World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* EVALUATION OF ANTIOXIDANT AND ANTICOAGULANT ACTIVITIES OF *LIPPIA MULTIFLORA* MOLDENKE LEAVES

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Article Received on 25/06/2022 **Article Revised on 15/07/2022** **Article Accepted on 05/08/2022**

ABSTRACT

Objective: This study aimed to evaluate the phytochemical composition and *in vitro* antioxidant and anticoagulant properties of *Lippia multiflora* Moldenke leaves. **Methods**: Decoction and ethanol extract of leaves of *L. multiflora* were tested for their chemical composition, radical scavenging activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and spectrophotometry methods, and anticoagulant activities (estimated by PT, APTT and fibrinogen values). **Results**: The phytochemical screening result showed that *L. multiflora* leaves contain alkaloids, tannins, flavonoids, and reducing sugars. All preparations have free-radical scavenging activities. Spectrophotometry analysis results showed that the lowest IC50 value was F3 ethanol fraction, with an IC50 value of 1.83 µg/ml ppm. The highest IC50 value was the F1 fraction, at 22.32 µg/ml ppm. All extracts caused an increase in TP, APTT, and a decrease in fibrinogen. **Conclusion**: All extracts of *L. multiflora* leaves possess considerable antioxidant activity and are rich in total polyphenols and flavonoids. They also act in the process of coagulation. This study shows that *L. multiflora* leaves may have the potential to be developed as a medicinal drug.

KEYWORDS: *Lippia multiflora*, antioxidant, anticoagulant activities.

INTRODUCTION

Medicinal plants are a vital resource for the majority of people living in Africa and are the main means by which people heal themselves.[1,2] According to the World Health Organization (WHO), more than 80% of the population in Africa resorts to traditional medicine to solve the problem of primary health. Plants have always been part of daily human life and are used for various purposes; food, health, and clothing, etc.[3,4] *Lippia multiflora* Moldenke (Verbenaceae) is one of those widely used plants. It's a widely distributed herbaceous plant in West and Central Africa. *L. multiflora* (LM) is an aromatic plant whose leaves and flowers are known to have the ability to treat several diseases, including malaria, fever, dysentery, high blood pressure, diarrhea, anemia, headache, constipation, fatigue, diabetes, ulcers, and hemorrhoids.[5,6,7,8] The multiple medicinal virtues of *L. multiflora* can be explained by its rich phytochemical composition.[9,10,11] Despite their incontestable efficacy in the treatment of venous thromboembolic diseases, current treatments with anticoagulants, present many limitations.[12] Non-steroidal anti-inflammatory drugs and heparins, administered only by injection, have been extensively documented by numerous clinical trials.[13,14] There is a need, therefore, to discover new anticoagulants that will have no undesirable effects. Medicinal plants are believed to be an important source for the discovery of potential anti-inflammatory and anticoagulant substances. Several plant extracts and different classes of phytochemicals have been investigated and shown potential anti-inflammatory and anticoagulant activity.[15]

Indeed, phytochemical data show that *L. multiflora* contains tannins, flavonoids, peptide saponins, caffeine, terpenes, and alkaloids.[16,17,18] Literature data reports that polyphenols and flavonoids possess anticoagulant and antithrombotic properties.[19]

The objectives of this study were to determine the chemical composition of *L. multiflora* leaves extract and its anticoagulant effects.

MATERIALS AND METHODS

Plant material

The leaves of the species *L. multiflora Moldenke* (Figure 1), purchased at the large market of Dolisie "department of Niari", were harvested between June and August 2021, according to information obtained from sellers. It was identified by the botanical expert of the National Institute of Health Sciences of Brazzaville.

Figure 1: *L. multiflora* Moldenke leaves (a) and its commercial form (b)

Preparation of the decoction

The dry leaves of L. multiflora were pulverized using a metal mortar. Then 100 g of *L. multiflora* leaf powder was introduced into 1000 ml of distilled water and boiled for about 30 minutes. After cooling, the mixture was filtered through cotton. The rotavapor was used to concentrate the decoction obtained. The dry extract obtained was used for the pharmacological analysis after dilution in distilled water (Figure 2).

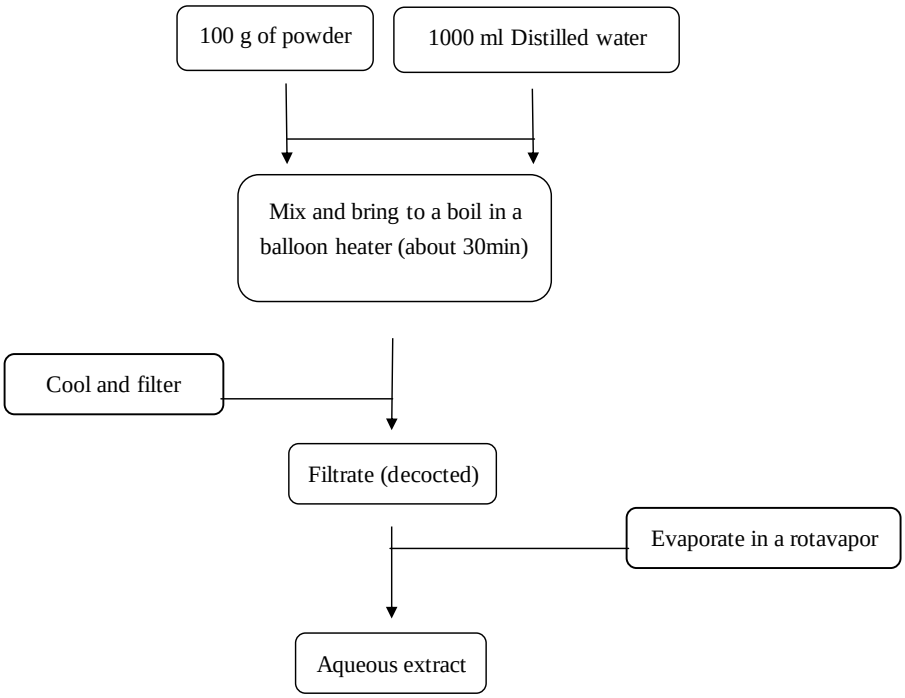

Figure 2: Summary of the steps for obtaining the decoction.

Fractionation of the aqueous extract

The resulting decoction was evaporated on a rotavapor to obtain an aqueous soluble extract. From this aqueous soluble extract, the fractions were obtained. The elution was carried out with a water-ethanol mixture of increasing polarity, in the following proportions: 100%

distilled H_2O, EtOH-H_2O (30:70 v/v), EtOH-H2O (70:30 v/v), and 100% EtOH. Indeed, the aqueous extract was chromatographed on an open column of Polyamide 6 (Fluka) 1.5 mm in diameter and 50 cm long.

Chemical analysis

The main chemical families (alkaloids, tannins, flavonoids, reducing sugars, and saponosides) were searched using tube tests.

Identification of alkaloids

5 ml of aqueous extract of *Lippia multiflora* was placed in a test tube. Then 1 ml of 1N hydrochloric acid and a few drops of reagents were added. The formation of a red precipitate (with Dragendorff's reagent) or a yellowish precipitate (with Mayer's reagent) indicates the presence of alkaloids.[20]

Identification of tannins

Tannins were identified by mixing 5 ml of an aqueous decoction of *Lippia multiflora* with 1 ml of an aqueous solution of iron chloride. In the presence of tannins, a greenish or blue-blackish color develops.[20]

Identification of flavonoids

5 ml of 5% decoction of *Lippia multiflora*, 5 ml of a solution of hydrochloric acid (HCL), 1 ml of iso-amyl alcohol, and a few shavings of magnesium were mixed. A color change indicates the presence of the following compounds[20]:

-Orange or yellow, for the flavones;
-Purplish pink, for the flavanones;
-Red for flavonols and flavanols.

Reduced sugar test.

To 5 ml of decoction of *Lippia multiflora* was added 1 ml of Fehling's liqueur. The formation of a brick-red precipitate indicates the presence of reducing sugars.[20]

Identification of saponins

5ml of aqueous extract of *Lippia multiflora* was mixed with 5ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was taken as an indication of the presence of saponins.[20]

Assay of total polyphenols (PPT).

The principle is based on the determination of the optical densities of the extracts compared to those of a standard solution of gallic acid of known concentration. The PPT test was performed using a spectrophotometer. To 0.1 ml of Lippia multiflora extract was added 0.9 ml of distilled water, 0.9 ml of Folin-Ciocalteu's reagent (1N), and immediately 0.2 ml of a solution of Na_2CO_3 (20%). The resulting mixture was incubated at room temperature for 40 minutes, protected from light. The absorbance was then measured with a spectrophotometer at 725 nm against a methanol solution used as a blank. It should be noted that a calibration straight line has been carried out beforehand before analysis with gallic acid under the same conditions as the samples to be analyzed. The results obtained were expressed in mg of gallic acid equivalent per gram of dry matter (mgEAG/gMs).[21]

Assay of total flavonoids

In a test tube, 250 µl of the extract and 1 ml of distilled water were introduced. At the initial time (0 minutes), 75 µl of a NaNO2 solution (5%) was added, followed by 75 µl of AlCl3 (10%) 5 minutes later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water are successfully added to the mixture. The absorbance of the mixture obtained was measured with a UV-visible spectrophotometer at 510 nm and the results were expressed in mg of rutin equivalent per 100 grams of dry matter (mgERu/100g Ms).[22]

Dosage of antiradical compounds

Test solutions containing 50, 100, and 200 µg/mL of *Lippia multiflora* in methanol were prepared and 0.3 ml of the solution was mixed with 3 ml of a mixture of H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes containing the reaction solutions were incubated at 95◦C for 90 minutes and then cooled to ambient temperature. The absorbance of the resulting solution was measured at 695 nm against a blank. A methanol solution (0.3 mL) was used as a control. Antioxidant activity was expressed as the number of grams equivalent to ascorbic acid. Antioxidant activity values were represented in standard graphs plotted with a DO of the standard compared to the different concentrations of ascorbic acid (10, 25, 50, 100, 250, and 500 µg/mL) treated similarly (Senguttuvan et al., 2014).

DPPH radical scavenging assay

The free radical scavenging property of the extracts was proven by the DPPH radical scavenging test.[22] The hydrogen atom donor capacity of the plant extracts was determined by the decolorization of the methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrasyl), which produces a violet/violet color in the methanolic solution and fades to shades of yellow in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 ml of this solution was mixed with 1.6 ml of the methanol extract at different concentrations (12.5 to 150 µg/mL). The reaction mixture was vortexed well and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The percentage of DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A0 - A1)/A0\} \times 100$$

Evaluation of anticoagulant activity

Blood donors

Blood samples were taken from 10 healthy volunteers (5 men and 5 women, with an average age of 24.9 ± 8.2 years), without chronic disease and not treated with medication. The volunteers did not use any special diets (vegetarian or vegan, etc.), or take salicylic acid or its derivatives, or nonsteroidal anti-inflammatory drugs for

at least 2 weeks. They were also non-smokers. All subjects gave their informed consent to inclusion before participating in the study. The study was carried out in accordance with the guidelines of the Declaration of Helsinki for Human Rights.

Preparation of a pool of plasma

Each blood sample was subjected to double centrifugation at 2500 revolutions for 15 minutes, to obtain platelet-poor plasma (PPP). A platelet-poor plasma pool was made by mixing all the PPPs.

Coagulation tests

200 µl of platelet-poor plasma was incubated for 15 min at 37°C, with 200 µl of distilled water, the pure decoction of *Lippia multiflora* leaves, dilutions 1/2, 1/4, polyphenolic fractions F1, F2, F3 and acetylsalicylic acid, respectively, before performing the analyses.

Human plasma clotting assays were performed. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen assays were performed according to the diagnostic kit manufacturer's instructions. The coagulation tests were carried out with a Helena C II optical coagulometer.

Statistical analysis

The qualitative variables were expressed as a percentage and the quantitative variables as a mean ± standard error on the mean. The results were analyzed using an ANOVA test followed by Dunnett's test on Prism version 8 Graphpad software. The retained significance level was $p \leq 0.05$. The comparison of the means was carried out by the Anova test.

RESULTS AND DISCUSSION

Chemical screening

The results of qualitative tests of the sample extracts were performed to detect the presence of various phytochemicals. The results are shown in Table I. These results revealed the presence of alkaloids, tannins, flavonoids, and reducing sugars. The appearance of the tube reactions is shown in Figure 3.

Table I: main phytochemical families of the decoction of the leaves of *Lippia multiflora*.

Test/Solvent	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Reducing sugars	+
Terpenoids	+
Saponins	-

+: positive result (presence); -: negative result (absence)

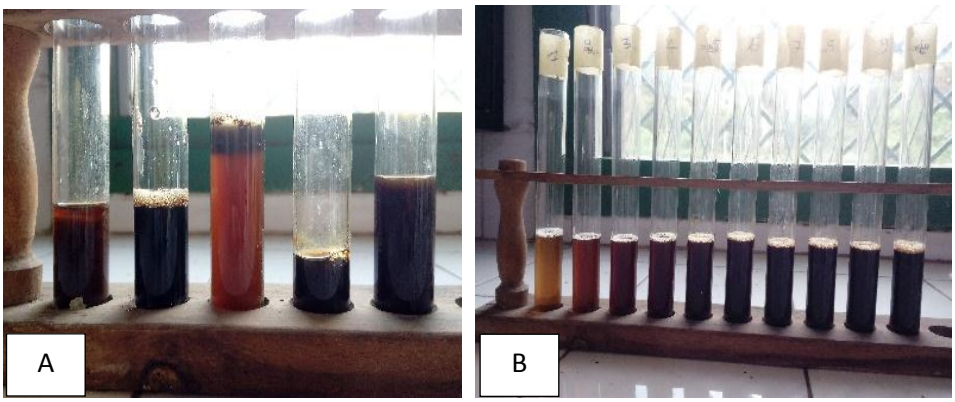

Figure 3: Chemical screening: A (alkaloids, tannins, flavonoids, reducing sugars, control, respectively) and B (saponosides).

Assays of polyphenols and total flavonoids

The results of the determination of the concentrations of polyphenols and flavonoids are presented in Table II. The analysis of these results shows high concentrations of polyphenols, particularly in the fractions F2 (37.02 µg/mL) and F3 (38.13 µg/mL).

Table II: the content of extracts of *Lippia multiflora leaves* in flavonoids and polyphenols.

Products	Polyphenols (mgEAG/gMS)	Flavonoids (mgEAG/gMS)
Aq extract	1.344	0.644
F1	12.348	1.6744
F2	37.023	3.836
F3	38,136	6.3056
F4	13.86	3.62

Scavenging activity

The study of the antiradical activity showed inhibitory concentrations of 22.16 µg/ml, 22.32 µg/ml, 3 µg/mL, 1.83 µg/ml and 6.59 µg/mL respectively, for the aqueous extract of *L. multiflora*, F1, F2, F3 and F4 fractions (Figure 4).

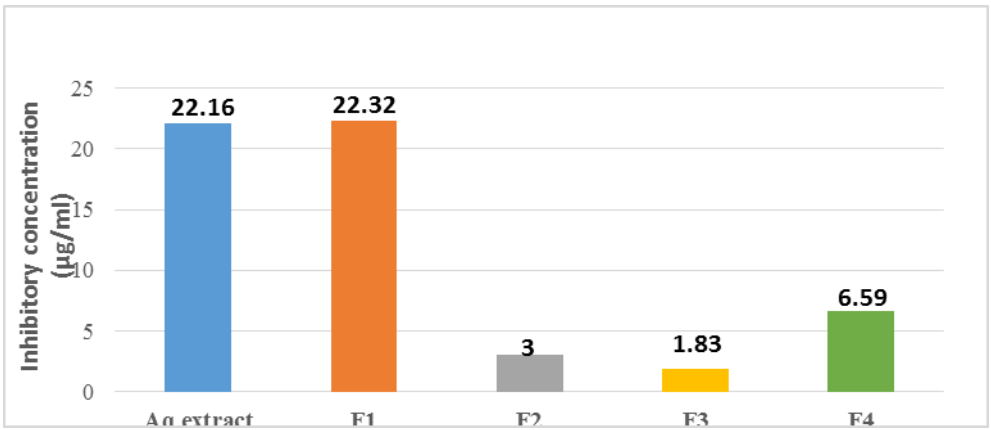

Figure 4*: free-radical scavenging activity of the extract and the fractions. Aq : aqueous and F : Fraction.*

Evaluation of anticoagulant activity

Effect of aqueous extract and fractions of *L. multiflora* leaves on PT

Figure 5 shows the results of the PT of the extracts studied, expressed as secondes.

Analysis of these results reveals a significant increase in PT values with all the extracts studied.

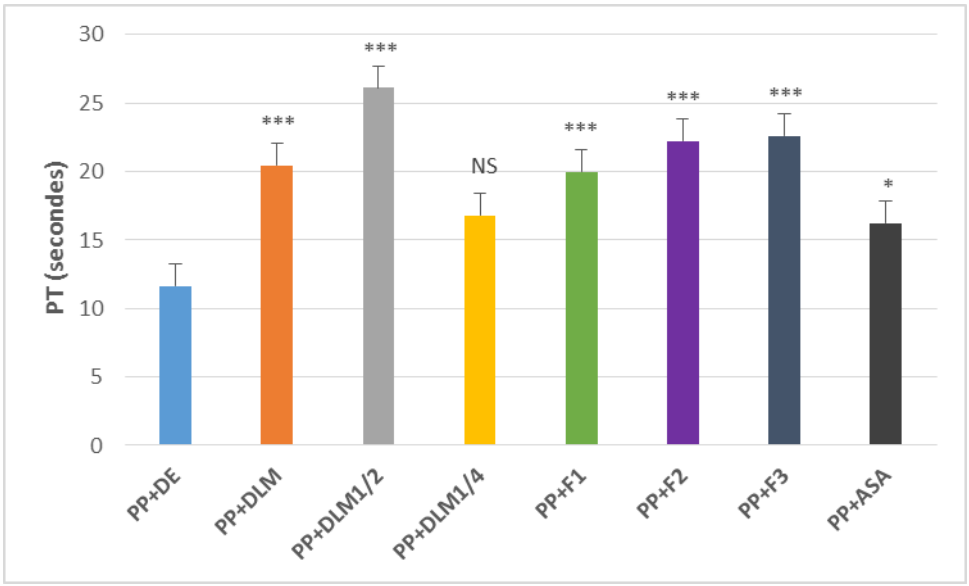

Figure 5: PT and INR values for different extract concentrations. Results are expressed as mean ± standard error. N=5; (*): p<0.005; (***): p<0.0001. ANOVA test followed by Dunnett's test. PT: prothrombin time; PP: plasma pool; DLM: Decoction of *Lippia multiflora*; F: fraction; ASA: Acetyl Salicylic Acid.

Effect of aqueous extract and fractions of *L. multiflora* leaves on APTT

The effects of pure decoctions, diluted to ¼ and ½, as well as the fractions (F1, F2, F3) on APTT are presented in Figure 6. Analysis of these results reveals a statistically significant increase in APTT with the different extracts studied.

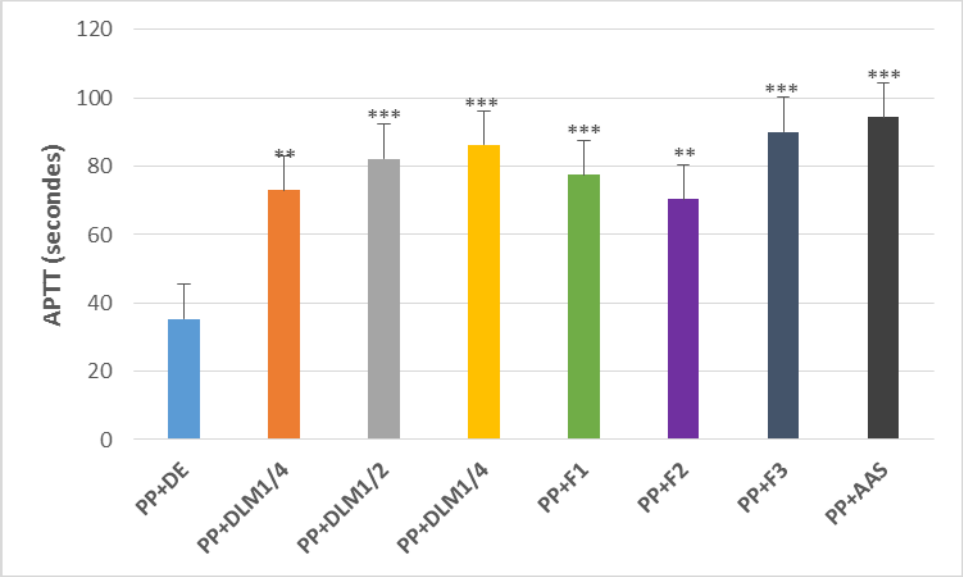

Figure 6: Effects of *L. multiflora* extracts on activated partial thromboplastin time in the presence of pooled plasma. Results are expressed as mean ± standard error. N=5; (*):p<0.03; (**): p<0.005; (***): p<0.0004; (NS): not significant. ANOVA test followed by Dunnett's test. PP: plasma pooled; DLM: Decoction of *L. multiflora*; F: fraction; ASA: Acetyl Salicylic Acid

Effect of aqueous extract and fractions of *L. multiflora* leaves on fibrinogen levels

Fibrinogen was used to estimate the total amount of prothrombin in the blood. The diagrams presented in Figure 7 shows the results of the fibrinogen level. Analysis of these results shows a significant decrease in fibrinogen level values with all the extracts studied.

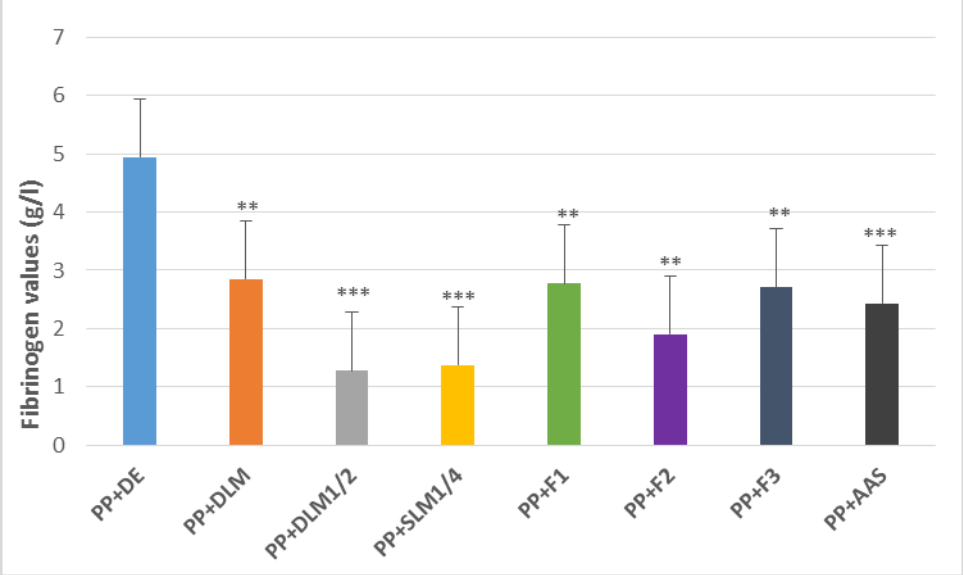

Figure 7: Effects of *L. multiflora* extracts on fibrinogen concentrations in the presence of pooled plasma. Results are expressed as mean ± standard error. N=5; (**): p<0.001; (***): p<0.0001; (NS): not significant. ANOVA test followed by test Dunnett.

Plants play an indispensable role in the discovery of natural products to meet the growing needs of populations in terms of health care. It's accepted that the pre-clinical evaluation of the antithrombotic potential of novel molecules requires the use of reliable and reproducible experimental models. PT, APTT, fibrinogen polymerization, and platelet aggregation are the most commonly used preparations to determine the efficacy of novel antithrombotic drugs.

The present study aimed to determine the phytochemical composition and evaluate the anticoagulant properties of the leaves of *Lippia multiflora*.

The chemical screening revealed the presence of alkaloids, tannins, flavonoids, and reducing sugars. However, we did not find saponosides. These results differ from those found by Gouollaly and al.[23], as well as Fulgence Yapo Allo and al.,[24] who reported the presence of saponosides. This difference could be explained by the geographical origin of the plant. The phytochemical composition of *Lippia multiflora* leaves could justify the main medicinal uses of this widely used plant.

The quantitative analysis of the total polyphenols and flavonoids showed that the polyphenols were found in a greater proportion than the flavonoids, whatever the extract was considered. Flavonoids are multifunctional bioactive compounds present in many plants whose consumption has been linked with the prevention of cardiovascular disease.[25] Several *in vitro* studies show that these components inhibit platelet aggregation, a major process contributing to both the development of atherosclerosis and acute platelet thrombus formation, followed by embolism-producing cyclic flow reduction in stenosed arteries.[25] The gradient of decreasing polarity used would favor a large number of fractions in phenolic compounds. The F3 fraction presented the highest concentrations of polyphenols. Analysis of antiradical activity revealed that the aqueous extract inhibits free radicals at a concentration of 22.16 µg/ml, compared to 1.83 µg/ml for the F4 fraction. Free radical activity is therefore associated with the polyphenol and flavonoid content. The lower IC value indicated the higher free radical scavenging activity of the sample plants with a low IC_{50} value may have the potential to be developed as a medicinal drug. The scientific literature reveals that polyphenolic compounds are known to exhibit significant free radical scavenging activities.[26] The antioxidant activity of polyphenols is due to their ability to scavenge free radicals, their potential to donate hydrogen atoms or electrons, and their power as metal chelators.[27] The position and the degree of hydroxylation are the determining factors of this activity.[28] Flavonoids inhibit the enzymes responsible for the production of superoxide anions, such as xanthine oxidase and protein kinase C. Flavonoids also inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase, which are all involved in the generation of reactive oxygen species.[29] The antiradical activity of the extracts is correlated with the content of polyphenols and flavonoids.

The anticoagulant properties of *L. multiflora* have been investigated by evaluating the effects on the prothrombin level (PT), the activator thromboplastin time (APTT), and the fibrinogen level of a protein-poor plasma pool. The results obtained show that the extracts of *L. multiflora* significantly increase the PT, APTT and decrease fibrinogen values. The mechanism of this phenomenon would be linked to the inhibition of the activity of thrombin. L. multiflora inhibits the proteolytic activity of thrombin, observed as inhibition of fibrinogen induced by thrombin polymerization, stabilized fibrin formation and platelet aggregation.[30]

The activity of different extracts of *L. multiflora* on the APTT (intrinsic pathway) and the PT (extrinsic pathway), could be explained by the presence of polyphenols (in large quantities) and flavonoids. Daily consumption of these compounds has been shown to inhibit platelet function.[31] Flavonoids have a real potential to be responsible for the anticoagulant activity observed, both by the significant presence in the most active extracts and fractions.[32] They are known to be anti-inflammatory and the inflammation pathway is closely linked to platelet aggregation and arachidonic acid coagulation. In addition, the latter (flavonoids), inhibit platelet adhesion, aggregation, and secretion, which activate blood coagulation through the release of mediators that act as triggers in the activation of the mechanical pathway of the signaling cascade.[33]

Narayana et al.,[34] reported the antithrombotic effect of flavonoids. This property is linked to their inhibitory effect by binding platelet cell receptors (ADP receptor and factor receptor von Willebrand).

CONCLUSION

This study focused on the phytochemical screening, antiradical, and anticoagulant activity of the leaves of *Lippia multiflora* Moldenke. It confirms the presence of some phytochemical compounds that are known to have medicinal properties, justifying the use of the plant in traditional medicine. The study also showed that leaf extract of *L. multiflora* increases PT, APTT and decreases fibrinogen levels in a pool of plasma. This anticoagulant activity could be attributed to the presence of polyphenols and flavonoids. However, other studies with more advanced methods might need to confirm these results.

Conflict of interest statement

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

Thanks at the Faculty of Health Sciences, particularly at the Biochemistry and Pharmacology Laboratory (Professeur Ange Antoine ABENA) for the award of the technical supplies.

Author Contributions: Conception LMM, JOM and CRDD; methodology, LMM, JOM, CRDD, GT and DGN; supervision, DM, EM, AAA; writing original design, JOM, AEMMM and CL; writing proofreading, LMM, JOM, and RBB; final revision, AAA. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Ngbolua KN, Shetonde OM, Mpiana PT, Inkoto LC, Masengo CA, Tshibangu DST, Gbolo ZB, Baholy R, Fatiany PR. Ethno-pharmacological survey and Ecological studies of some plants used in traditional medicine in Kinshasa city (Democratic Republic of the Congo). Tropical Plant Research, 2016a; 3: 413-27
2. Ngbolua KN, Mpiana PT, Mudogo V, Ngombe NK, Tshibangu DST, Ekutsu EG, Kabena ON, Gbolo BZ, Muanyishay L. Ethno-pharmacological survey and Floristical study of some Medicinal Plants traditionally used to treat infectious and parasitic pathologies in the Democratic Republic of Congo. International Journal of Medicinal Plants, 2014b; 106: 454- 67.
3. Gurib - Fakim Ameenah. Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. Molecular Aspects of Medicine, 2006; 27(1): 1-9.
4. Baba Aissa F. Encyclopedie des plantes utiles. Ed. Librairie moderne, Rouiba, 2000; 368 p.
5. Djengue, HW, Dansi, A, Adjatin, A, Dossou-Aminon, I, Dansi, M, Sanni, A. Ethnobotanical investigation of *Lippia multiflora* Moldenke, a local aromatic leafy vegetable under domestication in Benin. Int. J. Curr. Res. Biosci. Plant Biol, 2017; 4(5): 44-51. doi: https://doi.org/10.20546/ijcrbp.2017.405.005
6. Oladimeji FA, Orafidiya Lara O, Okeke IN. Physical properties and antimicrobial activities of leaf essential oil of Lippia multiflora Moldenke. The International Journal of Aromatherapy, 2004; 14: 162–8.
7. Abena AA, Diatewa M, Gakosso G, Gbeassor M, Hondi-Assah T, Ouamba JM. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Phytotherapy, 2003; 74: 231–6.
8. Pascual M.E., Slowing K, Carretero E, Sánchez Mata D, Villar A. Lippia: traditional uses, chemistry and pharmacology: a review. Journal of Ethnopharmacology, 2001; 76: 201-14.
9. Abena AA, Etou Ossibi AW, Gouolally Tsiba, Okemy Andissa N, Ouamba JM. Monographic study of *Lippia Multiflora* Moldenke (Verbenaceae). phytotherapy; Heidelberg, 2017; 15(1): 27-32.
10. Ahouansinkpo E, Atanasso J, Dansi A, Adjatin A, Orobiyi A, Sanni A. Ethnobotany, phytochemical screening and toxicity risk of *Cleome gynandra* and *Cleome viscosa*, two traditional leafy vegetables consumed in Benin. Int. J. Curr Microbiol App Ski, 2016; 5(2): 813-29.
11. Etou Ossibi AW, Elion Itou RDG, Nzonzi J, Nsonde Ntandou GF, Dimo T, Ouamba JM, Abena AA. Effects of aqueous extract of Lippia multiflora Moldenke (Verbenaceae) on blood pressure, heart rate and electrocardiogram waves in normotensive rats Revue CAMES – Série Pharm. Méd. Trad. Afr, 2014; 17(1): 1-9.
12. Tarik Khouya, Mhamed Ramchoun, Abdelbassat Hmidani, Souliman Amrani, Hicham Harnafifi, Mohamed Benlyas, Younes Filali Zegzouti, Chakib Alem Khouya T. Anti-inflflammatory, anticoagulant and antioxidant effects of aqueous extracts from Moroccan thyme varieties, Asian Pac J Trop Biomed, 2015; http://dx.doi.org/10.1016/j.apjtb.2015.05.011
13. Nazli Y, Çolak N, Colak S, Çakır O. Heparin induced thrombocytopenia. J Clin Exp Investig, 2014; 5(1): 137-44.
14. Coxib and traditional NSAID Trialists' (CNT) Collaboration, Bhala N, Emberson J, Merhi A, Abramson S, Arber N, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet, 2013; 382(9894): 769-79.
15. Stankovic N, Mladenovic M, Mihailovic M, Arambasic J, Uskokovi c A, Stankovic V, et al. Synthesis and toxicological studies of in vivo anticoagulant activity of novel 3-(1-aminoethylidene)chroman-2,4-diones and 4-hydroxy-3-(1-iminoethyl)-2H-chromen-2-ones combined with a structure-based 3-D pharmacophore model. Eur J Pharm Sci., 2014; 55: 20-35.
16. Jigam AA, Akanya HO, Ogbadoyi EO, Bukar ED, Egwim CE. In vivo antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippia multiflora* mold. J Med Plant Res., 2009; 3: 148–54.
17. Kunle OF, Jegede IA, Ibrahim H, Okogun JI. Pharmacognostic studies on the leaf of *Lippia multiflora* Moldenke. JOPAT, 2002; 7(1&2): 40-5.
18. Kunle O, Okogun J, Egamana E, Emojevwe E, Shok M. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. J. Phytomedicine, 2003; 10: 59-61.
19. Subramani Bhavani and Sathiyarajeswaran P. Current update on herbal sources of antithrombotic activity - a comprehensive review The Egyptian Journal of Internal Medicine, 2022; 34: 26. https://doi.org/10.1186/s43162-021-00090-9
20. Evans WC. In: Bailiere Tindall WB, editor. Trease and Evans Pharmacognosy. 14th ed. London: Sauders company ltd, 1996: 191–575.
21. Siddhurraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constutents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Chem*, *2003*; 51(8): 2144-55.
22. Senguttuvan Jamuna, Paulsamy Subramaniam, Karthika Krishnamoorthy Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities Asian Pac J Trop Biomed, 2014; 4(1): 359-367. doi:10.12980/APJTB.4.2014C1030
23. Gouollaly Tsiba. Phytochemical study and evaluation of antifungal, anticancer and antioxidant activities of extracts of domestic *Lippia multiflora*

moldenke. Thèse, Université Marien Ngouabi, 2010: 195p.
24. Allo Fulgence Yapo, Konan André Brou, Méité Souleymane, Datté Jacques Yao. Acute and Sub-acute Toxicity Studies of the Aqueous Leaf Extract of *Lippia multiflora*. Asian Journal of Emerging Research, 2020; 48-53. DOI: 10.21124/AJERPK.2020.43.53
25. Buck R, Pastore JJ, Giraud F, Sulpice J-C, Janmey PA. Flavonoid inhibition of platelet procoagulant activity and phosphoinositide synthesis. J Thromb Haemost, 2003; 1: 1820-8.
26. Hafid Achmad Fuad, Ismail, Wardiyanto Samuel, Tumewu Lidya, Rahman Abdul, Anti Aty Widyawaruy. Free-radical scavenging activity screening of some indonesian plants. Int J Pharm Pharm Sci, 2014; 6(6): 115-7.
27. Aberoumand A and Deokule SS. Comparison of Phenolic Compounds of Some Edible Plants of Iran and India. Pakistan Journal of Nutrition, 2008; 7(4): 582-5.
28. Jiménez-Atiénzar Mercedes, Escribano Josefa, Cabanes Juana, Herrero Fernando Gandía, Carmona Francisco García. Oxidation of the flavonoid eriodictyol by tyrosinase. Plant Physiology and Biochemistry, 2005; 43(9): 866-73.
29. Pietta PG. Flavonoids as Antioxidants. Journal of Natural Products, 2000; 63: 1035-42. http://dx.doi.org/10.1021/np9904509
30. Bijak Michał, Saluk Joanna, Ponczek Michał Błażej, Nowak. Antithrombin Effect of Polyphenol-Rich Extracts from Black Chokeberry and Grape Seeds, 2012; 27(1): 71-6. https://doi.org/10.1002/ptr.4682
31. Kale MA, Sha hi SR, Somani VG, Shamkuwar PB, Dhake A. Hemostatic activity of the leaves of *Tridax procumbens* Linn.International Journal of Green pharmacy (IJGP), 2008; 2(1). DOI: http://dx.doi.org/10.223 77//ijgp. V2i1,398.
32. Leite Paula Mendonça, Miranda Ana Paula, Nader Juliana Mendes Amorim, Letícia Barbosa Santos, Rita Carolina Figueiredo, Duarte Vinícius Gonçalves Maltarollo. Correlation of Chemical Composition and Anticoagulant Activity in Different Accessions of Brazilian *Lippia alba* (Verbenaceae). Journal of Herbal Medicine. *In press.*
33. Khan, H., Jawad, M., Kamal, M.A., Baldi, A., Xiao, J., Nabavi, S.M., Daglia, M., (2018). Evidence and prospective of plant derived flavonoids as antiplatelet agents : Strong candidates to be drugs of future. Food Chem. Toxicol, 2018; 119: 355-67. https://doi.org/10.1155/2019/7125162.
34. Narayana K, Sripal D Deddy, Chaluvadi, Krishna. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of Pharmacology, 2001; 33: 2-16.