Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 11(4): 1816-1828, August 2017

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Ethnobotanical, phytochemical and toxicity analysis of a Beninese antihypertensive plant: *Lippia multiflora*

Dossa Clément GANDONOU[1,*], Hyacinthe AHISSOU[1], Jean-MarieTOKOUDAGBA[2] and Christian DANSOU[1]

[1]*Laboratoire d'Enzymologie et de Biochimie des Proteines, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 01BP: 188, Cotonou, Bénin.*

[2]*Laboratoire de Chimie Pharmaceutique Organique, Ecole de Pharmacie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Campus du Champ de Foire, 01 BP: 188, Cotonou, Bénin.*

[*] *Corresponding author; E-mail:gandonoufils@gmail.com; Tél: (+229) 97 54 98 85; (+229) 99 09 18 20*

ABSTRACT

The ethnobotanical research carried out with 20 traditional healers in south of Benin, particularly in Ketou, Bohicon, Mono and Savalou, allowed to collect 30 medicinal plants belonging to 18 families, used mainly for the treatment of arterial hypertension and other gastric affections. *Lippia multiflora* is retained on the basis of its high use against arterial hypertension, in order to carry out laboratory analysis such as: phytochemical sifting and the general toxicity on the shrimps larvas. The phytochemical screening showed that the leafy stems of the plant contain Gallic tannins, terpenes and steroids, the leuco-anthocyanes, anthocyanes, mucilages and reducing compounds whatever the harvest region is. The LC50 obtained at the end of the larvary toxicity test showed that the water and the which are respectively 1.14± 0.18 mg/ml and hydro ethanolic extracts 3.56±1.53 mg/ml, no have toxicity on the shrimps larvas in principle on the human cells 9PS, KB, A-549 and HT-29.

Keywords: Arterial hypertension, *Lippia multiflora*, phythochemical screening, larvary toxicity.

INTRODUCTION

The use of traditional medicine is very widespread and has a sanitary and economic importance in developing countries (Tabuti et al., 2003). About 80% of the world population and more than 90% of the developing countries population use it for the primary health care (Jiofack et al., 2010). Despite the progress of biology and medicine of the time being, the majority of the populations of the developing countries don't have access to sufficient health care because of the weak economic systems (Singh et al., 2012). On the other hand, there is a danger that knowledge related to medicinal plants will be lost, even if the younger generations begin to take interest in it. These plants can be used to make improved traditional drugs/medicines thanks to bioactive molecules that they contain. The hypertensive affection is emerging with the lifestyle and the diet change. The rate of hypertension in Benin is 13.75%. The arterial

DOI : http://dx.doi.org/10.4314/ijbcs.v11i4.31

hypertension (AHT) is a cardiovascular pathology defined by a very high blood pressure (Organisation Mondiale de la Santé, 2002). In Africa, it contains some ethiopathological distinctive features like a low renin activity or its sodium-dependent character (whitfield, 2009). At present, 28% of the twenty year old Sub- Saharian adult populations are touched with some regional variations in particular, a predominance in the urban regions (Opie et al., 2005; Fourcade et al., 2007). In reference to, the normal blood pressure is inferior to 140/90 mmHg. The arterial hypertension would be responsible for at least 8 million of death per year in the world. The over proportion of people aged 60 and more who take antihypertensive drugs/medicines is established at 46% for the women and 38% for the men. Many factors of risk favor the arterial hypertension like the salt excessive in, food, the modification of food habits with the taking of hyper caloric meals, age, overweight, smoking or drinking alcohol. Often, this pathology is revealed during serious cardiovascular or renal accidents with sometimes, fatal ends (M'Buyanba-Kabangu et al., 2009). The arterial hypertension related diseases are among public health stakes of Benin (Fourn et al., 2005). This is the reason why the epidemiological studies was carried out in rural area of the southern part of the country in Oueme province (Bruneton et al., 2009) and some among then concerned more particularly pregnant women. In difficult economic environments, characterized of by high drug, prices, pharmacopoeia and traditional medicine become a significant alternative to health coverage, especially when the medication becomes permanent for the patient suffering with a chronic pathology. Priority was given to the traditional of medicinal plants knowledge in Southern Benin used against arterial hypertension. This us increasing evolution of the number of hypertension is a (Le Cardiologue et al., 2007) challenge for the scientific community. Although there have been important studies on the identification of antihypertensive species, and their biochemical composition (Farnsworthh et al., 2001). Very few studies are interested exclusively in antihypertensive plants in Benin. It is true that the hyperkalemia, hyperuricémie, and hyperglycéridémie were collected in Benin but this study was carried out only in three different districts in South of Benin. An ethnobotanical survey carried out in some Benin pytodistricts on the antihypertensive plants helped to collect 30 species divided up in 18 families. The most commonly used species is *Lippia multiflora*. We wish in to identify in the information (description and systematics, chemical composition, pharmacological data and toxicological data) available on the plant in order to help to reduce the damage caused by this disease.

MATERIALS AND METHODS

Antihypertensive plants inventory

An inventory of knowledge was carried out mainly from bibliographical data (Data base, reference work, publications) completed with survey, helping to list about thirty used plants in Beninto treat the hypertension. From this inventory, a selection of potentially interesting plants to be studied was carried out by applying diverse criteria. First of all the number of documented references, the relevance and the redundancy of the information were revealed. Therefore, a great number of bibliographical references on a frequently used plant in traditional medicine in different countries underpins a certain medicinal interest. The information is relevant, the reason why it is found in many references. However, its redundancy can also

be caused by the previous phytochemical studies and the possibility to highlight the original compounds is diminished. Then the chemical composition and the reported biological activities were also taken into account: Some plants which were subjects of inventory were previously studied and the chemical compounds and / or biological activities were already reported. Contrary to the absence of phytochemical work, were an important selection criterion because, it helped to look for the original chemical structures identification which are not described yet. It is the same as the specificity of the use of the plant in the treatment of hypertension.

Study scope

Our survey was carried out in 2015 in the main towns of the four departments of Benin which are chosen because of their demography and their varied geographical areas. We carried out the survey in the areas devoted exclusively to the marketing of medicinal plants in the markets of these towns. The concerned regions are: Plateau, Mono, Zou and Collines. Precisely, the survey was carried out in the towns of Kétou for the Plateau regions, Houéyogbé for Mono Bohicon and Djidja for Zou and Savalou for Collines.

Methods

The survey was carried out using the method of Tokoudagba et al., 2010. This study was performed with 20 herbal medicine doctors chosen from the agenda of ‘’Programme National de la Promotion de la Médécine Traditionnelle’’of Benin Ministry of Health. It was carried out in the selected areas in presence of people who master the mother tongues of these regions and the plants. The tools of the survey were composed of a questonnaire and interviews. During the survey, questions are asked are related to:

- Used plants to treat the hypertension / high blood pressure
- Vernacular names of the used plants.
- Parts of the used plants
- How to make the medicines, and dosage.
- Administration and the remedies

We asked questions to traditional herbal medicine doctors and the over-sixties people with traditional knowledge. A selection from the most used species and the helped pharmaco-chemistry point of view after consulting the different data banks, to retain only one species belonging to only one botanical family. This species was harvested from July to August 2015 in their natural habitat in Benin. The botanical determinations are carried out at ‘’Herbier National ‘’of University of Abomey Calavi (Benin). We must inderline that this harvested species presents either a similar use in the other traditional pharmacopoeias or other popular uses.

Phytochemical analysis

The phytochemical screening of the extracts was performed according to the method used by Houngbeme et al.(2014): Mayer’s and Dragendorff’s tests for alkaloids, Fehling’s test for free reducing sugars, Fehling’s test for glycosides, Liebermann-Burchard’s test for triterpenoids, Liebermann-Burchard’s test for steroids, frothy test for saponins, Shinoda’s and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard’s test for free cyanogenetics derived and Borntrager’s test for free anthraquinones.

Preparation of the crude extracts

The decoction and steeping method in conformity with the traditional method of preparation were used according to procedure

of Houngbeme et al., 2014. In this study, two (2) types of extracts: hydroethanolic and watery were prepared for each of the 4 plants so be it a total of 8 extracts. Fifty grams (50g) of the powder of each plant are put in five hundred milliliters (500mL) of solvent (water-ethanol (4:6; v/v) for the hydro –ethanol extracts). The mixture in left in continuous agitation during seventy-two hours (72hours) and the obtained macerate is filtered successively three times on hydrophilic cotton. An essay taking of 50g of vegetable powder was intruded in 500 ml of distilled water contained in a saucepan. All is brought to boil on electric sheets during 15 minutes. Then the substance obtained after filtering was evaporated direly at 40°Cwith a Rota vapor (Heidolph Laborata 4000 efficient) coupled with a cooler (Julabo FL 300) of water and the obtained residue is weighed for the yield determination according to the relationship:

$$\text{Yield (\%)} = \frac{\text{Dry obtained extract}}{\text{Initial of powder}} \times 100$$

Test of larvary toxicity

The test is performed against Artemia salina Leach by the method of Houngbeme et al*.,* 2014 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 sea water until hatching of young larvae (48 hours).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 solution containing no active substance were left under stirring for 24 hours. 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)] x 100. Data (dose-answer) are transformed by logarithm and the LC_{50} were determined by linear regression. Tests were carried out in triplicate.

Identification of vegetable species

The harvested plant was identified at ‘’National Herbal of Abomey-Calavi University’’ with analytical flora of Benin (Akoègninou et al., 2016).

$$LF = \frac{\text{Listing number for the considered plant}}{\text{Listing total number for all the plants}} X\ 100$$

RESULTS

Inventory of collected antihypertensive plants

During the survey, the great majority of traditional healers don’t quickly identify the arterial hypertension pathology because it doesn’t have its equivalent in the traditional system of thought. This observation is true again in the rural environment. On the other hand they cure the characteristic symptoms associated to them: headaches, the heart beat, the tiredness when making effort, dizziness’s, heavy sweating; and refer to diseases for a confirmation of their diagnostic in health centers before taken care of them. The causes of the diseases according to traditional doctors include the excessive consumption of animal fats and food salt. The results of this survey were inscribed in the Table1. In order to know the plants mostly used by traditional doctors, we calculated for each of them, the use frequency which represents the percentage of traditional doctors having listed a given species in comparison to the investigated population. The analysis of table1 shows cleanly that *Lippia multiflora* is a plant having the highest use frequency (50%). This result

enacted us to retain this species to carry out preliminary analysis at laboratory. The Table 2 presents the monograph of this selected plant. This species is harvested between July and august 2015 in the different areas taken into account by the ethno botanical survey. The identification of the species was carried out at Herbier National of university of Abomey Calavi. *Lippia multiflora* is an aromatic plant of 2,5 m height widespread in the sudano-zambezian savannas (Adjanohoun et al., 2002), The leaves put in the same groups of organs inserted at the same level on a stem by four or three, are oblong in form of spearhead or elliptical (Pascula et al., 2001). Their base is angular with slender point, finely toothed edge and covered with whitish little hair below, carrying 7 to 8 pairs of lateral veins. The cylindrical, protuding terminal top part of the plants are from 5 to 20 millimeter long and 4 to 8 millimeters wide arranged at the end of a terminal ramification of the stem. The flowers are small and white or yellowish. The small simple leaves are obtuse Growing in the central and southern countries of America and in tropical Africa, the plant is used in African traditional medicine to treat malaria and arterial hypertension; the characteristic aroma of its leaves gets people use it in from of infusion and tea, hence its name of Gambia tea (Kanko et al., 2004) points out that it is used as anti disinfectant, antipyretic and diuretic. In internal use, the leafy stems, the flowered or fruitful summits of *Lippia multiflora* are used to treat the liver affections (jaundice, hepatitis insufficiency, the beginning of cirrhosis inflammations of gall bladder), the lack of appetite, anemia, cardiac weakness, dizziness's, palpitations, weakness of nerves, melancholy, physical and intellectual overwork, pernicious access of malaria, the flu, asthma, blood pressure, menopause nervousness, severe jaundice, the chronic ulcers, cold, epilepsy, the bucco-anal and digestive candidoses are cholagogue, choleretic, antispasmodic and anti-inflammatory. The roots are antispasmodic and treat abdominal affections and rheumatism. In external use the leafy stems (vulneraire alcoolats 10-20%) cure rhinopharyngites affections, cataracts buccal affections, sprains, burns, wounds, wrinkles, rheumatic pains, and chronic ulcers. They are also antiseptic, tonic, analgesic and healing. The roots are anti-inflammatory and antiseptic.

Chemical composition of Lippia multiflora

The results of the phytochemical screening are summarized into Table 3.

Extraction yield

The yield of the obtained extracts is expressed in form dry extract percentage contained in 100g of the plant powder (Houngbeme et al., 2014). The obtained values are summarized in Table 4.

Cytotoxicity activity

LC_{50} values of tested extracts were summarized in Table 5below.

The LC_{50} vary from 0.81 to 1.32 mg/mL for the aqueous then from 2.03 to 5.10 mg/mL for the hydro-ethanolic extracts. In order to appreciate the toxicity degree of the extracts from the values of LC_{50}, we compared with the correspondence table (Table 6).

Table 1: List of selected plants during the survey and their frequency in the different areas.

N°	Kinds of species	Mono	Savalou	Kétou	Djidja	Number	Frequency	Families
1	*Lippia multiflora*	5	3	1	1	10	50	Verbenaceae
2	*Calotropis procera*	1	2	0	0	3	15	Asclépiadaceae
3	*Jatropha curcace*	0	2	1	0	3	15	Euphorbiaceae
4	*Spondias mombin*	2	0	1	0	3	15	Anacardiaceae
5	*Crateva religiosa*	0	1	2	0	3	15	Capparaceae
6	*Securinega virosa*	1	0	1	0	2	10	Euphorbiaceae
7	*Acanthospermum hispidum*	1	0	1	0	2	10	Asteraceae
8	*Newbouldia laevis*	0	1	0	1	2	10	Bignoniaceae
9	*Byrsocarpuscoccineus*	0	1	0	1	2	10	Connaraceae
10	*Monodora myristica*	1	0	1	0	2	10	Annonaceae
11	*Rauvolfia vomitoria*	0	1	0	1	2	10	Apocynaceae
12	*Ricinuscommunis*	0	1	0	1	2	10	Euphorbiaceae
13	*Hymenocardia acida*	0	0	1	1	2	10	Euphorbiaceae
14	*Pteleopsissuberosa*	0	1	1	0	2	10	Combretaceae
15	Hyptis suaveolens	0	1	1	0	2	10	Lamiaceae
16	Chenopodiumambrosioides	1	0	1	0	2	10	Acesineeae
17	*Carica papaya*	0	1	1	0	2	10	Caricaceae
18	*Nauclea latifolia*	0	1	0	0	1	5	Rubiaceae

19	*Opilia celtidifolia*	0	0	1	0	1	5	Opiliaceae
20	*Croton zambezicus*	1	0	0	0	1	5	Euphorbiaceae
21	*Piliostigma thonningii*	0	0	0	1	1	5	Caesalpiniaceae
22	*Daniellia oliveri*	0	0	1	0	1	5	Caésalpianiaceae
23	*Ageratum conyzoides*	1	0	0	0	1	5	Astéraceae
24	*Eucalyptus citriodora*	0	0	1	0	1	5	Myrtaceae
25	*Xylopia aethiopica*	0	1	0	0	1	5	Annonaceae
26	*Eucalyptus camaldulensis*	1	0	0	0	1	5	Myrtaceae
27	*Elaeis guineensis*	0	0	0	1	1	5	Myrtaceae
28	*Uvaria chamae*	0	0	1	0	1	5	Arecaceae
29	*Citrus aurantifolia*	1	0	0	0	1	5	Rutacées
30	*Lannea velutina*	0	1	0	0	1	5	*Anacardiaceae*

Table2: Monograph of *Lippia multiflora*.

Species	Family name	Vernacular	Morphology, geographical distribution	Ethnobotanical data	Identified substances	Described activity	References
Lippia multiflora	Verbenaceae	Fon : Aglala, Aklala Yoruba : Efintin, Kanhoun Mina : Avundati Sahoue: Vundati	A straight aromatic plant of 2,5m height widespread in the sudano-zambeziean and the subtropical africa savannas	The decoction of the whole plant is used against cough, toothache, anaemia, general weakness, hiver affection (jaundice, liver insufficiency, beginning of cirrhose gall bladder inflammations) loss of appetite, anaemia, cardiac, weakness, dizzinesses, palpitations, weakness of nerves, melancholy, physical and mental over work, perncious access ofMalaria, shivers flu, asthma, low blood pressure, Menopause nervousness, Severe jaundice, Sleepdisease, chronic, ulcers, cold,epilepsy bucco-anal and digestive candidoses	α-thujene; α-pinene; Camphene; Sabinene; β-pinene; Myrcene; α-phellandrene; α-terpinene; P-cymene; Limonene; Eucalyptol (E)-β-ocimene γ-Terpinene; Terpinolene Camphor Terpinene-4-ol α-terpineol Thymol Carvacrol Thymyle Acetate B-caryophyllene Germacrene-D Elemol Caryophyllene oxide Monoterpene hydrocarbon Sesquiterpene hydrocarbon Oxygenated terpenes _	Antibacterial Antiradicalar Haemolitic Antalgic Antipyretic Antioxydizing Antifungicidal Antimalaria	Arbonirier, 2009 et Malgras, 1992

Table 3: Results of phytochemical screening.

Harvest area	Al	TAC	TAG	Fl	Ant	Leu	Qn	SP	Tp	St	Cy	Mu	Cm	Cr	Hl	O-H	C-H	H-C
Savalou	-	-	++	++	++	++	-	-	+	++	-	++	-	++	-	-	-	-
Djidja	+	-	++	++	++	++	-	-	++	++	-	++	-	++	-	-	-	-
Kétou	-	++	++	++	++	++	-	-	++	++	-	++	-	++	-	-	-	-
Houéyogbé	+	-	++	-	++	++	-	-	++	++	-	++	++	++	-	-	-	-
Totaux	2	1	4	3	4	4	0	0	4	4	0	4	1	4	0	0	0	0

TAG : Tanins galliques ; TAC : Tanins catéchiques ; Al : Alcaloïdes ; Fl : Flavonoïdes ; St : Stéroïdes ; Tp : terpènes ; Leu : Leucoanthocyanes ; Ant : Anthocyanes ; Mu : Mucilages ; O-H : O-Hétérosides ; C-H : C-Hétérosides ; Hl : Hétérosides libres ; Cr : Composés réducteurs ; Cm : Coumarines ; Hc : Hétérosides cardiotoniques ; Qn : Quinones ; Sp : Saponosides ; Cy : dérivés cyanogéniques
++ : Réaction fortement positive ; + : réaction positive ; - : réaction négative

Table 4: Summary of the extractions yields.

Harvest area	Extraction yield (%)	
	Acqueous extract	Hydro-ethanolic extract
Savalou	10,58	24,77
Houéyogbé	14,94	15,01
Djidja	23,54	19,77
Kétou	7,24	11,54

Table 5: LC_{50}values of the different tested extracts.

Harvest area	(mg/mL)	
	Aqueous extract	**Hydro-ethanolic extract**
Savalou	1,28	2,03
Houéyogbé	0,81	5,10
Djidja	1,15	2,31
Kétou	1,32	4,81

Table 6*:* Correspondence between LC_{50} and toxicity.

LC_{50}	Toxicité
$LC_{50} \geq 100$ µg/mL	(non toxique)
100 µg/mL > $LC_{50} \geq 50$µg/mL	+ (faible)
50 µg/mL > $LC_{50} \geq 10$ µg/mL	+ + (modérée)
$LC_{50} < 10$ µg/mL	+ + + (forte)

- : atoxic; +: weak toxicity; ++: moderate toxicity; +++: high toxicity.

DISCUSSION

The secondary metabolites emphasized in the survey areas, are put in table 3. It emerges from this table that the tested plant is rich in phytochemical compounds. The leafy stems contain gallic tannins, terpenes and steroids, leuco-anthocyanes, anthocyanes, mucilages and reducing compounds no matter what the harvest area is. The species contain also flavonoids except the samples harvested at Houéyogbé. We can say that these chemical groups are the most present in the plant. Moreover, notice that the plant doesn't contain cyanogenic derivatives and carditonic heterosides which are toxic substances that its health security (N'guessan et al., 2010). The present chemical groups in the plant possess different pharmacological properties: anti-œdema, anti –inflammatory, antibacterial, antiviral, antifungicidal and antiseptic. The presence of terpenes in the plant corroborates with the works when the isolated terpenic molecules of *Lippia multiflora.* It was reported that the tanins present a weak antibacterial, activity antiviral, anti-inflammatory, anti-hypertensives, antimutagene, immuno-stimulating, antitumorous, anti-diarrhoea activity relatively high . The coumarines have also anticurdling, antioxydizing, and anti-inflammatory properties. We also notice antiviral, antitumorous, anti-inflammatory, antiallergic and anticancerous activities in flavonoids. The tannins and the flavonoids have a proved anti-hypertensive activity (Ndiaye et al*.,* 2004; Ndiaye et al., 2005); so their presence would justify that the plant is highly used by traditional healers to combat this pathology (Jouvelet et al., 2000). The tested plant containing these different chemical groups these pharmacological properties cited above, mainly anti-hypertensive activity of tannins and flavonoids. The yield rate is 17.77% for the hydro-ethanolic extracts and 13.77% for

the aqueous extracts. We conclude in view of these values that the mixture (water-ethanol) extracts the great part of chemical principles. Therefore, the great number of this plant compounds is polar. The LC_{50} values analysis of tested extracts compared with the correspondence table below, allows us to say that the tested extracts are not toxic in the range of the analysed concentrations because the LC_{50} obtained are superior to the fixed limit (0,1 mg/ml). The ataxic character of these extracts proved by the general test of toxicity confirms the phytochemical screening results which showed the absence of cardio tonic heterosides, cyanogenic derivatives and quinonic derivatives that are generally toxic compounds. When we consider the correlation between the cytotoxicity on the shrimps larvas and on the 9PS and KB cells (Human nasopharyngeal Carcinoma) on the one hand (Pelka et al., 2000), the A-549 cells of pulmonary carcinome and the HT-29 cells of colon on the other hand (Carballo et al., 2002), we can say subject to further the researches, that the tested extracts are free of cytotoxic activity. We notice for all the obtained graphs that the correlation coefficient R^2 is superior to 0.8. There is a good correlation between the applied concentrations and the obtained responses. Moreover, we notice that the larvas are sensitive to the tested extracts. The number of dead larvas grows with the concentration, so the sensitivity of the larvas to the extracts follous relationship of dose-response. The Table 6 below summarizes the different values of lethal half-concentrations obtained. These values show the concentrations necessary to 50% of larvary population survival in the tested solutions.

Conclusion

The ethnobotanic survey carried out in the main towns of four Benin regions helped to know the therapeutic use of the medical herbs/plants currently used by people to treat the arterial hypertension. Among the plants which have been object of inventory, only *Lippia multiflora* is frequently used. Our research enatted us to show that this plant is rich in chemical groups which justifies, in principle its anti-hypertensive potentiality. The shrimp's larvas are tolerant to aqueous and hydro-ethanolic extracts thus the leafy stems of the plant don't have in principle any noxious effects on9PS, 9KB, A-549 and HT-29 human cells. This preliminary study contributes to the development of Benin flora and is worth furthering in evaluating the anti-hypertensive power of the troop types of extracts and in qualifying the chemical groups proved responsible for this activity.

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.

ACKNOWLEDGEMENTS

We sincerely thank all the traditional doctors who accept to share their knowledge with us to reach the objective of this work. We also thank Dr. Alban Gout on HOUNGBEME and M. Gilda's HOUNKANRIN for their technical assistances.

REFERENCES

Adjanohoun EJ, de Souza S. 2002. Guide pratique de phytothérapie: la santé par les plantes, 100 plantes médicinales utiles au Bénin, Centre de Biodiversité, CENPREBAF, Cotonou (Bulletin Régional d'Information, 4).

Akoègninou A, vanderBurg WJ, Vander LJG. 2016. *Maesen, Flore Analytique du Bénin*. Backhuys Publishers; 1043p.

Bruneton J. 2009. *Pharmacognosie, Phytochimie, Plantes Médicinales* (4ème édn). Tec et Doc: Paris; 1120.

Carballo JL, Hernandez-Inda ZL, Pérez P, Garcia-Gràvalos MD. 2002. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnology,* **2**: 17.

Fabricant DN, Farnsworth. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives,* **109**: 69-75.

Fourn L. 2005. Principaux enjeux de la santé publique au Bénin, Conférence du 21 décembre 2005 de l'IREEP, Cotonou, Bénin.

Fourcade L, Paule P, Mafart B. 2007. Hypertension artérielle en Afrique subsaharienne actualité et perspectives. *Médecine Tropicale,* **67**: 559-567.

Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Sohounhloue D, Moudachirou M, gbaguidi FA. 2014. 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): 1739-1745

Jouvelet B, Kedzierska A. 2000. *Guérisseurs et Féticheurs la Medicine Traditionnelle en Afrique de l'Ouest.* Edition alternative : Paris ; 144P 2000.

Kanko C, Koukoua G, N'Guessan Y, Fournier J, Pradère JP, Toupet L. 2004. Contribution à l'étude phytochimique de *Lippia multiflora* (verbenaceae). *Comptes Rendus Chimie,* **7**: 1029-1032.

Le Cardiologue. 2007. Spécial HTA. Comité français de lutte contre l'hypertension artérielle, 306, Novembre 2007.

M'Buyamba-Kabangu JR, Biswika RT, Thijs Tshimanga L, Ngalula FM, Disashi T, Kayembe PK, Richart T, M'Buyamba-Kayamba JR, Lepira FB, Staessen JA. 2009. In-hospital mortality among black patients admitted for hypertension-related disorders in Mbuji

Mapongmetsem PM, SabangNT. 2010. Ethnobotanical uses of medicinal plants of two ethno ecological regions of Cameroon. *International Journal of Medicine and Medical Sciences*, **2**(3): 60-79.

Ndiaye M, Chataigneau M, Lobysheva I, Chataigneau T, Schini-Kerth VB. 2005. Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. *Faseb Journal*, **19**: 455-7.

N'guessan KA, Yao-Kouamé A. 2010. Filière de commercialization et usage des feuilles de *Lippia multiflora* en Cote d'ivoire. *Journal of Applied Biosciences*, **29**: 1743-1752.

Organisation Mondiale de la Santé. 2002. Médecine traditionnelle : Rapport du

secrétariat. Conseil exécutif. Cent onzième session. Point 5.7 de l'ordre du jour provisoire. EB111/9 du 12 décembre 2002.

Pascula M, Slowing E, Carretero K, Sanchez E, Villar DMA. 2001. Lippia traditional uses, chemistry and pharmacology: a review. *Journal of ethnopharmacology*, **76**: 201-2014.

Pelka M, Danzl C, Distler W, Petschelt A. 2000. A new screening test for toxicity testing of dental materials. *J. Dent.,* **28**: 341-345.

Singh B, Singh BK. 2012. Ethnomedicinal use of Pteridophytes in reproductive health of tribal women of Pachmarhi Biosphere Reserve, Madhya Pradesh, India. *International Journal of Medicine and Medical Researcher*, **3**(12): 4780-4790.

Tabuti JR, Lye KA, Dhillion SS. 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration**.** *J Ethnopharmacol.,* **88**(1): 19-44.

Tokoudagba JM, Chabert P, Anger C, N'Gom S, Gbenou J, Moudachirou M, Schini-kerth V, Lobstein A. 2010. Recherche de plantes à potentialities antihypertensives dans la biodiversité béninoise, *Ethnopharmacologia*, **44**: 32-41.

Whitfield KE, Yao XKB, Boomer GP, Vogler MD, Hayward DJ. 2009. Vandenbergh, Analysis of candidate genes and hypertension in African American adults. *Ethnicity of Disease*, **19**: 18-22.