Medicinal & Aromatic Plants
ISSN: 2167-0412

Mounguengui et al., Med Aromat Plants 2015, 5:1
DOI: 10.4172/2167-0412.1000220

Research Article Open Access

Antihypertensive Potential of Aqueous Extract of *Nephrolepis biserrata* Leaves on Toad Aorta

Ibrahim B[2], Attéké Nkoulémbéné C[2], Mounguengui S[1,*], Lépengué AN[3], Issembé Azizet Y[4,1], Akoué NSI G[2], Souza A[2] and M'batchi B[2]

[1]*Research Institute in Tropical Ecology, Gabon*
[2]*Laboratory of Animal Physiology/Electrophysiology-Pharmacology Unity Agrobiology-University of the Sciences and Technics of Masuku, Gabon*
[3]*Laboratory of Physiopathology Unity Agrobiology-University of Sciences and Technics of Masuku, Gabon*
[4]*Institute of Pharmacopoeia and Traditional Medicine, Gabon*

Abstract

Of our ethnobotanic investigations in the district of Franceville, on 30 requested people, 20 answered the survey questionnaire. The present study aims at evaluating the hypotensive activity of *Nephrolepis biserrata* (*NB*) used by Ndoumou in the province of Haut-Ogooué in Gabon to treat or prevent against arterial hypertension. The Phytochemical studies show that the aqueous foliar extract of *Nephrolepis biserrata* consists of phenolic constituents, terpeniques and nitrogenous. This extract was used to realize all the tests of our study. The contraction is recorded by a system of isolated organ.

Nb (5-200 mg/ml) provoke the decrease of the contraction of the aorta of the toad induced by the norepinephrine (10^{-6} M) in presence and in absence of the endothelium. The threshold of the effect is 5 mg/ml in the presence and 50 mg/ml in absence of endothelium; a maximal decrease is observed in 200 mg/ml.

The acetylcholine (10^{-11}-10^{-7} M) decreases the contraction of the aorta caused by the norepinephrine (10^{-6} M) in the presence of the endothelium; 3.10^{-11} M is the threshold of the effect and 10^{-7} M the maximum, whereas in the absence of endothelium, we observe an increase of the contraction.

Nephrolepis biserrata (5-200 mg/ml) cause the reduction of the contraction induced by the norepinephrine (10^{-6} M) of the aorta pretreated with a solution of L-NAME (10^{-5} M), in the presence of endothelium; the threshold of the effect is 5 mg/ml and maximum to 200 mg/ml, so highlighting the antihypertensive potential of *Nephrolepis biserrata*, and those this in presence and in absence of endothelium.

Keywords: Acetylcholine; Antihypertensor; *Nephrolepis biserrata*; L-NAME; Norepinephrine; Toad aortic rings; Vasoconstriction; Vasodilatation

Introduction

Nowadays, because of the weakness of the economic resources, the populations in developing countries often turn to the traditional therapeutic practices (Pharmacopeia).

To accompany these populations, the World Health Organization (WHO) in set up a development project of the strategies which would involve the role of the African traditional medicine (confer: Strategy of the African region AFR/RC50/9; 2010).

According to the WHO, 60% of the current diseases would be due to medicine of synthesis.

Supports of the WHO in the herbal medicine as the appeal possible for medicine current, opens new ways of studies for numerous plants. So, studies were led to highlight the use of certain plants in the treatment of the high blood pressure in Africa.

In Ivory Coast, the populations use the aqueous solutions of the leaves of *Citrus aurantifolia* (Rutaceae) to handle the high blood pressure [1-3].

The potential antihypertensive of certain plants was also highlighted in certain works.

Khan and Gilani [4] showed that fruits of *Terminalia bellerica* decreased the blood pressure to anesthetized rats. Nworgu and al. [5] reported that the aqueous solutions of the leaves of *Nauclea latifolia* caused the decrease of the blood pressure to the rat. Boobalan and et al. [6] noted that the aqueous extract of the leaves of *Melothria maderaspatana* reduced the blood pressure of the patients suffering from high blood pressure. Khalili et al. [7-9], showed respectively that the aqueous extract of *Elaeocarpus ganitrus*, *Vaccinium Arctostaphylus* and *Vitis coignetiae* caused the reduction of the blood pressure to rats spontaneously hypertensive.

Studies revealed that the therapeutic properties met to plants were due to the presence of certain bioactive substances.

This is the way, according to the works of Andriambeloson [10], the compounds polyphenolics (catechines, proanthocyanidines, anthocyanines, flavanols and phenolic acids) cause a vasorelaxation endothelium-depending on the thoracic aorta of rat.

In 2003, Chataigneau [11], showed that compounds polyphenolics some wine and some grape juice infer the relaxations dependent on the endothelium at the level of diverse isolated arteries of rat. See also the epidemiological studies realized by Renaud and Lorgeril [12] who suggested that the consumption of red wine stemming from grapes (who have in their composition polyphenols), could explain the

***Corresponding author:** Mounguengui S, Research Institute in Tropical Ecology, Gabon, Tel: 3330661379196; E-mail: pha106@yahoo.fr

Received November 04, 2015; **Accepted** December 02, 2015; **Published** December 07, 2015

Citation: Ibrahim B, Attéké Nkoulémbéné C, Mounguengui S, Lépengué AN,Issembé Azizet Y, et al. (2015) Antihypertensive Potential of Aqueous Extract of *Nephrolepis biserrata* Leaves on Toad Aorta. Med Aromat Plants 5: 220. doi:10.4172/2167-0412.1000220

incidence more limited by the coronary cardiac diseases in the French population: it is the theory of the "French Paradox".

It is necessary to note also Perez-Vizcaino [13] investigations which revealed that the effects hypotensor of fruits and vegetables at the level of the hypertensive rats would be owed to the presence of polyphenols in the composition of the extracts of fruits and plants, particularly the flavonoids which are the most plentiful compounds and having a more important biological activity.

Nair and Gupta [14] and the epidemiological investigations led by Erdman [15] support the hypothesis which announces that the "regular consumption of food rich in flavonoids can reduce considerably the risks of the mortality bound (connected) to the cardiovascular diseases". Herrera et al. [16] showed that flavonoids decrease the contraction of the aortic artery of rat induced by the noradrenaline, so highlighting the antihypertensives properties of the flavonoids. Furthermore, Khan and Gilani [4] indicated that the consumption of fruits of *Terminalia bellerica* decreased the blood pressure of rats under anesthesia and this reduction was due to the presence of flavonoids, sterols and tannins.

On the other hand, Segasaka-Mitane et al. [17,18] reported that the saponins of tea leaves caused the decrease of the blood pressure at the level of the hypertensive rats spontaneously. Khan and Gilani [4] indicated that fruits of *Terminalia bellerica* decreased the blood pressure of rats under anesthesia and that the reduction of this one would be due to the presence in its chemical composition of flavonoids, sterols and tannins.

The role of the endothelium in the relaxation of the smooth muscle of vessels due to the action of the acetylcholine was demonstrated by Furchgott and Zawadzki [19].

Furthermore, it was highlighted that the nitrogen oxide (Nitric Oxide or NO/Endothelium Derived Relaxing Factor: EDRF) was a factor mattering in the relaxation of the cells of the smooth muscles [20],while the prostacycline stemming from another one way metabolic was a vasorelaxant important [21].

With regard to the previous studies, it would seem that the endothelium-dependent relaxation would arise from two independent ways.

However, other factors would play an important role in the relaxation of the smooth muscles vascular as described by Félétou and Vanhoutte et al. [22-25].

Triggle et al. [26,27] who showed that an additional vasodilator mechanism observed mainly in the coronary circulation and in the peripheral vascular beds generally, would be associated with a hyperpolarization of the smooth muscle cells.

Studies showed a similarity of the mechanisms of action of certain substances involved in the phenomenon of the relaxation of vessels.

So, Komas [28] revealed that polyphenols cause the increase of the cyclic AMP and the cyclic GMP, which will have for consequence a relaxation of the aortic arteries without endothelium.

Andriambeloson et al. [10] revealed that the production of Nitric Oxide and the endothelium-dependent and independent relaxation at the level of the aorta of rat was led by the polyphenols of the red wine.

The in vitro studies led by Chataigneau et al. [11] showed that compounds polyphenolics some wine and grape juice lead relaxations dependent on the endothelium would imply the nitric oxide (NO) as well as Endothelium Derived Hyperpolarization Factor in diverse isolated arteries. It is necessary to note also Ndiaye [29], showed that the formation of NO is due to the increase of the activity of NO-synthase endothelial by polyphenols, involves two different mechanisms which lead the relaxation of vessels, dependent one of the concentration of the free calcium in the cytosolique and other one of the way PI3-kinase/Akt.

Other works revealed the implication of the Enzyme of Conversion of the Angiotensin (ACE).

Indeed, Sakat et al. [8], related that the aqueous extract of *Elaocarpus ganitrus* reduced the blood pressure by inhibiting the activity of the Enzyme of Conversion of Angiotensin.

On the other hand, Sakat et al. [8], related that the aqueous extract of *Elaocarpus ganitrus* reduced the blood pressure by inhibiting the activity of ACE. Jang et al. [9] obtained the similar results, because they note that the red wine of *Vitis coignetiae* causes its effect to antihypertensive by inhibiting the Enzyme of Conversion of Angiotensin at the level of the hypertensive rats spontaneously. See all the concepts proposed by Busse et al. [30].

It is from this perspective that we are going to approach the phytochemical studies and the biological effect of *Nephrolepis biserrata* which is a plant used by the populations of Franceville and its surroundings (Haut-Ogooué) in disease prevention and to handle the arterial high blood pressure which is at present a problem of Public Health [31].

So, to give a scientific basis to this traditional practice in the Gabon, the study of the effect of the aqueous foliar extract of *Nephrolepis biserrata* on the contraction of the aorta of the heart of toad was led to estimate the antihypertensives properties of this plant, *in vitro*.

Material and Methods

Animal preparation

Toads (*Bufalo regularis*) were caught locally. The animal (100 to 150 gr) was sacrificed after destruction of the central nervous system (the brain and the spinal cord) and opening of the rib cage; both arteries which constitute the aortic stick of the heart of toad are taken, cleaned and kept in the physiological solution (Locke Ringer of composition gr: NaCl 6,5; KCl 0,14; $CaCl_2$ 0,12; $NaHCO_3$ 0,20, in 1 liter of water distilled and bubble with 95% O_2 and 5% of CO_2). Two rings are obtained for every artery and six toads were used for every dose of the aqueous foliar extract of *Nephrolepis biserrata* and substance for the biological tests.

Vegetal preparation

The leaves of *Nephrolepis biserrata* were collected in the University Campus of the USTM in Franceville (Haut-Ogooué), in Lambaréné (Moyen-Ogooué) and in Oyem (Woleu-Ntem).

Nephrolepis biserrata (Sw). Schott (PL.IX) is a fern of the family of the Dryopteridaceae authenticated by Dr Mounzéo and Issembe, Department of Biology, University of Masuku (Gabon) was used. This plant had already described by Tardieu-Blot [32].

Preparation of extracts foliar aqueous s of *Nephrolepis biserrata*: Leaves are crushed by means of a crusher (Waring-R Commercial ARMOR) to obtain a powder. The initial solution of the foliar extract was obtained by adding 0,50 gr of powder to 20 ml of distilled water, which afterward was diluted in several concentrations (5-200 mg/ml) to make biological tests.

So, the aqueous extract of the leaves of *Nephrolepis biserrata* collected to Franceville was used to make the various tests, because the populations used it under this shape.

Phytochemical screening: The phytochemical tests of *Nephrolepis biserrata* were made on the aqueous extracts, chloroformic and ethanolic of all the plants provenance from three sources of harvests quoted above.

Two methods of extraction were used: the maceration and the decoction [33]. The phytochemical tests allow to highlight groups of compounds (sterols, polyterpenes, tannins, polyphenols, flavonoids, quinones and saponins) contained in extracts. They were made according to the classic methods described by Paris and Moyse [34,35].

Alkaloids: The highlighting of alkaloids was made by using the methods of reactive Dragendorff and Mayer. The test consisted in taking 2 ml of filtrat in 2 different tubes may add in tube 5 drops of reactive of Dragendorff and 5 drops of the reactive of Mayer in the other one. The appearance of an orange-colored red precipitate in the tube containing the reactive of Dragendorff indicates the presence of alkaloids. Also the presence of a tint yellowish white in the other tube also gives evidence of the presence of alkaloids.

Anthraquinones: The highlighting was realized by introducing into a test tube 2 ml of filtrat more a solution of NH_4OH (10%) may shake, the formation of a red tint gives evidence of the presence of the free anthraquinones.

Cyanidines (Flavones): The test of Cyanidines was made by introducing into a test tube 2 ml of filtrat then the addition of 1ml of hydrochloric alcohol more 1 ml alcohol isoamylic and some shavings of magnesium. A pink-orange tint indicates the presence of flavones; pink-purplish puts evidence of the presence of flavones and red that of the flavanones.

Flavonoid: The highlighting of flavonoids was realized by adding to 2 ml of filtrat 1ml of H2SO4 more 1ml Na_2CO_3. The appearance of a dark tint stressed after the addition of the acid indicates the presence of flavonoids. The development of a tint blue-made purple after the addition of the base gives evidence of the presence of anthocyanins.

Saponosides: The test of saponosides was made by introducing 2 ml of the filtrat into a test tube then shaken during 2 minutes and left then with the rest during 2 minutes. The obstinacy of the foam indicates the presence of saponosides.

Sterol and triterpens: The presence of sterols and triterpens was highlighted by the test of Libermann-Buchard. In 2 ml of filtrat were added 2 ml of H_2SO_4 concentrated slowly during the wall of the test tube. The training of a brownish red ring highlights the presence of sterols and triterpens.

Tannins:The presence of tannic compounds (hydrolysable and not hydrolysable) was detected by adding to 2 ml of filtrat, 1ml of an aqueous solution of trichloride of horseshoe (2%). The appearance of a black blue color indicates the presence of Gallic tannins; also, the appearance of a green-black color after addition of the ferric trichloride (1%) indicates the presence of tannins catechics.

The chemical and pharmacological products

Norepinephrine, Acetylcholine and L-N (ω) -nitro-arginine methyl ester (L-NAME) obtained to Sigma Chemical Company was diluted in the distilled water and used for all the biological tests.

Reactive of Dragendorff and Mayer, NH_4OH (10%), hydrochloric Alcohol, isoamylic Alcohol, H_2SO_4, Na_2CO_3,Trichloride of horseshoe NaCl, KCl, $CaCl_2$, and $NaHCO_3$, obtained at Bioblock, Sigma Chemical Company and Fisher Scientific via Medilab in Gabon.

Experimental protocol

Rings from 3 to 5 mm in lengths of arteries are cut and placed in a Pétri dish containing the physiological liquid. A ring isolated and gone up between two hooks is fixed to a capacity of constraint which allows to collect the contractile activity of the ring under a tension in the rest of 2 g; the set is placed in a tank with isolated organ of 20 ml containing the physiological solution oxygenated continues it by means of a bullor and maintained in a temperature of 37°C and in a pH 7,4 by a circulating bath thermostated (Julabo EM). The signal collected via a sensor (LCM Systems Ltd UF1 Forces Sensor) is amplified by means of an amplifier (Bionic INSTRUMENTS) and transcribed on a graphic recorder (Linseis L250E). The preparation is stabilized during 60 minutes before the realization of different tests.

During the period of balance, tissues were stimulated with 40 mM of potassium (40 mm K^+), to reach the maximal tension after 10 minutes; then a series of rinsing is made with the physiological solution; this operation (potassium stimulation and rinsing) is three times made, then for the balance, the basic tension was adjusted in 1 g.

After the period of balance, the ring was precontracted with the norepinephrine (10^{-6} mM) to reach an optimal isometric contraction. The relaxation caused by various concentrations in an increasing and cumulative way of the foliar extract of *Nephrolepis Biserrata* (5-200 mg/ml) and of the acetylcholine (10^{-11}-10^{-7}M) in presence and in absence of endothelium was expressed as the variation of the percentage of the contraction led by the norepinephrine (100%).

For the continuation of our study, the aqueous foliar extract of *Nephrolepis Biserrata* was used to make the biological tests.

To verify the absence of endothelium destroyed mechanically, a test was realized by an answer to the acetylcholine (10^{-6} M), according to the method of Furchgott and Zawadzki [19].

To avoid the influence of the nitric oxide (NO or Endothelium Derived Relaxing Factor: EDRF), the preparation was beforehand incubated during 30 min in a solution of L-NAME (10^{-5} M) to block the metabolic way of the synthesis of the nitrogen oxide, before the contraction of the preparation led by the norepinephrine (10^{-6} M), to verify the absence of endothelium destroyed mechanically, a test was realized by an answer to the acetylcholine (10^{-6} M), according to the method of Furchgott and Zawadzki [19].

To avoid the influence of the nitric oxide (NO), the preparation was beforehand incubated during 30 min in a solution of L-NAME (10^{-5} M) to block the metabolic way of the synthesis of the nitric oxide (NO; EDRF), before the contraction of the preparation led by the norepinephrine (10^{-6} M), then followed by the treatment of the same preparation by various concentrations of *Nephrolepis biserrata* (5-200 mg/ml).

Statistical analyses

The results are expressed are forms of the average values expressed in percentages of variations of the contraction of the aorta led by the norepinephrine (10^{-6} M), according to various concentrations of the aqueous foliar extract of *Nephrolepis biserrata* and the acetylcholine (Table 1). The contraction caused by the norepinephrine (10^{-6} M)

Chemical compound	Aqueous Extract	Chloroformic Extract	Ethanolic Extract
Free Anthraquinones	+++	-	-
Anthocyanine	-	+++	++
Cyanidines	+++	-	-
Flavonoids	-	+++	++
Saponosides	+++	-	-
Sterols et triterpens	+	++	+++
Tannins	+++	-	++
Alkaloide	+++	-	-

+ + +: abundant; + +: not very abundant; +: to the state of trace; - : negative test.
Table 1: Chemical compounds of the foliar extract of *Nephrolepis biserrata* according to the solvents used.

corresponds to a 100% percentage, was considered as reference.

The doses of the effects threshold, maximum and the ED_{50} were determined for *Nephrolepis biserrata* and the acetylcholine.

ED_{50} values: the concentration of test drug that causes a 50% relaxation of agonist-induced aortic contraction.

Results were expressed as percentage of relaxation: $\%\ \text{relaxation} = \frac{R1 - R2}{R1} \times 100$

Where R_1 is the response to Norepinephrine and R_2 is the response after adding the test drug. The obtained values were expressed by means ± SD. Statistical significance was tested using student's test.

Results

The results presented in the picture1 show that samples outcomes of three sites (Franceville, Lambaréné and Oyem) of harvests contain the phenolic compounds, terpenic and nitrogenous. The analysis of these results highlights the abundance (+++) in tannins, anthraquinones free, cyanidines, saponosides and a presence in the state of track of sterols and terpenes in the aqueous extract. The presence of the total flavonoids and the anthocyanins were revealed in extracts chloroformic and ethanolic.

However, flavonoids and anthocyanines are more plentiful in the chloroformic extract; and the extract ethanolic is rich in compounds terpenes. For the continuation of our study, it is the aqueous foliar extract which was used for the various biological tests.

Effect of the aqueous foliar extract of *Nephrolepis biserrata* on the contraction of the aorta induced by the norepinephrine (10^{-6} M) in presence and in absence of endothelium.

In the presence of endothelium, the aqueous extract of *Nephrolepis biserrata* decreases in a way measure increasing and cumulative (5-200 mg/ml) the contraction of the aorta of toad led by the norépinephrine (10^{-6} M); the threshold of the effect is observed in 5 mg/ml (0,30%) and maximum in 200 mg/ml (92,24%); the ED_{50} is 100 mg/ml, is a 53,70% decrease (Figure 1). The exposure time of the preparation for every dose of the foliar extract was 1 at 3 minutes (s).

In absence of endothelium, the aqueous extract of *Nephrolepis biserrata* decreases in a way measures increasing and cumulative (5-200 mg/ml) the contraction of the led aorta spoke norepinephrine (10^{-6} M); the threshold of the effect is observed in 50 mg/ml (14,20%) and maximum in 200 mg / ml (96,59%); the ED_{50} is 100 mg/ml, is 47,34% decrease (Figure 1).

However, it is necessary to note that in absence of endothelium, we observe a light increase of the contraction in the concentrations 5, 10 and 30 mg/ml; respectively of 7,70%, 6,85% and 2,02%.

Effect of the acetylcholine on the contraction of the aorta induced by the norepinephrine (10^{-6} M) in presence and in absence of endothelium.

In the presence of the endothelium, the acetylcholine of a way measures increasing and cumulative (10^{-11} M-10^{-7} M) decrease the contraction caused by the norepinephrine (10^{-6} M); the threshold of the effect was observed in 3.10^{-11} M (30,85%) and maximum in 10^{-7} M (88,14%). The exposure time of the preparation for every dose

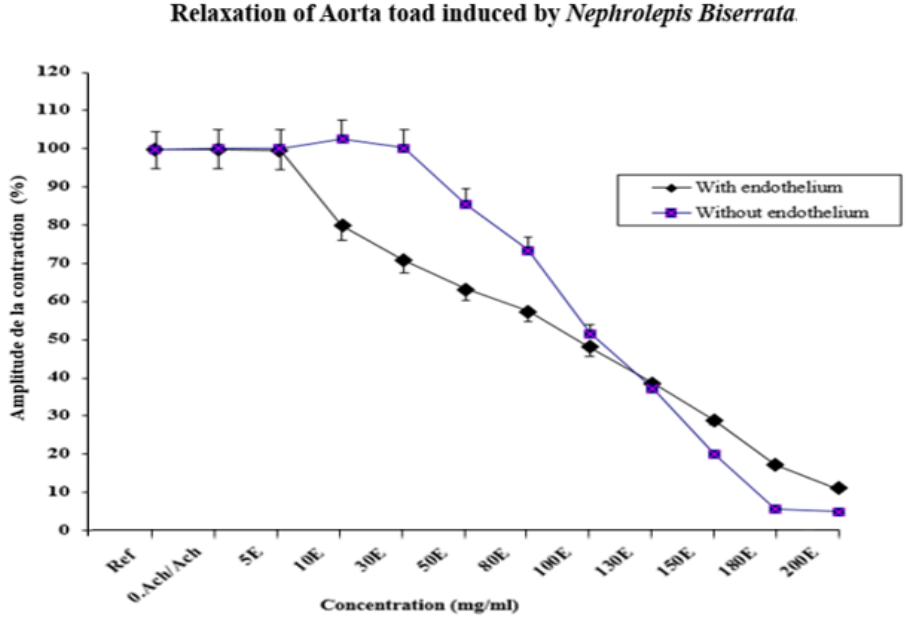

Figure 1: Effects of *Nephrolepis biserrata* on the contraction of the aorta of the heart of toad induced by the norepinephrine (10^{-6} M) in presence and in absence of endothelium.

concentration of acetylcholine was from 1 to 3 minute (s); the ED_{50} is in 10^{-10} M, is a 45,25% decrease (Figure 2).

In absence of endothelium, in the same concentrations as above indicated, the acetylcholine increases the contraction induced by the norepinephrine (10^{-6} M); the threshold of the effect was in 10^{-11} M (4,72%) and maximum in 10^{-7} M (28,35%); (Figure 2).

We notice that the threshold of the decrease of the aorta's contraction by *Nephrolepis biserrata* is observed in 5 mg/ml (0,30%) in the presence of endothelium and 50 mg/ml (14,20%) in its absence.

Effect of the aqueous extract of *Nephrolepis biserrata* on the contraction of the aorta induced by the norepinephrine (10^{-6} M) preincubated with a solution of L-NAME (10^{-5} M) in the presence of endothelium.

In the presence of the endothelium, the aqueous extract of *Nephrolepis biserrata* decreases in a way measure increasing and cumulative (5-200 mg/ml) the contraction led by the norepinephrine (10^{-6} M) the preparation of which was preprocessed by a solution of L-NAME (10^{-5} M). The threshold of the effect was observed in 5 mg/ml (1,70%) and maximum in 200 mg/ml (87,66%); the ED_{50} is 100 mg/ml, that is 51,49% of decrease Figure 3.

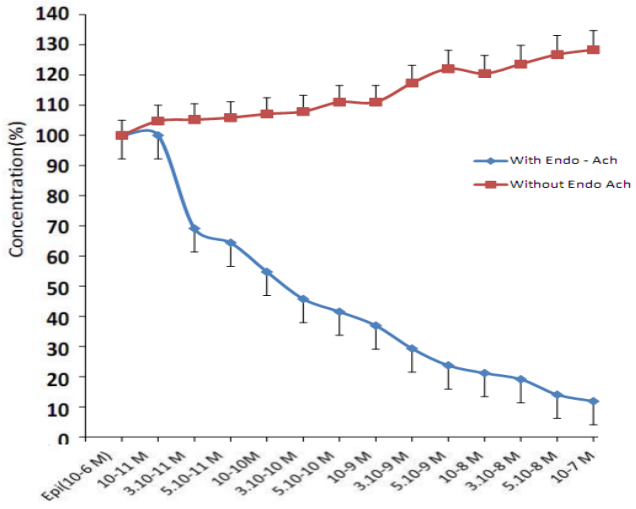

Figure 2: Effects of acetylcholine on the contraction of the aorta of the heart of toad induced by the norepinephrine (10^{-6} M) in presence and in absence of endothelium.

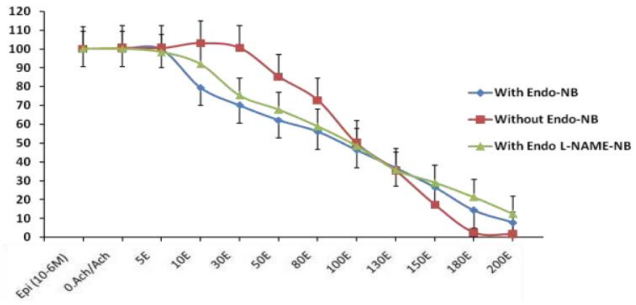

Figure 3: Effects of *Nephrolepis biserrata* on the contraction of the aorta induced by the norepinephrine (10^{-6} M) pretreated with the L-NAME (10^{-5} M) in the presence and in absence of endothelium.

Discussion

From our ethnobotanic studies led in a district of Franceville (Evéché) having 1.342 inhabitants, a questionnaire about the knowledge and the use of *Nephrolepis biserrata* was subjected to 30 of them, 20 wanted to participate in our survey.

It gets free that *Nephrolepis biserrata* called "milliélè" by the ethnic group Ndoumou used foliar of *Nephrolepis biserrata* infusion in traditional medicine to warn and/or deal the arterial high blood pressure and that the young leaves were consumed as vegetable in the province of the Haut-Ogooué in Gabon.

Our phytochemical studies on the samples of *Nephrolepis biserrata* collected to Franceville and its neighborhood (Haut-Ogooué), to Lambaréné (Moyen-Ogooué) and to Oyem (Woleu Ntem) present the same chemical groups.

Indeed, abundance in tannins, anthraquinones free, cyanidines, saponosides, alkaloids and a presence in the state of track of sterols and terpenes was observed in the aqueous extract. The presence of the total flavonoids and the anthocyanins were highlighted in extracts chloroformic and ethanolic.

Our phytochemical observations (Table1) are similar to those obtained by Adou et al. [36], who showed that the aqueous extract of *Nephrolepis biserrata* contains mainly flavonoids, tannins catechics and sterols. The biological tests (Figure 1) show that the aqueous foliar extract of *Nephrolepis biserrata* decreases the contraction of the aorta led by the norepinephrine (10^{-6} M); and this in a way measures increasing and cumulative (5-200 mg/ml) in presence and in absence of endothelium. Furthermore, the threshold of the effect (that is a 0,30% decrease) is observed in 5 mg / ml in the presence of endothelium and in 50 mg/ml (14,69%) in its absence; the effect is maximum in 200 mg / ml in both cases; 96,59% in the absence of endothelium is a 92,24% decrease in presence and 96,59% in the absence of endothelium.

These results highlight the effect relaxing of the foliar extract of *Nephrolepis biserrata*, thus the antihypertensives properties of a part of this plant at the level of the aorta of toad; the reduction of the amplitude of the contraction to 50% is observed in 100 mg/ml of *Nephrolepis biserrata* (ED_{50}) in presence and in absence of endothelium; let be a respective decrease of 53,70% and 47,34% (Figure 1).

Studies showed a correlation between cardiovascular risk and the consumption of polyphenols of natural origin [37] also reported that fruits of *Anacardium L.* of which the phytochemical studies revealed the presence of polyphenols were consumed and the leaves of this plant are used as antihypertensive in traditional medicine in Indonesia.

So, according to Khan and Gilani [4], the raw extract of the fruit of *Terminalia bellerica* containing flavonoids, sterols and tannins, to manage of a way dose-increasing cause the decrease of the blood pressure at the level of a rat anesthetized (*in vivo*), of the aorta having its endothelial layer intact and of the aorta in absence of endothelium (*in vitro*). These results indicate that *Terminalia bellerica* lowers the blood pressure through an opposing mechanism of the Ca^{2+}. Furthermore, for [37,38] the beneficial effects of polyphenols on the cardiovascular system could be partially owed to the direct action of these compounds on the vascular wall, and that polyphenols increase the production of the relaxing factors, especially the nitric oxide.

According to the studies of Martin et al. [39,40], polyphenols cause the increase of the calcium cytoplasmic cells endothelial which lead by a continuation of biochemical reactions the production of nitric oxide

implied in the mechanism of the vasorelaxation at the level of the aorta of bovine. Also let us indicate that Iwalokun et al. [2], revealed that *Loranthus micranthus* (*Lm*), used traditionally handle the arterial high blood pressure at the level of the aorta of rat by the increase of the production of the nitric oxide.

Other studies as that of the Chataigneau et al. [11], show that compounds polyphenolic are capable of increasing the concentration of the cytoplasmic calcium in endothelial cells to allow the activation of the NO-synthase endothelial (eNOS) by the complex calcium-calmoduline, and another way of eNOS's activation depends on that of the way phosphoinositide 3 (PI3) kinase/Akt and the consecutive phosphorylation of NO-synthase, Akt phosphoryled will be responsible for the activation of the eNOS.

According to Ndiaye et al. [41,42], this formation of NO is mainly due to the increase of the activity of NO synthase endothelial, a phenomenon involving two different mechanisms, one depending on the concentration of the free calcium in the cytosolic and the other one to the PI3-kinase/Akt's way.

According to Ndiaye et al. [29], compounds polyphenolic some red wine infer relaxations dependent on the endothelium are mediated by EDHF in the pig's coronary artery; and that these relaxations caused by EDHF, and led by compounds polyphenolic, are strongly reduced by inhibitors of the way PI3-kinase/Akt.

The use of the acetylcholine (10^{-11} M 10^{-7} M), shows a decrease of the contraction of the aorta of the heart of toad led by the norepinephrine (10^{-6} M) in the presence of endothelium in a way measure increasing and cumulative (Figure 2). The threshold of the effect was observed in 3.10^{-11} M (30,85%) and maximum in 10^{-7} M (88,14%). The ED_{50} is in 10^{-10} M, is a 45,25% decrease. These results are similar to those of certain authors who indicate that the acetylcholine causes the decrease the contraction of vessels in the presence of endothelium.

Indeed, Furchgott and Zawadzki [19] establishes the role of the endothelium in the relaxation of the smooth muscle of vessels due to the action of the acetylcholine; and that the nitric oxide (NO/Endothelium Derived Relaxing Factor or EDRF) according to Palmer [20], besides the prostacycline (PGI2) according to Moncada [21], identified as by endothelial-products were important vasorelaxants.

However, other factors would play an important role in the endothelium-depending hyperpolarization (Endothelium Derived Hyperpolarization Factor or EDHF) in the relaxation of the vascular smoother muscles [25,26].

It is necessary to remind also that the works of Eckly and Lugnier [43] revealed that polyphenols such as flavonoids cause the increase of the AMPc and the GMPc which cause the stimulation of certain potassium channels which will have for consequence a relaxation of the aortic arteries without endothelium.

Also let us note that the study of Ding [26], specify that the relaxation of vessels led by the acetylcholine is endothelium-dependent, through a very important factor, the nitrogen oxide (NO or Endothelium Derived Relaxing Factor: EDRF).

As a matter of fact, as the acetylcholine the aqueous extract of *Nephrolepis biserrata* provoke a vasodilatation of the aorta of the toad's heart.

In absence of endothelium, in the same concentrations (10^{-11} M-10^{-7} M), the acetylcholine increases the contraction led by the norepinephrine (10^{-6} M); the threshold of the effect was in 10^{-11} M (4,72%) and maximum in 10^{-7} M (28,35%); Figure 2.

Our results are similar to those of Ludmer et al. [44,45] who showed that in the absence of endothelium, the acetylcholine cause a human vasoconstriction of the coronary arteries of the heart, and that according to Nitenberg [46], the acetylcholine causes a vasoconstriction of the coronary arteries at the patients suffering from a coronary disease. These authors concluded that the vasoconstriction caused by the acetylcholine in absence of endothelium would be due to the dysfunction of the endothelium.

Contrary to the acetylcholine which causes a vasoconstriction with the absence of the endothelium, the aqueous foliar extract of *Nephrolepis biserrata* provoke a vasorelaxation in presence of endothelium with the amounts from 50 to 200 mg/ml. Let us note all the same that the threshold of the reduction in the contraction of the aorta by *NB* is observed to 5 mg/ml (0,30%) in the presence of endothelia and 50 mg/ml (14,20%) in its absence.

Andriambeloson [10] revealed that the relieving caused by polyphenols was observed on the level of the vessels with (to low dose) and without endothelium (with high amounts).

However, it should be noted that in absence of endothelium, we observe a light increase in the contraction to concentrations 5, 10 and 30 mg/ml; respectively of 7,70%, 6, 85% and 2, 02% (Figure 1).

In order to highlight the mechanism (s) implied in the presence of aqueous extracts of *NB*, we inhibited the metabolic way which produces nitric oxide (NO or EDRF), very important factor in the relieving of the vessels [19]. For that, as shows it the results of Figure 3, when the aorta of the heart of clamping plate in the presence of the endothelium is pretreated with a solution of L-NAME (10^{-5} M) inhibiting of synthesis of NO [4], then contracted by the norepinephrine (10^{-6} M) to have a contraction maximum and followed by a treatment by a solution of foliar extract aqueous of *Nephrolepis biserrata*, and this in manner proportions increasing and cumulative (5-200 mg/ml), we always observe a reduction in the contraction. The threshold of the effect is noted to 5 mg/ml (1,70%) and maximum with 200 mg/ml (87,66%); the ED_{50} is of 100 mg/ml, that is to say a reduction of 51,49%.

These results show that *Nephrolepis biserrata* causes its hypotensive effect by other mechanisms which would not imply only the way of the production of NO, because in spite of the inhibition of this one by L-NAME (10^{-5}M), the aqueous extract always causes by the action of its compounds, the reduction of the contraction induced by the norepinephrine (10^{-6} M).

Our work join thus those of Khan and Gilani [4], which reported that the rough extract of the fruit of *Terminalia bellerica* whose phytochimic studies reveal the presence of the flavonoïdes, of sterols and tannins like *Nephrolepis biserrata*, causes the reduction in the blood-pressure on the level of an anaesthetized rat (*in vivo*), aorta treated with a solution of L-NAME and aorta in absence of endothelium (in vitro). Moreover, work of Perez-Vizcaino [13], revealed that the injection of the quercetin which is a flavonoids, provoke in a manner proportions increasing; decrease the arterial pressure of the pretreated rats with a solution of L-NAME. These same authors observed this reduction of the blood pressure at various models of hypertensive rats.

Let us quote also work of Jaffri [47], which showed that in the hypertensive rats induced by the administration of L-NAME whose production of nitrogen oxide is defective, the extract of the palm oil (*Elaeis guineensis*) attenuated the increase in blood pressure of these animals.

Also let us recall according to the phytochimic studies on *Hibiscus sabdariffa* reported by Ojedaa [48], revealed the presence of polyphenols such as the anthocyanines and that these compounds cause the blood pressure decrease by inhibiting the activity of the Enzyme of Conversion of Angiotensin (ACE). Work of Jang [9], showed that the polyphenols of the red wine of *Vitis coignetiae* spontaneously provoke a hypotensive effect while inhibiting (ACE) on the level of the hypertensive rats. See the whole of the concepts suggested by Drank.

All in all, as work of Zisaki et al. [49,50] brings it back, the hypotensives substances are: inhibiters of the ACE, β and calci-blockers as well as the diuretic ones.

Our next investigations will relate to the metabolic ways implied in the mechanism of action of *Nephrolepis biserrata*.

Conclusion

This study shows that the aqueous extract of *Nephrolepis biserrata* decreases in manner proportions increasing, the contraction of the aorta of the heart of clamping plate; and this in presence and absence of endothelium.

This preliminary study shows that the antihypertensor effect of *Nephrolepis biserrata* seems dependant and independent endothelium to induce a vasodilatation of the vessels.

The acetylcholine taken as example of antihypertensive pharmacological substance of the arteries causes also the reduction in the contraction of the aorta in an amount-increasing way but dependant endothelium, because in absence of the endothelium, the acetylcholine provokes a vasoconstriction.

The action of antihypertensive *Nephrolepis biserrata* is observed (the reduction of the contraction) also when the preparation (aorta) is pretreated with a solution of L-NAME the inhibiter of the way of production the nitric oxide very important factor in the process of the relieving of the vessels, which lets consider that the chemical compounds of *Nephrolepis biserrata* mainly (polyphenols, alkaloid and terpenes), cause relieving by various metabolic ways which remain to be shown. The aqueous extracts of *Nephrolepis biserrata* present an antihypertensive property, which could justify and explain the use of this plant in the prevention and the treatment of arterial hypertension by the populations of Franceville and its surroundings.

Our studies must continue taking into consideration development of the Chronic Blood-Pressure at the young people and the people of the third age in various countries.

Acknowledgement

Mrs Collète Okassotou, secretary with the Vice-chancellorship of the USTM by which we learned the use of *Nephrolepis Biserrata* (*NB*) to treat Arterial Hypertension (HA) by the ethnos group Ndoumou of Franceville and its surroundings in the province of Haut-Ogooué in Gabon.

References

1. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AE (2008) Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. J Ethnopharmacol 115: 67-71.
2. Iwalokun BA, Hodonu SA, Nwoke S, Ojo O, Agomo PU (2011) Evaluation of the Possible Mechanisms of Antihypertensive Activity of Loranthus micranthus: An African Mistletoe. Biochem Res Int 2011: 159439.
3. Souza A, Lamidi M, Ibrahim B, Samseny RRR., Boukandou MM et al. (2011) Antihypertensive effect of an aqueous extract of citrus aurantifolia (Rutaceae) (Christm.) Swingle, on the arterial blood pressure of mammal. International Research of Pharmacy and Pharmacology 1: 142-148.
4. Arif-Ullah K, Gilani Anwarul Hassan (2008) Pharmacodynamic Evaluation of Terminalia bellerica for Its Antihypertensive Effect. Journal of Food and Drug Analysis 16: 6-14.
5. Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Ameachina JC, Ayinde BA (2008) Preliminary studies of blood pressure lowering effect of Naucleala tifolia in rats. In African Journal of Pharmacy and Pharmacology 2: 037-041.
6. Boobalan R, Pugalendi KV, Arjunan MM (2010) Aqueous extract of Melothria Maderaspatana (Linn.) leaf exerts antihypertensive effect and improves Mufa, Pufa and membrane fluidity of erythrocytes in patients with hypertension. An electron paramagnetic resonance investigation. Journal of Herbal Medicine and Toxicology 4: 133-139.
7. Khalili A, Khosravi MB, Zolghadr Asli AA, Nekooeian AA (2009) The Aqueous Extract of Vaccinium Arctostaphylus Berries Decreases Blood Pressure in Rat Model of Two-Kidney, One-Clip Renal Hypertension. Iranian Cardiovascular Research Journal 3.
8. Sakat SS, Wankhede SS, Juvekar AR, Mali VR, Bodhankar SL (2009) Antihypertensive effect of aqueous extract of Elaeocarpus ganitrus Roxb. seeds in renal artery occluded hypertensive rats. International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN: 0974-4304 Vol.1, No.3, pp 779-782 , July-Sept.
9. Hoon JJ, Yi SH, Kim JH, Lee DH, Lee JS (2011) Effects of Vitis coignetiae on the Quality and Antihypertension of Vitis hybrid Red Wine Korean J. Microbiol. Biotechnol. 39: 126–132.
10. Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, et al. (1997) Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br J Pharmacol 120: 1053-1058.
11. Thierry C, Ndiaye M, Valérie B, Kerth S (2003) Effets vasodilatateurs des composés polyphénoliques du vin : rôle de NO et EDHF. Mini-revue Sang Thrombose Vaisseaux; 15, n° 8 : 433–441.
12. Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339: 1523-1526.
13. Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A (2009) Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep 61: 67-75.
14. Nair S, Gupta R (1996) Dietary antioxidant flavonoids and coronary heart disease. J Assoc Physicians India 44: 699-702.
15. Erdman JW Jr, Carson L, Kwik-Uribe C, Evans EM, Allen RR (2008) Effects of cocoa flavanols on risk factors for cardiovascular disease. Asia Pac J Clin Nutr 17: 284-287.
16. Ojeda D, Jiménez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, et al. (2010) Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. J Ethnopharmacol 127: 7-10.
17. Sagesaka-Mitane Y, Sugiura T, Miwa Y, Yamaguchi K, Kyuki K (1996) [Effect of tea-leaf saponin on blood pressure of spontaneously hypertensive rats]. Yakugaku Zasshi 116: 388-395.
18. Peng WD (1999) Hypotensive effect of tenuifolic saponin and its mechanism. Zhongguo Yao Li Xue Bao 20: 639-642.
19. Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-376.
20. Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524-526.
21. Moncada S, Gryglewski R, Bunting S, Vane JR (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263: 663 - 665.
22. Feletou M, Vanhoutte PM (1988) Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 93: 515-524.
23. Félétou M, Vanhoutte PM (1996) Endothelium-derived hyperpolarizing factor. Clin Exp Pharmacol Physiol 23: 1082-1090.
24. Waldron GJ, Dong H, Cole WC, Triggle CR (1996) Endothelium-dependent hyperpolarization of vascular smooth muscle: role for a non-nitric oxide synthase product. Zhongguo Yao Li Xue Bao 17: 3-7.
25. Mombouli JV, Vanhoutte PM (1997) Endothelium-derived hyperpolarizing factor(s): updating the unknown. Trends Pharmacol Sci 18: 252-256.

26. Triggle CR, Dong H, Waldron GJ, Cole WC (1999) Endothelium-derived hyperpolarizing factor(s): species and tissue heterogeneity. Clin Exp Pharmacol Physiol 26: 176-179.

27. Ding H, Kubes P, Triggle C (2000) Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. Br J Pharmacol 129: 1194-1200.

28. Komas N, Lugnier C, Stoclet JC (1991) Endothelium-dependent and independent relaxation of the rat aorta by cyclic nucleotide phosphodiesterase inhibitors. Br J Pharmacol 104: 495-503.

29. Ndiaye M, Chataigneau T, Chataigneau M, Kerth SVB (2003) Red wine polyphenol induced endothelium-dependent NO-mediated relaxation is due to the PI3kinase/Akt-dependent phosphorylation of endothelial NO synthase in the isolated porcine coronary artery. Circulation 108: 4-101.

30. Busse R, Edwards G, Félétou M, Fleming I, Vanhoutte PM, et al. (2002) EDHF: bringing the concepts together. Trends Pharmacol Sci 23: 374-380.

31. Fourcade L, Paule P, Mafart B (2007) [Arterial hypertension in sub-Saharan Africa. Update and perspectives]. Med Trop (Mars) 67: 559-567.

32. Tardieu-Blot (1964) Flore du Gabon. Ptéridophytes 8: 85-86.

33. Bruneton J (1999) Pharmacognosie, Phytochimie, Plantes médicinales. Technique & Documentation, Paris, 721-741.

34. Paris R and Moyse H (1969) Précis de matière médicinale. Paris: Masson.

35. Bouquet M (1971) Travaux et documents de l'Orstom (Paris, no 13). Carlson C. F. et Riley T. V. Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. Journal of Applied Bacteriology 78: 264-269.

36. Lydie A, Dominique M, Awa T, Koffi C, Joseph II (2014) Nephrolepis Biserrata, une Ptéridophyte utilisée comme plante médicinale en Côte d'Ivoire. Journal of Applied Biosciences 81: 7298 – 7306.

37. Stoclet JC, Kleschyov A, Andriambeloson E, Diebolt M, Andriantsitohaina R (1999) Endothelial no release caused by red wine polyphenols. J Physiol Pharmacol 50: 535-540.

38. Cishek MB, Galloway MT, Karim M, German JB, Kappagoda CT (1997) Effect of red wine on endothelium-dependent relaxation in rabbits. Clin Sci (Lond) 93: 507-511.

39. Martin S, Andriambeloson E, Takeda K, Andriantsitohaina R (2002) Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. Br J Pharmacol 135: 1579-1587.

40. Sophie M, Giannone G, Andriantsitohaina R, Martinez MC (2003) Delphinidin, an active compound of red wine, inhibits endothelial cell apoptosis via nitric oxide pathway and regulation of calcium homeostasis. British Journal of Pharmacology 139: 1095–1102.

41. Ndiaye M, Chataigneau T, Kerth VBS (2003) Red wine polyphenolic compounds (RWPC) induce EDHF-mediated relaxation in the isolated porcine coronary artery via a redox-sensitive activation of the PI3kinase/Akt pathway in endothelial cells. Arch Mal Coeur Vaiss; 96: 375.

42. Ndiaye M, Chataigneau T, Andriantsitohaina R, Stoclet JC, Kerth VBS (2003) Red wine polyphenols cause endothelium-dependent EDHF mediated relaxations in porcine coronary arteries via a redox-sensitive mechanism. Biochem Biophys Res Commun; 310 : 371-377.

43. Eckly AE, Lugnier C (1994) Role of phosphodiesterases III and IV in the modulation of vascular cyclic AMP content by the NO/cyclic GMP pathway. Br J Pharmacol 113: 445-450.

44. Paul LL, Andrew SP, Thomas LS, Richard RW, Gilbert H et al. (1986) Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. N Engl J Med 315:1046-1051.

45. Steven WW, Joseph A, Walton, Henry H, Elizabeth G (1989) Evidence of Endothelial Dysfunction in Angiographically Normal Coronary Arteries of Patients with Coronary Artery Disease. Circulation 79: 287-291.

46. Alain N, Antony I, Foult JM (1993) Acetylcholine-induced coronary vasoconstriction in young, heavy smokers with normal coronary arteriographic findings. The American Journal of Medicine.

47. Jaffri JM, Mohamed S, Rohimi N, Ahmad IN, Noordin MM, et al. (2011) Antihypertensive and cardiovascular effects of catechin-rich oil palm (Elaeis guineensis) leaf extract in nitric oxide-deficient rats. J Med Food 14: 775-783.

48. Deyanira O, Ferrerb EJ, Zamilpab A, Arellanob AH, Tortoriello JB (2010) Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanines delphinidin- and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. Journal of Ethnopharmacology 127: 7–10.

49. Zisaki A, Miskovic L, Hatzimanikatis V (2015) Antihypertensive drugs metabolism: an update to pharmacokinetic profiles and computational approaches. Curr Pharm Des 21: 806-822.

50. Organisation Mondiale de la Santé (OMS) (2001) Promotion du rôle de la médecine traditionnelle dans le système de santé: Stratégie de la région africaine AFR/RC50/9. Harare : Bureau régional de l'Afrique, 2001 : 20. African Traditional Medecine Day.