

Studies of the Vasorelaxant Properties of *Gnetum africanum* Welw (Gnetaceae) on Isolated Rat Aorta

Reine Raïssa Rolande Aworet Samseny^{1*}, Justine Okome Essono², Sophie Aboughe Angone¹¹Pharmacopeia and Traditional Medicine Institute: National Scientific Research and Technology Center, P.O. Box: 1156, Libreville, Gabon²Higher Normal School, Libreville, GabonDOI: [10.36347/sajb.2022.v10i02.002](https://doi.org/10.36347/sajb.2022.v10i02.002)

| Received: 16.11.2021 | Accepted: 22.12.2021 | Published: 28.02.2022

*Corresponding author: Reine Raïssa Rolande Aworet Samseny

Pharmacopeia and Traditional Medicine Institute: National Scientific Research and Technology Center, P.O. Box: 1156, Libreville, Gabon

Abstract

Original Research Article

High blood pressure is a chronic cardiovascular disease caused by an increase in blood pressure above normal. Poorly treated high blood pressure can damage the arteries of the brain, heart and kidneys, leading to serious complications. Long considered a disease of the rich, hypertension is now a real public health problem in Africa. The treatment of this disease is not only long term, but it is also expensive for many patients. To overcome this, the African populations resort to herbal treatments. Among these plants we have *Gnetum africanum* which is a food plant from Gabon which has medicinal properties. Our work aims to validate the pharmacological effect of the aqueous extract of *Gnetum africanum* by a study on the isolated rat aorta. The rings aorta are kept in an oxygenated Mac Ewen solution and maintained at a temperature of 37°C. At the beginning of the experiment, the aortic band is subjected to a tension of 1 g force. We then balance the device for 1 hour to have stable contractile movements. The aqueous extract of the leaves showed a relaxing action on the smooth muscles of the isolated rat aorta. This relaxation of the isolated rat aorta is similar to that obtained by the action of amlodipine. These observations suggest that decoction of *Gnetum africanum* have a relaxant effect on rat isolated aorta. This justifies its use by traditional therapists in the treatment of arterial hypertension.

Keywords: Aorta, *Gnetum africanum*, hypertension, medicinal plants, vasorelaxing effect.**Copyright © 2022 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Hypertension is responsible for an estimated 9.4 million deaths each year. Since 2015 one in three adults suffers from it (OMS, 1999). It should be noted that in developing countries non communicable diseases such as hypertension, diabetes, heart disease and respiratory diseases, not only drain the budgets of the State but also that of the individuals who suffer from them and induce significant adverse effects on the quality of life of these people (Bura-Rivière and Boccalon, 2010). There are more cases of hypertension in Africa. More than 30% of adults in these countries suffer from it and this proportion continues to increase (OMS, 1999). HTA is a major cardiovascular risk factor representing a public health problem today in Africa, with prevalence in Gabon about 22.64 to 30% (Kearney *et al.*, 2004). High-income countries have started to reduce the prevalence of hypertension in their populations through strong public health policies, such as limiting the salt content in processed foods and establishing diagnostic and treatment services. Widely

available to tackle both hypertension and other risk factors. Many of them can cite examples of joint, multisectoral actions to effectively address the risk factors for hypertension. In contrast, many developing countries are seeing an increase in the number of people suffering from heart and stroke due to undiagnosed and uncontrolled risk factors such as hypertension (OMS, 2013). The treatment of arterial hypertension is a solution that indeed makes it possible to reduce cardiovascular mortality and morbidity, yet in developing countries, access to healthcare is a problem, which is a major factor in mortality. The population then resorts to traditional medicine. In Africa, the use of medicinal plants in traditional medicine constitutes a serious alternative to bioactive molecules (Kristo *et al.*, 2010). The difficulties faced such as the lack of essential medicines, the inadequacy of health care, the high cost of medicines and the socio-cultural habits of the populations explain their use of traditional medicine. It is in this context that we became interested in medicinal plants that act on arterial hypertension, in particular

Gnetum africanum, the local name of which is nkumu in Gabon. *Gnetum africanum* is a sarmentose, dioecious, creeping plant that can reach up to 10m in length and sometimes more. The twigs are slightly thickened at the nodes, glabrous. The leaves are decussate or sometimes whorled in threes. The *Gnetum africanum* has a range that extends from Central Africa to Nigeria and Angola. In Gabon, *Gnetum africanum* is quite common throughout the country. There are around 30 species of *Gnetum* across the tropics (Niangadouma, 2011). *Gnetum africanum* belongs to the Gnetaceae family. It has nutritional, medical and cultural virtues. The flexible and strong rods are used as snares to make traps. The seeds are eaten after cooking in some provinces of Gabon. The leaves are widely used as a vegetable and are part of the diet of all social classes. In addition to this food use, the leaves are part of the composition of traditional medicine recipes (bad mood or bad luck, purgative, against nausea, antidote against poisoning, facilitator of childbirth, against haemorrhoids and arterial hypertension, treatment sore throat and drunkenness). The leaves are also used to treat boils and finger fungus (Niangadouma, 2011). In fang it is called mekaghe, nkumu in obamba, téké. The leaves of *Gnetum africanum* are used as an antiseptic to treat wounds. *Gnetum africanum* is used to treat haemorrhoids. The fresh leaves are crushed to neutralize the effects of alcohol, and the "bulu" country uses the leaves to treat colds. *Gnetum africanum* leaves improve blood production in the human body. These leaves are also used for the treatment of the spleen (Niangadouma, 2011). The composition of the leaves of *Gnetum africanum* is of primary interest to humans. Indeed, the protein (Schippers and Besong, 2004) and vitamin content such as ascorbic acid (Ogunlesi *et al.*, 2010) are particularly high, which makes it a vegetable of choice; it is one of the most consumed leafy vegetables from the humid forests of Central Africa (Raponda and Sillans, 1995). It is a food plant of which we propose to study the pharmacodynamics properties on the contractile activity of the isolated rat aorta.

SUBJECTS

Plant material

The plant material is composed of the leaves of *Gnetum africanum* harvested from whole plants collected from the forest of Essassa (Gabon) during the month of February 2020.

Animal material

Male albino rats of the WISTAR strain with an average weight of 225g are used for the pharmacological tests. These rats are reared at the animal facility of our research institute Libreville (Gabon). They are pellet-fed and fasted 12 hours before the start of the experiment. Animals were cared for and treated according to the principles for the care and use of laboratory animals for biomedical research approved by the ethical committee of research.

METHODS

Preparation of the extract

100 g of fresh *Gnetum africanum* leaves are decocted for 1 hour. The solution is cooled to room temperature, filtered and then frozen for freeze-drying. The dry extract obtained is kept at 4°C.

Experimental device

The recording device consists of a 10ml insulated organ tank, contained in a thermostatically controlled water bath set at a temperature of 37°C. The biological preparation is immersed in an oxygenated isolated organ tank containing the physiological solution of the Mac Ewen glucose-type. The contractions are picked up by a strain gauge F30 HSE type 372 and transmitted to the HUGO SACHS amplifier. The contractile activity is observed using a RIKADENKI type graphic recorder.

Removal and assembly of the rings

An adult rat is sacrificed by cervical dislocation. A midline laparotomy is performed to remove the aorta. The aorta is freed of its connective veins, cut into 2mm rings, then attached to a hook, the top of which is connected to the strain gauge and the other end to the inside of the isolated organ tank. The rings are kept in an oxygenated Mac Ewen solution and maintained at a temperature of 37°C. At the beginning of the experiment, the aortic band is subjected to a tension of 1 g force. We then balance the device for 1 hour to have stable contractile movements.

Physiological solutions and pharmacodynamic substances used

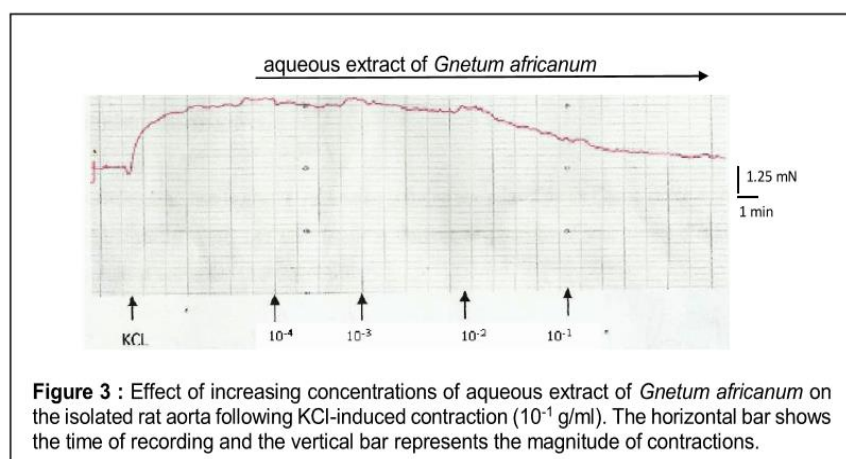
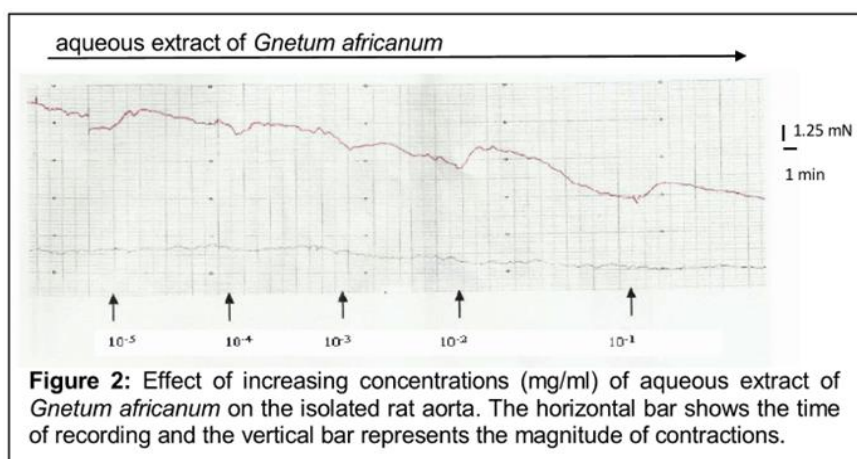
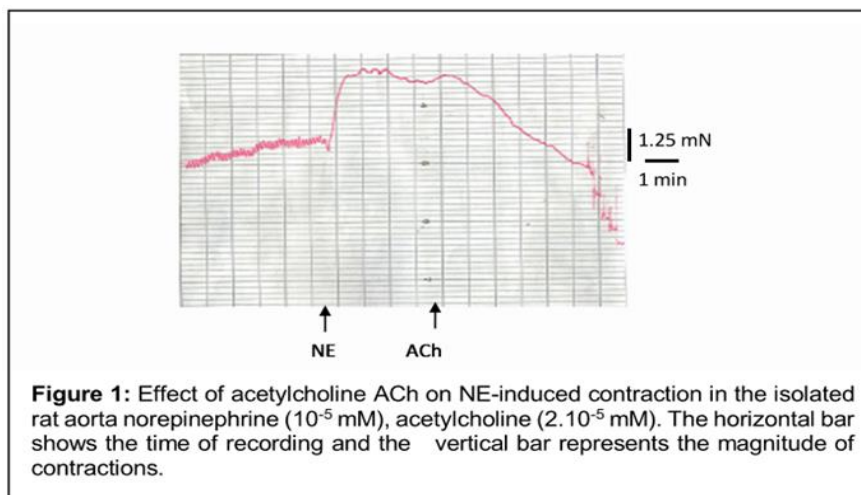
The physiological solution used for the experiments is the Mac - Ewen type physiological solution (NaCl: 130.05; KCl: 5.63; CaCl₂: 2.16; HPO₄Na₂: 0.91; HCO₃Na: 2.38; MgCl₂: 53, in mmol; distilled water qsp). In our various experiments we used norepinephrine (NE) and acetylcholine (Ach).

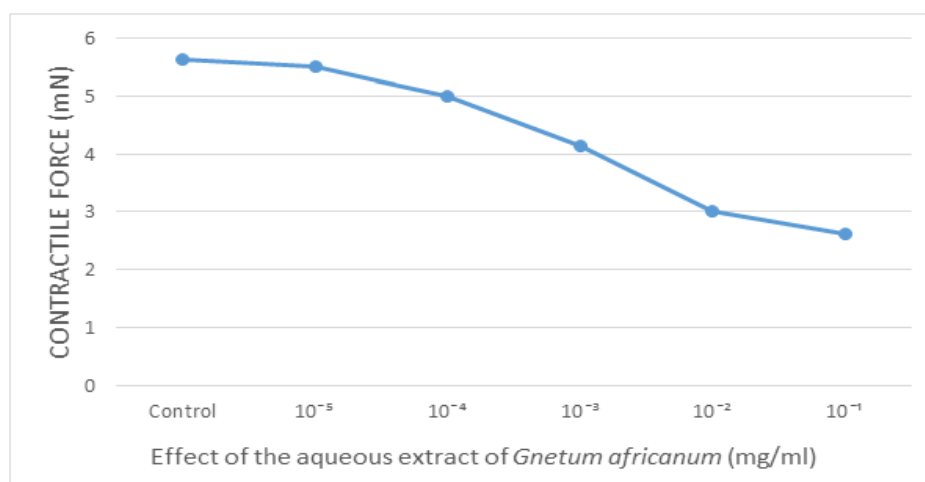
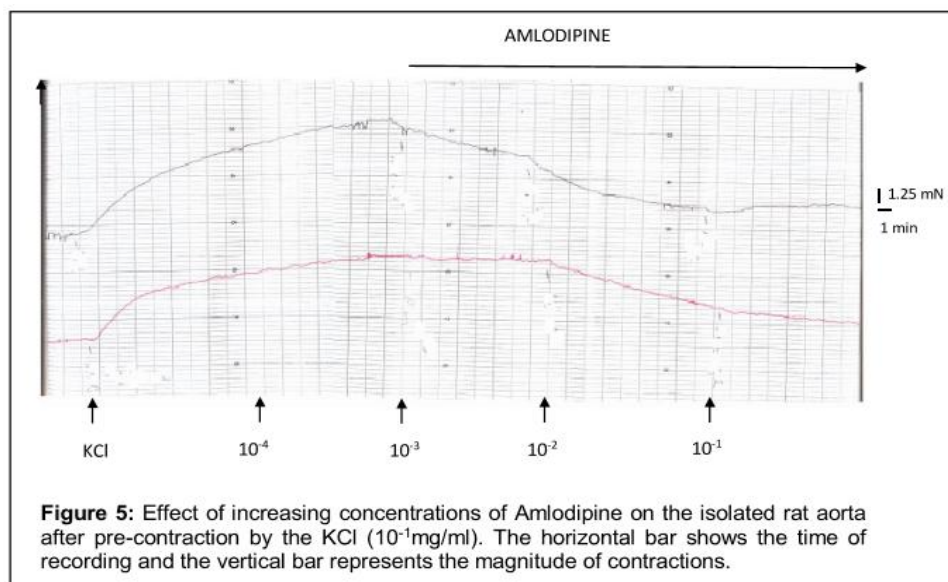
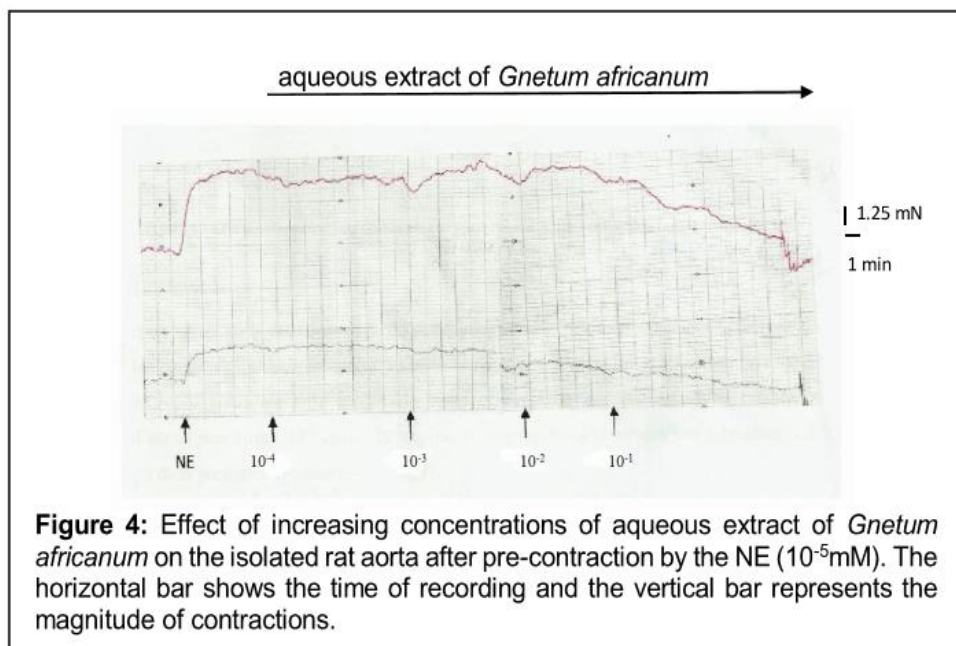
RESULTS

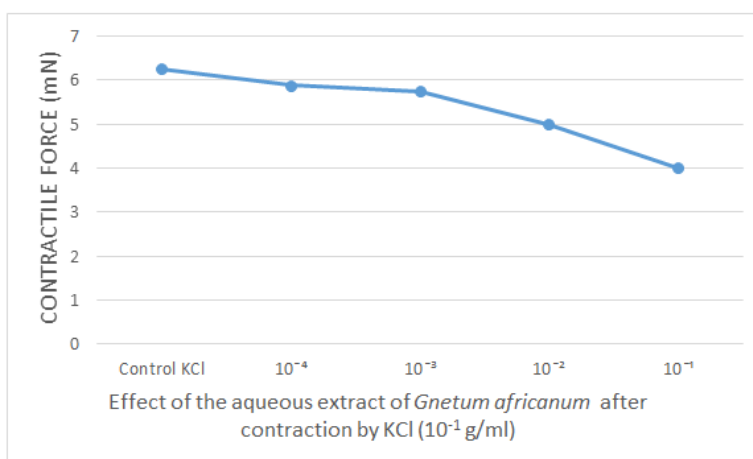
At the beginning of the experiment we check the integrity of the aorta by administering a dose of norepinephrine (10⁻⁵ mM) which causes a constriction which results in an increase in the curve, followed by the addition of a dose of acetylcholine (2.10⁻⁵ mM) which causes vasodilatation of the aorta which results in a decrease in the curve (figure 1). With increasing doses (10⁻⁵ mg/ml, 10⁻⁴ mg/ml, 10⁻³ mg/ml, 10⁻² mg/ml, 10⁻¹ mg/ml) of aqueous extract of *Gnetum africanum* (EAGA) on the uncontracted isolated rat aorta, relaxation is observed (figure 2). This relaxation goes from a normal value of 5.625 mN to 5.5 mN for a concentration of 10⁻⁵ mg/ml of aqueous extract of *Gnetum africanum*, to a contractile force of 5 mN for a concentration of 10⁻⁴ mg/ml of EAGA, to 4.125 mN for a concentration of 10⁻³ mg/ml, a force of 3 mN for a concentration of 10⁻² mg/ml and a force of 2.625 mN for a concentration of 10⁻¹ mg/ml (curve 1). Similarly

EAGA at increasing cumulative doses (10^{-4} mg/ml, 10^{-3} mg/ml, 10^{-2} mg/ml, 10^{-1} mg/ml) results in relaxation of the isolated rat aorta after KCl 10^{-1} g/ml induced contraction (Figure 3). The contractile force goes from 6.25 mN to 5.875 mN, 5.75 mN, 5 mN and 4 mN respectively (10^{-4} mg/ml, 10^{-3} mg/ml, 10^{-2} mg/ml, and 10^{-1} mg/ml: curve 2). Vasorelaxant activity is also observed when prior contraction of the isolated aorta is induced with norepinephrine (Figure 4, curve 3). To

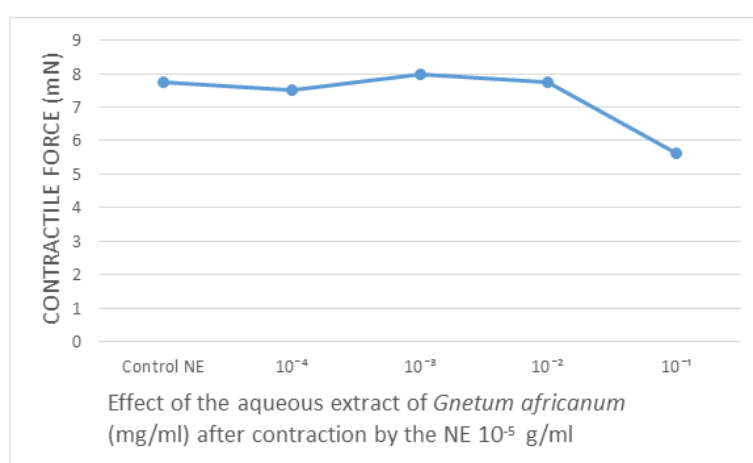
finish our experiment we contract the isolated aorta with KCl (10^{-1} g / ml). After the contraction we administer increasing concentrations of amlodipine (Am = 10^{-4} mg / ml, 10^{-3} mg / ml, 10^{-2} mg / ml, 10^{-1} mg / ml. From 10^{-2} mg / ml we observe a relaxation of the isolated rat aorta (Figure 5, curve 4). The contractile force decreases from 5.95 mN to 4.08 mN at 10^{-2} mg /ml concentration of amlodipine, and passes to 408 mN to 3.62 mN at 10^{-1} mg /ml concentration of amlodipine.



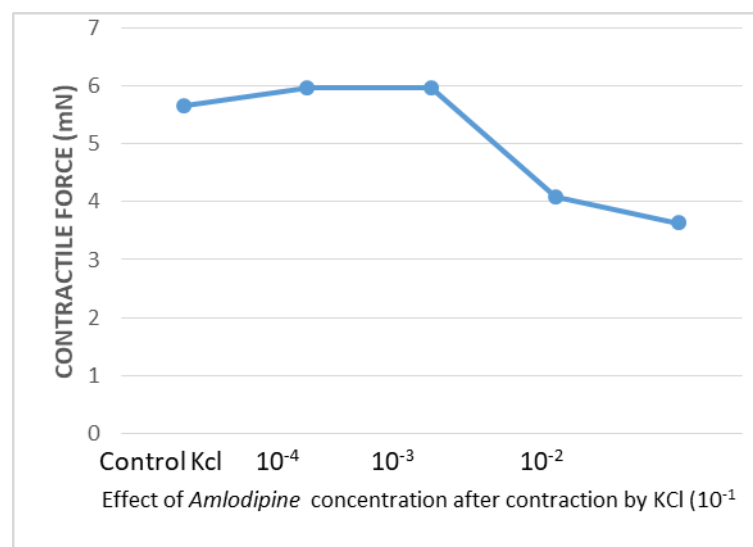




Curve-2: Effect of the aqueous extract of *Gnetum africanum* on the isolated rat aorta after contraction by KCl (10⁻¹ g/ml)



Curve-3: Effect of the aqueous extract of *Gnetum africanum* on the isolated rat aorta after contraction by norepinephrine (10⁻⁵ mM)



Curve-4: Effect of Amlodipine concentration on the isolated rat aorta after contraction by KCl (10⁻¹ g/ml)

DISCUSSION

Our experiment on the isolated rat aorta consisted first of all of checking the entire organ by injection in norepinephrine medium (Hideaki *et al.*, 1993), which caused a contraction of the aorta, followed by an administration of acetylcholine, which causes a

relaxation of the aorta. The muscle relaxant activity observed in the isolated aorta is similar to that described by Rameshrad *et al.* (2016), and is consistent with the effects of acetylcholine on the vessels; it leads to arteriolar vasodilatation as a result of the release of a vasodilating substance, nitric oxide, NO (Allain, 2000),

from the endothelium. Norepinephrine, on the other hand, causes physiological vasoconstriction by stimulation of the α_1 adrenergic receptors of the vascular smooth muscle, with an increase in peripheral vascular resistance, and therefore in blood pressure (Vidal, 2015). Our experiments then demonstrated the relaxing effect of *Gnetum africanum* on the isolated rat aorta. This vasodilatation is also observed with prior contraction of the aorta by KCl, as well as by norepinephrine. These different results show a vasorelaxing activity of *Gnetum africanum* which could have the same receptors as norepinephrine as its site of action, thus acting as an antagonist of the α_1 -adrenergic receptors. It is well established that the vasostimulatory action of norepinephrine, which is characterised by a phasic concentration and a tonic component, results from the mobilisation of intracellular calcium from internal storage sites and an intensification of the calcium influx (En Yin *et al.*, 2009). Whereas the vasostimulating effect of KCl results from the intensification of the calcium influx through the L-dependent calcium channels (En Yin *et al.*, 2009). The vasorelaxing effect could also be explained by the chemical composition of *Gnetum africanum*, which is rich in phenolic compounds including resveratrol (Mensah *et al.*, 2008, Fadi *et al.*, 2011). Resveratrol is a plant polyphenol produced by no less than 72 different plant species. Present in very high concentrations in the skin of red grape seeds, it has a very powerful antioxidant activity. It is also found in blueberries and peanuts (Jang *et al.*, 1997). Renaud and de Lorgeril (1992) show that moderate wine consumption can protect against coronary heart disease and Frankel *et al.* (1993) show that it is the resveratrol in wine which, by inhibiting the oxidation of LDL (Low Density Lipoprotein), must be responsible for its cardio protective effect. The consumption of *Gnetum africanum* also helps to reduce hypolipidaemia during therapy for cardiovascular diseases (Njoku *et al.*, 1997). This astonishing property is thought to be due to the presence of resveratrol, which helps to reduce the level of fat in fat cells. The ethanol leaf extract of *Gnetum africanum* used in the work of Eneh *et al.*, (2019) showed promise as a potential hypolipidemic agent by significantly decreasing the levels of total cholesterol and triglycerides. Other *Gnetum* also have vasorelaxant properties. *Gnetum gnemon*, a species of *Gnetum* native to Southeast Asia, is a small tree unlike many other *Gnetum* which are lianas. Consumption of its seed extract during lactation improved vasodilation and attenuated the development of hypertension in female offspring of pregnant rats fed on fructose (Uson-Lopez *et al.*, 2018). The antihypertensive potential of food plants has already been mentioned in the search for functional foods. Krasínska *et al.*, (2018) and Paran *et al.*, (2018) have indeed demonstrated that the addition of a tomato extract to a conventional hypertension treatment can significantly lower blood pressure values in patients. Other studies on garlic have shown its effectiveness in lowering blood pressure and reducing cholesterol levels (Majewski 2014). The final step in our experiment was

to contract the isolated rat aorta with KCl and give the biologic preparation amlodipine, which is a calcium channel blocker. Numerous studies show that amlodipine decreases the contraction induced by KCl on the coronary artery of dogs or the isolated aorta of rats (Yamanaka *et al.*, 1991, Matlib *et al.*, 1998). We notice that just like Amlodipine, our visibly released the isolated rat aorta from 10^{-2} mg / ml. This result is remarkable because it is a decoction. The outlook as a functional food is encouraging, clinical trials should be conducted to verify this relaxing effect in humans. These promising results on lowering blood pressure with the aqueous extract of *Gnetum africanum* are encouraging and confirm the traditional use of this plant.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Authors' contributions

RRRAS, JO, and SAA performed the experimental studies and drafted the manuscript. SAA and RRRAS played roles in the writing and editing of the manuscript.

REFERENCES

- OMS (Organisation Mondiale de la Santé). (1999). Dépistage et prise en charge de l'HTA. Available from: <https://www.who.int/fr/news-room/fact-sheets/detail/hypertension>.
- Bura-Rivière, A., et Boccalon, H. (2010). Physiologie et exploration de la circulation artérielle, *EMC Angéiologie*.
- Kearney P. M., Whelton M., Reynolds K., Whelton P. K., He J. (2004). Worldwide prevalence of hypertension: a systematic review. *Journal of Hypertension*, 22(1), 21-24.
- OMS (Organisation mondiale de la santé). (2013). Panorama mondial de l'Hypertension : Un « tueur silencieux » responsable d'une crise de santé publique mondiale. Journée mondiale de la santé 2013. Available from: <https://apps.who.int/iris/handle/10665/85334>.
- Kristo, A. S., Kalea, A.Z., Schuschke, D.A., Klimis-Zacas, D. J. A. (2010). Wild blueberry-enriched diet (*Vaccinium angustifolium*) improves vascular tone in the adult spontaneously hypertensive rat. *Journal of Agricultural and Food Chemistry*, 58(22), 11600-11605.
- Niangadouma, R. (2011). Gnetaceae, *Flore du Gabon*, 42, 17-22.
- Schippers, R. R., & Besong, M. T. (2004). *Gnetum africanum* Welw. Fiche de Protabase. Grubben, G.J.H. & Denton, O.A. (Editeurs). PROTA (*Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale*), Wageningen, Pays Bas.
- Ogunlesi, M., Okiei, W., Azeez, L., Obakachi, V., Osunsanmi, M., Nkenchor, G. (2010). Vitamin C

Contents of Tropical Vegetables and Foods Determined by Voltammetric and Titrimetric Methods and Their Relevance to the Medicinal Uses of the Plants. *International Journal of Electrochemical Science*, 5, 105-115.

- Raponda, W., et Sillans, R. (1995). Rites et Croyances des Peuples du Gabon. *Présence Africaine*.
- Hideaki, K., Sri, A. S., Masatoshi, H., Michihiro, T., Yoshihiro, U., Toshikazu, O. (1993). Induction of endothelium-dependent relaxation in the rat aorta by IRL 1620, a novel and selective agonist at the endothelin ETB receptor. *British Journal of Pharmacology*, 109, 486-490.
- Rameshrad, M., Babaei, H., Azarmi, Y., Fouladi, D. F. (2016). Rat aorta as a pharmacological tool for in vitro and in vivo studies. *Life Science*, 145:190-204.
- Allain P. (2000). Les médicaments, 3ème édition – Cdm Edition, broché, ISBN 29510288-1-4.
- Vidal. (2015). Le Dictionnaire, 140-141. 3287.
- En, Yin, L., Michael, F., Zufu, M., Orjan, K., Pontus, B. P., Andreas, P., Erik, G. P., Michael, H. (2009) Norepinephrine increases calcium sensitivity of mouse afferent arteriole, thereby enhancing angiotensin II-mediated vasoconstriction, *Kidney International*, 76(9), 953-959.
- Mensah, J. K., Okoli, R. I., Ohaju-Obodo, J. O., Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology*, 7 (14), 2304-2309.
- Fadi, A., Mafu, A. A., & Carole, R. (2011). *Gnetum africanum*: A wild food plant from the african forest with many nutritional and medicinal properties. *Journal of medicinal food*, 14(0): 1-9.
- Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C.F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, 275(5297), 218-220.
- Renaud, S., De, Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, 339, (8808), 1523-1526.
- Frankel, E. N., Waterhouse, A. L., Kinsella, J. E. (1993). Inhibition of human LDL oxidation by resveratrol. *The Lancet*, 341(8852), 1103-1104.
- Njoku, O. U., Eneh, F.U., Nwanguma, B. C. (1997). Investigation of test lipids of *Gnetum africanum*. *Journal of Pharmaceutical Research and Development*, 2:8-10.
- Eneh, F. U., Ugochukw, G. C., Okeke, C. R. (2019). Effect of Ethanol Extract of *Gnetum africanum* leaves on the Lipid Profile of Albino Rats. *IOSR Journal of Pharmacy and Biological Sciences*, 14(3 Ser III), 22-25.
- Uson-Lopez R. A., Kataoka, S., Mukai, Y., Sato, S., Kurasaki, M. Melinjo. (2018). Seed Extract (*Gnetum gnemon*) Consumption during Lactation Improved Vasodilation and Attenuated the Development of Hypertension in Female Offspring of Fructose-Fed Pregnant Rats. *Birth Defects Research*, 110(1), 27-34.
- Krasinśka, B., Osińska, A., Krasinśka, A., Osiński, M., Rzymiski, P., Tykarski, A., Krasinśki, Z. (2018). Favourable hypotensive effect after standardised tomato extract treatment in hypertensive subjects at high cardiovascular risk: a randomised controlled trial. *Kardiologia Polska*, 76(2), 388-395.
- Paran, E., Novack, V., Engelhard, Y. N., Hazan-Halevy, I. (2009). The effects of natural antioxidants from tomato extract in treated but uncontrolled hypertensive patients. *Cardiovascular Drugs and Therapy*, 23(2), 145-151.
- Majewski, M. (2014). Allium sativum: facts and myths regarding human health. *Roczniki Państwowego Zakładu Higieny*, 65(1), 1-8.
- Yamanaka, K., Suzuki, M., Munehasu, S., Ishiko, J. (1991). Effect of amlodipine, a new calcium antagonist on isolated blood vessels. *Nihon Yakurigaku Zasshi*, 97(3), 167-78.
- Matlib, M. A., French, J. F., Grupp, I. L., Van, Gorp, C., Grupp, G., Schwartz, A. (1998). Vasodilatory action of amlodipine on rat aorta, pig coronary artery, human coronary artery, and on isolated Langendorff rat heart preparations. *Journal of Cardiovascular Pharmacology*, 12 Suppl 7:S50-54.