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Comparison of the effects of aqueous extracts of *Persea americana* Mill (Lauraceae) leaves, root and trunk bark on isolated wistar rats aorta and phytochemical screening

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Abstract

Hypertension or elevated blood pressure is a serious medical condition that significantly increases the risks of heart, brain, kidney and other diseases. The aim of this study was to determine and compare the activities of avocado (*Persea americana*; family *Lauraceae*) leaves, root and trunk bark extracts on isolated aorta of wistar rats and perform phytochemical screening. The effects of the aqueous extracts of theselected plant materials were studied on the vasomotor activity of isolated rat aorta. Lastly, the vasodilatory effect of the extracts under study were reproduced in the laboratory using the adrenaline and potassium chloride solutions that are both well-known for their vasoconstrictor effect on smooth muscle cells constituting the aorta. The amplitudes of the responses varied depending on the type of extracts. Our results revealed that the leaf extracts was the part of the plant that had the highest effect on the isolated aorta. These extracts produced a continuous vasorelaxation effect in 22.22% of the cases studied against a transient vasorelaxation effect in 77.78% of all cases. The vasodilation observed in our study could be attributed to the presence of antihypertensive chemical compounds. However, the cause of the transient vasorelaxation effects remains to be determined.

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Introduction

Hypertension or elevated blood pressure is a serious medical condition that significantly increases the risks of heart, brain, kidney and other diseases. High blood pressure, one of the non-communicable pathologies, is one of the most widespread cardiovascular diseases in the world, since its progression has been spectacular in recent decades (WHO, 2019). In addition, studies have shown that this number is expected to increase with the aging of the population, urbanization and certain risky eating behaviors. If you take a closer look, you can see that high blood pressure is one of the most prevalent cardiovascular diseases in all rich countries, as well as in middle-income countries known as developing countries. An estimated 1.13 billion people worldwide have hypertension, with two-thirds of them living in low and middle income countries (WHO, 2019).

According to Lurbe I. *et al.* (2018) and Brewster LM *et al.* (2004), the care of a hypertensive patient requires permanent follow-up and costly lifelong treatment that combines several therapeutic routes in a hospital environment. This implies poverty stricken populations lacking essential infrastructures, logistics to care for patient and modern medicines (Western medicine) are left with traditional medicine as their only hope to recover into a good state of health (Kanjanahattakij N. *et al.* (2019) and Deb L. *et al.* (2015). Consequently, traditional medicine, which mainly relies on the use of medicinal plants, occupies an important place in the treatment of several so-called metabolic pathologies, including high blood pressure. However, physiological complications, sometimes fatal, followed the use of traditional medicine because of self-medication with dosage that had not been scientifically approved. In this study, the medicinal properties of a plant of Mexican origin widespread in Gabon (Tabeshpour J. *et al.*, 2017). The *in vitro* effects of the aqueous extracts from leaves, trunk barks and roots of avocado trees were evaluated on the vasomotricity of isolated aorta of Wistar rat. A phytochemical screening of these different extracts has led to the identification of some of the active ingredients.

Material and methods

Plant material

Our study focused on the use of aqueous extracts of three organs: trunk bark, root bark and leavesof anavocado treelocated in a private propertyin the Nzeng Ayong dragage district in the 6th arrondissement of Libreville municipalty in Gabon. These extracts were collected on 30thOctober 2018 and validated by Raoul Niangadouma, a botanist working at the national herbarium of Gabon where a reference sample *Persea americana* (NRJ 01) has been deposited.

Avocado originated from Central America, and is wide distributed in tropical and subtropical regions, according to Raponda Walker description [7]. The fresh drugs dried with a de humidifier (Bioblock Scientific LMS Cooled Incubator) at 35°C for one week were subsequently cut into small pieces and reduced into fine powder by grinding.

Pharmacological study

Preparation plant extracts

The plant extracts used in this study consisted of leaves, trunk and root bark. The selected plant materials were dried in an incubator for two weeks and then ground into powder using a pestle and mortar. 51 grams of root bark powder was infused in 450ml of distilled water for 15 minutes with a magnetic stirrer, preheated at 100°C and set at 500 rpm. The resulting decoctions obtained after filtration were frozen in glass jars and then subjected to freeze dryer for 72 hours and produced 1.35g of extract. 85 grams of trunk powder were macerated in 500ml of distilled water as described above. After lyophylisation 3.37g of extract were obtained. Finally, 155 grams of leaf powder are macerated in 1000ml. Then the supernatant produced 4.35g of extract.

Animal Models

Male and female Wistar strain albino rats (*Ratus ratus*), 3 to 4 months old, weighing on average 180 grams, from the pet store of the Institue de pharmacopée et de medicine traditionnelle (IPHAMETRA) were used for the *in vitro* tests of the different extracts.

Chemical solutions

The solutions used for the pharmacological study were Mac Ewen's solution, potassium chloride and adrenaline. These solutions were prepared in our lab from the substances supplied by CENAREST (Iphametra laboratory of phytochimistry and pharmacology).

We prepared 15% urethane in distilled water. Mac Ewen's solution prepared according to IPHAMETRA instruction.Pour 95ml of NaCl solution, Pour 21ml of KCl solution, pour 12ml of $CaCl_2$ solution, make up to 500ml with distilled water, pour 9.5ml of H_2PO_4Na solution, make up to 800ml with distilled water, pour 25ml of HCO_3Na solution, Pour 5ml of $MgCl_2$ solution, Add 2g of glucose, make up to 1000ml with distilled water and mix.

For the stock solutions, put in each tube, respectively 8g of NaCl, 20g of KCl, 20g of $CaCl_2$, 15g of H_2PO_4Na, 14g of HCO_3Na and finally 10g of $MgCl_2$ and complete each solution with 1000ml distilled water.

For the physiological solution, a NaCl solution is prepared at a concentration of 0.9%, ie 9g of NaCl in 1000ml of distilled water, in order to influence the salinity of the medium and therefore on homeostasis.

For pharmacological solutions, first dilute 10g of KCl in 100ml of distilled water in order to obtain a concentration of 10-1g /ml; then for the adrenaline solution a solution of 1 mg /ml (10-3g /ml) of Adrenaline is diluted in 10ml of distilled water in order to obtain 10-4g /ml of concentration which is then diluted to 10-5g /ml.

Solution of aqueous Extracts

From 0.5 g of extract of root bark powder, 5ml of distilled water is added in order to obtain a concentration of 10^{-1} g /ml which can then be diluted to 10^{-7} g/mL. We proceed in the same way with trunk bark and leaves extracts of *Persea americana*.

Dissection of the animal

Sampling of the thoracic aorta

The aorta was the organ removed during these dissections. The animal once acclimatized was weighed and anesthetized intra-peritoneally with urethane following a 1ml per 100g of body mass injected. Then, following an incision in the abdominal wall, the animal is opened longitudinally to the level of the neck in order to free the organs as much as possible to extract the aorta with ease. The organ was placed on a watch glass containing a solution of Mac Ewen before being stripped of the adherent tissues and cut into 3 to 5mm rings. Lastly, using scissors, small rings were taken from said organ to be mounted on the experimental device.

Preparation of aorta fragments

The aorta ring previously removed was kept alive in a Mac Ewen's solution thermostated at 37° C and oxygenated (95% O_2 and 5% CO_2). In addition, the different voltage variations due to the variations in diameter caused by the contraction and relaxation phases of the previously mounted ring were captured and then converted into electrical signals and were recorded on a graphic recorder.

Experimental protocol to determine the action of extracts on the isolated rat aorta

In order to study the action of plant extracts, increasing concentrations of extracts were added cumulatively in the organ tank just after a pre-contraction of the intact aorta ring with a given concentration of adrenaline or potassium chloride.

Single extract

After stabilization of ring aortic in Mac Ewen's solution for 60 minutes, an extract solution (root, trunk bark and leaves) is introduced into the cell of the experimental set-up using a syringe. To do this, 1ml of Mac Ewen's solution from the tank was replaced with the same volume of extract of increasing concentration ranging from 10^{-7} to 10^{-2}g /ml.

Effect of extracts on the isolated aorta in presence of Adrenalin or KCl

Potassium chloride and Adrenaline-Extract

After stabilization in Mac Even's solution for 60 min, the organ was stimulated with a 1ml KCl and AD solution respectively. An increase in the curve plot was observed until reaching a plateau before adding

the extract solution into the tank of the experimental setup using a syringe. This was done by removing 1ml of Mac Ewen's solution from the tank and replacing it with the same volume of extract in an increasing concentrations ranging from 10^{-7} to 10^{-2} gml^{-1}.

Phytochemical screening

Preparation of aqueous extracts

The aqueous extracts were made by decoction. 10g of each powder was put in contact with 100ml of distilled water and boiled for 1 hour. However, the volume of water was doubled (200ml) for the leaf powder because of the too high viscosity of the solution obtained. After cooling and filtration, the resulting aqueous extracts were stored for screening.

Statistical analyzes

Statistical analysis of the results was performed using analysis of variances (ANOVA), followed by the Tukey-Kramer multiple comparison tests. The curves with significance level at $p < 0.05$ were plotted with Excel 2010 software.

Results

The results of the phytochemical screening are summarized in (Table 1). Alkaloids, tannins, quinones, coumarines and sugars were found in various proportions in both organs. Tannins and reducing sugars appeared to be more in the three organs, while coumarines were concentrated in leaves and trunk bark. Flavonoids were detected in leaves and roots. Saponosides were detected in leaves and trunk bark. The presence of the various compounds in the plant was not homogeneous except for the reducing sugars of which we noted a strong presence in all the studied parts, in contrast to the gallic tannins which were remarkably absent. Furthermore, there were a non-negligible heterogeneous presence of the alkaloids, catechic tannins and coumarins. An important presence of flavonoids were observed in the leaves compared to other organs. Finally, the other listed compounds are present in traces or completely absent depending on the organ considered.

The graphs showed a considerable contraction increased following the addition of AD and KCl which are two powerful vasoconstrictors thus translating a contraction of the organ. Thus, indicating that it was functional (*Figure 1)*.Upon addition of the decoction from the root extract alone from the lowest (E7) to the highest concentration (E2), Figure 2b and c) shows that the extract caused temporary relaxations followed by a more pronounced contraction. Furthermore, when the extract is administered without prior pre-contraction (a), the organ gradually relaxed (*Figure 2a, b)*. Likewise the gradual administration from the lowest concentration (E7) to the highest concentration of the decoction from the trunk bark extract alone and after a preliminary pre-contraction of the organ had the same effects (*Figure 3)*.Regarding the effects of the decoction from the leaf extract, also administered alone from the lowest (E7)the highest concentration (E2), in figure 4b (a and b), it is observed that they a non-significant transient vasorelaxation on the organ followed by a more pronounced contraction. When the extract was administered after a preliminary pre-contraction of the organ (c), the organ gradually relaxes (*Figure 4a, b)*.

Table 1. Phytochemical coumpounds presents in roots, trunk bark and leaves of *Persea americana*.

Chemical group		Roots	Trunk bark	Leaves
Solvent			Water	
Alkaloïds		++	+	+
Tanins	Catechics	+++	+++	+++
	Gallics	−	−	−
Quinones		+	+	+
Flavonoïds		+	+	+
Coumarines		+	+++	+++
Terpenoïds	Sterols and Triterpenes	−	−	−
	Carotenoïd	+	−	−
Sugars		+	+	+
Reducing sugars		+++	+++	+++
Saponosides		−	+	

+++strongly positive reaction, ++moderately positive reaction, +weakly positive reaction, −not detected

+++Strong presence of reducing sugars throughout the plant while gallic tains are completely absent++ Significant presence of flavonoids in the leaves as well as in the other organs and an identical presence for the carotenoids in the roots. + non-negligible heterogeneous presence for alkaloids, catechic tannins and coumarins while the presence of other compounds is more or less negligible.

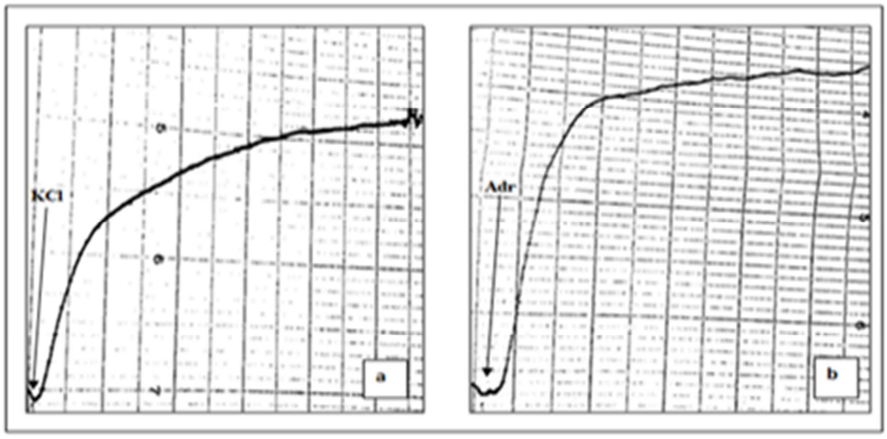

Fig. 1. Plot of the effects of potassium chloride (KCL) (a) adrenaline (AD) (b) on the isolated aorta. This plot shows considerable increase in the amplitude of the curves, because the organ contracts under the effect of AD or Adr and KCL, so it is functional.

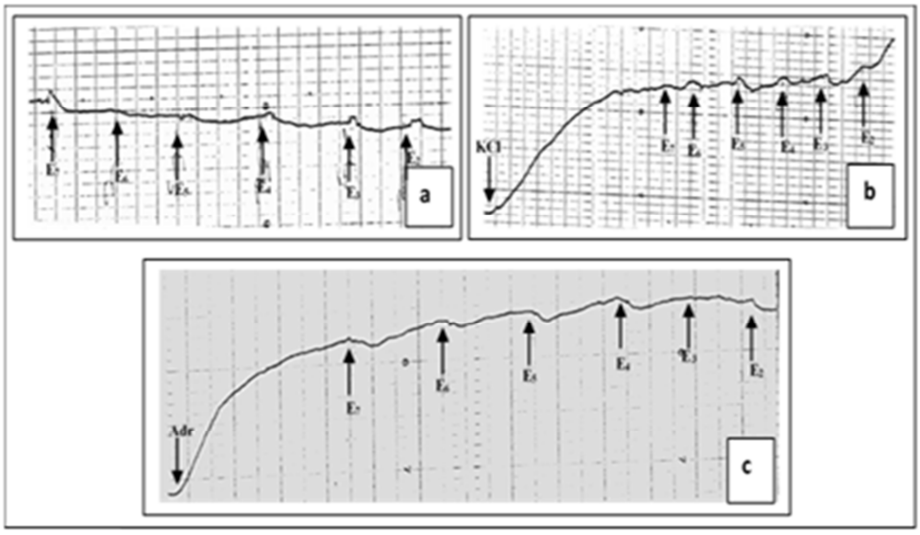

Fig. 2a. Trace of the effect of root extract on the acrta. Root extract alone (a), KCl + root extract (b), Adr + root extract (c).

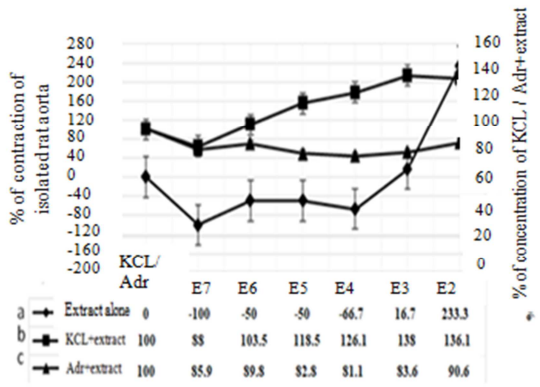

Fig. 2b. Combined graph of the root extract effect on the aorta.

Concentration (g/ml): E7 = 10^{-7}, E6 = 10^{-6}, E5 = 10^{-5}, E4 = 10^{-4}, E3 = 10^{-3}, E2 = 10^{-2}

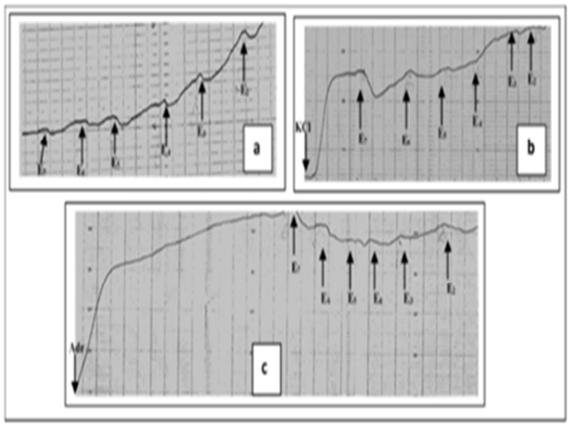

Fig. 3a. Effect of leaf extract on the aorta. Leaf extract alone (a), KCL + leaf extract (b), Adr+leaf extract (c).

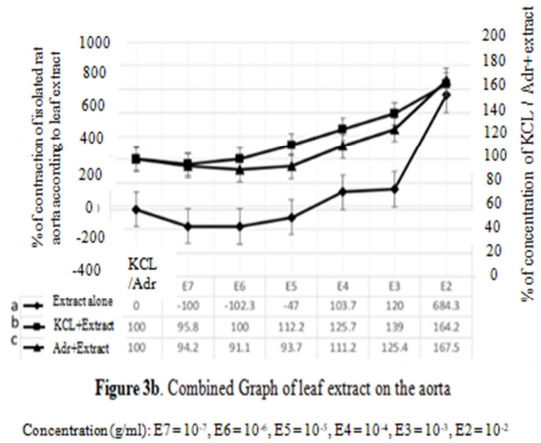

Figure 3b. Combined Graph of leaf extract on the aorta

Concentration (g/ml): E7 = 10^{-7}, E6 = 10^{-6}, E5 = 10^{-5}, E4 = 10^{-4}, E3 = 10^{-3}, E2 = 10^{-2}

Fig. 3b. Combined graph of leaf extract on the aorta. Concentration (g/ml): E7 = 10^{-7}, E6 = 10^{-6}, E5 = 10^{-5}, E4 = 10^{-4}, E3 = 10^{-3}, E2 = 10^{-2}

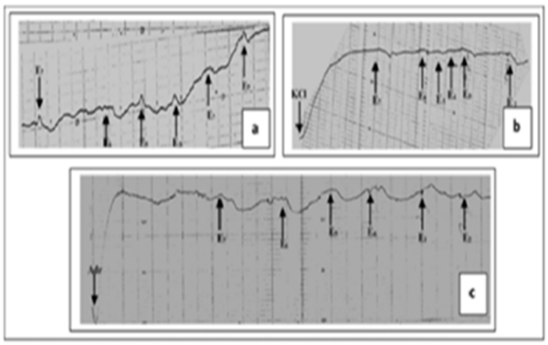

Fig. 4a. Effect of trunk extract on the aorta. Extract only (a), KCl + trunk extract (b), Adr+trunk extract (c).

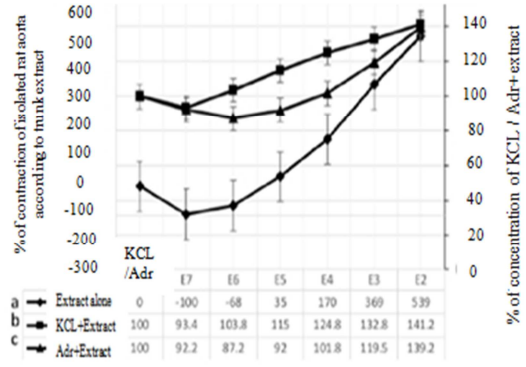

Fig. 4b. Combined graph of the effect of trunk extract on the aorta.

Concentration (g/ml): E7 = 10^{-7}, E6 = 10^{-6}, E5 = 10^{-5}, E4 = 10^{-4}, E3 = 10^{-3}, E2 = 10^{-2}

Discussion

Persea americana is a plant widely used in traditional medicine. According to Ojewole J. *et al.* (2006), Yasir M. *et al.* (2010), Mbang A. (2005) and Zague H. W. (2009), *Persea americana* has been used for the treatment of high blood pressure. It therefore seemed necessary to us to study the effect of this plant on arteries of the circulatory system like the aorta.

In the different series, the results are almost the same according to the general trend. Indeed, there is a relaxation of small amplitude and short duration followed by a contraction, amplitude and greater duration in response to the addition of extracts (cumulative concentrations). However, there are nevertheless disparities in the different results obtained. These disparities can be classified into three categories based on the intensity of the relaxation-contraction coupling. In the first category, there is a weak relaxation followed by a greater contraction while in the second category, the relaxation is progressive but relatively small. On the other hand, in the third category, relaxation is followed by a contraction of relatively similar intensity so that following the addition of the extract there is relaxation followed by a return to the initial state.

The results which translate an relaxation induced by the extract are in agreement with the results of Mbang *et al.* (2005) who demonstrated that hypotensive effects provoked by the extract of leaves of *Persea americana* on the isolated aorta of rats. Our extracts from leaves and roots induced, as in the study by Mbang *et al.* (2005), vasodilation at the level of the vessels by the release of nitrogen monoxide (Lamblin *et al.* (2005).

The slight relaxation observed is certainly due to the action of chemical compounds present in the extracts (Mbang A. *et al.*, 2005). Studies have shown that tannins have antioxidant and spasmolytic properties on smooth muscles (Tona *et al.*, 1999). Polyphenols have antioxidant properties but also vasodilatory properties and they are involved in the treatment of hypertension (Monika Szulińska *et al.*, 2017). Saponins have a hypertensive effect (Paulin Nyadjeu *et al.*, 2013). The vasorelaxing action of aqueous extracts of *Persea americana* could therefore have resulted from the action of one or the interaction of different chemical compounds.

Saponins also have a hypertensive effect (Paulin Nyadjeu *et al.*, 2013). The vasorelaxing action of aqueous extracts of *Persea americana* could therefore result from one or more of these interacting chemical compounds. Furthermore, the vasorelaxing activity of our extracts observed in this study are not the most representative of our work.

These results represent only 22.22% of our results. On the other hand, the results showing a transient relaxation effect (relaxation followed by a contraction) represent 77.78% of the results of our work. It then appears that our results only weakly confirm those obtained by our predecessors. This big difference could be due to the absence of certain substances allowing to check the capacity of relaxation of the organ like acetylcholine. Indeed, the organ's capacity for contraction has been clearly established, while its capacity for relaxation has not been clearly established. The concentrations used for the extracts and for the vasoconstrictors could have also been the cause.

We also conducted a study on the antioxidant potential of two of our extracts prepared in the laboratory: trunk and leaf extracts. The effective concentration EC50 is a measure to highlight the antioxidant properties. The EC_{50} corresponds to the halving of the concentration of DPPH in the reaction medium. The antioxidant capacity of a compound is even higher than its EC_{50} is small. Thus, we note that our extracts have an effective concentration significantly higher than that of the ascorbic acid control. The effective concentration of the trunk being higher than that of the leaf extract leads us to say that the antioxidant activity of the leaf extract is significantly greater than that of the trunk extract. However, it should be noted that the correlation coefficient showing the level of error in the manipulations was rather high during the experiment.

Conclusions

Our results of pharmacological and phytochemical analyses have revealed that the part of the plant that has the most effect on the isolated aorta among those that we have studied is the leaves compared to root and trunk. This could be explained by the fact that the leaves are the site of the biosynthesis of the secondary metabolites of the plant, including catechic tannins, alkaloids, quinones, flavonoids, coumarins, terpenoids (sterols, triterpenes, carotenoids), sugars (reducers and total) and saponosides (except in root extract). Some of these compounds have effects on smooth muscles. On the other hand, the study undertaken on the isolated rat aorta allowed us to observe a more or less slight vasorelaxation in 22.22% of the results but especially a transient relaxation marked by phases of relaxation followed by phases of contraction in 77.78% of the results. The mild vasorelaxation observed could be due to the presence of certain chemical compounds with antihypertensive properties.

Acknowledgement

All the authors declare that it has no conflict of interest of any kind whatsoever.

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