangifera indica*) and Avocado (*Persea americana*) were freshly harvested from the Wokeka farm of the Catholic University Insitute of Buea, Muea, South-West Region, Cameroon, in February 2018. All the chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of natural antioxidants

Polyphenols were extracted from plant materials using the maceration method, as described by [Womeni et al. \(2016\)](#). The fresh leaves (young and mature) were cleaned and cut into small pieces using a knife in order to facilitate the drying process. After this, the leaves were dried in an electric air-dried oven at 45 °C for 48 hours. The dried leaves were ground in a blender machine (Moulinex) and sieved (Diameter of pore: 1mm). About 20 g of each powder was extracted into 200 ml of Methanol, Ethanol, Water and boiled water respectively. The mixture was regularly subjected to shaking during the extraction. After the 48 hours of maceration, the mixture was filtered with a Wathman N°1 filter paper. The obtained filtrates were then subjected to rotatory evaporation at 45 °C under reduced

pressure for the removal of the solvent. The solvent residues was removed by drying the extract at 45 °C until the extract became solid and the weight constant. The dried extracts were stored at 4 °C for further analysis.

2.2.2. Determination of the total phenolic content

The total phenolic content of Mango and Avocado leaves was determined using the Folin-Ciocalteu colorimetric method, as described by [Gao et al. \(2000\)](#). In a test tube of 5 ml volume, 20 µl of a 2 mg/ml extract solution was added, followed by the Folin–Ciocalteu reagent (0.2 ml) and distilled water (2 ml). After 3 min incubation of the solution mixture at room temperature, 1 ml of 20% sodium carbonate solution was added and the mixture re-incubated for 20 min under the same conditions. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract.

2.2.3. Determination of the total flavonoid content

Aluminium chloride method was used for flavonoid determination using the method described by [Quettier et al. \(2000\)](#). 0.1ml of each extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415nm. Catechin (0.2mg/ml) was used as a standard.

Total flavonoid content was expressed as mg CAT/g of extract.

2.2.4. Determination of the antioxidant activity

2.2.4.1. DPPH radical scavenging assay

The radical scavenging ability of the extracts was determined according to the method of [Braca et al. \(2002\)](#). 4.5 ml of 0.002% alcoholic solution of DPPH was added to 0.5 ml of different concentrations (125, 250, 500, 1000 and 2000 µg/ml) of samples and standard solutions separately, in order to have final concentrations of products of 25-200 µg/ml. The samples were kept at room temperature in the dark and after 30 min, the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control and blank was measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful hydrogen donor, was used as positive control. The antiradical activity (AA) was determined using the following formula:

$$AA\% = [(Abs_{\text{control}} - Abs_{\text{sample}}) \times 100 / Abs_{\text{control}}]$$

Where Abs_{control} was the absorbance of control and Abs_{sample} the absorbance of the sample or standard.

2.2.4.2. Ferric reducing antioxidant power

The antioxidant potential of Mango and Avocado leaves extracts was also evaluated for their ability to reduce iron (III) to iron (II) following the method of [Oyaizu \(1986\)](#). An aliquot of 0.5 ml plant extract (125, 250, 500, 1000 and 2000 µg/ml) was mixed with 1 ml phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% aqueous $K_3Fe(CN)_6$ solution, well shaken and incubated at 50 °C for 30 min. After incubation, 1 ml of 10% TCA solution was added to stop the reaction and the mixture was

centrifuged at 3000 rpm for 10 min. 1.5 ml of supernatant, 1.5 ml of distilled water and 0.1 ml of 0.1% $FeCl_3$ solution were mixed and incubated for 10 min and absorbance read at 700 nm on spectrophotometer. A sample blank, containing all the reagents but no extract was prepared in the same conditions. Catechin, a recognized powerful ferric reducer, was used as a positive control to compare the reducing power of the extracts. A higher absorbance indicates a higher reducing power.

2.2.4.3. Hydroxyl radical scavenging ability

The hydroxyl radical scavenging capacity of the leaves extracts was evaluated by the method described by [Olabinri et al. \(2018\)](#). 60µl of $FeSO_4 \cdot 7H_2O$ (1 mM) was added to 90µl of aqueous 1,10 phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer pH 7.8 was added to the above mixture, followed by addition of 150 µl of hydrogen peroxide (0.17 mM) and 1.5ml of different concentrations of sample in sequence. The mixture was incubated for 5min at room temperature. The absorbance of the mixture was read at 560 nm against blank. All readings were taken in triplicate and Catechin was used as the standard. The percentage inhibition was calculated by the following equation.

$$\% \text{ Hydroxyl radical scavenging capacity} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of the sample or standard.

2.3. Statistical analysis

Results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Dunnett and Student-Newman-Keuls tests using Graphpad-InStat version

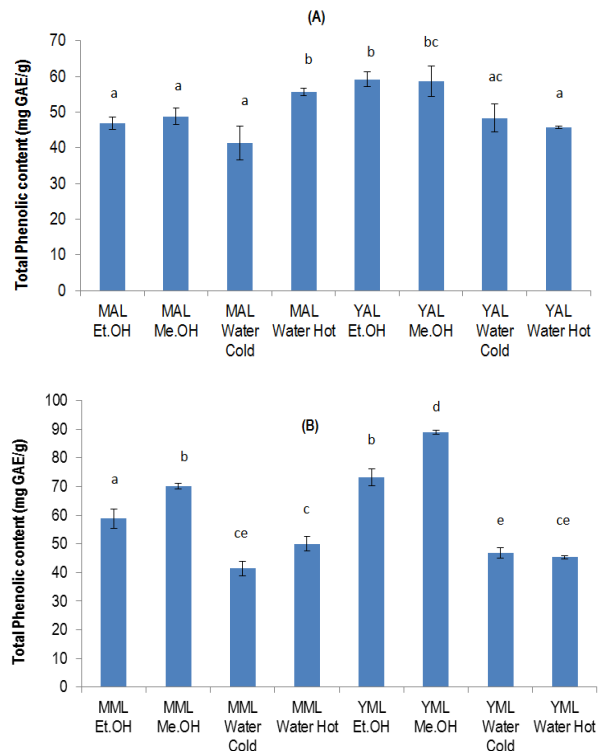
3.05, to evaluate the statistical significance of the data. A probability value at $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Total Phenolic content

Phenolic compounds are the major secondary metabolites found in plants which are used for their defence. In many studies the antioxidant activity of plant extracts has been attributed to these molecules (Womani *et al.*, 2016). The Total Phenolic content of Young and Mature *Mangifera indica* and *Persea americana* leaves measured by the Folin-Ciocalteu method is presented in Figure 1 (A-B). From Figure 1 (A) it is clearly observed that the methanolic and ethanolic extracts of Young Avocado leaves and the hot aqueous extracts of Mature Avocado leaves exhibited the highest phenolic content. No significant difference ($p > 0.05$) was recorded between these samples. However, their total phenolic content was significantly higher ($p < 0.05$) than that of the other extracts. No significant difference ($p > 0.05$) was registered between the phenolic content of the ethanolic, methanolic and cold aqueous extracts of Mature Avocado leaves and cold and hot aqueous extracts of Young leaves. In Figure 1 (B), it is clear that the ethanolic and methanolic extracts of Young and Mature *Mangifera indica* exhibited the highest total phenolic content compared to the cold and hot aqueous extracts of these same samples. However, the phenolic content of the ethanolic and methanolic extracts of Young Mango leaves was higher ($p < 0.05$) than that of the Mature leaves of the same plant.

Generally, the total phenolic content of all the extracts fell within 40-90 mg GAE/g. The fact that *Mangifera indica* and *Persea americana*



^{a-c}Values are presented as mean \pm Standard deviation. Means with different superscripts are significantly different ($p < 0.05$)

Figure 1(A-B): Total Phenolic content of Young and Mature leaves of *Persea americana* (A) and *Mangifera indica* (B). **MAL Et.OH:** Ethanolic extract of mature avocado leaves, **MAL Me.OH:** Methanolic extract mature of avocado leaves, **MAL water cold:** cold aqueous extract of mature avocado leaves, **MAL Hot water:** warm aqueous extract of mature avocado leaves, **YAL Et.OH:** Ethanolic extract of young avocado leaves, **YAL Me.OH:** Methanolic extract young of avocado leaves, **YAL water cold:** cold aqueous extract of young avocado leaves, **YAL Hot water:** warm aqueous extract of young avocado leaves; **MML Et.OH:** Ethanolic extract of mature mango leaves, **MML Me.OH:** Methanolic extract mature of mango leaves, **MML water cold:** cold aqueous extract of mature mango leaves, **MML Hot water:** warm aqueous extract of mature mango leaves, **YML Et.OH:** Ethanolic extract of young mango leaves, **YML Me.OH:** Methanolic extract young of mango leaves, **YML water cold:** cold aqueous extract of young mango leaves, **YML Hot water:** warm aqueous extract of young mango leaves.

extracts are rich in phenolic compounds has already been proven. Kaur *et al.* (2015) reported that the total phenolic content of *Mangifera indica* bark aqueous, ethanolic and methanolic extracts were respectively 128.6, 196.5, and 166.7 mg GAE/ml respectively. In the same line, Vinha *et al.* (2013) demonstrated that the total phenolic content of Algarvian Avocado (*Persea americana*) pulp, skin and seeds were respectively 410.2, 679.0,

and 704.0 mg/100 g respectively. The values obtained by Kaur *et al.* (2015) were significantly higher than those obtained in this study. However, the data obtained in this work with *Persea americana* was significantly higher than that obtained by Vinha *et al.* (2013). The difference observed between the total phenolic content obtained in this study and those reported in the literature can be attributed to genotypic and environmental differences (climate, temperature, location) between these plants, the choice of the part tested, the harvesting period, the extraction and characterization methods (Kim & Choe, 2004; Shan *et al.*, 2005). From this study, it was noticed that Young leaf extracts were richer in phenolic compounds than Mature ones. Similar results were previously reported by Habermann *et al.* (2016) with the young and mature leaves of *Blepharocalyx salicifolius*.

3.2. Total flavonoid content

Flavonoids are the most represented family of phenolic compounds. They have been proven to have good antioxidant activity through several mechanisms of action (D'Abrosca *et al.*, 2007). This has been attributed to their complex structures compared to that of phenolic acids. The flavonoid content of *Mangifera indica* and *Persea americana* extracts are illustrated in Figure 2 (A-B). The methanolic, cold and hot aqueous extracts of mature *Persea americana* leaves and the ethanolic, methanolic and hot aqueous extracts of the young leaves of this same plant have exhibited significantly higher ($p < 0.05$) flavonoid content compared to the other extracts. However, the highest value was recorded with the methanolic extract of its young leaves (Figure 2A). In Figure 2B, apart from the hot aqueous extracts of mature

Mangifera indica leaves and the cold and hot aqueous extracts of its young leaves which have presented the lowest ($p < 0.05$) flavonoid content, all the other extracts have exhibited significantly higher ($p < 0.05$) total flavonoid content.

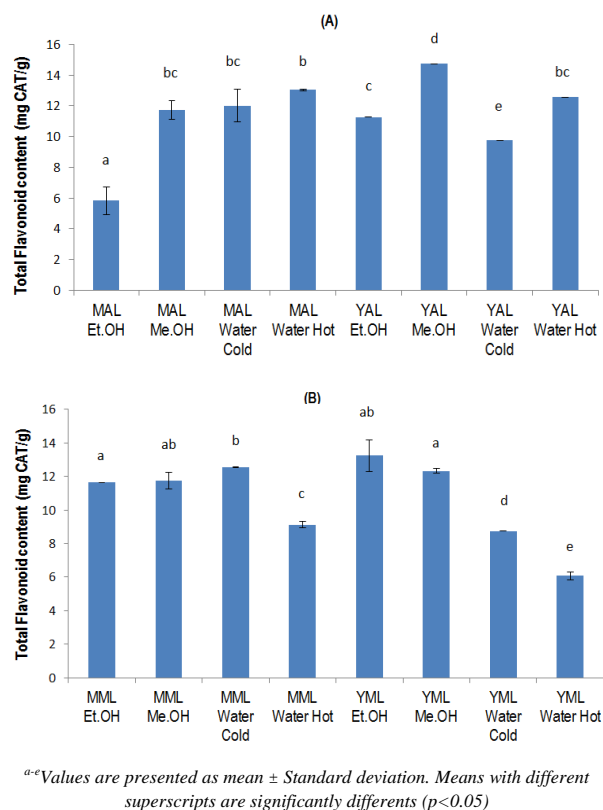


Figure 2 (A-B): Total Flavonoid content of Young and Mature leaves of *Persea americana* (A) and *Mangifera indica* (B). **MAL Et.OH:** Ethanolic extract of mature avocado leaves, **MAL Me.OH:** Methanolic extract mature of avocado leaves, **MAL water cold:** cold aqueous extract of mature avocado leaves, **MAL Hot water:** warm aqueous extract of mature avocado leaves, **YAL Et.OH:** Ethanolic extract of young avocado leaves, **YAL Me.OH:** Methanolic extract young of avocado leaves, **YAL water cold:** cold aqueous extract of young avocado leaves, **YAL Hot water:** warm aqueous extract of young avocado leaves; **MML Et.OH:** Ethanolic extract of mature mango leaves, **MML Me.OH:** Methanolic extract mature of mango leaves, **MML water cold:** cold aqueous extract of mature mango leaves, **MML Hot water:** warm aqueous extract of mature mango leaves, **YML Et.OH:** Ethanolic extract of young mango leaves, **YML Me.OH:** Methanolic extract young of mango leaves, **YML water cold:** cold aqueous extract of young mango leaves, **YML Hot water:** warm aqueous extract of young mango leaves.

From this analysis, it is clear that samples which presented good phenolic content also exhibited good flavonoid content. Globally, the total flavonoid content of this plant extracts varied from 6 to 14 mg CAT/g. The presence of flavonoids in *Persea americana* extracts has already been reported. [Vinha et al. \(2013\)](#) demonstrated that the total flavonoid content of *Persea americana* pulp, skin and seed were respectively 21.9, 44.3, and 47.9 mg/100 g. On the other hand [Arukwe et al. \(2012\)](#) showed that the total flavonoid content of *Persea americana*'s leaf, fruit and seed were respectively 8.11, 4.25 and 1.90 mg/100 g. In the same line, [Duresa \(2017\)](#) reported the presence of flavonoids in *Mangifera indica* and *Persea americana* fruits. The total flavonoid content obtained in this study was significantly higher than those reported by these authors. The environmental conditions, the part of the plant used, the nature of the extraction solvent and the age of the plant can explain these variations ([Kim & Choe, 2004](#); [Shan et al., 2005](#)).

3.3. Antioxidant activity

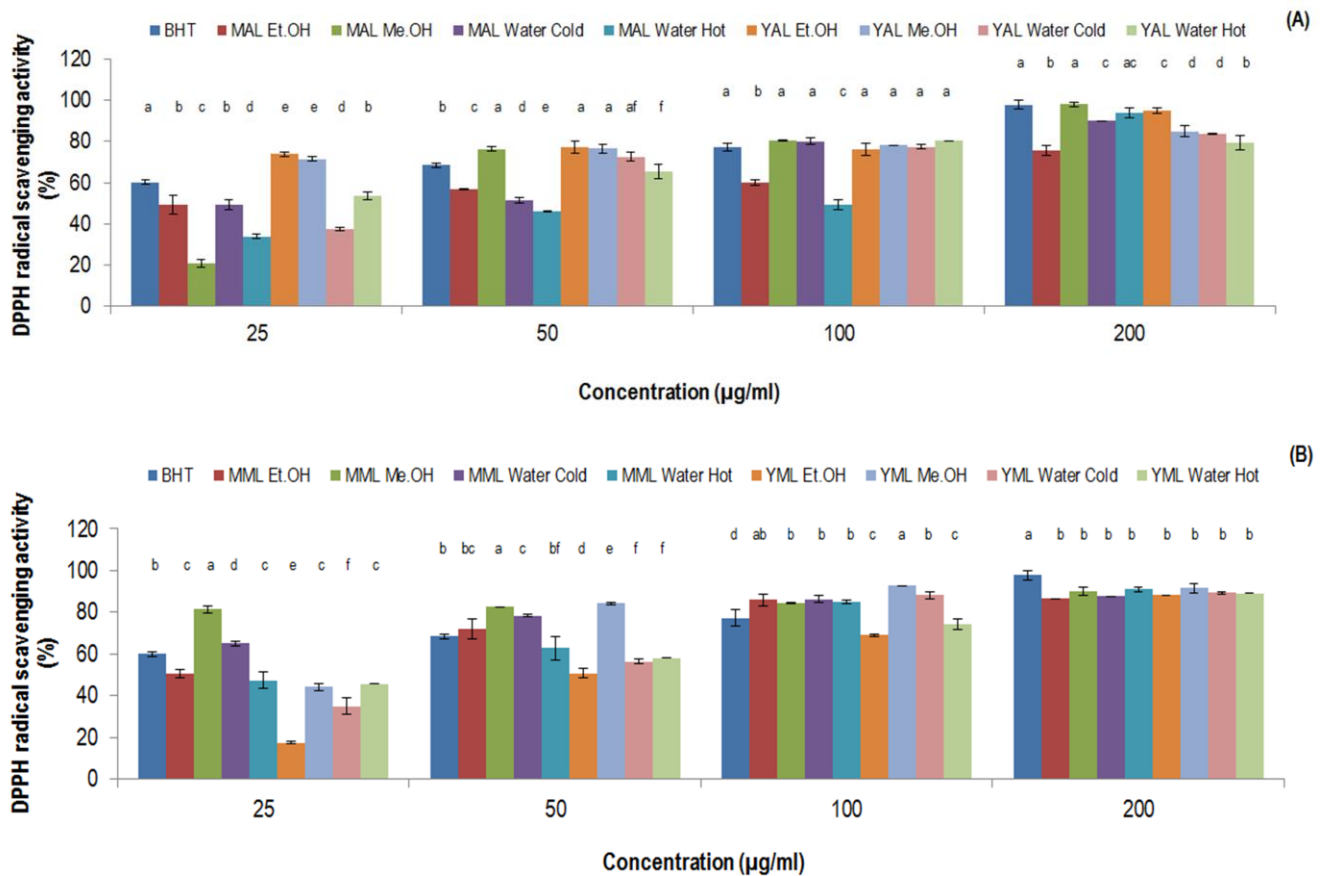
3.3.1. DPPH radical scavenging assay

In this study, the free radical scavenging capacity of *Mangifera indica* and *Persea americana* extracts was also evaluated and the results are presented in Figure 3 (A-B). Generally, the DPPH Radical Scavenging Activity of the extracts of both plants was significantly increasing ($p < 0.05$) with their concentration. In Figure 3 (A), at concentration 25 $\mu\text{g/ml}$, the activities of the ethanolic and methanolic extracts of young *Persea americana* leaves were significantly higher ($p < 0.05$) than that of BHT and all the other samples. At concentration 50 $\mu\text{g/ml}$, the

aqueous and organic solvent extracts of young Avocado leaves and the methanolic extracts of mature leaves of this same plant were significantly higher ($p < 0.05$) than that of the synthetic antioxidant tested. However, at concentration 100 $\mu\text{g/ml}$ apart from the methanolic and hot aqueous extracts of the mature leaves which presented significantly lower ($p < 0.05$) radical scavenging activity, the other extracts exhibited similar activity with the BHT. At concentration 200 $\mu\text{g/ml}$, all the extracts showed very good DPPH radical scavenging activities.

Concerning the activity of *Mangifera indica* leaves extracts, at concentration 25 $\mu\text{g/ml}$ the activity of the mature leaves was significantly higher ($p < 0.05$) than that of the young leaves (Figure 3B). The highest activities were recorded with the methanolic and cold aqueous extracts. At concentration 50 $\mu\text{g/ml}$, the mature leaves extracts still presented the best activity compared to the young leaves. However, the methanolic extract of young leaves alone exhibited the highest scavenging activity. At concentration 100 $\mu\text{g/ml}$, the activity of all the extracts were significantly higher or equal to that of BHT. At 200 $\mu\text{g/ml}$, the BHT exhibited the highest ($p < 0.05$) antioxidant activity and no significant difference ($p > 0.05$) was recorded between all the extracts.

The results obtained in this study globally showed that young Avocado leaves extracts are more active against the DPPH radical than mature ones. This result is in agreement with those reported by [Habermann et al. \(2015\)](#) who reported that, the aqueous extracts of young leaves of *Blepharocalyx salicifolius* has a good DPPH radical scavenging activity compared to mature leaves. On the other hand, the mature leaves of *Mangifera indica* were the best in



^{a-f}Values are presented as mean ± Standard deviation. Means with different superscripts for each concentration are significantly different (p<0.05)

Figure 3 (A-B): DPPH Radical Scavenging Activity of Young and Mature leaves of *Persea americana* (A) and *Mangifera indica* (B). **MAL Et.OH:** Ethanolic extract of mature avocado leaves, **MAL Me.OH:** Methanolic extract mature of avocado leaves, **MAL water cold:** cold aqueous extract of mature avocado leaves, **MAL Hot water:** warm aqueous extract of mature avocado leaves, **YAL Et.OH:** Ethanolic extract of young avocado leaves, **YAL Me.OH:** Methanolic extract young of avocado leaves, **YAL water cold:** cold aqueous extract of young avocado leaves, **YAL Hot water:** warm aqueous extract of young avocado leaves; **MML Et.OH:** Ethanolic extract of mature mango leaves, **MML Me.OH:** Methanolic extract mature of mango leaves, **MML water cold:** cold aqueous extract of mature mango leaves, **MML Hot water:** warm aqueous extract of mature mango leaves, **YML Et.OH:** Ethanolic extract of young mango leaves, **YML Me.OH:** Methanolic extract young of mango leaves, **YML water cold:** cold aqueous extract of young mango leaves, **YML Hot water:** warm aqueous extract of young mango leaves.

scavenging the DPPH radical compared to young leaves. This result is contradictory to those reported by Habermann *et al.* (2015). The fact that the ethanolic and methanolic extracts of *Mangifera indica* leaves exhibit better antioxidant activity than the aqueous extract has already been reported by Kaur *et al.* (2015). The interesting DPPH Radical Scavenging Activity of *Persea Americana*

leaves has also been previously reported by Vinha *et al.* (2013).

Generally, the plant extracts which have exhibited higher phenolic and flavonoid contents also presented the best antioxidant activities. These results are in agreement with those reported by Womeni *et al.* (2016), Bouba *et al.* (2010) and Womeni *et al.* (2013) who reported that plants with high phenolic content

generally exhibit high DPPH Radical Scavenging Activity.

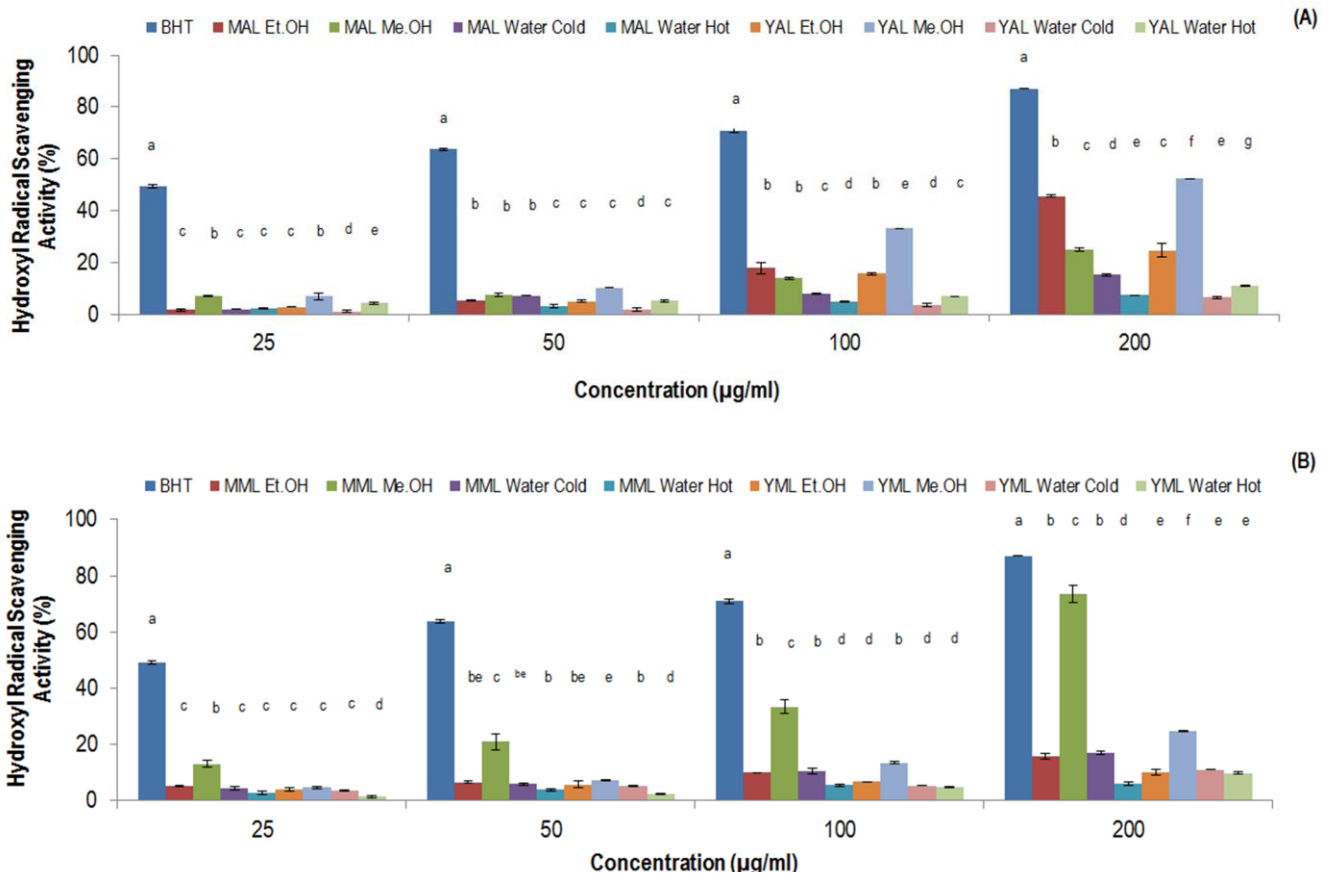
3.3.2. Hydroxyl Radical Scavenging Activity (HRSA)

The Hydroxyl Radical Scavenging Activity of *Mangifera indica* and *Persea americana* leaves extracts is presented in Figure 4 (A-B). The results obtained from this figure showed that the BHT exhibited the highest activity compared to the plant extracts. In Figure 4 (A) it is clearly observed that the young and mature ethanolic and methanolic extracts of *Persea americana* leaves presented a significantly higher ($p < 0.05$) Hydroxyl Radical Scavenging Activity compared to the aqueous extracts. However, the young leaves were the best in stabilizing this radical (OH^\cdot). However, in Figure 4 (B), the mature leaves, especially those extracted with methanol exhibited the highest HRSA compared to the other plant extracts. They were followed by the methanolic extracts of the young leaves. The results obtained in this study with *Persea americana* leaves are in agreement with those obtained from the DPPH test were the young leaves were the most active against the DPPH radical. The active molecule extracted from the leaves of these plants (phenolic antioxidants) may have several mechanisms of action. However, a different observation is made with *Mangifera indica* leaves extracts, as only the methanolic extract was the most active. This can be explained by the fact that the antioxidant having the ability to scavenge the Hydroxyl radical was only extracted by methanol. In many studies the best antioxidant activity of plant extracts was reported with methanol and this was related to their strong extraction power. It has the ability to extract at

the same time polar and non-polar molecules (as essential oil components) (Bouba *et al.*, 2010; Iqbal & Bhangar, 2007). The abundance in this extract of antioxidant molecules with different structures and mechanism of actions can explain the obtained results.

3.3.3. Ferric reducing antioxidant power (FRAP)

This test is generally used to evaluate the ability of a substance to reduce Ferric iron into ferrous iron, by donating its electron. This mechanism of action is known as a good indicator of the Antioxidant Activity of a substance. The Ferric Reducing Antioxidant Power of *Mangifera indica* and *Persea americana* leaves extracts are presented in Figure 5 (A-B). Almost all the extracts exhibited good Ferric Reducing Antioxidant Power. The highest activity was recorded with the cold aqueous extracts of young Avocado leaves (Figure 5A). Its activity was higher than that of the synthetic Antioxidant used (Vitamin C). However at concentration 100 and 200 $\mu\text{g/ml}$ the activity of all the other plant extracts was similar or slightly higher than that of Vitamin C. In Figure 5 (B), the lowest ($p < 0.05$) Ferric Reducing Antioxidant Power was recorded in cold and hot aqueous extracts of *Mangifera indica* leaves and this at all concentrations. However, the activity of the other extracts was similar to that of Vitamin C. This result confirms once again the fact that matures Mango leaves have good antioxidant activity than young ones. The interesting activity registered with these plant extracts can be attributed to their good total phenolic and Flavonoid contents. The highest antioxidant activity obtained with the cold aqueous extracts of Avocado leaves can be attributed to the presence of a powerful ferric reducer which

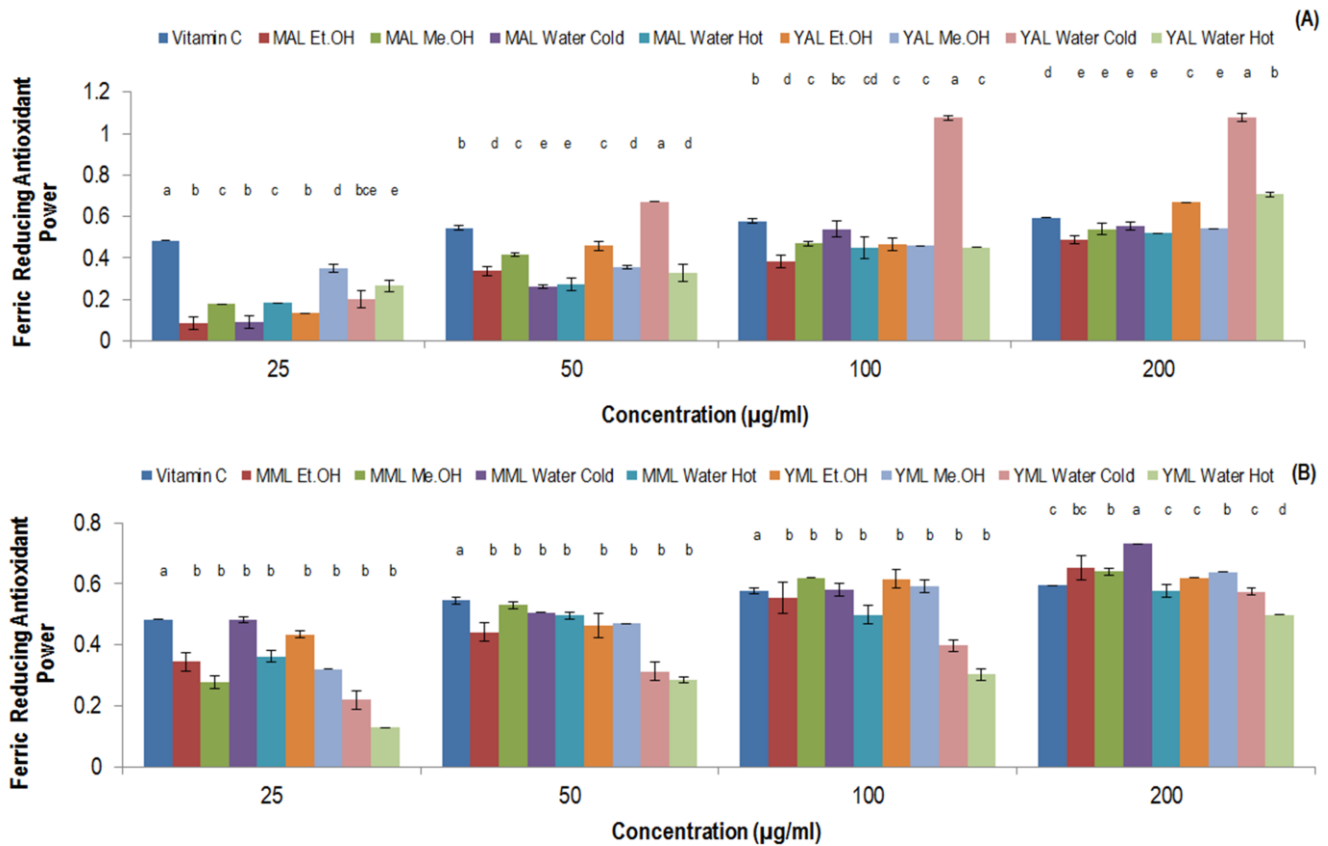


^{a-f}Values are presented as mean ± Standard deviation. Means with different superscripts for each concentration are significantly different (p<0.05)

Figure 4 (A-B): Hydroxyl Radical Scavenging Activity of Young and Mature leaves of *Persea americana* (A) and *Mangifera indica* (B). **MAL Et.OH:** Ethanolic extract of mature avocado leaves, **MAL Me.OH:** Methanolic extract mature of avocado leaves, **MAL water cold:** cold aqueous extract of mature avocado leaves, **MAL Hot water:** warm aqueous extract of mature avocado leaves, **YAL Et.OH:** Ethanolic extract of young avocado leaves, **YAL Me.OH:** Methanolic extract young of avocado leaves, **YAL water cold:** cold aqueous extract of young avocado leaves, **YAL Hot water:** warm aqueous extract of young avocado leaves; **MML Et.OH:** Ethanolic extract of mature mango leaves, **MML Me.OH:** Methanolic extract mature of mango leaves, **MML water cold:** cold aqueous extract of mature mango leaves, **MML Hot water:** warm aqueous extract of mature mango leaves, **YML Et.OH:** Ethanolic extract of young mango leaves, **YML Me.OH:** Methanolic extract young of mango leaves, **YML water cold:** cold aqueous extract of young mango leaves, **YML Hot water:** warm aqueous extract of young mango leaves.

was not extracted by other solvents and which was abundant in young leaves than mature leaves as it has been proven that young leaves generally exhibit good antioxidant activity compared to mature leaves (Habermann *et al.*, 2015). The results obtained in this study showing that the ethanolic, methanolic and aqueous extracts of *Mangifera indica* are in accordance with those reported by Kaur *et al.* (2015) who showed that the aqueous, ethanolic

and methanolic extracts of *Mangifera indica* have a ferric reducing antioxidant power of 40, 60 and 50 % respectively. In the same line Tremoccoli *et al.* (2018) showed that the Ferric Reducing Antioxidant Power of Avocado fruits (Hass and Fuerte varieties) were respectively 1.17, 656.9, 1.88 and 931.7 (µmole Fe²⁺ / g)³ for Hass peel, Hass seeds, Fuerte peel and Fuerte seeds respectively.



^{a-d}Values are presented as mean ± Standard deviation. Means with different superscripts for each concentration are significantly different ($p < 0.05$)

Figure 5 (A-B): Ferric Reducing Antioxidant Power of Young and Mature leaves of *Persea americana* (A) and *Mangifera indica* (B). **MAL Et.OH:** Ethanolic extract of mature avocado leaves, **MAL Me.OH:** Methanolic extract mature of avocado leaves, **MAL water cold:** cold aqueous extract of mature avocado leaves, **MAL Hot water:** warm aqueous extract of mature avocado leaves, **YAL Et.OH:** Ethanolic extract of young avocado leaves, **YAL Me.OH:** Methanolic extract young of avocado leaves, **YAL water cold:** cold aqueous extract of young avocado leaves, **YAL Hot water:** warm aqueous extract of young avocado leaves; **MML Et.OH:** Ethanolic extract of mature mango leaves, **MML Me.OH:** Methanolic extract mature of mango leaves, **MML water cold:** cold aqueous extract of mature mango leaves, **MML Hot water:** warm aqueous extract of mature mango leaves, **YML Et.OH:** Ethanolic extract of young mango leaves, **YML Me.OH:** Methanolic extract young of mango leaves, **YML water cold:** cold aqueous extract of young mango leaves, **YML Hot water:** warm aqueous extract of young mango leaves.

4. Conclusion

The objective of this study was to evaluate the total Phenolic content and Antioxidant Activity of the methanolic, ethanolic, aqueous and infusion extracts of young and mature *Mangifera indica* and *Persea americana* leaves. Generally the results of this investigation showed that the ethanolic and methanolic extracts of young and mature

leaves had the best phenolic and flavonoid content. However, the young leaves of *Persea americana* and the mature leaves of *Mangifera indica* were shown to have the best DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power and Hydroxyl Radical Scavenging Activity. Amongst the aqueous extracts, hot water was more efficient as antioxidant than cold water. The young leaves

of *Persea americana* and mature leaves of *Mangifera indica* are good sources of natural antioxidants that can be used to prevent the body against the damages caused by free radicals. They can be used as medicine or food preservatives, especially in foods containing polyunsaturated fatty acids. For those who generally use these leaves as traditional medicine, it is better to do the extraction in hot water than cold water.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Ethics

This Study does not involve Human or Animal Testing.

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