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INFLUENCE OF HARVESTING SITES ON THE ANTIHYPERTENSIF EFFECTS OF ESSENTIAL OILS OF LIPPIA MULTIFLORA MOLDENKE (VERBENACEAE) IN BENIN

Protein Biochemistry

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ABSTRACT

A comparative study of the major compounds was carried out despite the great diversity of the constituents characteristic of the essential oil of *Lippia multiflora*. It was interested to note the imprint that makes each locality with the chemical characters of the gasoline. The variation in the chemical composition of essential oils, was depended on many factors such as the method used, the parts of the plant used, the products and reagents used in the extraction, the environment, the genotype of the plant, the geographic origin, harvest period of the plant, degree of drying, drying conditions, temperature and drying time and the presence of pests, viruses and weeds. The effects of essential oils of *Lippia multiflora* were evaluated in Wistar rats on arterial pressure (BP) and on heart rate (HR). Administration of L-NAME was caused a significant increase in SBP in rats from 142.4 mmHg (D0) to 172.4 mmHg (D8) and from 122.8 mmHg (D0) to 138.6 mmHg (D8) respectively for the positive control batch and the batch subsequently treated with essential oil. Administration of the essential oil from D8 to D29 was caused a significant decrease in the SBP of the rats from 140 mmHg (D8) to 122.4 mmHg (D29). This decrease was markedly lower than that of the untreated rats which is 150.5 mmHg (D29). The administration of L-NAME was caused a significant increase in the DBP of the rats going from 93.8 mmHg (D0) to 137.5 mmHg (D8) and from 92.5 mmHg (D0) to 136.75 mmHg (D8) respectively for the positive control batch and the batch subsequently treated with essential oil.. The administration of the essential oil from D8 to D29 was caused a significant decrease in the DBP of the rats going from 136.75 mmHg (D8) to 99.8 mmHg (D29) and finally the administration of L-NAME caused a significant increase in the MAP of the rats going from 109.25 mmHg (D0) to 159.75 mmHg (D8) and from 99.8 mmHg (D0) to 151.25 mmHg (D8) respectively for the positive control group and the batch then treated with the raw extract). Administration of the essential oil from D8 to D29 was caused a significant decrease in the AMP of the rats, dropping from 151.25 mmHg (D8) to 114 mmHg (D29) ($p < 0.05$). These effects could be justify the use of this plant in traditional medicine in the treatment of high blood pressure.

KEYWORDS

Lippia multiflora, systolic blood pressure, diastolic blood pressure, mean arterial pressure, essential oil, losartan.

INTRODUCTION

Lippia multiflora Moldenke was an aromatic plant with a camphor scent, widely distributed in Africa and reaching 2.7 to 4 m in height[1]. It was not only consumed by the populations in theiform infusion but also, was used in traditional African medicine for its various therapeutic virtues [2; 7]. Preliminary phytochemical studies reveal the presence of flavonoids, leuco-anthocyanins, steroids or terpenoids and tannins in the aqueous extract of dry leaves of *L. multiflora* [8].

Flavonoids are compound recognized for their cardiovascular effects, particularly against atherosclerosis, coronary artery disease and against high blood pressure [9]. Indeed, it was offered a variety of uses in pharmacopoeia and medicine : infusions of its leaves are traditionally used for the treatment of malaria, diabetes, fevers, gastrointestinal disorders, cough, hypertension and, also, as a laxative[10;11]. Besides its medical potential, *L. multiflora* provides cosmetic adjuvants and pesticides[12] and [13]. Theses qualities are attributed to it mainly by the essential oil extractable from the leaves[14] and [6]. To this end, more than 42 volatile components have been identified in *L. multiflora* oil[15] with specific biomedical properties for each component[16]. The essential oil of *Lippia multijlora* was used by the food, pharmaceutical and cosmetic industries[17]. It also has been insecticidal[18] and bacteriostatic [19; 20] properties. Much work has been done on *Lippia multiflora* species. There is botanical and chemical variability within this species. The work was carried out by[21] defined several morphotypes. Some of the plants observed by these authors have been shiny, supple leaves, light green in color, elliptical in shape and varying in size from 3 to 6 cm, with a pleasant and pungent scent. Others have been oblong leaved, dark green in color, and 15 to 20 cm long, with a sweet and pleasant scent. From a chemical point of view, a parallel study of different essential oils of *Lippia multiflora* was helped to highlight the variability in the composition of this plant. This was the case with the various chemotypes which have been reported in the literature [22;23] and [18 ; 20]. Given the interest shown by the chemical components of the essential oil of *L. multiflora*, its use may be of additional benefit to the pharmaceutical and cosmetic industries in areas of its prevalence, such as in sub-Saharan Africa. This eventuality would be constituted a means of substantial poverty reduction and an additional asset for rural populations, dependent on a tertiary economy. To achieve this goal, it was necessary to produce essential oil in the quantity and quality required to sustainably support such industrialization. However, *L. multiflora* was remained above all an invasive plant and the quality of the essential oil was very variable[23] even within an ecological zone and for a given genotype with seasonal and environmental effects including those of the soil [23;24].

To this end, work was aimed at developing cultivation systems for *L. multiflora* was known[25] and[1] while the determination of chemotypes has only been descriptive for different ecological zones and[22] in lack of explanatory and comprehensive analysis that would be allowed control of the quantities and qualities of production. Such an analysis was possible because relationships have been established between certain chemical characteristics of the soil and the occurrence of certain weeds in certain ecosystems of sub-Saharan Africa[27].This was why a prospecting study in three areas of natural occurrence of *L. multiflora* in Benin was taken place for the picking of the leaves and the chemical analysis of the essential oil in relation to the same topographical segment of the landscape by considering the soil pH as well as the carbon (C), total nitrogen (N), total phosphorus (PT) and assimilable phosphorus (PA) contents of the soil. The objective is to identify the nutrients in the soil that would affect the occurrence of *L. multiflora* and the chemical nature of its essential oil. Ultimately, a soil fertility management strategy should be recommended for quantitative and qualitative production of *L. multiflora*. Previous studies have shown that the essential oils of L. multiflora cause negative inotropic and chronotropic effects on the isolated toad heart[8]. These effects could be contributed to the decrease in cardiac output and therefore blood pressure. Thus, the objectives of the present study were to assess the effects of essential oils from the dry leaves of *L. multiflora* on hypertension and to elucidate the mechanism of action of the observed effects.

MATERIALS AND METHODS

Plant material

It consists of plant material (*Lippia multiflora*), microbial strains (bacteria, yeast and mold), biological and chemical diagnostic equipment. Survey in current study was carried out in 2015 in the main towns of the four departments of Benin which are chosen because of their demography and their was varied geographical areas. Present study 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.

A specimen of the plant was deposited and identified by the national herbarium of the University of Abomey-Calavi (Benin)[28]. The plant materials used in present study were 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).

Biological and chemical diagnostic equipment :

The equipment was used in the laboratory consists of conventional glassware sterile single-use plates of 90mn diameter, a micropipette P100 Gilson brand and culture media. The culture media and reagents used in the microbiological diagnosis come from BIORAD, MERCK, OXOID, BASINGSTORE, BIOMERIEUX and DifCO laboratories. A 24-hour pure colony portion from the Mueller Hinton medium of each strain was emulsified in 5ml of physiological saline to obtain a turbidity of 0.5 on the Mc Farland scale. The chemical diagnostic equipment was a GC, a spectrometer, Clevenger for extraction of essential oils. Methods The survey was carried out using the method of[29]. This study was performed with[30] 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 questionnaire 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

Questions were asked 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). This harvested species presents either a similar use in the other traditional pharmacopoeias or other popular uses.

Extraction of oils and calculation of yields

About 200 g of plant material from each plant were each subjected to hydrodistillation with a Clevenger type apparatus for 3 hours [31], [32] and [33]. The essential oil thus obtained in an essencier was collected in a small dark bottle containing anhydrous sodium sulphate (Na_2SO_4) and stored at 4°C until use. The yield R was expressed relative to the fresh plant material and its calculation was as follows :

$$R = \frac{M(HE)}{M(MV)} \times 100$$

Where, R : yield; M (HE): mass of essential oil obtained; M (MV): Mass of extracted plant material.

Chemical composition of essential oils Analysis by gas chromatography

The oils were analyzed using a VARIAN CP.3380 gas chromatograph equipped with two capillary columns (apolar and polar) and connected to a Varian integrator (Model C-R4A°)[33].

Analysis by gas chromatography and mass spectrometry Technical characteristics of the equipment

All essential oils were analyzed by GPC / MS, which results in the separation of the constituents of the mixture on a column mounted in series with a mass spectrometer. Each component leaving the column passes directly into the ionization field of the spectrometer. The molecule subjected to the electronic impact is fragmented. The mass spectra obtained for each compound are compared with data from the literature as well as those from the laboratory data base. CPG / MS couplings were performed on a Hewlett Packard-Quadruple type device (Model 5970).

The chromatograph was equipped with a fused silica capillary column 30 m long and 0.25 mm inside diameter with a dimethylpolysiloxane (DB-1) type grafted phase 0.25 μm thick.

In vivo determination of hypertensive activity of essential oils of *Lippia multiflora*

This was a non-invasive blood pressure measurement performed on Wistar strain rats acclimatized to the ambient conditions of the breeding unit of the Teaching Unit and Research in Human Biology at the Faculty of Health Sciences of the University of Abomey-calavi. The present experimental type study was carried out in Wistars rats. It was carried out in a single phase : The study was carried out on 25 Wistars rats with a body weight of between 150 and 250g divided into three (03) groups and was carried out over a period of twenty-eight (28) days from D0 to D28. The rats were made hypertensive with the N (G) -Nitro-L-Arginine Methyl Ester (L-NAME). The arterial pressures of the rats were taken on D0, D8, D15, D22, and at D29. We were made a batch of 5 male rats. The treatment was carried out according to the following protocol:

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 μL / 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 μL / kg of body weight for 14 days then with losartan at a dose of 100 μL / kg of body weight for the following 14 days;
Group 4: batch of 5 rats treated with L-NAME at a dose of 40 μL / kg of body weight for 14 days then with essential oil of Lippia multiflora from the Djidja region at a dose of 500 μL / kg of weight bodily during the following 14 days; All administrations will be given orally using a gavage tube.

Losartan treatment

This is the antihypertensive tablet used as the reference drug. L-

NAME induction was performed in a batch of seven rats with an average body weight of 197.42 µL / kg, followed by treatment with losartan. This is dissolved in distilled water will be administered to the animals at a dose of 100 µL / kg of body weight, for a concentration of 19.74 µL / 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 was measured on the 30th day.

RESULTS AND DISCUSSION

Yields of the plants studied

The essential oil yields of plants of the family Verbenaceae, the yields of essential oil extracted from the leaves of *Lippia multiflora* (Kétou, Savalou, Mono and Bohicon) are relatively high and their yields are 0.58 %, 1.07 %, 1.70 % and 2.18 %, respectively. Yields, calculated from dry plant material, vary from sample to sample. The essential oil of Djidja has the highest yield (02.18 %) followed by that of Houéyogbé (01.70 %), Tchetti (01.07 %) then that of Ikpinlè (0.58 %). These essential oil contents are higher than those already obtained by [20] (0.53 %). For the same aromatic plant there is a different composition of essential oils, depending on the parts used, the period of picking, the geographical location and even according to the extraction protocol. These results have been confirmed by the work carried out by [7 ; 24] on essential oils of *Lippia multiflora*. This is explained by the concept of chemotype, which presents great variability, both quantitative and qualitative, which confirms the divergences in the results reported for a given plant.

Chemical composition of essential oils according to the sites

Table : Variation of the main EO compounds of *Lippia multiflora depending* on the place of harvest

		Harvest location		
Compounds	Savalou	Djida	Mono	Kétou
Linalol	0,3	91	/	/
terpinen-4-ol	0,3	/	12,5	/
α-terpinol	13,8	/	13	/
Sabinène	14,4	0,1	/	/
1,8-cinéol	50,6	0,2		13,5
Oxyde- caryophyllène	/	0,1	10,5	/
Beta-caryophyllène	/	/	/	28
Gamma-terpinène	/	/	13,65	47
Carvacrol	/	/	13	/
P-cymène	/	/	15	/

A comparative was studed of the major compounds was made in the table. Despite the great diversity of the characteristic constituents of *Lippia multiflora* essential oil, Linalool, 1,8-cineol, Gamma-terpinene and Beta-caryophyllene are still present. It was interested to note the imprint that makes each locality with the chemical characters of the gasoline. The table is shown the variation of the main compounds of *Lippia multiflora* essential oil depending on the place of harvest, highlighting the particular chemical features that characterize the essential oil of each locality.

For example, the essential oils of Djidja differ from those of Savalou, Mono and Kétou by the presence of Linalool (91 %), the oilessential from Savalou was also differentiated from that of Djidja, Mono and Kétou by the presence of 1,8-cineol (50.6 %), Sabinene (14.4 %) and α-terpinol (13.8 %). Likewise, the essential oil of Mono also differs that of Djidja, Savalou and Kétou by the presence of P-cymene (15 %), Carvacrol (13 %), terpinen-4-ol (12.5 %) and oxide-caryophyllene (10,5 %) and finally the essential oil of Ketou differentiates from Savalou, Djidja and Mono by the presence of Gamma-terpinene (47%) and Beta-caryophyllene (28 %). 1,8-cineol (50.6 %), Sabinene (14.4 %) and α-terpinol (13.8 %) can be identified with gasoline from the Colline department (Savalou). 1,8-cineol (50.6 %) is identified not only in the Colline department but also in the Plateau department. The α-terpinol (13.8 %) is identified not only in the department of Colline but also that of Mono. P-cymene (15%), Gamma-terpinene (13.65 %), Carvacrol (13 %), terpinen-4-ol (12.5 %) and caryophyllene oxide (10.5 %) can be found in the department of Mono (Houéyogbé). Gamma-terpinene (13.65 %) not only in the Mono department but also in the Plateau department. Gamma-terpinene (47 %), Beta-caryophyllene (28 %) and 1,8-cineol (13.5 %) are identified in the department of Plateau (Kétou). While Linalool (91 %) in general is much more characteristic of Zou essential oil even if we meets the *Lippia multiflora* type at Linalool in the Hill (Savalou). The variation in the chemical composition of essential oils depends on many factors such as the method used, the parts of the plant used, the products and the reagents used in the extraction, the environment, the genotype of the plant, the geographical origin, the harvest period of the plant, the degree of drying, the drying conditions, the temperature and the drying time and the presence of pests, viruses and weeds [34]. At the end of this campaign, we can confirm not only the existence of chemical polymorphism within the species Lippia multiflora in the flora of Benin, but also indicate the impact of terroir on this polymorphism. We distinguish four chemotypes of *L. multiflora* : α-terpinol (Savalou, Mono), 1,8-cineol (Savalou, Kétou), Gamma-terpinene (Mono, Kétou), and Linalol + 1,8-cineol (Djidja, Savalou). Ketou). The scientific interest of this study on Lippia multiflora from Benin was greatly asserted with the results obtained [35].

However, one aspect remains to be explored, to verify that the chemotypes keep their identity once transplanted.

Seeds should be collected from different localities, sown in neighboring plots and analyzed, depending on the period until flowering, the essential oils obtained. An essential contribution of this study is to identify components of plant nutrition that can influence the occurrence and variability of chemotypes of the essential oil of *L. multiflora* thus making an opening for the deepening of knowledge with a view to mastering the production strategies of this plant species with high economic potential which nevertheless remains a weed species.

Evaluation of the antihypertensive activity of essential oils of *Lippia multiflora*

Figures 1 and 2 show the systolic and diastolic arterial pressures of rats subjected to the study of the antihypertensive activity of essential oils of *Lippia multiflora*.

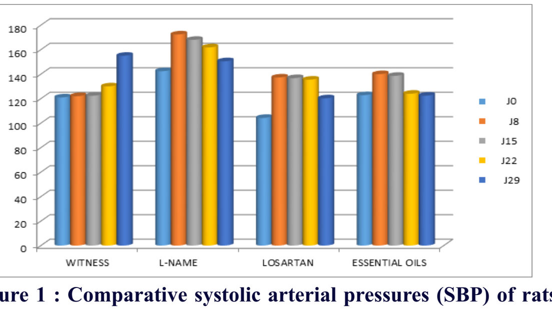

Figure 1 : Comparative systolic arterial pressures (SBP) of rats subjected to the study of the antihypertensive activity of essential oils of *Lippia multiflora* from Djidja (p-value <0.05)

The comparisons was made between J0 and J8, between J8 and J15, between J15 and J22 and between J22 and J29 to the same batch. Administration of L-NAME was caused a significant increase in SBP in rats from 142.4 mmHg (D0) to 172.4 mmHg (D8) and from 122.8 mmHg (D0) to 138.6 mmHg (D8) respectively for the positive control batch and the batch subsequently was treated with essential oil. Administration of the essential oil from D8 to D29 was caused a significant decrease in the SBP of the rats from 140 mmHg (D8) to 122.4 mmHg (D29). This decrease was markedly lower than that of the untreated rats which was 150.5 mmHg (D29).

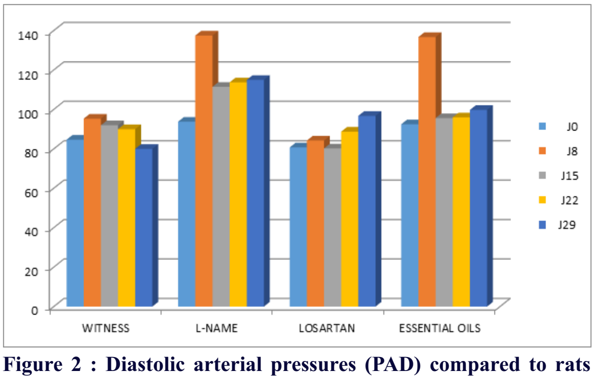

Figure 2 : Diastolic arterial pressures (PAD) compared to rats subjected to the study of the antihypertensive activity of essential oils of *Lippia multiflora* from Djidja (p-value <0.05)

The comparisons was made between J0 and J8, between J8 and J15, between J15 and J22 and between J22 and J29 to the same lot. The administration of L-NAME was caused a significant increase in the DBP of the rats going from 93.8 mmHg (D0) to 137.5 mmHg (D8) and from 92.5 mmHg (D0) to 136.75 mmHg (D8) respectively for the positive control batch and the batch subsequently was treated with essential oil. Administration of the essential oil from D8 to D29 was caused a significant decrease in the DBP of the rats from 136.75 mmHg (D8) to 99.8 mmHg (D29)

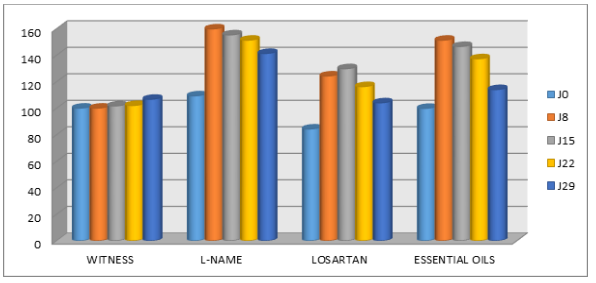

Figure 3: Comparative mean arterial pressures (MAP) of rats subjected to the study of the antihypertensive activity of essential oils of Lippia multiflora from Djidja (p-value <0.05)

Figure 3 was shawn that administration of L-NAME was caused a significant increase in rat PAM from 109.25 mmHg (D0) to 159.75 mmHg (D8) and from 99.8 mmHg (D0) to 151,25 mmHg (D8) respectively for the positive control batch and the batch subsequently was treated with the crude extract[7]. Administration of the essential oil from D8 to D29 caused a significant decrease in the rat's MAP, dropping from 151.25 mmHg (D8) to 114 mmHg (D29).

CONCLUSION

The principal component analysis was carried out on the monoterpenes and sesquiterpenes compositions of the essential oil of *Lippia Multiflora* plants grown in Benin and harvested over well-defined periods shows variability depending on the geographical origin. The study of variability was revealed four types of chemical profiles including four chemotypes of *L. multiflora* : α-terpinol (Savalou, Mono), 1,8-cineol (Savalou, Kétou), Gamma-terpinene (Mono, Kétou) and Linalool + 1,8-cineol (Djidja, Savalou, Kétou) depending on the geographical origin, including a grouping of the Savalou, Mono, Kétou and Djidja sitesopposed to samples from Mali. The study of the antihypertensive activity in vivo on a model of hypertensive rats caused by the L-NAME of essential oils of the plant in the region of Djidjashowed a significant drop in blood pressure to 137.25 mmHg on average after 14 days. The results of our research thus was obtained, offer a contribution to the enhancement of the plant resources of traditional Beninese medicine, which requires the implementation of scientific procedures. These results were confirmed the relevance of the traditional use of some of them, mainly the most active in the pharmacopoeia of Benin, and was shown the importance of the ethnopharmacological investigation in the search for new sources. medication for high blood pressure. The chemical composition would be proved to be the most interesting for considering a given pharmacological activity.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work

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