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Effects of the aqueous extract of *Persea americana* Mill (Lauraceae) leaves on the rat isolated aorta: evaluation of vasomotility and acute toxicity

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Abstract

Nowadays, hypertension is a real scourge for public health. *Objectives:* The study consisted in evaluating the effects of aqueous extracts of *Persea americana* Mill leaves (Leucaceae) on both the vasomotility and the acute toxicity of the isolated rat aorta. Study of the effects of the aqueous extract of *Persea americana* leaves on isolated rat aorta: qualitative approaches to vasomotility and acute toxicity. The animals used in this study were adult albino rats of the Wistar type *(Rattus Norvengicu, 150-250mg)*. The chemical composition of the extract was considered by phytochemical screening. The effect of the extract of the traditional healer pre-contracted with Potassium Chloride produces a slight temporary relaxation with each dose before contracting again and waiting for a value greater than the previous value. It is the same for this same extract of the traditional practitioner pre-contracted with Adrenaline, the organ relaxes then recontracts, and this with each dose of the extract administered. The DL_{50} showed that no signs of intoxication were observed in the treated animals. None mortality or oral toxicity was recorded in animals. Aqueous extracts prepared in the laboratory compared to traditional healer decoction cause relaxation of the aorta. Furthermore, the phytochemical screening revealed the presence of many antihypertensive components in the aqueous extract. Our study on the isolated rat aorta allowed us to observe a relatively more or less mild relative vasorelaxation. Others studies would be required for the plant to be designated for the development of an improved traditional medicine.

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Introduction

Nowadays, cardiovascular diseases are a real scourge for public health. Indeed, they are the leading cause of death in the world, with nearly 17.7 million deaths a year (Bey-Marrié *et al.*, 2019). This number is expected to increase with the aging of the population, urbanization and many eating behavior. Among cardiovascular diseases, high blood pressure (hypertension) is one of the most prevalent diseases in both developed and developing countries (WHO, 1996).

Formerly unknown, high blood pressure is a disease that is growing in size until it becomes a real public health problem. It is estimated that this disease currently affects 26% of the world's population and could reach 29%, or more than 1.5 billion people in 2025 (Kearney *et al.*, 2004). Even today, it is difficult to obtain accurate data on the prevalence of this disease in most developing countries. In Gabon, the prevalence is around 25 to 30% (Kearney *et al.*, 2004). According to (WHO, 1996), it is the leading cause of cardiovascular morbidity and mortality, so it is important to detect, treat and control hypertension.

Management of the hypertensive patient requires ongoing monitoring and treatment for life, which is expensive in a hospital setting, involving the combination of several therapies (Feuya Tchouya *et al.*, 2015). These prohibitive costs, especially for the poor, and the lack of produce in some localities, mean that many people resort to traditional medicine.

However, although medicinal plants play an important role in the treatment of hypertension, the fact remains that they are often the cause of accidents due to self-medication and lack of knowledge of dosages. Our study focused on the use of aqueous extracts obtained from *Persea americana* (Lauraceae). leaves. *Persea americana* is a specie originated from Central America, and wide distributed in tropical and subtropical regions (South and Central America, West Indies and Africa). According to (Raponda Walker *et al.*, 2003), *Persea americana* is a tree that has been introduced to Gabon for decades. The tree contains leathery leaves with little or no fragrance of dark green terminal or axillary inflorescences. Small fragrant flowers, arranged in panicles, pear-shaped or round fruit. The bark of the tree is used as a medicinal remedy in Gabon. The leaves are stomachic, pectoral, vulnerable. Also named avocado or alligator pear, the plant is a medium to large evergreen tree producing comestible fruits that are appreciated for their buttery and delicious pulp. Bark, fruits and leaves are used in traditional medicines for the treatment of several ailments: menorrhagia, hypertension, stomach ache, bronchitis, diabetes, anemia, gastritis, and gastroduodenal ulcer (Nigeria) (Liu *et al.*, 2003). In Gabon, the plant is used in the treatment of diabete mellitus (leaves) (Orji *et al.*, 2002); venereal diseases opportunistic of HIV/AIDS (bark and leaves) (Tjeck *et al.*, 2017).

This study therefore, was aimed to investigate in vitro the effects of aqueous extracts of *Persea americana* on the vasomotility of the isolated rat aorta and *in vivo* the acute toxicity of *Persea americana* leaves. The chemical composition of the extract was considered by phytochemical screening in order to apprehend potential constituents involved.We consisted in evaluating

Materials and methods

The study was conducted in the pharmacology/ toxicology and traditional medicine departments of the Institute of Pharmacopoeia and Traditional Medicine (IPHAMETRA) located in the Sibang district in the 6th district of Libreville.

Plant material

These are the dead leaves of *Persea americana* (Lauraceae) that are collected and used to prepare decoctions called recipes. A first recipe was taken from a traditional healer and a second was prepared in a laboratory.

The recipe was taken from a traditional healer from IPHAMETRA, after investigation of the content and preparation of the potions (see survey sheet). Indeed, the traditional healer uses only one recipe, containing only one plant; *Persea americana* whose leaves undergo a decoction.

For laboratory preparation, the leaves of Persea americana from a private concession at the Nzeng Ayong dredging district in the 6th arrondissement of Libreville commune were collected on 30/10/2018 at 6 am leaf treatment of *Persea americana* is validated by Raoul Niangadouma, a botanist of the national herbarium of Gabon where a reference sample *Persea americana* (NRJ 01) has been deposited.

Animals

The animals used in this study were adult albino rats of the Wistar type (*Rattus Norvengicus*) from the IPHAMETRA pet shop weighing between 150 and 250 g. They are raised under normal conditions of temperature (24 to 28 ° C) with access to appropriate water and food. The animals were acclimated and fasted on the eve of each experiment.

Aqueous extract

Two types of extracts

- First : decoction/brew made by a traditional healer
- Second: decoction prepared in laboratory.

Traditional healer decoction

Study of the effects of the aqueous avocado leaf extract of *Persea americana* on rat isolated aorta: qualitative approaches to vasomotility and acute toxicity. The potion taken with the traditional healer and which had been prepared according to his conditions (see investigation sheet), was put in jars and frozen, then lyophilized with a device (Christ Beta 1-8k, Bioblock Scientific, USA). After 72 hours of lyophilization, the powder is weighed and ready for handling. A 1.5 L extract gave a powder of 9.64g.

Laboratory decoction

The fresh leaves were washed with water and then dried at a temperature of 40°C for 5 days in an incubator (LMSTM 1200NP), then the leaves were crushed finely with a mixer (MOULINEX, France) and sieved with a colander very fine. The resulting powder (271g) was decoctionated in 2 liters of water for 45 minutes. After filtration, the solute was frozen for 48 hours and then lyophilized for 72 hours (Christ Beta 1-8k, Bioblock Scientific, USA). An amount of 26.5g of aqueous crude leaf extract (6% yield) was obtained and stored in a desiccation bell. A stock solution was prepared for each day of the experiment (Mbang A Owolabi, 2005).

Substances and solutions used

The crude extract of *Persea americana* (E) was diluted in distilled water to obtain a first concentration of 1g of powder in 10ml of distilled water (0.1g /ml or 10^{-1}g /ml), from which a series of 5 cascade dilutions was performed. The tests were performed using 1ml of each of the following concentrations; E-1 (10^{-1}g /ml), E-2 (10^{-2}g /ml), E-3 (10^{-3}g /ml), E-4 (10^{-4}g /ml) and E-5 (10^{-5}g /ml) in isolated organ chambers containing 10ml of McEwen saline (M composition: 130 NaCl, 5.63 KCl, 0.93 NaH_2 PO_4, 0.24mgCl_2, 5.52 CaCl 2; 11.9 $NaHCO_3$; 11 glucose). Adrenaline (AD, 10-6mm) (Alpha Aesar, Karlsruhe, Germany). Potassium chloride (KCl, 80mm) (VWR Prolabo, Leuven, Belgium) was prepared by dissolution in distilled water.

Prelevement of rat aorta

The rat was previously anesthetized with 15% urethane (Molekula, Shaftesbury, Dorset, UK) at 1 g /kg body weight (Konan *et al*., 2007), after abdominal incision and after clearing the rat's heart is sacrificed by exsanguination following carotid section. A segment of the aorta, emerging from the left ventricle and descending along the vertebral column, is removed as long as possible, then placed in a petri dish containing physiological McEwen solution. With the aid of two pairs of pliers, the vessels are freed of fatty adhesions and cut into segments of 5 to 6mm long. With scissors several rings of 5 to 6mm in length are taken. The tests are performed on fresh organs.

Measurement of contractile activity of aortic smooth muscle

The rat isolated aorta ring mounted in the device is suspended from two hooks, one is attached to the device and the other connected to a thin metal rod connected to the transducer. After being stretched with a force of about 1 g in order to mimic the tension exerted by the blood flow under in vivo conditions, the rings are immersed in the tank containing McEwen's physiological solution and stabilized for 90 minutes.

After the stabilization period, in order to check the smooth muscle responsiveness, a high concentration of KCl (80mM) is added to the organ cell. These results in a contraction which testifies to the good state of the smooth muscle fibers, then a washing is carried out in order to regain the basic tension. 30 minutes after washing, the experiment is repeated with adrenaline (1x10-3g /ml) (Sarr *et al*., 2009).

Finally, 30 minutes after washing, KCl (80mM) is added to the vat to contract the smooth muscle. Substances tested at different concentrations are added during the contraction plateau phase to see their potential relaxing effect on the aortic smooth muscle. Then it is washed, and 30 minutes after it, the same experiment is performed this time with adrenaline.

Phytochemical screening

Extraction

Phytochemical tests of extracts bioactive compounds was realized using standard procedures (Harborne *et al*., 1984), with small modifications (Nsi *et al*., 2013, 2019). An aqueous extract were realised to conduct the phytochemical screening consisting of 10g powdered leaves boiled in 100ml of distilled water for 1h. After cooling to room temperature and filtration the extractive solution were reserved.

Reagents

Many reagents were used for the characterisation of the various phytochemical groups: hydrochloric acid, sulphuric acid, acetic anhydride, ammonia 30%, isoamyl alcohol, sodium hydroxide, alpha-naphtol, mercuric chloride, potassium iodide and ferric chloride (Merck, Carlo Erba, Normapur). In addition, commercially available specific reagents were employed: Dragendorff and Fehling reagents (Carlo Erba, Prolabo). Magnesium chips were also used (Sigma). The identified chemical groups were alkaloids, tannins, flavonoids, quinones, coumarins, sterols and triterpenes, carotenoids, total sugars, reducing sugars and saponosides. The results involved visual observation of coloured reactions or training of characteristic precipitates (Nsi *et al*., 2019a). The detection of coumarin required a reading to 365nm with an ultraviolet lamp.

Assessment of acute toxicity

The toxicity test was conducted using the "dose adjustment" method in OECD line 425 (OECD, 2008) and consisted of testing Persea Americana extract at doses of 1000mg /kg; 3000mg /kg and 5000mg /kg. After 15 hours of fasting, the animals are divided so as to have 4 lots each containing 2 males and one female: - Batch 1 control receiving distilled water at a rate of 10ml /kg; - Lot 2 receiving the extract, at a rate of 1000mg /kg; - Lot 3 receiving the extract at 3000mg /kg and finally; Lot 4 receiving the extract at a dose of 5000mg /kg.

The behavior was observed as well as the number of deaths over a period of 14 days. A behavioral observation was performed 2h after administration of the substances. Then hydration and feeding were done daily for 14 days. During this period, signs of toxicity including change in coat, motility, tremor, grooming, sensitivity to noise after metal shock, stool appearance, mobility and death were noted.

Determination of LD50

No sign of intoxication was observed in the treated animals. No mortality was recorded in animals receiving up to a dose of 5000mg/kg body weight after 72 hours and then up to 14 days of observation.

Statistical analyzes

The results are presented as mean ± ESM (Standard Error on Mean) for N observations. The curves were plotted with Excel 2010 software.

Results

This study was made from the aqueous extract obtained in the laboratory and traditional healer decoction. The determination of DL50 showed that no sign of intoxication was observed in the treated animals. No mortality was recorded in animals receiving up to a dose of 5000mg /kg body weight after 72 hours and then up to 14 days of observation. None oral toxicity of the aqueous extract of *Persea americana* was found.

Fig. 1, A shows a significant increase in curve reflecting a considerable contraction (12.5mm) induced by potassium chloride (KCl) on the preparation of the isolated rat aorta.

Fig. 1,B shows a significant rise in curve reflecting a considerable contraction (18.75mm) induced by adrenaline (AD) on the preparation of the isolated rat aorta.

Fig. 1,C shows that when the extract is administered in increasing doses to the organ pre-contracted with KCl on laboratory extract, there is a temporary relaxation of the vessel with each dose, before the curve goes up. The black arrows translate the different increasing doses of the extract, from left to right.

Fig. 1,D illustrates after administering the first dose, the organ relaxes from a KCl concentration on tradipratician extract of 100% to a value of 99.1% then the curve rises. With each dose of the extract the organ relaxes slightly and then recontracts.

Fig. 1,E shows that when the organ pre-contracted with AD in laboratory extract is administered in increasing doses, there is a temporary relaxation of the vessel with each dose, before the curve goes up.
Fig. 1,F represents the average values of the activity of the organ in percentage at each administration of the tradipratician extract; the curve shows a relaxation followed by a contraction at each dose.

The extract developed by the traditional practitioner induces an increase in the percentage of vasoconstriction of the isolated aorta, pre-contracted with adrenaline and potassium chloride. Conversely, the decoction obtained at the end of the protocol developed in the laboratory, we obtain a decrease in the percentage of vasoconstriction. The extract from the laboratory therefore induces a vasodilatation than the extract from the traditional practitioner (Fig. 2).

Phytochemical analysis

The results of the phytochemical screening are presented in the following table. The (+) sign indicates a positive reaction and reflects the presence of the sought compounds while the sign (-) indicates a negative reaction.

The phytochemical screening revealed the presence of alkaloids, tannins, quinones, flavonoids, coumarins, sugars and saponins in the aqueous extract of *Persea americana* leaves (Table).

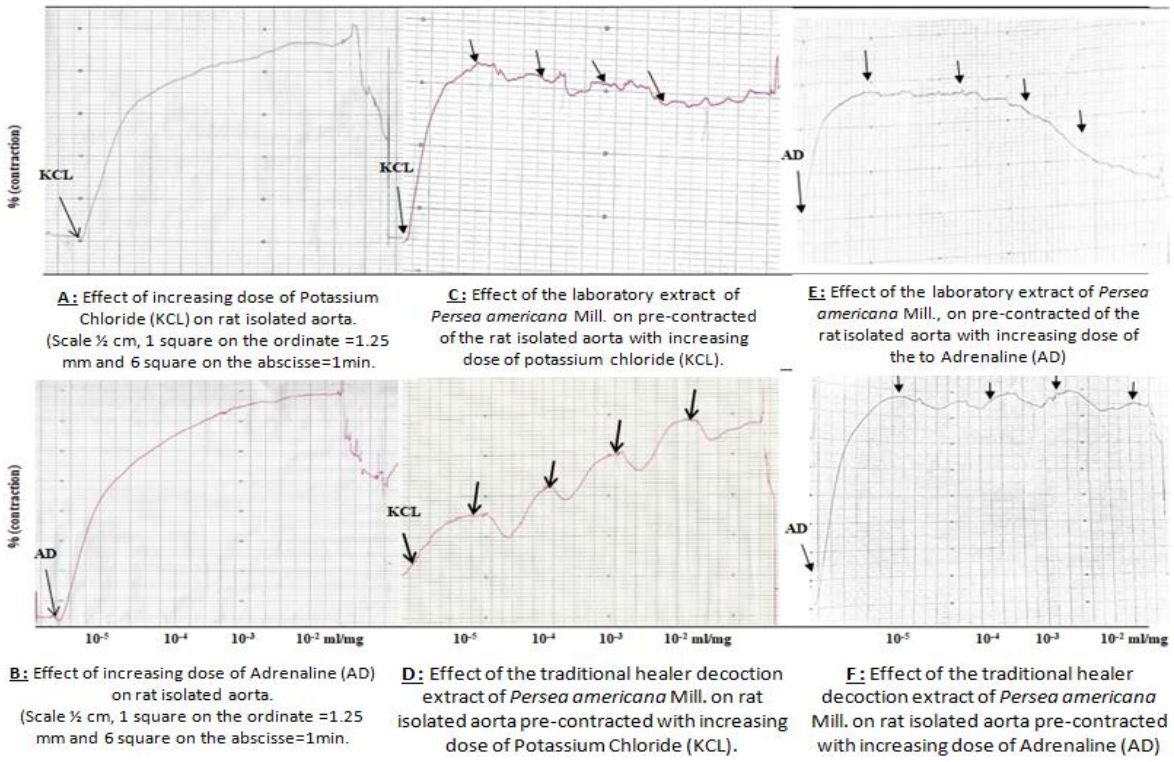

Fig. 1. Effect of *Persea americana* Mill. extract with regents solution (KCL and AD) on rat isolated aorta.

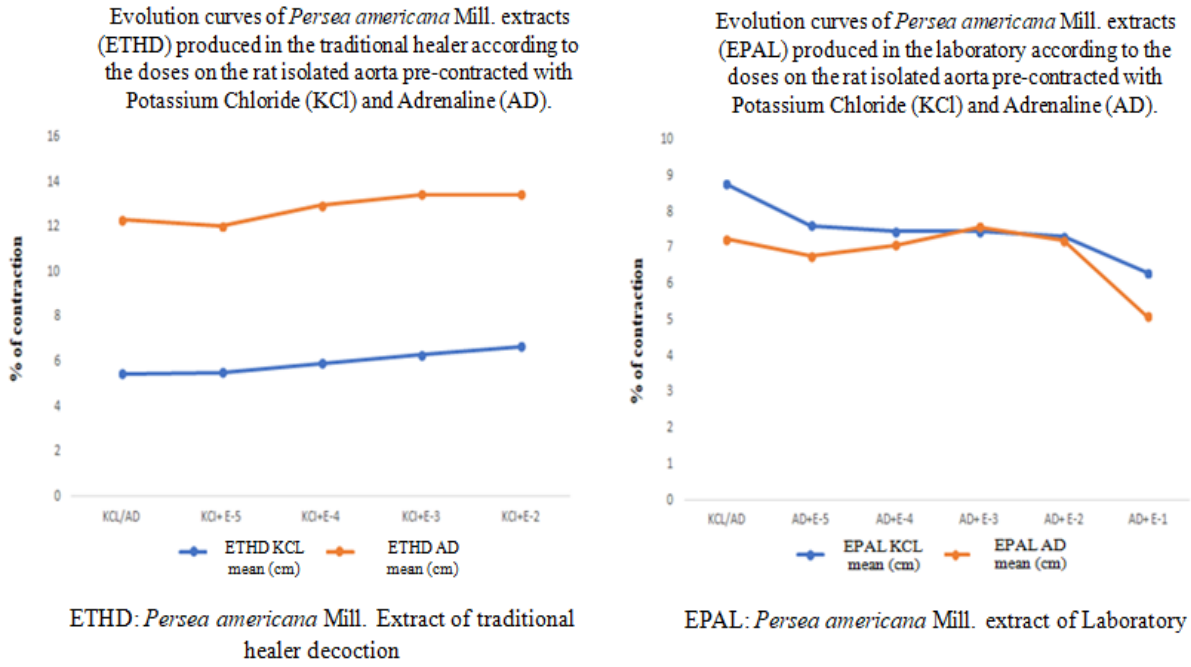

Fig. 2. Comparison of some effects of laboratory and traditional healer decoction extracts of *Persea americana* Mill.

Table 1. Phytochemical screening of *Persea americana* leaves.

	Chemical compounds										
	Alkaloids	Catechical tannins	Gallic tanins	Quinones	Flavonoïds	Coumarins	Carotenoïds	Sterols /triterpenes	Total sugars	Reducing sugars	Saponosids
Persea americana Mill. *leaves aqueous extract*	+	+++	-	+	+	+++	-	-	+	+++	+

The intensity of the staining observed was assimilated to the proportion of each type of compound and translated on a scale ranging from one to three by signs (+), (++) and (+++).

Discussion

The shape of the curves reveals that the KCl and AD exert a relatively muscular tension. The stretching of the organ to these values testifies to its integrity that is to say that the aorta used is well functional. It is well established that the vasostimulatory action of adrenaline which is characterized by a phasic concentration and a tonic component results from both the mobilization of intracellular calcium from internal storage sites and an intensification of the influx calcium (John *et al.*, 1978). While the vasostimulating effect of KCl results from the intensification of calcium influx through voltage-gated calcium channels of type L (John *et al.*, 1978).

The effect of the extract of the traditional healer pre-contracted with KCl produces a slight temporary relaxation with each dose before contracting again and waiting for a value greater than the previous value. It is the same for this extract of the traditional practitioner pre-contracted with AD, the organ relaxes then recontracts, and this with each dose of the extract administered.

The relaxation induced by the extract is comparable to the results of the work of (Mbang *et al.*, 2005). The latter has shown the hypotensive effects caused by the extract of *Persea americana* on the isolated rat aorta (Ojewole *et al.*, 2007).

Our extract would induce, as in the study by (Mbang *et al.*, 2005), a dependent endothelium vasodilation at the level of the vessels by the supposed release of nitric oxide (Lamblin *et al.*, 2005). In fact, the NO released releases the formation of cyclic guanosine monophosphate (cGMP), the increase of which favors the reuptake of calcium by the sarcoplasmic reticulum Ca^{2+} -ATPase thus causing the relaxation of the muscle fiber**.** The results of our study on the extract of the traditional practitioner are comparable to those obtained by Konan *et al.* (2006) on the isolated guinea pig aorta.

In these two cases of experience with the extract of the traditional practitioner, the relaxation was due to the action of endothelial NO, similar to the work of (Mbang *et al.*, 2005). The absence of considerable recontraction after each relaxation in this extract could be due to the inactivity of the enzyme responsible for inhibiting the production of NO.

Aqueous extracts of *Persea americana* leaves prepared in the laboratory cause relaxation of the aorta. For the organ pre-contracted with KCl, slight increasing relaxations occur, this relaxation of the extract tightened due to the action of the endothelium. For the pre-contracted organ in AD, there is a significant concentration at the last dose.

The conditions of preparation, conservation and the quality of the water used could be the reason for the differences in reactivity of the organ compared to the two extracts. But whatever the case, this extract, whether it comes from the traditional practitioner or the laboratory, is very reactive on the vascular tissue, here the aorta, and would be potentially effective under in vivo conditions.

In addition, some authors have highlighted the antihypertensive effect of certain chemical compounds found abundantly in the aqueous extract of *Persea americana*.

The antihypertensive effect of saponins has been demonstrated in the anesthetized rat (Peng, 1999; Jeon *et al.*, 2000).

These indications therefore suggest an antihypertensive power of the leaves of the plant as indicated by the traditional practitioner from these aforementioned chemicals.

Regarding the toxicity study, it should be noted that the aqueous extract of *Persea americana* administered orally in increasing doses (1000mg /kg; 3000mg /kg and 5000mg /kg) did not cause any death, compared to the scale of toxicity of a chemical according to the LD_{50} and the route of administration. According to the Hodge and Sterner (Hodge *et al.*, 1943) and (WHO, 1996) scales, such a Product would be classified almost in the category of non-toxic products.

Other work on the aqueous extracts of fruit of *Persea americana* at the dose 12500mg /kg did not cause a toxic effect (Germosén-Robineau, 2007). Our aqueous extract of *Persea americana* leaves may not be at the same dose.

These experimental conditions suggest better optimization and suggest that it would potentially be possible to produce improved traditional medicines of very good therapeutic quality.

The chemical composition of the leaves aqueous extract is close to that previously described (Yasir *et al.*, 2010). We can thus confirm the presence of alkaloids, tannins, quinones, flavonoids, reducing sugars, saponins and coumarines in these organs. Some of these constituents may be responsible for the antihypertensive activity of the plant. Several studies of plants used in traditional medicine have shown the involvement of these compounds.

Some studies reported hypotensive activity of alkaloids (N'do *et al.*, 2018). The alkaloid extract of *Moringa oleifera*, for example, showed a blocking effect of the calcium pump (Dangi *et al.*, 2008). While reserpin, has been identified as the substance responsible for the antihypertensive action of *Rauwolfia serpentina* (Ryan *et al.*, 2019). The flavonoid fraction of *Astragalus complanata* had antihypertensive activity in hypertensive rats (Paredes *et al.*, 2018). Quercetin and hesperidine have been studied (Perez-Vizcaino *et al.*, 2009).

The saponins would have vasodilatory properties. They would be precursors to the release of the dependent endothelium relaxing factor hypotensive action of tannins, by inhibition of the conversion enzyme, observed (Liu *et al.*, 2003). Coumarines whose auraptene, which is a coumarin monoterpene, would induce a drop in blood pressure in hypertensive rats (Razavi *et al.*, 2015)**.** The action of quinones was reported by (Lown, 1983)**.**

Conclusions

Our study on the isolated rat aorta allowed us to observe a relatively more or less mild relative vasorelaxation. The extract developed in the laboratory seems to have a better vasodilatation effect on the isolated aorta compared to that of the traditional practitioner. This last observation could be due to the presence of certain antihypertensive chemical compounds such as flavonoid, alkaloid, coumarins and saponins in plant extracts. Whatever extract used in this study, it revealed that it remains non-toxic at increasing doses up to 5000mg /kg. For greater safety and certainty, further more in-depth studies would be required for the plant to be designated for the development of an improved traditional medicine.

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