TOXICITY AND ANTIHYPERTENSIVE ACTIVITY OF LIPPIA MULTIFLORA EXTRACTS IN THE DIFFERENT REGIONS OF BENIN

Article *in* International Journal of Pharmaceutical Sciences and Research · July 2021

DOI: 10.13040/IJPSR.0975-8232.12(7).3933-42

9 authors, including:

Pascal Abiodoun Olounlade
National University of Agriculture
53 PUBLICATIONS **340** CITATIONS

SEE PROFILE

Lamine Baba-Moussa
University of Abomey-Calavi
580 PUBLICATIONS **3,400** CITATIONS

SEE PROFILE

Gbenou Joachim Djimon
University of Abomey-Calavi
129 PUBLICATIONS **1,544** CITATIONS

SEE PROFILE

IJPSR (2021), Volume 12, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 August 2020; received in revised form, 05 November 2020; accepted, 15 May 2021; published 01 July 2021

TOXICITY AND ANTIHYPERTENSIVE ACTIVITY OF *LIPPIA MULTIFLORA* EXTRACTS IN THE DIFFERENT REGIONS OF BENIN

Gandonou Dossa Clément [*1], Chodaton Marthe Dominique Épouse Zinsou [2], Agbodjogbe Kpèdétin Dieudonné Wilfrid [3], Olounlade Abiodoun Pascal [4], Baba-Moussa Lamine Saïd [4], Gbenou Joachim [2] and Ahissou Hyacinthe [1]

Laboratory of Enzymology and Biochemistry of Proteins [1], Faculty of Science and Technology, University of Abomey-Calavi, 01BP: 188, Cotonou, Benin.
Laboratory of Pharmacognosy and of Essentials oils [2], Faculty of Health Sciences and Technology, University of Abomey-Calavi, ISBA champ de Foire, 01BP: 918 Cotonou, Bénin.
Laboratory of Physiology of effort [3], School of INJES, Faculty of Health Sciences, University of Abomey-Calavi, BP: 169, Porto-Novo, Benin.
Zootechnical Research and Livestock System Unit [4], Laboratory of Animal and Fisheries Science. National University of Agriculture, 01 BP: 55 Porto-Novo, Bénin.

Keywords:

Aqueous decocty, Arterial hypertension, *Lippia multiflora*, L-NAME and Benin

Correspondence to Author:
Gandonou Dossa Clément

Doctor in Biochemistry and Pharmacognosy Products Teacher/ University of Abomey-Calavi; Benin, 01BP: 188 Cotonou, Benin.

E-mail: gandonoufils@gmail.com

ABSTRACT: The objective was to study the antihypertensive effects of the aqueous decoctycrude of *Lippia multiflora* from the region of Djidja at a dose of *the plant* as well as the effects of this extract on some biochemical parameters in the rat were made hypertensive by 500 mg/kg of body weight during the 14 days and were treated with losartan potassium at a dose of 100 mg/kg of body weight, of L-NAME at a dose of 40 mg/kg of body weight between 150 and 250g. Administration of L-NAME was caused a significant increase in PAS, PAD, and PAM in rats from 142.4±19.89 mmHg (J0) to 172.4±15.95 mmHg (J8) and 122.8±7.05 mmHg (J0) to 138.6±36.39 mmHg (J8), from 93.8±5.42 mmHg (J0) to 137.5±11.90 mmHg (J8) and from 92.5±4.55 mmHg (J0) to 136.75±7.68 mmHg (J8) and 109.25±15.52 mmHg (J0) to 159.75±27.80 mmHg (J8) and from 99.8±13.39 mmHg (J0) to 151.25±8.13 mmHg (J8) respectively for the positive control batch and the batch subsequently were treated with the raw extract). That of the crude extract from (J8) to (J29) was caused a significant decrease in SBP, PAM and PAD in the rats, dropping from 140±5 mmHg (J8) to 122.4±15.32 mmHg (J29), from 151.25±8.13 mmHg (J8) to 114±19.12 mmHg (J29) and from 136.75±7.68 mmHg (J8) to 99.8±14.82 mmHg (J29) respectively. These values are significantly lower than that of untreated rats, which was 150.5±10.40 mmHg (J29).

INTRODUCTION: Arterial hypertension was a pathology that affects not only the countries of the North but also those of the South, such as, for example, Africa [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3933-42

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3933-42

The African continent was in fact, particularly affected, as shown by the study on the prevalence of hypertension in seven populations originating from West Africa [2] but also in Ghana in the province of Ashanti [3]. The latest epidemiological data show that this disease was constantly increasing, as was type 2 diabetes [4]. High blood pressure in Africa has been ethiopathological features such as low renin activity or its sodium-dependent character [5]. Currently, 28% of the sub-Saharan adult population over the age of 20 were

affected with some regional variation, notably a predominance in urban areas [5, 6]. In recent years, the aging of the population and the number of obese or overweight subjects have contributed, along with urbanization, to the increase in the number of people affected. Overweight greater than 25 or obesity greater than 30 measured by the body mass index BMI expressed in kg/m^2 was directly related to the prevalence of high blood pressure. The latter was characterized by systolic blood pressure (PAS) and diastolic blood pressure (PAD) expressed in mm of mercury. In France, a subject was considered hypertensive when his blood pressure exceeds the values 140/90 (standards in the doctor's office) [6]. Several risk factors favor high blood pressure, such as excess salt in food, modification of eating habits with the consumption of high-calorie meals, age, overweight, smoking or even alcohol consumption. This disease was considered to be silent because many patients who are far from health centers do not benefit from an early diagnosis in the absence of blood pressure monitors available. This pathology was often revealed during serious cardiovascular or renal accidents, sometimes with fatal outcomes [7].

Nevertheless, suggestive is signed allow a diagnosis such as skin disorders of the neurofibro-matosis type, large palpable kidneys, an abdominal or precordial aortic breath, and a decrease in the femoral arterial pressure. The consequences of hypertension are damage to target organs such as the heart, kidneys, brain, and even the retina. Diseases linked to high blood pressure are among the public health challenges in Benin [8]. This was the reason why epidemiological studies have been carried out in rural areas in the South of the country in the province of Oueme [9] and some of them have been concerned more particularly pregnant women [10]. In the city of Cotonou, for example, access to care was geared towards self-medication in the absence of the seriousness of the disease in order to avoid the expenses linked to medical consultation. This study shows that self-medication accounts for 54% of therapeutic requests, while 23% of patients seek help from private clinics and 16% from health centers [11]. In a difficult economic environment characterized by the high cost of drugs, pharmacopeia and traditional medicine become a significant alternative in terms of health coverage, especially when the medication becomes permanent for the patient suffering from a chronic pathology. Our research program focused on demonstrating the therapeutic properties of African plants [12, 13] from Benin biodiversity [14, 15, 16]. We were primarily interested in traditional knowledge related to the use of medicinal plants with an antihypertensive reputation in Benin. Their activity were evaluated by measuring their vasodilator properties on a classic experimental model in cardiovascular pharmacology: the pig coronary artery. From this inventory, a selection of potentially interesting plants to study was made by applying various criteria. First of all, the number of documented references, the relevance, and the redundancy of the information were noted. Indeed, a large number of bibliographical references on a plant very frequently used in traditional medicine in different countries underlies a certain medicinal interest. The information was all the more relevant as it was found in several references. However, its redundancy can also be due to previous phytochemical studies, and the possibility of demonstrating original compounds was then reduced. Then the chemical composition and the reported biological activities were also taken into account: some of the inventoried plants have been studied previously, and chemical compounds and/or biological activities have already been reported.

Conversely, the absence of phytochemical work was an important selection criterion because it was made it possible to hope for the identification of original chemical structures, not yet described. It was the same with the specificity of the use of the plant in the treatment of hypertension.

MATERIALS AND METHODS:

Plan 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; 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) [17]. The plant material was used 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 was obtained, was sieved and was stored in a preservative for analysis.

Methods:

Preparation of Crude Extracts: 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 is 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 h), 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 was 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 was evaporated to dryness, and then was weighed for yield determination according to the relation [18]:

Yield = Ma of direct extract × 100 / mass of test sample

Test of Larvary Toxicity: The test was performed against Artemia salina Leach by the method of [19] and the literature as a simple bioassay method for assessment of preliminary toxicity of natural active products. The eggs of Artenia salina were incubated in seawater until hatching of young larvae (48 h). Then, series of solutions of each tested crud extract at varying and progressive concentrations were prepared. A defined number of larvae (16) were introduced into each solution. All solutions and control solutions containing no active substance were left under stirring for 24 h.

Counting under a microscope the number of dead larvae in each solution was used to evaluate the toxicity of the solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula:

% death = [(test - control) / control)] × 100

Data (dose-answer) are transformed by logarithm, and the LC_{50} is determined by linear regression. Tests were carried out in triplicate.

Toxicity Study:

Method for Assessing Acute Oral Toxicity: The toxicity test was carried out according to the guidelines of the Organization for Economic Cooperation and Development 20423 for testing chemicals. The substance was tested according to a process in which six rats, in particular females grouped into two lots of three weighings between 150-200g are used at each step. The absence or manifestation of substance-related mortality in a group that received a dose at a given stage determines the next stage; That was, three patterns can be observed: no further testing was necessary or additional animals are treated at the same concentration or additional animals are treated at the nearest higher or lower concentration. The initial dose was chosen from the following four 5, 50, 300, 2000 mg/kg. The dose was chosen that for which it was estimated that it can induce mortality in the treated animals. In the absence of such information on the test substance, the recommended starting dose for animal "welfare" reasons was 2000 mg/kg. The first batch of rats are received the extract at a dose of 2000 mg/kg of body weight. The second batch is served as a control and are received distilled water at the same dose. After force-feeding, the animals are observed carefully for the first four hours and then daily for 14 days. Their weight was taken at the start of the experiment and every 7 days thereafter. After 14 days, the following exams were done.

Haematological Examinations: Haematological examinations were performed using an SYSMEX KX 21N automated system according to the method used by [21, 22]. These examinations focus on the one hand on the count of red blood cells, white blood cells, and platelets and on the other hand on the determination of the hemoglobin level, the hematocrit, the Average Globular Volume (MCV), the content Corpuscular Average Hemoglobin (TCMH) and Corpuscular Average Hemoglobin Concentration (CCMH).

Biochemical Examinations: The biochemical examinations were carried out using the MIDRAY

BS 200 biochemical analyzer at the Biomedical Analysis laboratory of St Jean Hospital in Cotonou. These are the dosage of urea, creatinine and transaminases. Splitting the most active aqueous decoction. It was a liquid-liquid partition by different organic solvents.

The liquid-liquid partition consists of a transfer of material between two partially or immiscible liquid phases. The purpose of this separation was to isolate the molecule (s) present in one of the phases. The choice of solvents to be used depends on the solvating power of the molecules to be extracted.

Splitting Method: The fractionation was carried out by successively exhausting the aqueous extract with solvents of increasing polarity, which are: cyclohexane, dichloromethane, ethyl acetate, and butanol. 50 g of the aqueous extract obtained from the leaves of Lippia multiflora of Djidja, were introduced into a separating funnel of 2000 mL. To this amount of extract was added 500 mL of distilled water. The fractionation was carried out first, three times in a row, with 500 mL of cyclohexane (1500 mL). Each time, the whole was stirred and left to stand for decantation before the recovery of the organic phase. The fraction obtained was concentrated in a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300) at a temperature of 60 ° C, stored in an open flask, and placed under a hood for 24 h in order to eliminate any traces of solvent.

The raffinate was taken up in 3 × 500 mL of dichloromethane in accordance with the previous technique. The fraction obtained was stored in a flask. The raffinate obtained was then taken up in 3 × 500 mL of ethyl acetate. The fraction obtained was stored in a flask. The raffinate was finally obtained, taken up with 3 × 500 mL of butanol. After concentration under vacuum in a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300) at a temperature of 80 °C, the residue was recovered.

The remaining aqueous phase was also concentrated in a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300) under reduced pressure at a temperature of 80 °C. A total of five (5) fractions were obtained and were kept in vials stored at 4 °C.

Evaluation of the Vasodilator Activity of the Different Fractions of the Aqueous Decoctate: To verify the vasodilator activity of the extracts, we were used the model of pig coronary artery rings suspended in isolated organ vessels. These arteries are taken from freshly slaughtered pig hearts. The circumflex arteries are carefully removed, cleaned of adherent connective tissues, and rinsed with Krebs, avoiding damage to the endothelium. The coronary artery segments are 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 allowing the visualization and recording of variations in isometric voltages. The rings are placed in insulated organ tanks containing 10 ml of bicarbonate Krebs solution at 37 °C and oxygenated by a mixture of carbogen. The rings are subjected to a basic tension of 5 g and are then left to stand during a stabilization phase of 45 min. They are then contracted with a KCl solution (80 mM), making it possible, through maximum depolarization, to test the reactivity of the vascular smooth muscle. After obtaining the maximum effect, three successive washes are carried out. In order to test the integrity of the endothelium, the rings are contracted with the thromboxane analogue A2U46619, and to the contraction, plateau bradykinin was applied. After three successive washes, a stabilization phase of 45 min was observed at the end of which the rings were contracted again with the thromboxane analog A2U46619 before applying an increasing, and cumulative range of the different fractions of plant extracts were obtained.

Antihypertensive Activity of the Most Active Fraction: This was a non-invasive measurement of Blood Pressure carried out on Wistar strain rats acclimatized to the ambient conditions of breeding in the animal facility of the Human Biology Teaching and Research Unit of the Faculty of Sciences of Health of the University of Abomey-calavi. The present experimental study would be carried out in Wistar rats. It would be carried out in a single phrase: The study would be carried out on 25 Wistar rats of body weight between 150 and 250 g divided into three (03) groups and would be carried out over a period of twenty-eight (28) days going from J0 to J28. The rats were made hypertensive with the N (G) -Nitro-L-Arginine

Methyl Ester (L-NAME) [23]. The arterial pressures of the rats would be taken on J0, J8, J15, J22 and on J29. We were made up a batch of 5 male rats. The treatment would be carried out according to the protocol below:

Group 1: Batch of 5 rats treated with distilled water for 14 days;

Group 2: Batch of 5 rats treated with L-NAME at a dose of 40 mg/kg of body weight for 14 days and left to rest for the following 14 days;

Group 3: Batch of 5 rats treated with L-NAME at a dose of 40 mg/kg of body weight for 14 days then with losartan at a dose of 100 mg/kg of body weight for 14 days thereafter;

Group 4: Batch of 5 rats treated with L-NAME at a dose of 40 mg/kg of body weight for 14 days then with a crude aqueous decoction of Lippia multi-flora from the region of Djidja at a dose of 500 mg/kg of body weight for the following 14 days; All administrations would be made orally using a feeding tube.

Treatment with Losartan Potassium: It was the antihypertensive tablet used as a reference drug. L-NAME induction was performed on a batch of seven rats with an average body weight of 197.42 + 19.85 g, followed by treatment with losartan potassic.

This dissolved in distilled water would be administered to the animals at a dose of 100 mg/kg of body weight, a concentration of 19.74 mg/ml of distilled water for each rat. The hypertension induction and antihypertensive treatment all lasted for 15 days. Thus, the blood pressure of the animals were measured on the 30th day.

Statistical Analysis: The data were obtained for the two species were evaluated for significant differences in their means with the analysis of variance (Anova). The critical difference was estimated at $p \leq 0.05$. The differences between the means were separated using the turkey test still with the SPSS 17.0 software

RESULTS AND DISCUSSION:

Yield of Extractions: Using "Microsoft Excel 2010", we were made a preliminary statistical analysis by calculating and comparing the averages of the yields of each type of extract prepared. This was how the average of the yields is 17.77% for hydro-ethanolic extracts and 13.77% for aqueous decoctions. It was found that there was no significant difference between the yields of aqueous extracts and hydroethanolic extracts. This was all the more normal since water, and its mixture with ethanol have been similar polarities. It was concluded from these values that water or its mixture with ethanol extracts most of the chemical principal.

TABLE 1: SUMMARY OF EXTRACTION YIELDS

	Extraction yields (%)	
Harvest area	**Aqueous decoction**	**Hydro-ethanolic extract**
Tchettis	10.58	24.77
Houeyogbe	14.94	15.01
Djidja	23.54	19.77
Ikpinlè	7.24	11.54

Acute Larval and Oral Toxicity of Crude Extracts Larval Toxicity: Analysis of the LC_{50} values of the extracts that were tested with respect to the correspondence table above, allows us to say that the extracts tested do not be presented any toxicity in the range of concentrations analyzed because the LC_{50}s obtained are greater than the limit set (0.1 mg / mL).

The non-toxic nature of these extracts, proven by the general toxicity test, supports the results of phytochemical screening, which have been shown the absence of cardiotonic heterosides, cyanogenic derivatives, and quinone derivatives which are generally toxic compounds. Considering the correlation between cytotoxicity on shrimp larvae and on 9 PS and 9 KB cells on the one hand [24] the A-549 cells of pulmonary carcinoma and the HT-29 cells of the colon.

On the other hand, it could be said, subject to further investigation, that the extracts tested are free from cytotoxic activity [25]. It could be seen for all the graphs obtained that the correlation coefficient R^2 was greater than 0.8. There was, therefore a good correlation between the concentrations were applied, and the responses were obtained. In addition, we are noticed that the larvae are sensitive to all the extracts tested. The number of dead larvae increases with the concentration, so the sensitivity of the larvae to the extracts follows a dose-response relationship.

TABLE 2: RESULT OF LARVAL TOXICITIES

	LC50 (mg / mL)	
Harvest area	Aqueous decoction	Hydro-ethanolic extract
Tchettis	01.28	02.03
Houeyogbe	0.81	05.10
Djidja	01.15	02.31
Ikpinlè	01.32	04.81

TABLE 3: CORRESPONDANCE BETWEEN CL_{50} AND TOXICITY

LC_{50}	Toxicity
$CL_{50} \geq 0,1$mg/mL	-
0,1mg/mL > $CL_{50} \geq$ 0,05 mg/mL	+
0,05 mg/mL > $CL_{50} \geq 0,01$mg/mL	++
$CL_{50} < 0,01$mg/mL	+++

- : nontoxic; +: low toxicity; ++: moderate toxicity; +++: high toxicity

Table 2 above summarizes the different values of the lethal half-concentrations (LC_{50}) obtained. These values express the concentrations necessary for the survival of 50% of the larval population that was introduced into the solutions tested.

Acute Oral Toxicity: During the assessment of acute oral toxicity, no mortality was recorded in Rats have been given the aqueous extract of *Lippia multiflora* at the single dose of 2000 mg/kg body weight.

Evolution of the Weight of the Rats During the Toxicity Test: During the acute oral toxicity test, we noticed a change in the weight of rats treated with an aqueous and hydro-ethanolic decoction of *Lippia multiflora* during the 14 days of treatment **Fig. 1**.

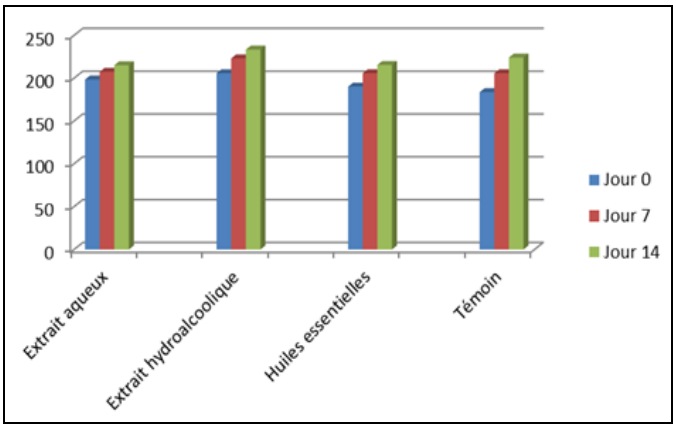

FIG. 1: EVOLUTION OF THE WEIGHT OF THE RATS DURING THE TOXICITY TEST

We note an increase in the weight of the rats having received our extract at the dose of 2000 mg/kg of body weight.

Haematological Parameter in Rats having Received the *Lippia multiflora* Extract: At the end of the 14 days of the experiment, we noticed no significant difference in the haematological parameters in the treated rats compared to the controls because p>0.05 (**Table 4** and **5**).

TABLE 4: EFFECT OF THE 2000 mg/Kg EXTRACT ON BLOOD CELL COUNT OR BLOOD CELL CONSTANTS

	HGB (g/dl)	HTE(%)	NGR (T/L)	VGM (fL)	TCMH(%)	CCMH (pg)
Aqueous	14.3±0.44	42.96±1.37	4.76±0.153	90.13 ±0.611	30 ± 0.2	33.3 ± 0.0
Hydro-Ethanolic	15.43±0.21	46.33±0.47	05.16±0.058	89.66±0.2329.	86±0.15	33.3 ± 0.1
Witness	14.63 ± 0.74	42.7±2.12	4.73±0.231	90.2 ± 0.27	30.07±0.231	33. 33±0.058

Renal and Hepatic Parameters in Rats Given the Aqueous Extract *of Lippia multiflora*: Following the administration of the aqueous extract of *Lippia multiflora* at a dose of 2000 mg/Kg of body weight, no significant difference was noted on the one hand between the renal parameters (Créat, Urea) and on the other hand between the hepatic parameters (ALAT and ASAT) compared to the control batch **Table 4**.

TABLE 5: EFFECT OF THE 2000 mg/Kg EXTRACT ON THE BIOCHEMICAL PARAMETERS

	ALAT(UI/L)	ASAT(UI/L)	CREAT(mg/l)	UREE(g/l)
Aqueous	14.29±3.51	13.84 ±2.50	14.70 ±0.11	0.23 ±0.01
Hydro-Ethanolic	14.30± 3.85	13.70 ±3.86	14.99 ±0.43	0.22 ±0.01
Witness	19.03±3.51	18.15 ±2.16	15.27 ±0.02	0.27 ±0.01
P value	0.206	0.189	0.090	0.006

According to the OECD26, an LC_{50} substance ≥ 2 g/kg was classified in category 5, grouping together substances with very low acute toxicity and therefore indicating that the drug could be considered safe **Table 5**.

These observations are in agreement with other studies. in particular those of Hussain, who arrived at the same conclusions from a methanolic extract of the aerial parts of *L. multiflora* then those of Araujo which confirm this absence of acute toxicity

of an extract this time from *L. multiflora* leaf administered intraperitoneally (LC_{50} = 2 g/kg). The toxicity test was carried out in accordance with OECD protocol 42327 allowed us to confirm that the aqueous and hydro-ethanolic extract of our plant was non-toxic. According to [28]. Any plant whose toxicity does not exceed 1000 mg/Kg was said to be non-toxic. But in the present study, the extract was administered at a dose of 2000 mg/Kg. This non-toxicity of the extract was easily understood because the plant was commonly used in many African countries to treat several ailments.

Our results agree with those of some authors who have been shown that the LC_{50} of the aqueous decoction of the leaves of *L. multiflora* was greater than 3000 mg/kg of body weight [29], and that of the trunk bark was greater than 5000 mg/Kg of body weight in the case of subchronic toxicity [30]. We were not found any significant difference as much on the various biochemical parameters measured as on those haematological. These results confirm the hepatoprotective [31] and nephroprotective properties of this plant.

Evaluation of the Vasodilator Activity of the Different Fractions of the More Active Extract:

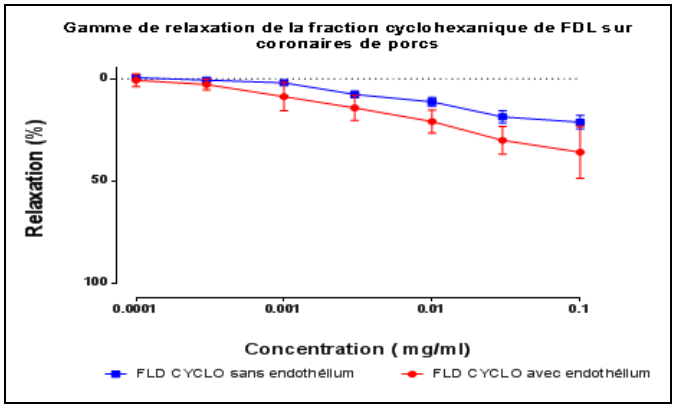

FIG. 2: VASORELAXANT EFFECT OF THE CYCLO-HEXANE FRACTION OF THE AQUEOUS DECOCTION OF *LIPPIA MULTIFLORA* FROM DJIDJA ON CORONARY ARTERIES WITH AND WITHOUT ENDOTHELIUM

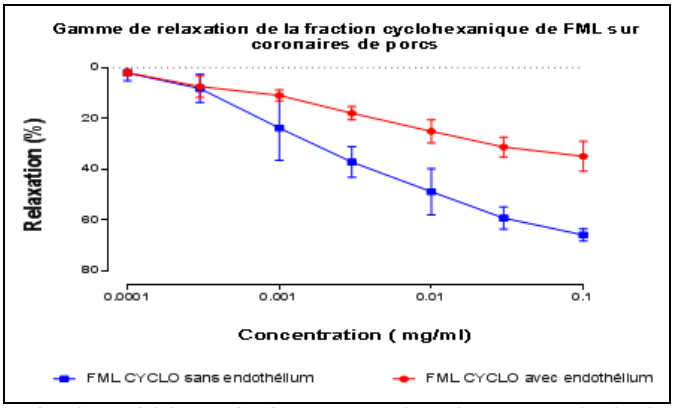

FIG. 3: VASORELAXANT EFFECT OF THE CYCLO-HEXANE FRACTION OF THE AQUEOUS DECOCTION OF *L. MULTIFLORA* FROM HOUEYOGBE ON CORONARY ARTERIES WITH AND WITHOUT ENDOTHELIUM

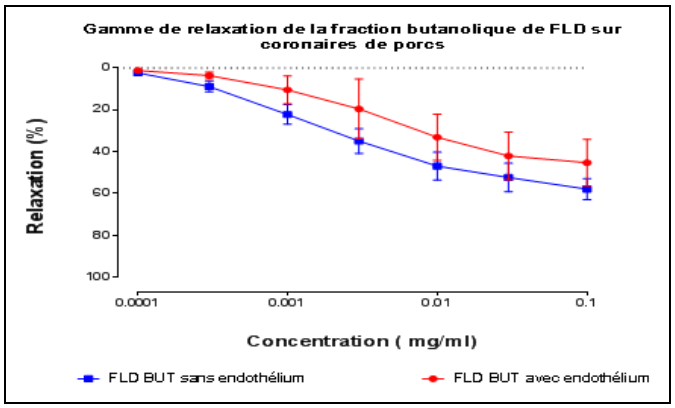

FIG. 4: VASORELAXANT EFFECT OF THE BUTANOLIC FRACTION OF THE AQUEOUS DECOCTION OF *LIPPIA MULTFLORA* FROM DJIDJA ON CORONARY ARTERIES WITH AND WITHOUT ENDOTHELIUM

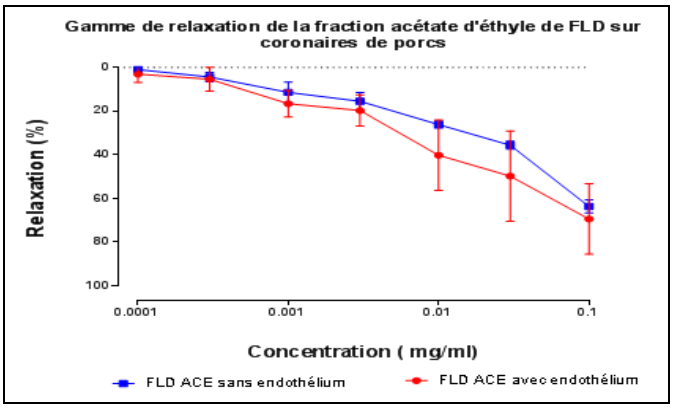

FIG. 5: VASORELAXANT EFFECT OF THE ETHYL ACETATE FRACTION OF THE AQUEOUS DECOCTION OF *LIPPIA MULTIFLORA* FROM DJIDJA ON CORONARY ARTERIES WITH AND WITHOUT ENDOTHELIUM

It was noted that the ethyl acetate fraction of the aqueous decoction of *Lippia multiflora* from Djidja relocated to 65% at a concentration of 25.81mg/mL compared to that of Mono which gave nothing. In total the aqueous decoction of *Lippia multiflora* from Djidja was the most active. These analyzes show that the vasorelaxant activity observed in the Djidja region was endothelium-dependent **Fig. 5** [32].

Antihypertensive Activity of the Most Active Fraction Extract: Tables 5 and **6** present the systolic and diastolic arterial pressures of the rats subjected to the study of the antihypertensive activity of the crude extract of the most active fraction.

TABLE 5: COMPARATIVE SYSTOLIC ARTERIAL PRESSURES (SBP) OF RATS SUBJECTED TO THE STUDY OF THE ANTIHYPERTENSIVE ACTIVITY OF THE CRUDE EXTRACT OF ETHYL ACETATE FRACTION OF THE AQUEOUS DECOCTION OF LIPPIA MULTIFLORA FROM DJIDJA

	J0	J8	J15	J22	J29
WITNESS	121±32.19	122.00±13.11	122.33±19.50	130±13.75	155±32.05
L-NAME	142.4±19.89	172.4±15.95*	168±18.38	161.8±9.49	150.5±10.40
LOSARTAN	104.25±27.5	137.25±9.22	136.75±5.44	135.5±23.69	120.25±23.16
EXTRACT	122.8±7.05	140±5**	138.6±36.39	124±9.49	122.4±15.32*

Data are expressed in mmHg and as an average +/- SEM

The comparisons are made between J0 and J8, between J8 and J15, between J15 and J22 and between J22 and J29 to the same batch. (*) = p-value < 0.05, (**) = p-value < 0.01, statistically significant difference.

Administration of L-NAME was caused a significant increase in rat SBP from 142.4±19.89 mmHg (J0) to 172.4±15.95 mmHg (J8) and from 122.8±7.05 mmHg (J0) at 138.6±36.39 mmHg (J8) respectively for the positive control batch and the batch subsequently treated with the crude extract. The administration of the crude extract from J8 to J29 caused a significant decrease in the SBP of the rats from 140±5 mmHg (J8) to 122.4±15.32 mmHg (J29). This significantly lower than that of untreated rats, which was 150.5 ± 10.40 mmHg (J29) [32].

TABLE 6: COMPARATIVE DIASTOLIC ARTERIAL PRESSURES (DBP) OF RATS SUBJECTED TO THE STUDY OF THE ANTIHYPERTENSIVE ACTIVITY OF THE CRUDE EXTRACT OF ETHYL ACETATE FRACTION OF THE AQUEOUS DECOCTION OF LIPPIAMULTIFLORA FROM DJIDJA

	J0	J8	J15	J22	J29
WITNESS	84.67±30.01	95.33±6.35	92±12.17	90±15.59	80±16.46
L-NAME	93.8±5.42	137.5±11.90	111.5±14.48	113.75±26.89	115±11.17
LOSARTAN	80.75±20.19	84.25±4.35	80.25±23.30	88.75±26.42	96.75±30.03
EXTRACT	92.5±4.55	136.75±7.68**	95.6±6.58	96±15.22	99.8±14.82*

Data are expressed in mmHg and as an average +/- SEM

The comparisons are made between J0 and J8, between J8 and J15, between J15 and J22 and between J22 and J29 to the same lot. (*) = p- value < 0.05, (**) = p- value < 0.01, statistically significant difference.

The administration of L-NAME was caused a significant increase in the DBP of the rats going from 93.8±5.42 mmHg (J0) to 137.5±11.90 mmHg (J8) and from 92.5±4.55 mmHg (J0) to 136.75± 7.68 mmHg (J8) respectively for the positive control batch and the batch subsequently treated with the crude extract. The administration of the crude extract from J8 to J29 was caused a significant decrease in the PAD of the rats passing from 136.75±7.68 mmHg (J) to 99.8±14.82 mmHg (J29) [32].

TABLE 7: COMPARATIVE MEAN ARTERIAL PRESSURES (MAP) OF RATS SUBJECTED TO THE STUDY OF THE ANTIHYPERTENSIVE ACTIVITY OF THE CRUDE EXTRACT OF ETHYL ACETATE FRACTION OF THE AQUEOUS DECOCTION OF *LIPPIA MULTIFLORA* FROM DJIDJA

	J0	J8	J15	J22	J29
WITNESS	100±2	100±2	101.67±3.06	102 ±8.89	106.67±7.64
L-NAME	109.25±15.52	159.75±27.80	155.25±10.18	151.25±8.13	141.25±35.65
LOSARTA	84.25±15.71	124.25±22.6	129.75±23.3	116.25±26.	104±26.4
EXTRACT	99.8±13.39	151.25±8.13	146.5±18.45	137.25±19.8	114±19.12

The data are expressed as an average +/- SEM.

Table 7 shows that the administration of L-NAME was caused a significant increase in the MAP of the rats passing from 109.25±15.52 mmHg (J0) to 159.75±27.80 mmHg (J8) and from 99.8 ± 13.39 mmHg (J0) to 151.25±8.13 mmHg (J8) respectively for the positive control lot and the lot subsequently was treated with the crude extract) [32]. Administration of the crude extract from J8 to J29 was caused a significant decrease in the MAP of the rats dropping from 151.25 ± 8.13 mmHg (J8) to 114±19.12 mmHg (J29).

CONCLUSION: The physiological study of the vasodilator activity of the aqueous decoctions of the powder of the dried leaves of this plant species showed a vasorelaxant activity dependent on the vasoactive factors NO and EDHF for the species *Lippia multiflora* of Djidja and for the species

Lippia multiflora from Houéyogbé. The study of the vasodilatory activity of the chemical fractions resulting from the liquid-liquid extraction of their aqueous extracts has been shown marked activity for the ethyl acetate fraction of the aqueous decoction of *Lippia multiflora* from Djidja. It relocated to 65% at a concentration of 0.1mg/mL compared to that of Houéyogbé, which gave nothing. The acute toxicity sought for on the two hydroalcoholic extracts made it possible to classify them in class III of who products. as slightly toxic extracts. The study of the antihypertensive activity *in-vivo* on a model of hypertensive rats provoked by the L-NAME of the aqueous extracts of the fraction of the most active plant showed a significant decrease in the value of the arterial pressure at 151.25±8.13 mmHg on average after 14. The results of our research were thus obtained. offer a contribution to the enhancement of the plant resources of traditional Beninese medicine, which requires the implementation of scientific procedures. These results confirm the relevance of the traditional use of some of them, mainly the most active in the pharmacopeia of Benin and show the importance of the ethnopharmacological survey in the search for new sources of drugs against high blood pressure.

ACKNOWLEDGEMENT: The authors sincerely thank all the actors of the laboratories involved in the success of this work. We also thank Mr. Richard FEYICHETAND. Dr. Jean-Marie TOKOUDAGBA and GANSE Hyppolyte for their technical assistance.

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.

REFERENCES:

1. Mendis S, Lindholm LH and Mancia G: WHO and ISH (International Society of Hypertension) risk prediction charts: assessment of cardiovascular risk for prevention and control of cardiovascular disease in low and middle-income countries. Journal of Hypertension 2007; 25: 1578-82.
2. Fauvel JP and Laville M: Arterial hypertension of the black subject. La Presse Médicale 2006; 35: 1067-71.
3. Fézan H, Tra B, Guy M, Kohué CC and N'Gaman Clejesson HB: Studies of some therapeutic plants used in the treatment of hypertension and diabetes: two emerging diseases in Côte d'Ivoire. Science & Nature 2008; 5: 39-48.
4. Cooper R, Rotini C, Ataman S, Osotimehin B, Kadiri S, Muna W, Kingue S, Fraser H, Forrester T, Bennett F and Wilks R: The prevalence of hypertension in seven populations of West African origin. American Journal of Public Health 1997; 87: 160-68.
5. Cappuccio FP, Micah FB, Emmett L, Kerry SM, Antwi S, Martin-Peprah R, Phillips RO and Eastwood JB: Prevalence, detection, management and control of hypertension in Ashanti. West Africa. Hypertension 2004; 43: 1017-22.
6. Fézan H, Tra B, Guy M, Kohué CC, N'gaman Clejesson HB: Studies of some therapeutic plants used in the treatment of hypertension and diabetes: two emerging diseases in Côte d'Ivoire. Science & Nature 2008; 5: 39-48.
7. Whitfield KE, Yao X, Boomer KB, Vogler GP, Hayward MD, Vandenbergh DJ: Analysis of candidate genes and hypertension in African American adults. Ethnicity of Disease, 2009; 19: 18-22.
8. Karadji H: Phytochemistry study and biology activity for two recipe using in the traditional treatment of arterial pressure to Mali, Bamako. PhD of Pharmacy FMPOS, 2005; 97.
9. Fourcade L, Paule P and Mafart B: Arterial hypertension in sub-Saharan Africa actuality and perspectives. Tropical Medicine 2007; 67: 559-67.
10. Mensah GA, Barkey NL and Cooper RS: Spectrum of hypertensive target organ damage in Africa: a review of published studies. Journal of Human Hypertension 1994; 8: 799-808.
11. Fourn L: Main public health issues in Benin, IREEP Conference of December 21, 2005, Cotonou, Benin.
12. Ouedraogo N: Arterial pressure in urbain middle of west African (Ouagadougou/Burkina Faso) The prevalence community transversaly study and associate factors, Ouagadougou: PhD medecine UFR/SDS; 2003, 90.
13. Okou C, Trebissou JND, Bahi C and Guede-Guina F: Effect of tobacoak, vegetable extract, on the arterial pressure carotidienn, the respiration and the duodenal of rabbit Rev Ivoir Sci Technol 2008; 11: 91-102,
14. RBPM: Recommandations for the good pratice medical, Adult arterial pressure, Long- life affection ALD 14/; 2012: 0,3,88,01, 83.
15. Savard S: The surexpression study *in-vivo* of the endothelial NO synthase at uremic rat: Endothelial dysfonction effect, Chapiter 2: arterial pression, University Laval, of medicine faculty 2006
16. Girerd X: Hypertension pratique, Paris, Editions Masson 2005; 209.
17. Adjanohoun EJ and de Souza S: Practical guide to phytotherapy: health through plants, 100 useful medicinal plants in Benin, Biodiversity Center, CENPREBAF, Cotonou (Regional Information Bulletin, 4) 2002
18. Tra Bi FH, GM, Irié, Kohué CC and N'gman Gaman: studies some therapeutic plant used in the arterial pressure treatment and diabetes: Two malaria in Cote d'Ivoire Sci, Nat, 2008; 5(1): 39-48
19. Gandonou DC, Ahissou H, Tokoudagba JM and Dansou C: Ethnobotanical, phytochemical and toxicity analysis of a Benin ese antihypertensive plant: *Lippia multiflora*. Int J Biol Chem Sci 2017; 11(4): 1816-28.

20. Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Moudachirou M and Gbaguidi FA: Phytochemical analysis, toxicity and antibacterial activity of benin medicinal used in the treatment of sexually transmitted infections associated with HIV/aids. Int J Pharm Sci Res 2014; 5(5): 1739-45.
21. Organisation de Cooperation et de Development Economique (OCDE) 2001: Bookshop, Test N ° 423: Acute oral toxicity, Method by toxicity class, URL http: www, Oedc, Org data oecg 17/50 / 1948370.
22. Mindiediba JB, Almaraz AN, Nag-Tiero MR, Mouhibatou YZ, Jeanne MZ and Germaine NO: *Lippia multiflora* a brief review of traditional uses, phytochemistry and pharmacology. International Journal of Drug Delivery 2013; 4(3): 289-96.
23. Dougnon TV, Bankole HS, Edorh P, Klotoe JR, Dougnon J, Fah L, Loko F and Boko M: Acute toxicity of *Solanum macrocarpon* linn (Solanaceae) on wistar rats: Study about leaves and fruits, American Journal of Biochemistry 2013; 3: 84-88.
24. Pelka M, Distler W, Petschelt A and Dent J: A new screening test toxicity testing of dental materials 2000; 28: 341-45.
25. Ba SH: Study of the phytochemistry and biological activities of *Zizyphus mauritiana* Lam (Rhamnaceae) used in the traditional treatment of diabetes and arterial hypertension in Mauritania, Bamako, FMPOS Pharmacy Thesis 2005; 120.
26. Taubert R, Berkels W, Klaus R and Roesen: Nitric oxide formation and corresponding relaxation of porcine coronary arteries induced by plant phenols: essential structural features. Journal of Cardiovascular Pharmacology 2002; 40: 701-13.
27. M'Buyamba-Kabangu JR, Biswika RT, Thijs L, Tshimanga GM, Ngalula FM, Disashi T, Kayembe PK, Richart T, M'Buyamba-Kayamba JR, Lepira FB and Staessen JA: In-hospital mortality among black patients admitted for hypertension-related disorders in Mbuji Mayi, Congo. American Journal of Hypertension 2009; 22: 643-48.
28. Le Cardiologue: Spécial HTA, French committee to fight high blood pressure, 306, November 2007.
29. Tokoudagba JM, Chabert P, Anger C, N'Gom S, Gbenou J, Moudachirou M, Schini-Kerth V and Lobstein A: Research of plants with antihypertensive potentialities in Beninese biodiversity. Ethnopharmacologia 2009; 44: 32-41.
30. Etou–Ossibi AW, Dimo T, Elion Itou RDG, NsondéNtandou GF, Nzonzi J, Bilanda DC, Ouamba JM and Abena AA: Effects of *Lippia multiflora* aqueous extract on DOCA salt-induced arterial hypertension in rats, Herbal Medicine 2012; 10(6): 363-68.
31. Etou-Ossibi AW, Nzonzi J, Mombouli JV, Nsondé-Ntandou GE, Ouanba JM and Abena AA: Phytochemistry and extract aqueous effect of *Lippia multiflora* on the isolated heard toad, J Phytothérapie 2005; 5: 193-99.
32. Ameyakpo Y: A growthregulator for the propagation of Lippia multiflora, a herbal for the management of mild hypertension in Ghana, Journal Medecine Plants Research 2009; 3(9): 681-85.
33. Ndiaye M, Chataigneau T, Chataigneau M and Schini-Kerth VB: Red wine polyphenols induce EDHF mediated relaxations in porcine coronary arteries through the redox-sensitive activation of the P13-kinase/Akt pathway. British Journal of Pharmacology 2004; 142: 1131-6.

How to cite this article:
Clément GD, Zinsou CMDE, Wilfrid AKD, Pascal OA, Saïd BML, Joachim G and Hyacinthe A: Toxicity and antihypertensive activity of lippia multiflora extracts in the different regions of Benin. Int J Pharm Sci & Res 2021; 12(7): 3933-42. doi: 10.13040/IJPSR.0975-8232.12(7).3933-42.