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Antioxidant and anti-gastroenteritis activities of *Funtumia elastica* (Apocynaceae) and *Caesalpinia bonduc* (Caesalpinaceae)

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ABSTRACT

Oxidative stress and infectious diseases are health problems on a global scale. The aim of this study was to evaluate the antioxidant and anti-gastroenteritis activities of hydroethanolic extracts of leaves of *Funtumia elastica* and *Caesalpinia bonduc*. After obtaining the hydroethanolic extracts, the phytochemical tests were carried out. The antioxidant activity was evaluated by different assays using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation inhibition (FTC and TBARS methods). The agar well diffusion method was used to determine the antibacterial activity to the plant extract. The triphytochemical study has revealed that these plants extracts contain a diversity of secondary metabolites. *F. elastica* gave a good anti-radical activity ($IC_{50} = 36 \mu\text{g/ml}$). *F. elastica* recorded the highest rate of inhibition of lipid peroxidation with 63% (FTC method) and 65% (TBARS method). *C. bonduc* extract (200 mg/ml) inhibited the *in vitro* growth of all the bacterial strains studied in this study with inhibition diameters that evolved between 9 mm and 13 mm. These plant extracts could be used if they are improved for the treatment of gastroenteritis related to *Shigella* and *Escherichia coli* and in the care of pathologies related to oxidative stress.

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Keywords: Antioxidant, anti-gastroenteritis, *Funtumia elastica*, *Caesalpinia bonduc*, hydroethanolic extracts.

INTRODUCTION

Oxygen reactive species (ORS) are involved in physiological processes at low levels. However, excess production of ORS can become toxic to major cell components, lipids, proteins and nucleic acids. However, cells use many antioxidant strategies to eliminate or minimize oxidative damage. Depending on the type, antioxidants may act by reducing or mutating ORS, trapping them

to form a stable compound, sequestering free transition metals, or generating antioxidant biological molecules of interest (Carole, 2008). Under certain conditions, these antioxidant systems cannot function effectively. The resulting dysfunction can be manipulated by supplementation with exogenous dietary antioxidants, either natural or synthetic. However, the use of these is restricted because of adverse effects on human

health (Tadhani et al., 2007). In addition, the control of bacterial infections becomes complex because many bacteria have developed resistance to most antibiotics, which is a public health problem on a global scale (Fernique et al., 2013). These bacterial infections include gastroenteritis caused by various pathogenic microorganisms (Amieva, 2005). It is estimated that 800,000 people worldwide die from gastroenteritis each year, including 500,000 children under five (Parashar et al., 2006). In view of all the above, the fight against oxidative stress and bacterial infections remain a major concern for the scientific world. The use of synthetic molecules against these evils has shown limits, it is imperative to direct the search for antidotes to the plant world with the hope of discovering new bioactive molecules. It is in this context that the present work was initiated with the aim of evaluating the antioxidant and anti-gastroenteritis activities of the hydroethanolic extracts of the leaves of *Funtumia elastica* and *Caesalpinia bonduc*, two plants of the Ivorian pharmacopoeia.

MATERIAL AND METHODS

Plant material

The leaves of *Funtumia elastica* and *Caesalpinia bonduc* were harvested in the forest region of Central West, in the Division of Daloa (Côte d'Ivoire) in January 2019. The plants were identified by the botanical laboratory of Jean Lorougnon University. Guede of Daloa.

Bacterial strains

Pathogenic bacterial strains responsible for gastroenteritis were used in this study. These are *Escherichia coli* 422, *Escherichia coli* 427, *Shigella* EEG and *Shigella* 1055. These clinical strains were provided by the bacteriology and virology department of the Regional Hospital Center of Daloa (Côte d'Ivoire).

Preparation of hydroethanolic extracts

The harvested leaves were washed, cut, dried out of the sun for two weeks (Figures 1 and 2) and made into powders using a

mechanical grinder type IKAMAG. These vegetable powders were used for the preparation of the various hydroethanol extracts. Thus, 100 g of vegetable powder of each plant was macerated in 1 L of an ethanol-water solvent (70:30, v / v) and then homogenized with magnetic stirring for 24 hours at 25 °C, using a magnetic stirrer type IKAR. The homogenate obtained was filtered successively twice on hydrophilic cotton and then once on Whatman n°2 paper. The filtrate was evaporated at reduced pressure at 60 °C, using a rotary evaporator and concentrated in an oven at 50 °C. The final powder obtained from each plant constituted the 70% hydroethanolic extract and was used for biological tests.

Phytochemical screening of different hydroethanolic extracts

The various chemical groups (alkaloids, total polyphenols, tannins, flavonoids, saponins, cardiotonic glycosides and sterols and polyterpenes) contained in the different extracts were identified by precipitation and tube-coloring tests (Bagre et al., 2007; Touré et al., 2011).

Total polyphenol content of hydroethanolic extracts

The method of Wood et al. (2002) was used for the determination of total polyphenols: a volume of 2.5 ml of Folin-Ciocalteu reagent diluted to the tenth was added to 30 µl of plant extract. The mixture was kept for 2 minutes in the dark at room temperature, then 2 ml of sodium carbonate solution (75 g/l) was added. Then, the mixture was placed for 15 minutes in a water bath at 50 °C and then rapidly cooled. Absorbance was measured at 760 nm, with distilled water as white. A calibration line was made with gallic acid at different concentrations. The analyses were carried out in triplicate, and the concentration of polyphenols was expressed in milligram gallic acid equivalent per gram of extract (mg EAG/g of plant extract).

Flavonoids content of hydroethanolic extracts

The flavonoid assay was performed according to the method described by Marinova et al. (2005). Indeed, in a 25 ml flask; 0.75 ml of 5% (w/v) sodium nitrite (NaNO_2) was added to 2.5 ml of extract. To the mixture, 0.75 ml of 10% (w/v) aluminum chloride (AlCl_3) was added and the whole was incubated for 6 minutes in the dark. Once this time had elapsed, 5 ml of sodium hydroxide (1N NaOH) was added and the volume was made up to 25 ml. The preparation was vigorously shaken before the determination of total flavonoids at 510 nm with the UV-visible spectrophotometer. The content of total flavonoids was expressed in milligram equivalent quercetin per gram of extract (mg EQ/g extract). Trials were conducted in triplicate.

Antioxidant activities of hydroethanolic extracts

2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The measurement of the anti-radical activity of the plant extracts was carried out by the 2,2'-diphenyl-1-picrylhydrazyl test (DPPH) according to the method of Parejo et al. (2000) with some modifications. A range of concentrations (0-1000 $\mu\text{g/ml}$) of plant extract or vitamin C (reference antioxidant) had been prepared. A volume of 2.5 ml of this solution was mixed with 2.5 ml of DPPH (100 μM) prepared in methanol. After homogenization, the mixture was incubated at room temperature (25 $^{\circ}\text{C}$), protected from light. After 15 minutes of incubation, the absorbance was read at 517 nm against a "white" which contains only methanol. The percentage inhibition of the DPPH radical was calculated according to the following equation:

$$\text{Inhibition DPPH (\%)} = (1 - (\text{DO}_{\text{trial}} / \text{DO}_{\text{white test}})) \times 100$$

where, DO_{trial} is the absorbance of the extract, $\text{DO}_{\text{white test}}$ is the absorbance of control test (no extract). All the tests were performed in triplicates ($n=3$).

The IC_{50} which is the concentration of the extract or vitamin C responsible for 50% inhibition of the DPPH radicals. It was determined on the graph representing the percentage inhibition of DPPH as a function of the concentrations of the extracts and vitamin C.

Inhibition of lipid peroxidation by the ferric thiocyanate (FTC) method

The antioxidant activity of plant extracts was determined by inhibiting the peroxidation of linoleic acid using the ferric thiocyanate method, according to the method described by Takao et al. (1994); the reaction mixture containing respectively 0.4 ml of extract (100 $\mu\text{g/ml}$), 0.4 ml of linoleic acid (2.52% in absolute ethanol) and 0.8 ml of phosphate buffer (pH 7.4) was incubated in a water bath for 1 hour at 40 $^{\circ}\text{C}$. An aliquot (0.1 ml) of this solution was then added to the mixture consisting of 5 ml of 70% ethanol and 0.1 ml of ammonium thiocyanate (30%). After 3 minutes, 0.1 ml of FeCl_2 prepared in 3.5% HCl (20 mM) was added to the reaction medium. A white test was carried out by replacing the extracts with distilled water. The absorbance of the red color resulting from the solution was read for 7 days at 500 nm in the spectrophotometer every 24 hours until the absorbance of the negative control (distilled water) reached its maximum. Vitamin C was used as a reference. The percent inhibition of lipid peroxidation was then calculated according to the following equation:

$$\text{Inhibition (\%)} = (1 - (\text{DO}_{\text{trial}} / \text{DO}_{\text{white test}})) \times 100$$

where, DO_{trial} is the absorbance of the extract, $\text{DO}_{\text{white test}}$ is the absorbance of control test (no extract). All the tests were performed in triplicates ($n=3$).

Inhibition of lipid peroxidation by the TBARS method

The method of Choi et al. (2002) using an induction of lipid peroxidation by the ascorbic acid/iron sulfate pair (Fenton reaction) was adapted for this test. 600 μl of plant extracts were added respectively 300 μl of Tris-HCl buffer solution (pH 7.5, 20 mM), 500 μl of linoleic acid (20 mM) and 100 μl of iron sulfate (4 mM). Peroxidation begins

after addition of 100 µl of ascorbic acid (5 mM). The reaction mixture obtained was incubated in a water bath at 37 °C, for 60 minutes. After this step, 2 ml of TCA (10%) were added to all the tubes. Then, to 1 ml of aliquot collected in each of the reaction mixtures prepared previously, was added 1 ml of TBA (1%). The reaction mixtures obtained were placed in a bath boiling at 95 °C for 20 minutes. Vitamin C was used as a reference molecule. A white test was performed by replacing the extract with distilled water. The absorbance was read spectrophotometer at 532 nm and the percent inhibition of linoleic acid was determined according to the following equation:

$$\text{Inhibition (\%)} = (1 - (\text{DO trial} / \text{DO white test})) \times 100$$

where, **DO** trial is the absorbance of the extract, **DO** white test is the absorbance of control test (no extract). All the tests were performed in triplicates (n=3).

Antibacterial activities

Antibacterial tests were performed using the solid medium diffusion method (agar) described by Kuete et al. (2004) and Bssaibis et al. (2009). To do this, a solution of hydroethanolic extract of concentration 200 mg/ml was prepared. Petri dishes containing

Müeller -Hinton agar were inoculated by flooding with the prepared bacterial inoculum. Then, 6 mm diameter wells were dug by pressing the large end of a Pasteur pipette into the agar. These wells were then filled with 50 µl of the hydroethanolic extract solution (200 mg/ml) to be tested. The whole was incubated at 37 °C for 24 h. After this time, the inhibition diameter around each well was measured. The sensitivity of the bacterial strains to each extract was assessed according to the criterion of Bssaibis et al. (2009). Thus, a bacterium is said to be resistant if the inhibition diameter is less than or equal to 8 mm. On the contrary, it is said to be sensitive, if the diameter is between 9 and 14 mm and very sensitive when the diameter is between 15 and 19 mm and then extremely sensitive if the diameter is greater than or equal to 20 mm (Bssaibis et al., 2009).

Statistical treatment

The data were presented as mean ± standard deviation. All data were analyzed by unidirectional ANOVA and the differences between the means were evaluated using Neuman-Keuls multiple comparison tests. Differences were considered significant at $p < 0.05$. All analyzes were performed using the Graph Pad software, version 5.01 (USA).



Figure 1 : Dried leaves of *Funtumia elastic*.



Figure 2: Dried leaves of *Caesalpinia bonduc*.

RESULTS

Extraction yields for different plant extracts

The extraction yield of *F. elastica* leaves was 11.56%. As for that of *C. bouduc*, it was 13.58%. The two hydroethanolic extracts obtained have presented the same appearance (pasty) with different colors (Table 1).

Triphytochemistry of different hydroethanolic extracts

The phytochemical screening of the various extracts of *F. elastica* and *C. bouduc* showed the presence of phenolic compounds (tannins, flavonoids), saponins and alkaloids in the leaves of both plants. However, the identified bioactive compounds were differently distributed in the different hydroethanolic extracts of each plant. The phytochemical tests make it possible to affirm that there are more phenolic compounds in the two extracts which were the object of study. Furthermore, this triphytochemical study showed that the extract of *F. elastica* contains alkaloids, cardiotonic glycosides and sterols and polyterpenes in small amounts unlike the *C. bouduc* extract. However, the hydroethanolic extract of *C. bouduc* remains that which contained the saponins in very large quantities (Table 2).

Phenolic compounds contents of the different hydroethanolic extracts

Table 3 showed a significant difference ($p < 0.05$) between the contents of phenolic compounds of the various hydroethanol extracts studied. This study showed that *F. elastica* has a total phenol content of 72.35 ± 1.67 mgEAG whereas *C. bouduc* has a content of 59.11 ± 1.89 mgEAG. Moreover, the same observation has been recorded with the flavonoid contents. In fact, the flavonoid content of *F. elastica* was 28.11 ± 2.71 mgEQ while that of *C. bouduc* was 13.45 ± 2.82 mgEQ.

Antioxidant activities of different hydroethanolic extracts

DPPH Radical Scavenging Activity

The results of the antiradical activity of the hydroethanol extracts of the two plants

studied as well as vitamin C were presented in Figure 3. Vitamin C has the strongest antiradical activity ($IC_{50} = 16 \mu\text{g/ml}$). This reference molecule (Vitamin C) is followed by the hydroethanolic extract of *F. elastica*, which has a good antiradical activity ($IC_{50} = 36 \mu\text{g/ml}$) compared to *C. bouduc* extract ($IC_{50} = 52 \mu\text{g/ml}$). Table 4 showed a significant difference ($p < 0.05$) between the antiradical activities of extracts.

Lipid peroxidation by the FTC method

Figure 4 revealed a significant difference ($p < 0.05$) between the activities of extracts in this method. The results indicate that Vitamin C inhibited lipid peroxidation with a high level of 80%. Moreover, the respective inhibition rates of 63% and 40% were obtained respectively with the extracts of *F. elastica* and *C. bouduc*.

Lipid peroxidation by the TBARS method

By this method, the results also showed that Vitamin C recorded the inhibition rate of 85%. The extract of *F. elastica* showed an inhibition rate of 65% against 43% for *C. bouduc*. Figure 5 showed that there was a significant difference ($p < 0.05$) between the activities of extracts.

Antibacterial activities of different hydroethanolic extracts

Table 5 showed the inhibition diameters (mm) on bacterial germs tested at a concentration of 200 g/ml (extract). There was a significant difference ($p < 0.05$) in the activity of the two extracts on each of the bacteria studied. Extract of *F. elastica* strongly inhibited the growth of bacterial strains of *Shigella* (*Shigella* EEG, *Shigella* 1055). On these strains, this extract gave average inhibition diameters of 25 mm. However, this same extract of *F. elastica* had no effect on *Escherichia coli* strains (*E. coli* 422, *E. coli* 427). As for the *C. bouduc* extract, it inhibited the *in vitro* growth of all the bacterial strains that were the subject of this study. For this extract, the diameters of the zones of inhibition on the strains of *Escherichia coli* 422 and 427 were respectively 13 mm and 9 mm. However, on both strains of *Shigella* (EEG; 1055), *C. bouduc* extract induced mean inhibition zone diameters of 12 mm.

Table 1: Extraction yield and appearance of different extracts.

Plante extracts	Extraction yield	Appearance/Color
<i>F. elastica</i>	11.56%	Pasty/brown
<i>C. bouduc</i>	13.58%	Pasty/green

Table 2: Phytochemical screening of different hydroethanolic extracts.

Chemical constituents	Hydroéthanolic extracts of différent plants	
	<i>F. elastica</i>	<i>C. bouduc</i>
Alkaloids	+	-
Total polyphenols	+++	+++
Tannins catechic	-	-
Tannins gallic	++	+
Flavonoids	++	++
Saponins	+	+++
Cardiotonic glycosides	+	-
Sterols and polyterpenes	+	-

-: absence, +: low presence, ++: average presence, +++: very strong presence

Table 3: Content of phenolic compound of the different hydroethanolic extracts.

Extracts of plants	Total phenol content (mgEAG)	Flavonoids content (mgEQ)
<i>F. elastica</i>	72.35 ± 1.67 ^a	28.11 ± 2.71 ^a
<i>C. bouduc</i>	59.11 ± 1.89 ^b	13.45 ± 2.82 ^b

Averages are expressed with standard deviations (±). Values with the different letter by exponent in each of the columns are significantly different (p < 0.05).

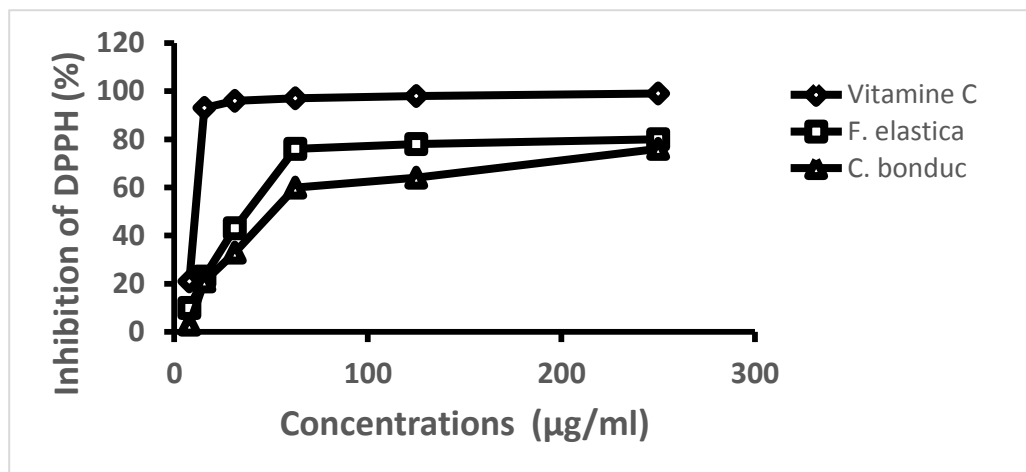
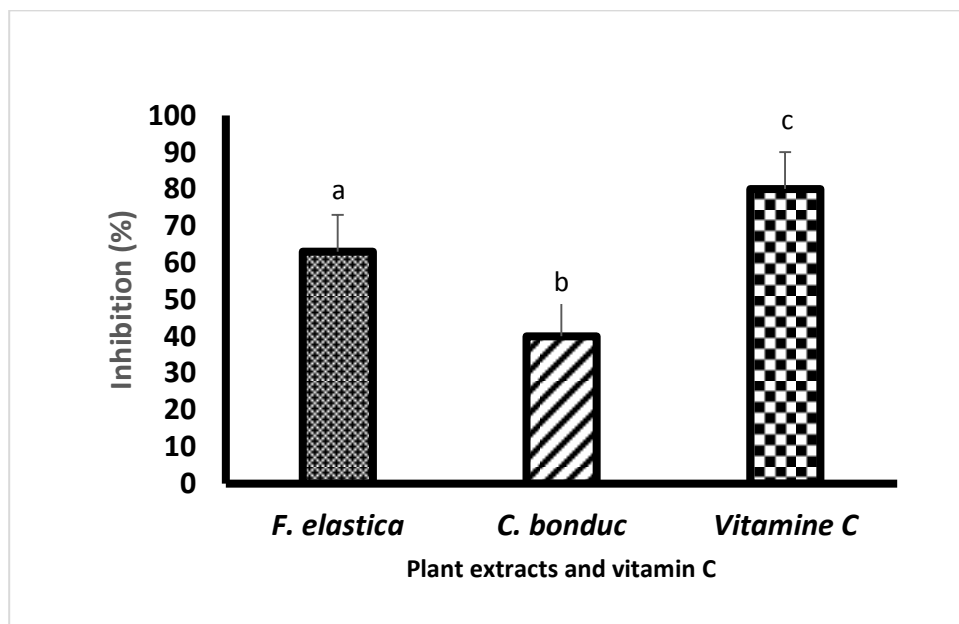


Figure 3: Evolution of the antiradical activity of hydroethanolic extracts of plants studied.

Table 4: IC₅₀ values of vitamin C and different hydroethanolic extracts studied.

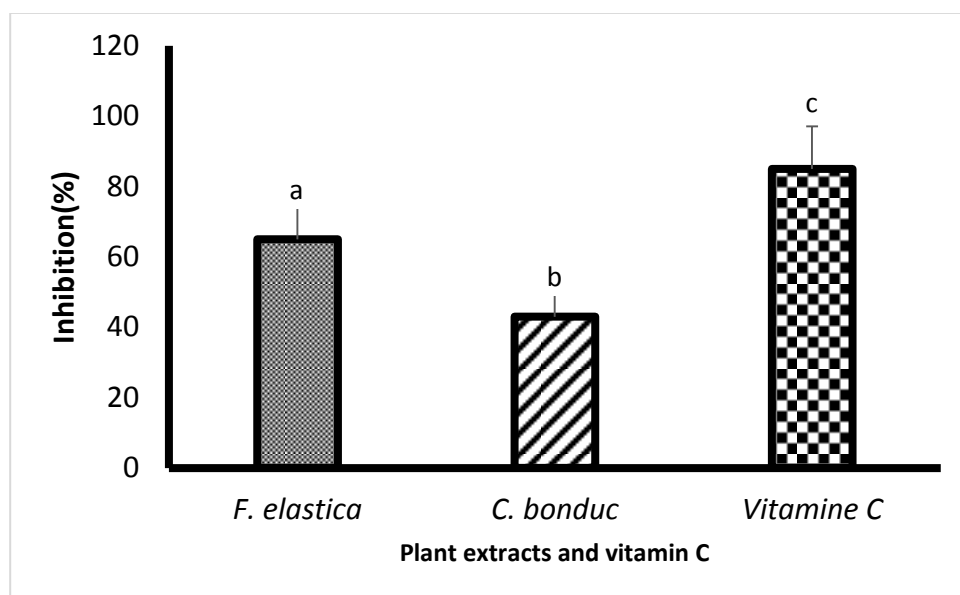
Extracts/reference molecule	IC ₅₀ (µg/ml)
<i>F. elastica</i>	36 ± 0.20 ^a µg/ml
<i>C. bouduc</i>	52 ± 0.55 ^b µg/ml
Vitamin C	16 ± 0.32 ^c µg/ml

Averages are expressed with standard deviations (±). Values with the different letter by exponent in each of the columns are significantly different (p < 0.05).



Averages are expressed with standard deviations (±) and are significantly different (p < 0.05).

Figure 4: Inhibition of lipid peroxidation by the FTC method.



Averages are expressed with standard deviations (\pm) and are significantly different ($p < 0.05$).

Figure 5: Inhibition of lipid peroxidation by the TBARS method.

Table 5: Inhibition diameters (mm) of the different hydroethanolic extracts.

Hydroéthanolic extracts (200 mg/ml)	Inhibition diameters (mm) to 200 mg/ml			
	<i>Shigella</i> EEG	<i>Shigella</i> 1055	<i>E. coli</i> 422	<i>E. coli</i> 427
<i>F. elastica</i>	25 \pm 1 ^a	24 \pm 1 ^a	0 ^a	0 ^a
<i>C. bonduc</i>	12 \pm 1 ^b	12 \pm 1 ^b	13 \pm 1 ^b	9 \pm 1 ^b

Averages are expressed with standard deviations (\pm). Values with the different letter by exponent in each of the columns are significantly different ($p < 0.05$).

DISCUSSION

The yield of the hydroethanolic extract of leaves of *Funtumia elastica* was 11.56% against 13.58% for leaves of *Caesalpinia bonduc*. These results show that the studied leaves contain chemical substances soluble in the ethanol-water solvent (70: 30, v/v). The higher yield of *Caesalpinia bonduc* (13.58%), unlike that of *Funtumia elastica*, can be explained by the fact that the extractable substances of *Caesalpinia bonduc* have more affinity for the ethanol-water binary solvent (70: 30, V/V) than those of *Funtumia elastica*.

The phytochemical analysis of the two extracts studied reveals that they contain

practically the same substances, with the exception that the extract of *F. elastica* additionally contains alkaloids, cardiotonic glycosides and sterols and polyterpenes. In the present study, the hydroethanolic extract of *C. bonduc* does not contain alkaloids and catechic tannins. However, the work of Adedapo et al. (2017) has shown that the aqueous extract of this same plant contains alkaloids and catechic tannins. This observation can be attributed to the choice of solvent used. Moreover, the method of study, the place of harvest and the nature of the organ used can be at the origin of this difference (Gisèle et al., 2018). However, the

chemical composition of the *Funtumia Elastica* extract confirms the results of Gnagne et al. (2017) who showed the presence of these same chemical groups.

The determination of the phenolic compounds shows that the hydroethanolic extract of *F. elastica* is richer in phenolic compounds than that of *C. bonduc*. The extract of *F. elastica* also has the most interesting antioxidant activities compared to *C. bonduc*. According to N'guessan et al (2007) and Ernest et al. (2018) there is a correlation between total phenol levels and antiradical activity. The phenolic compounds present in plants are natural antioxidants which are according to Min and Ebeler (2008) compounds capable of interfering with the free radicals permanently generated by the human organism.

The antioxidant activity of our plants is therefore linked to their high content of phenolic compounds (Ernest et al., 2018). These results are also consistent with the work of Adedapo et al. (2008). According to these authors, plants with good antioxidant activity contain high levels of phenolic groups. Moreover, in the present study, the IC_{50} recorded with the *C. bonduc* extract remains lower than that obtained by Elin et al. (2018) which was 79.80 $\mu\text{g/ml}$ for the leaves of the same plant. According to these authors, the roots and barks of *C. bonduc* gave IC_{50} of 135.77 $\mu\text{g/ml}$ and 169.92 $\mu\text{g/ml}$, respectively. Also, the work done by Sivasankari et al. (2011) on *C. bonduc* leaves gave an IC_{50} greater than 40 $\mu\text{g/ml}$ with the methanolic extract. Several reasons could explain the differences observed between these results, in particular the origin of the plant, the organ used, the methodology and the reagents used. However, the antioxidant activity of these two hydroethanolic extracts remains lower than that of vitamin C taken as the reference molecule. Indeed, vitamin C is the predominant antioxidant in the skin and has both intracellular and extracellular antioxidant potency (Zussman et al., 2010). Being water-soluble, vitamin C neutralizes free radicals in the electron compartments of the cell, as an electron donor, and protects intracellular structures from oxidative stress (Martini and Seiller, 2006). This justifies its use in our

study as a reference molecule. Also, the best activity of vitamin C compared to hydroethanolic extracts studied can be explained by its purity. Indeed, the more the molecule is purified, the more it has a good biological activity which is not the case of plant extracts studied. These extracts generally contain many impurities that can prevent the pharmacological action of the chemical groups they contain.

The antibacterial activity of the various extracts varies from one extract to another and from one bacterial strain to another. Indeed, the extract of *F. elastica* inhibits the growth of strains of *Shigella* (*Shigella* EEG and *Shigella* 1055), however, no inhibition is observed on *E. coli* strains (*E. coli* 422 and *E. coli* 427). As for the *C. bonduc* extract, it has inhibitory activity on the four strains. In addition, it appears from the results that among the two extracts studied, that of *F. elastica* is more active on *Shigella* sp. In fact, the inhibition diameter of this plant on *Shigella* strains is double that of *C. bonduc* for each bacterial strain. However, according to Fernique et al. (2013), the *Shigella* strains studied would be extremely sensitive to the hydroethanolic extract of *F. elastica*. If we stick to these same authors, the four strains of the present study remain sensitive to the hydroethanolic extract of *C. bonduc*. These hydroethanolic extracts could therefore be used in the treatment of diseases related to these bacterial strains, in particular gastroenteritis. The antibacterial activities of these hydroethanolic extracts are due to the presence of the chemical groups they contain whose antimicrobial properties have already been demonstrated by several authors (Irene et al., 2012; Innocent and Chinelo, 2018).

Conclusion

It emerges from this study that the various crude extracts tested have interesting antioxidant activities that would be dependent on the contents of phenolic compounds. The hydroethanolic extract from *Funtumia elastica*, the richest extract of total polyphenols and flavonoids was the most active. However, the hydroethanolic extract from *Caesalpinia bonduc* showed antibacterial activity on the pathological

strains of *Shigella sp* and *Escherichia coli* tested. As for the hydroethanolic extract derived from *Funtumia elastica*, it was active only on clinical strains of *Shigella sp*. These plant extracts could be used if they are improved for the treatment of gastroenteritis related to *Shigella* and *Escherichia coli* and in the care of pathologies related to oxidative stress. In perspective, it would be important to characterize the chemical molecules responsible for these antioxidant and antibacterial effects.

COMPETING INTEREST

No competing interest associated with this work.

AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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