Journal of Biosciences and Medicines, 2022, 10, 100-109
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Hypotensive Effects and Phytochemical Profile of Aqueous Extract and Fractions of *Desmodium velutinun* Leaves (Willd) D. C. (Fabaceae) in the Rat

Blondy Mboungou-Bouesse[1,2,3,4], Arnaud Wilfrid Etou Ossibi[1,3*], Elva Ngolo[1,2,3,4], Romaric De Garde Elion Itou[1,2,3], Béril Fabrice Mavouba[2,3], Dieu-Merci Bevel Gallo Mongo[2,3], Gouollaly Tsiba[2,3,4], Ange Antoine Abena[1]

[1]Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of Congo
[2]Department of Pharmacopeia and Traditional Medicine, National Institute of Health Science & Research, Delhi, India
[3]Laboratory of Chemistry, Organic Biomolecules and Pharmacodynamics, Science City (Ex-ORSTOM), Brazzaville, Republic of Congo
[4]Plant and Life Chemistry Unit, Faculty of Sciences and Techniques, Marien Ngouabi Univesity, Brazzaville, Republic of Congo
Email: *etouarnaud@yahoo.fr

How to cite this paper: Mboungou-Bouesse, B., Ossibi, A.W.E., Ngolo, E., Itou, R.D.G.E., Mavouba, B.F., Mongo, D.-M.B.G., Tsiba, G. and Abena, A.A. (2022) Hypotensive Effects and Phytochemical Profile of Aqueous Extract and Fractions of *Desmodium velutinun* Leaves (Willd) D. C. (Fabaceae) in the Rat. *Journal of Biosciences and Medicines*, **10**, 100-109.
https://doi.org/10.4236/jbm.2022.108009

Received: June 8, 2022
Accepted: August 7, 2022
Published: August 10, 2022

Abstract

Desmodium velutinum (*D. velutinum*) is a plant of the family Fabaceae used in traditional Congolese medicine for the treatment of high blood pressure (HBP) or hypertension (HT). The present work evaluated the hypotensive activity and determined the phytochemical profile of the aqueous extract and fractions of *Desmodium velutinun* (Willd) D. C. (Fabaceae) in the rat. The hypotensive effect of the aqueous extract and different fractions (F1, F2, F3 and F4) was evaluated at the dose of 2.5 mg/kg in normotensive rats anesthetized with 15% urethane at 1.5 g/kg, i.p. (intraperitoneally). Our results showed that the F3 fraction after administration caused a significant decrease in blood pressure and heart rate which remained higher than that of the aqueous extract and fractions (F1, F2 and F4). The richness in polyphenolic and flavonoid compounds as well as the separating capacity of the eluent used within the F3 fraction clearly justifies the beneficial effects of the latter on blood pressure and heart rate compared with the extract and fractions (F1, F2 and F4). In a second part we carried out the qualitative (thin-layer chromatography) and quantitative (total polyphenols and flavonoids determination) analysis. The chemical compounds remained dominated by the polyphenolic and flavonoid groups, the content of total polyphenols and total flavonoids meets the following in-

creasing order: (extract, F1, F2, F3 and F4). The results obtained could certify the use of *Desmodium velutinun* in traditional Congolese medicine.

Keywords

Hypotensive, Fraction, *Desmodium velutinun*, Phytochemistry

1. Introduction

The so called "non-communicable" diseases such as high blood pressure, diabetes and cancer are a serious threat to human health. The negative impact of this problem is increasing day by day with the evolution of modern societies. However, taking some antihypertensive drugs can reduce the blood pressure to too low values, below 120/70mmHg (hypotension) which reveals the risk of cardiovascular accident [1]. But also a too high pressure can lead to a hemorrhagic stroke due to a break in the vessels. Among the causes of death in France in 2008 published by Inserme, cardiovascular diseases as a whole are the second leading medical cause of death after cancers. They are unevenly distributed across continents and countries. Thus, 15% of the French population, 20% of the American population and 1% of the Chinese population are affected. Africa has the highest prevalence rate in the world, with 46% of adults over the age of 25 associated with other comorbidities, hence the high rate of mortality [2]. In the African region, 20 million people would be affected, its estimated prevalence rate, also varies by country: 15% in Algeria, 30% in Mauritius and Seychelles, 20% - 35% in Gabon, 9.5% in Gambia. In Congo-Brazzaville, the teaching hospital in Brazzaville records 26% of cerebrovascular accident (CVA) or strokes, according to statistics provided by the neurology department. Strokes are a real public health problem, more important than infectious diseases in adults. The prohibitive cost of certain drugs, especially for populations in poor countries who have difficulty accessing so called modern drugs, directs victims to traditional remedies. The application of extracts made from medicinal plants would thus be an encouraging lead in the fight against certain diseases. This is why the WHO is encouraging the intensification of research into new ways of combating these pathologies, based on those that use treatments based on medicinal plants (phytotherapy and pharmacognosy) [3]. The aim of this present work was to evaluate the hypotensive effect and determine the phytochemical profile of the aqueous extract and fractions of *Desmodium velutinun* (Willd) D. C. (Fabaceae) in rats.

2. Materials and Methods

2.1. Plant Material

In the present study, *Desmodium velutinum* (*D. velutinum*) leaves were used. These leaves were harvested from the village SOSSI, in the department of Niari in the month of November 2018. The samples of these leaves were identified at

the Institut national de Recherche en Sciences Exactes et Naturelles (IRSEN) at the national herbarium service at number 636. These samples were then dried at the laboratory of the Institut National de Recherche en Sciences de la Santé (IRSSA) for 12 days in the shade.

2.2. Animal Material

The animal material was consisted of wistar rats (males and females) aged 16 to 20 weeks and weighing between 250 and 270 g. These animals were raised in the animal house of the Faculty of Sciences and Techniques of Marien Ngouabi University at an ambient temperature of 27˚C ± 1˚C with free access to food and tap water, under a cycle of 12 hours of light and 12 hours of darkness.

2.3. Preparation of Fractions

The hydroethanol extract of *D. velutinum* leaves was fractionated in a bioguided manner. For this purpose, 1 g of the hydroethanolic extract of *D. velutinum* leaves was chromatographed on an open column of Polyamide 6 (Fluka) of 1.5 mm diameter and 50 cm long. Elution was carried out with a water ethanol mixture of decreasing polarity, in the proportions: 100% H_2O, EtOH-H_2O (30:70 v/v), EtOH-H_2O (70:30 v/v), and 100% EtOH.

2.4. Experimental Setup and Recording Technique for Blood Pressure

The blood pressure recording of the animals was done in this study following the invasive method after anesthetizing the rats with urethane 15% at 1 ml/100g. Blood pressure was assessed by the invasive method (bloody) or direct method. The technique consists in recording the blood pressure using a catheter introduced into the carotid artery of the anesthetized rat [4]. The catheter is linked to the transducer connected to the hemodynamic recorder Biopac Student Lab M35-type to visualize the different tracings recorded on the computer screen. After a period of stabilization of the cardiovascular parameters of approximately 30 minutes, all the substances were administered through the catheter attached to a syringe at the level of the femoral vein of the rat. These substances, dissolved in 0.9% NaCl solution, were administered at a rate of 0.1 ml/100g rat body weight [5].

2.5. Evaluation of the Hypotensive Effects of the Aqueous Extract and the Different Fractions of *D. velutinum* in Rats

15 normotensive rats were divided into 5 lots of 3 rats each, treated as follows:

- Lot 1 received the aqueous extract of the leaves of *D. velutinum* at a rate of 2.5/mg/kg per i.v (intravenous);
- Lots 2, 3, 4 and 5 received the different fractions of *D. velutinum* (F1, F2, F3 and F4) at a dose of 2.5 mg/kg, per i.v respectively.

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured for one hour during the experiment.

2.6. Statistical Analysis

The values expressed in the figures are the means of a series of values plus or minus Standard Error of the Mean (SEM). These values were calculated using Excel. The comparison of the means of the two series of values was done using the t-Student test. The significance threshold was set at $p < 0.05$.

2.7. Thin-Layer Chromatography (TLC)

Phenolic compounds can be characterized on the thin-layer chromatographic method (TLC). A small quantity of the mixture to be separated is deposited on the stationary phase and this phase is put in contact with the mobile phase (eluent). The mobile phase migrates upwards, by capillary action, along the fixed phase, carrying the components of the mixture. This is the phenomenon of elution, which allows the separation of the components of the mixture to be analyzed [6]. Each component migrates from a certain height (different components migrate at different speeds), characteristics of the substance, which is called frontal ratio or frontal retention (fr).

2.8. Determination of Polyphenols

The determination of total polyphenols was performed using a spectrophotometer. We determined the optical densities of our extract and fractions and compared them to that obtained by a gallic acid standard of known concentration. The extract and fractions were assayed as follows: to 0.1 ml of extract and fractions introduced in different test tubes, were added 0.9 ml of distilled water; 0.9 ml of Folin Ciocalteu reagent (1N); then immediately 0.2 ml of a $2CO_3$ solution (20%). The resulting mixture was incubated at room temperature for 40 minutes in the dark. The absorbance was then measured with a spectrophotometer at 725 nm against an ethanol solution used as the blank. Note that a calibration line was previously performed before the analysis with gallic acid under the same conditions as the samples to be analyzed [7]. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgEa/100gMs).

2.9. Determination of Total Flavonoids

Total flavonoids were also assayed using a spectrophotometer, as follows: 250 µl of the extract and fractions were placed in different test tubes, 1 ml of distilled water was successively introduced into each test tube. At the initial time (0 minute), 75 µl of a $NaNO_2$ (5%) was added, followed by 75 µl of $AlCl_3$ (10%) 5 minutes later. 6 minutes later, 500 µl l of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the resulting mixture was directly measured by UV-visible spectrophotometer at 510 nm and the results were expressed as mg quercetin equivalent per 100 grams of dry matter (mgEct/100g Ms). A calibration curve was developed with standard solutions of quercetin prepared at different concentrations.

3. Results

3.1. Hypotensive Effect of Aqueous Extract and Fractions of *D. velutinum* in Normotensive Rats

Intravenous administration of the aqueous extract (2.5 mg/kg) and the different fractions (F1, F2, F3, and F4) at the dose of 2.5mg/kg caused an immediate decrease in diastolic blood pressure (DBP), about 9 seconds after their administration [8] (**Figure 1**), and the respective decrease in DBP of −47.60% ± 0.52%; −27.59% ± 5.86%; −23.72% ± 2.74%; − 49.99% ± 0.75% and −26.88% ± 3.44% ($p < 0.001$) (extract, F1, F2, F3, and F4). This decrease was followed by a slight rise in DBP at the 5th minute not exceeding the initial value, followed by a progressive decrease in DBP until the 60th minute. Regarding systolic blood pressure (SBP), the extract and fractions caused an abrupt decrease in SBP about 9 seconds after their administration at the same dose (**Figure 2**), the decrease in SBP is −23.28% ± 0.65%; −11.99% ± 0.16%; −12.38% ± 0.94%; −56.03% ± 0.23% et −24.37% ± 0.39% ($p < 0.001$) for extract, F1, F2, F3, and F4, respectively. This decrease was followed by a slight rise in DBP at the 5th minute not exceeding the initial value except for the F1 fraction, followed by a progressive decrease in SBP until the 60th minute.

Concerning heart rate (HR), at the same dose, the F2 and F4 fractions caused a significant decrease of −9.71% ± 0.09%; et −9.98% ± 0.16%; ($p < 0.01$) respectively. This decrease is followed by a constant rise in HR at the 5th minute (**Figure 3**) not exceeding the initial value, this rise was again followed by a considerable decrease until the 60th minute unlike the F1 fraction. Only the extract and the F3 fraction showed respectively a very significant decrease of −47.99% ± 0.06%; −50.25% ± 2.13% ($p < 0.001$), followed by a rise in the 5th minute and then a progressive relapse until the 60th minute.

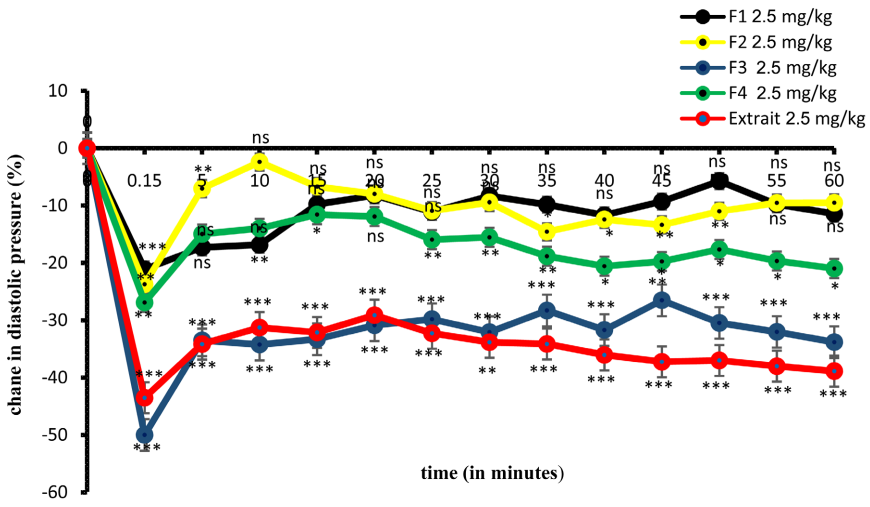

Figure 1. Effects of aqueous extract of *D. velutinum* leaves and different fractions on diastolic blood pressure (DBP) in normentensive rats. Each point is a mean ± ESM with n = 3. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ significant difference in relation to the DBP initial value.

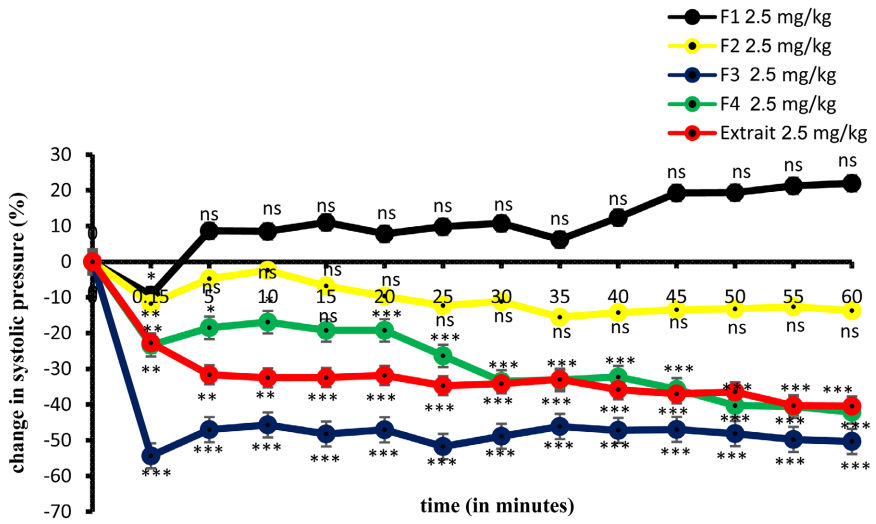

Figure 2. Effects of the aqueous extract of *D. velutinum* leaves and different fractions on systolic blood pressure (SBP) in normentensive rats. Each point is a mean ± ESM with n = 3. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ significant difference in relation to the SBP initial value.

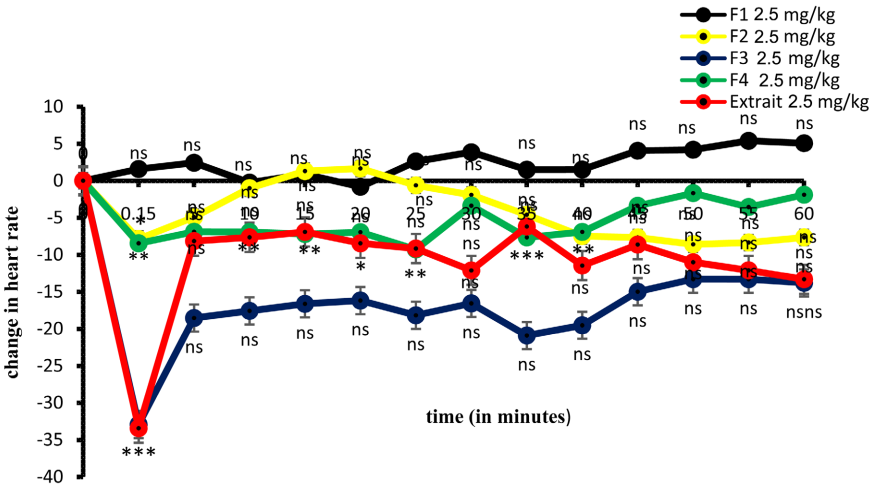

Figure 3. Effects of aqueous extract of *D. velutinum* leaves and different fractions on heart rate (HR) in normotensive rats. Each point is a mean ± ESM with n = 3. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ significant difference in relation to the HR initial value.

3.2. Thin-Layer Chromatography of the Fractions and the Extract of *D. velutinum*

The chromatogram in **Figure 4** shows the chromatographic profiles of the aqueous extract and the different fractions (F1, F2, F3 and F4) of the leaves of *D. velutinum*. This profile shows, after the sputtering of the plate with Neu and visualization at 366 nm, different streaks that can materialize the presence of several chemical families.

The orange-yellow fluorescences at the frontal ratios (0.15; 0.28; 0.85 and 0.58) highlighted in the extract and fractions, could be attributed to the derivatives of an ortho-di-hydroxylated flavonol at the 3' and 4' position in the flavonol

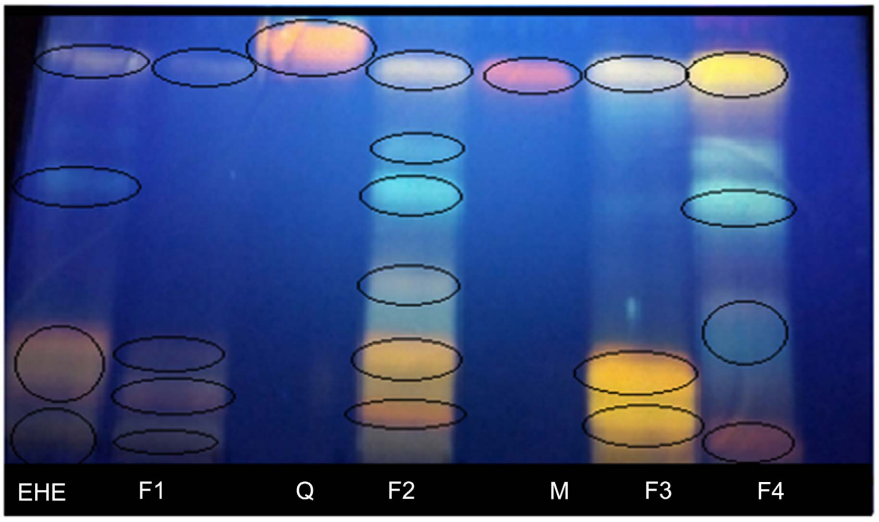

Figure 4. Chromatographic profiles of the aqueous extract and the different fractions of *Desmoduim elutinum* leaves and reference compounds: Eluent Ethyl acetate/formic acid/water (9.5/0.25/0.25); Developer: Neu; Observation: UV-366 nm. Reference compounds: Q: Quercetin and M: Myricetin. Hydroethanol extract: HEE.

ring, the blue to bluish-white fluorescent compounds at the ratio (0.72; 0.85; 0.28) on fractions F2, F4 and the extract could be attributable to phenolic acid on fractions F2, F4 and the extract could be attributable to phenolic acid derivatives, the light yellow compounds at the frontal ratios (0.87 and 0.84) their presence is characteristic to quercetin and myrtecin derivatives.

3.3. Determination of Total Polyphenols and Flavonoids in the Extract and Fractions of *D. velutinum*

The results of the quantitative analysis by UV-visible spectrophotometer of the extract and the different fractions of *D. velutinum* studied are represented in **Figure 5**. In this composition we can see that the fractions are quantitatively richer in phenolic compounds (polyphenols and total flavonoids) than the extract. The quantitative analysis of total polyphenols and flavonoids also shows that fractions F4, F3, and F2 (**Figure 5**) are quantitatively richer in flavonoids and total polyphenols than fraction F1. The contents of total polyphenols and total flavonoids in the extract are respectively 354.55 mgEAG/100g/Ms and 114.22 mgEQt/100gMs and the contents of total flavonoids in the fractions are respectively: 266.38; 221.9; 355.54 and 376.67 mgEQt/100gMs and those of polyphenolic compounds are: 404.27; 530.55; 566.76 and 612.25 mgEAG/100gMs for fractions F1, F2, F3 and F4 respectively.

4. Discussion

We conducted a study on the hypotensive effects of the extract and different fractions of *D. velutinum* leaves in normotensive rats. The experimental studies showed that the administration of the extract and different fractions at the dose of 2.5 mg/kg in rats caused an immediate decrease in blood pressure and heart

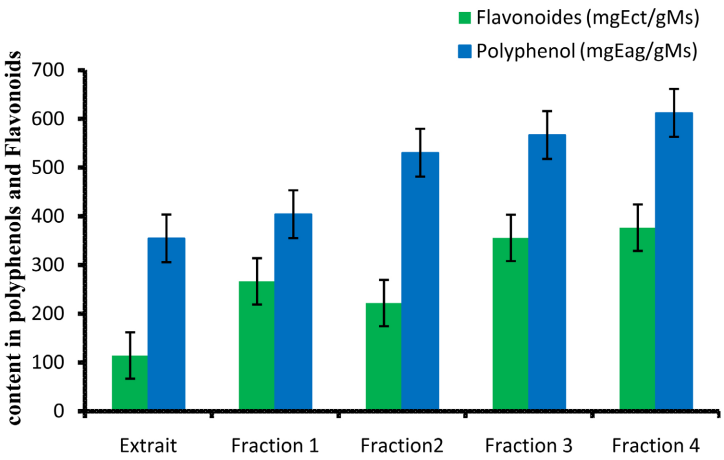

Figure 5. Content in total polyphenols et flavonoids.

rate. The significant decrease in blood pressure at the fractions varied as follows: F3 (−49.99% ± 0.75%); F4 (−27.59% ± 5.86%); F2 (26.88% ± 3.44%) and F1 (−23.72% ± 2.74%) and the extract −23.28% ± 0.65% ($p < 0.001$), at the same dose, the same trends were observed in heart rate. The F3 fraction showed a decrease in blood pressure and heart rate greater than that of the extract and all fractions. This decrease suggested that the extract and fractions could have hypotensive effects. Many previous works have shown the same results with other plants ([9] [10] [11] [12]) and on the other hand by the richness of polyphenols and flavonoids observed by thin-layer chromatography, also by the high content of total polyphenols and total flavonoids when assayed. In addition, it was demonstrated a positive correlation between the total polyphenolic content and the cardiovascular diseases. In this context, we can say that the latter, suspected to be the origin of the manifest pharmacological property, could probably be the combined action of polyphenols and flavonoids (quercetin, Murtecin and Rutin); saponins and sterols. Indeed, saponins and flavonoids of their diuretic effect, combat effectively high blood pressure. They are also likely to stimulate the production of nitric oxide (NO) which induces endothelium vasorelaxation which depends on arteries, thus causing a decrease in blood pressure [13]. As for polyphenols, they have a vasodilative and hypotensive effect [14]. However, flavonols and sterols in plants reduce the absorption of LDL-cholesterol, thus reducing the risk of atheromatous vascular plaque formation, a factor that triggers hypertension, and thus resulting in vascular protection leading to an increase in capillary resistance [15] [16] suggested that luteolin-8-C-glucoside is a flavonoid that protects the integrity of the vascular barrier by inhibiting hyperpermeability. Therefore, it is useful as a therapy for vascular inflammatory diseases. However, it is important to note that the significant decrease in blood pressure caused by the F3 fraction could be justified by the abundance of polyphenolic compounds observed on the F3 fraction during thin-layer chromatography, also by the presence of non-cholinomimetic hypotensive active principles next to cholinomimetic substances of muscarinic-type in this fraction. These substances could be calcium

channel blockers, similar results on the F3 fraction were obtained with *Terminalia superba* Englers et Diels (Combretaceae) on the rings of the aorta [17]. The decrease in blood pressure observed in the extract could be explained by the synergy of chemical compounds found in the aqueous extract unlike the fractions. Also, the high content of total polyphenols and total flavonoids in the fractions and extract could justify in part the traditional use of this plant on hypotension.

5. Conclusions

Our results allow us to attest to the hypotensive efficiency of the fractions and the aqueous extracts on normotensive rats.

Our work also shows the efficiency of the F3 fraction compared to the other fractions and the aqueous extract with regard to the reduction of blood pressure and heart rate.

Another part of this work was devoted to the phytochemical study of the fractions and the aqueous extract of the leaves of *D.velutinum* more precisely on the qualitative analysis (thin-layer chromatography) and the quantitative analysis (determination of total polyphenols and total flavonoids). Our analytical data showed the presence of different polyphenolic compounds both by fractionation and thin-layer chromatography (TLC) than by assay. This high presence of polyphenolic compounds in fractions F3, F4 and F2 confirms in pharmacology a hypotensive activity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Benvenuto, L. and Krakoff, L. (2011) Morbidity and Mortality of Orthostatic Hypotension: Implications for Management of Cardiovascular Disease. *The American Journal of Hypertension*, **24**, 135-144.

[2] Tankeu, A. and Noubiap, J. (2016) Oscillation Blood Pressure Therapeutic Target in Type 2 Diabetes Patients with Hypertension. *Annals of Translational Medicine*, **4**, Article No. 422.

[3] OMS (2002) Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. Genève, 135 p.

[4] Nguelefack, T. (2002) Effets antihypertenseurs des composes neutres isolés de l'extrait au chlorure methylene/ethanol des feuilles des feuilles de Bidenspilosa Linn. (*Astera cea*) chez le rat. These de Doctorat, 3eme cycle Faculté de Science université de Yaoundé Cameroun, Yaoundé, 136 p.

[5] Dimo, T., Nguelefack, T. and Tan, P. (2003) Possible Mechanisms of Action of the Neutral Extract from *Bidens pilosa* L. Leaves on the Cardiovascular System of Anaesthetized Rats. *Phytotherapy Research*, **17**, 1135-1139.

[6] Lagnika, L. (2005) Etude Phytochimique et Activité Biologique de Substances naturelles Isolées de plantes Béninoises. Thèse de doctorat, Université de Louis

Pasteur Strasbourg-France, Strasbourg, 280 p.

[7] Muanda, F. (2010) Identification de polyphénols, évaluation de leur activité antioxydante et étude de leurs propriétés boilogiques. Thèse de doctorat, Université Paul Verlaine, Metz, 106 p.

[8] Andzi Barhé, T., Ngoualali, T., Gallo Mongo, B., Amboyi, A. and Ongoka, R. (2020) Chemical Screening, Total Polyphenols and Flavonoids Content, Hypotensive and Anti-Radical Activities from the Extracts of Leaves of *Urena lobata* L. *International Journal of Green and Herbal Chemistry*, **9**, 532-542.

[9] Ngolo, E., Etou Ossibi, A., Epa, C., Loubanou, C., Samba Ndossi, A., Ouamba, J. and Abena, A. (2018) Effet hypotenseur de l'extrait aqueux de la recette à base de *Brillantaisia patula* T. Anderson (Acanthaceae) et de *Desmodium velutinum* (Willd) D.C (Fabaceae) chez le rat. *International Journal of Multidisciplinary and Current Research*, **6**, 1417-1418.

[10] Etou Ossibi, A.W., Elion Itou, R.D.G. and Nzonzi, J. (2014) Effets de l'extrait aqueux de *Lippia multiflora* Moldenke (Verbenaceae) sur la pression artérielle, la fréquence cardiaque et les ondes de l'électrocardiogramme chez le rat normotendu. *Revue CAMES-Série Pharmacopée et Médecine Traditionnelle Africaine*, **17**, 1-9.

[11] Titrikou, S., Eklu-Gadegbeku, K. and Aklikokou (2008) Effets de Biophytum petersianum (Oxalidaceae) sur la pression artérielle chez le rat Wistar. *Phytothérapie*, **6**, 215-218.

[12] Nguelefack, T. (2002) Effets analgésiques et cardiovasculaires des extraits de feuilles de *Kalanchoe crenata* (Andrews) Haworth. Thèse de Doctorat PhD, Université de Yaoundé I, Yaoundé, 162 p.

[13] Beneto, S., Lopez, D., Sàiz, M., Buxaderas, S., Sàanchez, J., Puig-Parellada, P. and Mitjavila, M. (2002) Flavonoïd-Rich Diet Increases Nitric Oxide Production in Rat Aorta. *British Journal of Pharmacology*, **135**, 910-916. https://doi.org/10.1038/sj.bjp.0704534

[14] Deliorman, D., Calis, L. and Ergur, F. (2000) Studies on the Vascular Effects of the Fractions and Phenolic Compounds Isolated from *Viscum album* ssp. *Journal of Ethnopharmacology*, **72**, 323-329. https://doi.org/10.1016/S0378-8741(00)00251-8

[15] Stoclet, J., Chataigneau, T., Ndiaye, M., Oak, M., El Bedoui, J., Chataigneau, M. and Schini-Kerth, V. (2004) Vascular Protection by Dietary Polyphenols. *European Journal of Pharmacology*, **500**, 299-313. https://doi.org/10.1016/j.ejphar.2004.07.034

[16] Lee, W., Ku, S. and Bae, J. (2014) Vascular Barrier Protective Effects of Orientin and Isoorientin in LPS-Induced Inflammation *in Vitro* and *in Vivo*. *Vascular Pharmacology*, **62**, 3-14.

[17] Ngo Lemba, E., Demougeot, C., Bopda, O., Dimo, T., Dzeufiet, P., Bilanda, D., Corinne, G. and Berthelot, A. (2011) The Aqueous Extract of *Terminalia superba* (Combretaceae) Prevents Glucose Induced Hypertension in Rats. *Journal of Ethnopharmacology*, **133**, 828-833.