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Antioxidative Effects and Mechanisms of Antihypertensive Potential of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC in Rats

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Abstract

Introduction: Hypertension is currently recognized as a global public health problem because of its frequency and the associated risks of cardiovascular and renal disease. In this view, medicinal plants such as *Croton gratissimus* Burch (Crt) and *Schrankia leptocarpa* DC (Sch) are known to have many and various metabolites possessing potential for the prevention and treatment of several diseases.

Methods: The present study was to evaluate the effects of ethanolic extracts of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC to assess their antioxidant activity and efficacy in the treatment of L-NAME induced hypertension in Wistar albino rats, heart rate, waves and to elucidate the mechanism of action of the observed effects. Total phenolic content was determined by using the Folin-Ciocalteu method while total flavonoids and condensed tannins were estimated using standard procedures. The antioxidant capacities in the forms of DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) were evaluated by spectrophotometric methods.

Results: The results showed that total phenolic, flavonoids and condensed tannins values were higher in *Schrankia leptocarpa* DC ethanolic extracts: 40.60 mg equivalent of Gallic acid/g of dry plant, 30.67 mg equivalent of rutin/g of dry plant, 17.50 ± 0.45 mg equivalent of catechin/g of dry Plant. IC50 value of *Croton gratissimus* Burch extract was 0.3638 mg/ml and *Schrankia leptocarpa* DC extract was 0.1215 mg/ml as opposed to that of ascorbic acid 0.040 mg/ml. The strongest ferric reducing ability was found in ethanol extract of *Schrankia leptocarpa* DC (1000 µmol AAE g^{-1}) followed by ethanol extract of *Croton gratissimus* Burch (800 µmol AAE g^{-1}). Treatment with L-NAME alone resulted in a progressive increase on mean arterial pressure. This increase was significant as early as the first week, as the mean arterial pressure increased from 121 ± 3 mmHg to 140 ± 4 mmHg ($p<0.05$). After four weeks of treatment, the mean arterial pressure reached a value of 190 ± 2 mmHg, whereas the group receiving L-NAME and *Croton gratissimus* Burch and *Schrankia leptocarpa* DC developed a blood pressure of: 170 ± 3 mmHg and 150 ± 4 mmHg only. These results show an antihypertensive effect of these extracts. Moreover, the study of the effect on heart rate of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC on the isolated and perfused rat heart model showed a negative inotropic effect which could explain in part the antihypertensive effect found. In summary, *Croton gratissimus* Burch and *Schrankia leptocarpa* DC (20 mg/kg) oppose the adrenaline-induced rise in mean arterial pressure throughout the experiment. This opposition to this elevation is more pronounced with *Schrankia leptocarpa* DC extract. These results suggest that the ethanolic extracts of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC has some hypotensive effects via the muscarinic receptors.

Lipid profile groups treated with *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts significantly ($p<0.005$) decreased. This study shows that these two plants have a hypoglycemic and hypolipidemic effects. We observed significant increases compared to the negative controls in

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total protein (p<0.005). For serum Na+ concentration in rats, all extracts showed highly significant decreases (p<0.005) when compared with control. For chloride ions, a significant decrease (p<0.005) was found compared with control for the 500 mg/kg dose of *Schrankia leptocarpa* DC. However, no significant increase was obtained for potassium.

Conclusion: The present study suggests that ethanolic extracts of *Croton gratissimus* Burch and *Schrankia leptocarpa* have a hypotensive effect mediated by muscarinic receptors. This effect may justify the use of this plant in the treatment of hypertension in traditional medicine.

Keywords: *Schrankia leptocarpa*; *Croton gratissimus*; Antioxidant; Blood pressure; Heart rate

Introduction

Hypertension is currently recognized as a global public health problem because of its frequency and the associated risks of cardiovascular and renal disease. More than a quarter (26.4%) of the world's adult population is hypertensive, and this proportion is expected to rise to 29.2% by 2025, representing nearly 1.6 billion hypertensive individuals [1,2]. In Africa, more than 20 million people suffer from hypertension; its prevalence is between 25 and 35% in adults aged 25 to 64 years, with a tendency to worsen over time [3,4].

Hypertension is currently one of the major risk factors for cardiovascular, neurological and renal events. Several studies demonstrated that excessive and chronic ingestion of ethanol causes cardiomyopathy, cardiac arrhythmias, heart failure and hypertension [5-6].

Similarly, numerous studies also indicate that diets high in carbohydrates, particularly sugars and even more particularly sucrose and fructose increase the risk of cardiovascular diseases including hypertension [7]. It has been reported that some metabolic abnormalities such as hyperinsulinemia, insulin resistance and hypertriglyceridemia as well as hyperactivity of the sympathetic nervous system and oxidative stress were frequently associated with the pathogenesis of both ethanol and sucrose induced-hypertension [8]. It is well known that hypertension can often lead to lethal complications if left untreated [9]. In spite of the large number of modern drugs, people largely use complementary and alternative medicine to prevent and cure illness [10].

In Benin, a West-African country, during the past decades, hypertension prevalence has been characterized by a progressive rise to reach 27.9% in 2008 [11].

Many hypertensive subjects are not aware of their hypertensive status, and are often diagnosed when complications arise. The low living standard and the relatively high cost of hypertension treatment make access to medical care difficult. Thus, a significant proportion of hypertensive patients used medicinal plants for their treatment. In West Africa, more than one hundred of medicinal plants used in the treatment of hypertension have been reported by ethnobotanical studies [12,13].

In response to the expansion of these highly managed diseases, the WHO, in its resolution AFR/RC50/R3 of August 31st, 2000, encouraged African countries to develop regional strategies on traditional medicine to undertake research on medicinal plants and promote their optimal use in health care delivery systems. For a long time, medicinal plants have been an inexhaustible source for African traditional healers to treat (or cure) certain often life-threatening conditions. Today, many traditional healers continue to draw on their secret by using the derivatives of these medicinal plants to treat several serious diseases [14,15]. In Benin, about 90% of the people depend on traditional medicines and traditional healers as their main source of health care [16].

Therefore, there is an urgent need to develop new and effective drugs for the treatment of hypertension. In this view, medicinal plants such as *Croton gratissimus* Burch (Crt) and *Schrankia Leptocarpa* DC (Sch) are known to have many and various metabolites possessing potential for the prevention and treatment of several diseases.

The medicinal value of these practices requires the isolation and identification of new chemicals [17]. For this purpose, phytochemical studies are being intensively investigated to help determine the chemical constituents contained in medicinal plants. Phytochemical screening plays an essential role in the characterization of chemical family groups in a given plant [15].

The objectives of the present study were to evaluate the effects of ethanolic extracts of *Croton gratissimus* Burch and *Schrankia Leptocarpa* DC to assess their antioxidant activity and efficacy in the treatment of L-NAME induced hypertension in Wistar albino rats, heart rate, waves and to elucidate the mechanism of action of the observed effects.

Materials and Methods

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 *Croton gratissimus* Burch and *Schrankia Leptocarpa* DC. They were collected in the agro-ecological zone of the Abomey-Calavi commune in July 2018 and brought to the Physiopathology/ Pharmacology and Molecular Toxicology/FAST-UAC laboratory for drying. Their certification was carried out at the National Herbarium of Benin by comparison with reference samples kept under the numbers AA 6745/HNB and AA 6744/HNB respectively for *Croton gratissimus* Burch and *Schrankia leptocarpa* DC. After an average drying period of four weeks at about 23°C, they were ground and green colored powders were obtained.

Ethanolic extract of *Croton gratissimus* Burch: 208.7 grams of powder were macerated in 1000 ml of 95° ethanol and sonicated before filtering. The maceration residues were macerated and sonicated twice more in 750 ml of 95° ethanol. The filtrates were passed through a rotavapor in order to isolate the solvent from the crude extract. Then, the crude extract obtained was placed in an oven at 45° for several days. At the end, a pasty, blackish-green extract was obtained. The obtained extracts were stored at 4°C until biological assay.

Ethanolic extract of *Schrankia Leptocarpa* DC: In 400 ml of 95° ethanol was macerated 130 grams of *Schrankia leptocarpa* DC powder which after sonification was passed to filtration. The residues obtained after this first maceration underwent a series of macerations, sonifications and filtrations with decreasing volumes ranging from

400 ml to 150 ml. The filtrates thus collected were rotavaporised to isolate the crude extract from the ethanol. The crude extract thus obtained was deposited in an oven at 45° for several days. At the end, a pasty, blackish- green extract was obtained. The obtained extracts were stored at 4°C until biological assay.

Phytochemical screening

Phytochemical screening of the plant was carried out according to the methods described by Wagner and Blat [18] and Bruneton [19] for the detection of plant secondary metabolites. Tannins, alkaloids, steroids, coumarins, saponins, naphthoquinones, triterpenes, lignans, pigments, anthracene derivatives, triterpenes, lignans, pigments, anthracene derivatives 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.

Quantification of some bioactive molecules Total phenolic content: Total phenolics of each extract were estimated by Folin-Ciocalteu reagent method [20]. This method is based on the reduction in alkaline media of phosphotungstic mixture ($WO4^{2-}$) phosphomolybdic ($MoO4^{2-}$) of Folin reagent by the oxidizable group of phenolic compounds, leading to the formation of blue reduction products. Latter have a maximum absorption at 765 nm whose intensity is proportional to the amount of polyphenols present in the sample. Then, 200 µl of diluted sample were added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µl of saturated sodium carbonate (75 g/l) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. The standard calibration curve was plotted using Gallic acid (y=0.043 x=0.051; R^2=0.994). The mean of three readings was used and the results expressed as mg of Gallic Acid Equivalents (GAE)/100 mg of extract.

Total flavonoid content: The determination of flavonoids was performed according to the colorimetric assay described previously [21]. To 1 ml of extract (100 µg mL^{-1}), 3 ml of methanol, 0.2 ml of 1 M potassium acetate, 0.2 ml of 10% aluminum chloride and 5.6 ml of distilled water was added and left at room temperature for 30 min. Absorbance of the mixture was read at 415 nm using UV spectrophotometer. Quercetin was used as reference compound to produce the standard curve (y=0.325 x=0.363; R^2=0.995) and the results were expressed as mg of Quercetin Equivalent (QE)/100 mg of extract.

Condensed tannins: Condensed tannins were estimated using the method of author [22] modified by author [23] vanillin reagent was prepared by mixing equal volume: 8%, methanol at 37% and 4% of vanillin in methanol. The mixture was maintained at 30°C before the assay. Two hundred (200) µl of each extract to be analyzed were added to 1000 µl of reagent of vanillin; the mixture was stirred and incubated in darkness at 30°C for 20 min. The absorbance was measured at 500 nm by a spectrophotometer UV (Perkin Elmer) against white consisting of a mixture of methanol (37%) and HCl (8%) with equal volume.

DPPH radical scavenging activity

The antioxidant activity was determined according to the method previously described [24]. In the presence of antioxidant which is typical for DPPH free radical decays, the change in absorbency at 517 nm is followed spectrophotometrically. Briefly, 1.5 ml of a freshly prepared methanolic solution of DPPH (2%) was mixed with 0.75 ml of extract solution (1-0.007 mg/ml). After 15 min of incubation in the dark, at room temperature, absorbencies were read at 517 nm against a blank sample consisting of a 1.5 ml of methanol and 0.75 ml of extract solution. All tests were performed in triplicate. DPPH radical inhibition percentage was calculated according to the following formula: inhibition (%) = [(AB–As)/AB] × 100 were as is the sample (tested extract solution) absorbance and AB is the blank absorbance.

Ferric-reducing antioxidant power (FRAP) assay

This method measures the reducing power of antioxidants in a mixture by their ability to reduce ferric tripyridyl-triazine (Fe^{3+}-TPTZ) to ferrous (Fe^{2+}-TPTZ) at acidic pH [25]. Test tubes are filled with 0.5 ml of extracts at a concentration of 10 mg/ml. To these solutions are added 1.25 ml of a phosphate buffer solution (0.2 M, pH 6.6) and 1.25 ml of potassium hexacyanoferrate 1% $K_3Fe(CN)_6$ (1%). After incubation time of 30 min, 1.25 ml of trichloroacetic acid (10%) was added to the mixtures and centrifuged at 2000 rpm for 10 min. To volumes of 0.625 ml of supernatant taken from each tube were added 0.625 ml of distilled water and 0.125 ml of 0.1% $FeCl_3$. The absorbances were read on a spectrophotometer at 700 nm.

Effects of ethanolic extracts of *Croton gratissimus* and *Schrankia leptocarpa* on cardiovascular parameters

Animal experiment: The study used adult Wistar rats of both sexes, weighing 195 g to 280 g, two to three months of age and normotensive, with blood pressure around 120 mmHg. These rats are fed a growth and maintenance diet, with a permanent water supply. The temperature of the animal house is around 25°C, with a 12-h light-dark cycle (light: 7 am to 7 pm).

Antihypertensive effect of *Croton gratissimus* and *Schrankia leptocarpa*

For this study, rats were divided into five groups of six rats each.

1. Control group: Rats of this group were orally administrated (gavage) with distilled water

2. L-NAME group: Rats were treated with L-NAME at 20 mg/kg/day;

3. L-NAME-Crt extract group: rats were treated with L-NAME at 20 mg/kg/day and with

Croton gratissimus ethanolic extract at 500 mg/kg/day.

4. L-NAME-Sch extract group: rats were treated with L-NAME at 20 mg/kg/day and with *Schrankia leptocarpa* ethanolic extract at 500 mg/kg/day.

5. L-NAME-Captopril group: Rats were treated with L-NAME at 20 mg/kg/day and with captopril, an angiotensin-converting enzyme inhibitor, given as reference treatment at 100 mg/kg/day.

These rats receive the extract dose daily by gavage. Blood pressure is measured at the beginning of treatment and then once a week during the treatment period by the plethysmographic method [26].

Effects of *Croton gratissimus* and *Schrankia leptocarpa* extracts on heart rate

The heart mounted in the Langendorff device [27], first perfused with Krebs-Henseleit (KHB) perfusion solution alone for a period of 20 min to stabilize it, and then in the presence of *Croton gratissimus* and *Schrankia leptocarpa* extracts at concentrations of 20 mg/ml. These were tested in each case for ten minutes. The change from one concentration to the other was always preceded by perfusion of the heart with KHB buffer alone until the heart returned to its original rhythm.

Effects of ethanolic extracts of *Croton gratissimus* and *Schrankia leptocarpa* on adrenaline- induced hypertension

1. Centro group: Rats of this group were orally administrated (gavage) with distilled water.

2. Adrenaline (Adr) (10 µg/kg) was administered to normotensive rats (n=6) as a single dose.

3. Adr-Crt extract group: *Croton gratissimus* extract (20 mg/kg, i.v) pretreated (5 min) as a single dose.

4. Adr-Sch extract group: *Schrankia leptocarpa* (20 mg/kg, i.v) pretreated (5 min) as a single dose.

Biochemical assay and ionogram

Characteristic markers of pathophysiology related to hypertension were measured. These included glucose, total protein, total cholesterol, LDL, HDL, triglycerides. In addition, the ionogram consisted of the determination of potassium, chlorine and sodium. The biochemical tests were carried out with the reagents of the spectrum kit and on the EMP-168 spectrophotometer.

Statistical analysis

The data collected were recorded in the Excel 2010 spreadsheet. Means (m) and Standard Deviations (SD) were calculated for each batch. Statistical analyses were performed using Minitab 16 and Stat view 5.0 software. The results obtained at the level of the different groups were compared using the analysis of variance ANOVA (one way) and the Fisher test. The differences were significant for p-value <0.005.

Results

Extraction yields

The extraction yield obtained for ethanolic extraction for the studied plants is shown in Table 1. From this table, it emerges that the ethanolic extract of *Croton gratissimus* Burch gave a better yield than ethanolic extract of *Schrankia leptocarpa* DC. Thus, yields of 8.56% for *Croton gratissimus* Burch and 6.78% for *Schrankia leptocarpa* DC were obtained.

Phytochemical screening

Table 1 presents the results of the phytochemical screening of samples of the plants studied. From this table, it should be noted that the plants' extracts present a varied richness in secondary metabolites.

From this Table 1, it should be noted that the plants' extracts present a varied richness in secondary metabolites. Thus, we note the presence of Sterol and Triterpene, Anthracene derivatives, lignan, tannins, flavonoid, Anthocyanins, Coumarin in all the samples of the plants studied with a strong presence in the ethanolic extracts of *Schrankia leptocarpa* DC. However, no saponosides, mucilages, Coumarin and alkaloids were found in the ethanolic extract of *Croton gratissimus* Burch.

After the phytochemical screening, three chemical compounds groups such as total phenolic compounds, flavonoids and tannins were measured on the ethanolic extracts. Table 2 presented values obtained.

Quantification of some bioactive molecules

Antioxidant activity: DPPH is stable free radical that accepts an electron or hydrogen to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1,1-diphenyl-2-picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug [28].

Table 1: Phytochemical Constituents extracts of ethanolic extract of *Croton gratissimus Burch.* and *Schrankia leptocarpa DC.*

Secondary metabolites	Phytochemical analysis of *Croton gratissimus Burch*	Phytochemical analysis of *Schrankia leptocarpa DC.*
Saponosides	-	+
Sterol and Triterpene	+	+
Anthracene derivatives	+	+
Coumarin	-	+
Mucilage	-	+
Lignin	+	+
Tanins	+	+
Flavonoid	+	+
Anthocyanins	+	+
Alkaloids	-	+

+ Present; - Absent

Table 2: Total phenolic compound, flavonoids and tannins content in *Croton gratissimus Burch* and *Schrankia leptocarpa DC.* Extracts.

Ethanolic extract	Total Phenolic compound[(a)]	Flavonoids[(b)]	Condensed tannins[(c)]
Croton gratissimus Burch	20.10 ± 0.80	18.51 ± 0.08	20.40 ± 0.14
Schrankia leptocarpa DC.	40.60 ± 0.54	30.67 ± 0.66	17.50 ± 0.45

[(a)]mg equivalent of Gallic acid/g of extract; [(b)]mg equivalent of Rutin/g of extract; [(c)] mg equivalent of Catechin/g of extract
Values are mean ± SE (n=6)

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC. Extracts is given in Figure 1. This activity was increased by increasing the concentration of the sample extract. The reduction of DPPH radical by antioxidants is evaluated by the decrease in absorbance at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation [29]. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant potential of medicinal plants [30]. In this study, the DPPH radical scavenging activities of extracts therefore increased gradually in a dose concentration dependent manner (0.002-1 mg/ml) (Figure 1). The results show that IC50 value of *Croton gratissimus* Burch extract was 0.3638 mg/ml and *Schrankia leptocarpa* DC extract was 0.1215 mg/ml as opposed to that of ascorbic acid 0.040 mg/ml.

Antioxydant activity obtained using the FRAP method

The reducing capacity of the extracts is another significant indicator of antioxidant activity. The presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron which is an important mechanism of phenolic antioxidant action [31]. In this assay, the compounds with reduction potential react with potassium Ferricyanide (Fe^{3+}) to form potassium Ferrocyanides (Fe^{2+}), which then react with ferric chloride to form ferric ferrous complex that is greenish in color [32].

The strongest ferric reducing ability was found in ethanol extract of *Schrankia leptocarpa* DC. (1000 µmol AAE g^{-1}) followed by ethanol extract of *Croton gratissimus* Burch (800 µmol AAE g^{-1}) (Figure 2).

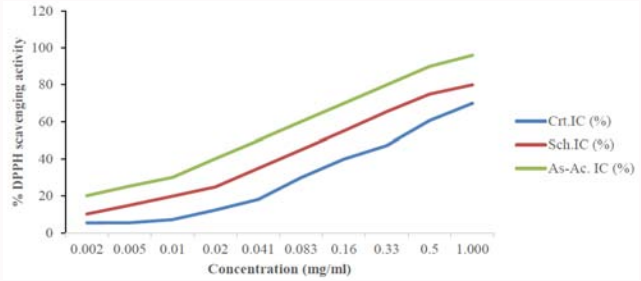

Figure 1: Radical scavenging activity of *Croton gratissimus Burch* and *Schrankia leptocarpa DC.* extracts.

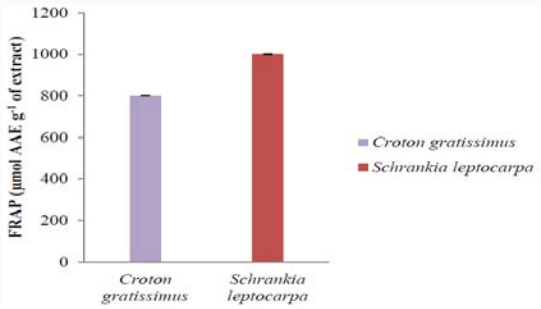

Figure 2: Antioxidant activity obtained using the FRAP method. Each value represents a mean ± SE (n=3).

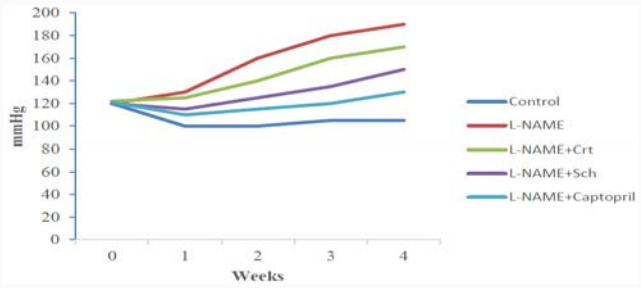

Figure 3: Effect of *Croton gratissimus Burch* and *Schrankia leptocarpa DC* extracts on mean arterial pressure. Values are mean ± SEM, n=6 rats/group.

Effect of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts on mean arterial pressure

The results obtained are shown in Figure 3. Treatment with L-NAME alone resulted in a progressive increase on mean arterial pressure. This increase was significant as early as the first week, as the mean arterial pressure increased from 121 ± 3 mmHg to 140 ± 4 mmHg ($p<0.05$). After four weeks of treatment, the mean arterial pressure reached a value of 190 ± 2 mmHg, confirming hypertension. The mean arterial pressure of the control group, on the other hand, remained around 100 mmHg.

The concomitant administration of L-NAME and *Croton gratissimus* Burch on the one hand, and of L-NAME and *Schrankia leptocarpa* on the other hand, showed that these plants slowed the increase in mean arterial pressure compared with the group treated with L-NAME alone. After four weeks of treatment, the mean arterial pressure reached 170 ± 3 mmHg for the L- NAME + *Croton gratissimus* Burch group and 150 ± 4 mmHg for the L-NAME + *Schrankia leptocarpa* group.

These values were significantly different compared with the arterial pressure of the group treated with L-NAME alone, which

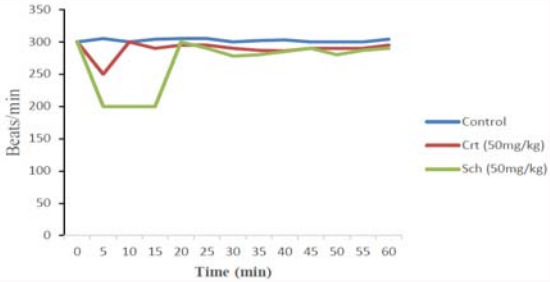

Figure 4: Effects of *Croton gratissimus Burch* (Crt) and *Schrankia leptocarpa DC* (Sch) extracts on heart rate of normotensive rats. Values are mean ± SEM, n=6 rats/group.

was about 190 ± 2 mmHg. It can be noted that the effects of *Croton gratissimus* Burch and *Schrankia leptocarpa* extracts only started to manifest themselves from the first week of treatment. The results of this study therefore show that *Croton gratissimus* Burch and *Schrankia leptocarpa* caused a significant slowing of the evolution of arterial pressure induced by L-NAME. However, arterial pressure remained significantly elevated compared to the control. The effect of captopril was a reduction of blood pressure to 130 ± 2 mmHg.

Effects of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts on heart rate of normotensive rats

The ethanolic extract (50 mg/kg) of *Croton gratissimus* Burch significantly decreased the heart rate from 300 ± 10 to 250 ± 6 beats/min ($p<0.005$) before returning to its approximate initial value 5 min later (Figure 4). The ethanolic extract (50 mg/kg) of *Schrankia leptocarpa* DC also significantly decreased the heart rate from 300 ± 12 to 200 ± 6 beats/min ($p<0.005$) before returning to its approximate initial value 10 min later (Figure 4).

Effects of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts on adrenaline- induced arterial pressure elevation

Administration of adrenaline (10 µg/kg) in normotensive rats caused an immediate increase in blood pressure from 120 ± 2 to 190 ± 4 mm Hg ($p<0.005$). This pressure subsequently decreased rapidly to below the initial value before stabilizing slightly above the initial value (Figure 5). Administered 5 min after ethanolic extract of *Croton gratissimus* Burch (20 mg/kg), adrenaline only caused an increase in blood pressure of 140 ± 3 mm Hg ($p<0.005$) (Figure 5). Administered 5 min after the ethanolic extract of *Schrankia leptocarpa* DC (20 mg/kg), adrenaline only caused an increase in blood pressure of 130 ± 2 mm Hg ($p<0.005$) (Figure 5). In summary, Figure 4 clearly shows that ethanolic extracts of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC (20 mg/kg) oppose the adrenaline-induced rise in mean arterial pressure throughout the experiment. This opposition to this elevation is more pronounced with *Schrankia leptocarpa* DC extract.

Biochemical data

The first aim of the exploration of the different values obtained for the biochemical parameters and the ionogram was to verify if there was indeed installation of arterial pressure after the induction phase. The second aim was to verify the efficacy of the extracts both in relation to captopril (reference molecule), the positive controls and the negative control.

Croton gratissimus Burch and *Schrankia leptocarpa* DC extracts decrease hyperglycemia (Figure 6) shows that arterial pressure

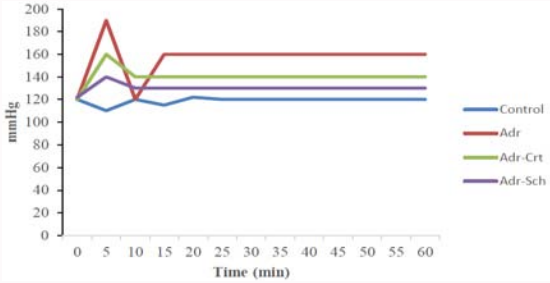

Figure 5: Effects of Adrenaline (Adr) on arterial pressure in normotensive rats not pretreated with *Croton gratissimus Burch* and *Schrankia leptocarpa DC* and pretreated with *Croton gratissimus Burch* and *Schrankia leptocarpa* D. Values are mean ± SEM, n=6 rats/group.

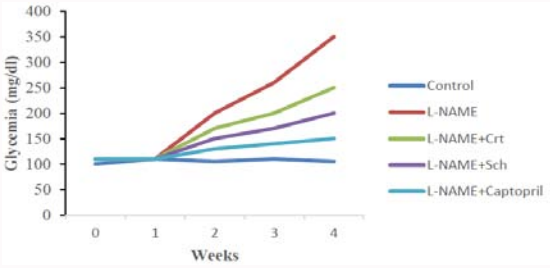

Figure 6: Effects of *Croton gratissimus Burch* and *Schrankia leptocarpa DC* extracts on blood glucose concentrations in control and rat made hypertensive by 4 weeks administration of L-NAME. Each value represents the mean ± SEM (n=6).

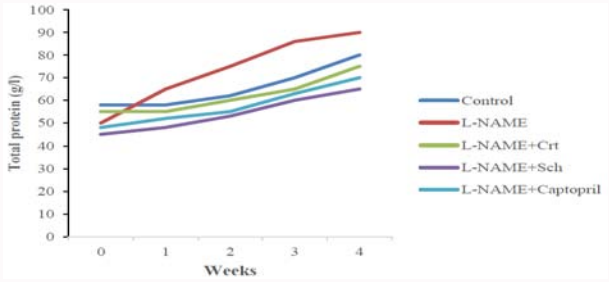

Figure 7: Effects of *Croton gratissimus Burch* and *Schrankia leptocarpa DC* extracts on total protein concentrations. Each value represents the mean ± SEM (n=6).

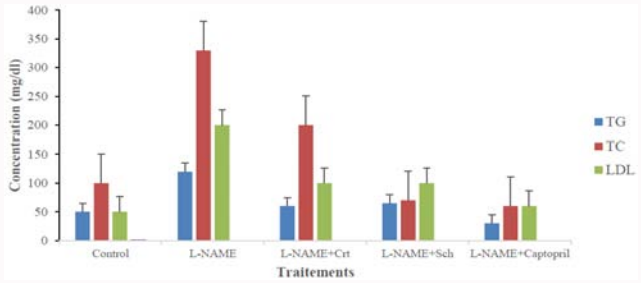

Figure 8: Anti-hyperlipidemic activities of *Croton gratissimus Burch* and *Schrankia leptocarpa DC* extracts on blood pressure, L-NAME-induced hypertension Wistar rats. Each value represents the mean ± SEM (n=6).

induced by L-NAME in rats. With the concentration of 500 mg/kg, significantly decreased ($p<0.005$) the glucose levels during 3 weeks suggesting that these extracts have hypoglycemic effect.

In the present study was also accompanied by the elevation in serum total protein in group treated with L-NAME administered rats

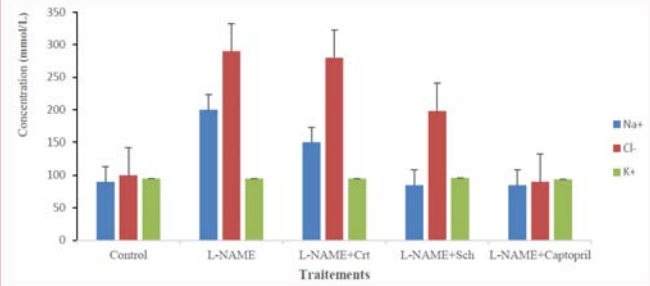

Figure 9: Effects of *Croton gratissimus Burch* and *Schrankia leptocarpa DC* extracts on Serum sodium ions, chloride ions and potassium ions levels of L-NAME-induced hypertension Wistar rats. Each value represents the mean ± SEM (n=6).

as compared with control ($p<0.005$). As shown in Figure 7.

We observed significant increases compared to the negative controls in total protein ($p<0.005$). Induction of arterial pressure in Wistar rats increased serum levels of Triglyceride (TG), Total Cholesterol (TC), Low-Density Lipoproteins (LDL). Administration of *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts on plasma lipid profile normalized the increase in TG, cholesterol, compared to control rats. Regarding the changes in HDL levels, arterial pressure decreased HDL levels. *Croton gratissimus* Burch and *Schrankia leptocarpa* DC extracts normalized it relative to control levels confirming their hypolipidemic action (Figure 8).

Ionic data

For serum Na^+ concentration in rats, all extracts showed highly significant decreases when compared with control ($p<0.005$) (Figure 9). For chloride ions, a significant decrease was found compared with control ($p<0.005$) for the 500 mg/kg dose of *Schrankia leptocarpa* DC (Figure 9). However, no significant increase was obtained for potassium.

Discussion

In the present study, the solvent used for the extractions was ethanol. This choice was motivated by the ability of this solvent to extract the bioactive substances present in the plants. Indeed, Siddhuraju et al. [33] already reported in a study they carried out on *Moringa olifera* that methanol (80%) and ethanol (70%) proved to be the best solvents for the extraction of antioxidant compounds [33]. The results we obtained also confirmed this conclusion, as large families of bioactive metabolites were identified.

The result obtained for 130 g of *Schrankia leptocarpa* was 6.78%. This result was lower than that obtained by Allangba et al. [34] which were 7.76% for 200 g. On for 200.8 g of *Croton gratissimus*, the result obtained (8.6%) was respectively higher than the result obtained for 100 g by Okokon et al. which was 3.81% and Njoha et al. [35,36] which was 6.23%. As the solvents used were ethanol in all these studies, we can explain these differences on the one hand by the quantities of powder used and on the other hand by the ecological factors that conditioned the growth of these plants in the different regions.

The phytochemical study of the two plants showed that they share certain secondary metabolites such as Saponosides sterols and triterpenes, anthracene derivatives, Coumarin, Mucilage, lignan, Tanins, Anthocyanins, Alkaloids and flavonoids. It should be noted that coumarins, saponosides, mucilages and alkaloids are exclusively present in *Schrankia leptocarpa* DC.

These results are similar to those obtained by Naidoo et al. [37] with the difference that she revealed the presence of alkaloid in *Croton gratissimus*. Lagnika et al. [38] for phytochemical screening of *Schrankia leptocarpa* had obtained results consistent with those described in the present study. The compound assays quantified phenolic compounds, condensed tannins and flavonoids. These flavonoid concentrations obtained are significant and reveal the bioactive character of both plants. Indeed, flavonoids are known for their antioxidant activities in general and their O^{2-} radical scavenging power and are XOR inhibitors [39]. Tannins are endowed with a great antioxidant capacity due to their phenol nuclei [40,41]. They have the particularity of inhibiting lipid peroxidation by acting as a proton donor and free radical acceptor thus stopping the self-oxidation mechanism [42,43].

In general, total polyphenols are involved in the antioxidant activities of plants. A study conducted by Evenamede et al. [43], already showed that there is a strong correlation between the phenolic compound content and the antioxidant activity of the species he studied. Thus, he was able to establish that the correlation coefficient between the polyphenol content of *C. Sieberiana* extracts and antioxidant activity was highly significant with R^2=0.996 indicating that 99.6% of the antioxidant capacity of the extracts is due to the contribution of phenolic compounds which are the dominant antioxidants in these extracts [43]. These results were also consistent with those reported by other authors who demonstrated a positive correlation between total phenolic content and anti-free radical activity [44,45]. The proportions of phenolic compounds revealed in our study thus abound in favor of their antioxidant powers.

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 [46,47]. Mechanism of DPPH that was electron transfer method and FRAP was redox essays.

Our plants thus have considerable antioxidant activity because the smaller the IC50 value, the greater the antioxidant activity of the extract [48]. The iron reduction study (FRAP) also confirmed that *Schrankia leptocarpa* had higher antioxidant power than *Croton gratissimus*. In our case, both methods not only confirmed the antioxidant activity of the two plants but also allowed us to see which one has the higher antioxidant activity.

The concomitant administration of L-NAME and extracts of *Schrankia leptocarpa* and *Croton gratissimus* shows that these plants slow down the increase in blood pressure compared to the group treated with L-NAME alone. The results of this study therefore show that extracts of *Schrankia leptocarpa* and *Croton gratissimus* caused a significant slowing of the evolution of blood pressure induced by L-NAME.

The antihypertensive effect observed could be related to the presence of flavonoids. Indeed, it was reported in this study that these plants are rich in flavonoids. Many studies report that flavonoids have an antihypertensive effect. Indeed, these phenolic compounds have vasorelaxant effects related to the increase in the production of Nitric Oxide (NO). Thus, it has been shown that the messenger Ribonucleic Acid (mRNA) of endothelial-type Nitric Oxide Synthase (NOSe) was overexpressed in patients on a diet rich in phenolic compounds [49] and that this increased NO production seemed to lead to vasorelaxation of the blood vessels [50]. It has also recently been shown that phenolic compounds from arbutus (*Arbutus unedo*) induce endothelial vasorelaxation through a mechanism related to the stimulation of guanylate cyclase [51].

Several plant extracts tested on the isolated and perfused heart report cardiac effects on the isolated heart model [52-54]. The cardiac effects of *Schrankia leptocarpa* and *Croton gratissimus* extracts were also evaluated, in particular the heart rate on the isolated and perfused heart of rats.

Intravenous administration of *Schrankia leptocarpa* and *Croton gratissimus* extracts caused an immediate decrease in mean arterial pressure and heart rate in normotensive rats. This extract thus causes hypotension which could be explained by the negative chronotropic effect of these extracts. Furthermore, these extracts (20 mg/kg, i.v) administered 5 min before administration of epinephrine (10 µg/kg, i.v) partially counteract the rise in blood pressure induced by the latter.

This result could be explained by the reduction of calcium influx that these extracts would cause by activating muscarinic receptors. There is therefore no direct antagonism between these extracts and adrenaline. These extracts could thus have antihypertensive properties. Indeed, it has been shown that these ethanolic extracts of *Schrankia leptocarpa* and *Croton gratissimus* prevent and treat arterial hypertension induced by L-NAME in rats.

The presence of certain chemical families (secondary metabolites), in particular anthocyanins which would have sedative cardiac properties [55] or other substances with anti-calcium effect, could explain the effects of *Schrankia leptocarpa* and *Croton gratissimus* extracts on the isolated rat heart.

In addition, arterial hypertension is accompanied by an increase in lipid parameters and in such cases there is a potential increased atherogenicity, characterized by a significant rise in total cholesterol, LDL and triglyceride levels [56]. The significance levels obtained for these different parameters in the present study confirm the occurrence of hypertension before the animals were subjected to the different treatments.

Work carried out by Stepein et al. [57] showed that the decrease in lipid parameters (total cholesterol and LDL) in Wistar rats made hypertensive by a diet rich in sodium reflected a beneficial effect of *Camellia sinensis* in the treatment of the pathology. Indeed, we obtained similar results with our extracts.

Schrankia leptocarpa and *Croton gratissimus* resulted in a very significant decrease in triglycerides. It seems that the extracts significantly regulate the lipid profile of rats, which is of great interest for the treatment of cardiovascular diseases, especially hypertension.

The lipid lowering potential of the extract may be attributed to the presence of phytochemical constituents like flavonoids, saponins and tannins [58-60]. Flavonoids are reported to lower LDL-cholesterol and increase HDL-cholesterol concentrations in hypercholesteremic animals [61]. Saponins are reported to inhibit pancreatic lipase activity in high fat diet fed mice leading to greater fat excretion due to reduced intestinal absorption of dietary fats [62]. Similarly, tannins are recognized for their ability to inhibit lipid absorption [63].

The improvement of blood glucose level induced by *Schrankia leptocarpa* and *Croton gratissimus* ethanolic extracts associated with

a reduction of serum triglycerides and total cholesterol as previously mentioned for most hypoglycemic treatments [64]. The glucose lowering effect of the extract may be due to its ability to improve the function of pancreatic β cells by increasing cells sensitivity to insulin and/or the rate of glucose transport and utilization [65]. In the present study was also accompanied by the elevation in serum total protein abnormalities such as vascular congestion, sinusoid dilatation and inflammation in the liver of hypertensive rats.

Very recently, a study reviewed all the analyses of potassium consumption and blood pressure and found an association between high potassium consumption and a reduction in blood pressure [66]. The results obtained in our study did not show an increase in potassium. However, Sodium ions (Na^+) decreased significantly at all doses. Indeed, recognized scientific groups have concluded that current levels of sodium in the diet are dangerous and cause hypertension [67,68]. Sodium acts on vascular stiffness, and reduces the adaptation of the vessels to the increase in blood volume that it induces and the consequence is an increase in blood pressure [69,70]. These significant decreases are therefore beneficial in maintaining normal blood volume on the one hand and slowing down vascular stiffness on the other. The 500 mg/kg dose of *Schrankia leptocarpa* was associated with a significant decrease in Chloride ions (Cl^-) [71]. Furthermore, some animal studies have shown that chloride plays a central role in modulating vasoconstrictive action, especially through angiotensin 2 [72,73]. The extract at this dose could therefore mimic the activity of ACE inhibitors that prevent the conversion of angiotensin I to angiotensin II and therefore have a beneficial effect on the arteries and the heart.

Conclusion

Schrankia leptocarpa and *Croton gratissimus* could thus act by reversing the inhibiting effect of L-NAME on NO synthase. Secondary metabolites detected in *Schrankia leptocarpa* and *Croton gratissimus*, have been shown to induce hypotensive effect in Wistar rat associated with alteration of cardiac function (decreased heart rate). Beneficial effects of polyphenol compounds on cardiovascular system have also been described. Thus, endothelium dependent vasorelaxant effect of many anthocyanins has been reported. It has also been shown that condensed tannins induced antihypertensive effect associated with endothelium dependent vasorelaxant activity. The observed antihypertensive effect could thus result from the synergistic action of pharmacological properties of alkaloids, tannins, Flavonoid, Coumarin and anthocyanin's present in *Schrankia leptocarpa* and *Croton gratissimus*. Thus, this study provides scientific validation of the empirical use of this medicine in the management of hypertension and could serve as a basis of the formulation of improved traditional drug for hypertension. However, more study need to be carried out in order to determine the exact mechanism of action of *Schrankia leptocarpa* and *Croton gratissimus*.

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