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RESEARCH ARTICLE

ANTIRADICAL ACTIVITY AND DETERMINATION OF PHENOLIC COMPOUNDS OF EXTRACTS OF LIPPIA MULTIFLORA (VERBENACEAE): A PLANT TRADITIONALLY USED AGAINST ARTERIAL HYPERTENSION IN BENIN

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ABSTRACT

The antiradical potential of Lippia multiflora evaluated by the DPPH radical method reveals that the hydroethanolic extract has a higher antioxidant capacity (957.90 ± 2.42 mmolEAA / mg) than the aqueous extract (489.35 ± 11.29 mmol EAA / mg) against 14.43 mg / mL for ascorbic acid used as reference antioxidant. The contents of the phenolic compound extracts have been determined and vary according to the extract. These values are between 104.68-957.90% for total phenols, 0.038-0.338% for flavonoids and 0.002-0.031% for condensed tannins. The hydroethanolic extract has high levels of these metabolites irrespective of the area of origin of the plant, which would justify their strong anti-radical activity.

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INTRODUCTION

High blood pressure (HTA) is a very common condition but has long been considered a rare and even non-existent disease in Africa. It is now a real public health problem and refers to a non-transitory rise in arterial blood pressure that is based on two points: elevation of tension and persistence (Arama *et al.,* 1988). HT is one of the leading causes of death in both sexes. All these complications, coupled with the inefficiency of modern medicine and the high cost of treatment have led some patients to use traditional medicine based on the use of medicinal plant extracts which represent a large source of antioxidants and have very few side effects. For the treatment of hypertension, about 800 plants have been used worldwide.

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An ethnobotanical study conducted in Benin on medicinal plants revealed the use of *Lippia multiflora* for the treatment of hypertension (Gandonou *et al.,* 2017). *Lippia multiflora* is a plant used in traditional African medicine to treat malaria and high blood pressure (Koffi *et al.,* 1985, Noamessi *et al.,* 1985). The characteristic aroma of its leaves makes it consume as an infusion (Kanko *et al.,* 1995) reports that it is used as an antitussive, disinfectant, antipyretic and diuretic.In Benin, traditional healers provide health coverage of the population especially at the rural level, traditional medicine responds better to our needs if it has easier access for our populations (WHO *et al.,* 2002). It is in the context of contributing to the study of traditional treatment of arterial hypertension from a plant that different parts of *Lippia multiflora* have been used such as leaf, bark or root. According to (Agnaniet *et al.,* 2005), this plant has antioxidant properties that could justify its use in the treatment of hypertension. (Tokoudagba *et al.,* 2012) noted a risk of toxicity when the extracts were used for a long time.

Lippia multiflora has also been used in decoction forms for the treatment of liver diseases (jaundice, liver failure, early cirrhosis, inflammation of the gall bladder), lack of appetite, anemia, heart weakness, dizziness, palpitations, nervous weakness, melancholy, physical and intellectual overwork, malignant access to malaria, chills, flu, asthma, hypotension, nervousness of menopause, severe jaundice, sleeping sickness, chronic ulcers, colds, headaches, epilepsy, oral and digestive candidiasis. Scientifically, the plant is reported to have insecticidal and pesticidal properties against body parasites, Otadimeyi *et al.,* 2000), Bassolé *et al.,* 2003), Owolabi *et al.,* 2009), have shown that it has remarkable antimicrobial and antiviral activity. The objective of the present study is to evaluate the antiradical power of the aqueous and hydro-ethanolic extracts of *Lippia multiflora* leaves and to quantify the chemical groups responsible.

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 hills. Specifically, the survey was made in the towns of Kétou for the department of Plateau; Houéyogbe for the department of Mono; Bohicon and Djidja for the Zou and Savalou for the hills. A specimen of the plant is deposited and authenticated at the national herbarium of the University of Abomey-Calavi (Benin). The leaf was air-dried in the laboratory before spraying with an electric grinder.

Methods

Preparation of raw extracts

The aqueous-ethanolic and aqueous extracts were prepared for each of the four samples from the four cities according to standard techniques (Houngbeme *et al.,* 2014) .For this, 50 g of powder are dissolved in 500 ml of solvent (water- ethanol (4: 6, V / V) for the hydro-ethanol extracts). The mixture is left stirring continuously for seventy-two hours (72 hours) and the macerate obtained is filtered successively on hydrophilic cotton 3 times. The filtrate was then evaporated to dryness at 40 ° C. using a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300). For the aqueous decoction, 50 g of powder are introduced into 500 ml of distilled water. The whole contained in a flask is brought to moderate boiling on an electric plate for 15 minutes. The mixture obtained is filtered and evaporated to dryness, and then weighed for yield determination according to the relation:
Yield = (mass of dry extract) / (test sample mass) X 100

Evaluation of the potential for antiradical extracts: The antiradical activity was determined by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) using the procedure described by Lamien-Medard *et al.,* 2008). This method is based on the reduction of the stable free radical DPPH by an H radical donor. The DPPH is a stable radical which has in solution a characteristic absorption at 517 nm giving it a purple coloration. This color disappears rapidly when the DPPH is reduced by a free radical scavenger (antioxidant), to give the DPPH.H reduced form (Molineux *et al.,* 2004). The reduction reaction of DPPH is according to the equation below:

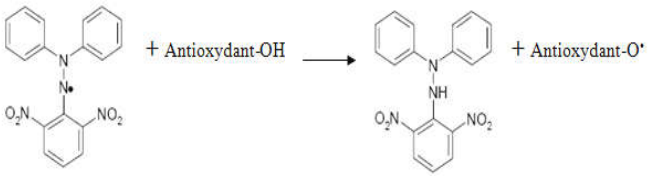

Figure 1. Diagram of the reaction of an antioxidant with the radical DPPH

The antiradical activity of the extract was determined using a calibration curve established with Ascorbic Acid (0-10mg / mL). Each test is made in triplets. The antiradical activity is expressed in mmol Equivalent Ascorbic acid per gram of extract (mmolEAA / g)

Quantification of compounds with phenolic structure

Determination of total phenols: Quantification of total phenols was performed according to the standard method (Singleton *et al.,* 1998). This method consists of 2 steps. On the one hand, a mixture of 125 µl of the extract solution and 625 µl of the Folin Ciocalteu reagent was incubated for 5 min at room temperature (25 ° C.). For the second step, 625 µL of the 75 g / L solution of Na2CO3 was added to the previous mixture, and incubated again for 2 h. Then the absorption of the mixture was measured at 760 nm using a spectrophotometer (Biomate3). Phenolic contents were determined using the calibration curve of gallic acid (0-100 mg / mL) used as a standard. The total phenol content is expressed in milligram equivalent of gallic acid per 100 mg of extract used.

Determination of total flavonoids: Total flavonoids were estimated by standard methods (Zhishen *et al.,* 1999 and Kim *et al.,* 2003). The procedure is to add 0.5 mL of 2% AlCl3 solution to 0.5 mL of the analyzed extract at 0.1 mg / mL. After 10 min of incubation, the optical density (OD) is read at 415 nm using a spectrophotometer (Biomate3). Calibration was performed with rutin in the 0-200 mg / mL concentration range.

Dosage of condensed tannins: The determination of condensed tannins was performed according to the method used by Broadhurst *et al.,* 1978) modified by Heimler *et al.,* 2006). The Vanillin solution is prepared by dissolving 4 g of Vanillin in 100 ml of methanol and that of the catechin is prepared from 20 mg of catechin in 4 ml of methanol, 3 mL of a methanolic solution of 4% vanillin and 1.5 mL of concentrated hydrochloric acid were added to 400 µL of each sample or standard. The mixture is incubated for 15 min and the absorbance read at 500 nm using a spectrophotometer (Biomate3). Concentrations of condensed tannins were deduced from calibration ranges established with catechin following the concentration range (0-300 µg / mL) and expressed in microgram equivalent of catechin per milligram of extract.

Statistical analysis

All data obtained were subjected to a statistical analysis using Minitab software version 1.0. The results were averaged with the Graph Pad Prism software. The differences between the means were recorded using the Kruskal-Wallis test. The results were considered statistically significant for $p < 0.05$

RESULTS AND DISCUSSION

Yield of extractions: The yield of preparation of the crude extracts is summarized in Table 1 below as a percentage of dry residue obtained from 100 g of powder.

Table 1. Summary of extraction yields

Harvesting area	Extraction yields (%)	
	Aqueous extract	Water-ethanolic extract
Savalou	10,58	24,77
Houeyogbe	14,94	15,01
Djidja	23,54	19,77
Ketou	7,24	11,54

It is noted that the yield is better when the extraction is made with the water-ethanol mixture and this independently of the harvesting zone, with the exception of the Djidja sample. The mixture of water and alcohol allows to extract most of the chemical principles of this plant. This result is consistent with that obtained by Muhammad *et al.,* 2012).

Antiradical activity of extracts: The various levels of antioxidant substances in the extracts are presented below in the form of a histogram (Figure 2) making it possible to see the difference in antiradical activities of the extracts according to the harvesting zones.

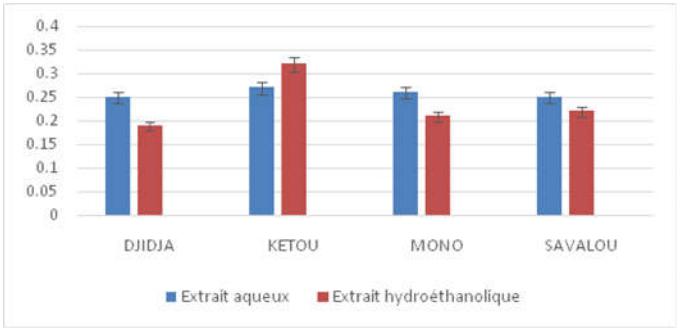

Figure 2. Antioxidant content of *Lippia multiflora* extracts from different regions

The results show regardless of the harvest area, all extracts possess antioxidant power. But it is the extracts of the plant from Ketou which reveal the strongest antioxidant activities with 375.47 ± 26.195mmolEqAA/g for the hydro-ethanolic extract, and 250.25 ± 10.6 mmolEqAA/g for the aqueous extract. For other cities, it is the aqueous extract that has a higher antioxidant activity, value determined from the calibration curve (Figure 3) established with ascorbic acid which is the reference antioxidant. The values are the average of three repetitions ± standard deviation. These results provide evidence that *Lippia multiflora* leaves are useful for treating various diseases caused by free radicals. The plant would be useful as a scavenger of free radicals and thus help in the treatment of many diseases caused by reactive oxygen species. These pathologies are among others aging, inflammation, cancer, atherosclerosis, diabetes. The entrapment action of the constituents of the plant has been shown to be related to polyphenolic compounds (Edamatsu *et al.,* 1989, Tani *et al.,* 1985).

Content of extracts of polyphenolic compounds: Using the absorbance of the molecules, certain families of compounds of therapeutic interest have been quantified to justify the antiradical activity exhibited by the extracts.

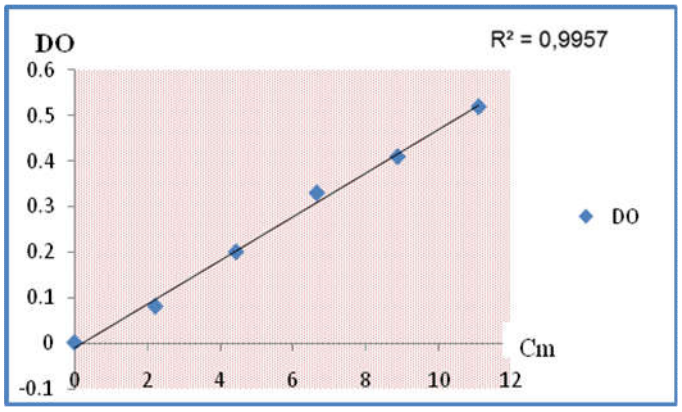

Figure 3. Ascorbic acid calibration curve for determination of antiradical activity

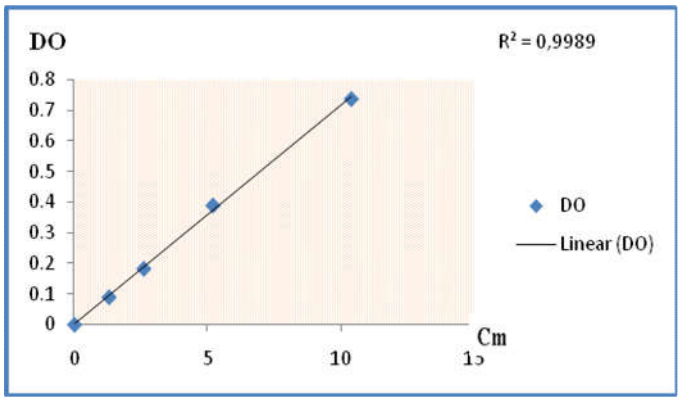

Figure 4. Gallic acid calibration curve for determination of total phenols

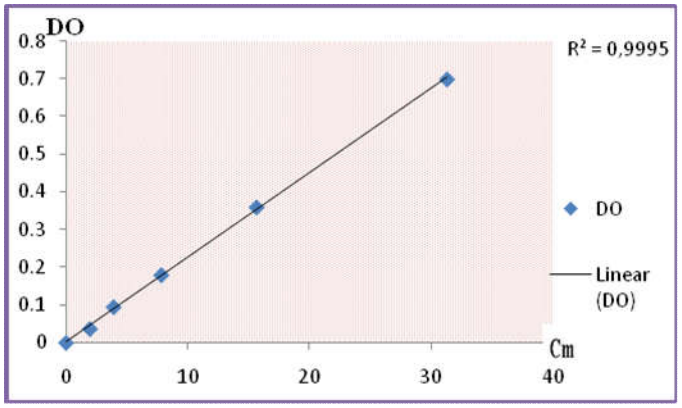

Figure 5. Rutin calibration curve for flavonoid determination

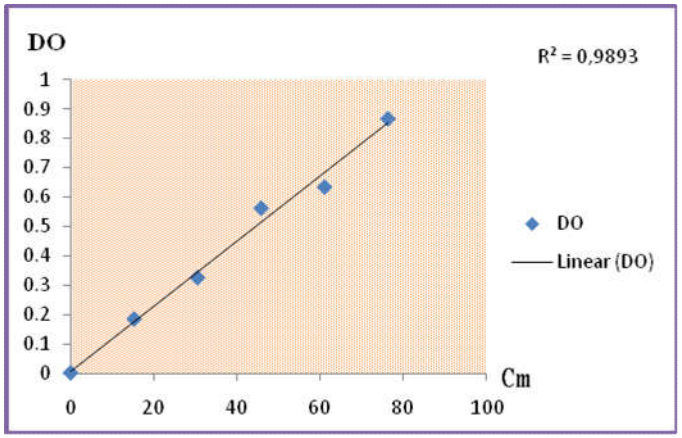

Figure 6. Catechin calibration curve for tannins determination

Table 2. Content of extracts of phenolic compounds, flavonoids and condensed tannins

Zone	Extrait	Teneur en phénols totaux (mgéq acide gallique/mg)	Teneur en Flavonoïdes (mgéq rutine/mg)	Teneur en tanins condensés (mgéq cathéchine/mg)
	Extrait aqueux	409,52±12,10[b]	0,212±0,002[d]	0,031±0,001
DJIDJA	Extrait hydro-éthanolique	957,90±2,42[a]	0,338±0,001[c]	0,009±0,001
	Extrait aqueux	335,32±2,42[b]	0,197±0,000[d]	0,030±0,001
MONO	Extrait hydro-éthanolique	396,61±7,26[a]	0,108±0,000	0,006±0,001
	Extrait aqueux	104,68±4,03[b]	0,038±0,000[e]	0,002±0,001
SAVALOU	Extrait hydro-éthanolique	199,03±11,29[a]	0,048±0,001[e]	0,009±0,001
	Extrait aqueux	417,58±7,26[b]	0,176±0,001[d]	0,002±0,002
KETOU	Extrait hydro-éthanolique	489,35±11,29[a]	0,248±0,001[c]	0,014±0,001

In a column, values with different letters are significantly different

Table 2 shows the contents of the extracts, respectively total phenols, flavonoids and condensed tannins, determined from the linear regression equations of each calibration curve (Figs. 4, 5 and 6). The quantification results showed that the hydro-ethanolic extracts mainly contain these chemical groups irrespective of the crop area of the plant. The major constituents of the extracts are the total phenols with a content of 957.90 ± 2.42 mg equivalents of gallic acid / mg of ethanolic extract of the DJIDJA plant. Our results are in agreement with the result of (Algeria et al, 2011) which reported that the aqueous extract of *Lippia multiflora* has a content of about 7.36 ± 0.86 mg eq cat / g of extract. In addition, the values found are very low for flavonoids and tannins compared with total phenols; we could then say that the demonstrated antiradical activity is mainly due to the total phenols but reinforced by the weak presence of flavonoids and condensed tannins. In addition, the essential oil of the plant harvested in Kétou reveals the highest content of flavonoids and total phenols.

Conclusion

This study indicates that aqueous-ethanolic and aqueous extracts of *L. multiflora* leaves exert an antiradical activity. It is important to mention that the hydro-ethanolic extract gave the most interesting results. These results confirm the traditional use of leaf extracts of *L. multiflora* for the control of arterial hypertension. Future research should be conducted to study the antihypertensive activity of the plant through the active extracts and isolate the active ingredients responsible for this activity.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: All the authors participed in writing, giving feedback on the manuscript, have read and approved the final manuscript.

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