International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 9 (9), pp. 001-007, September, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# Antihypertensive effect of an aqueous extract of *CITRUS AURANTIFOLIA* (Rutaceae) (Christm.) Swingle, on the arterial blood pressure of mammal

**[1,2]A. Souza*, [2]M. Lamidi, [1]B. Ibrahim, [2]R. R. R. Aworet Samseny, M. [2]Boukandou Mounanga, [1]B. M'Batchi**.

[1]Unité de Recherche d'Agrobiologie, Université de Sciences et Techniques de Masuku. BP. 913 Franceville Gabon. Tel/Fax : 631334

[2]Institut de Pharmacopée et de Médecine Traditionnelle, Centre National de la Recherche Scientifique et Technologique. BP: 1156 Libreville Gabon.

Accepted 07 September, 2020

***CITRUS AURANTIFOLIA*** **is used in African folk medicine for the management of hypertension. In order to provide a scientific basis for this use, we studied the effects of an aqueous extract of *CITRUS AURANTIFOLIA* (Ecita) on arterial blood pressure and on isolated heart and aorta activities. Rabbits were used for the study on the arterial blood pressure using a Ludwig manometer. Albino Wistar rats were used for the studies regarding the isolated heart and aorta activities using isolated organ bath systems. Ecita (4mg/kg-16mg/kg b.w) produced a dose-dependent and significant decrease in rabbit blood pressure ($p<0.05$). This hypotension was not prevented by atropine (2 mg/kg b.w, $p>0.05$). Ecita (4mg/kg-16mg/kg b.w) dose-dependently reduced hypertension evoked by adrenalin (30 µg/kg b.w.). Ecita ($10^{-8}$mg/ml-$10^{-2}$mg/ml) induced both negative inotropic and chronotropic effects on the heart contractile activity. The plant extract ($10^{-8}$mg/ml-$10^{-2}$mg/ml) induced a dose-dependent relaxation of contractions produced by adrenalin ($3.10^{-3}$mM) or by KCl (80mM). Ecita-evoked vasorelaxant effects were totally abolished by removal of the endothelium layer or by a pre-treatment with L-NAME (mg/ml). It was concluded that the extract possesses an antihypertensive activity which could be related to both cardiodepression and the vasorelaxation. Endothelium-dependent mechanisms might be involved.**

**Key words:** *Citrus aurantifolia*, blood pressure, heart, aorta, hypertension**.**

## INTRODUCTION

Hypertension remains a public health problem in developed countries (Foucarde *et al.,* 2007). Many people are still forced to use traditional plant remedies for the management of this disease, since antihypertensive medical treatments are long in duration and expensive. *Citrus aurantifolia* is a one of the most widely-used medicinal plants in the African folk medicine (Kerharo and Adam, 1974). The fruits of *Citrus aurantifolia* are the part of plant most used. However, in Ivory Coast (Western Africa), people also use stem bark to treat hypertension. An aqueous decoction of stem bark is prepared and taken orally two times per day. *Citrus aurantifolia* is used for several other diseases such as gastrointestinal disorders, fever, malaria, scabies and diabetes (Das *et al.,* 2008; Abo *et al.,* 2008).

Pharmacological investigations on *Citrus aurantifolia* extracts have shown laxative (Souza *et al*., 2002), anti-inflammatory, anticancer, antiviral and antibacterial properties (Ebana *et al.,* 1991; Ibukun *et al.,* 2007; Jaiprakash *et al.*, 2009). Phytochemical studies of the plant led to the isolation of many bioactive compounds such as flavonoids and limonoids which have potential health benefits (Ebana *et al*., 1991). Based on the traditional use of *Citrus aurantifolia* stem bark for treating hypertension and in order to validate the plants phytomedical properties, in this study, the effect of an aqueous extract of the plant on the arterial blood pressure was evaluated using isolated rat hearts and

* Corresponding author email : souzapg@yahoo.fr : Tél : 00241-07727754

aorta which are essential components of blood pressure regulation.

## MATERIALS AND METHODS

### Plant material

The aqueous extract was prepared from the dried stem bark of *Citrus aurantifolia* obtained at a local herb market in Abidjan, Ivory Coast. Plant material was authenticated by Prof. AKE ASSI Laurent, an expert botanist (Department of Botany, University of Cocody, Abidjan).

### Preparation of the plant extract

The bark was crushed into small pieces and was macerated in n-hexane under a magnetic stirrer for 24 hours to remove soluble substances. The supernatant was then removed and the solid remainder was completely dried. The dried part was extracted with distilled water for 24 hours. The extract obtained was filtered with cotton and Wattman paper. The solvent was evaporated using a Rotavapor manufactured by BUCCHI. The residue, soluble in water, was labelled "aqueous extract of *Citrus aurantifolia"* (Ecita) and stored at $5^{o}$C.

### Animal experiments

Rabbits (*Oryctologus cunuculus)* (2.5-3kg) and albinos Wistar rats (*Rattus norvegicus*) (150-200g) were used. Animals were raised at room temperature using natural light-dark cycles with food and water *ad-libitum*. All experiments were conducted in accordance with Gabonese government rules for biomedical research involving animals.

### Study of the effect of the plant extract on the arterial blood pressure

A rabbit, anesthetized with ethyl urethane (40%) at a dose of 1g/kg b.w, was placed in dorsal decubitus in a vat for dissection. Dissection and recording of the arterial blood pressure were performed as described by Konan *et al.* (2006). The thigh and the neck were dissected to expose the carotid and the saphenous veins. A polyethylene catheter, filled with heparinized saline and connected to a "U" tube of the Ludwig pressure gauge via a polyethylene tube, was inserted into the carotid. The "U" tube contained mercury which was surmounted by a float connected by a wire to an inscriptor stylet. The variations of arterial blood pressure transmitted to the mercury were collected by a float and registered on a kymograph. The saline solution and drugs were administered through the saphenous vein via a polyethylene canula.

### Study of the effect of the plant extract in rat isolated heart

A rat was anesthetized with ethyl urethane anaesthesia (20%) at a dose of 1g/kg b.w and was maintained under artificial respiration. The heart was quickly dissected and removed. The isolated heart was suspended on the exit of a tap through multiple connections to bottles placed at 50cm with the top of the equipment as described in detail by Datté-jacques *et al* (2006). The bottles contained the control and the test solutions were maintained at a temperature of 37°C and continuously aerated with air. The activity of the isolated heart was recorded on a Kymograph via an inscriptor stylet connected to the isolated heart apex.

### Study of the effect of the plant extract on the isolated aorta activity of rat

A rat was sacrificed by cervical dislocation and ex-sanguinated. The thoracic aorta was quickly removed, was cleaned of connective tissue and placed in Petri-dish containing an oxygenated Mac Ewen physiological solution with the following composition (mM): NaCl, 130; KCl, 5.6 $CaCl_2$, 2.6; $NaH_2PO_4$, 0.91; $NaCO_3H$, 11.9, $MgCl_2$, 0.24; glucose, 11). The organ was cut into rings of about 5mm width. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the inner lining of the rings using plastic tubing. The aortic ring was mounted in a 20ml jacketed tissue bath, containing the physiological solution, on a fine stainless rod which was connected to a force transducer (F30 HSE 372). The force transducer was connected to a grass polygraph recording system (Rikadenki). The rings were allowed to equilibrate during 90 minutes under a resting tension of 1g. To obtain a reproducible response, the ring was previously stimulated three times with KCl (80 mM). Subsequently, a tissue was exposed to KCl or to adrenalin (ADR) to induce a maximum response and then, the plant extract was cumulatively added into the organ bath solution. After each test, the aortic rings were washed three times with a fresh saline solution and allowed to equilibrate for 30 minutes. For experiments regarding endothelial-denuded aortic rings, the effectiveness of endothelial removal was verified, by the loss of the relaxant response to ACh ($2.10^{-5}$mM) on adrenalin-evoked contractions.

### Drugs

The drugs used were: Atropine (ATR) and Adrenalin

**Table 1**. Effect of the aqueous extract of *Citrus aurantifolia* in rabbit arterial blood pressure

| Drugs | Dose | Mean arterial blood pressure (mmHg) | |
|---|---|---|---|
| | | MABP before | MABP after |
| Ecita | 0.4 mg/kg | 78. 83 ± 2.05 | 75.69 ± 4.43 |
| | 4 mg/kg | | 65 ± 6.50 |
| | 8 mg/kg | | 33.91 ± 2.32** |
| | 16 mg/kg | | 27.98 ± 2.20** |
| | 32 mg/kg | | 13.69 ± 3.15** |
| Ecita followed by atropine | 8 mg/kg + 2 mg/kg | 77 ± 6,71 | 33.91 ± 2.32** |
| Atropine followed by Ecita | 2 mg/kg + 8 mg/kg | | 28.29 ± 4.57** |
| ADR | 30µg mg/ml | | 148 ± 3.21*** |
| ADR+EACA | 30µ g/kg + 4 mg/kg | 73 ± 3,96 | 114 ± 4.6†† |
| | 30µg/kg + 8 mg/kg | | 110.7 ± 1.43†† |
| | 30 µg/kg + 16 mg/kg | | 101.04 ± 3.32†† |

Drugs were administered intravenously. Values are expressed as Mean± SEM, ** P<0.01, *** P<0.001 *vs*. MABP before. †† P<0.01, ††† P<0.0001 *vs.* MABP after ADR (n= 4-5).

(ADR) from Sigma Chemical Company (St Louis, USA). Drugs were diluted at a desired concentration in 0.9% saline for *in vivo* or in Mac Ewen physiological solution just before use for *ex vitro* experiments.

### Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) obtained from separate experiments. Values were analysed using a One-way Analysis of Variance (ANOVA) followed by Dunnet's test. The difference between the concentrations were considered statistically significant when P<0.05.

## RESULTS

### Effect of Ecita on the blood pressure in rabbits

The mean arterial blood pressure (MABP) of anesthetised rabbits was 76 ± 5.22 mmHg. Ecita, at a dose of 0.4 mg/kg b.w., had no significant effect on rabbit blood pressure. However, at concentrations of 4, 8, 16 and 32 mg/kg b.w., Ecita produced a dose-dependent decrease in arterial blood pressure of 29 ± 3.02, 43.09 ± 3.6, 49.02 ± 2.2 and 54.31 ± 3.51% respectively. Figure 1a shows the typical effects of the increasing concentrations of the plant extract. Data from different experiments are consigned in table 1. Atropine applied at a dose of 1.5 mg/kg b.w. did not exert any significant effect on an Ecita-induced fall in MABP (p<0.05). Adrenalin ($5.10^{-4}$ mg/kg b.w) caused an increase of MABP of +76 ± 6,45mm Hg (hypertension) which was significantly reduced in the presence of increasing doses of Ecita (4 mg/kg-16 mg/kg b.w). The arterial blood pressure evoked by adrenalin was reduced to the values of 47 ± 8.41 (p<0.5), 40.07 ± 5.4 (p <0.01) and 16.04 ±6.83% (p<0.01) at Ecita doses of 4; 8 and 16 mg/kg b.w respectively.

### Effects of Ecita on the heart and the isolated aorta isolated from rat

Figure 2a represents the original tracing of the plant extract at a concentration of $10^{-3}$ mg/ml. Whereas Figure 2b represents the mean of variation of the force and rate of contractions from different experiments of Ecita ($10^{-7}$ - $10^{-1}$ mg/ml). Ecita at concentrations of $10^{-7}$ and $10^{-3}$ mg/ml produced a dose-related decrease in the isolated heart force (inotropic effect) of 7±1.6 % and 48± 3.7% respectively. In the same way, a decrease in the isolated Heart rate contractions (chronotropic effect) of 8± 0.93% and 28±2.4 % was recorded.

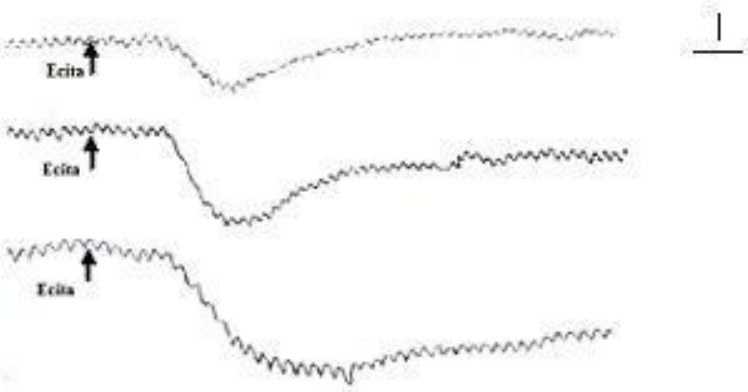


**Figure 1**. Typical tracings from a record of the rabbit arterial blood pressure. Arrows indicate the administration of Ecita (4, 8 and 16 mg/kg b.w.). Horizontal scale: 15 sec., Vertical scale: 20 mmHg.

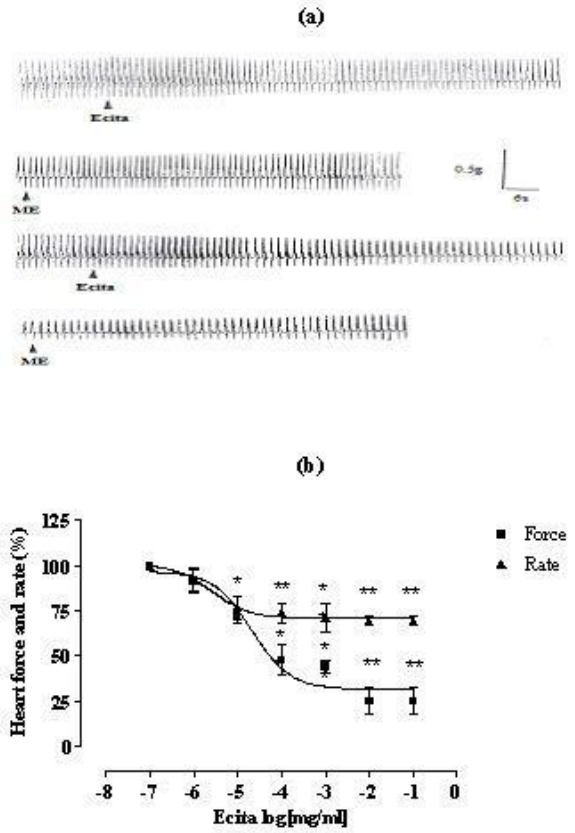


**Figure 2.** Negative inotropic and chronotropic effects induced by an aqueous extract of *Citrus aurantifolia* in rat isolated heart.
**(a).** Typical tracings from record of the mechanical activity in the rat heart. Arrowsindicate the administration of the plant

**(b).** Concentration-dependent diminution evoked by Ecita (10 to 10 mg/ml) on the isolated heart contractile activity. Values are expressed as a percentage of diminution to

Each value represents the mean ±SEM (n = 4-5) *p<0.5, **p<0.01 compared to control.

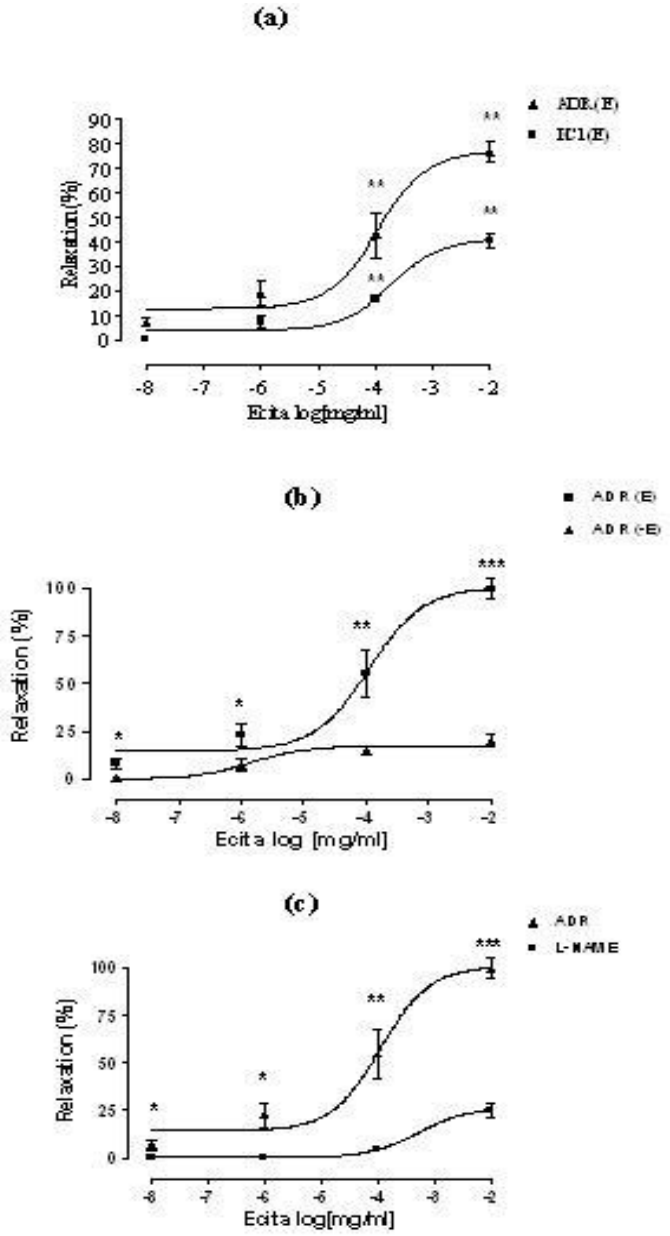


**Figure 3.** Effects of the aqueous extract *Citrus aurantifolia* in rat aorta contraction evoked by Adrenaline and by KCl.
**(a).** Concentration-dependent relaxation induced by Ecita (10 mg/ml – 10 mg/ml) in rat intact (E) or denuded (-E) aorta contractions evoked by adrenaline and by KCl.
**(b)** and **(c).** Concentration-dependent relaxation elicited by Ecita on the contractions evoked by Adrenaline in the presence and in the absence of L-NAME.
Each value represents the Mean ± SEM. (n= 4-5), *p<0.05, ** p<0.01, *** p<0.001.

Figure 3a shows the effects of Ecita on ADR or KCl pre-contracted aortic rings. The treatment of aortic rings with ADR or with KCl resulted in a contraction of the rings. In those conditions, Ecita, at concentrations ranging from $10^{-8}$mg/ml to $10^{-2}$mg/ml, produced a significant result ($p<0.01$) and concentration-dependent relaxation of ADR ($3.10^{-3}$mM) or KCl (40mM) pre-contracted rings. Ecita, at a concentration of $10^{-2}$mg/ml, produced a maximum vasorelaxation of 79.8±4.0% in ADR pre-contracted rings and 40.78±2.93% in KCl pre-contracted rings.

The $IC_{50}$ values were $1.12x10^{-4}$mg/ml in ADR pre-contracted aortic rings and $2x10^{-4}$mg/ml in KCl pre-contracted aortic rings. Furthermore, the relaxation induced by Ecita was endothelium-dependent, since, the effect of Ecita was significantly reduced in the endothelium denuded aortic rings. Indeed, the plant extract ($10^{-8}$mg/ml to $10^{-2}$mg/ml) induced a maximum relaxation of 17±2.33% vs. 79.8±4.0% (in aortic strips with endothelium) in aortic rings pre-contracted with ADR (figure 3b).

In the same way, the pre-treatment of aortic rings with the L-NAME, a NO synthase inhibitor, markedly inhibited ($p < 0.001$) the Ecita ($10^{-8}$mg/ml to $10^{-2}$ mg/ml)-induced relaxation of aortic strips (figure 3c).

## DISCUSSION

The stem bark of *Citrus aurantifolia* is used in folk medicine for the treatment of hypertension. The present study, performed in normotensive rabbits, showed that the aqueous extract of the plant produced a dose-dependent hypotension and, interestingly, elicited an antihypertensive action. However, since the effect of ACh was blocked by atropine (Kavoli *et al.,* 2002), this inhibitor of cholinergic muscarinic receptors did not prevent the hypotensive effect induced by the plant extract in rabbit arterial blood pressure, suggesting that the active ingredients in the plant extract did not act through cholinergic mechanisms to induce the hypotension observed. It is well established that blood pressure is the product of cardiac output and peripheral vessel resistance (Bowman and Rand, 1980). In order to determine the mechanism of action of the plant's active principles underlying its antihypertensive effect, experiments were performed on the isolated heart and on the isolated aorta of rats.

Our findings showed that the aqueous extract of *Citrus aurantifolia* induced negative inotropic and chronotropic effects, with the reduction of the heart force being more prominent. This experiment also revealed that the plant extract did not act through cholinergic receptors since atropine did not affect the pharmacological observed effect. Furthermore, it was observed that the aqueous extract of *Citrus aurantifolia* significantly reduced adrenalin and KCl-evoked vasoconstriction. These observations suggested a direct action of water soluble principles in the plant extract on the cardiac muscles and on the smooth muscles of the blood vessels.

Adrenaline is known to induce vasoconstriction through the activation of $\alpha_1$-adrenergic receptors leading to the influx of extracellular calcium and the release of intracellular calcium (Hashimoto *et al.,* 1986; Wang *et al.,* 2001). KCl-evoked contraction was due to an intensification of calcium influx through voltage-sensitive calcium channels. The inhibition of Adrenalin or KCl-induced contractions indicated that the pharmacological observed effect of the plant extract on aorta strips may be due to a blockade of calcium influx through receptor-operated calcium channels and voltage-sensitive calcium channels respectively. The inhibition of the intracellular calcium mobilization by the plant extract might be involved, since ADR evoked tonic contraction is due to the release of calcium from internal stores (Hashimoto *et al.,* 1986). Endothelial cells play a key role in the smooth muscle relaxation (Furchgott and Vanhoutte, 1989).

To see whether these vascular cells are involved in *C. autantifolia* extract-induced observed vasorelaxation, additional experiments were performed in endothelium-denuded preparations and in the presence of L-NAME, a NO synthase inhibitor (Arif-Ullah and Anwarul, 2008). Our study revealed that the effect of the *C. aurantifolia* was endothelium-dependent and the synthesis of Nitric oxide (NO), one of the major endothelium factors was involved in the plant mechanism of action, since the mechanical remove of endothelium as well as the presence of L-NAME, significantly and totally abolished the vasorelaxant effect of the plant extract.

Based on the nature of chemical compounds in *Citrus*, the presence of active principles, such as flavonoids are well documented to have beneficial effects on the cardiovascular system of mammals (Chan *et al.,* 2000; Arif-Ullah and Anwarul, 2008). Indeed, flavonoids, from a great variety of plants, have hypotensive and vasorelaxant activities (Aduragbenro *et al.,* 2009; Rosalia *et al.,* 2010) and their action, in vessels of mammals, involves endothelium-dependent NO/cGMP-mediated vasodilatation pathway. It is quite plausible that flavonoids might be responsible for the lowering of blood pressure produced by the plant extract. However further studies are required to elucidate this hypothesis.

It was concluded that the aqueous extract of *Citrus aurantifolia* contains a water soluble active compound with hypotensive activity that might involve the cardiac and vascular pathways. The active principles would activate the endothelial NOS to dilate the blood vessels which may explain the use of the stem bark of this plant in folk medicine for the treatment of hypertension.

## REFERENCES


Abo KA, Fred-Jaiyessimi AA, Jaiyesimi AEA (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. J. Ethnopharmacol. 115: 67-71.

Aduragbenro DAA, Yeside OO, Adeolu AA, Olanrewaju JM, Ayotunde SA, Olumayokun AO, Janet MM (2009). Blood pressure lowering effect of *Adenanthera pavonina* seed extract on normotensive rats. Record of Natural Products. 3 (2): 82-89.

Arif-Ullah Khan and Anwarul Hassan Giliani. (2008). Pharmacodynamic evaluation of *Terminalia bellerica* for its antihypertensive effect. J. Food Drug Anal. 16 (3): 6-14.

Bowman WC and Rand MJ (1980). Subcellular organization and cellular metabolism. *In*: Textbook of pharmacology. Blackwell Scientific 2 nd Ed. Pub London. pp 1928

Chan ECH, Pannangpetch P, Woodman OL (2000). Relaxation to flavones and flavonols in rat isolated aorta: Mechanism of action and structure activity relationships. J. Cardiovasc. Pharmacol. 35 (2): 326-

333.

Das FA, Barua I and Das DD (2008). Ethno-Medicinal practices: a case study among the Sonowal Kacharis of Dibrugarh, Assam. Ethno-Med. 2 (1): 33-37.

Datte JY, Yapo PA, Kouame-Koffi GG, Kati-Coulibaly S, Amoikon KE, Offoumou AM (2006). Leaf extract of *Caesalpinia bonduc* Roxb. (Caesalpiniaceae) induces an increase of contractile force in rat skeletal muscle in situ. Phytomed. 11(2-3): 235- 241.

Ebana RUB, Madunagu BE, Ekpe ED and Otung IN (1991). Microbiological exploitation of cardiac glycoside and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia*. J. Appl. Biotech. 71: 398-401.

Foucarde L, Paule P et Mafart B (2007). Hypertension artérielle en Afrique subsaharienne :

Actualité et perspectives. Med. Trop. 67: 559-567.

Furchgott RF and Vanhoutte PM (1989). Endothelium-derived relaxing and contracting factors. Fed Amer. Soc. Exptal Biol. 3: 2007-2018.

Hashimoto T, Hirata M, Itho T, Kanmura Y, Kuriyama H (1986). Inositol 1,4,5-triphosphate activates pharmaco-mechanical coupling in smooth muscle of rabbit mesenteric artery. J. Physiol. 370: 605-618.

Ibukun A, Tayo A, Toyin A, Tolu O and Tolu O (2007). Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally. Afr. J. Trad. CAM. 4 (2): 185-190.

Jaiprakash RP, Chidambara Murthy KN, Jayaprakasha GK, Mahadev BC and Bhimanagouda SP (2009). Bioactive compounds from maxican lime (*Citrus aurantifolia*) juice induce apoptosis in human pancreatic cells. J. Agric. Food Chem. 57(22)*:* 10933-10942.

Kavoli H, Pollock D, Fallah H (2002). Vasorelaxant effects of atropine: role of nitric oxide endothelium derived relaxing factor. Indian J. Pharmacol. 34: 244-250.

Kerharo J, Adam JG (1974). Pharmacopée sénégalaise traditionnelle. Plantes médicinales et

toxiques, Vigot et frères, Paris, pp 1011.

Konan BA, Datte-Jacques Y, Offoumou AM (2006). Action of the aqueous extract of *Sesamum radiatum* Schum, Tonn. (Pedaliaceae) on the cardiovascular system of mammalians: hypotensive effect. Current Bioactive Compounds. 2 (3): 263-267.

Rosalia C, Eva Sanz, Pilar P, Maria LM, Luis SR, Mario FG (2010). Mechanisms of relaxation induced by flavonoid ayanin in isolated aorta rings from Wistar rats. Colombia Medica. 41 (1): 10-16.

Souza A, Datte YJ and Abo KJC (2002). Effet myostimulant de l'extrait aqueux de *Citrus aurantifolia* sur l'activité contractile du muscle lisse : le taenia coli de cobaye. Rev. Med. Pharm. Afr. 16: 103-112.

Wang GJ, Shum AY, Lin YL, Liao JF, Wu XC, Ren J, Chen CF (2001). Calcium channel blockade in vascular smooth muscle cells: major hypotensive mechanism of S-petasin, a hypotensive sesquiterpene from petasites formosanus. J. Pharmacol. Exp. Ther. 297: 204-246.