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Comparative Evaluation of the Antioxidant and Anti-Inflammatory Properties of *Cajanus cajan* and *Crotaeva adansonii*

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Abstract

Antioxidants are the subject of extensive research because, in addition to their use as preservatives in foodstuffs as a replacement for synthetic antioxidants, they are also used in the treatment of many diseases. *Cajanus cajan* and *Crotaeva adansonii*, two medicinal plants used in Benin for their anti-hypertensive effects. The present study aimed to evaluate the antioxidant and anti-inflammatory activities of the hydroethanolic extract. The first part of this study concerns the extraction and quantification of total phenols, flavonoids and tannins. The second part studies the antioxidant activity of plant extracts, using the DPPH radical scavenging technique. The anti-inflammatory activity was studied using the carrageenan-induced rat paw edema. The results showed that total phenolic, flavonoids and condensed tannins values were higher in *Cajanus cajan* ethanolic extracts: 254.12 ± 5.37 mg of gallic acid equivalent per g of dry extract, 910.4 ± 2.45 mg of Quercetin equivalent per g of dry extract, 2.22 ± 0.05 mg of catechin equivalent per g of dry extract. Antioxidant activity methods show that all extracts from the two plants studied display antioxidant properties at different levels. IC50 value of *Cajanus cajan* extract was 0.03 mg/ml and *Crotaeva adansonii* extract was 0.09 mg/ml as opposed to that of ascorbic acid 0.01 mg/ml a powerful radical scavenger DPPH. The hydroethanolic extract of *Cajanus cajan* and *Crotaeva adansonii* significantly reduced ($p < 0.05$) the thickness of the rats' paws at 600 mg/kg body weight. With re-

gard to the percentage inhibition 6 hours after carrageenan injection, the samples of hydroethanolic extract of *Cajanus cajan* can be ranked in the following order: AAS (95% ± 2.4%) > Cc 600 (90% ± 2.6%) > Cc 400 (76% ± 2.2%) > Cc 200 (63.9% ± 2.1%). Meanwhile, samples of hydroethanolic extract of *Crotaeva adansonii* can be ranked in the following order: AAS (95% ± 1.8%) > Ca 600 (90% ± 2.3%) > Ca 400 (79.4% ± 1.2%) > Ca 200 (45.0% ± 2.8%). Further studies are needed to find the antioxidant and anti-inflammatory effects mechanism.

Keywords

Cajanus cajan, *Crotaeva adansonii*, Free Radical Scavenging Activity, Anti-Inflammatory

1. Introduction

There is a close, bidirectional, and self-perpetuating relationship between high blood pressure, oxidative stress, and inflammation, involving vascular, renal, and immune mechanisms responsible for cardiovascular complications. Recent studies are paying increasing attention to the importance of inflammation as a distinct cause of the development of hypertension, which is mediated by various mechanisms such as endothelial dysfunctionand hardening of the arteries [1]. To further explore this correlation, there is ample evidence to suggest that there is a close link between hypertension and increased oxidative stress in the vascular system, but the timing of this relationship—whether it is a cause or an effect—remains a subject of study arteries [2].

However, the majority of studies reflect that systemic inflammation and oxidative stress play a vital role in the pathogenesis of hypertension via the following processes: endothelial dysfunction, vascular remodeling, and arterial stiffness [3]. The complexity of this multiplicity creates a need to explore these avenues in depth in order to propose specific therapeutic interventions. In particular, the vascular health triad, which includes inflammation, reactive oxygen species, and endothelial dysfunction, plays an important role in regulating blood pressure arteries [4].

The unregulated functioning of these elements can lead to impaired vascularization and increased peripheral resistance, which are symptoms of hypertension [5] [6].

The polyphenol family became the starting point for all scientific research, particularly the discovery of natural molecules with very high antioxidant activity. Oxidants, whatever their origin, are a very serious problem not only for public health, but also for the food industry.

In the context of research into natural antioxidants, we were interested in evaluating some antioxidant properties of two plant species, namely: *Cajanus cajan* (L.) Mill sp. and *Crotaeva adansonii* DC. ssp.

The possibility of assessing the antioxidant properties of *Cajanus cajan* and *Crotaeva adansonii* using bioassays was used because, in the case of Benin in particular, it is possible to assess the antioxidant properties of *Cajanus cajan* and *Crotaeva adansonii* using bioassays, Today, it's important to learn from traditional pharmacopoeia, by testing the active principles of the medicinal plants we use, in order to obtain reliable, high-performance resources while preserving biodiversity.

There is our days, an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical and cosmetic) purposes. There are many cellular biochemical pathways and environmental toxins which produce reactive oxygen species (ROS) [7] and contribute to the development of diseases such as cancer, cardiovascular disorders, diabetes, cataracts and many neurodegenerative diseases [8].

Ordinarily, the levels of free radicals in living organisms are controlled by a complex set of antioxidant defenses, which minimize oxidative damage to important biomolecules, but in Oxidative stress circumstances, the endogenous antioxidants are not enough to deal with the increased levels of ROS [9] [10]. In contrast, the accumulation of excessive ROS, mainly due to external influences such as radiation, ultraviolet light, cigarette smoke, pathogens, drugs, etc., can inflict damage upon cellular macromolecules such as DNA, proteins and lipids [11]. This concept is supported by increasing evidence indicating that oxidative damage plays a role in the development of many chronic diseases. Further, oxidative stress may be associated with nearly 200 diseases, such as cardiovascular diseases, cancer, atherosclerosis, hypertension, ischemia, diabetes mellitus, neurodegenerative diseases (Alzheimer's and Parkinson's), rheumatoid arthritis [12]. Thus, in order for the level of excessive ROS to be reduced, and so the oxidative damage can be suppressed, the need for additional intake of exogenous antioxidants can be suggested [13]. Antioxidants are substances that when present in low concentrations, compared to those of an oxidisable substrate significantly delay or prevent oxidation of that substance [14].

Many studies have confirmed that plants and foods rich in polyphenolic content are effective scavengers of free radicals, thus helping in the prevention of these diseases through their antioxidant activity [15].

Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants which produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, *etc*. to prevent oxidation of the susceptible substrate. Natural products especially from plants sources have the ability to reduce oxidative stress by acting as antioxidants.

Given the widespread use of alcoholic and aqueous extracts in traditional medicine, the present research aimed to investigate the antioxidant and anti-inflammatory effects of hydroethanolic extracts of *Cajanus cajan* and *Crotaeva adansonii* leaves. In addition, the phytochemical composition was also evaluated.

2. Materials and Methods

2.1. Plant Material and Extraction

Two medicinal plants used in Benin for their anti-hypertensive effects constituted the plant material that we used in this study. These are *Cajanus cajan* and *Crotaeva adansonii*. They were collected in the agro-ecological zone of the Abomey-calavi commune in August 10, 2022 and brought to the Physiopathology, Pharmacology and Nutrition Research Unit, Laboratory of Biology and Molecular Typing in Microbiology Faculty of Science and Technology for drying. Their certification was carried out at the National Herbarium of Benin by comparison with reference samples kept under the numbers YH699/HNB and YH700/HNB respectively for *Cajanus cajan* and *Crotaeva adansonii*. After an average drying period of four weeks at about 23˚C, they were ground and green coloured powders were obtained.

2.2. Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium hexacyanoferrate [$K_3Fe(CN)_6$], trichloroacetic acid, gallic acid, ascorbic acid, quercetin, and $FeCl_3$ were purchased from Sigma Chemical; Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate (Na_2CO_3), aluminium chloride, potassium acetate and solvent methanol were obtained from Merck Chemical Supplies (Darmstadt, Germany). All the chemicals used, were of analytical grade.

2.3. Extraction Procedure

Two hundred and fifty grams (250 g) of finely ground plant material were extracted by maceration with hydroethanol (70% éthanol/30% eau (v/v)) for 72 h stirring. Each extraction is repeated three times. The macerates were filtered and concentrated using a rotary evaporator (BUCHI Rotavapor RII, Switzerland) at 40˚C - 50˚C. The obtained extracts were stored at 4˚C until biological assay.

2.4. Determination of Extraction Yield

The extraction yield was calculated using the following formula:

$$R\ (\%) = (\%) = [(\text{Mass of extract})/\text{Mass of powder of leaves}] \times 100$$

R is the yield of the extract.

2.5. Phytochemical Estimation

Phytochemical screening of the plant was carried out according to the methods described by Wagner and Blat [16] [17]. for the detection of plant secondary metabolites. Alkaloids, Tannins, Saponins, Leuco-anthocyanins, Mucilage, Flavonoids, Coumarins, Cyanogenic derivatives Anthocyanin pigments, Triterpenes, have been investigated using tube test. Each extract (10 mg/ml) were deposited on TLC plate to confirm the results. Each molecule family was then quantified after identification.

2.6. Quantification of Some Bioactive Molecules

1)Total Phenolics Content

The total phenol content of the plant extracts was determined using with the Folin–Ciocalteu reagent [18]. A volume of 200 µl of each extract was placed in test tubes and the mixture (1 ml of Folin Ciocalteu diluted 10 times and 0.8 ml of 7.5% sodium carbonate) was added. The tubes are shaken and stored for 30 minutes. The absorbance is measured at 765 nm using the spectrophotometer. A calibration curve with different concentrations of gallic acid was prepared. The total phenolic content in the extracts is expressed in milligrams (mg) of gallic acid equivalent per gram (g) of dry matter weight (mg EAG/g DM).

2) Total Flavonoid Content

Flavonoids were quantified using a colorimetric method [19]. A volume of 500 µl of methanolic catechin solution at different concentrations or diluted methanolic extract was added to 1500 µl of distilled water. At time zero, 150 µl of 5% sodium nitrite ($NaNO_2$) is added to the mixture. After 5 min, 150 µl of 10% (m/v) aluminum trichloride ($AlCl_3$) is added. After incubation for 6 min at room temperature, 500 µl of sodium hydroxide (NaOH) (1 M) is added. The mixture is immediately stirred thoroughly. The absorbance of the pinkish solution is measured at 510 nm against the blank. The total flavonoid content of medicinal plant extracts is expressed in milligrams (mg) of catechin equivalents per gram (g) of dry matter weight (EC/g). Each sample is repeated three times.

3) Condensed Tannins

The quantities of condensed tannins are estimated using the vanillin method in an acidic medium [20]. A volume of 50 µl of the crude extract is added to 1500 µl of the vanillin/methanol solution (4%, w/v) and then mixed using a vortex mixer. Next, 750 µl of concentrated hydrochloric acid (HCl) is added and left to react at room temperature for 20 min. The absorbance at 550 nm is measured against a blank. The concentration of tannins is estimated in milligrams (mg) of catechin equivalents per gram (g) of dry matter weight (EC/g) based on the calibration curve.

2.7. DPPH Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using DPPH [21].

In the presence of antioxidant which is typical for DPPH free radical decays, the change in absorbency at 517 nm is followed spectrophotometrically. The antioxidant activity was determined according to the method previously described [22]. Radical scavenging activity of extracts is expressed as radical scavenging percentage (RSP) and was calculated using the following equation [23].

$$\text{RSP (\%)} = (\%) = [(A_B - A_A)/A_B] \times 100,$$

where A_B is the absorbance of the blank ($t = 0$ min) and A_A is the absorbance of the tested extract solution ($t = 15$ min).

2.8. Anti-Inflammatory Activity: Carrageenan-Induced Paw Edema in Rats

The carrageenan-induced paw edema model was used to evaluate the in vivo anti-inflammatory effect of hydroethanolic extracts [24].

Studies have shown that salicylic acid, used as a standard, works in the second phase of inflammation while inhibiting the synthesis of these different mediators [25]. By inhibiting the production of prostaglandins through the inhibition of cyclooxygenase (COX2), it will limit the lowering of the pain threshold, hence its analgesic action, as well as inflammatory reactions, hence its antipyretic activity [26] [27].

The rats were divided into eight (8) groups of six (6) rats each. Before the test, they were deprived of food for 16 hours.

1) Groupe control: 6 rats receive only distilled water by gavage (10 ml/kg bw).

2) Groupe control (positive control): 6 rats were treated with a therapeutically used anti-inflammatory (acetylsalicylic acid: AAS) 100mg/kg bw, 30 minutes before injection of carrageenan (1%) into the fascia of the soles of the rats' feet.

3) Groupe test batch of *Cajanus cajan* (EthCc-200 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 200 mg/kg of hydroethanol extract of *Cajanus cajan*.

4) Groupe test batch of *Cajanus cajan* (EthCc-400 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 400 mg/kg of hydroethanol extract of *Cajanus cajan*.

5) Groupe test batch of *Cajanus cajan* (EthCc-600 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 600 mg/kg of hydroethanol extract of *Cajanus cajan*.

6) Groupe test batch of *Crotaeva adansonii* (EthCa-200 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 200 mg/kg of hydroethanol extract of *Crotaeva adansonii*.

7) Groupe test batch of *Crotaeva adansonii* (EthCa-400 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 400 mg/kg of hydroethanol extract of *Crotaeva adansonii*

8) Groupe test batch of *Crotaeva adansonii* (EthCa-600 mg/kg bw): 30 min. before carrageenan injection, 6 rats were orally administered 600 mg/kg of hydroethanol extract of *Crotaeva adansonii*

Leg thickness or volume was measured using a Pletysmometer just before carrageenan injection and 2, 4 and 6 hours after carrageenan injection. Mean volume (VT) was obtained from six (6) readings and calculated as follows:

$$VT\ (mL) = V_t - V_0,$$

where

- V_0: initial volume of the leg,
- V_t: paw volume at time t after carrageenan injection and extract treatment.

The percentage of inhibition of inflammation (Inh %) was obtained using the following equation:

$$(\%)\ \text{Inh} = [(V_{tc} - V_{tp})/V_{tc}] \times 100$$

where V_{tc} is the difference in paw volume of the control group, and V_{tp} is the difference in paw volume in the treated group (acetylsalicylic acid, extracts). A significant reduction in paw volume compared to the control batch was considered an anti-inflammatory effect.

2.9. Statistical Method

The data were expressed as means ± standard deviation (SD) of three replicate determinations. GraphPad Prism (version 8.0) was used to determine the correlation of the biological activities with polyphenol compounds by Pearson's correlation coefficient [28]. A two-way analysis of variance (ANOVA) followed by Sidak 's multiple comparison tests was performed using the GraphPad Prism (version 8.0) to compare differences between extracts. At the same time, a two-way analysis of variance (ANOVA) followed by Tukey's and Dunnett's multiple comparison tests was used to compare extracts to the control. The difference was considered statistically significant for a p-value < 0.05.

3. Results

3.1. Performance of Extraction

The best extraction yield was obtained with *Crotaeva adansonii* (5.406%). The lowest yield was obtained with *Cajanus cajan* (4.55%).

3.2. Phytochemical Screening

Qualitative tests revealed the presence of various secondary metabolites such as alkaloids, tannins, saponosides, leuco-anthocyanins, flavonoids, coumarins.

After the phytochemical screening, three chemical compounds groups such as total phenolic compounds, flavonoids and tannins were measured on the hydroethanolic extracts (Table 1) presented values obtained.

3.3. Quantification of Some Bioactive Molecules Family

Table 1. Total phenolic, flavonoid, and condensed tannins content of *Cajanus cajan* and *Crotaeva adansonii* (mean ± standard deviation).

Name of plants	Total phenolic[a]	Flavonoïde[b]	Condensed tannins[c]
Cajanus cajan	254.12 ± 5.37	910.4 ± 2.45	2.22 ± 0.05
Crotaeva adansonii	76.32 ± 1.34	277.84 ± 1.23	1.03 ± 0.08

[a] mg of gallic acid equivalent per g of dry extract, [b] mg of Quercetin equivalent per g of dry extract, [c] mg of catechin equivalent per g of dry extract.

Table 1 revealed the results of total phenolic content, total flavonoid and total tannins contents in the hydroethanolic extract of *Cajanus cajan* and *Crotaeva adansonii*. The highest content of total phenolics was detected in hydroethanolic extract of *Cajanus cajan* (254.12 ± 5.37 mg equivalent of gallic acid/g of extract)

compared to the hydroethanolic *Crotaeva adansonii* (76.32 ± 1.34 mg equivalent of gallic acid/g of extract). The *Cajanus cajan* extract had a higher flavonoid content (910.40 ± 2.45 mg equivalent of rutin/g of extract) and ethanolic extract of *Crotaeva adansonii* lowest (277.84 ± 1.23 mg equivalent of rutin/g of extract). The *Cajanus cajan* extract had the highest total tannin content (2.22 ± 0.05 mg equivalent of catechin/g of extract), while the *Crotaeva adansonii* (1.03 ± 0.08 mg equivalent of catechin/g of extract) extract had the lowest.

3.4. Antioxidant Activity DPPH Radical Scavenging Activity

The variations of the antioxidant power by the DPPH method, hydroethanol extracts of *Cajanus cajan*, *Crotaeva adansonii* leaves and standard ascorbic acid are presented in Figure 1.

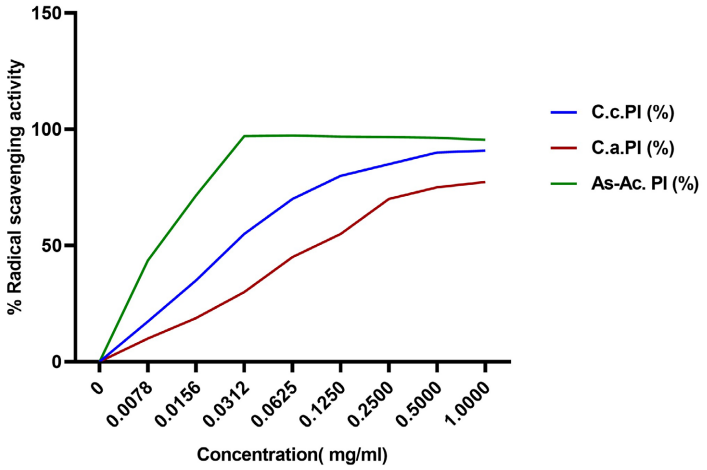

Figure 1. Radical scavenging activity of *Cajanus cajan*, *Crotaeva adansonii* leaves and standard ascorbic acid.

In this study, the DPPH radical scavenging activities of extracts therefore increased gradually in a dose concentration dependent manner (0.0078 - 1 mg/ml).

The result of the study expressed that ascorbic acid possessed PI: 95.468 ± 0.450; IC_{50} = 0.01 ± 0.017 mg·ml^{-1} radical inhibitory power at a concentration of 1mg/ml. The hydroethanolic extract of both the *Cajanus cajan* and *Crotaeva adansonii* leaves found to possess highest radical scavenging activity (PI): 90.838 ± 0.254; IC_{50} = 0.03 ± 0.01 mg·ml^{-1} and PI: 77.290 ± 0.37%; IC_{50} = 0.09 ± 0.03 mg·ml^{-1} respectively at 1 mg·ml^{-1} concentration.

The DPPH radical scavenging test of the hydroethanolic extract of *Cajanus cajan* showed greater activity than that of the hydroethanolic extract of *Crotaeva adansonii* (Cc I.P. > Ca I.P.).

3.5. Anti-Inflammatory Activity of Hydroethanol Extracts of *Cajanus cajan* and *Crotaeva adansonii*

The hydroethanolic extract of *Cajanus cajan*, *Crotaeva adansonii* showed a mark ed reduction in carrageenan-induced edema in rat paws (Figure 2 and Figure 3).

The anti-edematous activity was time- and dose-dependent. Compared to the control group, acetylsalicylic acid, the hydroethanol extract of *Cajanus cajan* (Figure 2), *Crotaeva adansonii* (Figure 3) significantly reduced ($p < 0.05$) the thickness of the rats' paws 6 hours after carrageenan administration.

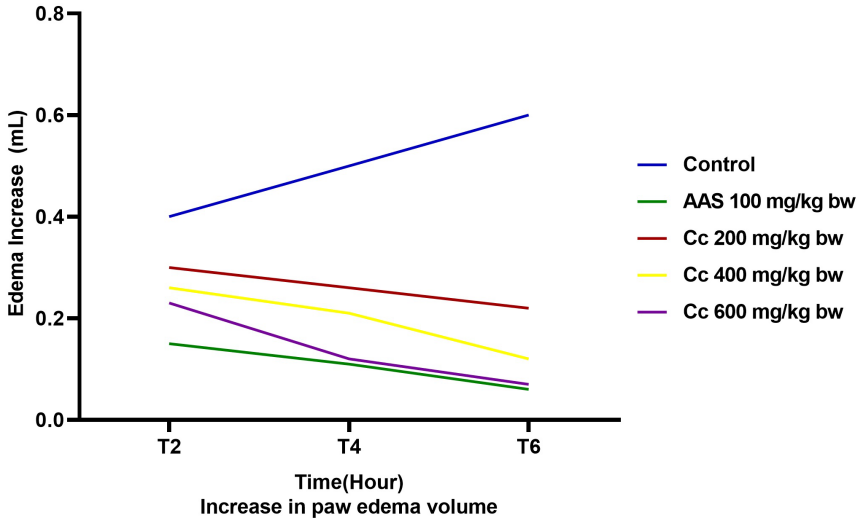

Figure 2. Effect of hydroethanolic extract of Cc on the increase in paw edema volume (mL) in rats over time. Legend: ASA: acetylsalicylic acid; Cc: *Cajanus cajan*.

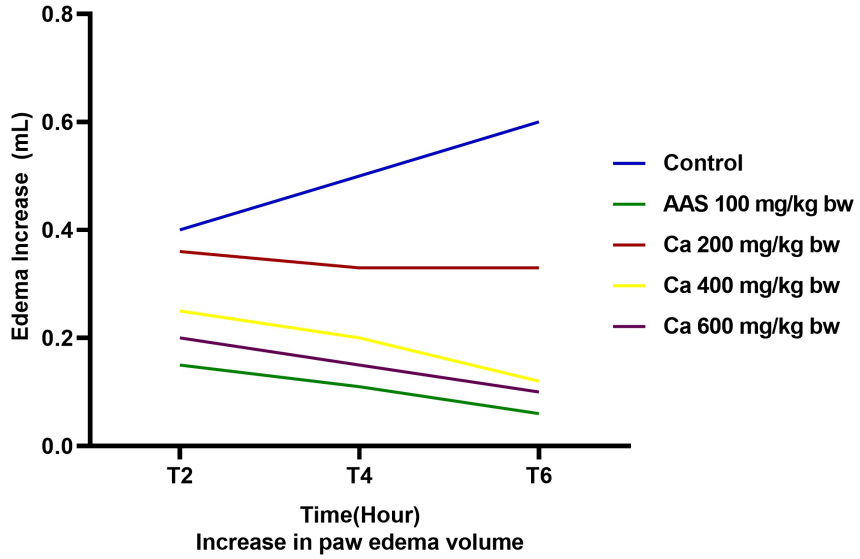

Figure 3. Effect of Ca hydroethanolic extract on the increase in paw edema volume (mL) in rats over time. Legend: ASA: acetylsalicylic acid; Ca: *Crotaeva adansonii*.

Two, Four, and six hours after administration of carrageenan (Figure 4 and Figure 5), no significant difference ($p > 0.05$) was observed between acetylsalicylic acid, hydroethanol extract of *Cajanus cajan* (Figure 4) and *Crotaeva adansonii* (Figure 5) at 600 mg/kg body weight regarding the inhibition percentage.

The percentage of inhibition 6 hours after carrageenan injection, the samples

can be ranked in the following order: acetylsalicylic acid (95% ± 1.6%) > *Cajanus cajan* (EthCc-600 mg/kg bw) (90% ± 1.9%) > *Crotaeva adansonii* leaves (EthCa-600 mg/kg bw) (84.1% ± 3.4%).

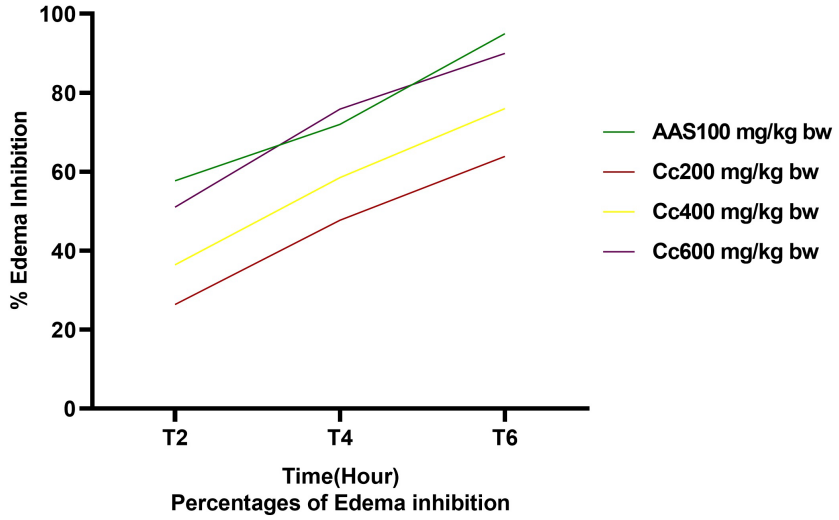

Figure 4. Percentage inhibition of hind leg edema in rats treated with hydroethanolic extract of Cc as a function of time. Legend: ASA: acetylsalicylic acid; Cc: *Cajanus cajan*.

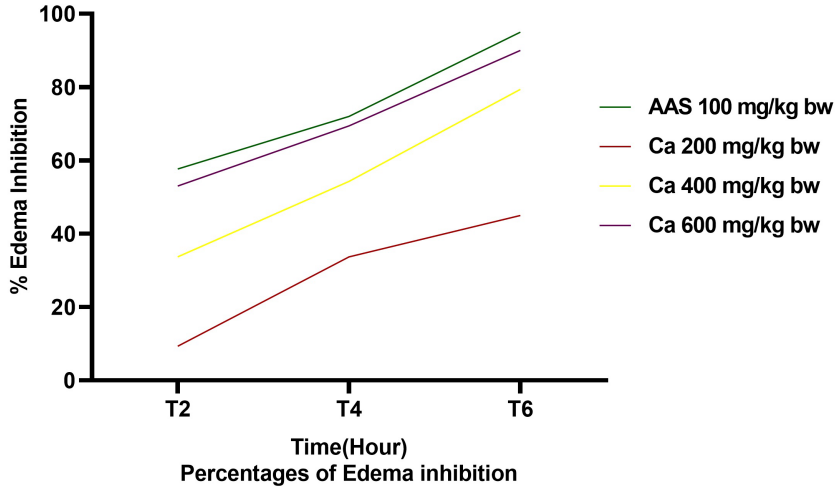

Figure 5. Percentage inhibition of hind paw edema in rat treated with hydroethanolic extract of Ca as a function of time. Legend: AAS: acetylsalicylic acid; Ca: *Crotaeva adansonii*.

4. Discussion

The reducing properties of plant extracts are generally associated with the presence of reductones [29], which have been shown to exert antioxidant activity by breaking the chain of free radicals through the donation of a hydrogen atom [30]. Reductones are also known to react with certain peroxide precursors, thereby preventing the formation of peroxide.

Oxidative stress results from an imbalance caused by the excessive production

of ROS or a reduction in the antioxidant defenses of the organism. Generally, the reducing properties of antioxidant are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom [29]. Phytochemical analysis confirmed the presence of important phenolic compounds such as tannins and flavonoids which were known to be natural bioactives substances and antioxydant properties.

Phenolic compounds such as condensed tannins and flavonoids were quantified. The flavonoid concentrations obtained are significant and reveal the bioactive nature of both plants. Flavonoids are known for their antioxidant activities in general and their ability to trap O_2 radicals, and are inhibitors of XOR [31]. Tannins are endowed with a great antioxidant capacity due to their phenol nuclei [32] [33]. They have the particularity of inhibiting lipid peroxidation by acting as a proton donor and free radical acceptor thus stopping the self-oxidation mechanism [34] [35].

Medicinal plants used in folk medicine are particularly interesting for investigation of their antioxidant effects. Some authors reported that the therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases [36].

The antioxidant ethanol extracts of *Cajanus cajan* and *Crotaeva adansonii* leaves and standard antioxidant (ascorbic acid) towards the DPPH radical was evaluated. This capacity reduction is determined by a decrease in absorbance induced antiradical substances.

This method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant, inducing a color change from purple to yellow at 517 nm. The degree of reduction in absorbance measurement indicates the radical scavenging (antioxidant) power of the extract.

The solubility of the antioxidant compounds was found to have a significant effect on the recovery of compounds during extraction. Thus, the polarity of solvents has an indirect function in the extraction process, because it can raise the solubility of antioxidant compounds [37].

The hydroethanolic extract of the two plants showed different percentages of inhibition of DPPH anti-radical activity depending on the concentration. Several studies have shown that DPPH anti-radical effects increase sharply with increasing sample concentration and standard concentration, up to a certain point and are therefore considered to be highly dependent on the concentration of the extract [38] [39]. The results obtained in this study indicate that hydroethanolic extracts from both plants have a remarkable ability to donate electrons to reactive free radicals, converting them into more stable, non-reactive species, to reduce oxidized intermediates, and to act as primary antioxidant substances.

It is reported that phenolics compounds and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms [40]. Polyphenols have the function to scavenge the free radicals in human body and to help maintain healthy body by scavenging or removing

the reactive oxygen species (ROS) [13].

The strong inhibition of the DPPH radical observed in the hydroethanolic extract of both plants, and particularly in the hydroethanolic extract of *Cajanus cajan*, could be linked to polyphenolic compounds capable of donating electrons or transferring hydrogen atoms in order to neutralize free radicals. It could therefore be a promising therapeutic agent for treating stress induced by pathological conditions. The antioxidant activities of hydroethanolic extracts of *Cajanus cajan* and *Crotaeva adansonii* may also be related to their total flavonoid content.

Several studies have reported the biological activity of flavonoids [41]. But the best-described property of almost every group of flavonoids is their capacity to act as antioxidants. As antioxidants, flavonoids have been reported to be able to interfere with the biochemical pathways involved in the generation of reactive oxygen species (ROS), quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction [42] [43]. It has been shown that the antioxidant molecules such as Ascorbic acid, flavonoids and tannins reduce and discoloured DPPH due to their ability to yield hydrogen [40]. Whatever the nature of the radical-scavenging power of our plants extracts, it is to see that there is a correlation between the polyphenolics compounds and the antioxidant activities of the extracts were complex. Several reasons could be resulted in the differences of the extracts in their compositions, and consequently their antioxidant activities [44] [45]. Mechanism of DPPH that was electron transfer method. So they may present differing results, each only partially reflecting the antioxidant activity [46] [47]. The Folin Ciocalteu Reagent method to measure the polyphenolics content could be disrupted by other soluble components in extracts such as proteins, peptides, polysaccharides, and pigments.

Interest in flavonoids as antioxidant therapy for cardiovascular disease originates from epidemiological data suggesting improved cardiovascular outcomes in individuals with high levels of intake of food and beverages with high flavonoid content [48] [49] as well as cellular work suggesting a strong antioxidant effect of these compounds [50]. The limited oral bioavailability of flavonoids suggests that cellular signaling mechanisms, rather than anti-free radical activity, are more likely to be responsible for the sustained cardiovascular benefits of flavonoids [51].

Carrageenan has been used to evaluate the anti-inflammatory activity of various products, including plant extracts. Intraplantar injection of carrageenan causes local inflammation when injected into the sole fascia [52]. Intraplantar injection of carrageenan resulted in a time-dependent increase in rat paw volume. The cause of this inflammatory response is a tissue lesion that induces the synthesis of histamine, prostaglandins, leukotrienes and other substances [53]. PAF (p1aqueta activating factor), cytokines, NO (nitric oxide), and TNF (tumor necrosis factor), are mediators that promote vasodilation, leading to redness and heat at the site of inflammation [54].

The maximum increase was obtained after 6 hours in the control group. However, administration of the extracts significantly reduced paw volume. The hy-

droethanolic extract of both plants inhibited edema in a dose-dependent manner, with the strongest inhibition observed six hours after carrageenan administration. The first phase of inflammation is characterized by the production of serotonin, histamine and bradykinin. The second phase is due to the biosynthesis of prostaglandins [55] associated with migration of leukocytes to the inflamed area [56].

On the basis of the above results, both plants inhibit both stages of the inflammation process, making them potential candidates for the treatment of inflammation-related diseases.

These mediators promote vasodilation, resulting in redness and heat at the site of inflammation.

This suggests that extracts of *Cajanus cajan* and *Crotaeva adansonii* act in the same way as salicylic acid. Studies have shown that salicylic acid, used as a reference, acts in the second phase of inflammation by inhibiting the synthesis of these different mediators [57]. By inhibiting the production of prostaglandins through the inhibition of cyclooxygenase (COX2), it limits the lowering of the pain threshold, hence its analgesic action, as well as inflammatory reactions, hence its antipyretic activity [25] [26].

These results suggest that extracts of *Cajanus cajan* and *Crotaeva adansonii* have an effect that opposes the action of endogenous pro-inflammatory mediators. This action is more likely to be exerted on cyclooxygenase, the enzyme responsible for prostaglandin synthesis [58].

The results presented indicate that hydroethanolic extracts of *Cajanus cajan* and *Crotaeva adansonii* alleviate oxidative stress thanks to their antioxidant properties. The antioxidant profile of these two plants can be exploited to treat radicals associated with pathological conditions. It has also been shown that the anti-free radical effects on the DPPH radical increased to a certain extent with increasing sample concentration and were therefore highly dependent on the concentration of the extract. This high activity is not limited to phenolic compounds, but may be due to the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, flavonoids, lignans, alkaloids, vitamins (ascorbic acid), etc. [35] [59].

5. Conclusions

This study reported on the phytochemical analysis, antioxidant and anti-inflammatory activities of *Cajanus cajan* and *Crotaeva adansonii*. The effectiveness of free radical scavenging increases with concentration, and this capacity depends on both the quantities and types of phenolic compounds present in the extracts. However, the industrial exploitation of the hydroethanolic extract of phenolic compounds from *Cajanus cajan* and *Crotaeva adansonii* appears promising. These two plants can be used as natural antioxidant supplements to prevent auto-oxidation and the degradation of foodstuffs. In addition, free radical scavenging activity is also useful as a disease-preventive property. The antioxidant activity exhibited by the hydroethanolic extract of *Cajanus cajan* and *Crotaeva adansonii* could jus-

tify the ethnotherapeutic use of these plants by traditional healers. In order to link each fraction to its biological activity, bio-guided fractionation of the extracts would be necessary (e.g., HPLC, LC-MS/MS, GC-MS) to isolate and identify the specific bioactive compounds responsible for the observed effects. It may also be possible to study the relationships between chemical structure and antioxidant/anti-inflammatory activity while comparing the activity of the isolated compounds to that of the crude extract in order to identify synergistic or antagonistic effects.

Research perspectives should evolve from a global approach towards precise identification of active compounds, in-depth mechanistic understanding, and biological and pharmacological validation, paving the way for innovative therapeutic or nutritional applications.

Conflicts of Interest

The authors have nothing to declare as far as the conflict of interest is concerned.

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