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MINERAL CONTENT AND VASORELAXANT ACTIVITY OF LIPPIA MULTIFLORA HARVESTED IN BENIN

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ABSTRACT
We determine the mineral composition and lave evaluate the vasorelaxant activity of the powders and extracts of Lippia multiflora from 4 zones of Benin: Kétou, Mono, Djidja and Savalou. The main minerals found in the leaves are sodium (Na), potassium (K), magnesium (Mg), calcium (Ca). The Lippia multiflora samples in the four Kétou, Savalou, Mono and Djidja regions have calcium contents of 2800.7624mg / 100g, 2200.6034mg/ 100g, 2800.6240 mg/100g and 2800.1238mg/100g, respectively. These values are higher than the limit recommended by the WHO (800mg / 100g / day). The potassium content of the samples is respectively 700.8545mg / 100g, 1600.5812mg / 100g, 700.1855 mg / 100g and 1100, 7123 mg / 100g. What is below the 2000mg / 100g / day limit. For magnesium, the amount in the samples is 504 mg / 100g, 307 mg / 100g, 326 mg / 100g, and 510 mg / 100g respectively, compared with a normal value of 350mg / 100g for adults. The sodium content of the samples is 4150.5014mg / 100g, 2270.2344 mg / 100g, 2570.6953mg / 100g and 2610.9309mg / 100g, respectively, for a recommended value of 500mg / 100g / day. The aqueous decoction of Lippia multiflora of Djidja gave a good relaxation on the coronary arteries at a concentration of 25.81mg / mL compared to the extracts of the other zones. In total, the aqueous decoction of Lippia multiflora from Djidja is the most active followed by that of the Mono sample with a concentration of 21.32 mg / mL. The vasorelaxant activity observed for the Djidja sample is endothelium-dependent and the vasoactive factor NO (nitric oxide) is involved in this relaxation.

Keywords: Lippia multiflora, Benin, Extract, Minerals, Vasorelaxant activity.

INTRODUCTION

Lippia multiflora is an aromatic plant traditionally used to treat bronchial conditions, malaria, conjunctivitis, gastric disorders, enteritis, cough and lerhum [1]. The plant also has antihypertensive, relaxing and diuretic properties [2]. It is also used as a substitute for tea and a disinfectant of the mouth. Clinical studies [3] have confirmed its use in the treatment of malaria caused by *Plasmodium falciparum* [4] in Ivory Coast, the plant is used to treat febrile attacks and jaundice and diarrhea. In the [5] countryside as in the city, the hot drink prepared from its leaves is very consumed to pass the fatigue and promote sleep after a day

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of hard work. However, despite its many virtues and the great interest it arouses, *Lippia multiflora* remains a wild plant and subject to gathering. Thus the development of new crops could be an early solution hence the interest of research studies conducted on *Lippia multiflora* for domestication and recovery. It is at least in this logic that the present work, whose main objective is to evaluate the mineral composition [6] of the *Lippia multiflora* samples from the different regions of Benin and their vasorelaxant activity on the models of coronary arteries of the pig.

MATERIAL AND METHOD

Plant material

The leaves of *Lippia multiflora* were harvested in 2015 in the main cities of 4 departments of Benin chosen for their demographic weight and their diversified geographical areas. The departments concerned are: Plateau, Mono, Zou and Collines. The investigation was made in the towns of Ketou for the department of Plateau; Houeyogbe for the department of Mono; Bohicon and Djidja for the Zou and Savalou for Collines. A specimen of the plant is deposited and identified by the national herbarium of the University of Abomey-Calavi (Benin) [7].The plant material we used is made of leaves or leaf stems of plant selected based on their frequency use against opportunistic infections and arterial hypertension. After harvesting the plants, their fresh samples were dried for ten to fourteen days in the dark in a constant-temperature cabinet (conditioned air). The dried leaves were reduced to powder with an electric grinder (Flour MILLS NIGERIA, El MOTOR No. 1827) and the ground matter obtained was sieved and stored in a preservative for analysis.

Preparation of crude extracts [8]

The hydro-ethanolic and aqueous extracts were prepared for each of the four samples collected from the four cities according to standard techniques. 50 g of powder are dissolved in 500 ml of solvent (water-ethanol (4: 6, V / V) for the hydro-ethanolic extracts, the mixture is left stirring continuously for seventy-two hours (72 hours) and the macerate obtained was filtered three times successively on hydrophilic cotton. Then the filtrate was evaporated to dryness at 40 ° C using a rotavapor (HeidolphLaborota 4000 efficient) coupled to a water cooler (Julabo FL 300). For the aqueous decoction, 50 g of powder were introduced into 500 ml of distilled water. The whole contained in a vial was brought to moderate boiling on an electric plate for 15 minutes. The mixture obtained was filtered and evaporated to dryness, and then weighed for yield determination according to the relation:

$$\text{Yield} = \frac{\text{Mass of dried extract}}{\text{mass of test sample}} \text{X } 100$$

Determination of minerals Content in total ash

It is a question of determining the nonvolatile residual substances contained in a drug after calcination of the previously dried powder. A test portion of 5 g of powder is introduced into a previously weighed quartz crucible and calcined at 600°C. in a muffle furnace. Then allow to cool and then weigh to evaluate the total ash content according to the expression:

$$\text{Percentage total ash} = \frac{\text{Massofash x 100}}{\text{Test mass}}$$

Test mass = mass before calcination – tare; Mass of ash = mass after calcination – tare

Determination of sodium and potassium [9]

It was performed using an automatic dilution flame photometer. Flame photometry is based on the fact that when the atoms of an element are excited by a flame, they emit the determined wavelength radiations whose intensity can be measured by a flame ionization photometer. The results are expressed in mEq / L, convertible in mg or in µg.

The sample must be in the form of an aerosol so that the solvent evaporates instantly in the flame. The photons emitted by the internal standard of potassium or sodium vaporized in the flame are sent through an interference filter on a photomultiplier thereby generating a reference voltage. The photons emitted by the sample to be assayed according to the same process generate a measurement voltage. The sodium or potassium concentrations are read on the screen of the device.

Determination of calcium (Ca^{2+}) [9]

The Ca-Kit allows the colorimetric determination of total calcium, without deproteinization, in the solutions to be analyzed. The calcium ion reacts with the methylthymol blue indicator (BMT) in an alkaline medium.

Ca^{++} + BMT ⟶ complexe Ca-BMT

The intensity of the Ca-BMT complex concentration, measured at 612 nm, is proportional to the amount of calcium present in the sample. Three reagents were prepared: Reagent 1 (R1): the standard (1x3 ml) and Ca2 + (2.50 mmol / l); Reagent 2 (R2): staining reagent (2x 80 ml) with methylthymol blue (0.092 mmol / l); Reagent 3 (R3): alkaline reagent (2x 80 ml), monoethanolamine (200 ml / l) and pH> 11. For the working solution, put the powder from the R1 flask into the empty flask and dissolve it with 5 ml of distilled water. After complete dissolution, add flask R2, then mix with stirring and wait 24 hours at 20 - 250 C. After stirring, put the samples in the photometer and wait 1 minute. The staining is stable for 1 hour at 20-250 C, then perform a calibration at each series of assays. The procedure of the assay is summarized in Table 1.

Table 1. Operating protocol for calcium determination

	Blanc réactif	**Etalon**	**Dosage**
Stallon	-	10 µl	-
Sample	-	-	10 µl
Working solution	1 ml	1 ml	1 ml

Table 2. Operating protocol for the determination of magnesium

	White Reagent	**Sample**	**Dosage**
Distilled Water	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl
Working solution	1 ml	1 ml	1 ml

Table 3. Operating protocol for iron determination

	White reagent	Stallon	White Sample	Dosage
Distilled water	200 µl	-	-	-
Stallon	-	200 µl	-	-
Sample	-	-	200 µl	200 µl
Reagent 2	-	-	1 ml	-
Working solution	1 ml	1 ml	1 ml	1 ml

After stirring, put the samples in the photometer and wait for 1 min. The staining is stable for one hour at 20-250°C, then perform a calibration at each series of assays.

Calculation: With the spectrophotometer, the calculations are performed as follows:

$$\text{Sample concentration} = \frac{\text{DO sample} \times N}{\text{Stallion}}$$

N = concentration of the standard

Determination of magnesium (Mg2+) [9]

The Mg-Kit allows the colorimetric determination of total magnesium, without deproteinization, in the solutions to be analyzed. The magnesium ion reacts with the calmagite in an alkaline medium to give a complex of pink color. Three reagents were prepared to do this manipulation: Reagent 1: it is the standard (5ml) then 25 mg / l of magnesium sulphate which we used as the white of the sample; Reagent 2: We used calmagite (160 mg / l) and a staining reagent (2 x 80 ml); Reagent 3: We used an alkaline (2 x 80 ml) and a reagent at pH 11. The optimal density (OD) of the working solution should be between 0.77 and 1.125 at 520 nm. The test was carried out with distilled water (20 µl), a wavelength (520 nm), the standard (20 µl), the sample (20 µl) and 1 ml as the working solution. The sample was mixed with the various reagents, then 1 minute at 20- 250°C was waited for. After that, put everything in the photometer already set and perform a calibration at each batch of assays. The interpretation of test results must be made taking into account the clinical context and possibly the results of other tests. The procedure is recorded in Table 2.

The concentration of the sample is made at the product of OD of the sample and the concentration of the standard by the ratio of the OD of the standard.

$$CE = \frac{\text{Sample DO} \times N}{\text{Standard DO}}$$

SC: sample concentration; OD: optical density; N: standard concentration.

Determination of iron (Fe2 +) [9]

The Ferrimat-Kit allows the colorimetric determination of iron in the solutions to be analyzed, in the presence of guanidine and in acid medium, with hydroxylamine as reducing agent and ferrozine as an indicator. Guanidine hydrochloride denatures the transport proteins and maintains them in acidic pH solution.Ferric iron is reduced to ferrous iron by hydroxylamine. The Fe2 + ion chelates to ferrozine to give a colored complex. The iron present in the sample was determined by the following reactions:

$$Fe^{+++}\text{transferrin} \xrightarrow{\text{Guanidine-HC}} Fe^{++}\text{ Hydroxylamin}$$

$$Fe^{+++} \longrightarrow Fe^{++}\text{ Ferrozine}$$

Ferrozine: (pyridyl-2) -3 bis- (phenyl-4-sulphonic acid) - 5.6 di-sulphonic acid-5 ', 5", triazine-1, 2, 4, monosodium salt.

We also used three types of reagents: Reagent 1 (R1): the standard (1 x 20 ml) and iron (35.5µmol / l or 2mg / l) that we used as the blank of the sample; Reagent 2 (R2): We took guanidine (2 x 80 ml), hydroxylamine (230 mmol / l), guanidine hydrochloride (4.5 mol / l) and acetate buffer at pH 5; Reagent 3 (R3): We used a staining reagent (1x 14 ml), ferrozine (44.4 mmol / l) and an acetate buffer

at pH 5. The test was carried out with 40 ml of reagent 2; 1.5 ml of R3, the sample blank (200µl and 1ml of R1); reagent blank (300 µl), ED (200 µl). The assay was made by 200 µl of R2 and 1 ml of the working solution. As in the previous method, the sample was mixed with the various reagents, then one minute was waited at 20-250 C. Afterwards, the whole was put in the photometer already set and a calibration was carried out at each series of assays. The procedure is summarized in Table 3.

As in the previous method, the sample was mixed with the various reagents, then one minute is waited at 20-250 ° C. After that, the whole is put in the photometer already set and a calibration is carried out at each series of dosages. The interpretation of test results must be made taking into account the clinical context and possibly the results of other tests.

The concentration of the sample is made at the OD product of the sample; the optimal density of the blank of the sample (DOBE) and the concentration of the standard by the ratio of the OD of the standard.

$$EC = \frac{\text{Sample DO} \times \text{DOBE} \times N}{\text{DO étalon}}$$

EC: sample concentration;
DOBE: optimal white density of the sample;
DO: optical density;
N: concentration of the standard

Vasorelaxant tests

This protocol was used by [10].To verify the vasodilator activity of the extracts, we used the model of the rings of pork coronary artery suspended in cells with isolated organs. These arteries were taken from freshly slaughtered pork hearts. The circumflex arteries were carefully removed, cleaned of adherent connective tissues and rinsed with krebs, avoiding altering the endothelium. The coronary artery segments were cut into rings of 3 to 4 mm and then mounted between two hooks, the first being fixed and the second connected to a voltage sensor itself connected to an amplifier and a computer for viewing and recording isometric voltage variations.

The rings were placed in isolated organ chambers containing 10 ml of krebs bicarbonate solution at 37 ° C and oxygenated with a mixture of carbogen (95% O2, 5% CO2). The rings were subjected to a base tension of 5 g and were then left standing during a stabilization phase of 45 minutes. They were then contracted with a solution of KCl (80mM) allowing, by a maximal depolarization, to test the reactivity of the vascular smooth muscle. After obtaining the maximum effect, three successive washes were performed. In order to test the integrity of the endothelium, the rings were contracted with the thromboxane analogue A2U46619 (10-8 M) and at the contraction plateau bradykinin (310-8 M) is applied. After three successive washes, a stabilization phase of 45 minutes was observed at the end of which the rings were contracted again with the thromboxane analogue A2U46619 (10-8 M) before applying a growing and cumulative range (10-4). 310-1 g / L) of the various extracts of the plants obtained.

Statistical analysis of the results

The statistical analysis was made by Student's ttest. The results are given as an average +/- SEM. Values of $p < 0.05$ are considered statistically different.

RESULTATS AND DISCUSSION

Extractions yield

The preparation yield of the crude extracts is summarized in Table 4 below as a percentage of dry residue obtained per 100 g of powder.

We note that the yield is better when the extraction is made with the water-ethanol mixture and this independently of the harvesting area, apart from Djidja sample (***Table 4)***. The mixture of water and alcohol allows to extract most of the chemical principles of this plant.

Minerals are important compounds because of their physiological and metabolic function in the body. The result shown in Table 5 shows that *Lippia multiflora* is a leaf plant rich in minerals. The main elements present in the leaves are sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca). Magnesium (Mg), and ashes were found in small quantities. The abnormal enzymatic activity and the ionic imbalance of the blood are related to the deficit of Na, K, and Mg because they are the vital elements of the living cells. The samples of *Lippia multiflora* in the four regions Ketou, Savalou, Mono and Djidja have calcium contents of 2800.7624mg / 100g, 2200.6034mg / 100g, 2800.6240 mg / 100g and 2800.1288mg / 100g respectively. The recommended daily calcium intake by WHO is 800mg /100g for adults and children. This study shows that the calcium and sodium content of *Lippia* species is above the standard recommended by WHO 800mg / 100g. Calcium is the most abundant mineral in the body and it is involved in blood coagulation, muscle contraction, neurological function, bone and tooth formation [11]. It is also an important factor in enzymatic metabolic processes [12]. The study showed that the potassium content of *Lippia multiflora* is 700.8545mg / 100g and 1600.5812mg / 100g, 700.1855 mg / 100g and 1100.7123 mg / 100g, respectively. As a result, it appears that potassium is the most abundant mineral in the *Lippia multiflora* plant. This observation is consistent with the results of alinnor et *al*.[2] that potassium is most abundant in agricultural products. Potassium is important in the regulation of cardiac rhythm, body water balance and neurotransmission [13]. High level of potassium in the body increases iron utilization and are beneficial for people taking diuretics to control high blood pressure. The who [14] recommended a daily intake of 2000mg / 100g of potassium for adults and 1600mg / 100g for children. This study revealed that the potassium content of the *Lippia*

species samples in the regions of Savalou and Djidja is accurate by the standards recommended by the WHO for children, this is not the case for *Lippia* species in the regions of Kétou and Mono. The magnesium content of *Lippia multiflora* is 504 mg / 100 g, 307 mg / 100 g, 326 mg / 100 g, 510 mg / 100 g of the four Ketou, Savalou, Mono and Djidja regions respectively.The recommended nutritional intake of magnesium is 350mg / 100g for adults and 170mg / 100g for children. Given the differences between varieties it can be concluded that *Lippia multiflora* species in all four regions have the required minimum magnesium content to meet the daily needs of adults and children. Magnesium is known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia (premature hair loss), dermatitis, dysfunction of the immune system, gonad atrophy, altered spermatogenesis, congenital malformations and disorders of coagulation [15]. According to, magnesium plays a vital role in calcium metabolism and bone formation and is also involved in the prevention of related diseases to the circulatory system. It helps regulate blood pressure and the secretion of insulin. Sodium is an important mineral that contributes to the regulation of blood fluidity and the maintenance of the potential of electrons in body tissues. The sodium contents of *Lippia multiflora* are respectively 4150.5014 mg / 100 g, 2270.2344 mg / 100 g and 2570.6953 mg / 100 g and 2610.9309 mg / 100 g of the regions of Ketou, Savalou, Mono and Djidja. The World Health Organization has recommended daily 500mg / 100g sodium intake for adults and 400mg / 100g for children. The result indicates that the sodium content in the leaves of *Lippia multiflora* harvested in the four regions, represent at least four times the standard daily intake, so *Lippia multiflora* from these regions are real sources of sodium and could be recommended to pregnant women and those with hypertension and renal disease whose direct intake of salt must be minimized [16]. The iron content of *Lippia multiflora* leaves found in this study is well above that recommended by the WHO of 10-15 mg / 100g perday. According to iron as a trace element plays many biochemical roles and it is a fundamental element in the metabolism of almost all living organisms. In humans, iron is an essential element of several types of proteins and enzymes.

It is important for the normal functioning of the central nervous system and facilitates the oxidation of carbohydrates, proteins and fats. Iron is needed for the formation of hemoglobin in the blood and it is an important part of the diet of pregnant women, breastfeeding mothers, infants, and the elderly who often have anemia and other diseases related to blood (Table 5).

Determination of vasorelaxant activity

We note that the aqueous decoction of *Lippia multiflora* from Mono was relaxed to 20% at a concentration of 22.11 mg /L for without endothelium and 21.32 mg/mL for with endothelium (Figure 1**)**.

We note that the aqueous decoction of *Lippia multiflora* of Djidja was relaxed to 25% at a concentration of 33,73mg/mL for without endothelium and 25,81mg/mL for with endothelium. In total, the aqueous decoction of *Lippia multiflora* of Djidja is more active followed by the decoction of Mono. It also results from these analyses that the vasorelaxant activity observed in the region of Djidja is endothelium dependent. The results show that an extract of the eight tested, has a good vasodilator activity. This is the aqueous decoction of *Lippia multiflora* leaves of Djidja (Figure 2). The vasorelaxant effect (25% relaxation) of the aqueous decoction of *Lippia multiflora* leaves is of 33,73mg/mL. The aqueous decoction of *Lippia multiflora* leaves of Mono is weakly active (Figure 1) while the other extracts are inactive. The relaxation of the aqueous decoction of Mono *Lippia multiflora* leaves is endothelium-dependent.

Determination of the mineral composition

Table 5. Composition in mineral elements of *Lippia multiflora* leaves harvested in four different regions in mg / 100g of dry matter

Composition (mg)	LK	LS	LM	LD
Cendre	0,1407	0,1197	0,1541	0,1224
Calcium (Ca)	2800,7624	2200,6034	2800,6240	2800,1238
Magnesium(Mg)	504	307	326	510
Potassium (K)	700,8545	1600,5802	700,1855	1100,7123
Sodium (Na)	4150,5014	2270,2344	2570,6953	2610,9309
Ion (Fe)	1121,0547	1267,0886	3998,0665	1205,6122
Na/K	58,5778	14,1377	36,6272	23,5830
Ca/Mg	557,0683	2061,7140	2227,1746	549,2624

Number of repetitions n=3; significant difference $p < 0.05$

Mechanisms of action involved

Flavonoids present in the aqueous extract of *Lippia multiflora* could act either by inhibiting the angiotensin I converting enzyme or by antagonizing angiotensin II because angiotensin II has been correlated with NO [17]. Tannins are also involved in the inhibition of

enzymes such as 5-lipooxygenase and Angiotensin converting enzyme. There may also be other mechanisms of action: these molecules act by opening the ATP-dependent potassium channels leading to cell hyperpolarization and a decrease in intracellular Ca2 + by calcium channel inhibition associated [18] with a reduction of release of Ca2 + by the sarcoplasmic reticulum; by inhibition of phospholipase C; by phosphorylation of proteins accelerating relaxation Taubert et *al.*[19] and inhibition of Rho kinase and by promoting vasorelaxation indirectly by decreasing the degradation of adenosine monophosphate (AMP) via the inhibition of phosphodiesterase II, by inhibiting the production of as well as the synthesis of renin by negative feedback and hence Ang II and deactivating O2- superoxide anions[19] (Figure 3 et Figure 4).

Fig 1. Vasorelaxing effect of the aqueous decoction of *Lippia multiflora* of Mono on the coronary arteries with and without endothelium

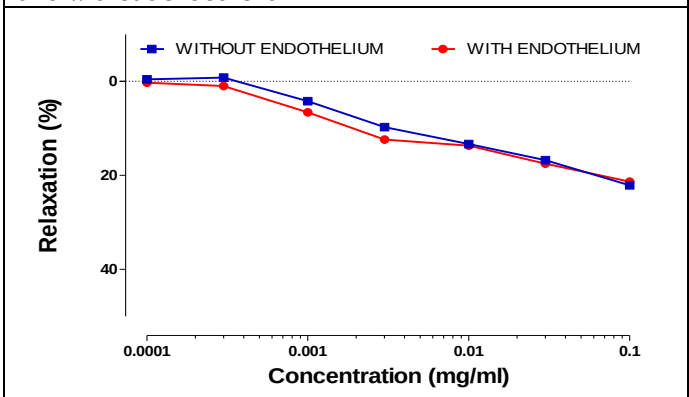


Fig 2. Vasorelaxing effect of the aqueous decoction of *Lippia multiflora* of Djidja on coronary arteries with and without endothelium

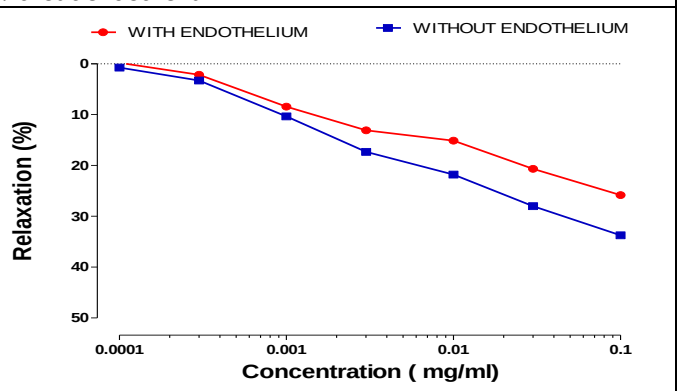


Fig 3. Effect of the aqueous decoction of Lippia multiflora of Mono on pig coronary arteries provided with endothelium and in the presence of inhibitors

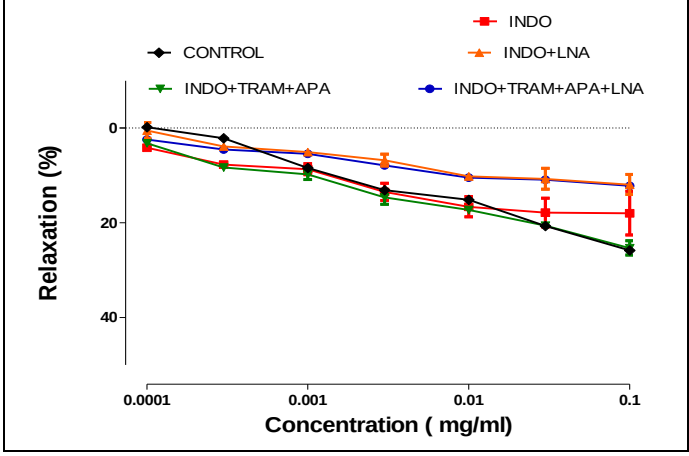
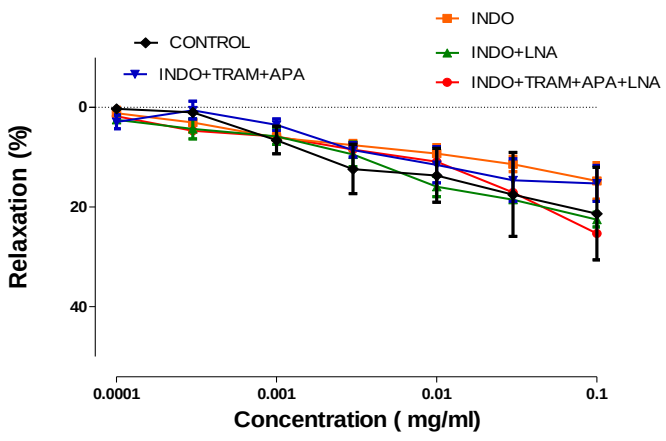


Fig 4. Effect of the aqueous decoction of Lippia multiflora of Djidja on pig coronary arteries provided with endothelium and in the presence of inhibitors



CONCLUSION

The hydroethanolic extracts of Djidja, Savalou, Kétou and that of the Kétou aqueous decoction of Lippia multiflora are not active on the coronary arteries. The aqueous decoction of Lippia multiflora leaf of Mono is weakly active. Alone the aqueous decoction of leaves of Lippia multiflora harvested in Djidja (Verbenaceae) proved to be active with a vasodilator activity (EC50 of 33.73 mg / mL) significantly greater than the activity of Mono. The physiological study of the vasodilator activity of the aqueous decoction of the dried leaf powder of Lippia multiflora showed a vasorelaxant activity dependent on the vasoactive factor NO (nitric oxide) while the EDHF vasoactive factor (Hyperpolarizing factor derived from endothelium) is not involved. The results of our research thus obtained, offer a contribution to the valorization of plant resources of traditional Beninese medicine. These results confirm the relevance of the traditional use of some of them, mainly the ones most active in Benin pharmacopoeia.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

All the authors' participle in writing, giving feedback on this manuscript, have read and approved the final manuscript.

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REFERENCES

1. Pascual ME, Slowing K, Carretero E, Sánchez Mata D, Villar A. *Lippia*: traditional for uses, chemistry and pharmacology, a review. *J.Ethnopharmacol*, 76, 2001, 201-214.
2. Kanco C, Koukoua G, N'Guessan YT, Fournier J, Pradère JP, Toupet L. Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). *C.R.Chimie*, 7, 2004, 1029-1032.
3. Menut C, Lamaty G, Samaté D, Nacro M, Bessière JM. Contribution à l'étude des *Lippia africaines*, Constituants volatils de trois espèces du Burkina Faso. *Rivista Italiana Eppos*, 11, 1993, 23-29.
4. Ajaiyeoba EO, Falade CO, Fawole OI, Akinboye NE, Gbotosho GO, Bolaji OM, AshidiJ S, Abiodun OO, Osowole OS, Itiola OA, Oladepo O, Sowunmi A. et. Oduola AM. L'efficacité des remèdes à base de plantes utilisées par les herboristes dans l'Etat d'Oyo au Nigeria pour le traitement des infections. 2004.
5. Bouquet A, Debray M, Kanko C, Koukoua G, N'guessan YT, Tomi F, Casanova J, Fopurnier J. Plantes médicinales de Côte d'Ivoire. ORSTOM, Paris in C. Composition de l'huile essentielle de *Lippia multiflora* (verbénacée). Comparaison des huiles essentielles de quelques espèces Africaines et Américaines de Lippia à celles de *Lippia multiflora*. *J.Soc.Afr.Chim*., 1, 1996, 51-58.
6. Emebu PK, Anyika JU. Proximate and mineral composition of kale (Brassicaoleracea) grown in Delta State, Nigeria. Pak. *J. Nutr*., 10, 2011, 190-194.
7. Gandonou DC, Ahissou H, Tokoudagba JM, DANSOU C. Ethnobotanical, phytochemical and toxicity analysis of a Benin ese antihypertensive plant: Lippia multiflora Int. *J. Biol. Chem. Sci*, 11(4), 2017, 1816-1828,
8. Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Moudachirou M, and gbaguidi FA. Phytochemical analysis, toxicity and antibacterial activity of benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with hiv/aids. *Int J Pharm Sci Res*, 5(5), 2014, 1739-1745.
9. Pinta M. Méthodes de référence pour la détermination des éléments minéraux dans les végétaux: azote, phosphore, potassium, sodium, calcium, magnésium, par les laboratoires membres du Comité Inter-Instituts pre.enté. 1974.
10. Tokoudagba JM, Eizawa H, Yui Y, Inoue R, Kosuga K, Hattori R, Aoyama T, Sasayama S. Lysophosphatidylcholine inhibits endothelium dependent hyperpolarization and N omega- nitro-L-arginine/indometacin-resistant endothelium-dependent relaxation in the porcine coronaryrtery. *Circulation*, 92, 1995, 3520-6.
11. Senga Kitumbe P, Opota Onya D, TambaVemba A, TonaLutete G, Kambu Kabangu O, Covaci A, Apers S, Pieters L, Cimanga K. Chemical composition and nutritive value study of the seed oil of Adenantherapavonina L. (Fabaceae) growing in Democratic Republic of Congo. *International journal of Pharm tech Research*, 5(1), 2013, 205-216.
12. Karau, Njagi NM, Machocho AK, Wangai LN. Phytonutrient, mineral composition and in vitro antioxidant activity of leaf and stem bark powders of pappeacapensis (L). Pakistan *Journal of Nutrition*, 11(2), 2012, 123-132.
13. Alinnor IJ, Oze R. Chemical evaluation of the nutritive value of Pentaclethra macrophyllabenth (African Oil Bean) Seeds. Pakistan *Journal of Nutrition*, 10(4), 2011, 335-359.
14. OMS. Recommandations diététiques basées sur l'approche alimentaire: élaboration et utilisation. FAO/OMS N°880, Genève, 1998, 125.
15. Andzouana M, Mombouli JB. Assessment of the Chemical and phytochemical Constituents of the Leaves of a Wild Vegetable Ochthocharisdicellandroides(Gild). Pakistan *Journal of Nutrition*, 11(1), 2012, 94-99.
16. Griendling KK, Sorescu D, Ushio-Fukai M. NDPH oxide: role in cardiovascular biology and disease. *Circ Res*, 86, 2000, 494 501.
17. Savard S. Etude de la surexpression in-vivo de la NO synthase endothéliale chez le rat urémique : effets sur la dysfonction endothéliale. Chapitre 2, Hypertension artérielle. Université Laval, faculté de médecine. 2006.
18. Karasu C. Time course of changes in endothelium-dependent and-independent relaxation of chronically diabetic a of reactive oxygen species. *Eur J Pharmacol*, 392, 2000, 163-173.
19. Taubert D, Berkels R, Klaus W, Roesen R. Nitric oxide formation and corresponding relaxation of porcine arteries induced by plant phenols: essential structural features. *Journal of Cardiovascular Pharmacology*, 40, 2002, 701-713.