



Research Paper

Comparative phytochemical analysis and antimicrobial activity of extracts of seed and leaf of *Persea americana* Mill.

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ABSTRACT

Persea americana is a fruit tree very well-known and appreciated in the Republic of Benin for its fruit but not for the pharmacological properties of its various organs. Sometimes the seeds contained in the fruits pose serious environmental problems. This study therefore focused on the phytochemical study and the determination of the antimicrobial properties of the ethanolic extracts of avocado seeds and leaves. The qualitative analysis of the ethanolic extracts of the seeds and leaves showed the presence of the major chemical groups, namely Reducing compound, Alkaloids, Flavonoids, Phenolic compounds, Leuco-anthocyanins, Saponins and Terpenoids. Free Anthracenics were present in the seeds and absent in the leaves. Quantitative analysis of 100 g of the extracts from the seeds and leaves showed that the seeds contain more phenolic compounds (7.68 g), alkaloids (2.14 g) and reducing compounds (1.72 g) than the leaves. Antimicrobial tests carried out on eleven bacterial strains indicated a larger inhibition diameter (25 mm) and a smaller minimum inhibitory concentration (0.3125 mg / ml) against three strains (*Micrococcus luteus*, *Streptococcus oralis*, *Escherichia coli* ATCC25922) with the seeds extract. The activity of the leaves was not negligible (minimum inhibitory concentration of 0.625 mg/ml against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Strept. oralis* and *Enterococcus faecalis* ATCC29212 strains). At the end of this study, the ethanolic extract of *P. americana* seeds remained more effective than the extract of the leaves, so subject to other tests, the seeds and leaves of *P. americana* could therefore contribute to antibacterial control.

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INTRODUCTION

The potential of higher plants being used for new drug is still largely unexplored. Plants are the most exclusive sources of drugs for the majority of the world as people in the developing countries use medicinal plants for their primary health care (Cowan, 1999; Adebayo-tayo and Adegoke, 2008; Maciel et al., 2002). The use of medicinal plants as sources of relief from illnesses can be traced back to several millennia (Hill, 1952; Eto, 2013). It is an art as old as mankind. Plants have the major advantage of still being used as the most effective and cheaper alternative

sources of drugs (Balandrin, 1985; Pacific Health Inform, 2005; Ilozue et al., 2014). Generally, plant parts (seeds, leaves, bark, fruits and stems) contain bioactive agents. These confer such plant parts with nutritive and antimicrobial properties which could contribute to the management of diseases. However, proper assessment on the possible dietary and therapeutic potentials of such plant parts are required for informed use in animals, including human (Egbonu, 2015, 2018). Several plants have been the focus of studies to scientifically prove their

many beneficial effects and promote better indication as an alternative therapy. *Persea americana*, known as avocado tree, is an evergreen tree belonging to Lauraceae family, native of Central America, and currently distributed in tropical and subtropical regions (Yasir et al., 2010).

The fruit of the plant is much loved by the population in Republic of Benin. So the plant is known and loved for its fruit. The leaves and seeds from the fruit are very little exploited by the population for their pharmacological properties. The seeds waste may represent a severe ecological problem in Republic of Benin. However, at the same time, it may be of interest to industry as a source of bioactive compounds. Beside, its biological activities such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, amoebicidal and giardicidal activities had been reported (Adeyemi et al., 2002; Ojewole and Amabeoku, 2006; Anaka, 2009; Alhassan et al., 2012; Chia et al., 2010; Adaramola et al., 2016; Adesina et al., 2016; Dennis and Wulandari, 2017; Kristanti et al., 2017; Sang et al., 2019). Literature had also showed numerous biological properties of the leaves of *P. americana* (Nahak et al., 2017; Kolawole et al., 2012; Egbuomwan and Inetianbor, 2017; Ogundare and Oladejo, 2014; Owusu Boadi et al., 2015). Separate studies on the phytochemical composition and antimicrobial properties of leaf and seed extracts have been carried out, but the simultaneous comparative study of the two organs of the plant remains unclear. This study therefore is a comparative study of the phytochemical composition and antimicrobial activities of ethanolic extracts from the leaves and seeds of *P. americana* in order to enhance the plant and more particularly the seeds.

MATERIALS AND METHODS

Extract preparation

Ethanol is a very little toxic solvent and according to some authors allows the extraction of maximum of chemical compounds (Umar et al., 2016). That is what motivated us to take an interest in the ethanolic extract of the two organs.

The leaves and fruits of the same tree were used during this experiment. The fresh leaves of *P. americana* were properly washed with distilled water to remove debris. The fruits were deseeded by removing the fleshy cover. The resultant seeds were washed with clean tap water, crushed into smaller pieces with the help of manual grater. Thereafter, the leaves and the seeds were air dried for 3 weeks under regular turning to enhance even drying. The dried leaves samples and seeds were separately grounded into fine powder using a mechanical grinder. The cold ethanol extraction was adopted for extraction.

50 g of powder of *P. americana* leaves or seed were crushed and recovered in 500 ml of ethanol at 96°C. After agitation and homogenization, the mixture is filtered on

Whatman paper and the filter is concentrated in a rotary evaporator at a temperature between 55 and 60°C with the help of vacuum pump to obtain the extract. The dry, watery triturated extract obtained was stored in a refrigerator at 4°C.

Phytochemical screening

The qualitative phytochemical screening was performed based on colouring or precipitation reactions. It is made directly on the ethanolic extract of *P. americana* leaves according to Houghton and Raman method (1998) and Simo et al. (2019). Quantitative phytochemical tests were carried out according to the method of Harborn (1973) and Umeaku et al. (2018).

Antimicrobial activity assessment methods

Eleven references strains such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* A24974, *Micrococcus luteus* ATCC10240, *Proteus vulgaris* A25015, *Streptococcus oralis*, *Enterococcus faecalis* ATCC 29212, *Salmonella typhi* R 30951401 and *Escherichia coli* O157 were used.

Sensitivity test

It was done according to the disc method inspired from the one described by Bauer et al. (1996). Briefly, 1 ml of pre-culture of 18-24 h (10^6 UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by flood. After seeding, the sterile Whatman paper discs (5 mm de diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 μ l of plant extract (20 mg/ml). The dishes were kept for 15-30 min at room temperature before incubation at 37°C. The inhibition zones diameters were measured after 24 to 48 h using a ruler graduated (Adesokan et al., 2007). For each extract, the experiment was performed induplicate.

Determination of the minimum inhibitory concentration (MIC)

The MIC has been determined using macrodilution method with Visual assessment of the growth of microorganisms (Delarras et al., 1998). Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 μ g/ml) were performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (106 UFC/ml). After 24 h of incubation, turbidity tubes were examined relative to the control tube containing

distilled water and the inoculum (10^6 UFC/ml).

Determination of the minimum bactericidal concentration (MBC)

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37°C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC (Farshori et al., 2013).

Data treatment and analysis

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data. Minitab (version 17) software was used for the variance analysis (ANOVA). Finally a structuring of the medium was made which allowed us to compare and identify the excerpt (s) most active on the various parameters through Student Newman and Keuls (SNK) test on the threshold of 5% of significance.

RESULTS AND DISCUSSION

Performance extraction

For 50 g of avocado seeds powder, 5.56 g of ethanolic extract were obtained. This extract was therefore obtained with a yield of 11.12%. That of the leaves was obtained with a yield of 9.15%. The extraction solvent being the same, it appears that the seeds have a higher extraction yield than that of the leaves.

Qualitative and quantitative composition of extracts

The phytochemical screening of the extracts of the leaf and the avocado seed qualitatively showed the presence of Reducing compound, Alkaloids, Flavonoids, phenolic compounds consisting of catechic and gallic tannins, Leuco-anthocyanins, Saponins and Terpenoids (Table 1). Free Anthracenics were present in the seeds and absent in the leaves. Other compounds such as Anthocyanins, Quinonics compound, Cartenoids, O-heterosides, Mucilages are not present in these extracts. The list of compounds present can vary depending on whether one is in the presence of another extract or even powder of the two substances. Quantitative analysis of 100 g of the ethanolic extract of the seed showed 7.68 g of Phenolic compounds, 5.33 g of Flavonoids, 2.14 g of Alkaloids and 1.72 g of reducing compounds. That of the leaf showed 5.38g of Phenolic compounds, 7.28 g of flavonoids, 1.98 g of alkaloids and 0.81 g of reducing compounds. Apart from the flavonoids,

the other compounds are higher in the seed than the leaf (Table 1). These values are slightly different from those observed in other studies (Omolaro et al., 2017; Egbuonu et al., 2018). This can be explained by the fact that the extracts studied are different, or even by the difference in certain natural parameters such as the nature of the soil, the climate, the season etc. (Kaiser et al., 2001).

The extracts inhibitory diameter zone with the reference strains

Figure 1 shows the inhibitory activity of extracts of seeds and leaves of *P. americana*. The sensitivity of the microbial strains tested varied significantly from one extract to another ($p < 0.001$). Of the two extracts, that obtained from *P. americana* seeds presented a very pronounced antagonistic effect by inhibiting the growth of more than 90% of the pathogenic strains (Figure 1). The highest inhibition diameter observed with this extract was (25 mm) against the *P. vulgaris* strain while the smallest inhibition diameter (0 mm) was obtained against the *S. typhi* strain (Figure 1). With the ethanolic extract of the leaf, the largest diameter of inhibition was (15.5 mm) obtained against the *E. coli* ATCC25922 strain. The smallest diameter observed was 0 mm, obtained against the *S. epidermidis* and *S. typhi* strains. The leaf extract inhibited approximately 80% of the microbial strains tested. In most cases, the diameters of inhibition of the extract of the seeds were greater than the diameters of inhibition of the extract of the leaves. The two cases where the opposite was noted were identified with *P. aeruginosa* ATCC 27853 and *M. luteus*. From these observations, it appears that the microbial strains are more sensitive to the ethanolic extract of *P. americana* seeds than to the extract of the leaves. This could be explained by the fact that apart from Flavonoids, the content of the other compounds is higher in the seeds than the leaves. In addition, the extract of the seeds contains an additional compound (Free Anthracenics) than that of the leaves. As compared with literature, the diameters of inhibitions obtained with seeds shows that they are very active on the strains tested (diameters around 25 mm) (Nounagon et al., 2017).

Minimum inhibitory concentrations (MIC) of *Persea americana* seeds and leaves extract

The two extracts inhibited the proliferation of most pathogenic bacteria with varying minimum inhibitory concentrations. According to the tests carried out, the smallest minimum inhibitory concentrations (MIC) (0.3125 mg/ml) was obtained with the extract of *P. americana* seeds against three strains, namely (*M. luteus*, *S. oralis*, *E. coli* ATCC25922). The highest MIC of 2.5 mg / ml was obtained against the *S. epidermidis* T22695 strain. The lowest

Table 1: Phytochemical constituents of *P. americana* leaf and seed (mg/100g).

Compounds	ES/QLA	ES/QNA	EL/QLA	EL/QNA
Reducing compound	+	1.72 ± 0.019	+	0.81 ± 0.028
Alkaloids	+	2.14 ± 0.012	+	1.98 ± 0.007
Flavonoids	+	5.33 ± 0.064	+	7.28 ± 0.054
Tanins catechic	+		+	
Tanins gallic	+	7.68 ± 0.027	+	5.38 ± 0.01
Anthocyanins	-		-	
Leuco-anthocyanins	+		+	
Quinonics compound	-		-	
Saponin	+		+	
Coumarin	+		+	
Terpenoids	+		+	
Mucilages	-		-	
Cartenoids	-		-	
Free Anthracenics	+		-	
O-heterosides	-		-	

(+) = Presence; (-) = Absence; **ES/QLA**: seeds extract/Qualitative analysis ; **ES/QNA** : Seeds extract/Quantitative analysis ; **EL/QLA** : leaves extract/Qualitative analysis ; **EL/QNA** : leaves extract/Quantitative analysis.

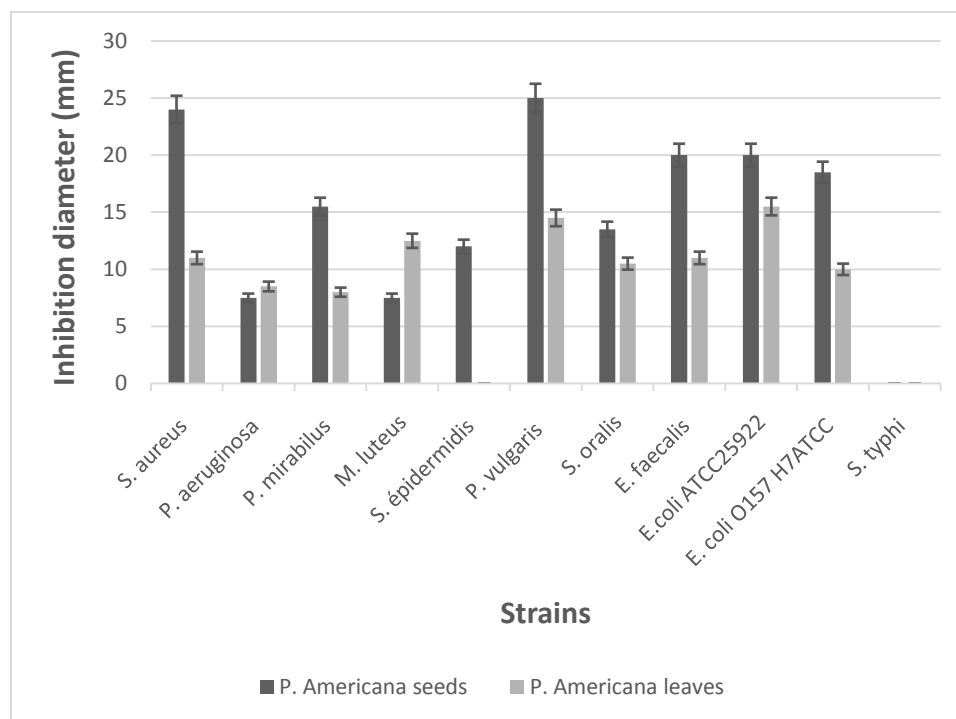


Figure 1: Diameters of inhibition of *Persea americana* seeds and leaves extracts.

minimum inhibitory concentration obtained with the extract of the leaves is 0.625 mg / ml against four strains (*S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *S. oralis*, *E. faecalis* ATCC29212 (Table 2). The highest MIC of 1.25 mg/ml was observed in the others cases. In most cases, the minimum inhibition concentrations of the extract of the seeds remain lower. The opposite trend was observed in

two cases (*Staph. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853). These two strains could be more sensitive to Flavonoids than to other quantified chemical compounds since Flavonoids are higher in the extract of the leaves. The determination of the inhibitory concentrations confirms the observations noted above. The inhibitory activities observed could be due to the presence of the chemical

Table 2: Minimum inhibitory concentrations (mg/ml) of the extracts on the studied reference strains.

STRAINS	<i>S. aureus</i> ATCC 29213	<i>P. aeruginosa</i> ATCC 27853	<i>P. mirabilis</i> A24974	<i>M. luteus</i>	<i>S. épidermidis</i> T22695	<i>P. vulgaris</i> A25015	<i>S. oralis</i>	<i>E. faecalis</i> ATCC29212	<i>E. coli</i> ATCC25922	<i>E. coli</i> O157 H7ATCC	<i>S. typhi</i> R 30951401
EL	0.625	0.625	1.25	1.25	0	1.25	0.625	0.625	1.25	1.25	0
ES	1.25	1.25	1.25	0.3125	2.5	0.625	0.3125	0.625	0.3125	1.25	0

ES : seeds extract; EL: leaves extract.

Table 3: Minimum Bactericidal Concentrations (mg/ml) of extracts with reference strains.

STRAINS	<i>S. aureus</i> ATCC 29213	<i>P. aeruginosa</i> ATCC 27853	<i>P. mirabilis</i> A24974	<i>M. luteus</i>	<i>S. épidermidis</i> T22695	<i>P. vulgaris</i> A25015	<i>S. oralis</i>	<i>E. faecalis</i> ATCC29212	<i>E. coli</i> ATCC25922	<i>E. coli</i> O157 H7ATCC	<i>S. typhi</i> R 30951401
EL	0	0	10	0	0	0	0	1.25	0	0	0
ES	0	0	0	0	10	0	0	1.25	0	0	0

ES : seeds extract; EL: leaves extract.

groups present in the two extracts. The antimicrobial properties of these different groups were event described in the literature (Ngoupayo et al., 2016; Karou et al., 2006; Cushnie et al., 2005). Absolutely, the lower concentrations observed with seeds are due to the higher levels of phenolic compounds, alkaloids and reducing compounds. The determination of the inhibitory concentrations confirms that the seeds are more active than the leaves on the strains of microbes tested.

Minimum bactericidal concentration (MBC) (mg/ml) *Persea americana* seeds and leaves extracts

Regarding the Minimum Bactericidal Concentration, the two extracts had the same effect on two different bacterial strains (Table 3). The smallest concentration noted in the two extracts was 1.25 mg/ml against the same strain *E. faecalis* ATCC29212. The highest concentration was 10

mg/ml for the two extracts. This concentration was obtained against *P. mirabilis* A24974 strain with the extract of the seeds and against *S. epidermidis* T22695 strain with the extract of the leaves. These results are in line with those obtained on the inhibition tests, because the two extracts have the same inhibitory effect on the strain *E. faecalis* ATCC29212 (Table 3). These extracts also inhibited other microbes on which the bactericidal power has been noted. At the end of these observations, the bactericidal power of these two extracts is almost the same and is generally weaker than the inhibitory power. However, these two extracts prove to be effective against *E. faecalis* ATCC29212.

CONCLUSION

Based on the findings of this study conducted on seeds and leaves of *P. Americana*, it appears that the seeds and leaves

are made up of large chemical groups such as the Reducing compound, the Alkaloids, the Flavonoids, and the phenolic compounds containing catechic tannins and gallic, Leucoanthocyanins, Saponins and Terpenoids. A comparative study of antimicrobial testing of seeds and leaves extract found that seeds are more active than leaves. Subject to other tests, avocado seeds could change from their status of environmental pollutants to the status of resources of molecules useful in the antibacterial fight in phytotherapy.

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