

Phenolic Profile and Antioxidant, Anti-Inflammatory Activity of *Annona senegalensis*, *Ipomoea batatas*, *Terminalia superba* and *Psidium guajava* Linn Extracts Used in Benin

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Abstract

The purpose of this study is to quantify the polyphenolic content and evaluated anti-inflammatory and antioxidant activity of four plants extracts of *Annona senegalensis*, *Ipomoea batatas*, *Terminalia superba* and *Psidium guajava*. Phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminum trichloride methods, respectively with ethanolic extract. The antioxidant activity was accessed by DPPH and FRAP methods. The results of the analysis of this study indicate that the ethanol extracts of the test plants (*A. senegalensis*, *I. batatas*, *P. guajava* and *T. superba*) are potential source of natural antioxidant and anti-inflammatory.

Keywords

Antioxidant, *In Vivo* Anti-Inflammatory, Polyphenolic, Ethanol Extracts

1. Introduction

Plants represent an inexhaustible and renewable source of bioactive compounds (alkaloids, saponosides, flavonoids...), whose traditional and medical use has been utilized by Men since antiquity [1] [2]. The latter are of increasing interest to

chemists, biologists, physicians and others because of their less toxic and use in the treatment of diseases against which synthetic drugs are sometime ineffective [3] [4]. Antioxidants are molecules that neutralize free radicals, molecules that are unstable and can damage the cell. Antioxidants are molecules that can slow, inhibit, or prevent the oxidation process, eliminating free radicals, molecules that are unstable and can damage the cell and decreasing oxidative stress [5]. Several studies have shown that some compounds of plant extracts including polyphenols exerted an antioxidant and anti-inflammatory properties [6] [7]. Phenolic compound are natural antioxidants as they retard the progress of many diseases, including cancer, cardiovascular and neurodegenerative diseases by protecting the body from free radicals [8]. Biological activities of extracts used have been attributed to secondary metabolic compounds, mainly to phenolic compounds [9] [10] [11] [12] [13]. Previous work revealed antioxidant and anti-inflammatory activities in the plants used [10] [11] [14] [15]. Synthetic antioxidant such as benzoic acid, BHA (Butylated Hydroxy Anisol) or BHT (Butylated Hydroxy Toluene) may cause side effect for our health. They may cause tumour for animal if it used in the long-term and harm the liver when over consumed [16]. Therefore, it important to research natural antioxidants which are able to decrease free-radicals in the body. The evaluation of antioxidant and anti-inflammatory potentials is, however, a crucial. In this study, the objectives were to determine the content of phenolic (total phenols and flavonoids). In addition, antioxidant activities using two methods (FRAP and DPPH) and *in vivo* anti-inflammatory were evaluated in four ethanolic extracts grown in Benin.

2. Material and Methods

2.1. Plant Material

The leaves of *Annona senegalensis*, *Ipomoea batatas*, *Terminalia superba* and the Bark of *Psidium guajava* plants were collected from Abomey-Calavi district in Benin. These plant parts were authenticated respectively under vouchers numbers YH626/HNB, YH624/HNB, YH625/HNB by Benin national herbarium, University of Abomey-Calavi, Benin.

2.2. Plant Extract Preparation

These four plants were dried at room temperature for two weeks and ground to fine powder. The powder (50 g) was macerated for 72 h under continuous stirring in 500 ml of ethanol. The macerates were then filtered 3 times with cotton wool and once with Whatman paper No. 1 (Qualitative Circles 150 mm Cat No 1001 150). The filtrates obtained were then evaporated using a rotary evaporator (IKA HB10S40, Germany) under reduced pressure. The concentrated was dried at 40°C in the oven until complete evaporation. The residues obtained after drying constitute the ethanolic extracts derived from each plant and were used for the study of different biological activities. The yield of the crude ethanolic extract of each plant was determined by the ratio of the mass of the dry extract obtained to the mass of the treated plant material.

2.3. Total Phenolic Contents

Total phenolic content in the plant extracts was determined by the Folin-Ciocalteu method [17] with some modifications. Briefly, 125 μL of Folin-Ciocalteu reagent (25%) was mixed with 50 μL of plant extracts (5 mg/mL) in water. After 5 min of incubation, 125 μL of Na_2CO_3 solution (20%), 700 μL of water was added. The absorbance was measured at 760 nm against blank without plant extract after incubation in the dark for 1 hour. Gallic acid was used as a reference substance. Total phenolic content was calculated from the calibration curve of gallic acid and expressed as gallic acid equivalents in milligrams per gram of the sample (mg/g of dry mass) and calculated as mean value \pm SD ($n = 3$).

2.4. Determination of Total Flavonoid

Total flavonoid content in the four plant extracts was determined by Aluminium chloride method described by [18] using quercetin as standard. Briefly, 500 μL of 2% Aluminium chloride solution was mixed with 500 μL of plant extracts (6.15 mg/ml). After 15 minutes incubation at room temperature, the absorbance of sample was read at 415 nm against the blank (mixture of 500 μL ethanolic extract solution and 500 μL of methanol) using a spectrophotometer. This experiment was repeated three times for precision and values were expressed in mean \pm standard deviation. Total flavonoid content was calculated from calibration curve of quercetin and expressed as quercetin equivalents (mg/g of dry mass).

2.5. Anti-Inflammatory Activity

2.5.1. Animals and Condition

The animal material used was Wistar rats of EOPS (Exempt from Specific Pathogenic Organisms) health status, approximately eight weeks old and weighing between 150 g and 200 g. The animals were housed in polypropylene cages integrated with water pots and under hygienic conditions with standard rat food and free access to water. After two weeks of acclimatization at a constant temperature of $22^\circ\text{C} \pm 2^\circ\text{C}$ under a 12/12h light/dark cycle, the rats were divided into groups for the different tests.

2.5.2. Formalin Oedema Induction

Rats were randomly assorted and bodily marked for indication. A number of 40 rats were divided into 8 groups of 5 rats each according to their body weight. All animals were provided with food and water ad libitum throughout the experimental period.

Group 1: Negative control, receiving normal saline (1 mL/kg).

Group 2: Positive control group, treated with 100 mg/kg standard drug (aspirin).

Group 3: Test groups, received ethanolic plant extract (200 mg/kg).

One hour after, inflammation was induced by injection to formalin (50 μL of 2% w/v) into the subplantar tissue of left hind paw of each animal [19]. Before the experimentation, the rats were starved overnight to allow for proper sample ab-

sorption into the blood stream [20]. Normal saline, aspirin and ethanolic plant extract were administered per os. The rat paw oedema was evaluated by change of volume using a vernier calliper before and after formalin solution injection at 1, 2, 3, 4 and 5 hours.

The percentage decrease in paw volume is calculated by following formular:

$$\% = \frac{(\text{change in volume in control group} - \text{Change in volume in treated group}) \times 100}{\text{change in volume in control group}} \quad (1)$$

2.6. Antioxidant Activity of Plants Extracts

The antioxidant activity of ethanol extracts was investigated using the DPPH scavenging assay and the ferric reducing power assay.

2.6.1. DPPH Radical Scavenging Activity of Plant Extracts

The radical-scavenging activity of plant extracts was measured using 1,1-diphenyl-2-picryl hydroxyl (DPPH). 1 mL of extract (5; 2.5; 1.25; 0.625; 0.312; 0.156 and 0.078) mg/mL was mixed with 1 mL of DPPH solution (prepared with methanol) and the mixture was maintained in dark at room temperature for 30 min. The reference positive controls were BHT, gallic acid and quercetin. The absorbance of the sample and positive control were measured against a blank control (1 mL of DPPH was added to 1 mL of methanol) at 517 nm. All experiments were performed in triplicate. The inhibition percentage was calculated using the following formula:

$$\% \text{Inh} = \frac{Ac - As}{Ac} \times 100 \quad (2)$$

Ac represent the absorbance of the control and as is the absorbance of the sample [21].

2.6.2. Ferric Reducing Antioxidant Power Assay

The capacity of the plant extracts to reduce the ferric-ferry cyanide complex to the ferrous-ferry cyanide complex was determined as described by [22] with slight modifications. Briefly, 500 µl of plant extracts (97.6 - 5000 µg/ml) were mixed with phosphate-buffered saline (PBS) (PH: 6.4) and 2.5 ml of 1% potassium ferry cyanide ($K_4Fe(CN)_6$). The mixture was incubated at 50°C for 20 minutes. After incubation 1 ml of 10%, Trichloroacetic Acid Reagent was added to the mixture and centrifuged at 3000 rpm for 10 min. Then, 1.5 ml of supernatant was mixed with 0.3 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm. Increment of the absorbance of the reaction mixture indicates increased reducing power. The same procedure was adopted for Ascorbic acid used as positive control. All the tests were performed in triplicate.

2.7. Cytotoxicity Essay

The cytotoxic effect of the extracts was evaluated following the method described by [23]. The test is a primary toxicity test carried out twice on 48 hours larvae of *Artemia salina*. 16 live larvae were contacted with the series of solutions

of progressive concentrations (100 mg/mL; 50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.125 mg/mL; 1.582 mg/mL; 0.781 mg/mL; and 0.391 mg/mL; 0.1955) of the ethanolic extracts of plants. These tests and controls were incubated under agitation for 24 h the number of surviving larvae is counted. These DL_{50} were calculated using the regression line obtained from the surviving larvae in function of the extracts concentration representation.

2.8. Statistical Analysis

Microsoft Excel 2016 spreadsheet was used for data processing. The differences between test in these experiments was assessed for significance using analysis of variance (ANOVA) and student t-test, where probability ($p < 0.05$) was considered significant.

3. Result

3.1. Total Phenolic and Flavonoid Content

Total phenolic and flavonoids content of the plant extracts are summarized in **Table 1**. *Psidium guajava* possessed the highest phenolic content (295.91 ± 1.72 mgGAE/g) while *Terminalia superba* recorded the lowest (231.67 ± 0.64 mgGAE/g). However, *Terminalia superba* has highest total flavonoids content (207.64 ± 3.68 mgQE/g) while *Annona senegalensis* has the lowest (64.71 ± 1.10 mgQE/g). Analysis show difference ($p < 0.05$) between polyphenols and flavonoids content.

Values are an average of three replications \pm SD; mg GAE/g (milligrams of gallic acid equivalents per grams of ethanolic extract and mg QE/g Milligrams of quercetin equivalents per grams of ethanolic extract). Intra-column means followed by the different letters differ significantly at the 5% level.

3.2. DPPH Radical-Scavenging Activity

Different inhibitory concentration (IC_{50}) was observed in this assay. *A. senegalensis* was showed a lowest IC_{50} (2.62 ± 0.26 mg/mL), followed by *T. superba* (3.67 ± 0.18 mg/mL), *I. batatas* (4.38 ± 0.64 mg/mL) and *P. guajava* (4.69 ± 0.72 mg/mL) respectively. The radical scavenging activity of the four plant extracts was compared with activity of synthetic antioxidants with IC_{50} values of the positive controls were Butylated Hydroxy Anisol ($IC_{50} = 114.3 \pm 0.16$ mg/mL),

Table 1. Total polyphenols and total flavonoid content from ethanolic plant extracts.

Ethanolic plant extracts	Total polyphenols (mg GAE/g)	Total flavonoids (mg QE/g)
<i>A. senegalensis</i>	$275.07 \pm 2.02b$	$64.71 \pm 1.10c$
<i>I. batatas</i>	$266.39 \pm 7.46c$	$113.69 \pm 2.21b$
<i>P. guajava</i>	$295.91 \pm 1.72a$	$46.95 \pm 5.33d$
<i>T. superba</i>	$231.67 \pm 0.64d$	$207.64 \pm 3.68a$

quercetin ($IC_{50} = 125.65 \pm 0.42 \mu\text{g/mL}$), and gallic acid ($IC_{50} = 233.87 \pm 0.18 \mu\text{g/mL}$). The test showed difference ($p < 0.05$) between radical scavenging activity of plants extracts. The same difference ($p < 0.05$) was observed with synthetic antioxidants molecule used.

3.3. Ferric Reducing Antioxidant Power Assay

The iron chelating activity for the plant ethanolic extracts was showed in **Table 2**. *A. senegalensis* has presented the best iron chelating activity, with the lowest IC_{50} value ($3.58 \pm 0.58 \text{ mg/ml}$), following to *T. superba* ($4.29 \pm 0.31 \text{ mg/mL}$), *P. guajava* ($4.69 \pm 0.65 \text{ mg/mL}$), and *I. batatas* ($4.88 \pm 0.22 \text{ mg/ml}$) mg/mL . The plant extracts have the low activity compared to ascorbic acid used as the reference molecule, which showed high scavenging activity with $IC_{50} = 24.08 \pm 0.51 \mu\text{g/mL}$. The data related to inhibitory percentage show that, an increase in concentration is linked with an increase in scavenging capacity.

DPPH (2,2-diphenyl 1-picrylhydrazyl); FRAP (Ferric reducing-antioxidant power); R² (Correlation of determination); IC_{50} (50% inhibitory concentration). In comparison with the reference molecules used, the extracts of the four plants are less active than gallic acid ($IC_{50} = 233.87 \pm 0.18 \mu\text{g/mL}$), BHA ($IC_{50} = 114.3 \pm 0.16 \mu\text{g/mL}$) and quercetin ($IC_{50} = 125.65 \pm 0.42 \mu\text{g/mL}$) considering the DPPH method as well as ascorbic acid ($IC_{50} = 24.08 \pm 0.51 \mu\text{g/mL}$) considering the FRAP method (**Table 3**).

Table 2. Antioxydant activity of plant extracts.

Methods	Ethanolic extracts	Equations	R ²	IC ₅₀ (mg/ml)
DPPH	<i>A. senegalensis</i>	$y = 6.70x + 32.43$	0.92	$2.62 \pm 0.26c$
	<i>I. batatas</i>	$y = 8.38x + 13.25$	0.98	$4.38 \pm 0.64b$
	<i>P. guajava</i>	$y = 9.48x + 5.49$	0.97	$4.69 \pm 0.72b$
	<i>T. superba</i>	$y = 8.47x + 18.95$	0.93	$3.67 \pm 0.18c$
FRAP	<i>A. senegalensis</i>	$y = 10.16x + 13.62$	0.99	$3.58 \pm 0.58b$
	<i>I. batatas</i>	$y = 11.81x - 7.65$	0.98	$4.88 \pm 0.22a$
	<i>P. guajava</i>	$y = 11.29x - 2.97$	0.99	$4.69 \pm 0.65a$
	<i>T. superba</i>	$y = 11.16x + 2.04$	0.99	$4.29 \pm 0.31ab$

Intra-column means followed by the different letters differ significantly at the 5% level.

Table 3. The antioxidant activity of standards by DPPH and FRAP method.

Standards	DPPH (IC ₅₀ $\mu\text{g/mL}$)	FRAP (IC ₅₀ $\mu\text{g/mL}$)
Gallic acid	$233.87 \pm 0.18b$	-
Ascorbic acid	-	$24.08 \pm 0.51a$
BHA	$114.3 \pm 0.16b$	-
Quercetin	$125.65 \pm 0.42a$	-

3.4. Anti-Inflammatory Activity

The evolution of the edema through the increase in the volume of the pate of the rats is presented in **Figure 1**. The comparison of the pate volume shows a difference ($p < 0.05$) between the action of distilled water and those of aspirin used as reference molecule. Similarly, Analysis of variance shows a difference ($p = 0.03$) between the action of the extracts compared to that of distilled water.

The plant extracts anti-inflammatory activity evaluated by the paw edema method induced by formalin is shown in **Table 4**. Edema inhibition percentage of the injected footpad increased with the hours' evolution and reached a peak after 5 hours. In this study, aspirin was used as the standard anti-inflammatory drug reduced formalin-induced edema in rats with inhibition percentage range from $19.25\% \pm 0.27\%$ to $88.91\% \pm 0.56\%$. Among the test plants extracts, *A. senegalensis* extract demonstrated the best anti-inflammatory property, which was upheld and increased between 1 h ($12.84\% \pm 0.13\%$) and 5 hours ($82.2\% \pm 0.20\%$). The lowest anti-inflammatory activity was recorded with *I. batatas* extract

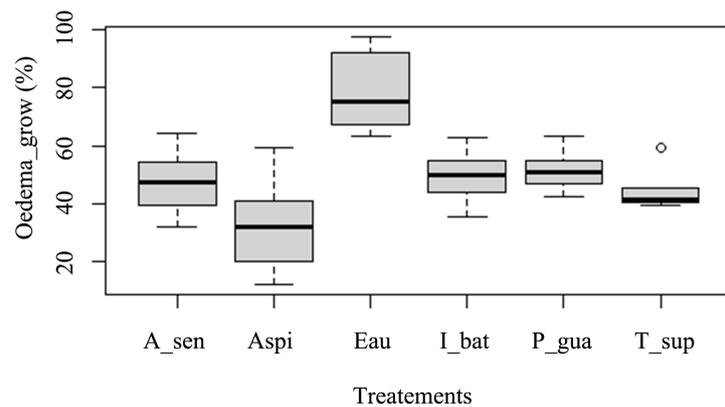


Figure 1. Boxplot oedema evolution of antiinflammatory activity of treatments. *A_sen* (*Annona senegalensis*), *I_bat* (*Ipomoea batatas*), *P_gua* (*Psidium guajava*), *T_sup* (*Terminalia superba*), *Aspi* (*Aspirin*), *Eau* (*Water*).

Table 4. Anti-inflammatory activity of ethanolic plant extracts.

Plant extracts	Inhibition percentage (%) (mean \pm SD)				
	1 h	2 h	3 h	4 h	5 h
<i>A. senegalensis</i>	12.84 \pm 0.13b	24.94 \pm 0.79b	55.87 \pm 0.45a	71.59 \pm 0.61b	82.2 \pm 0.20b
<i>I. batatas</i>	6.25 \pm 0.01e	25.96 \pm 0.64b	42.81 \pm 0.46d	62.83 \pm 0.33d	73.19 \pm 0.44e
<i>P. guajava</i>	9.25 \pm 0.53c	29.4 \pm 0.91a	48.55 \pm 0.30c	67.28 \pm 1c	78.4 \pm 0.77d
<i>T. superba</i>	8.66 \pm 0.41d	20.03 \pm 0.19c	53.58 \pm 0.56b	72.32 \pm 0.84b	80.84 \pm 0.57c
Aspirin	19.25 \pm 0.27a	26.7 \pm 0.32b	57.53 \pm 0.54a	76.55 \pm 0.33a	88.91 \pm 0.56a

h (hour), *A. Senegalensis* (*Annona senegalensis*), *I. Batatas* (*Ipomoea batatas*), *Psidium guajava* (*P. guajava*), *Terminalia superba* (*T. superba*). Intra-column means followed by the different letters differ significantly at the 5% level.

who inhibit paw edema from $6.25\% \pm 0.01\%$ (1 h) to $73.19\% \pm 0.44\%$ (5 h). The comparative analysis of anti-inflammatory data taking into account the overall inhibition percentage without taking into account the time effect shows the same trend was observed. Indeed, aspirin is more active than all the extracts (**Figure 2**).

3.5. Cytotoxicity Effect of Plant Extracts

Figure 3 shows the variation in the number of surviving larvae as a function of the concentration of the extracts. It is noted that the extract of *A. senegalensis* showed the low rate (62.5%) of dead larva at 100 mg/mL while the *P. guajava* extract recorded the high rate (100%) of dead larva at 100 mg/mL. The extracts therefore exhibited dose-response activity. The interaction between the concentration of extracts and the mortality rate shows a difference in mortality between the types of plant ($p < 0.0001$) and the concentration of extract ($p = 0.03$). The determined LC_{50} vary from 28.67 mg/mL to 51.06 mg/mL (**Table 5**).

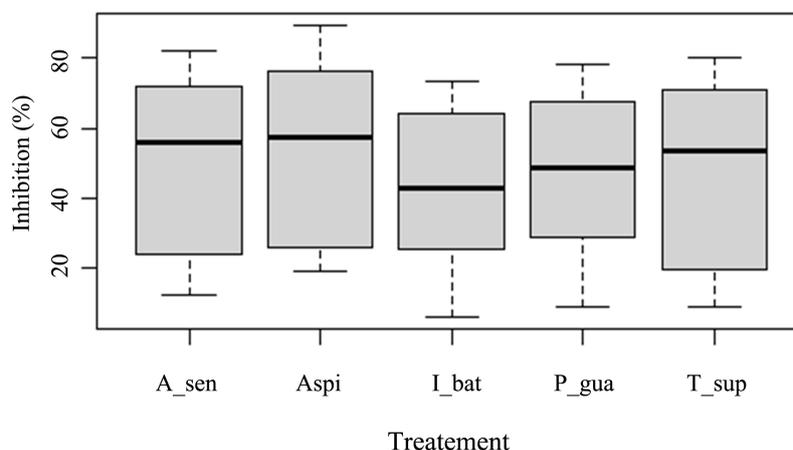


Figure 2. Box plot inhibition rate of inflammatory activity. *A_sen* (*Annona senegalensis*), *I_bat* (*Ipomoea batatas*), *P_gua* (*Psidium guajava*), *T_sup* (*Terminalia superba*), *Aspi* (*Aspirin*).

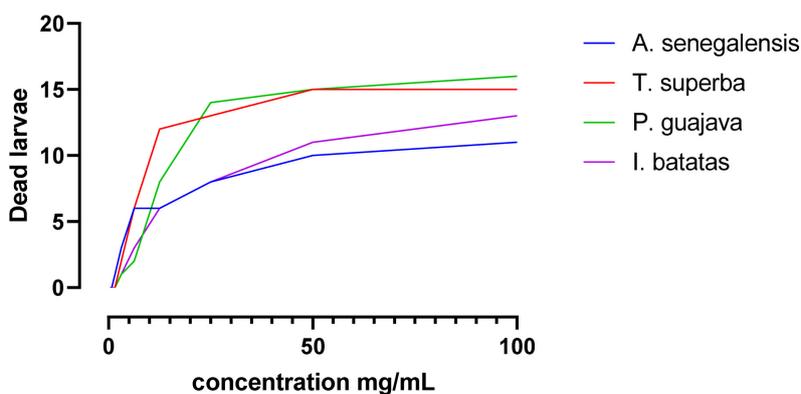


Figure 3. Larvae mortality rate according to the plants extracts concentration. *A. Senegalensis* (*Annona senegalensis*), *I. Batatas* (*Ipomoea batatas*), *P. guajava* (*Psidium guajava*), *T. superba* (*Terminalia superba*).

Table 5. Lethal dose (mg/mL) of the plant extracts.

<i>Ethanollic plant extracts</i>	LC ₅₀ (mg/mL)	R ²
<i>A. senegalensis</i>	51.06	0.677
<i>I. batatas</i>	46.60	0.813
<i>P. guajava</i>	32.24	0.699
<i>T. superba</i>	28.67	0.583

LC (Lethal concentration), R² (Correlation of determination), Intra-column means followed by the different letters differ significantly at the 5% level.

4. Discussion

Medicinal plants exhibit a wide variety of therapeutic effects, including anti-inflammatory, antioxidant activities [24]. These beneficial effects of plants are linked with bioactive compounds. Among the molecules found in medicinal plants are polyphenols [25]. In this study, the determination of polyphenols in the ethanolic plant extracts shows that *P. guajava* extract contents a higher amount of polyphenols following to *A. senegalensis*, *I. batatas*, and *T. superba*. Otherwise *T. superba* who has the lowest polyphenols content recorded a higher amount total flavonoid among the four ethanolic extracts. This results shows that this plant (*T. superba*) is richer in flavonoids than other compounds of polyphenols group. Polyphenolic compounds subdivided into subclasses like phenolic acids, phenolic alcohols, flavonoids, stilbenoids and lignans [26]. Out of these, most of the isolated, identified compounds are from the class of flavonoids. Flavonoids as earlier mentioned, flavonoids included the most isolated, identified and diversified class of polyphenolic compounds [27]. Recent research on secondary metabolites is very advanced, especially in the fields of herbal medicine and food hygiene, because of their various biological properties: antioxidant, anti-inflammatory, etc. [28]. Therefore, in the present study, *A. senegalensis*, *I. batatas*, *P. guajava* and *T. superba* were demonstrated notable antioxidant effect. However, antioxidant power of the plants extracts was lower than those of BHA, quercetin and gallic acid used in DPPH assay. The result of FRAP scavenging ability showed also a similar to DPPH assay. However, *A. senegalensis* extract showed higher ability to reduce ferric ion compared to *I. batatas*, *P. guajava* and *T. superba* extracts. Other authors like Kengni showed strong antioxidant activity of *A. senegalensis* leave extracts [29]. This activity was more remarkable with ferric ion chelation than with DPPH radical scavenging. Moreover, Ahmed reported that the H₂O₂ scavenging activity and the ferric reducing assay power of *A. senegalensis* extracts was significantly greater ($p < 0.05$) than ascorbic acid. The same author reported that the ferric thiocyanate scavenging activity and thiobarbituric acid (TBA) activity of all the extracts of *A. senegalensis* were significantly greater ($p < 0.05$) than that of ascorbic acids [30].

The above antioxidant potentials of leaves ethanol extracts of *A. senegalensis* can relate to the phenolic content. In fact, the phytochemical screening and

HPLC analysis made by Kengni revealed the presence of several phenolic compounds including phenol (o/p-coumaric acid; syringic acid), anthocyanins, tannins, and flavonoids (vanillic acid) [29]. These different compounds are known for their strong antioxidant activity [31].

Among the four plant extracts tested, antioxidant property was highest in *A. senegalensis* and lowest in *P. guajava*. Kougnimon *et al.* [11] reported the good antioxidant activity of leaves ethanolic extract of *T. superba* with IC₅₀ value of 34.72 µg/mL. Based on the results, the all ethanolic plant extracts contained phenolic compounds. *I. batatas* extract was showed antioxidant effect with IC₅₀ values (4.88 ± 0.22 mg/mL; 4.38 ± 0.64 mg/mL) which are lows than those reported by Prasanth *et al.* [32] and Koncic *et al.* [33] respectively 54.1 µg/mL and 54.1 µg/mL. Likewise, IC₅₀ values found by Dewijanti *et al.* [34] for the the yellow and purple varieties of leaves of *I. batatas* were 47.65 µg/mL and 87.402 µg/ml respectively. Braga *et al.* [35] reported that leaves ethanolic extract of *P. guajava* have a good content of total phenolic (766.08 µg/mg) and flavonoids (118.90 µg/mg). Rika *et al.* [36] makes the same observation regarding leaves ethanolic extract of *P. guajava* (49.55 µg-GAE/100g). In the previous work, the values of the total phenolic and flavonoids of methanol leaves extract of Pakistan guajava were 83.34 µg-GAE/mg and 53.39 µg-QCE/mg respectively [37]. These TPC reported was lower than those obtained in our study. In the same way, total phenolic content (266.39 ± 7.46 mgGAE/g) and flavonoids (113.69 ± 2.21 mg QE/g) content of *I. batatas* extract were higher than those obtained in the previous research [33] [38].

Biotechnology generates different bioactive secondary metabolites from plant tissues or organs culture by various strategies such as genetic transformation of plants for improve of production of bioactive compounds of secondary metabolic origin including alkaloids, flavonoids, phenolics and tannins. These bioactive compounds are economically important as drugs and flavors [39]. The studie *in vitro* culture was showed more efficient than whole plant for the production of different bioactive secondary metabolites such as anthraquinones, benzyloquinoline, alkaloids, berberine, ginsenoside compared to whole plant production following agronomic method [40]. Thangaraj was found that biological activities of the extract show a significant variation depending on the extraction methods [41]. Indeed, Rajan and Thangaraj [42] revealed higher anti-radical property for both macerated and Soxhlet methanol extracts compared to fractionation extraction from whole plant of *Osbeckia parvifolia* [42].

Apart from the remarkable antioxidant activity observed, all the extracts showed good anti-inflammatory activity. It was evaluated by Formalin edema induction. Injected of formalin into the subplantar surface of the right hind paw causes local inflammation. The same observation was showed with the molecule of carrageenn and albumin [43] [44] [45]. The formation and release of autocooids and neurogenically induced by stimulation of C nerve fibers contribute to the development of inflammation [46]. The plant extracts studied had a progressive inhibitory effect on formalin-induced odema gradually. However, this effect is

more remarkable from the 3 hours. Yeo has shown that *Annona senegalensis* has anti-inflammatory properties. The increase in the percentage of inhibition observed with the ethanolic extract of these plant extracts is due to the increase in the number of inflammatory cells including mast cells, neutrophils cells and macrophages cells. In addition, flavonoids decrease the permeability of blood capillaries and make them more resistant [47]. The results of this previous work have shown that flavonoids and phenolic compounds including coumarins possess anti-inflammatory properties [48]. We have just seen above that the study plant extracts have a good composition in polyphenols and total flavonoids. These compounds are recognized for their remarkable anti-inflammatory activities [49]. In the present study, all extracts of the four plants have a good content of Flavonoids. *T. superba* leave extracts has highest total flavonoids content (207.64 ± 3.68 mgQE/g). Several studies have revealed the anti-inflammatory properties of extracts of *Terminalia* species [45] [50]. Cytotoxicity evaluation showed no toxicity with reference to the toxicity scale established [51]. All the extracts from the four plants had $LD_{50} > 0.1$ mg/mL.

5. Conclusion

This study showed that the ethanolic extract of *A. senegalensis*, *I. batatas*, *P. guajava* and *T. superba* viewed as a potential source of natural antioxidant and anti-inflammatory. In addition, these plants can provide for the prevention of diseases related oxidative stress. Furthermore, the assays of total polyphenols and total flavonoids in these plant extracts revealed that the ethanolic plant extracts contained phenols and flavonoids. Indeed, these phenolic compounds could be responsible for these observed activities. Moreover, the extracts of these plants do not show any toxicity.

Conflicts of Interest

The authors declare no conflict of interest.

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